Management of Unresectable Pancreatic Ductal Cancer

*The SSAT, AGA, AASLD, ASGE, AHPBA Consensus Panel**

General Summary

1. The median duration of survival for patients with unresectable pancreatic ductal cancer is 6 to 10 months.
2. Given this modest duration of survival, quality of life is an important clinical end point in the management of patients with unresectable pancreatic ductal cancer. Relief or palliation of biliary tract and gastroduodenal obstruction and pain are the major clinical issues affecting quality of life.
3. Multiple therapeutic approaches have been evaluated for the management of biliary tract and gastroduodenal obstruction. Choice of therapeutic approach is dictated by the temporal recognition of unresectability. Two broad groups of patients with unresectable pancreatic cancer are (1) the majority of patients with unresectable pancreatic ductal cancer recognized nonoperatively (by imaging) and (2) the minority of patients with unresectable pancreatic ductal cancer recognized intraoperatively.
4. The preponderance of controlled and uncontrolled studies strongly support the consensus that endoscopic biliary stenting provides optimal relief of jaundice in patients with unresectable pancreatic ductal cancer recognized nonoperatively. Percutaneous transhepatic stenting is reserved for patients in whom endoscopic stenting has failed or is precluded technically. When unresectable pancreatic ductal cancer is recognized intraoperatively, controlled and uncontrolled data support operative bilioenteric bypass, regardless of prior biliary stenting.
5. Accumulated data support open gastroenterostomy for palliation of gastroduodenal obstruction. Data are insufficient for consensus on prophylactic gastroenterostomy during operative bilioenteric bypass.
6. Pain adversely affects quality of life in a majority of patients with unresectable pancreatic ductal cancer; pain control improves quality of life. Both controlled and uncontrolled data support the consensus that both operative and percutaneous neurolytic celiac plexus block are more effective than oral analgesia for relief of pain.
7. Antineoplastic therapy—irradiation, chemotherapy, and immunotherapy—currently have limited impact on overall duration of survival. Controlled data for combined irradiation and systemic chemotherapy and chemotherapy alone show overall survival is improved by a few months. Sparse data on immunotherapy do not support a survival benefit. There are insufficient controlled or uncontrolled data to support a consensus on a standard antineoplastic therapeutic regimen for patients with unresectable pancreatic ductal cancer.
8. Future studies of patients with unresectable pancreatic ductal cancer should address objective measures of quality of life and survival. Stage of disease and specific symptoms should be carefully defined for patients in studies.

Palliation of Jaundice and Gastroduodenal Obstruction

The panel agrees that biliary trace and gastroduodenal obstructions are major clinical problems facing patients with unresectable pancreatic ductal cancer. Quality of life improves substantially with relief of either or both obstructions.

Endoscopic Stenting for Palliation of Jaundice. If unresectable pancreatic ductal cancer is recognized preoperatively, endoscopic biliary stenting is preferred. Biliary stenting is technically feasible in 95% of patients. Current data support the use of either a plastic stent or an expandable metallic stent. Metallic stents, although not exchangeable, are preferred when life expectancy exceeds 3 months. Occlusion of either type of stent by ductal debris or tumor remains a problem. Further study of the composition and design of stents with regard to quality of life and cost efficiency are needed.

Percutaneous Transhepatic Stenting. Percutaneous transhepatic biliary stenting is reserved for those patients with unresectable pancreatic ductal cancer in whom endoscopic biliary stenting fails, or in the absence of endoscopic expertise. Technical feasibility ex-

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ceeds 95%. The durability of transhepatic biliary stenting is similar to that of endoscopic stenting.

Operative Bilioenteric Bypass. When unresectable pancreatic ductal cancer is established intraoperatively, bilioenteric bypass for biliary obstruction is recommended even if prior nonoperative stenting has been employed successfully. Technical feasibility approaches 98% unless precluded by carcinomatosis or extensive local disease. Operative bypass of the hepatic or common duct is preferred over the gallbladder. Recurrence of jaundice is less common after operative than nonoperative methods. Laparoscopic bilioenteric bypass is yet unestablished.

Gastric Outlet Obstruction. When gastroduodenal obstruction occurs in patients with unresectable pancreatic ductal cancer, operative gastrojejunostomy is indicated. In patients with unresectable pancreatic ductal cancer established intraoperatively without gastroduodental obstruction, no objective intraoperative criteria reliably predict subsequent gastric outlet obstruction. Although prophylactic gastrojejunostomy in these patients does not increase mortality, morbidity is increased. Current data are insufficient to recommend routine prophylactic gastrojejunostomy in such patients. Endoscopic or radiologic gastrointestinal stenting is yet unestablished.

Pain Management

Pain is a significant clinical problem in most patients with unresectable pancreatic ductal cancer and warrants specific treatment. Most patients who have pain are candidates for management through a stepwise pharmacologic progression of oral or transdermal analgesia. However, side effects can significantly impair quality of life.

Recent controlled data suggest that a percutaneous neurolytic splanchnic plexus block provides better initial pain relief and improved quality of life, compared with oral analgesia, in patients with unresectable pancreatic ductal cancer. Although duration of response is limited, repeat blocks can be successful. Transthoracic (thorascopic) splanchnicectomy and endoscopic ultrasonographic splanchnic plexus blocks are yet unestablished. Further studies comparing neurolytic blocks—either operative or nonoperative—to a progressive pharmacologic pain management ladder for efficacy of pain relief and quality of life are warranted.

Current controlled data support intraoperative chemical neurolytic splanchnic block in patients found unresectable at operation. Benefit has been demonstrated in intensity and duration of pain relief, quality of life, and perhaps survival. Intraoperative chemical splanchnicectomy should be strongly considered in all patients who are found to be unresectable at operation.

Antineoplastic Therapy

Irradiation. Irradiation has been employed to control tumor growth in patients with locally unresectable pancreatic cancer with a good clinical performance status. External-beam radiation with 5-fluorouracil provides a median survival of 10 months and a 2-year survival of 12%. External-beam irradiation plus intraoperative irradiation plus 5-florouracil provides a median survival of 12 months and a 2-year survival of 20%. External-beam irradiation with newer radiation sensitizers warrants further study. Current data do not suggest that there is a significant enough survival benefit for intraoperative irradiation to warrant that procedure in patients with unresectable pancreatic ductal cancer except in controlled trials.

Chemotherapy. Currently the results of chemotherapy for patients with unresectable pancreatic ductal cancer remain disappointing with respect to survival. Alternative measures of tumor control and quality of life are under evaluation. Presently the two most active agents against pancreatic ductal cancer are 5-fluorouracil and gemcitabine. Median survival, however, remains approximately 6 months. To date, combination chemotherapy has not improved survival over single-agent chemotherapy in patients with unresectable pancreatic ductal cancer.

Immunotherapy. Regardless of type, immunotherapy remains unestablished for patients with unresectable pancreatic ductal cancer. Results of immunotherapy on survival, tumor regression, and quality of life are few. Further studies, however, are warranted in well-defined subgroups of patients with unresectable pancreatic ductal cancer.

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Endoscopic Palliation of Pancreatic Cancer

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Endoscopic palliation of jaundice associated with pancreatic cancer was introduced by Soehendra and Reynders-Frederix[1] in 1979. Despite great enthusiasm for this technique and its success in relieving jaundice, few studies have addressed whether or not this intervention actually improves the patient's quality of life; it has always be *assumed* that it does. To date no randomized, prospective trials have been reported in which treatment with stents was compared to no therapy. Ballinger et al.[2] were the first to report a systematic assessment of quality of life in 19 patients with malignant bile duct obstruction in which the patients themselves were used as historical controls. These investigators reported that patients experienced significant improvement in anorexia and indigestion. However, mood, physical health, and level of activity were unchanged 12 weeks after stent placement. In another study of patients with malignant obstructive jaundice, Luman et al.[3] used the European Organization for Research and Treatment of Cancer Quality of Life (EORTC QLQ-30) questionnaire to evaluate the effect of stenting on quality of life in 38 patients with malignant obstructive jaundice. In that study there was a significant improvement in emotional, cognitive, and global health scores in addition to the expected relief of jaundice and pruritus. Additionally, anorexia, diarrhea, and sleep patterns were significantly improved. Finally, Sherman et al.[4] used the Functional Assessment of Cancer Therapy (FACT) as the instrument to measure quality of life in 53 patients with malignant obstructive jaundice. The questionnaire was administered before biliary stenting and 30 days after stenting. In the subset of 30 patients with pancreatic cancer, there was a statistically significant improvement in energy levels, coping with illness, nervousness, fear of dying, acceptance of illness, sleep, abdominal cramps, weight, aches and pains, and pruritus. The authors concluded that stenting in malignant obstructive jaundice results in improvement in physical and functional well-being and is therefore a justifiable treatment that improves quality of life. Although these studies all have some methodologic problems, a consistent conclusion from each is that endoscopic stenting in malignant obstructive jaundice relieves symptoms and improves quality of life compared to the time before stenting.

ENDOSCOPIC STENTING

Stent Diameter

Many issues regarding endoscopic stenting in malignant obstructive jaundice have been addressed yet many more remain. The first duodenoscopes had small working channels, which limited the size of the stent that could be placed. Relatively early on, the following question was posed regarding efficiency of stent size: "Is bigger better?" The issue of stent size was addressed in part in a study by Speer et al.[5] in which 8 Fr and 10 Fr stents were compared. In that study the incidence of cholangitis was significantly lower in the 10 Fr group (5%) than in the 8 Fr group (34%). Stent patency was significantly longer for the 10 Fr stents as well (median 32 weeks vs. 12 weeks). This study prompted mandatory use of a large-channel duodenoscope for palliation of jaundice associated with malignant bile duct obstruction. The 10 Fr polyethylene stent has become the standard. The usual biopsy channel size for therapeutic duodenoscopes is 4.2 mm. This channel will accommodate up to an 11.5 Fr stent. With this capacity the question was raised as to whether the 11.5 Fr stent would be better than the 10 Fr stent. Interestingly, however, in a comparison of 10 Fr and 11.5 Fr stents in 212 patients with malignant obstructive jaundice, there was a trend toward improved patency for the 11.5 Fr size, but no reduction in the number of stent-related interventions or days spent in the hospital for stent dysfunction.[6] The current standard of endoscopic practice is placement of 10 Fr plastic stents.

Plastic Stents

Plastic stents (polyethylene and Teflon) are relatively inexpensive but have a tendency to clog. Stud-

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ies of stent clogging have revealed that bacteria first adhere to the inner surface of the stent and then secrete a glycoprotein matrix called biofilm as protection.[7] Subsequently calcium bilirubinate precipitates, and cellular debris and food particles, which reflux through the stent from the duodenum, are trapped within the biofilm forming sludge. The material slowly builds until the stent clogs. The key to avoiding this sequence of events is to delay or prevent the adherence of bacteria to the inner surface of the stent. The mean time to stent blockage for a 10 Fr stent is approximately 4 to 5 months. As a result, many patients will require at least one stent exchange before dying of their cancer. Stent exchange increases costs and diminishes the quality of life for these terminally ill patients. Several approaches have been tried in an attempt to prolong stent patency, such as rotating antibiotics to kill bacteria, ursodeoxycholic acid to alter bile composition, and new materials (Teflon) to render the inner surface nonadherent.

Stents have been impregnated with antibiotics or silver (a bactericide) to prevent bacterial adherence. A recent randomized study compared patients with a "slicker" Teflon (Tannenbaum-Wilson Cook) stent to those with a standard polyethylene stent.[8] A total of 106 patients (54 with Tannenbaum and 52 with polyethylene stents) were entered into the study, and the randomization was equitable. Stent placement was successful in 96% and 100% of patients receiving polyethylene and Tannenbaum stents, respectively. The median time to stent occlusion was 100 days for the polyethylene and 104 for the Tannenbaum group (no difference). Thus, at least in this trial, no advantage was seen for the slicker Teflon stent over the standard polyethylene stent.

Although Teflon is inherently smoother than polyethylene, when it is extruded in the manner used to make stents, pits and irregularities develop on the inner surface where bacteria can adhere. Results of a preliminary study evaluating a new double-layer stent have been recently been reported.[9] This stent has the following two theoretical advantages: (1) It has a double-layer construction and, although it has flaps to prevent proximal and distal migration, there are no side holes. It has been known for some time that side holes provide an excellent site for bacterial adherence; thus if these side holes are eliminated, the stent should remain patent for a longer period. (2) This stent has a coating of Teflon applied to the inner surface of the stent rather than the whole stent being extruded from a piece of Teflon, which allows for a much smoother inner surface. In the first study to report on its effectiveness, a group of Japanese investigators found that the median patency period of the new double-layer stent was 180 days, which was longer than that for standard plastic stents (114 days) and uncovered expandable metal stents (142 days) but shorter than that for covered metal stent (261 days).[9] Antibiotic and silver impregnation of stents has also been tried and tested in vitro, but such stents have not been tested clinically.

Metal vs. Plastic Stents

The "holy grail" of endoscopic stenting is the development of a stent that will stay predictably patent for the remainder of the patient's life. Plastic stents clog and the diameter of plastic stents is limited by the working channel of the duodenoscope. In the early 1990s, expandable metal stents were introduced into the practice of endoscopy. Their advantage lay in the fact that they could be confined to a catheter that was smaller than 10 Fr but when released, they would automatically expand to 10 mm (30 Fr). When they were introduced, they were heralded as the long-awaited "savior" for endoscopic stenting with the prediction that their patency would far exceed that of plastic stents. Unfortunately they have not entirely lived up to their billing. Although they are largely free of clogging by sludge, tumor ingrowth occurs without stent coating and when stents are coated with a plastic, cancers tend to obstruct either end of the stent or the stent migrates. An additional drawback for metal stents is their cost. Moreover, in many areas of the United States, expandable stents are not reimbursed separately from the facility fees. When this is the case, the hospital "cost" of performing the endoscopic retrograde cholangiopancreatography (which must include all accessories *and* the metal stent) will exceed reimbursement and the hospital will loose money.

Despite these drawbacks, several randomized studies have compared stent patency and costs of expandable metal vs. plastic stents. The first large randomized trial was reported in 1992 by Davids et al.[10] In this study, 105 patients were randomized to receive either a metal stent (Wallstent, Schneider Stent, Minneapolis, Minn.) or a plastic stent. Median stent patency was significantly longer in patients with metal stents (273 vs. 126 days). Median survival of patients was 149 days and did not differ between groups. An incremental cost-effectiveness analysis showed that the initial placement of a metal stent resulted in a 28% cost decrease for endoscopic procedures.

A second large randomized multicenter study was conducted in the United States, but the data have been reported in abstract form only.[11] In this study, 163 patients with malignant obstructive jaundice were randomized to receive either a Wallstent or a polyethylene stent. Wallstents and plastic stents were suc-

cessfully placed in 98% and 95% of patients, respectively (no difference). Median time to stent obstruction was 62 days for plastic stents vs. 111 days for Wallstents (significant difference). This study was complicated by the fact that patients who presented for a stent change could also be included.

In a more recent study, 105 patients were randomized to groups receiving either metal stents or 11.5 Fr plastic stents, where half of the plastic stent group underwent stent exchange on a scheduled basis while the other half had their stents exchanged only when symptoms or signs of obstruction occurred.[12] Symptom-free survival and cost of therapy were compared among the three groups. Patients with metal stents and plastic stents who had their stents exchanged on demand were symptom free significantly longer than those undergoing scheduled stent changes. The cost for the metal stent group was $904 less than the cost for the exchange-on-demand plastic stent group and $2,127 less than the cost for the scheduled-exchange plastic stent group. However, plastic stents were more cost-effective if the patient lived less than three months. Herein lies the key to decisions concerning endoscopic stenting in 1998; plastic stents are most cost-effective in patients with short life spans (<3 months), whereas metal stents are more advantageous in more fit patients who will outlive the patency of a plastic stent. The problem is that we do not have a "crumple index," or an accurate measure of life expectancy at the time of presentation. Consequently we cannot predict, at the time of the initial stent placement, how long the patient will live. In light of this deficiency, endoscopists are relegated to making decisions based on their intuition concerning patient survival, but they are also influenced by practical issues such as reimbursement for metal stents.

ENDOSCOPIC STENTING VS. SURGICAL BYPASS

In the mid-1980s, the surgical literature advocated open bypass as the best palliation for pancreatic carcinoma. The surgical literature cited low morbidity and mortality and relatively long intervention-free survival. This spawned the famous editorial by Dr. Peter Cotton[13] entitled "Apples and Oranges." In this frequently quoted editorial, Cotton exposed the problems of comparing different treatments by reviewing results of case series rather than conducting well-designed prospective, randomized trials. It was evident that endoscopists were performing stent placement on elderly, frail patients with comorbid illnesses that rendered them unfit for surgical intervention. Alternatively, patients who were relatively young with few comorbid illnesses and good nutritional status were undergoing surgical bypass. These two disparate groups precluded clinical comparisons of treatment outcomes.

To compare surgical vs. endoscopic palliation of jaundice in patients with pancreatic cancer, a randomized comparative trial was conducted at the Middlesex Hospital in London.[14] In that study 204 patients were randomized to either surgery (n = 103) or stent placement (n = 101), and technical success was achieved in 94% and 95% of surgical and stent patients, respectively. Stented patients had a lower procedure-related mortality (3% vs. 14%; $P = 0.01$), major complication rate (11% vs. 29%; $P = 0.02$), and median total hospital stay (20 vs. 26 days; $P = 0.001$). However, recurrent jaundice occurred in 36 stented patients and only two surgical patients. Median survival was similar in the two groups (surgical = 26 weeks, stent = 21 weeks; $P = 0.065$). Regrettably, no economic analysis was performed on the results from this study. Practitioners are left to conclude that endoscopic stenting is easier and safer but surgical bypass provides more durable relief of jaundice. These data suggest that fit patients with unresectable pancreatic cancer benefit from surgery, whereas the others should be treated with endoscopic stenting.

ENDOSCOPIC VS. PERCUTANEOUS STENT PLACEMENT

In the mid-1980s, both endoscopic and percutaneous techniques were the two main alternatives to relieve jaundice in patients with pancreatic cancer. A prospective, randomized trial comparing these techniques was completed in 1986.[15] Seventy-five patient with malignant obstructive jaundice were randomized to percutaneous or endoscopic stenting with plastic stents being used in both groups. The endoscopic approach was significantly more successful (81% vs. 61%; $P = 0.017$) and had a significantly lower 30-day mortality (15% vs. 33%; $P = 0.016$). Outcomes of the percutaneous approach were influenced by trial design requiring a large-caliber (10 to 12 Fr) stent. Although large stents were considered necessary to achieve a reasonably durable stent patency, large stents caused complications (e.g., bile leaks, bleeding, etc.) as they were advanced through the liver. Consequently further comparative trials of these techniques with different stent compositions are warranted.

SUMMARY

There are many approaches to palliation of jaundice in patients with pancreatic cancer. Each stenting technique has merit. Regrettably, an insufficient number of randomized comparative trials using the latest

technology have been performed. Moreover, economic analyses of techniques to aid in clinical decision making are few. Currently data support endoscopic stenting as the standard for most patients with obstructive jaundice from pancreatic cancer. Patients with an expected survival of more than 6 months should undergo placement of an expandable metal stent, whereas patients with a limited life expectancy (<3 months) are best served with a plastic stent. Randomized comparative studies are needed between laparoscopic techniques and endoscopically placed expandable metal stents. Percutaneous techniques may prove safer and more effective when metal expandable stents are used.

REFERENCES

1. Soehendra N, Reynders-Frederix V. Palliative gallengange drainage. Dtsch Med Wochenschr 1979;104:206-209.
2. Ballinger AB, McHugh M, Catnatch SM, et al. Symptom relief in quality of life after stenting for malignant bile duct obstruction. Gut 1994;35:467-470.
3. Luman W, Cull A, Palmer KR. Quality of life in patients stented for malignant biliary obstruction. Eur J Gastroenterol Hepatol 1997;9:481-484.
4. Sherman S, Lehman G, Earle D, et al. Endoscopic palliation of malignant bile duct obstruction: Improvement in quality of life [abstr]. Gastrointest Endosc 1995;41:414A.
5. Speer AG, Cotton PB, MacRae KD. Endoscopic management of malignant biliary obstruction: Stents of 10 French gauge are preferable to stents of 8 French gauge. Gastrointest Endosc 1988;34:412-417.
6. Sherman S, Lehman G, Earle D, et al. Are the patency rates for 10 French and 11.5 French stents different for common duct obstruction and hilar obstruction? Randomized, prospective study [abstr]. Gastrointest Endosc 1996;43:395.
7. Libby ED, Leung JW. Prevention of biliary stent clogging: A clinical review. Am J Gastroenterol 1996;91:1301-1308.
8. Meyerson SM, Geenen JE, Catalano MF, et al. "Tannenbaum" Teflon stents vs traditional polyethylene stents for treatment of malignant biliary strictures: A multicenter, prospective randomized trial. Gastrointest Endosc 1998;47: AB122.
9. Uno K, Nakajima M, Yasuda K, et al. Evaluation of a new plastic stent (double-layer stent) for inoperable malignant biliary obstruction. Gastrointest Endosc 1998;47:AB131.
10. Davids PH, Groen AK, Rauws EA, et al. Randomized trial of self-expanding metal stents versus polyethylene stents for distal malignant biliary obstruction. Lancet 1992;340:1488-1492.
11. Carr-Locke DL, Ball TJ, Connors PJ, et al. Multicenter randomized trial of Wallstent biliary endoprosthesis versus plastic stents [abstr]. Gastrointest Endosc 1993;39:310.
12. Prat F, Chapat O, Ducot B, et al. A randomized trial of endoscopic drainage methods for inoperable malignant strictures of the common bile duct. Gastrointest Endosc 1998;47:1-7.
13. Cotton PB, Endoscopic management of bile duct stones (apples and oranges). Gut 1984;25:587-597.
14. Smith AC, Dowsett JF, Russel RC, et al. Randomized trial of endoscopic stenting versus surgical bypass in malignant low bile duct obstruction. Lancet 1994;344:1655-1660.
15. Speer AG, Cotton PB, Russell RC, et al. Randomized trial of endoscopic versus percutaneous stent insertion in malignant obstructive jaundice. Lancet 1987;2:57-62.

Palliation of Jaundice in Unresectable Pancreatic Carcinoma: Radiologic-Guided Interventions

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The percutaneous transhepatic approach for biliary stenting is a well-established technique that affords reliable access to the dilated intrahepatic biliary tree and highly effective palliation of obstructive jaundice.[1] The relief of symptoms and improvement in quality of life are much appreciated by these patients despite their limited survival.

INDICATIONS

Percutaneous transhepatic biliary drainage (PTBD) is indicated in patients with complete or partial biliary obstruction. In most institutions, PTBD is reserved for palliative drainage in those patients in whom retrograde endoscopic drainage is not possible. Endoscopic drainage is the first choice because of its lower complication rate and because it can often be performed on an outpatient basis and completed in a single intervention. PTBD involves at least an overnight stay in the hospital but can almost always be performed successfully in 96% to 100% of patients with dilated biliary ducts if endoscopic stenting fails.[2] Failure of endoscopic stenting may occur in patients with advanced pancreatic lesions with complete biliary obstruction or in patients who have altered upper gut anatomy that precludes a retrograde approach because of prior surgery, for example, following a Whipple procedure, or as a result of gastroduodenal obstruction. PTBD may be favored in particular centers because of local differences in expertise or unavailability of alternative techniques. In patients with obstructive biliary sepsis, the percutaneous approach may be preferable to the endoscopic route because it can be performed simply and rapidly on the supine patient. Relative contraindications to PTBD are coagulopathies, which should be addressed by suitable infusions of blood products, and significant ascites, which may require preliminary tapping.

TECHNIQUES

The usual percutaneous approach is by the right intercostal route. Some centers advocate a left substernal approach, which is thought to be less painful than the intercostal route. It allows the patient free movement and comfort when lying in bed, minimizes the catheter dislodgment that sometimes occurs with respiratory movements, and eliminates the danger of puncture of the pleural space.[3] Hayashi et al.[3] combined the left-sided approach with the use of real-time sonographic guidance to achieve a near 100% success rate with an average of 1.1 needle passes and the elimination of radiation to the operator's hands. The technique of sonographic guidance for either right or left approaches is attractive and is becoming more widely used; however, no convincing data have been published showing its advantage over fluoroscopic techniques. The different choices of routes, needles, catheters, and puncture techniques have strong advocates who all base their decisions on personal experience; however, comparative data confirming the optimal method are scant.

STENT OPTIONS AND RESULTS

With established percutaneous biliary access, three options exist for drainage of the obstructed biliary system and for providing palliation of jaundice and sepsis secondary to obstruction. These options include (1) implantation of different endoprostheses, either plastic or expandable metallic, (2) placement of indwelling internal-external catheters, and (3) in the ex-

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treme situation of an impassable complete obstruction, placement of external drainage catheters.

Endoprostheses

Both tubular plastic and expandable metallic stents are prone to obstruction and have a limited duration of patency. However, many patients with pancreatic carcinoma die within 4 to 6 months and 12 Fr plastic stents often remain patent for the remainder of the patient's life providing good palliation.[4] Metallic stents were anticipated to prolong stent patency significantly as they enabled a stent with a lumen of 10 mm to be introduced using an introducer system of only 8.5 Fr in outer diameter. In practice it was disappointing to find that even metallic stents were prone to occlusion as evident in 24% of our series of 50 Wallstents.[2] Although the expected benefits of metallic stents have not been completely realized, prospective randomized trials have shown advantages of metallic over plastic stents, which seems to offset the 8- to 10-fold greater cost of the metallic stents.[5] In the comparative trial by Lammer et al.[5] the data for plastic vs. metallic stents are, respectively, 30-day mortality 24% vs. 10%, median survival 98 days vs. 123 days, and median time to obstruction or death 81 days vs. 122 days.

The cause of occlusion of metallic stents is not well established.[6] Sludge formation is common and suggested approaches such as the use of choleretics and antibiotics have been tried with conflicting results. With low pancreatic lesions, stenting across the sphincter of Oddi is almost always needed, but theoretically, by leaving the stent above the sphincter and not breaching the integrity of the sphincter, reflux of enteric contents into the bile ducts can be minimized. The reduction of contamination of the biliary tree may reduce the resultant sludge formation. Most interventional radiologists agree that tumor overgrowth is also a significant factor in stent occlusion. Therefore stents long enough to extend well beyond the tumor margins are recommended and should be beneficial.[6] Filling defects demonstrated on cholangiograms are not necessarily the result of tumor ingrowth despite frequent supporting statements in the endoscopic literature. These defects are much more likely to result from sludge or debris. Histologic examination is required for differentiation. Tumor ingrowth through the mesh interstices of the metallic stent does occur, but its relative clinical importance is debatable as shown by two postmortem studies and by direct endoscopic inspection.[7-9] In the first study, there was ingrowth in only 1 of 15 patients. The tumor was a hepatoma causing 50% narrowing of the stent but no occlusion.[7] In the other autopsy study of 24 patients, nonobstructive tumor ingrowth was found in only one patient.[8] Work continues in an attempt to discover a pharmacologic stent coating that would inhibit sludge formation and tumor growth.[6] Initial work with Wallstents covered by a layer of polyurethane has shown no improvement in patency compared to bare metal stents.[10] There was considerable sludge formation and tumor overgrowth, and tumor was even found within the stent as a result of breakdown of the coating. Migration of coated stents has been a reported problem in early animal studies and pilot clinical trials. In the United States a limited pilot trial of silicone-coated Wallstents is underway. Potential problems with covered stents include obstruction of biliary drainage at the confluence of the major bile ducts and cystic duct or pancreatic drainage at the sphincter of Oddi. One of our early patients who was treated with a covered stent had to undergo cholecystectomy because of blocked drainage from the cystic duct. There are a number of different metallic stents in use with more in development. At present the most popular stent for palliation of malignant biliary obstruction is the Wallstent, which appears to be better than the Gianturco Z stent.[11] The Strecker stent used in Europe also appears effective.

In several large series reporting malignant obstructions of different etiologies, the average duration of patency for metallic stents was between 5.8 and 8.3 months and occlusion rates ranged from 7% to 24%.[1,2,11] In the multicenter European study by Rossi et al.[11] of 240 patients using life-table analysis, the overall survival rates were 42% at 25 weeks and 16% at 50 weeks. The 30-day mortality rate was 14.6%. The 25-week patency rate for nitinol Strecker stents was 78% and for Wallstents was 67%.[11] In the study by Hausegger et al.[10] of 30 patients with polyurethane-covered Wallstents, the 30-day mortality rate was 20% and the stent occlusion rate was 37%. The patency rates after 1, 3, 6, and 12 months were 96%, 69%, 47% and 31%, respectively.

Internal-External Drains

In patents in whom temporary drainage is required before surgery to allow improvement in the overall condition or for biopsy confirmation of malignancy, an internal-external drain can be positioned across the obstruction with external drainage to a bag. Capping of the tube allows internal biliary drainage. These tubes are easily exchanged over a wire and provide an effective alternative to implanted endoprostheses.

External Drains

If it is impossible to manipulate a stent through the biliary obstruction, a transhepatic catheter positioned in the bile ducts above the blockage provides good palliation.

COMPLICATIONS

Major complications associated with PTBD or percutaneous stent placement as reported in the Quality Improvement Guidelines of the Society of Cardiovascular and Interventional Radiology include bleeding (2.5%), sepsis (2.5%), localized inflammation (1.2%), pleural perforation (0.5%), and death (1.7%). These rates are an average for all patients and vary in the reports from different centers. Bleeding from portal or hepatic veins is almost always self-limited. Bleeding from hepatic arteries is successfully treated by selective arterial embolization.[12] Patients with continuing external drainage should be monitored for excessive fluid and electrolyte loss.

CONCLUSION

The percutaneous transhepatic placement of an uncovered expandable metallic stent is an effective and safe means of palliating the symptoms of obstructive jaundice resulting from unresectable carcinoma of the pancreas. This approach is indicated when endoscopic placement is not possible.

REFERENCES

1. Kaufman SL. Percutaneous palliation of unresectable pancreatic cancer. Surg Clin North Am 1995;75:989-999.
2. Gordon RL, Ring EJ, LaBerge JM, Doherty MM. Malignant biliary obstruction: Treatment with expandable metallic stents—Follow-up of 50 consecutive patients. Radiology 1992; 182:697-701.
3. Hayashi N, Sakai T, Kitagawa M, Kimoto T, Inagaki R, Ishii, Y. US-guided left-sided biliary drainage: Nine-year experience. Radiology 1997;204:119-122.
4. Dick BW, Gordon RL, LaBerge JM, Doherty MM, Ring EJ. Percutaneous transhepatic placement of biliary endoprostheses: Results in 100 consecutive patients. J Vasc Interv Radiol 1:97-100.
5. Lammer J, Hausegger KA, Fluckiger F, Wionkelbauer FW, Wildling R, Klein GE, Thurnher SA, Havelee L. Common bile duct obstruction due to malignancy: Treatment with plastic versus metal stents. Radiology 1996;201:167-172.
6. Libby ED, Leung JW. Prevention of biliary stent clogging: A clinical review. Am J Gastroenterol 1996:91:1301-1308.
7. Hausegger KA, Kleinert R, Lammer J, Klein GE, Fluckiger F. Malignant biliary obstruction: Histologic findings after treatment with self-expandable stents. Radiology 1992;185: 461-464.
8. Boguth L, Tatalovic S, Antonucci F, Heer M, Sulser H, Zollikofer CL. Malignant biliary obstruction: Clinical and histopathologic correlation after treatment with self-expanding metal prostheses. Radiology 1994;192:699-674.
9. Hausegger KA, Mischinger HJ, Karaic R, Klein GE, Kugler C, Kern R, Uggowitzer M, Szolar D. Percutaneous cholangioscopy in obstructed bilary metal stents. Cardiovasc Intervent Radiol 1997;20:191-196.
10. Hausegger KA, Thurnher S, Bodendorfer G, Zollikofer CL, Uggowitzer M, Kugler C, Lammer J. Treatment of malignant biliary obstruction with polyurethane-covered Wallstents. Am J Roentgenol 1998;170:403-408.
11. Rossi P, Bezzl M, Adam A, Chetty N, Roddle ME, Iacarl V, Cwikiel W, Zollikofer CL, Antonucci F, et al. Metallic stents in malignant biliary obstructions: Results of a multicenter European study of 240 patients. J Vasc Interv Radiol 1994;5:279-285.
12. Burke DR, Lewis CA, Cardella JF, Citron SJ, Drooz AT, Haskal ZJ, Husted JW, McCowan TC, van Moore A, Oglevie SB, Sacks D, Spies JB, Towbin RB, Bakai CW. Quality improvement guidelines for percutaneous transhepatic cholangiography and biliary drainage. J Vasc Interv Radiol 1997;8: 677-681.

Palliation of Jaundice: Operative Bypass

Hans G. Beger, M.D., F.A.C.S., Andreas Schwarz, M.D.

Palliation of jaundice can be achieved in patients with unresectable pancreatic cancer by operative biliary bypass, endoscopic transpapillary biliary stenting, or percutaneous transhepatic biliary stenting. This synopsis shows the results of controlled clinical trials and large multicenter studies and attempts to answer the following questions: (1) Do operative biliary bypass procedures show any advantage over endoscopic or percutaneous transhepatic stent insertion with regard to morbidity, mortality, quality of life, readmission rates, recurrent jaundice, later gastric outlet obstruction, reoperation rates, survival time, and costs; (2) does preoperative biliary stent insertion reduce morbidity and mortality; and (3) should the initial operative palliation consist of a biliary bypass alone or should a combined biliary and gastric bypass be undertaken?

BILIARY BYPASS OPERATION VS. ENDOSCOPIC BILIARY STENTING

A comparison of biliary bypass operation and endoscopic biliary stenting was undertaken in three randomized studies[1-3] (Table I). Regarding hospital stay, subsequent gastric outlet obstruction, and duration of survival, there were no significant differences between the two approaches. Recurrent jaundice, however, was present more frequently in patients with endoscopic stents. Procedure-related complication and 30-day mortality rates were significantly lower in patients with endoscopic stenting only in the study by Smith et al.[3]

BILIARY BYPASS OPERATION VS. PERCUTANEOUS BILIARY STENTING

Biliary bypass operation and percutaneous transhepatic biliary stenting were compared in only one prospective randomized study by Bornman et al.[4] Total complication rate, 30-day mortality, and median duration of survival were similar. Bleeding complications (20% vs. 4%), recurrent jaundice (38% vs. 16%), and subsequent gastric outlet obstruction requiring reoperation (14% vs. 0%) were significantly greater after percutaneous biliary stenting than after biliary bypass operation.

DOES PREOPERATIVE BILIARY STENTING REDUCE MORBIDITY AND MORTALITY?

Preoperative biliary drainage before surgery vs. surgery alone without preoperative stenting was compared in four prospective randomized studies.[5-8] The preoperative biliary drainage was performed percutaneously and transhepatically[5-7] in three studies (Table II) and endoscopically[8] in one study. All studies demonstrated that preoperative biliary drainage did not decrease morbidity and mortality compared to surgery alone[5-8] but was associated with a significantly longer hospital stay.[6,7]

SHOULD INITIAL OPERATIVE PALLIATION CONSIST OF BILIARY BYPASS ALONE OR COMBINED BILIARY AND GASTRIC BYPASS?

The influence of the type of procedure on morbidity and mortality was analyzed in the first national multicenter study[9] Table III of palliative operations for pancreatic cancer in the United States (N = 1180 patients). Survival time after gastric bypass alone was significantly shorter than after biliary bypass alone or combined biliary and gastric bypass. The reoperation rate after gastric bypass alone (12%) was significantly higher than after biliary and gastric bypass ($P < 0.001$) because of the higher incidence of reoperative gastric bypass in the group undergoing gastric bypass alone. Similar complications occurred with all types of bypass. There was a 30-day mortality rate of 25% for reoperation. The French multicenter study[10] of 2493 patients revealed a significantly higher postoperative mortality rate (20% vs. 14%) and a significantly shorter duration of survival (3.2 vs. 5.2 months) after

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Table I. Biliary bypass operation vs. endoscopic biliary stenting: Results of prospective randomized clinical trials

	Shepherd et al.[1]		Andersen et al.[2]		Smith et al.[3]		
	Bypass	Stent	Bypass	Stent	Bypass	Stent	*P* value
No. of patients	25	23	19	25	101	100	
Successful drainage (%)	92	91	76	96	94	95	NS
Median total hospital stay (days)	13	8	27	26	26	20	NS
Complication rate (%)	40	22	—	—	29	11*	0.02*
Recurrent jaundice (%)	0	43†	16	28	2	36†	<0.01†
30-day mortality (%)	20	9	31	20	14	3‡	0.01‡
Gastric outlet obstruction (%)	4	9	0	0	7	17	NS
Median survival (wk.)	18	22	14	12	26	21	NS

*$P = 0.02$.
†$P < 0.01$.
‡$P = 0.01$.

Table II. Preoperative biliary drainage vs. surgery alone without stenting: Results of prospective randomized clinical trials

	Hatfield et al.[5]		McPherson et al.[6]		Pitt et al.[7]	
	Preoperative PTBD	Surgery alone	Preoperative PTBD	Surgery alone	Preoperative PTBD	Surgery alone
No. of patients	28	27	34	31	37	38
Successful preoperative PTBD (%)	89.3	—	—	—	87	—
Total hospital stay (days)	—	—	40	23*	31	23†
Overall morbidity (%)	14	15	33.3	41.9	56.8	52.6
Postoperative mortality (%)	14	15	32	19	8.1	5.3

PTBD = percutaneous transhepatic biliary drainage.
*$P < 0.05$.
†$P < 0.005$.

Table III. Comparison of different bypass procedures: Biliary bypass alone vs. combined biliary and gastric bypass[9]

	Bypass			Biliary bypass technique	
	GO	BO	BG	CD	CC
No. of patients	176	474	530	479	444
Age (yr)	64.6	66.3	64.7	65.6	64.9
Mean survival (yr)	208*	279	259	290	254
30-day mortality (%)	17	13	13	12	14
Complication rate (%)	26	27	25	25	25
Reoperation rate (%)	8	12†	5	5	10
Later gastric bypass (%)	4	7	3	3	6
Later biliary bypass (%)	4	6	3	2	4

GO = gastric bypass only; BO = biliary bypass only; BG = combined gastric and biliary bypass; CD = choledochoenterostomy; CC = cholecystoenterostomy.
*$P < 0.05$.
†$P < 0.0001$.

cholecystoenteric bypass procedures (N = 237 patients) than choledochoenteric bypass procedures (N = 1770 patients). The American study,[9] as well as the French multicenter study,[10] supported combined biliary and gastric bypass as the initial procedure, thus minimizing reoperation and its attendant morbidity and mortality.

SUMMARY

The initial success rate for palliation of jaundice is similar after endoscopic stenting and biliary bypass operation. Morbidity and 30-day mortality rates are greater after the bypass operation. Biliary stenting is associated with a higher rate of hospital readmission and reintervention for recurrent jaundice (28% to 43%) and subsequent gastric outlet obstruction (up to 17%). The outcomes of the initial operative bypass procedure favor combined biliary and gastric bypass.

REFERENCES

1. Shepherd HA, Royle G, Ross April, Diba A. Endoscopic biliary endoprosthesis in the palliation of malignant obstruction of the distal common bile duct. A randomized trial. Br J Surg 1988;75:1166-1168.
2. Andersen JR, Sorensen SM, Kruse A, et al. Randomised trial of endoscopic endoprosthesis versus operative bypass in malignant obstructive jaundice. Gut 1989;30:1132-1135.
3. Smith AC, Dowsett JF, Russel RCG, et al. Randomised trial of endoscopic stenting versus surgical bypass in malignant low bile duct obstruction. Lancet 1994;344:1655-1660.
4. Bornman PC, Harries-Jones EP, Tobias R, et al. Prospective controlled trial of transhepatic biliary endoprosthesis versus bypass surgery for incurable carcinoma of head of pancreas. Lancet 1986;I:69-71.
5. Hatfield ARW, Terblanche J, Fataar S, et al. Preoperative external biliary drainage in obstructive jaundice. Lancet 1982; 2:896-899.
6. McPherson GAD, Benhamin IS, Hodgson HJF, et al. Pre-operative percutaneous transhepatic biliary drainage: The results of a controlled trial. Br J Surg 1984;71:371-375.
7. Pitt HA, Gomes AS, Lois JF, et al. Does preoperative percutaneous biliary drainage reduce operative risk or increase hospital cost? Ann Surg 1985;201:545-553.
8. Lai ECS, Mok FPT, Fan ST, et al. Preoperative endoscopic drainage for malignant obstructive jaundice. Br J Surg 1994; 81:195-1198.
9. Neuberger TJ, Wade TP, Swope TJ, et al. Palliative operations for pancreatic cancer in the hospitals of the U.S. Department of Veteran Affairs from 1987 to 1991. Am J Surg 1993;166:632-637.
10. Huguier M, Bumel H, Manderscheid J-C, et al. Surgical palliation for unresected cancer of the exocrine pancreas. Eur J Surg Oncol 1993;19:342-347.

Palliation of Jaundice: Gastrointestinal Obstruction

Michael G. Sarr, M.D.

Less than 5% of patients with pancreatic cancer *present* with mechanical duodenal obstruction—but because pancreatic cancer is a locally aggressive invasive neoplasm, development of duodenal obstruction is well known to occur. This raises the question of whether the addition of a prophylactic gastroenterostomy (in the absence of duodenal obstruction) improves overall palliation in patients with unresectable pancreatic cancer?

The following four questions will be addressed herein:

1. How often does duodenal obstruction occur?
2. Does prophylactic gastroenterostomy prevent future gastric outlet obstruction?
3. Does prophylactic gastroenterostomy increase mortality and morbidity or improve survival?
4. Does prophylactic gastroenterostomy improve palliation?

1. How often does duodenal obstruction occur?

This question has been addressed retrospectively by many surgical reports[1-4] and prospectively by several comparisons of management of unresectable pancreatic cancer by biliary endoprosthesis.[5-7] Before the introduction of nonoperative endobiliary stent placement, palliation of extrahepatic biliary obstruction required operative biliary decompression. Many reports, a few of which are quoted here,[1-4] retrospectively determined how often future duodenal obstruction developed in patients without any signs of "impending" duodenal obstruction who underwent a biliary bypass alone. In these studies, duodenal obstruction *requiring reoperation* (gastroenterostomy) occurred in 10% to 15% of patients prior to death; operative mortality at this time in the nature history of the disease was quit high (≥20%). What is not known is the number of other patients dying with or because of duodenal obstruction who are unappreciated or untreatable because of the advanced stage of the disease. More recently, several groups have prospectively evaluated nonoperatively placed endobiliary stents in patients with known unresectable disease.[5-7] Prior to their death (mean survival 5 to 6 months), the patients had a similar incidence of the development of duodenal obstruction (9% to 15%). In summary, from these and other studies, duodenal obstruction can be expected to develop in at least 10% to 15% (and maybe more) of patients with unresectable pancreatic cancer in whom no evidence of impending duodenal obstruction is noted at presentation.

2. Does prophylactic gastroenterostomy prevent gastric outlet obstruction?

The answers can be found in many of the abovementioned studies and others. Among the retrospective series that adequately address this topic, studies by Sarr et al.[1,2] Schantz et al.[4] Lillemoe et al.,[3] and Watanapa and Williamson[8] have convincingly shown that mechanical gastric outlet obstruction is prevented by gastroenterostomy in more than 96% of patients. The prospective randomized studies of nonoperative palliation by biliary endoprosthesis vs. operative biliary bypass and gastroenterostomy[5-7] showed a very low incidence of gastric outlet obstruction in the surgically treated group (<4%). In summary, prophylactic gastroenterostomy does effectively prevent future mechanical gastric outlet obstruction. To be fair to this topic, one group[9] recently questioned the ability of gastroenterostomy to relieve or prevent future symptoms (from a patient's perspective) of gastric outlet obstruction (functional or chemical)—their study was small but provocative.

3. Does prophylactic gastroenterostomy increase mortality and/or morbidity or improve survival?

Most surgeons acknowledge that duodenal obstruction may develop in patients with unresectable pancreatic cancer and that gastroenterostomy will prevent this problem; however, morbidity is definitely increased by prophylactic gastroenterostomy. Studies specifically examining this question[1-4,8-11] have shown that although operative mortality is not increased, a poorly understood delay in gastric emptying (defined

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as inability to eat by postoperative days 8 to 10) occurs in 8% to 20% of patients, thereby increasing the length of hospitalization, cost, and "morbidity"; the risk of bleeding or anastomotic leakage is small. Also, all studies have failed to show any impact on survival by prophylactic gastroenterostomy. A prolonged hospitalization after a prophylactic, nontherapeutic gastroenterostomy cannot be considered optimal "palliation." In summary, prophylactic gastroenterostomy does increase morbidity in 10% to 20% of patients and does not improve survival.

4. Does prophylactic gastroenterostomy improve palliation?

This question has always been (and remains) difficult to answer for several reasons. First, no prospective randomized study has addressed this question or included a satisfactory patient-based assessment of quality of life. Second, should the surgeon add a "nontherapeutic" gastroenterostomy to the therapeutic biliary bypass knowing that it will prolong hospitalization (and possibly presence of the uncomfortable nasogastric tube) in 10% to 20% of patients to prevent the need for reoperation gastroenterostomy in 10% to 15% of patients in the future?

IS PROPHYLACTIC GASTROENTEROSTOMY WARRANTED IN 1999?

Management of *unresectable* pancreatic cancer has changed in the past decade with the introduction of nonoperative palliation of extrahepatic biliary obstruction by endobiliary stents. Few surgeons would advocate operative "palliation" in patients with known liver or peritoneal metastases (in the absence of duodenal obstruction) because survival is short, jaundice can be palliated by a biliary stent, and the operative mortality and morbidity of a celiotomy, including the requisite pain and convalescence of the celiotomy, can be avoided. Therefore the question of prophylactic gastroenterostomy in 1999 becomes relevant only in that much smaller subset of patients with a "clinically" resectable pancreatic cancer according to preoperative criteria who undergo exploration for potential curative resection and are found to be unresectable. Recent data in this group of patients show that mean survival is 6 to 7 months if occult liver or peritoneal disease is found, but 11 to 13 months if distant nodal or vascular involvement is present.[12] This latter group lives longer, the risk of duodenal obstruction might be greater, and these patients might best benefit from a prophylactic gastroenterostomy. Although this reasoning seems appropriate, no prospective randomized studies or quality-of-life studies exist to provide objective data for review.

REFERENCES

1. Sarr MG, Gladen HE, Beart RW Jr, van Heerden JA. Role of gastroenterostomy in patients with unresectable carcinoma of the pancreas. Surg Gynecol Obstet 1981;152:597-600.
2. Sarr MG, Cameron JL. Surgical management of unresectable carcinoma of the pancreas. Surgery 1982;91:123-133.
3. Lillemoe KD, Sauter PK, Pitt HA, Yeo CJ, Cameron JL. Current status of surgical palliation of periampullary carcinoma. Surg Gynecol Obstet 1993;176:1-10.
4. Schantz SP, Schickler W, Evans T, Coffey RJ. Palliative gastroenterostomy for pancreatic cancer. Am J Surg 1984;147: 793-796.
5. Bornman PC, Harries-Jones R, Van Stiegman G, Terblanche J. Prospective controlled trial of transhepatic biliary endoprosthesis versus bypass surgery for incurable carcinoma of the head of the pancreas. Lancet 1986;1:69-71.
6. Shepherd HA, Royle G, Ross APR, Arthur M, Colin-Jones D. Endoscopic biliary endoprosthesis in the palliation of malignant obstruction of the distal common bile duct: A randomized trial. Br J Surg 1988;75:1166-1168.
7. Smith AC, Dowsett JF, Russell RCG, Hatfield ARW, Cotton PB. Randomized trial of endoscopic stenting versus surgical bypass in malignant low bile duct obstruction. Lancet 1994;344:1655-1659.
8. Watanapa P, Williamson RCN. Single loop biliary and gastric bypass for irresectable pancreatic carcinoma. Br J Surg 1993; 80:237-239.
9. van der Schelling AD, van den Bosch RP, Klinkenbijl JHG, Mulder PGH, Jeekel J. Is there a place for gastroenterostomy in patients with advanced cancer of the head of the pancreas. World J Surg 1993;17:128-133.
10. Doberneck RC, Berndt GA. Delayed gastric emptying after palliative gastrojejunostomy for carcinoma of the pancreas. Arch Surg 1987;122:827-829.
11. de Rooij PD, Rogatko A, Brennan MJ. Evaluation of palliative surgical procedures in unresectable pancreatic cancer. Br J Surg 1991;78:1053-1058.
12. Luque-de Léon E, Tsiotos GG, Balsiger B, Barnwell J, Burgart LJ, Sarr MG. Staging laparoscopy for pancreatic cancer should be used to select the best means of palliation and not only to maximize the resectability rate. J GASTROINTEST SURG 1999;3:111-118.

Palliation of Pain: Operation

Keith D. Lillemoe, M.D.

Pancreatic cancer has long been regarded as one of the most painful forms of malignancy. In a review of 19 studies evaluating the incidence and various features of pain in more than 2200 patients with pancreatic cancer, Saltzburg and Foley[1] identified that pain was present at the time of diagnosis in 50% to 97% of patients, with most studies noting an incidence of 80% or more. Pain occurred at some point in the course of the disease in up to 89% of patients in those studies in which pain was specifically evaluated. More recently, however, because of a greater awareness of the diagnosis, the percentage of patients with pancreatic cancer presenting with substantial pain has decreased. A prospective analysis from Memorial Sloan-Kettering Cancer Center demonstrated that 40% of patients with pancreatic cancer reported no pain at the time of referral and another 30% had only minimal complaints of pain.[2] Moderate-to-severe pain was present in only 30% of patients, with only 10% of patients in this latter category. Similarly, prospective data collected from The Johns Hopkins Hospital have also demonstrated that only 20% of patients reported clinically significant pain, as assessed by a visual analogue scale.[3]

MANAGEMENT OF PANCREATIC CANCER PAIN—SURGICAL APPROACHES

The surgical options for management of pancreatic cancer include resection (if appropriate based on the extent of the tumor) and surgical palliation for obstruction of the biliary tree or gastrointestinal tract. Pancreatic resection in which all gross tumor is removed offers not only relief of symptoms, including pain, but also offers the chance of long-term survival. Palliative resection, in which gross and/or microscopic tumor is left behind, has recently been demonstrated to offer some survival advantage; however, the long-term effect and quality of life, and specifically pain control, have not been well defined.[4]

Conventional palliation of pancreatic cancer includes biliary bypass and, in most cases, a gastrojejunostomy. Biliary bypass will relieve the component of a patient's pain that is due to biliary obstruction. Gastric outlet obstruction is seldom a cause of pain but can cause early satiety, nausea, and vomiting. There is no role for surgical bypass of an obstructed pancreatic duct due to pancreatic cancer. Nonoperative palliation of obstructive jaundice can also be accomplished by the placement of percutaneous transhepatic or endoscopic biliary stents. As with surgical bypass, these procedures are likely to eliminate pain due to biliary obstruction. Although attempts at endoscopic placement of pancreatic duct stents for pancreatic cancer have been reported, in general, these techniques have not shown a major long-term benefit in relief of pain.

INTRAOPERATIVE CHEMICAL SPLANCHNICECTOMY

Chemical splanchnicectomy, employing principles of neurolysis of celiac ganglia, can be performed at the time of laparotomy for unresectable pancreatic cancer. Chemical splanchnicectomy for unresectable pancreatic cancer was first described by Copping et al.[5] in 1969. In the 1978 update of their experience with this technique, which included 41 patients, 88% of patients with pain due to pancreatic cancer experienced relief of pain postoperatively.[6] Most of these patients underwent palliative biliary and gastrointestinal bypass at the same operation. These results were compared with those from a group of historic control subjects in which only 21% of patients had pain control after similar palliative procedures. There were no reported complications of chemical splanchnicectomy.

Although widely advocated, intraoperative chemical splanchnicectomy was first objectively assessed by a randomized, placebo-controlled study in 1993. Pre- and postoperative pain was assessed by the use of questionnaires including a visual analogue scale. Patients with histologically proved pancreatic cancer were randomized at the time of laparotomy to receive either chemical splanchnicectomy with 50% alcohol or a placebo saline injection. The two groups were

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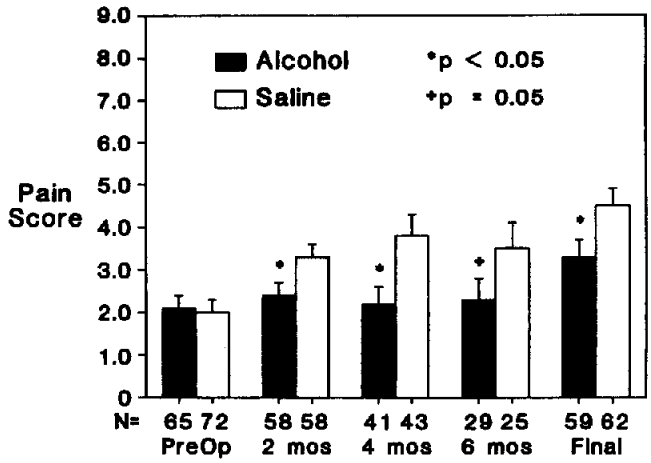

Fig. 1. Mean postoperative pain scores for all patients and controls.

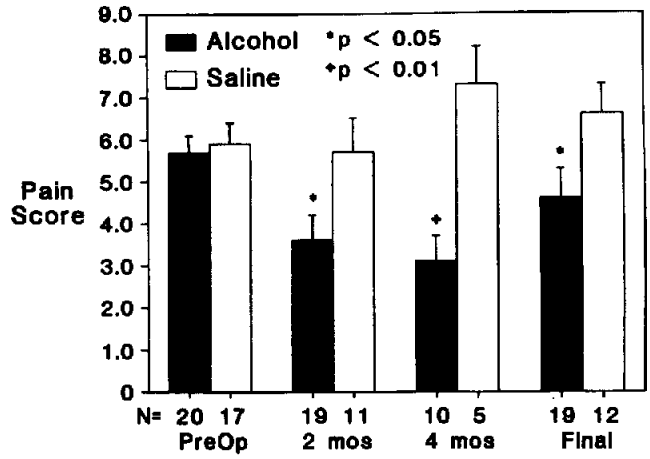

Fig. 2. Mean postoperative pain scores for subgroup of patients with preexisting pain.

similar with respect to age, sex, location and stage of tumor, operation performed, use of postoperative chemotherapy and radiation therapy, and initial assessment scores for pain, mood, and disability. There was no difference between the patients receiving chemical splanchnicectomy and the control group with respect to perioperative length of stay. Mean postoperative scores at all assessment points for all patients randomized were significantly lower following chemical splanchnicectomy with alcohol than those of control patients (Fig. 1). The subgroup of patients with preexisting pain had a statistically significant decrease in postoperative pain scores compared with control patients (Fig. 2). Some 70% of patients with preexisting pain who received alcohol decreased their narcotic requirements compared with none of the patients in the placebo group ($P < 0.001$). Patients receiving alcohol experienced a mean of 3.3 pain-free months compared with 0.8 pain-free months for the control patients ($P < 0.05$).

Patients with no preexisting pain also had a statistically significant decrease in mean postoperative pain scores compared with the placebo controls. The average number of months without substantial pain was 7.2 months in the alcohol group and only 3.0 months in the placebo group ($P < 0.0001$). Only 46% of patients receiving alcohol block ever required significant doses of narcotic pain medications compared with only 60% of patients in the placebo group ($P < 0.05$). Finally, 56% of patients receiving alcohol splanchnicectomy never reported having severe pain compared with only 34% of patients in the placebo group ($P < 0.05$).

An unexpected finding in this study was a highly significant increase in survival among patients with severe preoperative pain who underwent chemical

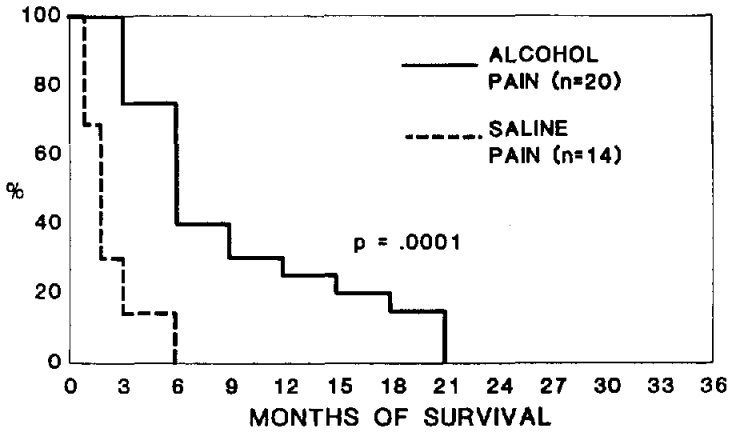

Fig. 3. Increased survival in patients with severe preoperative pain undergoing chemical splanchnicectomy.

splanchnicectomy, compared with similar patients in the control group (Fig. 3). These two subgroups were analyzed with respect to age, tumor location, tumor stage, operation performed, the use of chemotherapy and radiation therapy, baseline mood, and disability. No significant difference was apparent in any of these comparisons, suggesting that adequate pain control may improve survival in patients with this disease.

Intraoperative chemical splanchnicectomy can be performed as part of any surgical procedure performed for palliation of pancreatic cancer. After it has been determined at laparotomy that the patient's tumor is unresectable, intraoperative chemical splanchnicectomy should be performed whether the patient has severe pain or is pain free at the time of surgery. The technique is applicable whether the cancer arises in the head, body, or tail of the pancreas. There would appear to be no need for chemical splanchnicectomy following pancreaticoduodenectomy or distal pancreatectomy if resection is complete with grossly clear margins. Laparotomy is not indicated, however, if its sole purpose is to perform intraoperative chemical splanchnicectomy. The availability of percutaneous celiac block has eliminated the need for this procedure in almost all patients.

THORACOSCOPIC PANCREATIC DENERVATION

The recent advances in laparoscopic and thoracoscopic surgery have led to a technical description of the thoracoscopic pancreatic denervation performed via the left chest. This technique appears to be most applicable for patients in whom percutaneous celiac block cannot be performed. Patients requiring thoracoscopy must be suitable candidates for general anesthesia; however, they require no other special preparation. The patient is provided with adequate pain control via a minimally invasive technique with laparotomy, and the associated postoperative pain is avoided.

No prospective, randomized trials have been performed to evaluate thoracoscopic pancreatic denervation. A number of authors, however, have reported significant pain control following the procedure with minimal perioperative morbidity.[7] A number of authors, however, have reported significant pain control following this procedure with minimal perioperative morbidity when performed for either pancreatic cancer or chronic pancreatitis.[8]

REFERENCES

1. Saltzburg D, Foley KJ. Management of pain in pancreatic cancer. Surg Clin North Am 1989;69:629-649.
2. Hudis C, Kelsen D, Niedzwiecki D, et al. Pain is not a prominent symptom in most patients with early pancreas cancer [abstr]. Proc Am Soc Clin Oncol 1991;10:326.
3. Lillemoe KD, Cameron JL, Kaufman HS, et al. Chemical splanchnicectomy in patients with unresectable pancreatic cancer. Ann Surg 1993;217:447-457.
4. Lillemoe KD, Cameron JL, Yeo CJ, et al. Pancreaticoduodenectomy: Does it have a role in the palliation of pancreatic cancer? Ann Surg 1996;223:718-728.
5. Copping J, Willix R, Kraft R. Palliative chemical splanchnicectomy. Arch Surg 1969;98:418-420.
6. Flanigan DP, Kraft RO. Continuing experience with palliative chemical splanchnicectomy. Arch Surg 1978;113:509-511.
7. Worsey J, Ferson PF, Keenen RJ, Julian TB, Landreneau RJ. Thoracoscopic pancreatic denvervation for pain control in irresectable pancreatic cancer. Br J Surg 1993;80:1051-1052.
8. Bradley EL III, Reynhout JA, Peer GL. Thoracoscopic splanchnicectomy for "small duct" chronic pancreatitis: Case selection by differential epidural analgesia.

Palliation of Pain in Pancreatic Cancer

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Pain is a common symptom in patients with pancreatic cancer at the time of diagnosis.[1] Typically the tumor is surgically resectable in only a fraction of cases.[2] The efficacy of chemotherapy and radiation therapy in the treatment of these patients is also limited. As a result, the palliation of pain to maintain a satisfactory quality of life is one of the primary concerns during the remaining existence of patients with pancreatic cancer.

PANCREATIC CANCER PAIN

Pain associated with pancreatic cancer is typically located in the epigastric area and radiates to the thoracolumbar back region. The pain is often characterized as constant, gnawing, and visceral in quality. In a recent study of pancreatic cancer patients awaiting exploratory laparotomy or chemotherapy, 29% had moderate-to-severe pain, 34% had mild pain, and 37% had no pain.[1] However, in more advanced pancreatic cancer, pain occurs in 80% to 85% of patients.[3]

The mechanism of pancreatic cancer pain may have different etiologies including stretching of nerves by the tumor, invasion of nerves by the tumor, or pancreatic ductal obstruction by tumor contributing to a form of pancreatitis.[4] Most pancreatic cancer pain is communicated by the celiac plexus, which is primarily a sympathetic nervous system structure containing both visceral afferent and efferent fibers. The celiac plexus is located anterolateral to the aorta at the superior border of the first lumbar vertebral body, and receives contributions from the greater (origin at T5 to T10 vertebral levels), lesser (T10 and T11), and least splanchnic nerves (T12).

PHARMACOLOGIC THERAPY

The Agency for Health Care Policy and Research recommends the World Health Organization (WHO) analgesic ladder as an effective and validated approach in the pharmacologic treatment of cancer pain[5] (Fig. 1). This simple method uses a stepwise approach beginning with nonopioids and progressing to weak and then strong opioids, according to need.[5,6] Some examples of weak opioids are codeine and propoxyphene; strong opioids may include morphine and fentanyl. Medications are prescribed on a time-scheduled basis with additional as-needed doses available. Key principles for successful pharmacologic pain management involve individualizing the pharmacologic regimen to the patient with great attention to detail and close follow-up. Oral administration is the preferred route because of its simplicity and cost-effectiveness. However, parenteral and rectal routes can be acceptable alternatives.

Pain management with opioid medications can be acceptable for many patients but may have certain dis-

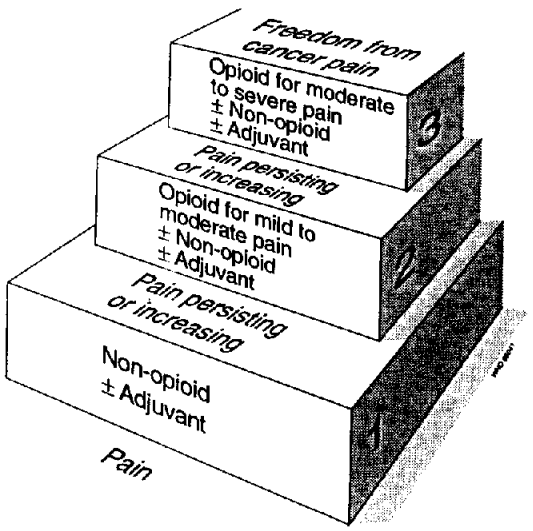

Fig. 1. World Health Organization (WHO) three-step analgesic ladder. (From World Health Organization: Cancer Pain Relief and Palliative Care: Report of a WHO Expert Committee [World Health Organization Technical Report Series, No. 804]. Geneva: World Health Organization, 1990.)

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advantages. In a study evaluating the WHO analgesic ladder in patients, the most frequent side effects were dry mouth (39% of the days of follow-up), drowsiness (38%), constipation (35%), and nausea and vomiting (22%); no side effects were noted in only 24% of the days of follow-up.[6] In addition, there is increasing evidence that chronic high doses of opioids may inhibit immune function including activity of natural killer cells, which are thought to scavenge tumor cells.[7] Theoretically this immune suppression may be especially important in those patients who require increasingly large doses of opioids to treat their pain.

NEUROLYTIC CELIAC PLEXUS BLOCK

Neurolytic celiac plexus block is the most commonly used interventional procedure for the relief of pancreatic cancer pain.[8] Neurolytic celiac plexus block is typically performed by placing needle tips at the anterolateral border of the first lumbar vertebra from a posterior bilateral percutaneous approach (Fig. 2). The injection of a neurolytic agent such as alcohol can provide analgesia for durations ranging from 3 to 6 months, and often for the remainder of the patient's life.

The efficacy of neurolytic celiac plexus block for treatment of upper abdominal cancer pain has been previously evaluated by meta-analysis.[8] At the time, that particular methodologic approach was identified in 24 reports: two randomized controlled trials, one prospective case series, and 21 uncontrolled retrospective case series. The authors concluded that neurolytic celiac plexus block has long-lasting benefit for 70% to 90% of patients with pancreatic and intraadominal cancers.[8]

There are several randomized controlled trials comparing neurolytic celiac plexus block to pharmacologic therapy in the management of pancreatic cancer pain.[9-11] All of these studies involved a limited number of subjects (10 to 12 patients in each randomized treatment arm), with study designs not utilizing a double-blind system. The significant findings of these studies suggest that neurolytic celiac plexus block provides more effective analgesia during the first four weeks following the procedure.[10,11] Also, patients randomized to neurolytic celiac plexus block have significantly decreased opioid consumption, which can last from up to 50 days following the procedure[9,10] to the time of death.[11] Also, patients randomized to celiac plexus block may have less deterioration in quality-of-life estimates as a function of time compared to patients with pharmacologic therapy.[10]

In a recent investigation, patients with unresectable pancreatic cancer who were randomized to intraoperative chemical splanchnicectomy (splanchnic neurolysis—similar to celiac plexus block) had improved pain control until they died compared to the placebo control group.[12] There was also a significantly decreased opioid requirement in the chemical splanchnicectomy group compared to the placebo group. Particular strengths of this investigation were the large number of subjects (N = 137) and the use of a double-blind method of study. In a subgroup of patients with significant pain, there was prolonged sur-

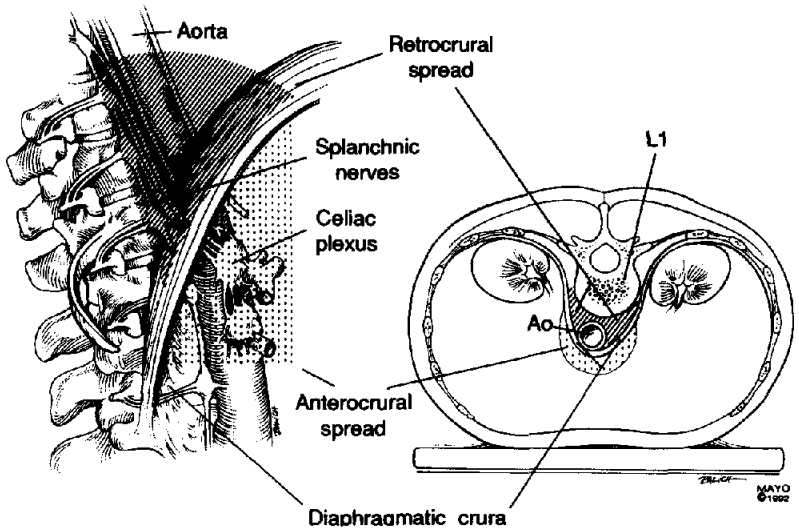

Fig. 2. Neurolytic celiac plexus block is accomplished by spread of injectate anterior to the diaphragmatic crura. Bilateral needles are placed from the thoracolumbar back region to approach the anterolateral border of the first lumbar vertebra.

vival time in patients randomized to the chemical splanchnicectomy treatment group.[12]

Neurolytic celiac plexus has been determined to be a relatively safe procedure.[8] Mild adverse effects such as bowel hypermotility and orthostatic hypotension are common (38% to 44% of cases) but tend to be transient.[8] Serious adverse effects, such as neurologic deficits (lower extremity paresis or paraplegia, or loss of bowel or bladder control), are extremely uncommon and occurred in 4 (0.15%) of 2730 patients in one study.[13]

There has been little consensus in the timing of neurolytic celiac plexus block for the treatment of unresectable pancreatic cancer pain. Traditionally, neurolytic celiac plexus block has been reserved for patients who were receiving inadequate analgesia or were suffering with untreatable side effects from pharmacologic therapy. However, evidence suggests that earlier implementation of neurolytic celiac plexus block may be advantageous. There may be an important relationship between pain and stress and survival in cancer patients.[14] Lillemoe et al.[12] found that a subgroup of patients who received chemical splanchnicectomy had improved survival time as compared to a placebo control group. Other studies have shown that pain and stress can inhibit immune function, accelerate tumor growth, and decrease survival after tumor challenge.[14] In addition, chronic high doses of opioids may inhibit immune function including activity of natural killer cells, which are thought to scavenge tumor cells.[7] Applying these findings to pancreatic cancer pain management, potential survival time might be optimized with earlier implementation of neurolytic celiac plexus block by providing improved analgesia and decreasing opioid requirements.

CONCLUSION

The most commonly used techniques in the palliation of pain associated with unresectable pancreatic cancer are pharmacologic therapy and neurolytic celiac plexus block. Existing studies suggest that neurolytic celiac plexus block is a well-tolerated intervention that may improve analgesia, decrease opioid requirements, and minimize deterioration of quality of life. A shift in timing toward earlier implementation of neurolytic celiac plexus block may be advantageous.

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REFERENCES

1. Kelsen DP, Portenoy RK, Thaler HT, et al. Pain and depression in patients with newly diagnosed pancreas cancer. J Clin Oncol 1995;13:748-755.
2. Warshaw AL, Fernandez-del Castillo C. Pancreatic carcinoma. N Engl J Med 1992;326:455-465.
3. Kalser MH, Barkin J, MacIntyre JM. Pancreatic cancer: Assessment of prognosis of clinical presentation. Cancer 1985; 56:397-402.
4. Nakagawa T, Mori K, Nakano T, et al. Perineural invasion of carcinoma of the pancreas and biliary tract. Br J Surg 1993; 80:619-621.
5. Jacox A, Carr DB, Payne R, et al. Management of Cancer Pain. Clinical Practice Guideline No. 9. AHCPR Publication No. 94-0592. Rockville, Md.: Agency for Health Care Policy and Research, U.S. Department of Health and Human Services, Public Health Service, March 1994.
6. Ventrafridda V, Tamburini M, Caraceni A, De Conno F, Naldi F. A validation study of the WHO method for cancer pain relief. Cancer 1987;59:850-856.
7. Yeager MP. Morphine inhibits spontaneous and cytokine-enhanced natural killer cell cytotoxicity in volunteers. Anesthesiology 1995;83:500-508.
8. Eisenberg E, Carr DB, Chalmers TC. Neurolytic celiac plexus block for treatment of cancer pain: A meta-analysis. Anesth Analg 1995;80:290-295.
9. Polati E, Finco G, Gottin L, Bassi C, Pederzoli P, Ischia S. Prospective randomized double-blind trial of neurolytic celiac plexus block in patients with pancreatic cancer. Br J Surg 1998;85:199-201.
10. Kawamata M, Ishitani K, Ishikawa K, et al. Comparison between celiac plexus block and morphine treatment on quality of life in patients with pancreatic cancer pain. Pain 1996; 64:597-602.
11. Mercadante S. Celiac plexus block versus analgesics in pancreatic cancer pain. Pain 1993;52:187-192.
12. Lillemoe KD, Cameron JL, Kaufman HS, Yeo CJ, Pitt HA, Sauter PK. Chemical splanchnicectomy in patients with unresectable pancreatic cancer. Ann Surg 1993;217:447-457.
13. Davies DD. Incidence of major complications of neurolytic coeliac plexus block. J R Soc Med 1993;86:264-266.
14. Liebeskind JC. Pain can kill. Pain 1991;44:3-4.

Irradiation for Unresectable Pancreatic Cancer

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Approximately 40% of the 28,900 patients diagnosed with ductal adenocarcinoma of the pancreas in 1999 will present with unresectable but nonmetastatic disease. Inasmuch as these patients are unresectable by a Whipple procedure or total pancreatectomy because of tumor invasion of the portal or mesenteric vessels, and they have no clinically demonstrable metastases, radiotherapeutic approaches have been frequently employed for these patients. The approaches have included external-beam irradiation with and without 5-fluorouracil (5-FU) chemotherapy, intraoperative irradiation, and more recently external-beam irradiation with new chemotherapeutic agents.

EXTERNAL-BEAM IRRADIATION: RANDOMIZED TRIALS

With the exception of one study, conventional external-beam irradiation for unresectable pancreatic cancer has been shown to improve survival when combined with 5-FU chemotherapy compared to irradiation alone or chemotherapy alone (Table I). From this data, the "best" median survival and 2-year survival figures for external-beam irradiation and 5-FU are approximately 10 months and 12%, respectively.

Because of the limited tolerance of normal tissue in the upper abdomen (liver, kidney, spinal cord, and bowel) to external-beam irradiation, total doses of only 45 to 54 Gy, in 25 to 30 fractions, have usually been given. For an unresectable lesion this dose of irradiation is inadequate, which is reflected in the treatment results from both prospective and retrospective studies, with high rates of tumor progression and poor survival. The Mayo Clinic reported a local failure rate of 72% for 122 patients with unresectable pancreatic cancer who were treated with 40 to 60 Gy of external-beam irradiation.[5]

Because of these high local failure rates, preoperative irradiation has been studied to assess its ability to convert locally unresectable pancreatic cancer to resectable disease. In one representative study, 16 patients with locally unresectable pancreatic cancer were treated with 45 Gy of external-beam irradiation and

Table I. Prospective randomized studies of radiation therapy and chemotherapy for unresectable pancreatic cancer

Investigation	No. of patients	Median survival (mo)	Local failure in evaluable patients (%)	2-year survival rate (%)
Mayo Clinic[1]	32	6.3	—	—
EBRT (35-37.5 Gy/4 wk) only	32	10.4	—	—
EBRT (35-37.5 Gy/4 wk)/5-FU				
Gastrointestinal Tumor Study Group[2]	25	5.2	24	5
EBRT (60 Gy/10 wk) only	83	9.6	26	10
EBRT (40 Gy/6 wk)/5-FU	86	9.2	27	10
EBRT (60 Gy/10 wk)/5-FU				
Gastrointestinal Tumor Study Group[3]	73	8.4	58	12
EBRT (60 Gy/10 wk)/5-FU	72	7.5	51	6
EBRT (60 Gy/10 wk)/doxorubicin				
Gastrointestinal Tumor Study Group[4]	31	6.5	38	41 (1 yr)
EBRT (54 Gy/6 wk)/5-FU/SMF	26	5.1	29	19 (1 yr)
SMF only				

EBRT = external-beam radiation therapy; 5-FU = 5-fluorouracil; SMF = streptozotocin, mitomycin-C, and 5-FU.

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infusional 5-FU to enhance resectability.[6] Of these 16 patients, only two (13%) subsequently underwent resection. This study and others indicate that neoadjuvant chemotherapy combined with radiation therapy will infrequently convert an unresectable lesion to a resectable tumor and thus improve outcome.

INTRAOPERATIVE IRRADIATION

Because of the poor local control and results achieved with conventional external-beam irradiation and chemotherapy, specialized radiation therapy techniques that increase the radiation dosage to the tumor volume have been used to improve local tumor control without increasing normal tissue morbidity. These include iodine-125 implants or intraoperative electrons as a booster dose in combination with external-beam irradiation and chemotherapy. A lower incidence of local failure in most series and improved median survival in some have been reported with these techniques when compared with conventional external-beam irradiation, but it is uncertain whether this is due to superior treatment or case selection (Table II).

In the Massachusetts General Hospital and Mayo Clinic studies combining external-beam and intraoperative irradiation, local tumor control has been improved but median survival is only approximately 12 months and the 2-year survival rate remains approximately 20%. Most patients have progression of their disease with liver metastases, peritoneal seeding, or both. Although slight gains in survival may be achieved by improving local tumor control, the high incidence of metastases precludes significant improvements in long-term survival.

NEW AGENTS WITH IRRADIATION

Because of the high incidence of hepatic and peritoneal metastases and the poor results with standard chemotherapy, current and future therapeutic efforts now include evaluation of external-beam irradiation with new agents (paclitaxel and gemcitabine). There is interest in these agents not only because of their systemic effects but also because of their radiosensitizing properties. In radiobiologic models it appears that paclitaxel results in enhanced radiosensitization by tumor reoxygenation after apoptotic clearance of paclitaxel-damaged cells. In a phase I trial from Brown University evaluating paclitaxel and 50 Gy of external-beam irradiation in patients with unresectable pancreatic and gastric cancer, the maximally tolerated dose of weekly paclitaxel with conventional irradiation was 50 mg/m^2.[11] The response rate was 31% for 13 evaluable patients with pancreatic cancer. These data have led to the initiation of an intergroup phase II study evaluating paclitaxel with external-beam irradiation for patients with unresectable pancreatic cancer.

Gemcitabine has been the focus of a recent investigation concerning the treatment of patients with advanced pancreatic cancer. Burris et al.[12] randomized 160 previously untreated patients with advanced and metastatic pancreatic cancer to receive either gemcitabine or 5-FU. Patients who were given gemcitabine had a statistically improved median survival, 1-year survival, and clinical benefit compared to patients who received 5-FU. In radiobiologic models gemcitabine has also been observed to be a potent radiosensitizer, likely because of depletion of intracellular deoxynucleoside triphosphates. At the present time, numer-

Table II. External-beam and intraoperative radiation therapy for unresectable carcinoma of the pancreas

Investigation	No. of patients	Median survival (mo)	2-year actuarial local failure (%)	2-year actuarial survival rate (%)
Radiation Therapy Oncology Group[7]	51	9	—	9 (18 mo)
Massachusetts General Hospital[8,9]				
^{125}I + EBRT (40-45 Gy/5 wk) ± Chemotherapy	12	12	33	20
EBRT (45-50 Gy/6 wk) ± Chemotherapy/IOERT (15-20 Gy)	41	12	55	20
Mayo Clinic[5,10]				
EBRT (40-60 Gy/6 wk) ± Chemotherapy	122	12.6	80	16
Preoperative EBRT (50.4-54 Gy) ± Chemotherapy/IOERT (20 Gy)	27	14.9	32	27
Postoperative EBRT (45-55 Gy/6 wk) ± Chemotherapy/IOERT (20 Gy)	56	10.5	35	6

EBRT = external-beam radiation therapy; CT = chemotherapy; IOERT = intraoperative electron irradiation.

ous investigators are pursuing phase I and II studies combining external-beam irradiation with gemcitabine. At Massachusetts General Hospital, the Dana Farber Cancer Institute, and Brigham and Women's Hospital, we are combining preoperative irradiation to the pancreas (50.4 Gy) with continuous-infusion 5-FU and weekly gemcitabine followed by restaging 3 to 4 weeks after completion of external-beam irradiation. If there is no evidence of distant metastases, intraoperative electron irradiation to the primary pancreatic lesion will be given. With this approach we hope to improve locoregional control, as well as reduce the incidence of hepatic and peritoneal metastases.

CONCLUSION

At present, the treatment of unresectable nonmetastatic pancreatic cancer remains palliative. In selected patients, aggressive treatment programs of irradiation and chemotherapy may result in median survivals of approximately 12 months and 2-year survival rates of 20%. Long-term survivors are rare. Future efforts will be directed toward evaluation of irradiation with new agents such as paclitaxel and gemcitabine.

REFERENCES

1. Moertel CT, Childs DS Jr, Reitemeir RJ, et al. Combined 5-fluorouracil and supervoltage radiation therapy of locally unresectable gastrointestinal cancer. Lancet 1969;2:865-867.
2. Gastrointestinal Tumor Study Group. Therapy of locally unresectable pancreatic carcinoma: A randomized comparison of high dose (6000 rads) radiation alone, moderate dose radiation (4000 rads + 5-fluorouracil), and high dose radiation + 5-fluorouracil. Cancer 1981;48:1705-1710.
3. Moertel CG, Frytak S, Hahn RG, et al. Radiation therapy combined with adriamycin or 5-fluorouracil for the treatment of locally unresectable pancreatic carcinoma: Gastrointestinal Tumor Study Group. Cancer 1985;56:2563-2568.
4. Gastrointestinal Tumor Study Group. Treatment of locally unresectable carcinoma of the pancreas: Comparison of combined-modality therapy (chemotherapy plus radiotherapy) to chemotherapy alone. J Natl Cancer Inst 1988;80:751-755.
5. Roldan GE, Gunderson LL, Nagorney DM, Martin JK, Ilstrup DM, Holbrook MA, Kvols LK, McIlrath DC. External beam versus intraoperative and external beam irradiation for locally advanced pancreatic cancer. Cancer 1988;61:1110-1116.
6. Jessup JM, Steele G, Mayer RJ, et al. Neoadjuvant therapy for unresectable pancreatic adenocarcinoma. Arch Surg 1993;128:559-564.
7. Tepper JE, Moyes D, Krall JM, et al. Intraoperative radiation therapy of pancreatic carcinoma: A report of RTOG-8505. Int J Radiat Oncol Biol Phys 1991;21:1145-1149.
8. Shipley WU, Nardi GL, Cohen AM. Iodine-125 implant and external beam irradiation in patients with localized pancreatic carcinoma: A comparative study to surgical resection. Cancer 1980;45:709-714.
9. Tepper JE, Shipley WU, Warshaw AL, Nardi GL, Wood WC, Orlow EL. The role of misonidazole combined with intraoperative radiation therapy in the treatment of pancreatic carcinoma. J Clin Oncol 1987;5:579-584.
10. Garton GR, Gunderson LL, Nagorney DM, et al. High-dose preoperative external beam and intraoperative irradiation for locally advanced pancreatic cancer. Int Radiat Oncol Biol Phys 1993;27:1153-1157.
11. Safran H, King T, Choy H, et al. Paclitaxel and concurrent radiation for locally advanced pancreatic and gastric cancer: A phase I study. J Clin Oncol 1997;15:901-907.
12. Burris H, Moore M, Anderson J, et al. Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreatic cancer: A randomized trial. J Clin Oncol 1997;6:2403-2413.

Chemotherapy for Pancreatic Adenocarcinoma

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A discussion of the role of chemotherapy for pancreatic cancer must initially address the definition of efficacy. Traditional end points for efficacy in any cancer therapy have included the objective response rate (significant tumor shrinkage), disease-free survival, overall survival, and quality of life. For many diseases an objective response appears to correlate well with prolonged survival (i.e., in those who achieved a response) and a high objective response rate often correlates well with an improved median survival. For solid tumors an objective response is generally accepted to reflect a 50% or greater reduction in the sum of the products of all bidimensionally measurable lesions. Although these criteria were originally developed by the World Health Organization and applied to bidimensional nodules on chest x-ray films or palpable masses on physical examination, they are now commonly applied to lesions observed with three-dimensional imaging techniques such as computerized tomography (CT) or magnetic resonance imaging (MRI). Applying these criteria to pancreatic cancer is difficult. Because pancreatic adenocarcinoma is often associated with a profound desmoplastic reaction, the measurements of a given tumor mass can vastly overestimate the malignant cell mass. Associated pathologic conditions in the pancreas such as varying degrees of pancreatitis or adjacent pseudocysts can cause architectural changes that may be radiographically difficult to distinguish from the malignant border. Furthermore, the radiographic interpretation of masses in the pancreas is often confounded by adjacent unopacified or poorly opacified small bowel.[1] For these reasons it is becoming more widely accepted that accurate disease measurements, at least of the primary tumor mass, are difficult to achieve.[2,3] In fact, there is growing interest in identifying alternate end points to gauge effective therapy. Studies have suggested that serial measurements of CA 19-9, a tumor-associated antigen highly expressed in pancreatic cancer, can be predictive of survival. At least two studies using chemoradiotherapy[4] and chemotherapy[5] have suggested that a decrease in the CA 19-9 level correlates well with improved survival. However, serial levels of CA 19-9 have not yet been accepted as a screening tool for antitumor efficacy.

Despite the known association of disabling symptoms such as pain or weight loss, global quality-of-life or symptom assessment has not routinely been used to measure efficacy of therapy. However, some recent studies have suggested that careful attention to these efficacy end points may identify a benefit of therapy that is underestimated by the objective response rate. For instance, Glimelius et al.[6] studied a validated quality-of-life assessment tool developed by the European Organization for Treatment of Research and Cancer (EORTC) in patients with pancreatic cancer who were receiving either best supportive care or chemotherapy. In this study a significantly higher percentage of patients who received chemotherapy reported an improved quality of life (36% vs. 10%). A somewhat different approach was taken in the seminal studies of the role of gemcitabine in pancreatic cancer. Using a symptom assessment tool developed especially for patients with pancreatic cancer, Rothenberg et al.[7] reported fewer tumor-associated symptoms (clinical benefit response) in 20% of the patients studied. Similarly Burris et al.[8] reported on a randomized trial comparing gemcitabine to "standard" therapy with 5-fluoruracil (5-FU). In this study 24% of the patients treated with gemcitabine reported improved symptoms compared to 5% of those treated with 5-FU. In this trial the objective response rates of the two arms of the study were indistinguishable (5% vs. 0%). These studies emphasize that the objective response rate may underestimate the true benefit of therapy.

Another powerful measure of efficacy in cancer therapy is survival. In the trial noted above by Burris et al.,[8] a small but significantly improved median survival was identified with gemcitabine therapy compared to 5-FU (5.7 vs. 4.5 months), and the 1-year survival was also significantly improved (18% vs. 2%). If one reviews the literature on single-agent chemotherapy treatment in pancreatic cancer, only four

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drugs have been reported to result in a reproducible median survival of longer than 5 months. In addition to gemcitabine, these include 5-FU,[9] goserelin,[10] and tamoxifen.[11] The latter two hormonal agents have never become accepted into the standard of care for pancreatic adenocarcinoma.

A variety of combination regimens have been promoted as promising. These have included 5-FU, adriamycin, and mitomycin (FAM)[12] with or without streptozotocin[13] and a modification of this regimen, 5-FU, mitomycin-C, and streptozotocin (SMF).[14] A randomized trial conducted by the North Central Cancer Treatment Group (NCCTG) demonstrated that combination therapy with FAM was not superior to 5-FU monotherapy based on median survival.[15] Two randomized trials have failed to demonstrate an advantage of SMF over FAM.[16,17] To date, there have not been any trials comparing gemcitabine to combination regimens.

Three studies have suggested that combination chemotherapy may improve survival when compared to best supportive care. Palmer et al.[18] randomized patients to FAM vs. best supportive care and noted a prolonged median survival (33 vs. 15 weeks) in the treated patient population. The trial noted earlier by Glimelius[6] also reported an improved median survival with 5-FU and leucovorin with or without etoposide. Finally, Mallinson et al.,[19] using a complicated five-drug regimen that included 5-FU, mitomycin-C, methotrexate, vincristine, and cyclophosphamide, reported a statistically significant improved survival over best supportive care. Although a subsequent NCCTG trial[20] did not demonstrate an advantage of the Mallinson regimen over monotherapy with 5-FU, the fact remains that there may be some survival advantage with 5-FU–based chemotherapy in pancreatic cancer.

The availability of an active agent, even a modestly effective agent such as gemcitabine, has spawned additional studies seeking to optimize the use of this drug. Because there may be a pharmacokinetic advantage for somewhat more prolonged infusion (10 mg/m^2/min) of gemcitabine,[21] a randomized trial is currently in progress comparing high-intensity dosing strategies of gemcitabine over short and prolonged infusions. In addition, there are preclinical mechanistic studies favoring synergy between cisplatin and gemcitabine[22]; this doublet combination is also under active investigation. Future therapeutic strategies in pancreatic cancer will undoubtedly take advantage of known molecular, biochemical, or biologic perturbations unique to this disease. Since a molecular hallmark of pancreatic cancer is a k-ras mutation at the twelfth or thirteenth codon, drugs that inhibit farnysel transferase or decrease farnyselated ras levels (such as isoprenoids) are in development or have already entered clinical trials. Unique matrix metalloproteinases have been identified as being associated with pancreatic cancer, and extensive studies are just now being completed with a matrix metalloproteinase inhibitor (BB25160).[23] The association of pancreatic cancer with unique mucin-associated antigens has raised the possibility of immunobiologic approaches including vaccines or radioimmunotherapy using antibodies against selected antigens.

In summary, pancreatic cancer remains a relatively chemoresistant disease. However, chemotherapy may offer an advantage compared to best supportive care. Combination chemotherapy does not appear to improve the results that can be achieved with 5-FU monotherapy. Gemcitabine may be superior to 5-FU treatment, but any true improvement in median survival with gemcitabine or any other treatment is slight. A better understanding of the biology of pancreatic cancer may indeed lead to improved therapeutic approaches.

REFERENCES

1. Freeny PC. Radiologic diagnosis and staging of pancreatic ductal adenocarcinoma. Radiol Clin North Am 1989;27:121-128.
2. Rothenberg ML, Abbruzzese JL, Moore M, Portenoy RK, Robertson JM, Wanebo HJ. A rationale for expanding the end points for clinical trials in advanced pancreatic carcinoma. Cancer 1996;78:627-632.
3. Ahlgren JD. Chemotherapy for pancreatic carcinoma. Cancer 1996;78:654-663.
4. Ishii H, Okada S, Sato T, Wakasugi H, Saisho H, Furese J, Ishikawa O, Matsuno S, Yokoyama S. CA 19-9 in evaluating the response to chemotherapy in advanced pancreatic cancer. Hepatogastroenterology 1997;44:279-283.
5. Willet CG, Daly WJ, Warshaw AL. CA 19-9 is an index of response to neoadjuvant chemoradiation therapy in pancreatic cancer. Am J Surg 1996;172:350-352.
6. Glimelius B, Hoffman K, Sjödén PO, Jacobsson G, Sellström H, Enander LK, Linné T, Svensson C. Chemotherapy improves survival and quality of life in advanced pancreatic and biliary cancer. Ann Oncol 1996;7:593-600.
7. Rothenberg ML, Moore MJ, Cripps MC, Andersen JS, Portenoy RK, Burris HA, Green MR, Tarassoff PG, Brown TD, Casper ES, Storniolo AM, Von Hoff DD. A phase II trial of gemcitabine in patients with 5-FU refractory pancreas cancer. Ann Oncol 1996;7:347-353.
8. Burris HA, Moore MJ, Andersen J, Green MR, Rothenberg ML, Modiano MR, Cripps MC, Portenoy RK, Storniolo AM, Tarassoff P, Nelson R, Dorr FA, Stephens CD, Von Hoff DD. Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreatic cancer: A randomized trial. J Clin Oncol 1997;15:2403-2413.
9. Ahlgren JD. Pancreatic cancer: Chemotherapy of advanced disease. In Ahlgren JD, MacDonald JS, eds. Gastrointestinal Oncology. Philadelphia: JB Lippincott, 1992, pp 227-235.
10. Philip PA, Carmichael J, Tonkin K, Buamah PK, Britton J, Dowsett M, Harris AL. Hormonal treatment of pancreatic

carcinoma: A phase II study of LHRH agonist goserelin plus hydrocortisone. Br J Cancer 1993;67:379-382.
11. Keating JJ, Johnson PJ, Cochrane AMG, Gazzard BG, Krasner N, Smith PM, Trewby PN, Wheeler P, Wilkinson SP, Williams R. A prospective randomised trial of tamoxifen and cyproterone acetate in pancreatic carcinoma. Br J Cancer 1989;60:789-792.
12. Smith FP, Hoth DF, Levin B, Karlin DA, MacDonald JS, Woolley PV, Schien PS. 5-Fluorouracil, adriamycin and mitomycin-C (FAM) chemotherapy for advanced adenocarcinoma of the pancreas. Cancer 1980;46:2014-2018.
13. Bukowski RM, Schacter LP, Groppe CW, Hewlett JS, Weick JK, Livingston RB. Phase II trial of 5-fluorouracil, adriamycin, mitomycin-C, and streptozotocin (FAM-S) in pancreatic carcinoma. Cancer 1982;50:197-200.
14. Wiggans RG, Woolley PV, MacDonald JS, Smythe T, Ueno W, Schein PS. Phase II trial of streptozotocin, mitomycin C and 5-fluorouracil (SMF) in the treatment of advanced pancreatic cancer. Cancer 1978;41:387-391.
15. Cullinan SA, Moertel CG, Fleming TR, Rubin JR, Krook JE, Everson LK, Windschitl HE, Twito DI, Marschke RF, Foley JF, Pfeifle DM, Barlow JF. A comparison of three chemotherapeutic regimens in the treatment of advanced pancreatic and gastric carcinoma. JAMA 1985;253:2061-2067.
16. Oster MW, Gray R, Panasci L, Perry MC. Chemotherapy for advanced pancreatic cancer: A comparison of 5-fluorouracil, adriamycin, and mitomycin (FAM) with 5-fluorouracil, streptozotocin, and mitomycin (FSM). Cancer 1986;57:29-33.
17. Gastrointestinal Tumor Study Group. Phase II studies of drug combinations in advanced pancreatic carcinoma: Fluorouracil plus doxorubicin plus mitomycin C and two regimens of streptozotocin plus mitomycin C plus fluorouracil. J Clin Oncol 1986;4:1794-1798.
18. Palmer KR, Kerr M, Knowles G, Cull A, Carter DC, Leonard CF. Chemotherapy prolongs survival in inoperable pancreatic carcinoma. Br J Surg 1994;81:882-885.
19. Mallinson CN, Rake MO, Cocking JB, Fox CA, Cwynarski MT, Diffey BL, Jackson GA, Hanley J, Wass VJ. Chemotherapy in pancreatic cancer: Results from a controlled, prospective, randomized, multicentre trial. Br Med J 1980;281:1589-1591.
20. Cullinan S, Moertel CG, Wieand HS, Schutt AJ, Krook JE, Foley JF, Norris BD, Kardinal CG, Tschetter LK, Barlow JF. A phase II trial on the therapy of advanced pancreatic carcinoma: Evaluations of the Mallinson regimen and combined 5-fluorouracil, doxorubicin, and cisplatin. Cancer 1990;65:2207-2212.
21. Plunkett W, Huang P, Searcy CE, et al. Gemcitabine: Preclinical pharmacology and mechanisms of action. Semin Oncol 1996;10:3-15.
22. Kawai Y, Yang L-Y, Plunkett W. Inhibition of repair of cisplatin-induced DNA damage in human colon carcinoma cells by gemcitabine [abstr]. AACR Proc 1998;39:156.
23. Bramhall S. The matrix metalloproteinases and their inhibitors in pancreatic cancer. Int J Pancreatol 1997;2:1-12.

Diagnosis and Management of Cholangiocarcinoma in Primary Sclerosing Cholangitis

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Cholangiocarcinoma remains difficult to diagnose and is a major cause of death in patients with primary sclerosing cholangitis. Recently serum carcinoembryonic antigen and carbohydrate antigen 19-9 (CA 19-9) levels have been reported to improve diagnostic accuracy in patients with cholangiocarcinoma and primary sclerosing cholangitis. We reviewed our experience with cholangiocarcinoma complicating primary sclerosing cholangitis to identify clinical factors associated with cholangiocarcinoma in patients with primary sclerosing cholangitis and to determine the appropriate management of patients with confirmed or suspected cholangiocarcinoma. Between 1984 and 1997, 25 patients (18%) were diagnosed with cholangiocarcinoma among 139 patients with primary sclerosing cholangitis. The diagnosis of primary sclerosing cholangitis was made coincident with the diagnosis of cholangiocarcinoma in 12 patients and preceded it by a mean of 62 months in the remaining 13 patients. The incidence of inflammatory bowel disease was higher ($P < 0.05$) in patients with cholangiocarcinoma (80% vs. 61%). Nine patients (36%) with cholangiocarcinoma were managed with either extrahepatic bile duct resection and/or partial hepatic resection (n = 5) or liver transplantation (n = 4), and the remaining 16 patients were unresectable at presentation. Serum CA 19-9 was elevated in all six patients with cholangiocarcinoma who were analyzed and in none of the eight patients without cholangiocarcinoma who were tested ($P < 0.01$). Actuarial 1- and 3-year survival rates in the resected patients (56% and 28%, respectively) were significantly longer ($P < 0.02$) than in the unresected patients (13% and 0%, respectively). The 10-year actuarial mortality rates for cholangiocarcinoma among all 139 patients was 25%. In summary, cholangiocarcinoma was the leading cause of death in patients with primary sclerosing cholangitis and was often diagnosed concurrently with or within months of its diagnosis. Early liver transplantation for patients with primary sclerosing cholangitis will not reduce the incidence of cholangiocarcinoma-related mortality in these patients. (J GASTROINTEST SURG 1999;3:357-368.)

KEY WORDS: Primary sclerosing cholangitis, cholangiocarcinoma, liver transplantation, biliary tract malignancies

Cholangiocarcinoma develops frequently in patients with primary sclerosing cholangitis (PSC) and is currently a leading cause of death in patients with this disease.[1-5] The widespread intra- and extrahepatic biliary strictures associated with PSC make the diagnosis of cholangiocarcinoma difficult in this setting. Most patients are diagnosed with advanced unresectable disease, and the median survival in patients with cholangiocarcinoma complicating PSC has been poor.[1,2] Recently several studies have reported encouraging results using serum tumor markers (carcinoembryonic antigen [CEA] or carbohydrate antigen 19-9 [CA 19-9]) or newer radiologic techniques in the early diagnosis of cholangiocarcinoma in patients with PSC.[6-8] Liver transplantation has produced excellent long-term survival in patients with end-stage liver disease from PSC and has been advocated early in the course of the disease to avoid the mortality associated with cholangiocarcinoma.[2,9-13] The aim of the present study was to review our experience in 25 patients with cholangiocarcinoma complicating PSC to identify clinical and radiologic factors associated with cholan-

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giocarcinoma and to determine the appropriate management of patients with known or suspected cholangiocarcinoma.

MATERIAL AND METHODS

Patient Characteristics

The records of 139 patients with PSC managed at the Johns Hopkins Hospital between January 1, 1984, and December 31, 1997, were retrospectively reviewed. All patients had cholangiographic findings consistent with a diagnosis of PSC including multifocal stricturing of the intrahepatic or extrahepatic bile ducts, or both.[14] Cholangiographic findings were supported by a clinical history and histologic specimens consistent with PSC. From this group of patients with PSC, 27 patients were identified with a primary cancer originating in the liver, biliary tract, or pancreas. Twenty-five patients (18%) had a histologically or cytologically confirmed diagnosis of cholangiocarcinoma. Two additional patients had an unresectable malignancy (one adenocarcinoma of the pancreas and one small cell carcinoma of the gallbladder) that was not consistent with cholangiocarcinoma and were not included in further data analysis.

Patient characteristics are shown in Table I for PSC patients with cholangiocarcinoma and for the control group of 112 patients without a biliary or pancreatic malignancy. Cholangiocarcinoma was diagnosed in the 25 patients at a mean age of 47 ± 2 years (range 23 to 62 years). The diagnosis of PSC was made coincident with the diagnosis of cholangiocarcinoma or during the initial evaluation of biliary symptoms (within 90 days of PSC diagnosis) in 12 patients and preceded it by a mean of 62 months in the remaining 13 patients. Fifty-two percent of the patients with cholangiocarcinoma were women, whereas only 32% of the 112 patients with PSC alone were women (not statistically significant).

Inflammatory bowel disease was present significantly more often ($P < 0.05$) in patients developing cholangiocarcinoma than in patients with PSC alone (80% vs. 61%). Inflammatory bowel disease was present a mean of 10 ± 2 years prior to the diagnosis of cholangiocarcinoma. Only five patients (20%) had established cirrhosis at the time cholangiocarcinoma was diagnosed.

Thirteen (52%) of the 25 patients had undergone an invasive operative or nonoperative procedure to improve biliary drainage before the diagnosis of cholangiocarcinoma. Six patients underwent one open biliary tract operation, whereas one patient had had two prior operations. Four patients had a previous simple cholecystectomy, three underwent a cholecys-

Table I. Patient characteristics and symptoms

	PSC with cholangiocarcinoma (n = 25)	PSC without cholangiocarcinoma (n = 112)
Mean age (yr) at diagnosis of PSC	45 ± 2	46 ± 2
Age (yr) at referral to Johns Hopkins Hospital		
Mean	46 ± 2	47 ± 2
Range	(23-62)	(7-89)
Sex		
Male	12	76
Female	13	36
Race		
White	23	94
African-American	2	16
Asian	0	2
Associated diseases (%)		
Inflammatory bowel disease	80*	61
Ulcerative colitis	52	43
Crohn's disease	20	14
Nonspecific inflammatory bowel disease	8	4
Cirrhosis	20	28
Symptoms (%)		
Jaundice	70	71
Pain	57	38
Pruritus	32	33
Cholangitis	23	22

*$P < 0.05$ vs. PSC patients without cholangiocarcinoma.

tectomy and common bile duct exploration, and one patient was managed with a bile duct resection and choledochojejunostomy. The most recent biliary operation was performed a mean of 78 months (range 1 to 180 months) before the diagnosis of cholangiocarcinoma. Seven patients (28%) had undergone one or more nonoperative procedures to dilate biliary strictures before the diagnosis of cholangiocarcinoma. Four patients were managed with a series of endoscopic balloon dilations starting a mean of 25 months (range 17 to 39 months) before the diagnosis of cholangiocarcinoma. Three patients had percutaneous transhepatic stents placed through dominant biliary strictures 1, 6, and 44 months before being diagnosed with cholangiocarcinoma.

Common symptoms in patients developing cholangiocarcinoma in the setting of PSC included jaundice (70%), weight loss (35%), pruritus (32%), nausea and vomiting (27%), cholangitis (23%), and anorexia (18%). Abdominal pain was present more often in patients with cholangiocarcinoma (57% vs. 38%), but this difference failed to reach statistical significance.

Management

The management of 25 patients with both cholangiocarcinoma and PSC is outlined in Table II. Nine patients underwent operative exploration and resection of all gross tumor. Resection of the extrahepatic biliary tract was performed in four patients as previously described and included the hepatic duct bifurcation (n = 3) or the left hepatic lobe (n = 1).[15] All four patients managed with resection had transhepatic biliary stents placed preoperatively. The diagnosis of cholangiocarcinoma was suspected preoperatively in three of the four patients undergoing biliary resection. A left hepatic lobectomy was performed in one patient with an intrahepatic cholangiocarcinoma. Four patients were managed with a total hepatectomy and liver transplantation. The diagnosis was suspected preoperatively in only one of the four patients undergoing liver transplantation.

Seven patients underwent operative exploration for a planned curative resection and were found to be unresectable because of peritoneal or liver metastases (n = 5), celiac lymph node metastases (n = 2), and/or locally extensive tumor (n = 2). Partial tumor resection and Roux-en-Y choledochojejunostomy were performed in two patients. Cholecystectomy was performed in three patients and biopsy without cholecystectomy in two patients (prior cholecystectomy [n = 1]). Chemical splanchnicectomy was also performed in three unresectable patients. One patient with a locally extensive tumor was unresectable at staging laparoscopy and underwent operative exploration after receiving radiation and chemotherapy. Metastatic disease was found to be present, and a cholecystectomy was performed. Eight patients were deemed unresectable based on radiographic studies and did not undergo operative exploration. Four of these patients were palliated with transhepatic stents, and one patient had an endoscopic stent placed. Three patients received no further therapy.

Table II. Management of 25 patients with primary sclerosing cholangitis and cholangiocarcinoma

	No. of patients
Resection (n = 9)	
Biliary tract resection	3
Biliary tract and partial hepatic resection	1
Partial hepatic resection	1
Total hepatectomy and liver transplantation	4
*Palliative operation (n = 8)**	
Roux-en-Y choledochojejunostomy	2
Cholecystectomy	4
Splanchnicectomy	3
Biopsy	1
Nonoperative management (n = 8)	
Transhepatic biliary stent	4
Endoscopic biliary stent	1
None	3

*More than one procedure was performed in two patients.

Tumor Characteristics

Cholangiocarcinomas were classified as previously described.[16] Intrahepatic tumors were confined to the liver, did not involve the extrahepatic biliary tree, and showed no evidence of a primary tumor elsewhere. Perihilar tumors involved or required resection of the hepatic duct bifurcation. Patients with a significant intrahepatic component with involvement of the hepatic duct bifurcation were included with the perihilar rather than the intrahepatic tumors. Nineteen tumors (76%) were classified as perihilar, four tumors (16%) were intrahepatic, and two tumors (8%) involved the gallbladder primarily.

Endoscopic brushings were taken from dominant biliary strictures before the definitive diagnosis of cholangiocarcinoma in 10 (40%) of the 25 patients. Cytologic examination was diagnostic for carcinoma in three patients and suspicious for carcinoma in one patient. The remaining six samples yielded a benign diagnosis (marked atypia [n = 2] and reactive changes [n = 4]). Biopsy samples were taken from dominant strictures in four additional patients using the percutaneous choledochoscope inserted through a trans-

Table III. Laboratory evaluation

	PSC with cholangiocarcinoma (n = 25)	PSC without cholangiocarcinoma (n = 112)
Bilirubin (mg/dl)	8.5 ± 2	5.8 ± 0.7
Risk score	3.67 ± 0.3	3.62 ± 0.2
CEA (ng/ml)*	24 ± 11	2.8 ± 2
CA 19-9 (IU/ml)†	590 ± 360	12 ± 2

*Mean values in 36 patients.
†Mean values in 14 patients.

hepatic stent tract. These biopsy specimens demonstrated a cholangiocarcinoma and a biliary adenoma in one patient each and were benign in two patients. In addition, one patient had metastatic cholangiocarcinoma diagnosed on the basis of peritoneal fluid cytologic findings later confirmed by autopsy. The sensitivity of endoscopic biliary cytologic examination and/or biopsy in the diagnosis of cholangiocarcinoma was 43% in all patients, 25% in patients with resectable tumors, and 50% in patients with unresectable tumors.

Following resection, microscopic residual tumor was present at the surgical margin in three of the five patients undergoing biliary and/or hepatic resection and in one of four patients managed with total hepatectomy and liver transplantation. Sixteen patients were not resectable at the time of diagnosis. Metastatic disease was present in 13 patients (liver [n = 6], peritoneum [n = 6]), and celiac lymph nodes [n = 3]). Two patients were not operative candidates because of coexistent medical problems and uncompensated cirrhosis, respectively, and one patient had a locally unresectable tumor.

Laboratory Evaluation

Serum bilirubin and a multicenter risk score was determined at the time of referral for the PSC patients without cancer and at the time of cancer diagnosis in the patients with cholangiocarcinoma[17] (Table III). Risk scores were calculated from the patient's age, serum bilirubin level, histologic stage according to the Ludwig criteria,[18] and the presence or absence of splenomegaly. Serum bilirubin levels were higher in the patients with cholangiocarcinoma, but this difference did not reach statistical significance. Multicenter risk scores were similar in patients with and without cholangiocarcinoma.

Serum CEA levels were determined in 18 of the 21 PSC patients with cholangiocarcinoma managed since January 1989 and in 18 of 78 patients without cholangiocarcinoma managed during the same time period (Table III and Fig. 1). Median serum CEA levels were 5.3 ng/ml (range 0.7 to 186 ng/ml) in patients with cholangiocarcinoma and 1.8 ng/ml (range 0.6 to 11.8 ng/ml) in patients without cholangiocarcinoma (P = 0.08). Serum CEA levels were elevated (normal <5.0 ng/ml) significantly (P = 0.01) more often in patients with cholangiocarcinoma than in control patients (56% vs. 11%). Patients with resectable cholangiocarcinoma had elevated CEA levels as frequently as patients with unresectable tumors (60% vs. 55%). The sensitivity and specificity of serum CEA for the diagnosis of cholangiocarcinoma in PSC were 56% and 89%, respectively.

Serum carbohydrate antigen 19-9 (CA 19-9) levels were determined in only six patients with cholangiocarcinoma and in eight control patients (Fig. 1). Median CA 19-9 levels were 195 U/ml (range 74 to 2340 U/ml) in the patients with cholangiocarcinoma and 10 U/ml (range <8 to 23 U/ml) in the control group (P = 0.08). Serum CA 19-9 levels were elevated in all six cancer patients (one resectable and five unresectable) and in none of the eight control patients (P <0.01). The sensitivity and specificity of serum CA 19-9 levels in the diagnosis of cholangiocarcinoma were 100% and 100%, respectively.

No significant differences were observed among resected and unresected patients in hematocrit, white blood cell count, or serum creatinine, total bilirubin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, or albumin.

Radiologic Evaluation

CT was performed in the evaluation of 15 patients with cholangiocarcinoma and demonstrated a mass lesion in six (40%). MRI scans were obtained in 12 patients, and a mass lesion was identified in five of them (42%). Visceral angiography was performed in 16 patients at the time of cancer diagnosis and was normal in only two patients (12%). Occlusion or encasement was present in the left portal vein in eight patients (50%), the main portal vein in six patients (38%), the right portal vein or main hepatic artery in three patients (19%) each, and either the right or left he-

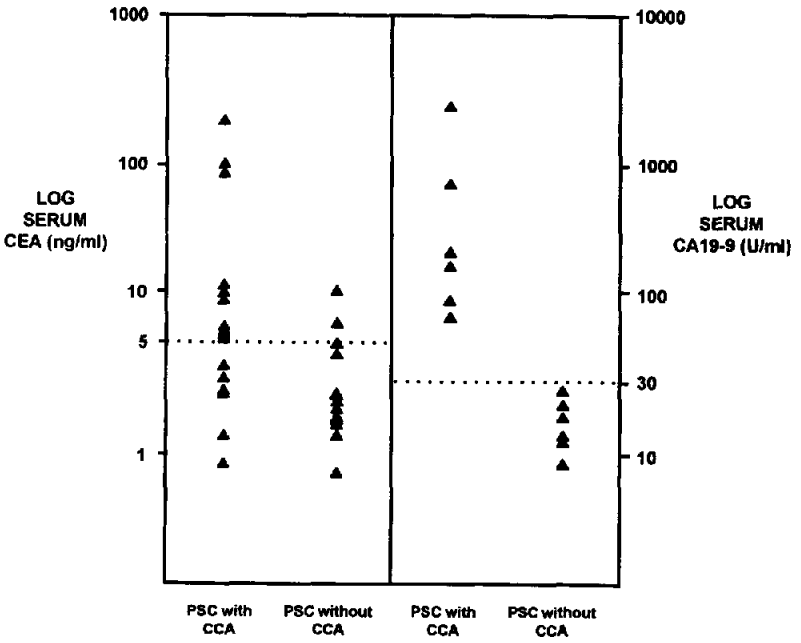

Fig. 1. Serum carcinoembryonic antigen *(CEA)* and carbohydrate antigen 19-9 *(CA 19-9)* levels in primary sclerosing cholangitis *(PSC)* patients with and without cholangiocarcinoma *(CCA)*. Both serum CEA and CA 19-9 levels were elevated significantly ($P < 0.05$) more often in patients with cholangiocarcinoma.

Table IV. Accuracy of radiologic evaluation in predicting resectability of cholangiocarcinoma

	Sensitivity (%)	Specificity (%)	Accuracy (%)
CT	75	67	69
MRI	80	57	67
Visceral angiography	100	58	71

patic artery in two patients (12%). The sensitivity, specificity, and overall accuracy of CT, MRI, and visceral angiography at predicting resectability in patients with PSC and cholangiocarcinoma are shown in Table IV.

Endoscopic retrograde cholangiography and/or percutaneous transhepatic cholangiography was performed in 22 patients during the initial diagnostic evaluation for cholangiocarcinoma. Percutaneous transhepatic stents were inserted or were in place in 15 patients including all of the patients undergoing biliary resection and reconstruction (n = 6) and two of the patients at the time of liver transplantation. Hemobilia occurred in three patients after transhepatic stent placement and required hepatic artery embolization in two patients.

Six patients were followed at Johns Hopkins for more than 6 months before being diagnosed with cholangiocarcinoma. Three of these patients had incidental cholangiocarcinomas detected at liver transplantation. CT scans and cholangiograms were obtained in two of these three patients and MRI scans were obtained in all three patients during the year preceding transplantation. None of the studies were read as suspicious for cholangiocarcinoma at the time of the study. Three patients were unresectable at the time of diagnosis. One of these two patients had a filling defect visible on endoscopic cholangiography, and two of three CT scans demonstrated a mass lesion at the time of diagnosis.

Statistical Analysis

All data are presented as percentage of patients or mean ± standard error of the mean (SEM). Percentages were compared using Fisher's exact test or chi-square test where appropriate, and means were compared using Student's *t* test. Survival curves were constructed using the Kaplan-Meier technique and were compared by the log-rank test.[19]

RESULTS

Mortality and Morbidity

Two (12%) of the 17 patients died after surgical exploration without being discharged from the hospital. One (25%) of four patients managed with liver transplantation died of hemorrhage. One of two patients undergoing a palliative resection and Roux-en-Y choledochojejunostomy died of sepsis. No operative deaths occurred in patients undergoing biliary and/or hepatic resection or laparotomy without resection. Three (38%) of the eight patients managed nonoperatively with unresectable tumors died within 30 days of the diagnosis of sepsis and liver failure.

Postoperative complications developed in 10 patients (59%). The complication rate was 71% in patients undergoing biliary and/or hepatic resection, 75% in patients managed with liver transplantation, and 33% in patients undergoing laparotomy without resection. The most common complications in the operatively managed patients included cholangitis (23%) and perihepatic abscess (18%). The mean postoperative stay was 17 ± 4 days for all of the operatively managed patients, 25 ± 7 days in the patients managed with a biliary and/or hepatic resection, 21 ± 6 days following liver transplantation, and 7 ± 2 days following laparotomy without resection.

Survival

The 1-, 3-, and 5-year survival rates for all of the 25 patients with cholangiocarcinoma complicating PSC were 28%, 9%, and 0%, respectively. Two patients are still alive with no evidence of disease 10 and 53 months, respectively, after liver transplantation. The remaining 23 patients have died between 0 and 58 months after diagnosis. Median survival for all PSC patients with cholangiocarcinoma was 7 months (Table V).

The 1-, 3-, and 5-year survival rates for the nine resected patients were 56%, 28%, and 0%, respectively (Fig. 2). Five patients were managed with resection of the extrahepatic biliary tree and/or hepatic resection, and all patients died of recurrent cancer. The one patient with known cholangiocarcinoma preoperatively died of recurrent disease 22 months after transplantation. Three patients had incidental (not suspected preoperatively and discovered in the explant liver) cholangiocarcinoma. One of these three patients died in the perioperative period of hemorrhage, and the other two are still alive. The actuarial survival of patients with cholangiocarcinoma managed with liver transplantation was significantly lower ($P < 0.05$) than in the 26 PSC patients without cholangiocarcinoma managed since 1990 (Fig. 3).

None of the unresectable patients lived longer than 17 months after diagnosis. Eight patients were palliated operatively with a 1-year survival rate of 25% and a median survival of 6.5 months (Fig. 2). None of the nonoperatively managed patients lived longer than 9 months with a median survival of 2.5 months in this group. The actuarial survival following resection of cholangiocarcinoma in PSC was significantly longer than for patients who were unresectable ($P < 0.01$), underwent operative exploration but did not undergo resection of all gross tumor ($P < 0.02$), or were managed nonoperatively ($P < 0.01$) (Fig. 2).

The 10-year actuarial mortality for all 139 patients with PSC is shown in Fig. 4. The mortality from all causes 10 years after the diagnosis of PSC was 47%. Twenty-three (92%) of 25 patients with cholangiocarcinoma died leading to a 10-year cholangiocarcinoma-related mortality of 25% among all 139 patients with PSC. Most of the deaths in the first year following the diagnosis of PSC were due to cholangiocarcinoma leading to a 1-year actuarial mortality from cholangiocarinoma of 9%. Following the initial

Table V. Actuarial survival of primary sclerosing cholangitis patients with cholangiocarcinoma

Patients	No.	Mean survival (mo)	Median survival (mo)	1-year (%)	3-year (%)	5-year (%)
All patients	25	11 ± 3	7	28	9	0
Resected	9	21 ± 7	18	56	28	0
Hepatic and/or bile duct resection	5	20 ± 10	9	40	20	0
Liver transplantation	4	21 ± 11	22	75	38	0*
Unresected	16	6 ± 1†	5	13	0	0
Operative palliation	8	8 ± 2‡	6.5	25	0	0
Nonoperative palliation	8	3 ± 1†	2.5	0	0	0

*Two patients still alive at 10 and 53 months, respectively, following liver transplantation.
†$P < 0.05$ vs. resected patients.
‡$P < 0.05$ vs. nonoperative palliation.

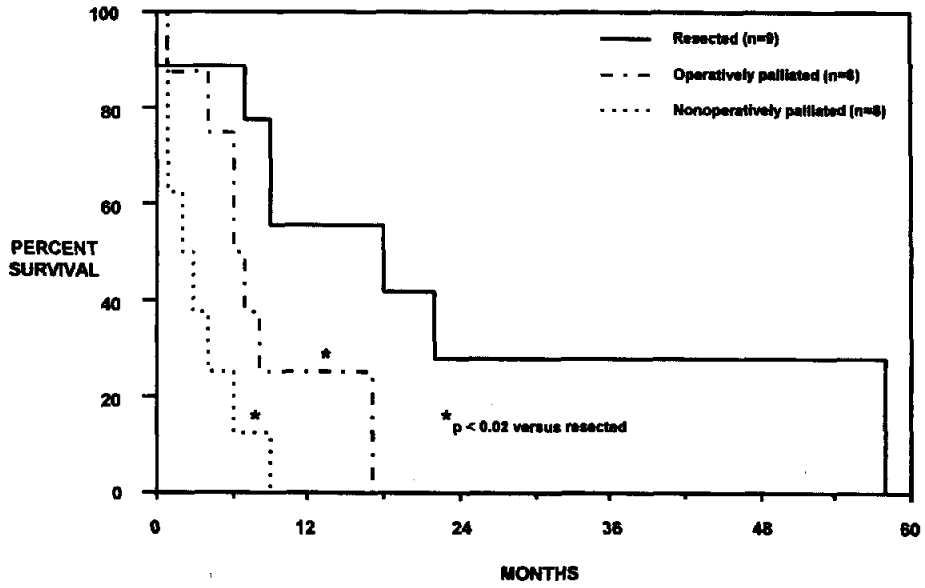

Fig. 2. Overall survival for patients with primary sclerosing cholangitis and cholangiocarcinoma. Overall survival for resected patients was significantly ($P < 0.02$) longer than for operatively or nonoperatively palliated patients.

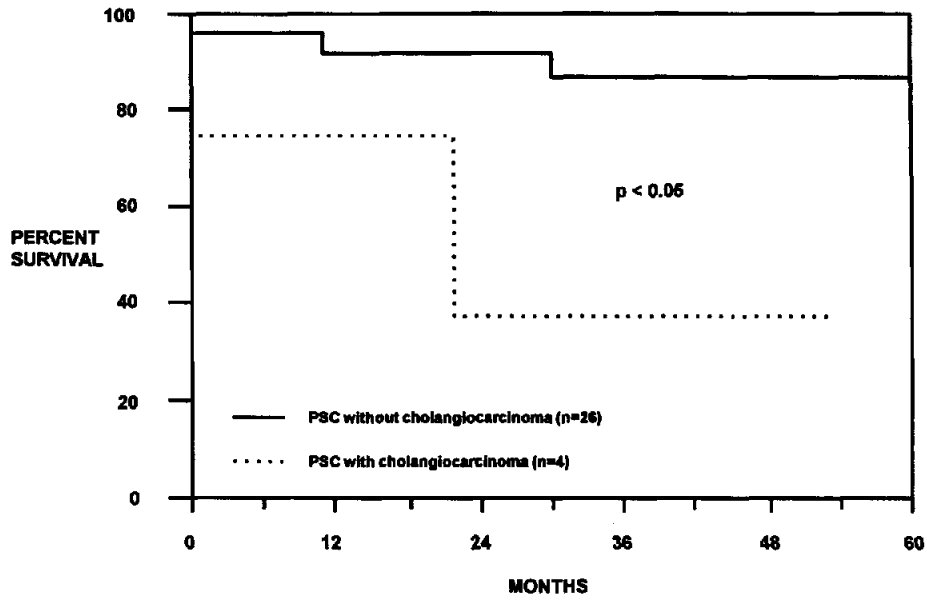

Fig. 3. Overall survival following liver transplantation in patients with primary sclerosing cholangitis *(PSC)*. Overall survival was significantly ($P < 0.05$) longer in patients without cholangiocarcinoma.

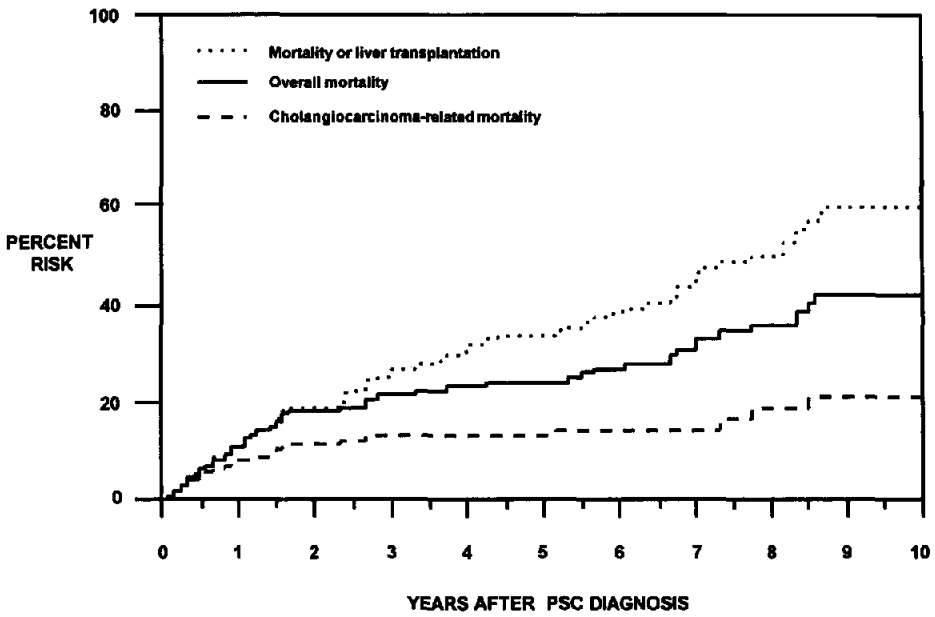

Fig. 4. Actuarial mortality during the 10-year period following the diagnosis of primary sclerosing cholangitis *(PSC)* in all 139 patients managed at Johns Hopkins Hospital between 1984 and 1997. Most of the deaths during the first year after the diagnosis of cholangiocarcinoma were related to cholangiocarcinoma. After the first year, the number of deaths from other causes increased. Overall 10-year mortality was 47% and cholangiocarcinoma-related mortality was 25% at 10 years. Sixty-six percent of the 139 patients had either died or received a liver transplant within 10 years of being diagnosed with primary sclerosing cholangitis.

year after the diagnosis of PSC, most deaths were due to causes other than cholangiocarcinoma. Sixty-six percent of patients had died or received a liver transplant 10 years after the diagnosis of PSC. The overall 10-year risk of developing cholangiocarcinoma among PSC patients at Johns Hopkins Hospital who had not yet received a liver transplant was 27%.

DISCUSSION

Cholangiocarcinoma occurs frequently in patients with PSC and was detected in 18% of the patients with this disease managed at Johns Hopkins during the past 14 years. The diagnosis of cholangiocarcinoma was often made coincident with the diagnosis of PSC and occurred more commonly in patients with inflammatory bowel disease. Patients with cholangiocarcinoma complicating PSC were similar with regard to symptoms, age, serum bilirubin, and multicenter risk scores to PSC patients without cancer. Serum CA 19-9 was elevated in all six patients with cholangiocarcinoma analyzed and in none of the patients without cholangiocarcinoma who were tested ($P < 0.01$). Serum CEA, CT and MRI scans, and cytologic findings were less sensitive at detecting cholangiocarcinoma. Surgical resection was possible in nine patients (36%). The median survival for all patients with cholangiocarcinoma was 7 months, and long-term disease-free survival was seen only in patients with unsuspected cholangiocarcinomas detected at the time of liver transplantation.

Cholangiocarcinoma was initially reported as a frequent complication of PSC by Rosen et al.[1] The reported incidence of cholangiocarcinoma in PSC ranges from 7% to 42%.[1-5] A recent population-based study of 125 patients in Sweden reported a cumulative cholangiocarcinoma risk of 11.2% within the first 10 years following the diagnosis of PSC.[5] The overall 10-year survival following the diagnosis of PSC was 69% in this group of patients, and cholangiocarcinoma was the second leading cause of death accounting for 31% of the deaths. In the present study, the cumulative 10-year risk of developing cholangiocarcinoma was 27%, and cholangiocarcinoma caused 44% of the deaths. The higher incidence of cholangiocarcinoma at our institution likely reflects the greater tendency to refer patients with this diagnosis than with uncomplicated PSC. Overall survival 10 years after the diagnosis of PSC was only 53%, in large part because of the relatively high incidence of cholangiocarcinoma at our institution.

No definite risk factors for developing cholangiocarcinoma in PSC have been identified to date. In our series, inflammatory bowel disease was present signif-

icantly more often in patients with cholangiocarcinoma and was present on average for 10 years before the diagnosis of cholangiocarcinoma. In other studies, inflammatory bowel disease was commonly present for a prolonged period in patients with cholangiocarcinoma but did not increase the risk of developing cholangiocarcinoma.[3-5] In the present study, the incidence of cholangiocarcinoma also was higher in women than in men (26% vs. 14%). Although this difference did not reach statistical significance, a similar trend has been observed in several other studies.[5,6]

The duration of clinically evident PSC was often quite brief prior to the diagnosis of cholangiocarcinoma. More than half of the patients in this series were diagnosed with PSC less than 1 year before they presented with cholangiocarcinoma, and often both diagnoses were made at the same time. Rosen et al.[1] also reported the simultaneous diagnosis of PSC and cholangiocarcinoma in 30% of their patients, and the diagnosis of cholangiocarcinoma was made within 1 year of the diagnosis of PSC in half of the patients in the Swedish study.[5] Furthermore, the severity of hepatic parenchymal injury also does not correlate with the risk of cholangiocarcinoma. Only 20% of the patients in this series had cirrhosis at the time of diagnosis, and other studies have also seen no correlation between histologic stage and the risk of developing cholangiocarcinoma.[1]

Identifying clinical, laboratory, or radiologic signs diagnostic or suggestive of cholangiocarcinoma in PSC has been difficult. Rosen et al.[1] suggested that cholangiocarcinoma was heralded by rapid clinical deterioration with progressive jaundice, weight loss, and pain. However, a deteriorating course was not observed in this series or in several other studies, despite the fact that most patients present with advanced, unresectable tumors.[3,5] Abdominal pain has been significantly associated with cholangiocarcinoma in the setting of PSC in other series, and we also observed a similar trend in patients with cholangiocarcinoma.[3,4] Progressive jaundice was also not uniformly observed in the present study, and no significant difference was observed in serum bilirubin values or results of other liver function tests between patients with and without cholangiocarcinoma.

Serum CEA and CA 19-9 may have a role in the diagnosis of cholangiocarcinoma in PSC. Serum CA 19-9 has a higher sensitivity than CEA in diagnosing cholangiocarcinoma, and Nichols et al.[7] reported an 89% sensitivity and 86% specificity for detecting cholangiocarcinoma in the setting of PSC. Ramage et al.[6] also reported an index using both serum CA 19-9 and serum CEA levels with a sensitivity and specificity for diagnosing cholangiocarcinoma of 66% and 100%, respectively. This series of 74 patients with PSC included 11 with occult and four with clinically evident cholangiocarcinoma. Serum CA 19-9 was also a better tumor marker for cholangiocarcinoma in the present study with a sensitivity and specificity of 100% in the small number of patients examined. Moreover, the serum CA 19-9 level was elevated (90 U/ml) in the one patient with an occult tumor that was analyzed in our series and in 54% of the occult tumors reported by Ramage et al.[6] CA 19-9 is excreted in the bile, and the serum level is often mildly elevated in benign hepatobiliary disorders including PSC.[20] However, marked elevations in serum CA 19-9 are only rarely associated with benign disease.

The diagnostic accuracy of current radiologic techniques and cytologic findings for detecting cholangiocarcinoma in the setting of PSC remains poor.[6] The sensitivity of CT and MRI at detecting cholangiocarcinoma at a resectable stage was less than 50%, and endoscopic cholangiography with brush cytology or biopsy also missed cholangiocarcinoma in its early stages. Four patients with cholangiocarcinoma in the current series had biliary strictures endoscopically dilated within 2 years of being diagnosed with cholangiocarcinoma, and this problem has been observed in other series of patients managed with endoscopic dilation.[21-23] Three of these patients had metastatic disease when diagnosed with cholangiocarcinoma. In addition, biliary cytologic findings or brushings from suspicious strictures were often benign in patients with resectable lesions. Campbell et al.[8] retrospectively reviewed ultrasound, CT, and MRI images and cholangiograms from 30 patients with PSC and cholangiocarcinoma and reported the presence of probable or definite tumors in 83% of these patients. However, as in our experience, many of these tumors were not appreciated at the time of the radiologic study.

For patients with a definite preoperative diagnosis of cholangiocarcinoma, exploration and surgical resection should be attempted if imaging studies indicate that the tumor may be resectable. Survival was significantly prolonged in patients undergoing resection and was similar to survival in other patients with perihilar cholangiocarcinoma.[16] Similar mean survival was observed with resection of the extrahepatic biliary tract and/or hepatic resection or total hepatectomy and liver transplantation. Long-term survival following resection of occult cholangiocarcinomas identified at transplantation has been comparable to that in patients without cholangiocarcinoma, and both patients surviving the transplant in the current series are still alive.[9]

For the PSC patient with a suspected cholangiocarcinoma based on cholangiographic appearance, mildly elevated tumor markers, or suspicious or atyp-

ical cytologic findings, the appropriate management is more controversial. We strongly believe that patients with a dominant stricture that recurs following one or at most two endoscopic balloon dilations and/or has associated dysplasia or cytologic atypia on brushings or biopsy should be resected rather than managed with further cholangiography with repeat cytologic examination, brushings, biopsies, and/or balloon dilation, or measurement of serum tumor markers. We have recently reported that extrahepatic biliary resection does not adversely affect patient survival and, in fact, prolonged both overall survival and survival until death or liver transplantation when compared to patients whose biliary strictures were managed with endoscopic and/or percutaneous techniques.[24] Furthermore, the recent experience at UCLA in 127 patients with PSC demonstrated no effect of prior biliary tract surgery on survival after liver transplantation.[9] A lower threshold for proceeding to biliary resection may improve the dismal resectability rate for patients with cholangiocarcinoma and PSC.

Early liver transplantation has been recommended for patients with PSC to decrease the mortality from cholangiocarcinoma.[2,11-12] Criteria for liver transplantation in patients with PSC have recently been proposed to achieve this goal and include the following: (1) a Mayo risk score >4.8; (2) cirrhosis and complications of portal hypertension; and (3) disabling symptoms.[11] Once a patient is diagnosed with PSC and considered and referred for liver transplantation, the process includes a pretransplant evaluation, activation for transplantation when the preceding criteria are met, and transplantation when an organ is available. However, only 20% to 30% of patients who develop cholangiocarcinoma have cirrhosis and would meet these criteria for liver transplantation.[1] Furthermore, the mean time from the diagnosis of PSC to transplant was 5 years in a recent series, and the shortest time from diagnosis to transplant in the 30 patients undergoing liver transplantation in the present series was 18 months (see Fig. 4).[13] Proponents of early liver transplantation suggest that most cases of cholangiocarcinoma would be prevented by transplantation earlier in the course of the disease. However, since the greatest risk for developing cholangiocarcinoma is within a year of being diagnosed with PSC, most patients developing cholangiocarcinoma would not benefit from a policy of early liver transplantation in PSC. In fact, only 2 of the 25 patients in this series met the preceding criteria for liver transplantation, had PSC long enough to be a transplant candidate, and would have benefited from early transplantation. One of these two patients did have a liver transplant and had a small occult cholangiocarcinoma.

Cholangiocarcinoma remains a devastating complication in patients with PSC. The diagnosis of cholangiocarcinoma is often made early in the course of PSC minimizing the value of an effective screening tool unless the diagnosis of PSC can be made earlier in asymptomatic patients with inflammatory bowel disease. Serum CA 19-9 is the most useful screening test to date. For patients with known cholangiocarcinoma, surgical resection significantly prolongs survival. Liver transplantation is associated with excellent long-term survival in patients with incidental cholangiocarcinoma. Early liver transplantation in patients with PSC is unlikely to lower the mortality rate, as most patients present too soon after the diagnosis of PSC or too early in the course of their disease to be transplant candidates. A more aggressive surgical approach to suspicious strictures may increase the rate of early detection of cholangiocarcinoma and survival in patients with PSC.

REFERENCES

1. Rosen CB, Nagorney DM, Wiesner RH, Coffey RJ Jr, LaRusso NF. Cholangiocarcinoma complicating primary sclerosing cholangitis. Ann Surg 1991;213:21-25.
2. Farges O, Malassagne B, Sebaugh M, Bismuth H. Primary sclerosing cholangitis: Liver transplantation or biliary surgery? Surgery 1995;117:146-155.
3. Bergquist A, Glaumann H, Persson B, Broome U. Risk factors and clinical presentation of hepatobiliary carcinoma in patients with primary sclerosing cholangitis: A case-control study. Hepatology 1998;27:311-316.
4. Broome U, Olsson R, Loof L, Bodemar G, Hultcrantz R, Danielsson A, Prytz H, Sandberg-Gertzen H, Wallerstedt S, Lindberg G. Natural history and prognostic factors in 305 Swedish patients with primary sclerosing cholangitis. Gut 1996;38:610-615.
5. Kornfeld D, Ekbom A, Ihre T. Survival and risk of cholangiocarcinoma in patients with primary sclerosing cholangitis. Scand J Gastroenterol 1997;32:1042-1045.
6. Ramage JK, Donaghy A, Farrant JM, Iorns R, Williams R. Serum tumor markers for the diagnosis of cholangiocarcinoma in primary sclerosing cholangitis. Gastroenterology 1995; 108:865-869.
7. Nichols JC, Gores GJ, LaRusso NF, Wiesner RH, Nagorney DM, Ritts RE Jr. Diagnostic role of serum CA 19-9 for cholangiocarcinoma in patients with primary sclerosing cholangitis. Mayo Clin Proc 1993;68:874-879.
8. Campbell WL, Ferris JV, Holbert BL, Thaete FL, Baron RL. Biliary tract carcinoma complicating primary sclerosing cholangitis: Evaluation with CT, cholangiography, US, and MR imaging. Radiology 1998;207:41-50.
9. Goss JA, Shackleton CR, Farmer DG, Arnaout WS, Seu P, Markowitz JS, Martin P, Stribling RJ, Goldstein LI, Busuttil RW. Orthotopic liver transplantation for primary sclerosing cholangitis: A 12-year single center experience. Ann Surg 1997;225:472-481.
10. Abu-Elmagd KM, Malinchoc M, Dickson ER, Fung JJ, Murtaugh PA, Langworthy AL, Demetris AJ, Krom RAF, Van Thiel DH, Starzl TE. Efficacy of hepatic transplantation in patients with primary sclerosing cholangitis. Surg Gynecol Obstet 1993;177:335-344.
11. Wiesner RH, Porayko MK, Hay JE, LaRuffo NF, Steers JL, Krom RA, Dickson ER. Liver transplantation for primary sclerosing cholangitis: Impact of risk factors on outcome. Liver Transpl Surg 1996;2:99-108.

12. Nashan B, Schlitt JH, Tusch G, Oldhafer KJ, Ringe B, Wagner S, Pichlmayr R. Biliary malignancies in primary sclerosing cholangitis: Timing for liver transplantation. Hepatology 1996;23:1105-1111.
13. Narumi S, Roberts JP, Emond JC, Lake J, Ascher NL. Liver transplantation for sclerosing cholangitis. Hepatology 1995;22:451-457.
14. MacCarty RL, LaRusso NF, Wiesner RH, Ludwig J. Primary sclerosing cholangitis: Findings on cholangiography and pancreatography. Radiology 1983;149:39-44.
15. Cameron JL, Pitt HA, Zinner MJ, Herlong HF, Kaufman SL, Boitnott JK, Coleman J. Resection of hepatic duct bifurcation and transhepatic stenting for sclerosing cholangitis. Ann Surg 1988;207:614-622.
16. Nakeeb A, Pitt HA, Sohn TA, Coleman J, Abrams RA, Piantadosi S, Hruban RH, Lillemoe KD, Yeo CJ, Cameron JL. Cholangiocarcinoma: A spectrum of intrahepatic, perihilar, and distal tumors. Ann Surg 1996;224:463-475.
17. Dickson ER, Murtaugh PA, Wiesner RH, Grambsch PM, Fleming TR, Ludwig J, LaRusso NF, Malinchoc M, Chapman RW, Kaplan MM, Maddrey WC, Williams R, Farrant M, Langworthy A. Primary sclerosing cholangitis: Refinement and validation of survival models. Gastroenterology 1992;103:1893-1901.
18. Ludwig J, Barham SS, LaRusso NF, Elveback LR, Wiesner RH, McCall JT. Morphologic features of chronic hepatitis associated with primary sclerosing cholangitis and chronic ulcerative colitis. Hepatology 1981;1:632-640.
19. Kaplan EL, Meier P. Non-parametric estimation from incomplete observations. J Am Stat Assoc 1958;53:457-481.
20. Maestranzi S, Przemioslo R, Mitchell H, Sherwood RA. The effect of benign and malignant liver disease on the tumour markers CA 19-9 and CEA. Ann Clin Biochem 1998;35:99-103.
21. Johnson GK, Geenan JE, Venu RP, Schmalz MJ, Hogan WJ. Endoscopic treatment of biliary tract strictures in primary sclerosing cholangitis: A large series and recommendations for treatment. Gastrointest Endosc 1991;37:38-43.
22. Gaing AA, Geders JM, Cohen SA, Siegel JH. Endoscopic management of primary sclerosing cholangitis: Review, and report of an open series. Am J Gastroenterol 1993;88:2000-2008.
23. Lee JG, Schutz SM, England RE, Leung JW, Cotton PB. Endoscopic therapy of sclerosing cholangitis. Hepatology 1995;21:661-667.
24. Ahrendt SA, Pitt HA, Kalloo AN, Venbrux A, Klein AS, Herlong JF, Coleman J, Lillemoe KD, Cameron JL. Primary sclerosing cholangitis: Resect, dilate, or transplant? Ann Surg 1998;227:412-423.

Discussion

Dr. J. Roslyn (Philadelphia, Pa.). One of the greatest challenges is how to identify those patients with PSC who have already developed cholangiocarcinoma. How did you select the patients for operation? What was it about that clinical course that led to the decision to operate? Since this is a retrospective study, we do not know how many of your other patients with PSC are harboring a cholangiocarcinoma. Although you are an advocate of resection, only five patients underwent resection, and three of those patients had positive margins. The only long-term survivors are really those patients who underwent liver transplantation. Your conclusion was that most of those patients would not be candidates for liver transplantation based on conventional criteria. Should we reevaluate the criteria for liver transplantation? I am not convinced that conventional resection is going to provide a long-term cure. In terms of diagnosis, we have become great fans of magnetic resonance cholangiography, and there are some new agents on the horizon that are better than gadolinium in terms of identifying the bile duct. Do you have any experience with or thoughts on improved magnetic resonance technology in helping you identify those patients who are developing cholangiocarcinomas? Finally, do you have any information about biliary levels of CEA or CA 19-9?

Dr. S. Ahrendt. We evaluated these 25 patients to decide whether performing a liver transplant using the conventional criteria for selecting patients for transplantation would have had any impact on their survival. Fourteen of the 25 presented with cholangiocarcinoma too early to receive a liver transplant. They had cancer at the time of diagnosis of PSC, so a liver transplant would not have helped in most of these patients. The criteria currently in use to select patients for transplantation include a Mayo risk score of 4.8, complications of portal hypertension, or recurrent cholangitis not responding to conventional therapy. In applying these criteria in the remaining 11 patients, only two would have come to liver transplantation earlier in their course, and of those two, one did receive a liver transplant and had one of the incidental cholangiocarcinomas. I do not think that early liver transplantation is going to be effective unless the diagnosis of PSC can be made earlier and that brings us to your last question about new imaging modalities. Two things need to happen to make the diagnosis of PSC at an earlier stage. First, PSC needs to be suspected in asymptomatic patients with inflammatory bowel disease, usually on the basis of abnormal liver function tests. Second, the diagnosis needs to be confirmed by cholangiography. Until recently the diagnosis of PSC required an invasive endoscopic cholangiogram, but perhaps the availability of newer noninvasive techniques such as magnetic resonance cholangiography will at least make it easier to evaluate these asymptomatic patients. Recent studies suggest in some cases a diagnosis of primary sclerosing cholangitis can be made using half-Fourier acquisition single shot turbo spin-echo (HASTE) magnetic resonance cholangiography. It is true that, with surgical resection, positive margins were common and most patients did poorly. These patients had a known cholangiocarcinoma preoperatively and had they had a liver transplant, they would have done poorly as well. The key is to make the diagnosis of cholangiocarcinoma early. We were struck by the number of patients in our series who had dominant strictures managed with endoscopic or percutaneous balloon dilation and were then diagnosed a year or two later with cholangiocarcinoma. Had they un-

dergone resection of that stricture when it was initially identified, the tumor may have been at a stage where a curative resection could have been performed.

Dr. M. Callery (Worcester, Mass.). Was there any change over time in your use of hepatic resection? How did you select patients for hepatic resection and what was the extent of the resections?

Dr. Ahrendt. Two patients in the resection group underwent a partial hepatectomy. One patient had a negative right hepatic duct margin and a positive left hepatic duct margin and underwent a left hepatectomy in addition to resection of the extrahepatic biliary tract and hilum. The other patient had an intrahepatic tumor that was resected with a left hepatic lobectomy. The indication for surgery was suspicion of cholangiocarcinoma in four of the five patients who were resected, and the presence of a dominant stricture and jaundice in the fifth patient. In the 1980s, at our institution, dominant strictures of the hilum were managed with surgical resection of the hilum and extrahepatic biliary tract, and several of those patients had unsuspected cholangiocarcinoma detected postoperatively in the specimen.

Dr. K. Kelly (Scottsdale, Ariz.). In those patients who had liver transplants, were there any multicentric tumors found in the specimens? Can you build a case for transplantation based on the multicentricity of the disease?

Dr. Ahrendt. The four patients managed with liver transplantation did not have multicentric tumors. Patients can have diffuse cholangiocarcinoma. In addition, molecular abnormalities, dysplasia, and carcinoma in situ have been identified in hepatic ducts away from the primary tumor in patients with PSC who have had a liver transplant.

S4a + S5 With Caudate Lobe (S1) Resection Using the Taj Mahal Liver Parenchymal Resection for Carcinoma of the Biliary Tract

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Recently we have been performing S4a + S5 with total resection of the caudate lobe (S1) by using a dome-like dissection along the root of the middle hepatic vein at the pinnacle, which we refer to as the Taj Mahal liver parenchymal resection, for carcinoma of the biliary tract. This procedure offers the following advantages: (1) It allows total resection of the caudate lobe, including the paracaval portion (S9), and (2) because the cut surface of the liver is large, it allows intrahepatic jejunostomy to be performed more easily with a good field of view. The indications for this procedure include hilar bile duct carcinoma, gallbladder carcinoma, and choledochal cyst (type IVA). Because of the high rate of hilar liver parenchyma and caudate lobe invasion associated with hilar bile duct carcinoma, the liver must be resected. The Taj Mahal procedure is indicated in cases where extended liver resection is impossible. The dissection limits of this procedure are, on the left side, the B2+3 bifurcation at the right margin of the umbilical portion of the portal vein and, on the right side, the B8 of the anterior branch and the B6+7 bifurcation of the right posterior branch. This procedure could also be described as a reduced form of extended right hepatectomy and extended left hepatectomy. For gallbladder carcinoma, this procedure is indicated to ensure an adequate surgical margin and eradicate transvenous liver metastasis, particularly in cases of pT2 lesions. Hilar and caudate lobe invasion also occurs in liver bed–type gallbladder carcinoma, and bile duct resection and caudate lobe resection are required for the surgery to be curative. We performed this procedure in four cases of hilar bile duct carcinoma, five cases of gallbladder carcinoma, and one case each of choledochal cyst (type IVA) with carcinoma of the bile duct and gallbladder adenomyomatosis. Curative resection was possible in all except the patient with adenomyomatosis, and all of the patients are alive and recurrence free 10 to 37 months postoperatively. This procedure, in addition to preserving liver function, provides a wide field of view and facilitates reconstruction of multiple intrahepatic bile ducts. Thus it can be said to be a curative operation not only in patients considered high risk but also in those whose hilar bile duct carcinoma is limited to the bifurcation area (Bismuth type IIIa and IIIb) and in gallbladder carcinoma up to pT2 with slight extension on the hepatic side. (J GASTROINTEST SURG 1999;3:369-373.)

KEY WORDS: Biliary tract carcinoma, Taj Mahal liver resection, surgical technique, curative resection

Major hepatic resection is currently being performed in Japan as curative treatment for bile duct carcinoma and gallbladder carcinoma.[1] However, when extended resection is performed to treat these carcinomas, the volume of remnant liver parenchyma is very small, and as a result postoperative hepatic insufficiency and hepatic failure are not infrequent. Although many surgeons are performing preoperative portal vein embolization for hypertrophy of the remnant liver,[2,3] the incidence of complications after such operations remains quite high.[1,4] Reports from other countries have also shown high morbidity and mortality rates after hepatic resection for biliary tract carcinoma.[5,6] In hopes of resolving this dilemma, we

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have developed the Taj Mahal parenchymal resection (S4a + S5 with S1 resection) to minimize parenchymal loss and provide curative resection in these difficult cases. We hypothesized that resection of a smaller volume of hepatic tissue during radical surgery should be accompanied by a lower incidence of postoperative complications. We describe our experience in 11 cases in which the operative technique of S4a + S5 with S1 resection was applied as a new method for treating biliary and gallbladder tumors.

INDICATIONS

Hilar bile duct carcinoma, advanced gallbladder carcinoma, particularly in pT2-type lesions, and choledochal cyst (type IVA) are the most important indications for S4a + S5 with S1 resection. Because hilar carcinoma, pT2-type gallbladder carcinoma, and carcinoma located in the neck of the gallbladder may invade or spread to the hilar liver parenchyma and/or caudate lobe via the hilar branch of the portal vein, curative resection should be attempted.

SURGICAL TECHNIQUE

Incision

Thoracoabdominal or bilateral subcostal incisions are used. The thoracoabdominal incision is started in the left subcostal region, 2 cm below the costal margin at the lateral border of the rectus muscle, and is continued to the right side and then upward, by cutting the tenth costochondral junction, to the midaxillary line. The diaphragm is then severed with a linear cutter at the ninth intercostal space, as shown in Fig. 1. This technique is used in all hepatectomies except lateral segmentectomy. The incision is advantageous in caudate lobectomy because the right and left hepatic vein roots are well visualized and dissection can be performed with a *wider* field of view than with a midline or paramedian incision.

Dissection of the Hepatoduodenal Ligament and Hepatic Hilus

The lesser omentum is incised by means of electrocautery, and the anterior layer of the hepatoduodenal ligament is divided. After exposing the proper hepatic artery and encircling it with vascular tape, the duodenal edge of the bile duct is tracked down into the pancreas as distally as possible to obtain a disease-free surgical margin. The common bile duct is divided, ligated, and transected immediately above the upper margin of the pancreas, and a transfixing suture ligature is applied to the stump of the bile duct on the duodenal side. The lymph nodes around the common

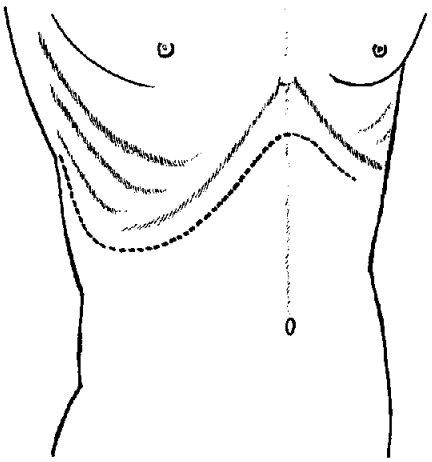

Fig. 1. Skin incision.

hepatic artery and root of the celiac trunk along with the lymph nodes in the hepatoduodenal ligament are dissected. The left and right hepatic arteries are encircled with vascular tape. The main trunk of the portal vein is then exposed, and the dissection is carried out to free the bifurcation of the portal vein from the surrounding tissue. The hepatic artery is skeletonized toward the hepatic hilus, and the root of the cystic artery is exposed and divided with a transfixing suture ligature.

Mobilization of the Caudate Lobe

Rather than mobilizing the left lobe, it is lifted upward and the loose connective tissue anterior to the inferior vena cava is dissected on the left side of the caudate lobe. The lowest short hepatic veins draining the caudate process are serially divided with transfixing suture ligatures on the inferior aspect of the liver, anterior to the vena cava. If a thick posterior inferior segmental branch of the right hepatic vein is found draining the posterior inferior segment of the liver, the surgeon should be careful not to damage it. The left and right portal veins are each encircled with vascular tape at the hepatic hilus. Small branches of the bifurcation of the portal vein draining the caudate lobe are ligated and divided. The left portal vein is exposed toward the umbilical portion of the portal vein. The branches of the left portal vein draining the caudate lobe are also ligated and cut. Mobilization of the caudate lobe is then completed by serially ligating and dividing the short hepatic veins draining the caudate lobe and the paracaval portion of the caudate lobe. The Arantius ligament is also ligated with a transfixing suture and divided. The upper margin of the caudate lobe is dissected with an ultrasonic scalpel.

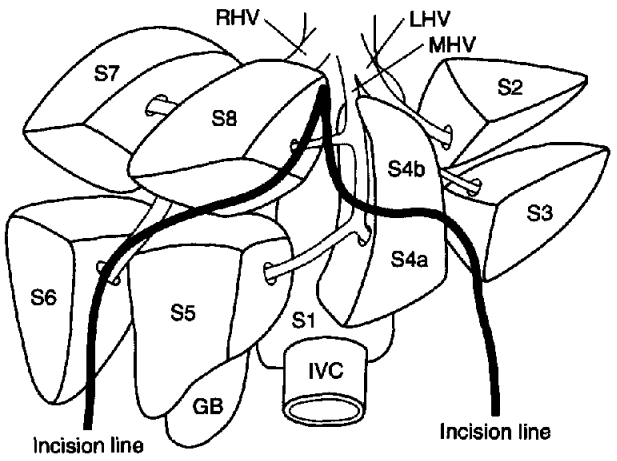

Fig. 2. Taj Mahal parenchymal incision line. GB = gallbladder; IVC = inferior vena cava; LHV = left hepatic vein; MHV = middle hepatic vein; RHV = right hepatic vein.

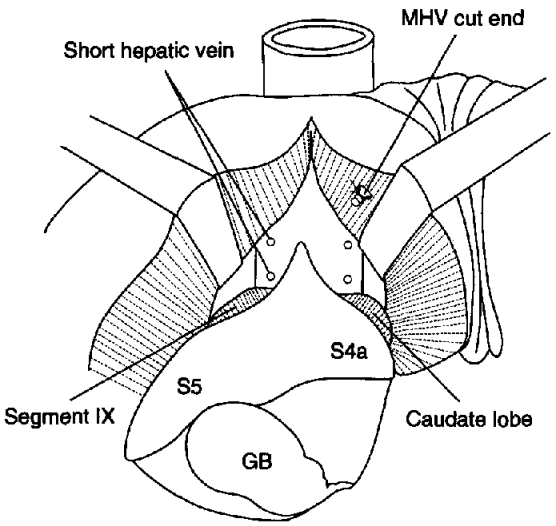

Fig. 3. Completed resection of S5 + S4a and caudate lobe. GB = gallbladder; MHV = middle hepatic vein.

Mobilization of the Right Lobe

The right coronary ligament is divided, and the roots of the right, middle, and left hepatic veins are exposed. The right triangular ligament and hepatorenal ligament are also separated to complete the mobilization of the right hepatic lobe.

Taj Mahal Parenchymal Resection

Intraoperative echocardiography is then performed to identify the right and middle hepatic veins. A parenchymal incision line is marked by electrocautery along the left margin of the right hepatic vein up to its midpoint, then to the left side toward the middle hepatic vein in a dome-like fashion, and finally down to the medial margin of the middle hepatic vein, keeping the S4b segment intact, as shown in Fig. 2. This resection line lies on Cantlie's line (which passes from the left side of the gallbladder fossa to the left side of the inferior vena cava to divide the liver into right and left lobes) on the left and approximately 3 cm away from the gallbladder fossa on the right, with the dome situated above; because the incision resembles the contour of the Taj Mahal, we refer to this procedure as "Taj Mahal" liver resection. Transection of the hepatic parenchyma is begun on the left side with an ultrasonic surgical aspirator along the demarcated line. The portal branches of the medial segment draining into the umbilical portion of the portal vein are ligated and cut. The hepatic parenchymal transection is continued, the middle hepatic vein is exposed, and the branches of the middle hepatic vein from S4a are ligated and cut. The left hepatic duct is divided at the bifurcation of the lateral superior (B2) and lateral inferior (B3) segmental bile duct, and the medial hepatic duct is divided at the medial superior (S4b) segment. After transection of the hepatic parenchyma on the left side is almost complete, the caudate lobe, which has already been mobilized, is brought up on the superior side of the bifurcation of the portal vein. The hepatic parenchyma on the right side is transected along the previously demarcated line. The branches of the right hepatic vein draining into the anterior inferior segment (S5) are serially ligated and cut very carefully so as not to injure the right hepatic vein. The posterior segmental (B6+7) and anterior superior segmental (S8) branches of the intrahepatic bile duct are exposed and divided. Last, the junction of the caudate process and the posterior segment of the liver are transected, and resection of S4a + S5 with combined resection of the caudate lobe, gallbladder, and extrahepatic bile duct is completed (Fig. 3).

Biliary Tract Reconstruction

The cut surface of the remnant liver is shown in Fig. 4. The biliary tracts are reconstructed by intrahepaticojejunostomies with a Roux-en-Y loop brought up in antecolic fashion and anastomosed with the posterior segmental duct (B6+7), anterior superior segmental duct (B8), lateral segmental duct (B2+B3) or (B2, B3), and/or middle superior segmental duct (B4b; sometime B4b drains into B3) (Fig. 5). Retrograde transhepatic biliary drainage tubes are inserted into each of the cut segmental ducts, and the tubes are brought out through the surface of the liver for stent-

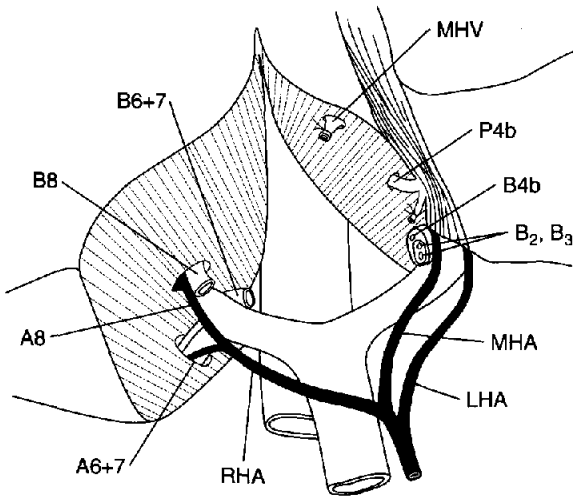

Fig. 4. Cut surface of the liver. RHA = right hepatic artery; LHA = left hepatic artery; MHA = middle hepatic artery; A = artery; **B** = bile duct; P = portal vein.

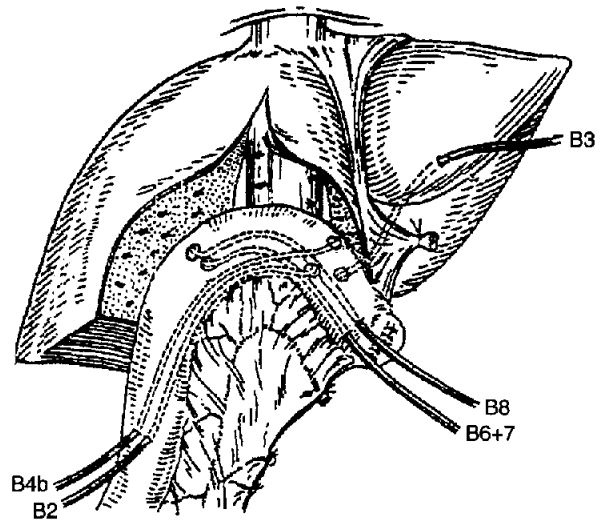

Fig. 5. Biliary tract reconstruction. B = bile duct.

ing and for performing postoperative intrahepatic cholangiography. A closed soft Penrose-style drainage tube is placed in the bilateral resection planes of the hepatic parenchyma. A chest tube is inserted into the right pleural cavity. All of the drainage tubes are brought to the surface of the body through separate wounds and connected to a sterile container and a Pleur-Evac. The wound is closed in layers.

Recent Cases of S4a + S5 With S1 Resection

Between September 1994 and April 1998, a total of 94 patients underwent hepatic resection at our institution and S4a + S5 with S1 resection was performed in 11 of them (11.7%) (Table I). The demographic data for patients undergoing S4a + S5 with S1 resection are presented in Table II. There were eight men and three women. The average age was 67 years (range 56 to 79 years). The preoperative indocyanine green retention rate at 15 minutes was greater than 10% in all cases (excretory function of the liver, normal value <10%). The indications for S4a + S5 with S1 resection were hilar bile duct carcinoma in four patients, gallbladder carcinoma (pT2 type) in five patients, choledochal cyst type IVA with adenocarcinoma in one patient, and gallbladder adenomyomatosis in one. We did not encounter any serious complications or major leakage in our patients. Curative resection was possible in all cases, and all of the patients are alive and recurrence free 10 to 37 months postoperatively.

Table I. Surgical procedures performed during 94 liver resections

Type of resection	No. (%)
Right or left trisegmentectomy	4 (4.2)
Right or left extended hepatectomy	18 (19.2)
Right or left hepatectomy	20 (21.3)
S4a + S5 with S1 (Taj Mahal)	11 (11.7)
Lateral segmentectomy	13 (13.8)
Nonanatomic resection	28 (29.8)
TOTAL	94 (100)

DISCUSSION

Hepatic resection has been used in the treatment of hilar bile duct carcinoma and advanced gallbladder carcinomas. The most appropriate operation for these carcinomas is extended hepatectomy (e.g., left or right trisegmentectomy or right or left extended hepatectomy).[1,5-8] However, major hepatic resection is not indicated in elderly patients or in patients with poor liver function because of the high surgical morbidity and mortality rates associated with it.[1,4-6] The segment S4a + S5 and S1 resection for hilar bile duct carcinoma and advanced gallbladder carcinoma that is described in this report offers the advantage of preserving adequate hepatic mass so that the patient is better able to tolerate the surgical stress, as compared with major hepatic resection. We performed this procedure in 11 patients and encountered no serious complications. Furthermore, because the hilar bifurcation of the bile duct and gallbladder is adjacent to the hepatic parenchyma of segments S4a +S5 with S1, resection of these segments resulted in surgical

Table II. Demographic data for patients undergoing Taj Mahal liver resection

Patient	Age (yr)	Sex	ICGR15 (%)	Indication	Complications	Outcome (up to September 1998)
1	64	M	14.2	Gallbladder adenomyosis	None	Alive; 37 mo
2	77	M	15.4	Hilar duct carcinoma	Aspiration pneumonia	Alive; 32 mo
3	62	M	12.7	Hilar duct carcinoma	None	Alive; 32 mo
4	70	M	11.5	Gallbladder carcinoma	None	Alive; 30 mo
5	78	M	12.0	Gallbladder carcinoma	None	Alive; 27 mo
6	60	F	11.0	Choledochal cyst (type IVA)	None	Alive; 27 mo
7	79	M	17.2	Hilar duct carcinoma	None	Alive; 24 mo
8	56	F	11.2	Gallbladder carcinoma	Endotoxemia	Alive; 17 mo
9	72	M	13.3	Gallbladder carcinoma	None	Alive; 16 mo
10	61	M	10.5	Hilar duct carcinoma	None	Alive; 11 mo
11	63	F	10.6	Gallbladder carcinoma	None	Alive; 10 mo

ICGR15 = indocyanine green retention rate at 15 minutes (value less than 10% indicates good liver function).

curability in hilar bile duct carcinoma and gallbladder pT2 carcinoma accompanied by direct liver invasion, and provided a good operative field for viewing the numerous stumps of the intrahepatic bile ducts; thus it was easier to perform the anastomosis. In order to resect segments S4a + S5 with S1, the liver must be transected in two different planes, and many intrahepatic bile ducts have to be anastomosed (usually four, sometimes even more). These are the disadvantages of this procedure. In addition, there is a slight increase in technical difficulty in performing the anastomosis between the intrahepatic bile duct and the jejunum. However, this procedure does not require transfusion of a larger volume of blood. In 1980 Hart and White[9] reported their experience with central hepatic resection for hilar cholangiocarcinoma. We also performed central hepatic resection in two patients at our institution during the same period.[10] Recently Miyazaki et al.[11] reported resection of segments 1 and 4 for hilar cholangiocarcinoma. The purpose of their operation was similar to ours, but our procedure can be used to treat pT2-type gallbladder carcinoma as well as hilar bile duct carcinoma. With this procedure it is easier to anastomose the intrahepatic bile ducts, B6+7, B8 on the right side, B2+3 or (B2, B3) and/or B4b on the left side separately with the Roux-en-Y jejunal loop. However, this procedure is contraindicated when the portal vein and hepatic arteries have been invaded.

CONCLUSION

Resection of hepatic segments S4a + S5 with S1 is a useful choice as a surgical procedure for hilar carcinoma when liver function is poor or the carcinoma is located within both hepatic ducts but has not invaded the hepatic artery and portal vein; it can also be used for pT2-type gallbladder carcinoma and choledochal cyst (type IVA) with or without carcinoma. However, we treated only a small number of patients and the follow-up period was limited. To determine the true value of this procedure will require longer follow-up and a greater number of patients.

REFERENCES

1. Nimura Y, Hayakawa N, Kamiya J, et al. Hepatic segmentectomy with caudate lobe resection for bile duct carcinoma of the hepatic hilus. World J Surg 1990;14:535-544.
2. Tanaka H, Kinoshita H, Hirohashi K, et al. Preoperative percutaneous transhepatic portal vein embolization to extend the indications for hepatectomy and to increase the safety of extended hepatectomy for hepatocellular carcinoma [in Japanese]. Nippon Geka Gakkai Zasshi 1992;93:1317-1323.
3. Nagino M, Nimura Y, Hayakawa N. Percutaneous transhepatic portal embolization using newly devised catheters: Preliminary report. World J Surg 1993;17:520-524.
4. Lai ECS, Tompkins RK, Roslyn JJ, Proximal bile duct cancer. Quality of survival. Ann Surg 1987;205:11-15.
5. Blumgart LH, Hadjis NS, Benjamin IS, Beazley R. Surgical approaches to cholangiocarcinoma at confluence of hepatic ducts. Lancet 1984;1:66-69.
6. Boerma EJ. Research into the results of resection of hilar bile duct cancer. Surgery 1990;108:572-580.
7. Tsuzuki T, Ueda M, Kurmochi S, et al. Carcinoma of the main hepatic duct junction: Indications, operative morbidity and mortality, and long-term survival. Surgery 1990;108:495-501.
8. Bismuth H, Nakache R, Diamond T. Management strategies in resection for hilar cholangiocarcinoma. Ann Surg 1992;215: 31-38.
9. Hart MJ, White TT. Central hepatic resection and anastomosis for stricture or carcinoma at the hepatic bifurcation. Ann Surg 1980;192:299-305.
10. Kawarada Y, Mizomoto R. Surgical treatment of hepatic hilar carcinoma [in Japanese]. Jpn J Gastroenterol Surg 1981;36: 245-250.
11. Miyazaki M, Ito H, Nakagawa K, et al. Segment I and IV resection as a new approach for hepatic hilar cholangiocarcinoma. Am J Surg 1998;175:229-231.

Fas Expression Prevents Cholangiocarcinoma Tumor Growth

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Cholangiocarcinoma continues to have a dismal prognosis with an overall survival rate of less than 10%. An increased understanding of the molecular oncogenesis of this tumor is needed. Fas/APO-1 (CD95) receptor and Fas ligand have been implicated as key factors in apoptosis. In this study we have examined the role of the Fas receptor in the growth of cholangiocarcinoma. The purpose of this study was to evaluate the role of the Fas receptor in the induction of apoptosis in cholangiocarcinoma and to assess the role of the Fas receptor in cholangiocarcinoma tumorigenesis. Human cholangiocarcinoma cells, SK-ChA-1, were evaluated for Fas receptor expression using fluorescence-activated cell sorting (FACS). Distinct cell populations (Fas-positive and Fas-negative) were isolated by FACS and cloned from single cell dilutions. Fas expression was assessed by FACS and reverse transcriptase-polymerase chain reaction (RT-PCR). Cell populations were further characterized by their sensitivity to anti-Fas monoclonal antibody at 72 hours. Cell viability and apoptotic index were evaluated by trypan blue cell count and terminal deoxynucleotidyl transferase–mediated deoxyuridine triphosphate nick end–labeling (TUNEL) assay, respectively. Distinct cell populations were evaluated for their ability to form tumors in BALB/c nude mice (2.5×10^6 cells per subcutaneous injection). After 4 weeks, tumors were evaluated for tumor area by caliper measurement and Fas expression by RT-PCR. Maintenance of biliary phenotype was assured by means of AE-1 (cytokeratin) immunohistochemistry. Populations of Fas-positive and Fas-negative cells were identified, isolated, and confirmed by FACS and RT-PCR. Treatment of Fas-positive cells with anti-Fas monoclonal antibody produced an 80% reduction in cell viability compared to no decrease in viability in Fas-negative cells by trypan blue cell count. TUNEL staining showed an apoptotic index of 75% for Fas-positive cells incubated with anti-Fas monoclonal antibody and no significant evidence of apoptosis in the Fas-negative cells. When cholangiocarcinoma cells were subcutaneously injected into nude mice, only Fas-negative cells formed tumor nodules; Fas-positive cells failed to form tumor nodules. The analyzed tumors lacked Fas messenger RNA by RT-PCR but maintained the biliary cytokeratin AE-1 by immunohistochemistry. Fas receptor expression is an important mediator of apoptosis in cultured human cholangiocarcinoma cells and appears to be a critical determinant of cholangiocarcinoma tumor growth in nude mice. (J GASTROINTEST SURG 1999;3:374-382.)

KEY WORDS: Fas, CD95, apoptosis, cholangiocarcinoma

The Fas/APO-1 (CD95) and Fas ligand system is a key regulator of apoptosis (programmed cell death).[1-3] The Fas/APO-1 (CD95) cell surface receptor is a member of the tumor necrosis factor receptor superfamily.[4-8] Fas exists as an inactive monomer that is aggregated to an active trimer when associated with Fas ligand. Fas is expressed in various human tissues including lymphocytes, heart, liver, lung, kidney, and ovary.[2,9,10] The expression level of Fas in cells may modulate cell death in both normal and pathologic states. In normal cell populations at steady state, the rates of cell proliferation and cell death approximate each other. In cancer, however, increases in cell number predominate over cell death. Malignancy may not be exclusively associated with enhanced cell proliferation but may also be linked to decreased cell death (i.e., apoptosis).[11,12]

Apoptosis is programmed cell death. Ultrastructurally apoptosis has a characteristic morphology. Initially there is loss of cell junctions and specialized

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membrane proteins with the formation of surface blebs. This process is followed by DNA fragmentation with characteristic condensation of these irregular, large DNA fragments (these DNA changes provide the basis for apoptosis detection methods such as terminal deoxynucleotidyl transferase–mediated deoxyuridine triphosphate nick end–labeling [TUNEL] assay). This process usually leads to cellular disruption producing proteolysis-resistant, membrane-bound apoptotic bodies that are phagocytosed by neighboring cells or shed into an adjacent lumen.[13] Apoptosis occurs as a normal regulatory mechanism in fetal and adult tissues; thus it is not a phenomenon peculiar to disease. As an example of a normal regulatory mechanism, apoptosis is vital for the elimination of autoreactive thymic T-lymphocytes during development. Apoptosis has been reported to play an important role in the pathogenesis of numerous diseases. For example, apoptosis is observed in deletion of CD4 cells in acquired immunodeficiency infection,[14] in allograft rejection,[15] and in tumor destruction following anticancer therapy. Many chemotherapeutic and radiotherapeutic agents eliminate cells by triggering apoptosis.[16] Consequently considerable effort is being focused on elucidating genes that encode apoptosis repressor and inducer proteins for the malignant phenotype.

The apoptosis-inducing Fas pathway has been implicated in malignant transformation. In comparison to normal cells, some malignant cells are characterized by abnormal phenotypes of Fas expression including abnormal expression of functional Fas,[17] mutant Fas incapable of intracellular signaling,[18] cellular release of soluble Fas,[19] and deficiency of Fas transduction pathway.[20] The aberrant expression of Fas by various tumors with poor prognosis has attracted interest in Fas as a potential target for induction of apoptosis in cancer therapy.[21,22] In this report we have isolated Fas-negative and Fas-positive phenotypes of SK-ChA-1 cholangiocarcinoma. Using this model we have shown that the Fas pathway is a mechanism for inducing apoptosis in cholangiocarcinoma and exemplified the significance of Fas expression in the tumorigenesis of cholangiocarcinoma.

MATERIAL AND METHODS

Reagents

The following reagents were used: Human Fas monoclonal antibodies (mAb) CH11 and GH4 (Upstate Biotechnology Inc., Lake Placid, N.Y.), phycoerythrin-conjugated anti-Fas polyclonal antibody (Pharmingen, San Diego, Calif.), AE-1 mAb (Biogenex, San Ramone, Calif.), alkaline phosphatase–conjugated antidigitonigen antibody (Boehringer, Mannheim, Germany), RNAzol reagent (Biotech Lab, Houston, Tex.), RNA PCR Core Kit (Clontech Lab, Palo Alto, Calif.), and Fas primers (Pathology Core Facility, Birmingham, Ala.).

Cell Culture and Isolation of Subpopulations

Human cholangiocarcinoma cells (SK-ChA-1) were provided by Dr. A. Knuth (Ludwig Institute for Cancer Research, London, U.K.). Cells were grown in RPMI 1640 (Life Technologies, Inc., Gaithersburg, Md.) supplemented with 2 mmol/L L-glutamine, penicillin (5 U/ml), streptomycin (5 mg/ml), and 10% heat-inactivated fetal calf serum. The cells were incubated at 37° C in 95% air/5% carbon dioxide.

Fas-negative and Fas-positive subpopulations were isolated by flow cytometry. The human cholangiocarcinoma cells were rinsed in cold phosphate-buffered saline solution (8 g/L NaCl, 0.2 g/L KCl, 1.44 g/L Na_2HPO_4, and 0.24 g/L KH_2PO_4), once in Visuon (Gibco, Gaithersburg, Md.), incubated for 3 minutes at 37° C, and harvested into complete medium containing 10% fetal calf serum by vigorous pipetting. The cells were centrifuged at 1200 rpm for 5 minutes at 4° C, resuspended (10^7 cells/50 ml) in complete medium, and labeled with 20 ml commercial phycoerythrin-conjugated antihuman Fas antibody at 4° C for 30 minutes and then washed with RPMI 1640 medium twice. Murine phycoerythrin-IgG1 was used as an isotype control. The stained cells were sorted in-to Fas-negative and Fas-positive subsets. Fas-negative and Fas-positive cells were continuously cultured in RPMI 1640 complete medium with or without 0.1 μg/ml anti-Fas antibody, respectively, for 2 weeks.

The sorted Fas-negative and Fas-positive cholangiocarcinoma cells were diluted to 1000 cells/ml. A diluted cell suspension volume of 1, 3, or 5 μl was added to wells containing 200 μl of medium in a 96-well plate and then incubated for 1 week. Wells with a single cell were selected and grown in the medium until enough cloned cells were available for study.

In Vitro Inhibition of Cholangiocarcinoma Cell Growth by Anti-Fas Monoclonal Antibody

Fas-positive and Fas-negative cells were grown to a subconfluent monolayer in RPMI 1640 with 10% fetal calf serum. Anti-Fas mAb at 1 and 2 μg/ml concentrations was added to the culture medium. Cells were grown in anti-Fas mAb–supplemented medium for 72 hours; the medium was changed daily. Cells were counted in triplicate by light microscopy for viability by trypan blue exclusion.

Determination of Cell Death

Cell Count and Trypan Blue Dye Exclusion. Cell pellets were resuspended in 1 ml phosphate-buffered saline (pH 7.4). A 0.1 ml aliquot of cell suspension was stained with an equal volume of 4% trypan blue for 5 minutes and subsequently counted. Blue-stained dead cells and unstained clear living cells were counted.

Determination of Apoptosis

TUNEL Staining. Cells (1×10^5/200 ml phosphate-buffered saline) were collected by cytospinning onto slides precoated with poly-L-lysine and fixed in 10% formalin for 1 hour. After being rinsed with water, slides were incubated with 20 μg/ml proteinase K for 15 minutes and washed four times with water. Endogenous peroxidase was blocked by methanol containing 1% hydrogen peroxide, and the slides were washed with water. They were subsequently immersed in terminal deoxynucleotidyl transferase (TdT) buffer (30 mmol/L Trizma base, pH 7.2, 140 mmol/L sodium cacodylate, 1 mmol/L cobalt chloride) containing TdT (0.3 ml), and digitonigen-modified deoxyuridine triphosphate was added. The slides were incubated in a humidified atmosphere at 37° C for 1 hour. The reaction was terminated by washing the slides with phosphate-buffered saline. After the slides were incubated in 10% fetal calf serum in phosphate-buffered saline for 30 minutes and dried, they were covered with 1:10 diluted alkaline phosphate–conjugated antidigitonigen antibody and incubated at 24° C for 1 hour. The slides were then washed with phosphate-buffered saline and stained with nitroblue tetrazolium-bromochloroindolyl phosphate at 24° C for approximately 30 minutes. The apoptotic index was determined under light microscopy by counting 500 cells and expressed as a percentage of positive cells.

Reverse Transcriptase-Polymerase Chain Reaction

Total cellular RNA was extracted using RNAzol reagent. cDNA was generated using RNA PCR Core Kit reagents and a 4800 GeneAmp thermocycler (Perkin-Elmer, Foster City, Calif.). The cDNA primers for human Fas were 5′CAGCTCTTCCACCTACAG3′ (sense) and 5′TCATGCTTCTCCCTCTTTCACATGG3′ (antisense). The reaction conditions were as follows: denaturing at 94° C for 1 minute, annealing at 52° C for 1 minute, and extension at 72° C for 1 minute for 30 cycles. Agarose gel electrophoresis confirmed the 500 base-pair DNA product for Fas.

Mice

Six- to 8-week-old athymic (nu/nu) female BALB/c mice were purchased from Charles River Laboratories, Inc. (Wilmington, Mass.) for tumor inoculation. All animals were maintained in a sterile environment. Cages, bedding, food, and water were autoclaved, and animals were maintained on a daily 12-hour light/12-hour dark cycle.

Tumor Xenograft in Nude Mice

Cloned Fas-negative and Fas-positive cholangiocarcinoma cells (1×10^6/ml) were trypsinized, washed, and resuspended in Dulbecco's phosphate-buffered saline solution (Cellgro). Twelve nude mice were anesthetized with isoflurane inhalation and 2.5×10^6 cells/0.2 ml/site were inoculated subcutaneously into bilateral flanks of mice using 22-gauge needles. A 2-week period was allowed for tumor engraftment; after this time, tumor sizes were measured using calipers. Tumor area was calculated by multiplying horizontal diameter by vertical diameter (both in millimeters). Tumor growth statistics were calculated using analysis of variance. After 4 weeks, the mice were anesthetized (ketamine, 10 mg/100 g, and xylazine, 1.5 mg/g) and killed by cervical dislocation.

Immunohistochemistry

The tumors were surgically removed, fixed in 10% formalin, embedded in paraffin, cut into sections, and immunohistochemically stained for AE-1. Sections were pretreated with protease I (Ventana, Tucson, Ariz.) for 8 minutes and subsequently incubated in AE-1 antibody for 32 minutes. Antibody was detected using the Ventana detection kit per manufacturer's protocol. Skin tissue was used as a positive control.

RESULTS

Isolation of Fas-Negative and Fas-Positive Subpopulations

Flow cytometric analysis was used to assess human cholangiocarcinoma expression of Fas antigen. Flow cytometric analysis revealed that approximately 20% of the analyzed cholangiocarcinoma cells were Fas positive, thus indicating that the cultured human cholangiocarcinoma cells heterogeneously express Fas. Fas-negative and Fas-positive subsets were separated by flow cytometric sorting. After sorting, Fas-negative cells were continually incubated in the presence of a small concentration of Fas antibody (0.1 μg/ml) for 1 to 2 weeks.

Although the cell-sorting technique isolated Fas-

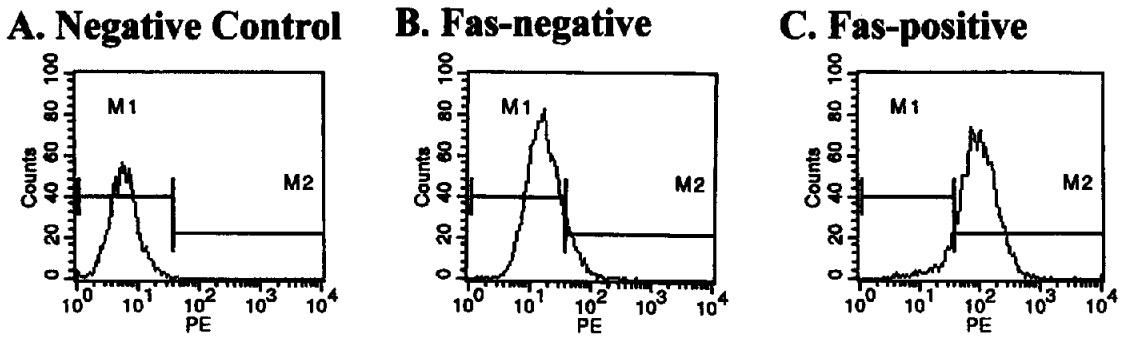

Fig. 1. Isolation of Fas-negative and Fas-positive cholangiocarcinoma populations. SK-ChA-1 cholangiocarcinoma was found to heterogeneously express Fas by fluorescence-activated cell sorting (FACS). FACS was subsequently used to separate Fas-positive and Fas-negative cells. Sorted cells were subsequently diluted to single cell populations and cloned. Using human cholangiocarcinoma cells incubated in murine phycoerythrin *(PE)*–conjugated antibody as a negative control **(A)**, Fas-negative clones were shown to have less than 10% Fas expression **(B)**, and Fas-positive clones were shown to have greater than 95% Fas expression **(C)**. Fluorescence intensity is plotted on the X axis; cell counts are plotted on the Y axis.

negative and Fas-positive cells, there was still overlap in Fas expression between these two populations. To further improve the purification of cells, several clones of Fas-negative and Fas-positive cells were generated by diluting to single cells. Fas expression by these clones was then determined by flow cytometry. Compared to the negative control (Fig. 1, *A*), Fas expression of the Fas-negative clone was less than 10% (Fig. 1, *B*); in contrast, Fas expression of the Fas-positive clone increased to greater than 95% (Fig. 1, *C*). The difference in the two populations was confirmed by RT-PCR for Fas, which revealed a high level of Fas PCR product only in Fas-positive clones compared to none or weak level of Fas PCR product in Fas-negative clones (data not shown).

Sensitivity of Fas-Positive and Fas-Negative Cells to Anti-Fas Monoclonal Antibody

Fas-negative and Fas-positive human cholangiocarcinoma cells respond differently when treated with anti-Fas mAb, which is known to activate the Fas pathway to produce apoptosis. Anti-Fas MAb stimulated cell death in Fas-positive cells only; Fas-negative cells were resistant. The reduction in cell viability was quantitated by trypan blue cell count (Fig. 2). Fas-positive cells had 75% and 90% reduction in cell viability with 1 and 2 μg/ml anti-Fas mAb treatment, respectively, whereas there was no significant reduction in viability in Fas-negative cells. Counts were compared to those in untreated controls. Microscopic changes were consistent with the characteristic features of apoptosis including nuclear condensation, cell rounding, and plasma membrane blebbing. Apoptosis was confirmed by TUNEL assay. TUNEL assay is a histochemical method of labeling DNA breaks that are characteristic of apoptotic cell death. Terminal deoxyribonucleotidyl transferase catalyzes the addition of biotinylated d-UTP to free 3′-OH ends of DNA fragments, with the synthesis of a polydeoxynucleotide polymer. The signal is amplified by avidin (antibiotin) peroxidase, and diaminobenzidine is used as chromagen.[13] According to the results of

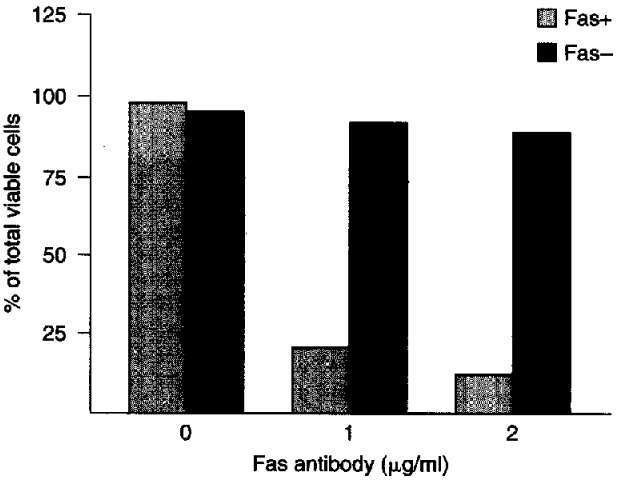

Fig. 2. Cell death induced by anti-Fas monoclonal antibody in Fas-negative and Fas-positive cells. Fas-negative and Fas-positive cells were incubated in the presence of medium alone (control), 1 μg/ml anti-Fas mAb, and 2 μg/ml anti-Fas mAb. Cells were harvested, stained with 4% trypan blue, and counted. There was a 75% and 90% reduction in cell viability in Fas-positive cells treated with 1 and 2 μg/ml anti-Fas mAb, respectively, but no significant reduction in treated Fas-negative cells.

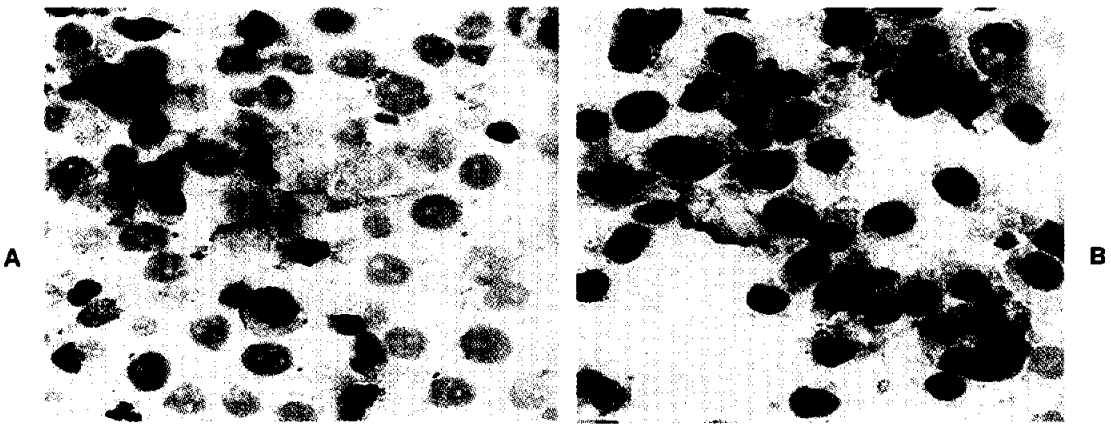

Fig. 3. Apoptosis induced by anti-Fas monoclonal antibody in human cholangiocarcinoma cells. Fas-negative **(A)** and Fas-positive **(B)** human cholangiocarcinoma cells were cultured in the absence (control) and presence of anti-Fas mAb (1 μg/ml and 2 μg/ml) and stained with terminal deoxynucleotidyl transferase stain (TUNEL). Dark blue cells indicate apoptotic cells. The apoptotic index is approximately 8% in Fas-negative cells vs. 70% in Fas-positive cells.

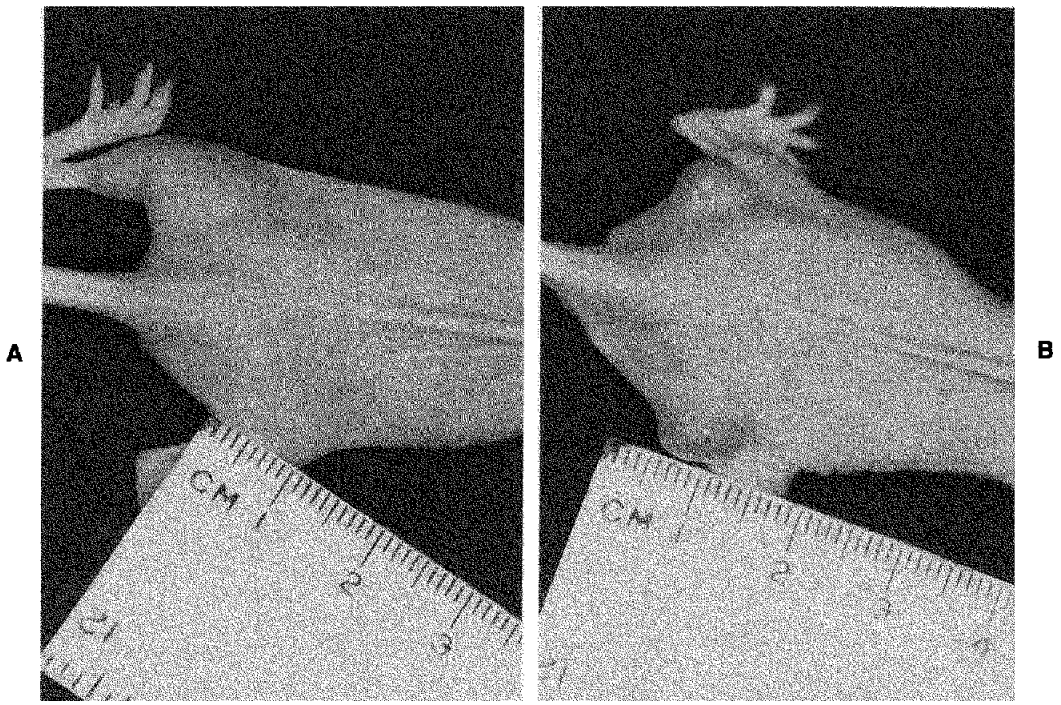

Fig. 4. Tumorigenesis of Fas-negative and Fas-positive human cholangiocarcinoma cells in nude mice. Fas-negative and Fas-positive cells (2.5×10^6/0.2 ml/site) were inoculated subcutaneously into the bilateral flanks of nude mice. Two weeks were allowed for tumor engraftment. **A,** Lack of tumor in an animal injected with Fas-positive cells. **B,** Typical tumors in an animal injected with Fas-negative cells.

TUNEL assay, less than 8% of Fas-negative cells underwent apoptosis when treated with anti-Fas mAb, although more than 70% of Fas-positive cells underwent apoptosis following treatment with anti-Fas mAb (Fig. 3).

Growth of Fas-Negative and Fas-Positive Cell Xenografts in Nude Mice

The tumorigenicity of cloned Fas-negative and Fas-positive cholangiocarcinoma cells was determined by creating xenographs in nude mice. Fas-negative and Fas-positive cells (2.5×10^6/site) were subcutaneously injected into bilateral flanks of female nude mice (N = 6 for each subgroup). After a 2-week engraftment period, Fas-negative cells were noted to have developed tumors in all six nude mice; tumors ranged from 1 to 2 cm in greatest diameter. In contrast, Fas-positive cells did not form appreciable tumors. Fig. 4 shows typical tumors bilaterally in the flanks of animals injected with Fas-negative cells (Fig. 4, *A*); in contrast, a typical animal injected with Fas-positive cells is shown with no tumor in either flank (Fig. 4, *B*). The mean area of tumor (tumor area = horizontal diameter [mm] × vertical diameter [mm]) produced by injected Fas-positive and Fas-negative cells at various time intervals is presented in Fig. 5; analysis of variance indicates a statistically significant F value of 95.33 and an equally significant *P* value of 0.0001. RT-PCR was used to assess the harvested tumors for Fas. Using synthetic Fas DNA as a positive control, a representative sample of tumors (N = 4) was tested and shown to lack Fas messenger RNA by RT-PCR (Fig. 6). As evidence of tumor differentiation from biliary epithelium, the tumors were immunohistochemically stained for AE-1, which is a cytokeratin

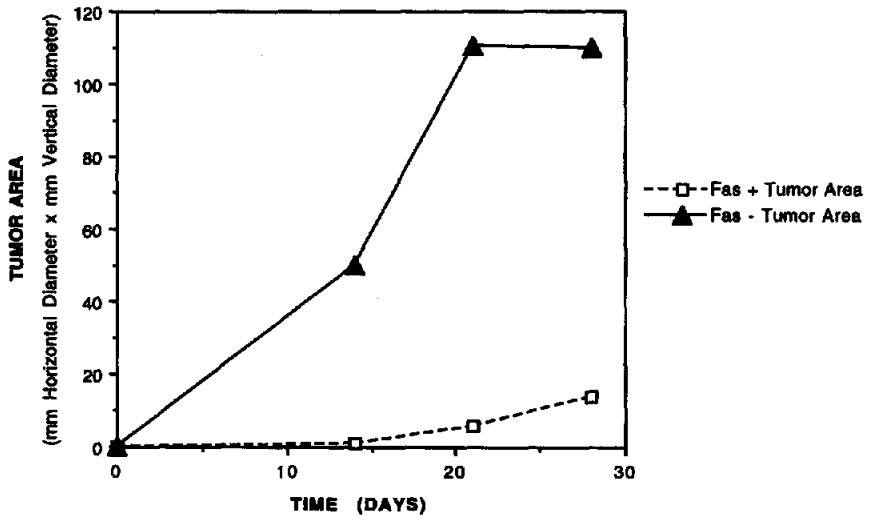

Fig. 5. Tumor area. Tumor area at various time points indicates a statistically significant difference in tumor production by injected Fas-negative cells vs. injected Fas-positive cells. Fas-negative cells produce significant tumors, whereas Fas-positive cells do not.

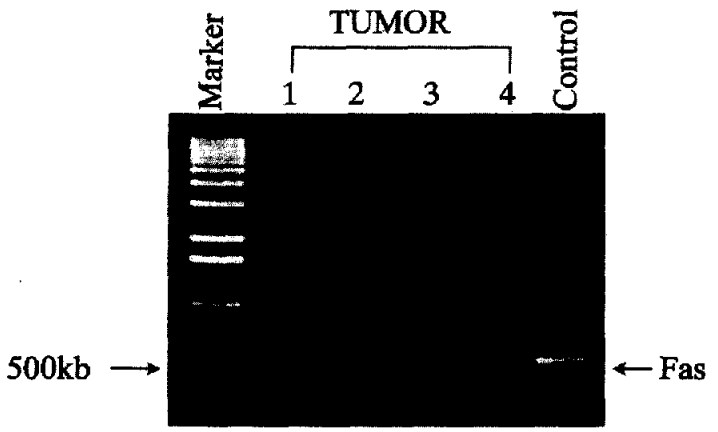

Fig. 6. RT-PCR of tumors. RT-PCR was performed to assess representative tumors for Fas using manufactured Fas cDNA of approximately 500 kb as a positive control. All analyzed tumors lacked Fas messenger RNA.

Fig. 7. AE-1 immunohistochemistry. Harvested tumors were stained for AE-1 cytokeratin as evidence of biliary epithelial differentiation. All tumors were AE-1 positive as indicated by brown staining of malignant epithelial cells.

that is characteristic of biliary epithelium. All tumors were AE-1 positive (Fig. 7). The glandular carcinoma cells stained brown (positive), whereas the interglandular fibrous tissue remained clear (negative).

DISCUSSION

Cholangiocarcinoma is a malignant tumor of the biliary ducts with less than a 10% 5-year overall survival.[23-26] Currently there are minimal opportunities for medical or surgical cure; therefore new modalities of treatment are needed.[6] Here we show that cultured human cholangiocarcinoma cells heterogeneously express Fas, a potential proapoptotic receptor. Most (80%) cells fail to express Fas or only weakly express this receptor. This low level or lack of expression of Fas, a major inducer of apoptotic cell death, may result in the failure of human cholangiocarcinoma to respond to current treatments. Consequently this may be responsible for the poor prognosis of this malignancy.[19,27,28] To further explore this hypothesis, we isolated and cloned Fas-negative and Fas-positive subpopulations of cultured human cholangiocarcinoma cells (SK-ChA-1). Using this two-phenotype model, we confirmed the sensitivity of the Fas-positive cells to anti-Fas mAb–induced apoptosis in vitro, whereas the Fas-negative cells were resistant. It appears that the presence of the Fas receptor in these cholangiocarcinoma cells conveys sensitivity to anti-Fas mAb–induced apoptosis. The anti-Fas mAb induction of apoptotic death of only Fas-positive cells suggests the importance of the Fas pathway in suppression of cholangiocarcinoma. Furthermore, when Fas-negative and Fas-positive cells were subcutaneously inoculated into nude mice, only Fas-negative cholangiocarcinoma cells survived to produce tumors.

These studies indicate that the deficiency of Fas expression may be associated with the pathogenesis of this tumor and its resistance to antitumor therapy. These findings suggest that Fas-positive cells, but not Fas-negative cells, were killed when injected subcutaneously. Fas ligand (the natural ligand for Fas) may be the in vivo biologic mediator stimulating apoptosis. Fas ligand is expressed on activated T cells, natural killer cells, thyroid cells,[29] epithelial cells,[30] and Sertoli cells[31]; it may also be present in a soluble form.[32] Nude mice are capable of producing Fas ligand; therefore endogenous Fas ligand is a likely natural mechanism for killing the Fas-positive cholangiocarcinoma cells and consequently suppressing tumor growth. These Fas-expressing tumor cells may also be activated in vivo by currently undescribed mechanisms, thus killing Fas-positive tumor cells (suicide apoptosis), leaving only Fas-negative tumor cells. Consistent with this hypothesis, some tumors spontaneously regress, and they often have large lymphocyte infiltrates. These findings support the concept of crucial involvement of the Fas pathway in tumorigenesis.

Finally, the heterogeneous expression of Fas in cholangiocarcinoma cells may be used to prognosticate both malignant potential and responsiveness to therapy. Understanding the underlying molecular events and responses to therapeutic agents may provide opportunities for new therapeutic modalities. Considering this concept of Fas expression being important in the tumorigenesis of cholangiocarcinoma, perhaps novel gene therapy techniques can be used to deliver Fas expression to Fas-negative cholangiocarcinoma to improve apoptotic destruction. For exam-

ple, cholangiocarcinoma sensitivity to tamoxifen treatment might be enhanced by Fas expression, considering the fact that tamoxifen appears to induce apoptosis by activation of the Fas pathway.[33] Other chemotherapeutic agents may utilize the Fas pathway to induce destruction of cholangiocarcinoma. These hypotheses will be explored in future experiments focused on molecular mechanisms, tumorigenesis, and underlying therapy.

REFERENCES

1. Perry RR, Kang Y, Greaves B. Effect of tamoxifen on growth and apoptosis of estrogen dependent and independent human breast cancer cells. Ann Surg Oncol 1995;2:238-245.
2. Kang Y, Cortina R, Perry RR. Role of c-myc in tamoxifen-induced apoptosis estrogen-independent breast cancer. J Natl Cancer Inst 1996;88:279-284.
3. Frankfurt OS, Sugarbaker EV, Robb JA, et al. Synergistic induction of apoptosis in breast cancer cells by tamoxifen and calmodulin inhibitors. Cancer Lett 1995;97:149-154.
4. Love RR. Tamoxifen therapy in primary breast cancer: Biology, efficacy, and side effects. J Clin Oncol 1989;7:813-815.
5. Butta A, Maclennan K, Flanders KC, et al. Induction of transforming growth factor beta 1 in human breast cancer in vivo following tamoxifen treatment. Cancer Res 1992;52:4261-4264.
6. Sampson LK, Vickers SM, Ying W, et al. Tamoxifen-mediated growth inhibition of human cholangiocarcinoma. Cancer Res 1997;57:1574-1579.
7. Taylor OM, Benson EA, McMahon MJ. Clinical trial of tamoxifen in patients with irresectable pancreatic adenocarcinoma. The Yorkshine Gastrointestinal Tumor Group. Br J Surg 1993;80:384-386.
8. Pollack IF, Randall MS, Kristofik MP, et al. Effects of tamoxifen on DNA synthesis and proliferation of human malignant glioma lines in vitro. Cancer Res 1990;50:7134-7138.
9. Couldwell WT, Weiss MH, DiGiorgio LM, et al. Clinical and radiographic response in a minority of patients with recurrent malignant gliomas treated with high-dose tamoxifen. Neurosurgery 1993;32:485-490.
10. Gelmann EP. Tamoxifen for the treatment of malignancies other than breast and endometrial carcinoma. Semin Oncol 1997;24(Suppl 1):65-70.
11. Ward RL, Morgan G, Dalley D, et al. Tamoxifen reduces bone turnover and prevents lumbar spine and proximal femoral bone loss in early postmenopausal women. Bone Miner 1993;22: 87-94.
12. Recker RR. Clinical review 41: Current therapy for osteoporosis [review]. J Clin Endocrinol Metab 1993;76:14-16.
13. Stanton MJ, Gaffney EF. Apoptosis: Basic concepts and potential significance in human cancer. Arch Pathol Lab Med 1998;122:310-319.
14. Pan ZQ, Radding W, Zhau T, et al. Role of calmodulin in HIV-potentiated Fas-mediated apoptosis. Am J Pathol 1996; 149:903-910.
15. Laine J, Etelamaki P, Holmberg C, Dunkel C. Apoptotic cell death in human chronic renal allograft rejection. Transplantation 1997;63:101-105.
16. Bellamy COC, Malcomson R, Wyllie AH. The role of p53 in apoptosis and cancer. In Martin SJ, ed. Apoptosis and Cancer. Basel: Karger Landes Systems, 1997, pp 132-133.
17. Wu JX. Apoptosis and angiogenesis: Two promising tumor markers in breast cancer [review]. Anticancer Res 1996;16: 2233-2240.
18. Thompson CB. Apoptosis in the pathogenesis and treatment of disease. Science 1995;267:1456-1462.
19. Trauth BC, Klas C, Peter AM, et al. Monoclonal antibody-mediated tumor regression by induction of apoptosis. Science 1989;245:301-305.
20. Dhein J, Daniel PT, Trauth BC, et al. Induction of apoptosis by monoclonal anti-APO-1 class switch variants is dependent on cross-linking of APO-1 cell surface antigens. J Immunol 1992;149:3166-3173.
21. Leithauser F, Dhein J, Mechtersheimer G, et al. Constitutive and induced expression of APO-1, a new member of the nerve growth factor/tumor necrosis factor receptor superfamily in normal and neoplastic cells. Lab Invest 1993;69: 415-429.
22. Midis GP, Shen Y, Owen-Schaub LB. Elevated soluble Fas (sFas) levels in nonhematopoietic human malignancy. Cancer Res 1996;56:3870-3874.
23. Pan ZQ, Radding W, Zhou T, et al. Role of calmodulin in HIV-potentiated Fas-mediated apoptosis. Am J Pathol 1996;149:903-910.
24. Kawamura K, Grabowski D, Krivacic K, et al. Cellular events involved in the sensitization of etoposide-resistant cells by inhibitors of calcium-calmodulin-dependent processes: Role for effects on apoptosis, DNA cleavable complex, and phosphorylation. Biochem Pharmacol 1996;52:1903-1909.
25. Nagata S, Golstein P. The Fas death factor. Science 1995;267: 1449-1456.
26. Inbal B, Cohen O, Polak-Charcon S, et al. DAP kinase links the control of apoptosis to metastasis. Nature 1997;390:180-184.
27. Itoh N, Yonehara S, Ishii A, et al. The polypeptide encoded by the cDNA for human cell surface antigen Fas can mediate apoptosis. Cell 1991;66:233-243.
28. Melino G, Annicchiarico-Petruzzeli M, Piredda L, et al. Tissue transglutaminase and apoptosis: Sense and antisense transfection studies with human neuroblastoma cells. Mol Cell Biol 1994;14:6584-6596.
29. Martinez-Lorenzo MJ, Alava MA, Anel A, et al. Release of preformed Fas ligand in soluble form is the major factor for activation-induced death of Jurkat T cells. Immunology 1996;89:511-517.
30. Mori T, Xu JP, Mori E, et al. Expression of Fas-ligand system associated with atresia through apoptosis in murine ovary. Horm Res 1997;48(Suppl 3):11-19.
31. Wilson SE, Li Q, Weng J, et al. The Fas-Fas ligand system and other modulators of apoptosis in the cornea. Invest Ophthalmol Vis Sci 1996;37:1582-1592.
32. Martinez-Lorenzo MJ, Alava MA, Anel A, et al. Release of performed Fas ligand in soluble form is the major factor for activation-induced death of Jurkat T cells. Immunology 1996;89:511-517.
33. Pan G, Vickers SM, Pickens A, et al. Apoptosis and tumorigenesis in human cholangiocarcinoma cells: Involvement of Fas/APO-1 (CD95) and calmodulin. Am J Pathol (in press).

Discussion

Dr. S. Raper (Philadelphia, Pa.). You showed that the effect of tamoxifen was not estrogen mediated. Are there other types of tumors that are Fas positive that would likely be inhibited by the tamoxifen paradigm?

Dr. A. Pickens. We have identified subpopulations of pancreatic cell lines that appear to be Fas negative and Fas positive; we have incubated these pancreatic cells in various concentrations of tamoxifen as well, but they do not appear to be as responsive to tamoxifen treatment. I note in the literature that there is an agent called FAP-I that is said to inhibit the Fas pathway in pancreatic cells.

Dr. J. Peters (Los Angeles, Calif.). It seems that you have picked out two very different subpopulations of this tumor. They differ in Fas expression, as you have shown, but they probably differ in many other ways. What direct evidence do you have that tamoxifen is acting through the Fas receptor?

Dr. Pickens. Concerning the origin of the two tumor subpopulations, we have looked at AE-1 immunohistochemical staining, which is a cytokeratin stain that indicates biliary epithelial phenotype. Both subpopulations appear to be of biliary origin. To determine whether cells are responding to tamoxifen via the Fas receptor, we have attempted to transfect sense and antisense cDNA into these cells and essentially reverse the effects of tamoxifen. These transfection data, along with the inhibition of the tamoxifen effect by the anti-Fas antibody, leads us to believe that the Fas receptor is important.

Dr. J. Drebin (St. Louis, Mo.). I was very impressed with the difference in biologic behavior of your Fas-negative and Fas-positive tumors. Is there any correlation with prognosis in humans? In humans, are Fas-positive tumors more indolent or Fas-negative tumors more aggressive? Second, as you look at other cell lines, do you continue to find that Fas-negative lines are more aggressive in nude mouse models? Getting human tumors to grow in mice can be problematic, and perhaps sorting by Fas would be a technical advance.

Dr. Pickens. With regard to prognosis, we are in the process of obtaining specimens from previous cases at the University of Alabama at Birmingham and staining for Fas expression; then we will review charts for treatment response and survival in order to assess prognosis according to Fas expression. To address your second question concerning the aggressiveness of other tumors, we are in the process of injecting pancreatic cell lines into mice, but beyond that we have no other information.

Dr. D. Morris (Albuquerque, N.M.). I believe you stated that only Fas-positive cell lines formed tumors in mice. Is this phenomenon observed in any other system, for example, in melanoma, where some cell lines would be receptor positive? Have you looked at any melanoma cell lines?

Dr. Pickens. We have not looked at melanoma cell lines. As to which cancer grew in the animal model, we found that Fas-negative cells grew, not Fas-positive cells. This suggests that Fas-negative cells are less sensitive to apoptotic destruction and are more tumorigenic.

Clinical Outcome and Quality of Life After Gastric and Distal Esophagus Replacement With an Ileocolon Interposition

Jürg Metzger, M.D., Lukas Degen, M.D., Christoph Beglinger, M.D., Markus von Flüe, M.D., Felix Harder, M.D.

Mainly because of the loss of reservoir function, loss of sphincter function, and exclusion of the duodenal route, patients who undergo gastrectomy suffer from many adverse effects postoperatively. The ileocecal interpositional graft is an attractive method to use as a gastric substitute after gastrectomy and distal esophagectomy. A pedunculated ileocecal graft is placed between the esophagus and the duodenum. The cecum acts as a reservoir while the ileocecal valve protects against enteroesophageal reflux. The duodenal passage is also preserved. Fourteen patients underwent this operation. The technique-related morbidity was low and the quality of life was good. During a mean follow-up of 6 months, no evidence of severe dumping syndrome or reflux esophagitis was observed. Further prospective randomized studies are warranted to compare this technique with the standard methods of gastric reconstruction. (J GASTROINTEST SURG 1999;3:383-388.)

KEY WORDS: Gastrectomy, gastric replacement, ileocolon interposition, quality of life

Cardia cancer type II and III and gastric cancer usually require a gastrectomy. In regard to the proximal spreading of the tumor, a distal esophagectomy is necessary in some cases. The two standard procedures used for reconstruction are Roux-en-Y esophagojejunostomy and jejunal pouch construction. Although Roux-en-Y esophagojejunostomy reduces reflux of duodenal juice, it offers neither reservoir function nor duodenal passage. The various jejunal pouch reconstructions do offer the advantage of maintaining reservoir function and also the duodenal passage. The disadvantages of these techniques are bile reflux and the fact that, because of the limited mesenteric pedicle, a reconstruction is not always feasible. To overcome these problems, we have evaluated the ileocecal interpositional graft for replacing the distal esophagus and the stomach.

Lee[1] and Hunnicutt[2] first described the reconstruction of the stomach by means of ileocolon interposition in the early 1950s. Although the concept and the initial experiences were promising, use of this technique had not been reported in the surgical literature since 1952. Recently a large study was published in which the same ileocolon segment was used to replace the stomach after total or proximal gastrectomy.[3] Between October 1995 and December 1997, we performed an ileocolon reconstruction in 14 patients after gastrectomy and/or distal esophagectomy.

MATERIAL AND METHODS

All patients who had a diagnosis of either gastric or distal esophagus cancer were included in this study. Preoperative assessment included chest x-ray examination, gastroscopy with biopsy, colonoscopy, CT scan, and endoluminal ultrasonography. Patients who had evidence of distant metastatic disease or pathologic findings in the right hemicolon were excluded from this study. Postoperatively patients underwent gastroscopy and were questioned regarding dumping

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Fig. 1. If reconstruction below the diaphragm is advised, the ileocolon segment is isolated and pedunculated at the ileocolonic artery.

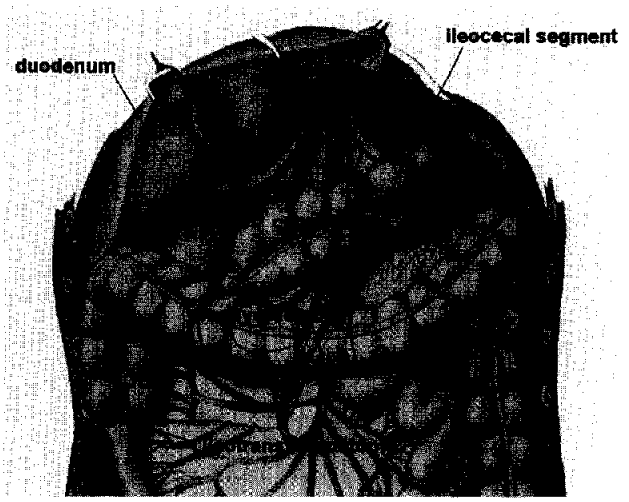

Fig. 2. Abdominal reconstruction. The ileocolon segment is rotated 180 degrees clockwise upward. The colonic end is sewn end to end to the duodenal stump and the ileum to the distal esophagus.

Fig. 3. If intrathoracic reconstruction is advised, the ileocolon segment is isolated and pedunculated at the right colonic artery.

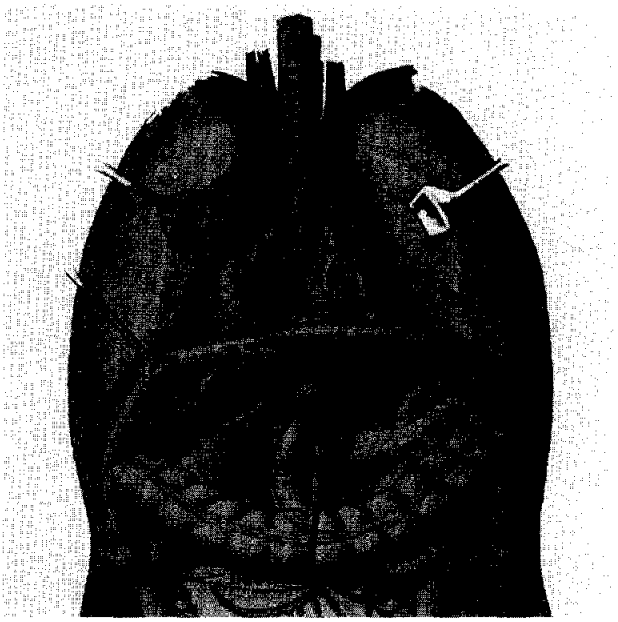

Fig. 4. Ileocecal interposition as esophagogastric replacement.

and reflux symptoms; in addition, gastrointestinal quality-of-life index scores[4] were recorded 3 to 6 months after the operation. This index is a recognized means of assessing quality of life in patients with gastrointestinal disease. A standard questionnaire contains 36 questions, each with five response categories. The response to the questions are summed to give a numeric score. The most desirable option is given a value of 4 points, whereas the least desirable option is given a value of 0. Scores were validated by comparing them with scores in normal individuals, who reached an average score of 125.8 points.

OPERATIVE TECHNIQUE

The details of the operative technique have been described previously.[5] After a total gastrectomy with compartment 2 dissection, the ileocolon is isolated (ileum, 7 cm; cecum and ascending colon, 17 to 20 cm) and pedunculated at the ileocolonic artery, if reconstruction below the diaphragm is advised (Fig. 1). First an appendectomy is performed and then, after transection of the ascending colon and ileum, the ileocecal segment is rotated 180 degrees clockwise upward. The colonic end is drawn and placed in the subhepatic space, avoiding any twisting of the vascular pedicle. This end is sewn to the end of the duodenal stump using a single-layer transmural running suture for the posterior wall and an extramucosal running suture for the anterior wall. The orad end of the ileum is anastomosed with the aborad end of the ascending colon using a single-layer extramucosal running suture (Fig. 2). In the case of an intrathoracic reconstruction, the ileocolon segment is pedunculated at the right colonic artery (Fig. 3). The isolated and dissected ileocolon segment is then placed in a plastic bag and pushed through the hiatus of the diaphragm. After the abdominal wound is closed, the patient is placed in a left lateral position. Through the right sixth intercostal space, the esophagus is resected up to the level of the azygos vein. The ileocecal segment is drawn into the right mediastinum and after removal of the plastic bag, the iliac end is anastomosed end to end with the distal end of the esophagus (Fig. 4). The anastomosis is checked for leaks using water-soluble contrast medium on postoperative day 6.

RESULTS

Patient data are summarized in Table I. In five patients an intrathoracic reconstruction was necessary and in the other nine patients a single abdominal reconstruction was indicated. The operation was well tolerated by all patients. Mean operating time was 360 minutes (range 250 to 510) minutes. One patient required drainage for an intra-abdominal infection. A 64-year-old man who underwent an abdominal reconstruction developed an acute abdomen on postoperative day 6. Laparotomy revealed a small leak in the ileoesophageal anastomosis, which was sealed by oversewing. Subsequently a pancreatic fistula occurred. The patient had to remain in the hospital for 118 days but he eventually made a good recovery. At the first clinical follow-up examination there was no evidence of any local or general problems. Overall mortality rate was zero. Patients were discharged 11 to 118 days (mean 20 days) after operation.

Two patients were lost to follow-up. One patient died of a myocardial infarction 6 months postoperatively and another patient died of malignant metastasis. Nine of 14 patients have been followed-up thus far. Three months after the operation, body weight stablized or began increasing. One of nine patients complained of early dumping symptoms and symptomatic reflux. In all other patients there was no evidence of dumping or reflux. Esophagogastroduodenoscopy revealed normal findings in all nine patients. Gastrointestinal quality-of-life index (GIQLI) scores were recorded an average of 6.5 months after the

Table I. Background information on patients undergoing ileocolon interposition

No. of patients	14
Sex	
Male	9
Female	5
Age (yr)	
Median	58
Range	41-77
Distribution of tumors	
Distal esophagus	1
Cardia	4
Proximal stomach/corpus	6
Antrum	3
Type of operation	
Intrathoracic reconstruction	5/14
Abdominal reconstruction	9/14

	Intrathoracic	Abdominal
Mortality	0	0
Morbidity		
Intra-abdominal abscess	0/5	1/9
Leakage, pancreatic fistula	0/5	1/9*
Hospital stay (days)		
Median	19	27
Range	14-52	11-118*

*One patient, a 64 year-old man, developed a pancreatic fistula and leakage of the ileoesophageal anastomosis. He underwent a laparotomy for oversewing of the leak and intra-abdominal washout on postoperative day 6. He was discharged on postoperative day 118. At the first clinical follow-up visit he had no local or general complaints.

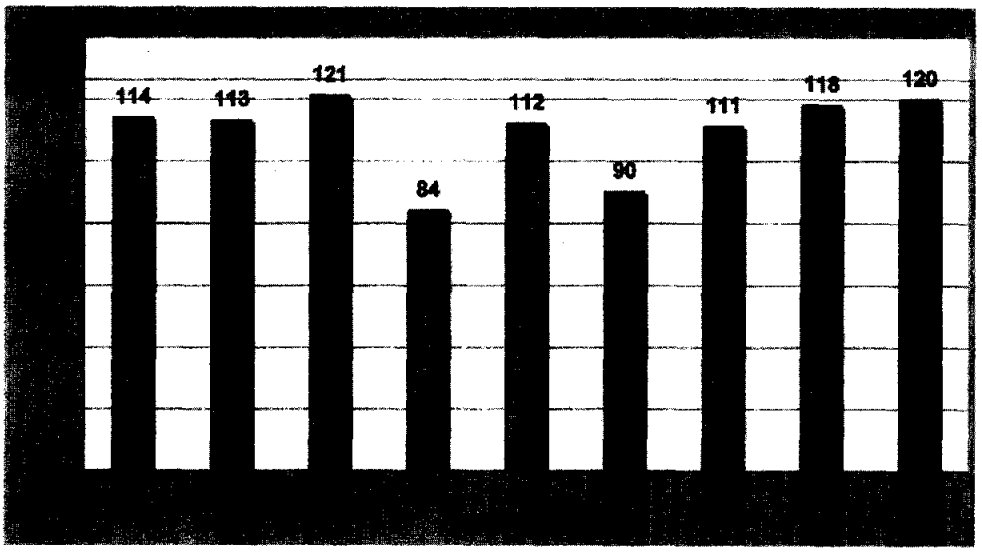

Fig. 5. Gastrointestinal quality-of-life index scores (n = 9).

operation. Seven of nine patients had excellent GIQLI scores (∅ 115 points for abdominal and 106 points for intrathoracic reconstruction) (Fig. 5). In the two patients who had low scores of 84 and 90 points, the main complaint was related to general symptoms such as exhaustion, depression, and nausea.

DICUSSION

Patients undergoing gastrectomy present problems associated with the loss of reservoir function, loss of lower esophageal sphincter, and bypass of the physiologic duodenal passage. Various types of reconstructions have been described in the surgical literature. Nakane et al.[6,7] and Iivonen et al.[8] showed the advantages of a pouch reconstruction compared to a standard Roux-en-Y reconstruction. Schwarz et al.[9] emphasized the better quality of life and normal glucose tolerance in patients undergoing an Ulm pouch reconstruction including preservation of the duodenal passage. On the other hand, several authors could not find any advantages to preservation of the duodenal passage or creation of a pouch compared to a simple Roux-en-Y procedure.[10-12] The value of a complicated reconstruction remains controversial.

Encouraged by the good results that we have achieved using the ileocecal segment for replacement of the rectum,[13-15] we considered using the same anatomic unit to replace the stomach after gastrectomy. Our hypothesis is summarized in Fig. 6. The ileocecal segment offers a physiologic reservoir with a valve that can sustain a pressure up to 80 cm H_2O.[16] Kumar and Phillips[16] demonstrated the mechanical significance of the ileocecal junction. Similar to the

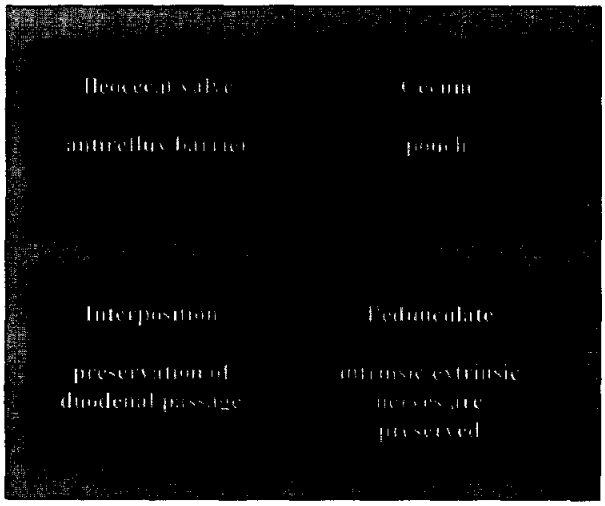

Fig. 6. Presumed advantages of ileocecal interposition.

angle of His, external ligaments maintain an angle between the distal ileum and the cecum. Manometry reveals a high-pressure zone on the human ileocecal junction.[17] The function of the ileocecal valve is controlled by neurologic, humoral, and pharmacologic influences.[18-20] Given the fact that the intrinsic and extrinsic nerves are preserved, it seems most likely that the ileocecal valve can function as effectively as the esophagogastric cardia in preventing the regurgitation of bile and pancreatic juice into the lower esophagus. Quigley et al.[21] examined the myoelectric activity and intraluminal pressure of the canine ileocolonic sphincter and reported that the ileocecal sphincter has little effect on transit or flow. Therefore

no evidence could be found to prove the hypothesis that the ileocecal sphincter delays the passage of food. Indeed, we were recently able to prove that ileocecal segment transposition does not alter whole-gut transit.[15] The cecum has a reservoir-like capacity of 300 to 400 ml, with an excellent compliance.[13] Pediatric surgeons have used the same ileocecal segment for esophageal replacement in children with esophageal atresia with convincing results.[22]

It has been shown that transposition of the ileocecal segment does not result in persistent diarrhea,[23-27] as long as the dissection of the ileum is not too extensive. The idea of using the ileocecal segment as a gastric substitute is not new. In 1951 Lee[1] published the first results of an experimental study in dogs. From a technical standpoint the operation was feasible. But because of some anatomic disadvantages in dogs, such as a very small right colon and a different type of blood supply, the results were not very convincing. However, Hunnicutt[2] performed the first four operations in humans in 1950. The physiologic results were remarkable. Although the concept and initial experiences in humans were already promising at that time, this technique had not been reported in the surgical literature since 1952.

To our knowledge there have been only three published reports[3,28,29] concerning this technique since we began using the ileocecal segment as a gastric substitute in 1995. But replacement of the partial esophagus and total stomach with the same graft between the intrathoracic esophagus and the duodenum has not yet been described in the surgical literature.

Sakamoto et al.[3] performed ileocolon interposition in 47 patients who underwent either total or proximal gastrectomy. They evaluated the function of the substitute using esophagoscopy, manometry, pH-metry, emptying time, body weight changes, and oral glucose tolerance test. There were no cases of direct operative death. These investigators concluded that this technique has the advantage of successfully preventing postoperative reflux esophagitis and providing functional replacement of the stomach as a reservoir for ingested food. Uras et al.[28] published their findings in a smaller series of six patients who underwent ileocecal interposition after pylorus-preserving near-total gastrectomy. None of the patients complained of dumping syndrome, weight loss, or reflux esophagitis.

CONCLUSION

We suggest that ileocolon interposition for gastric or distal esophagus replacement is an attractive restorative technique. The technique-related morbidity is low and our patients maintained an excellent quality of life. Further prospective randomized studies are necessary to compare this technique with the standard procedures for reconstruction after gastrectomy.

REFERENCES

1. Lee MC. Transposition of a colon segment as a gastric reservoir after total gastrectomy. Surg Gynecol Obstet 1951;92: 456-465.
2. Hunnicutt AJ. Replacing stomach after total gastrectomy with right ileocolon. Arch Surg 1952;65:1-11.
3. Sakamoto T, Fujimaki M, Tazawa K. Ileocolon interposition as a substitute stomach after total or proximal gastrectomy. Ann Surg 1997;226:139-145.
4. Eypasch E, Williams JI, Wood-Dauphinee S, et al. Gastrointestinal Quality of Life Index: Development, validation and application of a new instrument. Br J Surg 1995;82:216-222.
5. von Flüe M, Metzger J, Harder F. Ileocecal interpositional graft as gastric replacement after total gastrectomy and distal esophagectomy. Arch Surg 1997;132:1038-1042.
6. Nakane Y, Akehira K, Okumura S, Okamura S, Osawa T, Okusa T, Hioki K. Emptying of the jejunal pouch as a gastric substitute after total gastrectomy for cancer. Hepatogastroenterology 1997;44:901-906.
7. Nakane Y, Okumura S, Akehira K, et al. Jejunal pouch reconstruction after total gastrectomy for cancer. A randomized controlled trial. Ann Surg 1995;222:27-35.
8. Iivonen M, Matikainen M, Nordback I. Jejunal pouch reconstruction diminishes postoperative symptoms after total gastrectomy. Dig Surg 1997;14:260-266.
9. Schwarz A, Buchler M, Usinger K, et al. Importance of the duodenal passage and pouch volume after total gastrectomy and reconstruction with the Ulm pouch: Prospective randomized clinical study. World J Surg 1996;20:60-67.
10. Liedmann B, Andersson H, Bosaeus I, Hugosson I, Lundell L. Changes in body composition after gastrectomy: Results of a controlled, prospective clinical trial. World J Surg 1997;21: 416-421.
11. Svedlund J, Sullivan M, Liedmann B, Lundell L, Sjödin I. Quality of life after gastrectomy for gastric carcinoma: Controlled study of reconstructive procedures. World J Surg 1997;21:422-433.
12. Fuchs KH, Thiede A, Engemann R, Deltz E, Stremme O, Hamelmann H. Reconstruction of the food passage after total gastrectomy: Randomized trial. World J Surg 1995;19:698-705.
13. von Flue MO, Degen LP, Beglinger C, Hellwig AC, Rothenbuhler JM, Harder FH. Ileocecal reservoir reconstruction with physiologic function after total mesorectal cancer excision. Ann Surg 1996;224:204-212.
14. von Flue M, Harder F. New technique for pouch-anal reconstruction after total mesorectal excision. Dis Colon Rectum 1994;37:1160-1162.
15. Degen L, von Flue M, Collet A, Hamel C, Beglinger C, Harder F. Ileocecal segment transposition does not alter whole gut transit in humans. Ann Surg 1997;6:746-752.
16. Kumar D, Phillips SF. The contribution of external ligamentous attachments to function of the ileocolonic junction. Dis Colon Rectum 1987;30:410-416.
17. Cohen S, Harris LD, Levitan R. Manometric characteristics of the human ileocecal junctional zone. Gastroenterology 1968;54:72-75.
18. Soper NJ, Chapman NJ, Kelly KA, Brown ML, Phillips SF, Go VL. The "ileal brake" after ileal pouch-anal anastomosis. Gastroenterology 1990;98:111-116.

19. Philips SF, Quigley EM, Kummer D, Kamath PS. Motility of the ileocolic junction. Gut 1988;29:390-406.
20. McKirdy HC, Marshall RW, Taylor BA. Control of the human ileocaecal junction: An in vitro analysis of adrenergic and non-adrenergic non-cholinergic mechanisms. Digestion 1993;54:200-206.
21. Quigley EM, Phillips SF, Dent J, Taylor BM. Myoelectric activity and intraluminal pressure of the canine ileocolonic sphincter. Gastroenterology 1983;85:1054-1062.
22. Touloukian RJ, Tellides G. Restrosternal ileocolic esophageal replacement in children revisited. Antireflux role of the ileocecal valve. J Thorac Cardiovasc Surg 1994;107:1067-1072.
23. Ben-Chaim J, Shenfeld O, Goldwasser B, Shemesh E. Does the use of the ileocecal region in reconstructive urology cause persistent diarrhea? Eur Urol 1995;27:315-318.
24. Ecker KW, Pistorius G, Harbauer G, Feifel G. Bacterial clearance of the terminal ileum in relation to the ileocolic connection. Zentralbl Chir 1995;120:336-342.
25. King CE, Taskes PP. Small intestine bacterial overgrowth. Gastroenterology 1979;76:1035-1055.
26. Neale G, Gompertz D, Schönsby H, Tabagchali S, Booth C. The metabolic and nutritional consequence of bacterial overgrowth of the small intestine. Am J Clin Nutr 1972;25:1409-1417.
27. Drasar BS, Shiner M. Studies on intestinal flora. II. The bacterial flora of the small intestine in patients with gastrointestinal disorders. Gut 1969;10:812-819.
28. Uras C, Yigitbasi R, Erturk S, Hamzaoglu I, Sayman H. Restorative caecogastroplasty reconstruction after pylorus-preserving near-total gastrectomy: A preliminary study. Br J Surg 1997;84:406-409.
29. Shibata J, Naitoh H, Kawaguchi A, Terata N, Kodama M. Evaluation of ileocolon interposition after total gastrectomy. Nippon Geka Gakkai Zasshi 1997;98:549-554.

Variability in the Composition of Physiologic Duodenogastric Reflux

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Duodenogastric reflux has long been associated with various diseases of the foregut. Even though bile is often used as a marker, duodenogastric reflux consists of other components such as pancreatic juice and duodenal secretions. The aim of this study was to investigate the occurrence of duodenogastric reflux, its components, and the variability of its composition in normal subjects. Twenty healthy volunteers (7 men and 13 women) whose median age was 24 years underwent combined 24-hour bilirubin and gastric pH monitoring and intraluminal gastric aspiration. All probes were placed at 5 cm below the lower border of the lower esophageal sphincter. Aspiration was performed hourly and at any time when bilirubin and/or pH monitoring showed signs of duodenogastric reflux. Elastase and amylase were measured in the aspirate. All volunteers had episodes of physiologic duodenogastric reflux. A total of 70 episodes of duodenogastric reflux were registered with a median of three episodes (range 1 to 8) per subject. Most bile reflux occurred separately from pancreatic enzyme reflux. Pancreatic enzyme aspirate was significantly more often associated with a rise in pH in comparison to bile reflux ($P < 0.01$). Duodenogastric reflux is a physiologic event with varying composition. Both bile and pancreatic enzyme reflux frequently occur separately. These findings could explain the disagreement regarding assessment and interpretation of duodenogastric reflux in the past. Thus monitoring of duodenogastric reflux requires more than the detection of just one component. (J GASTROINTEST SURG 1999; 3:389-396.)

KEY WORDS: Duodenogastric reflux, bile reflux, pancreatic enzymes, reflux disease

Duodenogastric reflux was first observed and assessed by Beaumont[1] in 1833. During the past three decades duodenogastric reflux has been associated with a number of pathologic conditions of the foregut such as gastric ulcer,[2-4] gastritis,[5,6] gastric cancer,[7] and dyspepsia. Experimental studies have shown the destructive effects of bile and pancreatic juice on the mucosa.[8-10] Recent experimental and clinical evidence indicates that duodenogastric reflux could be involved in the development of Barrett's esophagus and adenocarcinoma of the distal esophagus.[11-14] However, duodenogastric reflux is a physiologic phenomenon.[15-18] In upper gastrointestinal endoscopy, it is not unusual to find bile in the gastric lumen in healthy subjects.

A number of different detection methods have been described to assess duodenogastric reflux, such as scintigraphy, technetium-HIDA scanning, gastric aspiration, and gastric pH monitoring.[17,19-21] The recent addition of long-term fiberoptic bilirubin monitoring has widened the diagnostic spectrum. The latter technique allows for intraluminal assessment of a marker of bile in the stomach as well as in the esophagus.[18,22] In validation studies on 24-hour gastric pH and bilirubin monitoring, a discrepancy between pH elevation and increasing or decreasing bilirubin absorption values led to the hypothesis that duodenogastric bile reflux sometimes occurs separately from other forms of duodenogastric reflux, causing a rise in intragastric pH, which had been suspected years ago.[23] That earlier observation thus prompted the current investigation. The aim of our study was to evaluate physiologic duodenogastric reflux with regard to the different components of refluxing duodenal juice and their changing composition during a 24-hour period.

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MATERIAL AND METHODS

Twenty healthy individuals volunteered, after giving written informed consent, for foregut functional investigations in our laboratory in the department of surgery at the University of Würzburg. The 13 women and seven men had a median age of 24 years (range 23 to 28 years), height 172 cm (range 163 to 191 cm), and weight 70 kg (range 50 to 100 kg). The selection criteria were absence of any upper gastrointestinal symptoms and exclusion of upper gastrointestinal disease. The volunteers underwent upper gastrointestinal endoscopy with intragastric biopsies and C-13 breath tests for *Helicobacter pylori* contamination, abdominal ultrasound investigation, esophageal manometry, and 24-hour esophageal pH monitoring.

In this study three different diagnostic assessment methods were combined to evaluate the subjects for signs of physiologic duodenogastric reflux, which included elevated intragastric pH, bilirubin exposure, and intragastric evidence of pancreatic enzymes. The protocol was approved by the local ethics committee. Testing was begun in the morning after an overnight fast of at least 6 hours. A combination of three probes were introduced transnasally into the gastric lumen between 10 AM and 12 AM. The tips of the probe system were placed at the previously measured position of 5 cm below the lower border of the lower esophageal sphincter. The probes were fixed on the nose of each volunteer. For the purpose of the study (i.e., for comparison of the different detection systems), the subjects remained in the laboratory during the 20-hour recording period. They were allowed to move around in the laboratory only between the hourly aspiration periods. All activities were recorded in each volunteer's diary for further evaluation and comparison with the recorded data.

During the investigation period, one meal was given at 6 PM. No aspiration was performed within 2 hours after the start of the meal to avoid any artifacts caused by aspiration of food. The meal was semi-standardized and consisted of cheese, oilspread toast, and vanilla or banana Biosorb energy drinks (Fresenintz, Bad Nanheim, Germany). Besides this meal, no other food or fluid intake was allowed to keep the intragastric conditions as standardized as possible and to minimize artifacts influencing the aspiration technique. The investigation was discontinued at 8 AM the next morning.

For pH recording a glass probe was used (Ingold, Urdorf, Switzerland), which was connected to a Digitrapper data logger (Medtronic, Düsseldorf, Germany). For bilirubin monitoring a Bilitec probe was simultaneously inserted through the same nostril and connected to the Bilitec 2000 data logger system (Medtronic). In addition, a double-lumen 12 Fr nasogastric tube with a single perforation at its tip (modified Vygon probe [Vygon, Aachen, Germany]) was positioned at the same level. Aspiration was performed on a regular basis, either hourly or when the data loggers showed signs of a reflux event on the pH recording device (pH >3) or the Bilitec recording device (absorption >0.25). During the aspiration phase, one sample of approximately 2 ml was collected for the detection of amylase and lipase. Another 2 ml of aspirate was collected for the detection of pancreatic elastase 1. Immediately after aspiration and collection, the samples were frozen in dry ice at −25° C and transported to the laboratory of Arndt and Keeser (Hamburg, Germany) at this temperature.

Results of gastric pH monitoring were analyzed according to previously published methods.[17] Prandial and postprandial changes within 2 hours were excluded from the analysis. A rise in the intragastric pH above 3 was considered a possible sign of a duodenogastric reflux episode. Analysis of the bilirubin monitoring was based on previous investigations and validation studies.[18] A rise in the absorption value above 0.25 was considered a sign of intragastric increased bilirubin exposure due to an episode of duodenogastric reflux. Pancreatic elastase 1, amylase, and lipase in the gastric aspirate were analyzed in the laboratory of Arndt and Keeser. A pancreatic elastase concentration above 10 ng/ml was considered positive for elastase 1 (enzyme-linked immunosorbent assay, Schebo Tech, Wittenberg, Germany). Alpha-amylase was assessed by means of the CNPG3 test (2-chloro-4-nitrophenyl-α-D 25 maltotrisoid substrate, Olympus art. No. OSR 6106). A value above 130 U/L was considered borderline. Pancreatic lipase was measured by means of the triolein method (Nobis) and was considered positive above 200 U/L.

The composition of the duodenogastric reflux was analyzed according to the following combinations of signs of duodenogastric reflux that are theoretically possible: bile only, bile and a rise in pH, bile and a rise in pH plus positive pancreatic enzyme aspirate, bile and positive pancreatic enzyme aspirate, pH and positive pancreatic enzyme aspirate, isolated pH, and isolated positive pancreatic enzyme aspirate. These episodes were assessed with regard to their duration and whether they occurred during the day or at night (between 10 PM and 7 AM). For statistical comparison Fisher's exact test was used.

RESULTS

All 20 volunteers showed signs of physiologic duodenogastric reflux. However, there was no uniform spectrum of physiologic duodenogastric reflux among the persons studied, but there was considerable interindividual variation in composition and duration of

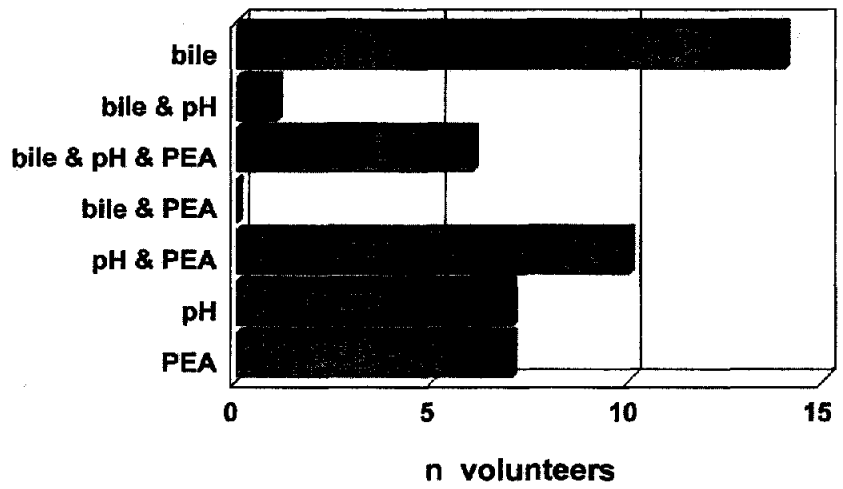

Fig. 1. Distribution of various signs of duodenogastric reflux among 20 volunteers. Note that one volunteer can have more than one tye of reflux event (pH = pH rise >3; bile = bilirubin absorption >0.25; PEA = pancreatic enzyme aspirate positive for pancreatic enzymes in the gastric lumen). In individuals with isolated bile or isolated PEA, the intragastric pH is below 3.

Fig. 2. Distribution of various types of reflux events among 70 episodes with signs of duodenogastric reflux in 20 volunteers. Note that pH is significantly more often associated with positive pancreatic enzyme aspirate than with bile reflux (* = significantly different by Fisher's exact test; pH = pH rise >3; bile = bilirubin absorption >0.25; PEA = pancreatic enzyme aspirate positive for pancreatic enzymes in the gastric lumen).

the presenting signs. Fig. 1 shows the distribution of the types of episodes within the volunteer population. Fourteen volunteers had signs of isolated bile reflux, and 10 volunteers showed signs of a rise in pH along with reflux of pancreatic juice during the test. The distribution of the episodes with various compositions suggesting duodenogastric reflux is presented in Fig. 2. A total of 70 episodes with signs of duodenogastric reflux were identified. The median number of signs of reflux episodes was three per individual with a range of one to eight. Only three volunteers had more than five reflux episodes. The most frequently occurring episode was isolated bile reflux (n = 25), as detected by bilirubin monitoring, followed by 15 episodes of positive pancreatic enzyme aspirate in combination with a rise in pH. The combination of bile and positive pancreatic enzyme aspirate without a rise in pH never occurred in any of the volunteers. In addition, positive pancreatic enzyme aspirate is significantly ($P < 0.01$) more often associated with an intragastric pH elevation above 3 than with intragastric bile reflux (i.e., bilirubin detection). This indicates

Table I. Distribution of signs of duodenogastric reflux episodes between day and night

	Day	Night	Total
Bile	8	17	25
Bile and pH	0	1	1
Bile, and pH, and PEA	4	2	6
Bile and PEA	0	0	0
pH and PEA	11	4	15
pH	8	6	14
PEA	6	3	9

pH = pH rise >3; bile = bilirubin absorption >0.25; PEA = pancreatic enzyme aspirate positive for pancreatic enzymes in the gastric lumen.

Table II. Distribution of signs of prolonged duodenogastric reflux episodes (duration longer than 1 hour) between day and night

	Day	Night	Total
Bile	0	6	6
Bile and pH	0	0	0
Bile, pH, and PEA	2	2	4
Bile and PEA	0	0	0
pH and PEA	2	1	3
pH	1	0	1
PEA	1	0	1

pH = pH rise >3; bile = bilirubin absorption >0.25; PEA = pancreatic enzyme aspirate positive for pancreatic enzymes in the gastric lumen.

that duodenal juice containing predominantly pancreatic juice is able to change the gastric pH environment more extensively in contrast to bile reflux.

The measured concentrations of pancreatic enzymes, that is, elastase 1, amylase, and lipase, were not all positive in each reflux episode. In 12 of 30 episodes in which signs of pancreatic reflux were demonstrated, all three enzymes were detected. Isolated amylase was found in eight episodes and isolated elastase in two. The combination of elastase and amylase was found in four episodes and the combination of amylase and lipase in three episodes. Pancreatic elastase and amylase are among the most important enzymes in this regard that can be detected in the gastric lumen. It is important to note that the intragastric concentrations of pancreatic enzymes measured were usually strongly positive and well above the borderline values. For elastase the median value was 2252 ng/ml (range 0 to 118,950 ng/ml). For amylase the median value was 13,388 U/L (range 0 to 44,352 U/L). For lipase the median value was 572 U/L (range 0 to 10,5000 U/L). The pH level during reflux episodes showed great variability with a median value of 4.1.

Another interesting observation can be made when results are classified according to whether events occurred during the day or at night (Table I). Bile reflux, isolated or in association with other signs of duodenogastric reflux, occurred twice as frequently at night as during the day. Isolated positive pancreatic enzyme aspirate is a rare finding during the night and isolated or in association with other signs of duodenogastric reflux, it occurs twice as often during the day as during the night. Even though these differences between day and night did not reach statistical significance in this series, they showed a clear trend toward bile reflux being a more frequent event during the night compared to pancreatic juice reflux. There was no correlation between physical activities such as walking around and sitting and reflux episodes.

Further analysis focusing on prolonged reflux episodes (i.e., duration longer than 1 hour [range 2 to 7 hours]) supported the previous finding (Table II). Isolated bile reflux lasting longer than 1 hour was recorded only during the night. Prolonged bile reflux associated with other signs of duodenogastric reflux was far more often detectable during the night than during the day. In contrast, prolonged episodes of positive pancreatic aspirate lasting longer than 1 hour were a rare finding during the night and occurred more often during the day.

DISCUSSION

A major problem in determining the role of duodenogastric reflux in foregut diseases is the limited number of available diagnostic procedures. Initially we used 24-hour gastric pH monitoring to register changing levels of acidity or alkalinity in the gastric lumen as a possible indicator of duodenogastric reflux.[17] Subsequently we reported that the discovery of an elevated intragastric pH and an increased bilirubin exposure frequently is not coincidental.[18] Furthermore, in the present study, by combining gastric pH monitoring, bilirubin monitoring, and intragastric fluid aspiration, we were able to document a wide variation in the composition of duodenogastric reflux. In addition, our results show that pancreatic enzymes, bilirubin exposure, and pH elevation can occur in the proximal stomach for several hours. On the other hand, there are reports that bile and pancreatic secretions physiologically have a digestive or postprandial pattern or occur in the interdigestive period in coordination with the migrating motor complex (MMC).[15]

A complex system regulates the digestion process during and after a meal by releasing pancreatic juice

and bile into the duodenum coordinated with gastric secretions and upper gastrointestinal motility.[24] Keane et al.[15] investigated the correlation between digestive secretion, duodenogastric reflux, and MMC in six volunteers. During the interdigestive cycle, which can be separated into three phases, the release of bile and pancreatic juice starts early in phase 2 and reaches its maximum shortly before the onset of phase 3. In contrast, gastric secretion peaks at the beginning of phase 3. Also, bicarbonate secretion in the duodenum occurs with peak levels during phase 3 in coordination with gastric acid secretion. Late in phase 2 physiologic duodenogastric reflux increases, whereas it decreases at the onset of phase 3. Duodenal contents that had refluxed earlier as well as remaining food particles were cleared, in this study, from the stomach with the "housekeeping" function of phase 3 of the MMC. This is in contrast to several recent reports showing bilirubin exposure and/or pH elevation for several hours in the proximal stomach.[18,22,25] This is also true for pancreatic juice since we measured pancreatic enzymes in high concentrations as well as bile in the stomach for up to 5 hours.

We must therefore assume that physiologic duodenogastric reflux is a phenomenon that can be associated with the interdigestive MMC; however, the duodenogastric refluxate is obviously not always cleared by the housekeeping function of phase 3. In contrast, different components of the duodenal juice can reach the proximal stomach and stay there for several hours without clearance. Thus there is a possibility of a prolonged exposure of the gastric lumen with pancreatic and biliary secretions if the interdigestive phase is long enough. These findings clearly show that in the physiologic state the gastric mucosa must be resistant to pancreatic enzymes and bile since a physiologic exposure can last for several hours. On the other hand, a normal acidic gastric environment will inactivate pancreatic enzymes and bile acids will precipitate in a gastric pH environment with an acidic pH.[24,26] Any artificial elevation of the intragastric pH over a long-lasting period creates the possibility of having activated pancreatic enzymes and unprecipitated bile in the gastric lumen. Reflux episodes that reach up into the proximal stomach are probably of more clinical relevance.

The most important finding in this study is the variability in the composition of duodenogastric reflux. There are several reasons for these differences in exposure of acid, bile, and pancreatic enzymes in the gastric lumen. First, technical factors such as artifacts or certain limitations must be considered. The probe placement in the proximal stomach influences the detected composition of the refluxate since not every reflux episode reaches this area. However, this probe position has been shown to be the best placement for reproducible results in long-term intragastric pH monitoring, and reflux episodes that reach up into the proximal stomach are probably of greater clinical relevance.[27] Another technical consideration is the possible influence of probe placement through the lower esophageal sphincter on duodenogastric reflux. There is evidence, however, that this probe placement through the lower esophageal sphincter does not influence the esophageal and gastric environment.[28] In addition, probe placement even through the pylorus does not influence the gastric recordings.[29]

A second argument is the general finding in recent studies that long-term monitoring of physiologic gastrointestinal function has always shown a large interindividual variability in gastrointestinal motility and pH environment.[17,18,30,31] The third and most important explanation for this variability must be the physiologic element. Motility of the antroduodenal segment and the pylorus is an important factor in the regulation of physiologic duodenogastric reflux.[32] It is modulated by complex neurohumoral components in response to the composition of food and chyme. A selective action of pyloric motility, whether or not reflux of bile or pancreatic juice does occur, is very unlikely. As a consequence, the measured differences in the appearance of bile, pancreatic enzymes, and acid exposure must be related to variations in secretion release of bile and pancreatic juice as well as variations in the intraduodenal and intragastric mixture of the refluxate.

Secretion of pancreatic juice can vary remarkably during a 24-hour period. The daily volume of pancreatic secretions can reach 2.5 L with a flow rate ranging from 0.2 ml/min at rest to 4.0 ml/min during stimulation.[33-35] Bicarbonate concentration of the secreted fluid varies between 25 and 120 mEq/L depending on the flow rate.[36] Similar to gastric secretion, exocrine pancreatic secretion follows a cephalic phase in which pancreatic enzyme secretion increases up to 50% of the maximal secretory state. When acid also enters the duodenum, this rate increases to 90% for enzymes and bicarbonate secretions.[37] When acid is not entering the duodenum, bicarbonate secretion is not emerging. This could explain why duodenal juice can contain varying concentrations of both pancreatic enzymes and bicarbonate. With a low concentration of bicarbonate, the buffering capacity of the refluxate is low, and therefore pancreatic enzymes might be detected in combination with low intragastric pH. Discrepancies in the concentrations of measured pancreatic enzymes are possibly related to the secretory state of the pancreas. Amylase, lipase, and

proteases such as elastase have different functions and actions in the digestive process.[36] Therefore it is understandable why these components could appear in different concentrations in the proximal stomach.

Isolated increased intragastric bile exposure, especially as assessed during the night, can be explained by minor pressure resistance of the sphincter of Oddi at night and during the early morning hours with a filled gallbladder and release of bile into the duodenum, whereas pancreatic enzyme secretion is lowest at 0.2 ml/min.[36,38-41] Bile does have a very limited buffering capacity, which explains the lack of correlation between intragastric bile and pH elevation.[26,41]

In summary, a detailed analysis of physiologic duodenogastric reflux reveals no specific pattern of refluxing duodenal components into the gastric lumen. With the exception of bile in combination with pancreatic enzyme aspirate, all possible combinations were detected in this study. Even though four individuals showed only isolated bile reflux and four individuals showed only a rise in pH and pancreatic enzyme activity lacking any other combination, most individuals showed some combination without any regularity. The pH level after an episode of pancreatic or bile reflux in the physiologic state usually reached a pH value of just 4.1, which allows for only limited activation of the refluxing agents.

There is increasing evidence from recent investigations that refluxing bile into the esophagus is involved in Barrett's esophagus.[14,42,43] Increased bilirubin exposure is associated with columnar-lined epithelium with intestinal metaplasia. There are reports of the ability of pancreatic juice to cause damage in an experimental esophagitis model as well as in the carcinogenesis of esophageal carcinoma.[11,12] Not every patient with columnar-lined epithelium is found to have an associated increase in bilirubin exposure.[14,42] This discrepancy could be explained by the changing composition of duodenogastric reflux. Thus far, quantitative assessment of pancreatic refluxate has not been introduced into routine practice, since it is an expensive, time-consuming, and invasive procedure. On the other hand, the results of this study suggest that almost half of all healthy individuals show signs of pancreatic-associated reflux, which could also be responsible for the development of disease when mucosal resistance and other functional defects allow an increased exposure.

CONCLUSION

This study shows that all investigated healthy individuals have signs of physiologic duodenogastric reflux. The composition of duodenogastric reflux varied throughout the interdigestive recording period of 20 hours showing great interindividual variability among the different components. Even though bile is the most frequently occurring reflux component, pancreatic juice is also involved in almost half of all reflux episodes. Pancreatic enzyme reflux is significantly more often associated with a rise in intragastric pH compared to bile reflux. On the other hand, bile exposure occurred more often at night than during the day, whereas pancreatic enzyme exposure occurred more often during the day. This was especially true for prolonged reflux episodes lasting for more than 1 hour and up to 6 hours, whereas isolated bile reflux occurred only during the night. Because both bile and pancreatic enzymes have the potential for mucosal destruction in the stomach and in the esophagus, further investigation of these important components, including pathophysiologic studies, are warranted. Assessment of duodenogastric reflux requires more than monitoring of just one component.

REFERENCES

1. Beaumont W. Experiments and observations on the gastric juice and the physiology of digestion. Plattsburgh: FP Allen, 1833.
2. Du Plessis DH. Pathogenesis of gastric ulceration. Lancet 1965;1:974-978.
3. Rhodes J. Etiology of gastric ulcer. Gastroenterology 1972; 63:171-182.
4. Schumpelick V, Stemme D, Begemann F. Ulkuskrankheirt und duodenogastraler Reflux. Z Gastroenterol 1983;21:11-20.
5. Ritchie WP. Alkaline reflux gastritis: An objective assessment of its diagnosis and treatment. Ann Surg 1980;92:288-298.
6. Alexander-Williams J. Alkaline reflux gastritis: A myth or a disease? Am J Surg 1981;143:12-17.
7. Werner B. Historische Daten und Betrachtungen zur Entwicklung der Refluxkrankheiten des Magens. In Schumpelick V, Begemann F, Werner B (eds.) Refluxkrankheit des Magens. Stuttgart: Ende, 1985, pp 3-15.
8. Wickbom G, Bushkin FL, Linares C, Dragstedt LR. On the corrosive properties of bile and pancreatic juice on living tissue. Arch Surg 1974;108:680-684.
9. Duane WC, Weigand DM. Mechanism by which bile salt disrupts the gastric mucosal barrier in the dog. J Clin Invest 1980;66:1044-1049.
10. Begemann F, Schumpelick V. Problems in duodenogastric reflux assessment using bile acids and lysolecithin measurement. Scand J Gastroenterol 1981; 16(Suppl):51-54.
11. Johnson LF, Harmon JW. Experimental esophagitis in a rabbit model. Clinical relevance. J Clin Gastroenterol 1986;8: 26-44.
12. Pera M, Grande L, Gelabert M, Figueras X, et al. Epithelial cell hyperproliferation after biliopancreatic reflux into the esophagus of rats. Ann Thorac Surg 1998;65:779-786.
13. Ireland AP, Clark GWB, DeMeester TR. Carcinoma of the cardia: Role of short-segment Barrett's esophagus and columnar metaplasia. Dis Esophagus 1996;9:159-164.
14. Fein M, Ireland AP, Ritter MP, Peters JH, Hagen JA, Bremner CG, DeMeester TR. Duodenogastric reflux potentiates the injurious effects of gastroesophageal reflux. J GASTROINTEST SURG 1997;1:27-33.

15. Keane FB, Dimagno EP, Malagelada J-R. Duodenogastric reflux in humans: Its relation to fasting antroduodenal motility and gastric, pancreatic and biliary secretion. Gastroenterology 1981;81:726-731.
16. Müller-Lissner SA, Fimmel CJ, Sonnenberg A, Will N, Müller-Duysing W, Heinzel F, Müller R, Blum AL. Novel approach to quantify duodenogastric reflux in healthy volunteers and in patients with type I gastric ulcer. Gut 1983;24:510-518.
17. Fuchs KH, DeMeester TR, Hinder RA, Stein HJ, Barlow AP, Gupta NC. Computerized identification of pathological duodenogastric reflux using 24-hour gastric pH monitoring. Ann Surg 1991;213:13-20.
18. Fein M, Fuchs K-H, Bohrer T, Freys S, Thiede A. Fiberoptic technique for 24-hour bile reflux monitoring—Standards and normal values for gastric monitoring. Dig Dis Sci 1996;41(1):216-225.
19. Warshaw AL. Intragastric alkali infusion: A simple, accurate provocative test for diagnosis of symptomatic alkaline reflux gastritis. Ann Surg 1981;194:297-304.
20. Wickremesinghe PC, Dayrit PQ, Manfredi OL, et al. Quantitative evaluation of bile diversion surgery utilizing ^{99m}Tc HIDA scintigraphy. Gastroenterology 1983;84: 354-363.
21. Ritchie WP. Alkaline reflux gastritis: A critical reappraisal. Gut 1984;25:975-987.
22. Bechi P, Pucciani F, Baldini F, Cosi F, Falciai R, Mazzanti R, Castagnoli A, Passeri A, Boscherini S. Long-term ambulatory enterogastric reflux monitoring—Validation of a new fiberoptic technique. Dig Dis Sci 1993;38:1297-1306.
23. Müller-Lissner SA, Fraass C. Dissociation of duodenogastric marker reflux and bile salt reflux. Dig Dis Sci 1985;30: 733-738.
24. DiMagno EP. Human exocrine pancreatic enzyme secretion. In Go VLW, et al., eds. The Exocrine Pancreas: Biology, Pathobiology, and Diseases. New York: Raven Press, 1986, pp 193-211.
25. Romagnoli R, Bechi P, Salizzoni M, Collard JM. Combined 24-hour pH and bile monitoring of the denervated whole stomach as an esophageal substitute. Hepatogastroenterology, 1997 (in press).
26. Barthlen W, Liebermann-Meffert D, Feussner H, Stein HJ. Influence of pH on bile acid concentration in human, pig and commercial bile: Implications for "alkaline" gastroesophageal reflux. Dis Esophagus 1994;7:127-130.
27. Bartlow AP, Hinder RA, DeMeester TR, Fuchs KH. Position of pH-probes in the gastric lumen. In Fuchs KH, Hamelmann H, eds. (Hrsg) Gastrointestinale Funktionsdiagnostik in der Chirurgie. Berlin: Blackwell, 1997;140-146.
28. Emde C, Garner A, Blum A. Technical aspects of intraluminal pH-metry in man. Current status and recommendations. Gut 1987;28:1177-1188.
29. Longstreth GF, Malagelada IR, Go VLW. The gastric response to a transpyloric duodenal tube. Gut 1975;16: 777-780.
30. Emde C, Armstrong D, Castiglione F, Ciluffo T, Riecken EO, Blum AL. Reproductibility of long-term ambulatory combined pH/manometry. Gastroenterology 1991;100: 1630-1637.
31. Heimbucher J. Manometric evaluation of antroduodenal dysfunction. In Stein HJ, Fuchs KH, Bonavina L, eds. Functional Foregut Disorders. Heidelberg: JA Barth, QMP, 1998, pp 183-192.
32. Malagelada JR, Azpiroz F, Mearin F. Gastroduodenal motor function in health and disease. In Sleisenger GH, Fordtran, JS eds. Gastrointestinal Disease. Philadelphia: WB Saunders, 1993, pp 486-508.
33. Zimmermann MJ, Janowitz HD. Water and electrolyte secretion in the human pancreas. In Go VLW, Gardner JD, Brooks FP, Lebenthal E, DiMagno EP, Scheele G, eds. The Exocrine Pancreas. Biology, Pathobiology, and Disease. New York: Raven Pres, 1986, p 275.
34. Dreiling DA, Janowitz HD. The secretion of electrolytes by the human pancreas. Gastroenterology 1956;30:382.
35. Bro-Rasmussen F, Kellman SA, Thaysen JH. The composition of pancreatic juice as compared to sweat, parotid saliva and tears. Acta Physiol Scand 1956;37:97.
36. Pandol SJ. Pancreatic physiology. In Sleisenger GH, Fordtran JS, eds. Gastrointestinal Disease. Philadelphia: WB Saunders, 1993, pp 1585-1600.
37. Anagnostides A, Chadwick US, Selden AC, Maton PM. Sham feeding and pancreatic secretion. Gastroenterology 1984;87:109.
38. Nebel OT. Manometric evaluation of the papillae of Vater. Gastrointest Endosc 1975;21:126-128.
39. Funch-Jensen P, Kruse A, Ravensback J. Endoscopic sphincter of Oddi manometry in healthy volunteers. Scand J Gastroenterol 1987;22:243-249.
40. Guelrud M, Mendoza S, Rossiter G, Villegas MI. Sphincter of Oddi manometry in healthy volunteers. Dig Dis Sci 1990;35:38-46.
41. Chuttani R, Carr-Locke DL. Motility and dysmotility of the biliary tract and sphincter of Oddi. In Sleisenger GH, Fordtran JS, eds. Gastrointestinal Disease. Philadelphia: WB Saunders, 1993, pp 1777-1787.
42. Kauer WKH, Burdiles P, Ireland AP, Clark GWB, Peters JF, Bremner CG, DeMeester TR. Does duodenal juice reflux into the esophagus of patients with complicated GERD? Evaluation of a fiberoptic sensor for bilirubin. Am J Surg 1995;169:98-104.
43. Vaezi MF, Richter JE. Synergism of acid and duodenogastroesophageal reflux in complicated Barrett's esophagus. Surgery 1995;117:699-704.

Discussion

Dr. D. Castell (Philadelphia Pa.). About 3 years ago, we examined bile and pH at the same placement below the upper sphincter with the Bilitec absorbance and pH probes, and just performed overnight analysis. We were very frustrated because there was a terrible correlation between the two. We postulated that it was bicarbonate secretion or something coming from the pancreas. You have demonstrated that there is a more complex series of events, and I agree with your observation.

Dr. S. Attwood (Manchester, England). The message from your presentation seems to be that the Bilitec system underestimates duodenogastric reflux. Were you

able to determine from your volunteer studies whether there was any consistency in how much it is underestimated? Is it underestimated by a lot in one patient and a little in another? In other words, could we just develop a means of calculating an average amount by which the Bilitec system underestimates duodenogastric reflux, or is there too much individual variation?

Dr. K.-H. Fuchs. There is a great deal of interindividual variability. We may see some persons, for example, who have two episodes of duodenogastric reflux during a 24-hour period with positive pancreatic enzyme values and then see another individual who, during the night, has 6 hours of an elevated pH in combination with bilirubin exposure. We must realize that we measure the exposure in the proximal stomach. However, duodenogastric reflux episodes reflux through the pylorus and the refluxate is moving all the way up into the proximal stomach. It is surprising and interesting that we had healthy volunteers with the ability to keep pancreatic enzymes and bile in the proximal stomach for hours. It would be very difficult to find a difference in patients where this process is obviously dangerous.

Dr. J. Peters (Los Angeles, Calif.). I wonder if you could clarify two things for me. Bile does not seem to have much buffering capacity. Did you check in vitro to see whether that is indeed true? Second, was the pancreatic reflux associated with a meal? Was it mostly postprandial?

Dr. Fuchs. Professor Liebermann-Meffert from Munich has described the buffering capacity of bile as being very low. In answer to your second question, pancreatic reflux was not associated with meals. I also thought there might be a correlation between the level of pH increase and pancreatic enzyme reflux, but that was not the case, at least in our 20 healthy volunteers. We have studied five patients now, for example, a patient with a Billroth II resection and massive enterogastric reflux. All values for bile and pancreatic enzymes go way up and, again, there is no good method of quantification.

Barrett's Esophagus: A Surgical Disease

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Barrett's metaplasia can develop in patients with gastroesophageal reflux disease (GERD), and metaplasia can evolve into dysplasia and adenocarcinoma. The optimal treatment for Barrett's metaplasia and dysplasia is still being debated. The study reported herein was designed to assess the following: (1) the incidence of Barrett's metaplasia among patients with GERD; (2) the ability of laparoscopic fundoplication to control symptoms in patients with Barrett's metaplasia; (3) the results of esophagectomy in patients with high-grade dysplasia; and (4) the character of endoscopic follow-up programs of patients with Barrett's disease being managed by physicians throughout a large geographic region (northern California). Five-hundred thirty-five patients evaluated between October 1989 and February 1997 at the University of California San Francisco Swallowing Center had a diagnosis of GERD established by upper gastrointestinal series, endoscopy, manometry, and pH monitoring. Thirty-eight symptomatic patients with GERD and Barrett's metaplasia underwent laparoscopic fundoplication. Eleven other consecutive patients with high-grade dysplasia underwent transhiatal esophagectomies. Barrett's metaplasia was present in 72 (13%) of the 535 patients with GERD. The following results were achieved in patients who underwent laparoscopic fundoplication (n = 38): Heartburn resolved in 95% of patients, regurgitation in 93% of patients, and cough in 100% of patients. With regard to transhiatal esophagectomy (n = 11), the average duration of the operation was 339 ± 89 minutes. The only significant complications were two esophageal anastomotic leaks, both of which resolved without sequelae. Mean hospital stay was 14 ± 5 days. There were no deaths. The specimens showed high-grade dysplasia in seven patients and invasive adenocarcinoma (undiagnosed preoperatively) in four (36%). These results can be summarized as follows: (1) Barrett's metaplasia was present in 13% of patients with GERD being evaluated at a busy diagnostic center; (2) laparoscopic fundoplication was highly successful in controlling symptoms of GERD in patients with Barrett's metaplasia; (3) in patients with high-grade dysplasia esophagectomy was performed safely (invasive cancer had eluded preoperative endoscopic biopsies in one third of these patients); and (4) even though periodic endoscopic examination of Barrett's disease is universally recommended, this was actually done in fewer than two thirds of patients being managed by a large number of independent physicians in this geographic area. (J GASTROINTEST SURG 1999;3:397-404.)

KEY WORDS: Barrett's esophagus, gastroesophageal reflux disease, laparoscopic antireflux surgery, esophageal cancer, esophagectomy

In approximately 8% of patients with gastroesophageal reflux disease (GERD), the esophageal squamous epithelium is replaced by columnar epithelium,[1] a transformation thought to result from irritation by refluxed gastric and duodenal juices.[2] This metaplasia, known as Barrett's esophagus, may progress to high-grade dysplasia and eventually adenocarcinoma.[3,4] Thus adenocarcinoma represents the final step of a sequence in which a benign disease (GERD) evolves into a preneoplastic disease and eventually into cancer. A patient with adenocarcinoma that develops within Barrett's esophagus might be considered to represent a failure of the medical care system, since diagnosis of the premalignant mucosa offers an op-

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portunity to take action to prevent cancer. There is no consensus, however, about the best treatment for patients with Barrett's esophagus and two questions remain: (1) Is it better to continue use of proton pump inhibitors or perform fundoplication, and (2) for high-grade dysplasia, is it better to perform esophagectomy or rely on endoscopic surveillance?

The goal of this study was to assess (1) the incidence of Barrett's esophagus among patients with GERD, (2) the ability of laparoscopic fundoplication to control symptoms in patients with Barrett's metaplasia, (3) the results of esophagectomy in patients with high-grade dysplasia, and (4) the features of endoscopic follow-up programs for patients with Barrett's disease in one large geographic region (northern California).

PATIENTS AND METHODS

Between October 1989 and February 1997, a total of 535 patients studied at the Swallowing Center of the University of California, San Francisco (UCSF) had a diagnosis of GERD. Their evaluation included a clinical history and upper gastrointestinal series along with several additional tests. Each patient was questioned regarding the presence, duration, and severity of symptoms suggestive of GERD such as heartburn, regurgitation, dysphagia, and cough. Symptoms were rated prospectively on a scale of 0 (none) to 4 (very severe). Scores were obtained by interviewing the patients and asking them to grade their symptoms. The same interviewers carried out the pre- and postoperative assessments. All patients underwent a barium x-ray examination of the esophagus and stomach.

Esophagogastroduodenoscopy

The degree of esophagitis was graded according to the Savary-Miller classification.[5] Barrett's metaplasia was defined as the presence of specialized intestinal epithelium on biopsy specimens taken above the squamocolumnar junction.[2] High-grade dysplasia was defined as marked abnormalities in architectural and cellular morphology, without invasion beyond the basement membrane.[4] High-grade dysplasia and carcinoma in situ were considered synonymous. If invasion was present beyond the basement membrane, the disease was considered to be invasive adenocarcinoma.

Four-quadrant biopsies were taken at 1 to 2 cm intervals throughout the length of the Barrett's mucosa. When the endoscopic examination had been performed at another hospital, UCSF pathologists reviewed the biopsy slides. Before a patient underwent esophagectomy, the diagnosis of high-grade dysplasia was confirmed independently by two experienced pathologists.

Esophageal Manometry

Medications that interfere with esophageal and gastric motility were discontinued 3 days before the study. The patients were studied after an overnight fast. We used an eight-lumen manometry catheter (Zinectics Medical, Inc., Salt Lake City, Utah), with continuous perfusion at a rate of 0.5 ml/min by a pneumohydraulic capillary infusion system (Arndorfer Medical Specialties, Greenfield, Wis.) connected to a polygraph (Medtronic Synectics, Shoreview, Minn.). Pressure, length, and relaxation of the lower esophageal sphincter (LES) were measured using the stationary pull-through technique, with 0.5 cm increments between stations. Esophageal body function was assessed by giving 10 wet swallows of 5 ml of water at 30-seconds intervals. The data were analyzed by computer using a commercial software program (Gastrosoft, Medtronic Synectics).

Twenty-Four-Hour Esophageal pH Monitoring

Acid-suppressing medications were discontinued 3 (H_2-blocking agents) to 14 (proton pump inhibitors) days before the study. During the study, patients consumed a normal diet and took no medications that could affect the results. Ambulatory pH monitoring was performed by placing a pH probe 5 cm above the upper border of the manometrically determined LES. The probes were calibrated in a standard buffer solution at pH 7 and 1 before and after monitoring. Results were expressed as a DeMeester score,[6] a widely used composite number reflecting the magnitude of reflux. A score of more than 15 is considered abnormal and was required for a diagnosis of GERD.[6]

Laparoscopic Fundoplication

Thirty-eight of the 72 symptomatic patients with GERD who had Barrett's metaplasia underwent a laparoscopic fundoplication. There were 28 men and 10 women whose average age was 54 years (range 31 to 89 years). The patients had been symptomatic for an average of 194 months (range 12 to 480 months, median 144). At the time of referral they all were taking proton pump inhibitors, and 21 were taking prokinetic agents. In addition to the presence of Barrett's metaplasia, other indications for fundoplication were surgical therapy preferred over medical therapy (28 patients, 74%), dissatisfaction with the results of proton pump inhibitors (9 patients, 24%) (e.g., control of symptoms was fragile, control of symptoms re-

quired increasing doses of proton pump inhibitors, and miscellaneous side effects of the drug such as psychological depression), more than 75% temporal correlation between symptoms and episodes of gastroesophageal reflux during 24-hour pH monitoring, and GERD-induced respiratory symptoms incompletely controlled by medical therapy (10 patients, 25%).

Seventy-six percent of patients had a hiatal hernia as shown by the barium swallow. Preoperative esophageal manometry showed an average LES pressure of 7 mm Hg and an average maximal amplitude of peristalsis in the distal esophagus of 51 mm Hg (normal 100 ± 40 mm Hg). The average reflux score was 103 ± 68 (normal <15[6]).

Between October 1992 and June 1993, the operation in seven patients involved a 360-degree Nissen-Rossetti fundoplication with the short gastric vessels left intact. Subsequently the short gastric vessels were divided in all patients and the type of wrap was selected according to the strength of esophageal peristalsis.[7] A total (360-degree Nissen) fundoplication was performed in 12 patients with normal esophageal peristalsis, and a partial (240-degree Guarner) fundoplication was performed in 19 patients whose maximal peristaltic amplitude was less than 50 mm Hg. With the exception of the extent of the wrap, these last two procedures were technically similar and included reduction of the hiatal hernia, division of the short gastric vessels, narrowing of the esophageal hiatus using interrupted sutures placed behind the esophagus, and fixation of the wrap to the esophagus and the crus.[8] There was no case in which the gastroesophageal junction could not be reduced below the diaphragm (i.e., "short esophagus").

Esophagectomy

Eleven men with high-grade dysplasia underwent transhiatal esophagectomy. Their mean age was 66 years (range 47 to 76 years). The average duration of symptoms was 180 months (range 48 to 480 months, median 150 months). Eight patients complained of heartburn (score 3.1 ± 0.2) and six patients of regurgitation (score 2.3 ± 0.7). Of the five patients in this group who experienced dysphagia (score 2.9 ± 0.8), only one had a mechanical cause (i.e., a benign esophageal stricture shown on barium swallow). In the other four patients, dysphagia was thought probably to be secondary to abnormalities of esophageal peristalsis.

Statistical Analysis

Student's *t* test, Mann-Whitney U test, analysis of variance, Student-Newman-Keuls test, and Wilcoxon signed rank-sum test were used for statistical evaluation of the data. All of the results are expressed as mean ± standard deviation of the mean. Differences were considered significant at $P < 0.05$.

RESULTS

Incidence of Barrett's Metaplasia Among Patients With GERD

Barrett's metaplasia was identified in 72 (13%) of 535 consecutive patients with GERD. These 72 patients had been symptomatic for an average of 135 months (range 1 to 480 months, median 144 months). At the time of presentation, 60 (83%) of the 72 patients were taking omeprazole (20 mg/day, 40 patients; 40 mg/day, 20 patients) and 30 patients were taking cisapride in addition to omeprazole. Twelve patients (17%) were taking H_2-blocking agents (5 patients were also taking cisapride).

Table I shows the incidence and severity of symptoms in these 72 patients, and compares them with 138 patients with GERD but no visible esophagitis, and 325 patients with GERD and grade I to III esophagitis. There was no difference in the severity of heartburn, regurgitation, and dysphagia among the three groups of patients. Cough was more severe in patients with Barrett's esophagus than in patients with

Table I. Incidence (% of patients) and severity of symptoms (score 0 to 4) among 535 patients with gastroesophageal reflux disease

	Group I No esophagitis (n = 138)	Group II Grade I to III esophagitis (n = 325)	Group III Barrett's esophagus (n = 72)
Heartburn	3.0 ± 0.1 (81%)	3.1 ± 0.8 (86%)	3.2 ± 0.9 (89%)
Regurgitation	2.5 ± 1.0 (72%)	2.6 ± 1.1 (76%)	2.7 ± 1.2 (78%)
Cough*	1.8 ± 0.7 (27%)	2.3 ± 1.0 (27%)	2.5 ± 1.3 (32%)
Dysphagia	2.6 ± 1.0 (37%)	2.5 ± 1.0 (39%)	2.7 ± 1.0 (30%)

There was no difference in the severity of heartburn, regurgitation, and dysphagia among the three groups of patients.
*Cough was more severe in patients with Barrett's esophagus than in patients with GERD but no esophagitis. No difference was found in the incidence of symptoms among the three groups of patients.

Table II. Esophageal manometry and pH monitoring among 535 patients with gastroesophageal reflux disease

	Group I No esophagitis (n = 138)	Group II Grade I to III esophagitis (n = 325)	Group III Barrett's esophagus (n = 72)
LES pressure (mm Hg)	11 ± 6	9 ± 5	8 ± 4
LES length (cm)	2.5 ± 1	2.3 ± 1	1.9 ± 1
DEA (mm Hg)	75 ± 34	67 ± 38	55 ± 33
% Time pH <4	9 ± 5	19 ± 23	25 ± 20
GER episodes >5 min	4 ± 4	8 ± 7	11 ± 9
Reflux score (normal <15)	35 ± 16	68 ± 53	89 ± 64

LES = lower esophageal sphincter; DEA = distal esophageal amplitude; GER = gastroesophageal reflux.
A statistically significant difference was found between groups I and II, groups I and III, and groups II and III for all the above-listed parameters with one exception—there was no difference in LES pressure between group II and group III.

Table III. Severity of symptoms in 38 patients with Barrett's esophagus before and after laparoscopic fundoplication (score 0-4)

	Preoperatively	Postoperatively	*P* value
Heartburn	3.5 ± 0.5	0.2 ± 0.7	<0.0001
Regurgitation	2.9 ± 0.9	0.1 ± 0.7	<0.0001
Cough	2.7 ± 1.1	0	<0.0001
Dysphagia	2.7 ± 0.9	0.3 ± 0.7	<0.0001

Heartburn resolved in 95% of patients, regurgitation and dysphagia in 93% of patients, and cough in 100% of patients.

GERD and no endoscopic signs of esophagitis. There was no correlation between the severity of symptoms and the degree of mucosal injury.

A radiographic finding of hiatal hernia was present in 39% of patients with GERD and no esophagitis, 64% of patients with grade I to III esophagitis, and 80% of patients with Barrett's metaplasia ($P < 0.005$).

Table II shows the results of esophageal manometry and pH monitoring in these three groups of patients. Esophageal function (LES pressure and amplitude of peristalsis) deteriorated, esophageal acid exposure increased (% time pH <4 and reflux score), and esophageal acid clearance (number of reflux episodes longer than 5 minutes) worsened with increasingly severe mucosal injury.

Laparoscopic Fundoplication

All operations were completed laparoscopically. Average duration of the operation was 156 minutes. The only technical complications were pneumothorax in two patients (one required chest tube drainage). The patients were consuming an unrestricted diet after an average of 21 hours and left the hospital after an average of 35 hours. Average duration of follow-up was 23 ± 14 months. Table III shows the severity of symptoms before and after laparoscopic fundoplication.

Esophageal manometry, which was repeated in 15 patients 2 months after the operation, showed an increase in LES pressure (6 ± 3 mm Hg preoperatively to 14 ± 7 mm Hg postoperatively; $P < 0.0001$) and LES length (1.7 ± 0.7 cm preoperatively to 3.5 ± 0.9 cm postoperatively; $P < 0.0001$). The peristaltic amplitude in the distal esophagus did not change (50 ± 35 mm Hg preoperatively, 54 ± 25 mm Hg postoperatively; P = NS).

Three patients had persistent gastroesophageal reflux; each of them had a herniated fundoplication diagnosed by barium swallow. One patient underwent a second operation with resolution of the abnormal reflux, whereas the other two patients will probably undergo a second operation within the next 6 months.

Postoperative surveillance endoscopy was performed at intervals of 12 to 18 months in 21 (55%) of the 38 patients. Four-quadrant biopsies were taken at 1 to 2 cm intervals throughout the length of Barrett's mucosa. In none of these patients did the Barrett's disease regress, progress to high-grade dysplasia, or change in length. No endoscopic follow-up has been performed in seven patients (18%), even though that had been intended. Nine patients (24%) were lost to follow-up. One patient died 1 year after the operation of causes unrelated to GERD or the operation.

We were able to obtain follow-up information (mean 46 months; range 18 to 115 months) on 20

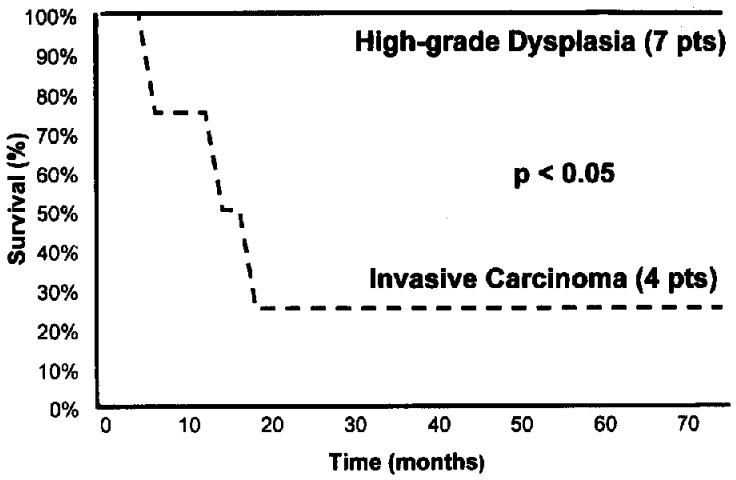

Fig. 1. Actuarial postoperative survival of seven patients with high-grade dysplasia and four patients with invasive adenocarcinoma.

of the 34 patients who continued on medical therapy after the diagnosis of GERD and Barrett's metaplasia had been established at our institution. Five of these patients have been treated at UCSF, whereas the remaining 15 patients have been treated elsewhere. Eleven of these 20 patients ultimately had a fundoplication performed at another hospital. Five patients have undergone surveillance endoscopy, and in each of them there was no change in the metaplasia. Six patients have had no postoperative endoscopic surveillance.

Eight patients have remained on medical therapy. Three of them have undergone endoscopy, and the Barrett's disease is unchanged. Five patients have had no follow-up endoscopy.

In one patient metaplasia was first detected in 1990. He was treated with proton pump inhibitors, and endoscopy was repeated annually. High-grade dysplasia was discovered 66 months after the initial diagnosis, and a transhiatal esophagectomy was performed. No invasive carcinoma was found.

Transhiatal Esophagectomy

The average duration of the operation was 339 ± 89 minutes (median 345 minutes), and the average blood loss was 575 ± 295 ml (median 600 ml). The patients required mechanical ventilation for approximately 2 days and remained in the intensive care unit for about 4 days. Average hospital stay was 14 ± 5 days (median 13 days). A leak of the cervical anastomosis developed in two patients, but each closed spontaneously without further incident. There were no postoperative deaths.

The esophagectomy specimen showed high-grade dysplasia in the Barrett's mucosa in seven patients and invasive adenocarcinoma in four patients (36%). The pathologic staging in these four patients was as follows: T1N0M0, T2N0M0, T1N1M0, and T2N1M0. The seven patients with high-grade dysplasia and the one patient with the T1N0M0 tumor are all alive without evidence of recurrent disease after an average of 40 months (range 11 to 73 months). The remaining three patients with invasive adenocarcinoma died 5, 14, and 16 months postoperatively from recurrent tumor (Fig. 1).

DISCUSSION

These findings show the following: (1) 13% of patients with GERD seen in a busy diagnostic center had Barrett's metaplasia; (2) laparoscopic fundoplication was highly successful in controlling symptoms in patients with Barrett's metaplasia; (3) in patients with high-grade dysplasia, esophagectomy was performed safely, and invasive cancer had eluded the preoperative biopsies in one third of such patients; and (4) despite a consensus that patients with Barrett's metaplasia should be followed endoscopically, this was actually done in fewer than two thirds of patients.

Incidence of Barrett's Metaplasia in Patients With GERD

Endoscopy showed columnar metaplasia in 72 (13%) of 535 patients in whom the diagnosis of GERD was confirmed by pH monitoring. Symptoms alone did not distinguish these patients from other GERD patients with less severe esophagitis (see Table I). However, esophageal manometry showed that an increasing degree of mucosal injury was accompanied by decreasing pressure and length of the LES, and by

decreased amplitude of esophageal peristalsis (see Table II). In addition, hiatal hernia was more common among patients with Barrett's metaplasia. These results parallel the accepted idea that Barrett's metaplasia constitutes an advanced stage of GERD[9,10] in which acid reflux is greater and esophageal acid clearance is slower than in GERD with less severe esophagitis.

Laparoscopic Fundoplication

Laparoscopic fundoplication dramatically improved symptoms (see Table III). Heartburn resolved in 95% of patients, regurgitation and dysphagia in 93% of patients, and cough in 100% of patients. The type of wrap was based on the preoperative esophageal function tests.[7,8,10] A 240-degree wrap was used in the patients whose esophageal peristalsis was weak. Although none of these patients developed de novo dysphagia postoperatively, dysphagia occurred in one patient (3%) after a Nissen-Rossetti fundoplication.

Several reports have shown that open antireflux operations can control symptoms in patients with Barrett's disease.[11-13] Our data indicate that the same is true of laparoscopic surgery, but with less morbidity, shorter hospitalization, and faster return to work.[7,8,14,15] Longer follow-up, however, is needed before concluding that the long-term results of laparoscopic and open fundoplication are equivalent.

At present, there is no consensus about the best treatment for Barrett's disease. The most common strategy is to give proton pump inhibitors, reserving surgery for patients whose symptoms are imperfectly controlled. This approach is questionable because elimination of symptoms, the goal of medical therapy, does not guarantee control of acid reflux in Barrett's disease. For instance, Katzka and Castell[16] performed 24-hour esophageal pH monitoring in five patients with Barrett's esophagus rendered asymptomatic by omeprazole and found that four (80%) still had abnormal acid exposure. Second, the damaging effects of gastroesophageal reflux may involve refluxed duodenal juice, not just acid. Using a fiberoptic probe to measure intraluminal bilirubin (a marker for duodenal juice), Kauer et al.[2] showed that 58% of patients with GERD had increased reflux of gastric and duodenal juice, and mucosal injury was greater with the mixture than with acid alone. In addition, bilirubin exposure was greater in patients with Barrett's metaplasia than in GERD patients without Barrett's metaplasia. Since most of the bilirubin reflux was seen when the pH in the esophagus was between 4 and 7, these reflux episodes would have been missed by standard pH monitoring.[2]

The concern is that medical therapy only decreases acid reflux while allowing reflux of duodenal contents to persist. A fundoplication, however, stops all reflux. If duodenal juice really does contribute to mucosal injury, surgery should be more effective than medical therapy in preventing the development of Barrett's metaplasia and its progression to dysplasia and cancer. In support of this concept, one randomized study found that both medical and surgical treatment of Barrett's disease controlled symptoms, but only surgery prevented progression to dysplasia.[17] Similar results were reported by McCallum et al.[18]

As reflux can persist even in asymptomatic patients, we would prefer to perform postoperative pH monitoring as a routine, but it is difficult to persuade asymptomatic patients to undergo the test. Reflux was identified in three of our patients, two of whom still complained of heartburn. One was asymptomatic.

Only 30 (62%) of 48 patients with Barrett's disease who were available had periodic follow-up endoscopic examinations. This was surprising, for all experts recommend surveillance endoscopic inspections and biopsies. In fact, periodic endoscopy is a cornerstone of medical management. Since there is no proof that fundoplication will block the progression to dysplasia, endoscopic surveillance is considered to be indicated.[19-21] Only 21 (75%) of the 28 patients followed up after laparoscopic fundoplication had any postoperative endoscopy, and only 9 (45%) of the 20 patients maintained on medical therapy or referred for surgery elsewhere were ever examined again by endoscopy.

Transhiatal Esophagectomy

We consider high-grade dysplasia an indication for esophagectomy, because occult invasive adenocarcinoma is often found after thorough inspection of the specimen.[20-24] Four (36%) of the 11 patients in whom we performed esophagectomy had invasive adenocarcinoma. Tumor had already spread to lymph nodes in two, both of whom died of recurrent cancer. Lymph node status is the major determinant of survival after surgery in this disease, and better survival rates can only be achieved if the operation is performed before the lymph nodes become involved.[25]

CONCLUSION

It is reasonable to consider Barrett's esophagus to be a surgical disease at any stage, from metaplasia to high-grade dysplasia. Laparoscopic antireflux surgery controls symptoms and may prevent progression to dysplasia. The data in this study, which includes patients being followed by many physicians throughout northern California, show that regular endo-

scopic monitoring of patients with Barrett's esophagus was actually performed in fewer than two thirds of patients. The logistical difficulties in implementing a more complete program appear to add another reason in favor of more liberal use of surgery for this disease.

REFERENCES

1. Sarr MG, Hamilton SR, Marrone GC, Cameron JL. Barrett's esophagus: Its prevalence and association with adenocarcinoma in patients with symptoms of gastroesophageal reflux. Am J Surg 1985;149:187-193.
2. Kauer WKH, Peters JH, DeMeester TR, Ireland AP, Bremner CG, Hagen JA. Mixed reflux of gastric and duodenal juices is more harmful to the esophagus than gastric juice alone. The need for surgical therapy re-emphasized. Ann Surg 1995;222: 525-544.
3. Spechler SJ, Goyal RK. Barrett's esophagus. N Engl J Med 1986;315:362-371.
4. Haggitt RC. Barrett's esophagus, dysplasia, and adenocarcinoma. Hum Pathol 1994;25:982-993.
5. Savary M, Miller G. The Esophagus: Handbook and Atlas of Endoscopy. Solothurn, Switzerland: Gassman AG, 1978, p 135.
6. Jamieson JR, Stein HJ, DeMeester TR, Bonavina L, Schwizer W, Hinder RA, Albertucci M. Ambulatory 24-h esophageal pH monitoring: Normal values, optimal thresholds, specificity, sensitivity, and reproducibility. Am J Gastroenterol 1992;87: 1102-1111.
7. Patti MG, De Pinto M, de Bellis M, Arcerito M, Tong J, Wang A, Mulvihill SJ, Way LW. Comparison of laparoscopic total and partial fundoplication for gastroesophageal reflux. J GASTROINTEST SURG 1997;1:309-315.
8. Patti MG, Arcerito M, Feo CV, De Pinto M, Tong J, Gantert W, Tyrrell D, Way LW. An analysis of operations for GERD: Identifying the important technical elements. Arch Surg 1998;133:600-607.
9. Stein HJ, Hoeft S, DeMeester TR. Functional foregut abnormalities in Barrett's esophagus, J Thorac Cardiovasc Surg 1993;105:107-111.
10. Stein JH. Eypasch EP, DeMeester TR, Smyrk TC, Attwood SEA. Circadian esophageal motor function in patients with gastroesophageal reflux disease. Surgery 1990;108:769-778.
11. DeMeester TR, Attwood SEA, Smyrk TC, Therkildsen DH, Hinder RA. Surgical therapy in Barrett's esophagus. Ann Surg 1990;212:528-542.
12. Williamson WA, Ellis FH Jr, Gibb SP, Shahian DM, Aretz HT. Effect of antireflux operation on Barrett's mucosa. Ann Thorac Surg 1990;49:537-542.
13. Attwood SEA, Barlow AP, Norris TL, Watson A. Barrett's oesophagus: Effect of antireflux surgery on symptom control and development of complications Br J Surg 1992;79:1050-1053.
14. Wu JS, Dunnegan DL, Luttmann DR, Soper NJ. The influence of surgical technique on clinical outcome of laparoscopic Nissen fundoplication. Surg Endosc 1996;10:1164-1170.
15. Hunter JG, Trus TL, Branum GD, Waring JP, Wood WC. A physiologic approach to laparoscopic fundoplication for gastroesophageal reflux disease. Ann Surg 1996;223:673-687.
16. Katzka DA, Castell DO. Successful elimination of reflux symptoms does not insure adequate control of acid reflux in patients with Barrett's esophagus. Am J Gastroenterol 1994; 89:989-991.
17. Ortiz A, Martinez de Haro LF, Parrilla P, Morales G, Molina J, Bermejo J, Liron R, Aguilar J. Conservative treatment versus antireflux surgery in Barrett's oesophagus: Long-term results of a prospective study. Br J Surg 1996;83:274-278.
18. McCallum RW, Polepalle S, Davenport K, Frierson H, Boyd S. Role of antireflux surgery against dysplasia in Barrett's esophagus. Gastroenterology 1991;100:A121.
19. Streitz JM Jr, Andrews CW Jr, Ellis FH Jr. Endoscopic surveillance of Barrett's esophagus. Does is help? J Thorac Cardiovasc Surg 1993;105:383-388.
20. Peters JH, Clark GWB, Ireland AP, Chandrasoma P, Smyrk TC, DeMeester TR. Outcome of adenocarcinoma arising in Barrett's esophagus in endoscopically surveyed and nonsurveyed patients. J Thorac Cardiovasc Surg 1994;108:813-822.
21. McDonald ML, Trastek VF, Allen MS, Deschamps C, Pairolero PC. Barrett's esophagus: Does an antireflux procedure reduce the need for endoscopic surveillance? J Thorac Cardiovasc Surg 1996;111:1135-1140.
22. Altorki NK, Sunagawa M, Little AG, Skinner DB. High-grade dysplasia in the columnar-lined esophagus. Am J Surg 1991;161:97-100.
23. Edwards MJ, Gable DR, Lentsch AB, Richardson JD. The rationale for esophagectomy as the optimal therapy for Barrett's esophagus with high-grade dysplasia. Ann Surg 1996;223:585-591.
24. Heitmiller RF, Redmond M, Hamilton SR. Barrett's esophagus with high-grade dysplasia. An indication for prophylactic esophagectomy. Ann Surg 1996;224:66-71.
25. Lerut T, Coosemans W, Van Raemdonck D, Dillemans B, De Leyn P, Marnette JM, Geboes K. Surgical treatment of Barrett's carcinoma. Correlations between morphologic findings and prognosis. J Thorac Cardiovasc Surg 1994;107:1059-1066.

Discussion

Dr. D. Rattner (Boston, Mass.). Your paper implies that Barrett's esophagus is a surgical disease. Does that mean that the patient with well-controlled symptoms on medical therapy who has no evidence of high-grade dysplasia should be operated on as a routine and, if so, do you have any evidence that fundoplication prevents progression of Barrett's esophagus any better than medical therapy does?

Dr. M. Patti. We consider Barrett's metaplasia an indication for surgery, even in patients who are asymptomatic on medical therapy, for two reasons. First, the data published by Katzka and Castell[16] showed that when pH monitoring is performed in patients who are asymptomatic on high doses of omeprazole, abnormal reflux is still present in 80% of them. Second, as shown by Kauer et al.,[2] 60% of patients with GERD have reflux of both acid and duodenal contents; omeprazole will control acid reflux only. Evidence that surgery is better than medical therapy for patients with metaplasia is supported by two prospective, randomized studies that have compared medical and surgical treatment for patients with Barrett's metaplasia. Ortiz et al.[17] reported progression from metaplasia to high-grade dysplasia in 3% of patients treated by fundoplication but in 22% of patients

who received medical therapy. These data confirm the findings of the earlier study by McCallum in 1991.[18]

Dr. J. O'Leary (New Orleans, La.). You had a group of patients in your study who were treated medically. Over the duration of the study, did those patients come to you with dysplasia or with Barrett's esophagus? Do you know how many of these patients were treated medically and how many progressed to get a surgical consultation?

Dr. Patti. Of the 34 patients who decided initially to undergo medical therapy, we have long-term follow-up data for 20. Eleven of those patients were still symptomatic and eventually decided to undergo surgical treatment. Only half of them underwent endoscopic surveillance, and neither regression of the metaplasia nor progression to dysplasia was found. In one patient who had the diagnosis of metaplasia established in 1990, high-grade dysplasia was diagnosed on follow-up in 1995. He subsequently underwent an esophagectomy.

Dr. T. Gadacz (Augusta, Ga.). In those patients who had the Nissen procedure, did you see any progression of the metaplasia to dysplasia and what is the length of your follow-up?

Dr. Patti. The average length of follow-up is about 2 years. We saw neither regression of the metaplasia nor progression to dysplasia in our patients.

Dr. D. Castell (Philadelphia, Pa.). You appropriately identified the fact that when we looked at patients with asymptomatic Barrett's disease who were on proton pump inhibitor therapy, there still could be some reflux, particularly overnight. It makes the point that when these patients are treated by fundoplication, some postoperative pH studies must be carried out. I am not sure that you have done that, but I think that is an important observation because these people are not as sensitive. The other point I want to make is that it is time to stop talking about the McCallum abstract; the article will never be published in its entirety because the data collected were from a hodgepodge of patients, and I do not think the findings are scientifically valid.

Dr. Patti. I agree entirely with the importance of postoperative pH monitoring to identify patients who might still have abnormal reflux even if they are asymptomatic. We studied only 15 of the 38 patients we operated on because it is really difficult to bring back asymptomatic patients. We found a herniated wrap in three patients and one of them was asymptomatic, but pH studies showed a lot of reflux. Barium swallow showed a herniated wrap, and eventually the patient underwent a second operation with resolution of the symptoms and correction of the abnormal reflux.

Role of the Lower Esophageal Sphincter and Hiatal Hernia in the Pathogenesis of Gastroesophageal Reflux Disease

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The relative importance of the lower esophageal sphincter (LES) and hiatal hernia in the pathogenesis of gastroesophageal reflux disease is controversial. To identify the role of hiatal hernia and LES in reflux disease, 375 consecutive patients with foregut symptoms and no previous foregut surgery were evaluated. All patients underwent upper endoscopy, stationary manometry, and 24-hour esophageal pH monitoring. Hiatal hernia was diagnosed endoscopically, when the distance between the crural impression and the gastroesophageal junction was ≥2 cm. The LES was considered structurally defective when the resting pressure was ≤6 mm Hg, the overall length was less than 2 cm, and/or the abdominal length was less than 1 cm. Factors predicting abnormal esophageal acid exposure (composite score >14.7) were analyzed using multivariate analysis. The presence of a hiatal hernia and a defective LES were identified as independent predictors of abnormal esophageal acid exposure. LES pressure and abdominal length were reduced in patients with hiatal hernia by 4 mm Hg and 0.4 cm, irrespective of the presence of gastroesophageal reflux disease. It is concluded that both a structurally defective LES and hiatal hernia are important factors in the pathogenesis of reflux disease. It is hypothesized that in the presence of a structurally normal LES, the altered geometry of the cardia imposed by a hiatal hernia facilitates the ability of gastric wall tension to pull open the sphincter. (J GASTROINTEST SURG 1999;3:405-410.)

KEY WORDS: Hiatal hernia, lower esophageal sphincter, gastroesophageal reflux disease, predictor

In 1951 Allison[1] emphasized the association between esophagitis and a hiatal hernia, and hiatal hernia became synonymous with gastroesophageal reflux disease (GERD). In 1956 esophageal manometry was introduced by Fyke et al.[2] and attention shifted to the lower esophageal sphincter (LES). Soon thereafter, investigators related sphincter function to the presence of GERD and its complications.[3-8] In 1979 Liebermann-Meffert et al.[9] performed a detailed anatomic dissection of the cardia and described a zone of thickened muscle that represented the muscular correlate to the LES. It subsequently became evident that in patients with hiatal hernia, the altered geometry at the cardia could potentially affect LES function. This has stimulated a renewed interest in the interaction between LES resistance to reflux and the altered geometry of the cardia imposed by a hiatal hernia.[10,11] The present study investigates this relationship in light of recent concepts[12] regarding the pathogenesis of GERD.

PATIENTS AND METHODS

Study Population

The study population consisted of 375 consecutive patients with foregut symptoms and no previous esophageal or gastric surgery. There were 230 men and 145 women who had a median age of 55 years (range 14 to 86 years). All patients were studied by upper endoscopy, stationary esophageal manometry, and 24-hour esophageal pH monitoring.

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Endoscopy

Upper gastrointestinal endoscopy was performed in all patients. Three landmarks were recorded: the position of the crural impression, the gastroesophageal junction, and the squamocolumnar junction. The gastroesophageal junction was defined as the level where the gastric rugal folds end and the tubular esophagus begins. A hiatal hernia was diagnosed when there was a difference of 2 cm or more between the position of the crural impression and the gastroesophageal junction.

Manometry

Stationary manometry was performed with a water-perfused catheter. The pressure profile of the LES was measured by the stationary pull-through technique. The resting sphincter pressure was measured at midinspiration at the respiratory inversion point. A structurally defective LES was identified by the presence of one or more of the following: a resting sphincter pressure less than or equal to 6 mm Hg, an overall length of less than 2 cm, and/or an abdominal length of less than 1 cm.[6]

Esophageal body function was assessed by placing the most proximal pressure port of the motility catheter 1 cm below the lower border of the upper esophageal sphincter, with the other four pressure ports trailing at 5 cm intervals. Wave progression and contraction amplitude (in mm Hg) were evaluated after each of ten 5 ml wet swallows. Contraction amplitudes in the distal esophageal segment were evaluated using the lowest pressure port in the esophagus that was located at least 3 cm above the upper border of the LES, and the mean value for 10 swallows was recorded.

Twenty-Four–Hour Esophageal pH Monitoring

A glass pH probe (Ingold, Urdorf, Switzerland) was positioned 5 cm above the upper border of the LES, and standard 24-hour pH monitoring was performed.[13,14] Abnormal esophageal acid exposure was identified when the 24-hour pH score exceeded 14.7 (95th percentile of healthy volunteers.[13])

Data Analysis

To investigate the relationship between LES and hiatal hernia in the pathogenesis of GERD, abnormal esophageal acid exposure was used as the most reliable marker for the presence of the disease. Predictors of abnormal esophageal acid exposure were identified using univariate and multivariate analysis of several variables, namely, age, sex, presence of a hiatal hernia, size of the hiatal hernia, contraction amplitudes in the distal esophagus, a structurally defective LES, and its components, which include resting pressure, overall length, and abdominal length. The effect of a hiatal hernia on the components of the LES and the esophageal contraction amplitudes were also assessed in patients with normal and abnormal esophageal acid exposure.

Statistics

Data are reported as mean ± standard deviation unless otherwise stated. Fisher's exact test was used to compare proportions between the two groups. The Mann-Whitney U test was used to compare continuous data. Multivariate analysis was done using logistic regression with backward elimination of variables. A P value <0.05 was considered significant.

RESULTS

Relative Importance of Hiatal Hernia and Lower Esophageal Sphincter in the Pathogenesis of Gastroesophageal Reflux Disease

Table I shows the results of the univariate analysis of the variables that were compared between patients with normal and abnormal esophageal acid exposure. All variables except age were significantly different between the two groups. Fig. 1 shows the extent to which a structurally defective LES or a hiatal hernia was related to a higher prevalence of abnormal esophageal acid exposure.

To assess the relative importance of the variables identified in the univariate analysis, a multivariate analysis was done. For the purpose of the analysis, only the status of the LES was used. If all components, that is, pressure, overall length, and abdominal length, were normal, the LES was considered structurally normal. If one or more of the components were abnormal, the LES was considered structurally defective. The multivariate analysis showed that a hiatal hernia and the presence of a structurally defective LES were identified as the main predictors of abnormal esophageal acid exposure (Table II). Hiatal hernia size and contraction amplitudes in the distal esophagus were not identified as independent predictors of abnormal esophageal acid exposure.

Fig. 2 shows the effect of a hiatal hernia, or altered geometry of the cardia, on a structurally normal or defective LES. The prevalence of abnormal esophageal acid exposure was highest when both a structurally defective LES and hiatal hernia were present and lowest when both were absent. Of particular note, the presence of a hiatal hernia altered the effective-

Table I. Predictors of abnormal esophageal acid exposure: Univariate analysis (N = 375)

	Esophageal acid exposure		
	Normal (n = 154)	Abnormal (n = 221)	*P* value
Age (yr)	55.8 ± 15.4	55.6 ± 14.8	NS
Male sex (%)	49.4	69.7	<0.0001
LES pressure (mm Hg)	14.2 ± 9.5	7.7 ± 6.2	<0.0001
LES overall length (cm)	2.9 ± 1.0	2.4 ± 1.2	<0.0001
LES abdominal length (cm)	1.5 ± 0.9	0.9 ± 0.7	<0.0001
Presence of defective LES (%)	37	71	<0.0001
Contraction amplitude (mm Hg)	90.5 ± 51.9	71.2 ± 47.0	<0.0001
Presence of hiatal hernia (%)	39	75.6	<0.0001
Hiatal hernia size (cm)	1.5 ± 1.9	2.9 ± 1.9	<0.0001

LES = lower esophogeal sphincter.

Table II. Predictors of abnormal esophageal acid exposure: Multivariate analysis (N = 375)

	Odds ratio (95% CI)	*P* value
Presence of hiatal hernia	3.8 (2.4-6.0)	<0.0001
Presence of a structurally defective LES	3.3 (2.1-5.2)	<0.0001
Male sex	1.9 (1.2-3.1)	<0.01

CI = confidence interval.

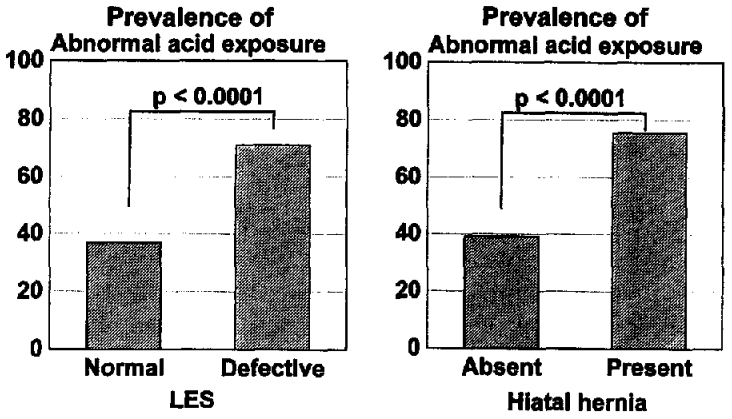

Fig. 1. Influence of a structurally defective lower esophageal sphincter or the presence of hiatal hernia on the prevalence of abnormal esophageal acid exposure on 24-hour pH monitoring.

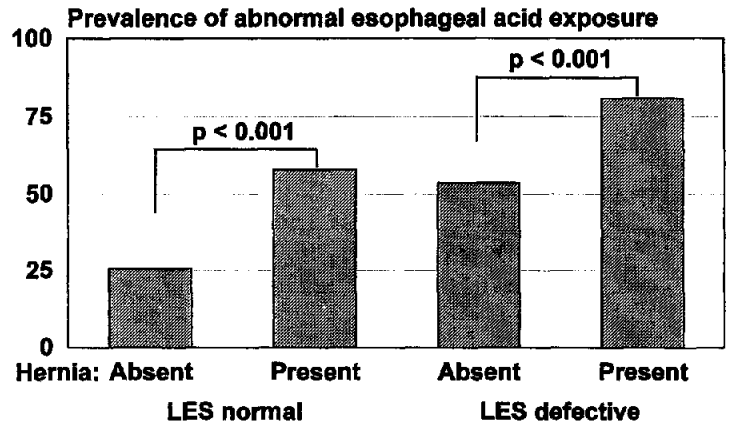

Fig. 2. Effect of a hiatal hernia on esophageal acid exposure in patients with a structurally normal or defective lower esophageal sphincter.

Table III. Effects of hiatal hernia on structural components of the lower esophageal sphincter (LES) in patients with normal esophageal acid exposure (N = 154)

	No hernia (n = 94)	Hernia (n = 60)	Δ	*P* value
LES pressure (mm Hg)	15.8 ± 9.9	11.7 ± 8.3	4.1	<0.005
LES overall length (cm)	3.0 ± 0.9	2.8 ± 1.3	0.2	NS
LES abdominal length (cm)	1.7 ± 0.7	1.3 ± 1.0	0.4	<0.001

Table IV. Effects of hiatal hernia on structural components of the lower esophageal sphincter (LES) in patients with abnormal esophageal acid exposure (N = 221)

	No hernia (n = 54)	Hernia (n = 167)	Δ	*P* value
LES pressure (mm Hg)	10.3 ± 7.4	6.9 ± 5.5	3.4	<0.001
LES overall length (cm)	2.6 ± 0.9	2.3 ± 1.3	0.3	<0.05
LES abdominal length (cm)	1.2 ± 0.7	0.8 ± 0.7	0.4	<0.001

Table V. Effects of hiatal hernia on esophageal contraction amplitudes (N = 375)

	Contraction amplitudes (mm Hg)		
	No hernia	Hernia	*P* value
Normal acid exposure (n = 154)	94.6 ± 57.1	84.2 ± 42.1	NS
Abnormal acid exposure (n = 221)	90.4 ± 59.8	64.9 ± 40.2	<0.005

ness of a structurally normal sphincter to function as a barrier and contributed to the inefficiency of a structurally defective sphincter.

Effects of Hiatal Hernia on the Structural Components of the Lower Esophageal Sphincter

The effect of a hiatal hernia on the structural components of the LES was analyzed in patients with normal and abnormal esophageal acid exposure (Tables III and IV). Hernia size did not differ between patients with normal and abnormal esophageal acid exposure. In patients with normal esophageal acid exposure, the abdominal length of the sphincter decreased by 4 mm and LES pressure was reduced by 4.1 mm Hg by the presence of a hiatal hernia. There was no difference in overall sphincter length indicating that the respiratory inversion point was moving caudad in the LES. In patients with abnormal esophageal acid exposure, the presence of a hernia was associated with a decrease of the abdominal length by 4 mm, the overall length by 3 mm, and the pressure by 3.4 mm Hg. Of interest, the presence of hiatal hernia was associated with the same reduction in LES abdominal length and pressure in patients with and without disease, that is, normal or abnormal esophageal acid exposure.

Effects of Hiatal Hernia on Esophageal Contraction Amplitude

Table V shows the effects of hiatal hernia on contraction amplitudes in the distal esophagus. In patients with abnormal esophageal acid exposure, the presence of hiatal hernia was associated with a significant reduction of the contraction amplitudes in the distal esophagus, whereas there was no significant effect of hiatal hernia on the contraction amplitudes in patients with normal acid exposure.

DISCUSSION

Multivariate analysis of patients with foregut symptoms identified both the altered geometry of the cardia resulting from a hiatal hernia and a structurally defective LES as important factors associated with GERD. Each factor increased independently the prevalence of abnormal esophageal acid exposure and when both were present the effect was additive. Consequently the effect of a hiatal hernia on the LES is similar irrespective of the presence of increased esophageal acid exposure. The difference is that if the LES was already deteriorated, the presence of a hiatal hernia added an additional insult.

In patients with normal acid exposure, the altered geometry of the cardia resulting from a hiatal hernia

was associated with a shortening of the LES abdominal length by 4 mm and a drop in pressure by 4 mm Hg. The abdominal length of the LES is defined as the distance between the respiratory inversion point and the lower border of the LES. Occasionally, in patients with a large hiatal hernia, the respiratory inversion point can be located below the LES. For this to occur the hernia sac is compartmentalized from the abdominal cavity by a narrow hiatus. This results in the absence of any measurable abdominal length to the sphincter. If there is free communication between the hernia sac and the abdominal cavity, then abdominal pressure can be transmitted throughout the hernia sac[10] and the respiratory inversion point will be located within the LES, even though the LES appears to be within the chest. As a consequence, a measurable abdominal length is commonly observed in patients with hiatal hernia.

The principal mechanism allowing gastroesophageal reflux to occur is the loss of LES resistance, either transiently or permanently. The transient loss of resistance of a normal LES is caused by gastric distension.[15] When the stomach is distended, gastric wall tension vectors pull the bottom of the LES open and reduce its length. This progresses up the sphincter with greater gastric distension until a critical length is reached, usually between 1 and 2 cm, when the sphincter opens, its pressure drops, and reflux occurs. This mechanism is responsible for physiologic postprandial reflux and can be augmented by the ingestion of large volumes of food. This mechanism is likely to cause increased esophageal acid exposure in those 25% of patients with no hiatal hernia and a structurally normal sphincter. Permanent loss of resistance occurs when the components of the LES are defective, that is, a low resting pressure, a short abdominal length, and/or a short overall length. As a result, reflux occurs throughout the day when the body is in the upright or supine position as well as during the postprandial period.[16]

When a hiatal hernia occurs, the altered geometry of the cardia allows the gastric wall tension to act more adversely on the LES. Ismail et al.[17] measured the intragastric pressure at which the cardia endoscopically opens in response to gastric distension with air. There was a close relationship between the intragastric pressure required to open the sphincter and the morphology of the cardia, that is, a higher pressure was necessary in patients with an intact angle of His compared to patients with a hiatal hernia. It was concluded that the ease at which the sphincter can be opened with gastric distension is greatly influenced by the anatomy of the cardia.

An important fact is that antireflux surgery can correct the abnormalities of a structurally defective sphincter and repair the hernia. Further, a Nissen fundoplication prevents the transient losses of LES length associated with gastric distension.[18] For these reasons, surgical therapy has been shown to be the most effective therapy for GERD.[19]

Hiatal hernia has also been implicated as a cause for reducing esophageal body contractility.[10] In the present study the contraction amplitudes in patients with normal esophageal acid exposure were not significantly reduced by the presence of a hiatal hernia, whereas this did occur in patients with abnormal esophageal acid exposure. This implies that esophageal mucosal injury rather than a hiatal hernia is more likely responsible for the reduction of esophageal contraction amplitudes in patients with GERD.

In conclusion, both the geometry of the cardia and the status of the LES are independent predictors of abnormal esophageal acid exposure and their combined effect is additive.

REFERENCES

1. Allison PR. Reflux esophagitis, sliding hiatal hernia, and the anatomy of repair. Surg Gynecol Obstet 1951;92:419-431.
2. Fyke FE, Code CF, Schlegel JF. The gastroesophageal sphincter in healthy human beings. Gastroenterologia (Basel) 1956;86:135-150.
3. Winans CS, Harris LD. Quantitation of lower esophageal sphincter competence. Gastroenterology 1967;52:773-778.
4. Cohen S, Harris LD. Does hiatus hernia affect competence of the gastroesophageal sphincter? N Engl J Med 1971;284: 1053-1056.
5. Dodds WJ, Dent J, Hogan WJ, Helm JF, Hauser R, Patel GK, Egide MS. Mechanisms of gastroesophageal reflux in patients with reflux esophagitis. N Engl J Med 1982;307:1547-1552.
6. Zaninotto G, DeMeester TR, Schwizer W, Johansson KE, Cheng SC. The lower esophageal sphincter in health and disease. Am J Surg 1988;155:104-111.
7. Stein HJ, Barlow AP, DeMeester TR, Hinder RA. Complications of gastroesophageal reflux disease: Role of the lower esophageal sphincter, esophageal acid and acid/alkaline exposure, and duodenogastric reflux. Ann Surg 1992;216:35-43.
8. Fuchs KH, Freys SM, Heimbucher J, Fein M, Thiede A. Pathophysiologic spectrum in patients with gastroesophageal reflux disease in a surgical GI-function laboratory. Dis Esophagus 1995;8:211-217.
9. Liebermann-Meffert D, Allgöwer M, Schmid P, Blum AL. Muscular equivalent of the lower esophageal sphincter. Gastroenterology 1979;76:31-38.
10. DeMeester TR, Lafontaine E, Joelsson BE, Skinner DB, Ryan JW, O'Sullivan GC, Brunsden BS, Johnson LF. Relationship of a hiatal hernia to the function of the body of the esophagus and the gastroesophageal junction. J Thorac Cardiovasc Surg 1981;82:547-558.
11. Sloan S, Rademaker AW, Kahrilas PJ. Determinants of gastroesophageal junction incompetence: Hiatal hernia, lower esophageal sphincter, or both? Ann Intern Med 1992;117: 977-982.
12. DeMeester TR, Ireland AP. Gastric pathology as an initiator and potentiator of gastroesophageal reflux disease. Dis Esophagus 1997;10:1-8.

13. Jamieson JR, Stein HJ, DeMeester TR, Bonavina L, Schwizer W, Hinder RA, Albertucci M. Ambulatory 24-h esophageal pH monitoring: Normal values, optimal thresholds, specificity, sensitivity, and reproducibility. Am J Gastroenterol 1992;87:1102-1111.
14. Kahrilas PJ, Quigley EMM. Clinical esophageal pH recording: A technical review for practice guideline development. Gastroenterology 1996;110:1982-1996.
15. Pettersson GB, Bombeck CT, Nyhus LM. The lower esophageal sphincter: Mechanisms of opening and closure. Surgery 1980;88:307-314.
16. Fein M, Hagen JA, Ritter MP, DeMeester TR, De Vos M, Bremner CG, Peters JH. Isolated upright gastroesophageal reflux is not a contraindication for antireflux surgery. Surgery 1997;122:829-835.
17. Ismail T, Bancewicz J, Barlow J. Yield pressure, anatomy of the cardia and gastro-oesophageal reflux. Br J Surg 1995;82: 943-947.
18. Mason RJ, Lund RJ, DeMeester TR, Peters JH, Crookes P, Ritter M, Gadenstatter M, Hagen JA. Nissen fundoplication prevents shortening of the sphincter during gastric distension. Arch Surg 1997;132:719-726.
19. Spechler SJ. Comparison of medical and surgical therapy for complicated gastroesophageal reflux disease in veterans. The Department of Veterans Affairs Gastroesophageal Reflux Disease Study Group. N Engl J Med 1992;326:786-792.

Thrombospondin-1 and Transforming Growth Factor Beta-1 Upregulate Plasminogen Activator Inhibitor Type 1 in Pancreatic Cancer

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Controlled degradation of the extracellular matrix by proteases is crucial in tumor cell invasion. We have shown that thrombospondin-1 (TSP-1), through activation of transforming growth factor beta-1 (TGF-β1), regulates the plasminogen/plasmin protease system in breast cancer. To determine whether this occurred in other epithelial neoplasms, we studied the role of TSP-1 and TGF-β1 in the regulation of the plasminogen/plasmin system in pancreatic cancer. ASPC-1 and COLO-357 pancreatic cancer cells were treated with TSP-1 or TGF-β1 at varying concentrations. The TSP-1 and TGF-β1–treated cells were also treated with either anti–TSP-1, anti–TSP-1 receptor, or anti–TGF-β1 antibodies. Urokinase plasminogen activator (uPA) and plasminogen activator inhibitor-1 (PAI-1) expression was determined by enzyme-linked immunosorbent assay. TSP-1 and TGF-β1 promoted a dose-dependent upregulation of ASPC-1 and COLO-357 PAI-1 expression. The TSP-1 effect could be blocked with anti–TSP-1 or anti–TGF-β1 antibodies. The TGF-β1 effect could be blocked only with anti–TGF-β1 antibody. Anti–TSP-1 receptor antibody blocked the TSP-1 effect on PAI-1 expression but had no effect on TGF-β1–mediated PAI-1 expression. Neither TSP-1 nor TGF-β1 had an effect on uPA production. We conclude that TSP-1, in a receptor-mediated process that involves the activation of TGF-β1, upregulates PAI-1 expression in pancreatic cancer without an effect on uPA production. (J GASTROINTEST SURG 1999;3:411-417.)

KEY WORDS: Thrombospondin-1, transforming growth factor beta-1, urokinase plasminogen activator, pancreatic neoplasms, plasminogen activator inhibitor

Tumor cell metastasis is a complex, multistep process that is believed to involve the controlled degradation of the tumor cell–associated extracellular matrix and basement membrane by proteases. The plasminogen/plasmin system is one of the main protease systems involved in malignancy.[1] Activation of plasminogen to plasmin on tumor cells is performed by the urokinase-type plasminogen activator (uPA).[2] By binding to a specific receptor on the cell surface, the proteolytic activity of uPA becomes more efficient and localized to the tumor cell surface. Localization of enzymes to the tumor cell surface increases pericellular proteolysis and enhances the invasive potential of the tumor cells.[3-6]

Although degradation of the extracellular matrix is necessary for tumor cell invasion, excessive matrix degradation has been shown to inhibit tumor cell migration and prevent invasion.[7-10] Plasminogen activator inhibitor-1 (PAI-1), the main inhibitor of uPA, has been shown to localize to the extracellular matrix in several malignancies in association with matrix proteins such as vitronectin.[11,12] PAI-1 has been shown to promote breast tumor cell invasion by preventing excessive matrix degradation by tumor-associated plasmin-mediated proteolytic activity.[9] Considerable evidence indicates that increased levels of tumor-associated PAI-1 are a strong, independent prognostic indicator of poor clinical outcome in cancer patients.[13,14]

A growing body of evidence points to the extracellular matrix as a key component in modulating plasminogen activation by providing molecules that reg-

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ulate cell growth and expression of proteases.[15,16] Thrombospondin-1 (TSP-1), an adhesive glycoprotein that localizes to the extracellular matrix in a variety of tumors,[17,18] regulates the plasminogen/plasmin system in lung and breast cancer.[8,9,19] TSP-1 has been implicated in cancer progression.[20] We have previously shown that TSP-1 promotes tumor cell invasion in vitro and the development of metastasis in animal models.[21] We have shown that patients with various malignancies have significantly elevated serum levels of TSP-1 compared with non–tumor-bearing human control subjects.[22] A major cell adhesive domain in the TSP-1 molecule is composed of the amino acid sequence cysteine-serine-valine-threonine-cysteine-glycine (CSVTCG).[23] This hexapeptide is now known to partly mediate TSP-1 adhesive interactions involved in cell-substratum adhesion and tumor cell metastasis.[9,21] Another growth factor present in the extracellular matrix, transforming growth factor beta-1 (TGF-β1), is an important regulator of the secretion of uPA in both benign and malignant cell lines.[24,25] It has been shown that TSP-1 can activate endogenously produced latent TGF-β1.[8,9,26-29] Specifically, we have provided evidence in support of the hypothesis that the upregulation of uPA and PAI-1 by TSP-1 in breast and lung cancer is mediated by activation of TGF-β1.[8,19] Furthermore, we have previously demonstrated that TSP-1 promotes breast cancer cell invasion through activation of endogenously produced TGF-β1.[9]

The present study was performed to determine the role of TSP-1 on the regulation of the two main regulators of the plasminogen/plasmin system, PAI-1 and uPA, in pancreatic cancer. Furthermore, based on our previous work in breast cancer, we also studied TGF-β1 as a possible mediator of TSP-1 in the regulation of the plasminogen/plasmin system.

METHODS

Materials

All reagents, unless otherwise specified, were purchased from Sigma Chemical Co. (St. Louis, Mo.). The human pancreatic adenocarcinoma cell lines ASPC-1 and COLO-357 were purchased from the American Type Culture Collection (HTB 26, Rockville, Md.). These cell lines were established from ascites fluid of patients with metastatic pancreatic cancer. These cells form invasive tumors in nude mice consistent with a pancreatic primary lesion. Tissue culture supplies were purchased from Fisher Scientific Co. (Pittsburgh, Pa.). All of the antibodies used in this study were immunoglobulin G. TGF-β1 was purchased from Collaborative Biomedical Products, (Chicago, Ill.).

Cell Culture

ASPC-1 or COLO-357 cells were cultured in Dulbecco's modified eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS), 300 μg/l L-glutamine, 100 units/ml of penicillin, 100 μg/ml of streptomycin, and 50 μg/ml of gentamicin sulfate. The cultures were maintained on plastic and incubated in 5% carbon dioxide/95% air at 37° C in a humidified incubator. Cell cultures were noted to be pathogen free. Tumor cells were harvested from subconfluent cultures (80% to 90% confluence) by short exposure to 0.02% ethylenediamine-tetraacetic acid (EDTA) solution and resuspended in serum-free DMEM before use.

TSP-1 Purification

TSP-1 was purified from Ca^{++} ionophore A23187–activated platelets as previously described.[30] Purified TSP-1 preparations contained a total of 60.06 pg of total TGF-β1 antigen and 2.85 pg of active TGF-β1 per microgram of purified TSP-1, as determined by a human TGF-β1 immunoassay (Quantikine, R&D Systems, Minneapolis, Minn.).

Antibodies

The TSP-1 antibody was a neutralizing affinity purified polyclonal antihuman TSP-1 goat IgG previously used in our laboratory.[31] Polyclonal antihuman CSVTCG-specific TSP-1 receptor antibody was raised in a rabbit from TSP-1 receptor isolated from human lung carcinoma as previously described.[23] The TSP-1 receptor was previously characterized as a binding protein specific for a CSVTCG hexapeptide sequence present in the type 1 repeat domain of TSP-1. The TGF-β1 antibody was a neutralizing monoclonal antihuman TGF-β1 turkey IgG (Collaborative Biomedical Products). With each antibody group, the respective control IgG was also evaluated for control purposes.

Quantitation of PAI-1 and Urokinase Plasminogen Activator

Approximately 50,000 cells were plated per well of a microtiter plate and allowed to attach and grow to 80% to 90% confluency on FCS-containing medium. The cells were weaned of the FCS-containing medium to a serum-free medium over 24 hours. The cells were then incubated for 48 hours at 37° C in 300 μl of serum-free DMEM with the addition of: (a) medium alone (control); (b) TSP-1 (10, 20, 40, or 50 μg/ml); or (c) TGF-β1 (1, 2.5, 5, or 10 ng/ml). Additionally, both the TSP-1 (40 μg/ml)– and TGF-β1

(5 ng/ml)–treated groups were treated with the addition of one of the following: (a) anti–TSP-1 antibody (100 μg/ml); (b) anti–TSP-1 receptor antibody (100 μg/ml); or (c) anti–TGF-β1 antibody (10 μg/ml). Appropriate buffer and IgG control were performed. At the end of the incubation period, the cells were washed with phosphate-buffered saline solution, and tumor cell extracts and media were harvested. The tumor cell extracts were obtained by adding 200 μl of cold 1% Triton X-100 in triethanolamine-buffered saline, pH 8.5, with the addition of protease inhibitors (AEBSF and leupeptin, 1:1000 concentration). The extracts were centrifuged at 10,000 rpm to remove cell debris. uPA and PAI-1 concentration in the cell extracts and media from the different treatment groups were measured using the IMUBIND total uPA and PAI-1 enzyme-linked immunosorbent assay kits purchased from American Diagnostica, Inc. (Greenwich, Conn.). The lower detection limits of the assays are 0.1 ng total protein/ml of sample. Assay procedures were performed according to the vendor's instructions. Total uPA and PAI-1 levels were quantitated by measuring solution absorbances at 450 nm and comparing the values with those of a standard curve. Results shown are the average of three separate determinations.

Statistical Analysis

All experiments were done in triplicate and performed at least twice. Values are expressed as the mean ± standard deviation. Statistical analysis was performed using Sigmastat software (Jandel Scientific, San Rafael, Calif.). Where several groups were compared to a control group, analysis of variance and the Student–Newman-Keuls method were used. *P* values less than 0.05 were considered significant. Dose-dependent response was evaluated by linear regression analysis.

RESULTS

Effect of TSP-1 and TGF-β1 on PAI-1 Expression

Enzyme-linked immunosorbent assay showed that both TSP-1 and TGF-β1 produced a significant increase in PAI-1 expression in the ASPC-1 tumor cell media compared to control cultures (Fig. 1). This effect was dose dependent as demonstrated by linear regression analysis (R = 0.853 and 0.824 for the TSP-1– and TGF-β1–treated cells, respectively). TSP-1, at 10, 20, 40, and 50 μg/ml, induced the ASPC-1 cells to express 28.7 ± 0.4, 28.8 ± 1.2, 35.3 ± 0.1, and 32.1 ± 1.2 ng/ml of PAI-1, respectively. TGF-β1, at 1, 2.5, 5, and 10 ng/ml, induced the ASPC-1 cells to express

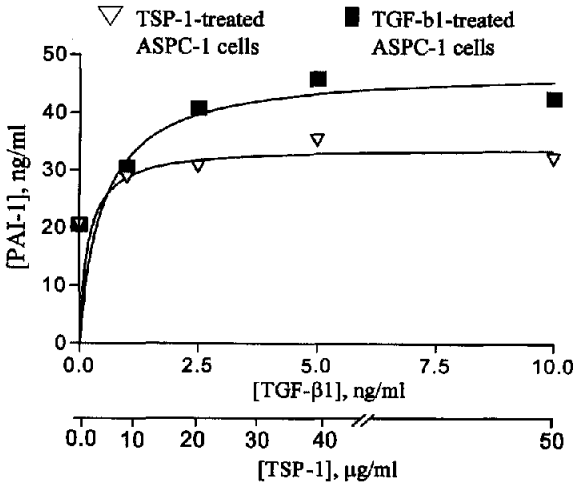

Fig. 1. Effect of thrombospondin-1 *(TSP-1)* and transforming growth factor beta-1 *(TGF-β1)* on ASPC-1 pancreatic cancer cell plasminogen activator inhibitor type 1 *(PAI-1)* expression.

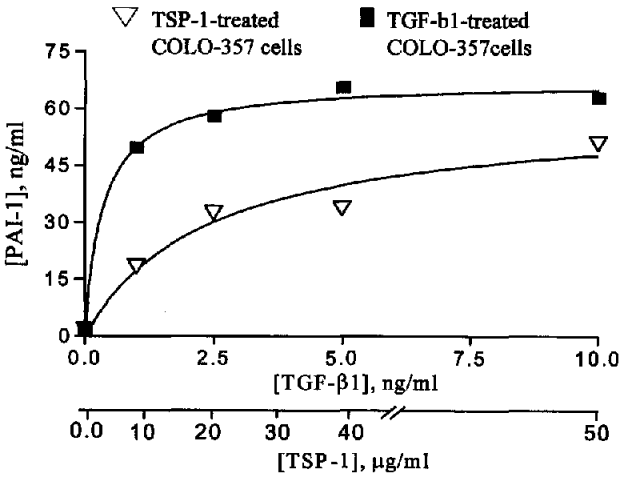

Fig. 2. Effect of thrombospondin-1 *(TSP-1)* and transforming growth factor beta-1 *(TGF-β1)* on COLO-357 pancreatic cancer cell plasminogen activator inhibitor type 1 *(PAI-1)* expression.

30.6 ± 0.5, 40.9 ± 0.1, 46.0 ± 0.7, and 42.6 ± 2.1 ng/ml of PAI-1, respectively. Untreated cells expressed only 20.5 ± 0.5 ng/ml of PAI-1 in the tumor cell media. Linear regression analysis showed that the differences in the median values among the treatment groups were statistically significant ($P = 0.003$). The differences between the TSP-1– or TGF-β1–treated cells and the control group were analyzed by the Student–Newman-Keuls method, and the differences were found to be statistically significant ($P < 0.05$). TSP-1 and TGF-β1 also increased PAI-1 expression in the COLO-357 tumor cell media (Fig. 2). This effect was also dose dependent, as demonstrated by lin-

Table I. Effect of neutralizing antibodies against TSP-1 and TGF-β1 on TSP-1–mediated PAI-1 expression (ng/ml ± SD) as determined by enzyme-linked immunosorbent assay

	Control	TSP-1	TSP-1 + α-TSP-1	TSP-1 + α-TGF-β1
ASPC-1	18.5 ± 0.5	34.0 ± 0.1	22.3 ± 0.2	20.1 ± 0.5
COLO-357	3.2 ± 0.8	46.5 ± 0.2	25.1 ± 0.3	22.8 ± 0.1

Table II. Effect of neutralizing antibodies against TSP-1 and TGF-β1 on TGF-β1–mediated PAI-1 expression (ng/ml ± SD) as determined by enzyme-linked immunosorbent assay

	Control	TGF-β1	TGF-β1 + α-TSP-1	TGF-β1 + α-TGF-β1
ASPC-1	18.5 ± 0.5	44.0 ± 0.8	45.1 ± 0.9	24.2 ± 0.5
COLO-357	3.2 ± 0.8	60.6 ± 0.3	58.5 ± 0.6	28.8 ± 0.1

ear regression analysis (R = 0.922 and 0.800 for the TSP-1– and TGF-β1–treated cells, respectively). TSP-1, at 10, 20, 40, and 50 μg/ml, induced the COLO-357 cells to express 18.4 ± 0.1, 32.6 ± 0.4, 33.9 ± 0.2, and 51.0 ± 0.3 ng/ml of PAI-1, respectively. TGF-β1, at 1, 2.5, 5, and 10 ng/ml, induced the COLO-357 cells to express 49.8 ± 0.1, 58.1 ± 0.1, 65.8 ± 0.7, and 63.0 ± 0.2 ng/ml of PAI-1, respectively. Untreated cells expressed only 2.0 ± 0.1 ng/ml of PAI-1. Linear regression analysis showed that the differences in the median values among the treatment groups were statistically significant ($P = 0.002$). The differences between the TSP-1– or TGF-β1–treated cells and the control group were analyzed by the Student–Newman-Keuls method, and the differences were found to be statistically significant ($P < 0.05$). Neither TSP-1 nor TGF-β1 had a significant effect on PAI-1 expression in tumor cell lysates.

Role of TGF-β1 in TSP-1–Promoted PAI-1 Expression

PAI-1 expression in response to TSP-1 or TGF-β1 was also determined with the addition of specific neutralizing antibodies against either TSP-1 or TGF-β1. The TSP-1 activity on PAI-1 upregulation was blocked with antibodies against either TSP-1 or TGF-β1. The TGF-β1 activity on PAI-1 expression was blocked with antibodies against TGF-β1 but was not significantly affected by anti–TSP-1 antibody (Tables I and II). Linear regression showed that the differences in the median values among the treatment groups were statistically significant ($P = 0.007$). The Student–Newman-Keuls method showed that addition of either anti–TSP-1 or anti–TGF-β1 antibody to the TSP-1–treated cells significantly blocked the TSP-1–mediated upregulation in PAI-1 expression in both the ASPC-1 and COLO-357 cells ($P < 0.05$). Whereas anti–TGF-β1 antibody blocked the TGF-β1–promoted upregulation of PAI-1 expression ($P < 0.05$ vs. TGF-β1 alone), the addition of anti–TSP-1 antibody had no effect on the TGF-β1–promoted upregulation of PAI-1 expression ($P > 0.05$ vs. TGF-β1 alone).

Table III. Effect of neutralizing antibody against the CSVTCG-specific TSP-1 receptor on TSP-1–mediated PAI-1 expression (ng/ml ± SD) as determined by enzyme-linked immunosorbent assay

	Control	TSP-1	TSP-1 + α-TSP-1 receptor
ASPC-1	18.5 ± 0.5	34.0 ± 0.1	20.5 ± 0.3
COLO-357	3.2 ± 0.8	46.5 ± 0.2	5.2 ± 0.6

Role of CSVTCG-Specific TSP-1 Receptor in TSP-1–Induced PAI-1 Expression

Enzyme-linked immunosorbent assay showed that neutralizing antibody against the CSVTCG-specific TSP-1 receptor blocked the effect of TSP-1 on PAI-1 expresssion in both the ASPC-1 and COLO-357 pancreatic cancer cells by 76% to 88% (Table III). The differences in the median values among the treatment groups are greater than would be expected by chance ($P = 0.028$). The difference in PAI-1 expression between the cells treated with TSP-1 alone and the cells treated with TSP-1 plus anti–CSVTCG–specific

TSP-1 receptor was analyzed by the Student–Newman-Keuls method, and the differences were found to be statistically significant ($P < 0.05$). Neutralizing antibody against the CSVTCG-specific TSP-1 receptor had no effect on TGF-β1–mediated PAI-1 expression.

Effect of TSP-1 and TGF-β1 on Urokinase Plasminogen Activator Expression

Neither TSP-1 nor TGF-β1 had an effect on uPA production by the ASPC-1 cells. uPA production in response to 10, 20, 40, and 50 μg/ml of TSP-1 was 4.9 ± 0.0, 5.0 ± 0.1, 5.1 ± 0.1, and 5.2 ± 0.0 ng/ml, respectively. uPA production in response to 1, 2.5, 5, and 10 ng/ml of TGF-β1 was 5.3 ± 0.2, 5.2 ± 0.1, 5.0 ± 0.0, and 4.9 ± 0.1 ng/ml, respectively. Nonstimulated cells expressed 4.9 ± 0.1 ng/ml of uPA. COLO-357 uPA production was below the detection limits for the assay (<0.1 ng/ml), with or without treatment with TSP-1 or TGF-β1.

DISCUSSION

Pericellular proteolysis by tumor cell–associated proteases is hypothesized to play a crucial role in tumor cell invasion.[1,3-5] The controlled degradation of the extracellular matrix in the pericellular environment allows tumor cells to detach from the main colony and to invade surrounding tissues. By preventing excessive plasmin-mediated degradation of the extracellular matrix, PAI-1 has been shown to play a crucial role in tumor cell invasion and metastasis.[9,11,13,14]

Our data demonstrate that both TSP-1 and TGF-β1 upregulate PAI-1 expression in pancreatic cancer cells. This effect was dose dependent and saturable, which suggested a receptor-mediated process. Furthermore, a neutralizing antibody against the CSVTCG-specific TSP-1 receptor blocked this upregulation in PAI-1 expression, thus confirming the involvement of the CSVTCG-specific TSP-1 receptor in the TSP-1–promoted upregulation of PAI expression. Although we did not specifically determine the mechanism underlying the increased PAI-1 expression in response to TSP-1 and TGF-β1 (i.e., increased protein synthesis vs. decreased internalization/degradation), there is an extensive body of literature showing that TGF-β1 upregulates PAI-1 production at the transcriptional level.[32-34] Further work is needed to determine the mechanism underlying the increased PAI-1 expression seen in response to TSP-1.

Our data also showed that although the TSP-1–mediated upregulation in PAI-1 production could be blocked with neutralizing antibodies against either TSP-1 or TGF-β1, the TGF-β1–mediated effect in PAI-1 production could only be blocked with antibody against TGF-β1. Since our affinity-purified TSP-1 contains only trace amounts of TGB-β1 (nonbiologically active), as determined by a human TGF-β1 immunoassay, these data show that TGF-β1 mediates the TSP-1 effect on PAI-1 expression in pancreatic cancer cells. This finding is consistent with previous work in our laboratory that showed TSP-1 could activate endogenously produced latent TGF-β1. Although in our experience the activation of latent TGF-β1 by TSP-1 depends on the presence of tumor cells, several investigators have reported that the arginine-phenylalanine-lysine sequences in the type I repeats of the TSP-1 molecule can directly activate TGF-β1 by inducing conformational changes in the inactive form of TGF-β1.[8,9,26-29]

PAI-1, the main inhibitor of uPA in malignancies, is stored in the matrix where it binds with other matrix proteins such as vitronectin.[11,12] By neutralizing uPA, PAI-1 decreases the formation of plasmin and therefore decreases the plasmin-associated proteolytic activity.[1] By decreasing the proteolytic activity in the extracellular matrix, PAI-1 could prevent excessive degradation of the matrix scaffold through which the tumor cells need to migrate in order to invade. This is consistent with work previously performed in our laboratory, which showed that although matrix degradation is necessary for tumor invasion, excessive degradation of the matrix by plasmin arrests tumor cell migration and invasion, presumably by preventing the cell-matrix interaction that tumor cells need for these processes to occur.[7,9] Additionally, plasmin is a known activator of several matrix metalloproteases (i.e., MMP-9). Thus downregulation of the matrix-bound plasmin activity could decrease the activation of metalloproteases and therefore further prevent excessive matrix degradation by tumor-associated proteases. Furthermore, increased PAI-1 expression in response to TSP-1 and TGF-β1 could contribute to the dense stromal reaction that is usually seen in pancreatic carcinomas. Additionally, increased PAI-1 expression by pancreatic tumors can lead to elevated PAI-1 levels in the systemic circulation, which could contribute to the systemic hypercoagulability seen in patients with this malignancy.

Pericellular proteolysis has been shown to be important for tumor cell invasion.[35] Our data show that neither TSP-1 nor TGF-β1 had an effect on uPA production by the ASPC-1 tumor cells. However, the ASPC-1 cells showed a high level of baseline (nonstimulated) uPA production. We have previously demonstrated that in ASPC-1 cells TSP-1 and TGF-β1 upregulate pericellular proteolysis by increasing uPA receptor expression and therefore cell surface–associated uPA activity. This results in an increase in

tumor cell–associated plasmin-generated pericellular proteolytic activity in response to TSP-1 and/or TGF-β1, despite the lack of effect on total uPA production in response to TSP-1 or TGF-β1. We have previously demonstrated that upregulation of tumor cell–bound plasmin activity by TSP-1 and TGF-β1 promotes pancreatic and breast tumor cell invasion.[36]

The COLO-357 cells did not produce any measurable levels of uPA with or without stimulation with TSP-1 or TGF-β1. These data correlate with the observation that interleukin-1β, a known stimulant for uPA production in other malignancies, does not induce uPA production in the COLO-357 cells (unpublished observation). We hypothesize that the lack of uPA production on either a basal or stimulated state by the COLO-357 cells is related to the previously reported low Raf-1 activity (a signaling molecule known to mediate uPA production) in these tumor cells.[37] Further work is needed to corrobate this hypothesis. Furthermore, a paracrine interaction between tumor cells and stromal cells has been described by which some non–uPA-producing tumor cells bind uPA produced by stromal cells.[38-40]

We conclude that TSP-1, through activation of TGF-β1, regulates the plasminogen/plasmin system in pancreatic cancer. Further insight into this system could allow for intervention at different levels in an effort to prevent TSP-1-mediated tumor cell invasion and metastasis.

REFERENCES

1. Kwaan HC. The plasminogen-plasmin system in malignancy. Cancer Metastasis Rev 1992;11:291-311.
2. Testa JE, Quigley JP. The role of urokinase-type plasminogen activator in aggressive tumor cell behavior. Cancer Metastasis Rev 1990;9:353-367.
3. Blasi F, Behrendt N, Cubellis MV, Ellis V, Lund LR, Masucci MT. The urokinase receptor and regulation of cell surface plasminogen activation. Cell Differ Dev 1990;32:247-254.
4. Ellis V, Behrendt N, Dano K. Plasminogen activation by receptor bound urokinase. A kinetic study with both cell-associated and isolated receptor. J Biol Chem 1991;226:12752-12758.
5. Blasi F. Molecular mechanisms of protease-mediated tumor invasiveness. J Surg Oncol Suppl 1993;3:21-23.
6. Blasi F. Urokinase and urokinase receptor: A paracrine/autocrine system regulating cell migration and invasiveness. Bioessays 1993;15:105-111.
7. Hosokawa T, Muraishi A, Rothman V, Papale M, Tuszynski GP. The effect of thrombospondin on invasion of fibrin gels by human A549 lung carcinoma. Oncol Res 1993;5:183-189.
8. Albo D, Arnoletti JP, Castiglioni A, Granick MS, Solomon MP, Rothman VL, Tuszynski GP. Thrombospondin (TSP) and transforming growth factor beta 1 (TGF-beta) promote human A549 lung carcinoma cell plasminogen activator inhibitor type 1 (PAI-1) production and stimulate tumor cell attachment in vitro. Biochem Biophys Res Commun 1994;203:857-865.
9. Albo D, Berger DH, Wang TN, Hu X, Rothman V, Tuszynski GP. Thrombospondin-1 and transforming growth factor-beta 1 promote breast tumor cell invasion through up-regulation of the plasminogen/plasmin system. Surgery 1997;122:493-499; discussion 499-500.
10. Krishnamurti C, Alving B. Plasminogen activator inhibitor type 1: Biochemistry and evidence for modulation of fibrinolysis in vivo. Semin Thromb Hemost 1992;18:67-80.
11. Bianchi E, Cohen RL, Dai A, Thor AT, Shuman MA, Smith HS. Immunohistochemical localization of the plasminogen activator inhibitor 1 in breast cancer. Int J Cancer 1995;60:597-603.
12. Seiffert D, Ciambrone G, Wagner NV, Binder BR, Loskutoff DJ. The somatomedin B domain of vitronectin. Structural requirements for the binding and stabilization of active type I plasminogen activator inhibitor. J Biol Chem 1994;269:2659-2666.
13. Costantini V, Sidoni A, Deveglia R, Cazzato OA, Bellezza G, Ferri I, Bucciarelli E, Nenci GG. Combined overexpression of urokinase, urokinase receptor, and plasminogen activator inhibitor-1 is associated with breast cancer progression: An immunohistochemical comparison of normal, benign, and malignant breast tissues. Cancer 1996;77:1079-1088.
14. Grondahl-Hansen J, Hilsenbeck SG, Christensen IJ, Clark GM, Osborne CK, Brunner N. Prognostic significance of PAI-1 and uPA in cytosolic extracts obtained from node-positive breast cancer patients. Breast Cancer Res Treat 1997;43:153-163.
15. Flaumenhaft R, Rifkin DB. Extracellular matrix regulation of growth factor and protease activity [review]. Curr Opin Cell Biol 1991;3:817-823.
16. Flaumenhaft R, Rifkin DB. The extracellular regulation of growth factor action [review]. Mol Biol Cell 1992;3:1057-1065.
17. Tuszynski GP, Nicosia RF. Localization of thrombospondin and its cysteine-serine-valine-threonine-cysteine-glycine-specific receptor in human breast carcinoma. Lab Invest 1994;70:228-233.
18. Clezardin P, Frappart L, Clerget M, Pechoux C, Delmas PD. Expression of thrombospondin (TSP1) and its receptors (CD36 and CD51) in normal, hyperplastic, and neoplastic human breast. Cancer Res 1993;53:1421-1430.
19. Arnoletti J, Albo D, Granick MS, Soloman MP, Castiglioni A, Rothman VL, Tuszynski GP. Thrombospondin and transforming growth factor-beta 1 increase expression of urokinase-type plasminogen activator and plasminogen activator inhibitor-1 in human MDA-MB-231 breast cancer cells. Cancer 1995;76:998-1005.
20. Tuszynski GP. The role of thrombospondin in malignancy and tumor cell metastasis. In Lahav J, ed. Thrombospondin. Boca Raton: CRC Press, 1993, pp 209-225.
21. Wang TN, Qian XH, Granick MS, Solomon MP, Rothman VL, Berger DH, Tuszynski GP. Inhibition of breast cancer progression by an antibody to a thrombospondin-1 receptor. Surgery 1996;120:449-454.
22. Tuszynski GP, Smith M, Rothman VL, Capuzzi DM, Joseph RR, Katz J, Besa EC, Treat J, Switalska HI. Thrombospondin levels in patients with malignancy. Thromb Haemost 1992;67:607-611.
23. Tuszynski GP, Rothman VL, Papale M, Hamilton BK, Eyal J. Identification and characterization of a tumor cell receptor for CSVTCG, a thrombospondin adhesive domain. J Cell Biol 1993;120:513-521.
24. Laiho M, Keski-Oja J. Growth factors in the regulation of pericellular proteolysis. Cancer Res 1989;49:2533-2553.

25. Keski-Oja J, Koli K, Lohi L, Laiho M. Growth factors in the regulation of the plasminogen plasmin-system in tumor cells. Semin Thromb Hemost 1991;17:231-239.
26. Schultz-Cherry S, Murphy-Ullrich JE. Thrombospondin causes activation of latent transforming growth factor-β secreted by endothelial cells by a novel mechanism. J Cell Biol 1993;122:923-932.
27. Schultz-Cherry S, Ribeiro S, Gentry L, Murphy-Ullrich JE. Thrombospondin binds and activates the small and large forms of latent transforming growth factor-β in a chemically defined system. J Biol Chem 1994;269:26775-26782.
28. Schultz-Cherry S, Lawler J, Murphy-Ullrich JE. The type 1 repeats of thrombospondin 1 activate latent transforming growth factor-β. J Biol Chem 1994;269:26783-26788.
29. Schultz-Cherry S, Chen H, Mosher DF, Misenheimer TM, Krutzsch HC, Roberts DD, Murphy-Ullrich JE. Regulation of transforming growth factor-β activation by discrete sequences of thrombospondin 1. J Biol Chem 1995;270:7304-7310.
30. Tuszynski GP, Srivastava S, Switalska HI, Holt JC, Cierniewski CS, Niewiarowski S. The interaction of human platelet thrombospondin with fibrinogen. J Biol Chem 1985; 260:12240-12245.
31. Wang TN, Qian X, Granick MS, Solomon MP, Rothman VL, Berger DH, Tuszynski GP. Thrombospondin-1 (TSP-1) promotes the invasive properties of human breast cancer. J Surg Res 1996;63:39-43.
32. Jakowlew SB, Mariano JM, You L, Mathias A. Differential regulation of protease and extracellular matrix protein expression by transforming growth factor-beta 1 in non-small cell lung cancer cells and normal human bronchial epithelial cells. Biochim Biophys Acta 1997;1353:157-170.
33. Jakowlew SB, Mariano JM, You L. Effects of transforming growth factor-beta 1 and phorbol ester on PAI-1 and PA genes in human lung cells. Growth Factors 1997;14:177-197.
34. van Waarde MA, van Assen AJ, Kanpinga HH, Konings AW, Vujaskovicz Z. Quantification of transforming growth factor-beta in biological material using cells transfected with a plasminogen activator inhibitor-1 promoter-luciferase construct. Anal Biochem 1997;247:45-51.
35. Mohanam S, Sawaya RE, Yamamoto M, Bruner JM, Nicholson GL, Rao JS. Proteolysis and invasiveness of brain tumors: Role of urokinase-type plasminogen activator receptor. J Neurooncol 1994;22:153-160.
36. Albo D, Berger DH, Tuszynski GP. The effect of Thrombospondin-1 and TGF-beta 1 on pancreatic cancer cell invasion. J Surg Res 1998;76:86-90.
37. Berger D, Jardines L, Chang H, Ruggieri B. Activation of Raf-1 in human pancreatic adenocarcinoma. J Surg Res 1997; 69:199-204.
38. Grondal-Hansen J, Ralfkiaer E, Kirkeby LT, Kristensen P, Lund LR, Dano K. Localization of urokinase type plasminogen activator in stromal cells in adenocarcinomas of the colon in humans. Am J Pathol 1991;138:111-117.
39. Pyke C, Kristensen P, Ralfkiaer E, Grondal-Hansen J, Eriksen J, Blassi F, Dano K. Urokinase-type plasminogen activator is expressed in stromal cells and its receptor in cancer cells at invasive foci in human colon adenocarcinomas. Am J Pathol 1991;138:1059-1067.
40. Pyke C, Kristensen P, Ralfkiaer E, Eriksen J, Blassi F, Dano K. The plasminogen activation system in human colon cancer: Messenger RNA for the inhibitor PAI-1 is located in endothelial cells in the stroma. Cancer Res 1991;51:4067-4071.

Characterization of a Novel Model of Pancreatic Fibrosis and Acinar Atrophy

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Significant fibrosis and acinar atrophy are characteristics of chronic pancreatitis; however, because of the lack of a reproducible model, early phases of these changes are poorly understood. We have developed a model of severe hyperstimulation and obstruction pancreatitis (SHOP) to better define the mechanisms of early pancreatic fibrogenesis. Sprague-Dawley rats were used and SHOP was induced by complete pancreatic duct obstruction and daily cerulein hyperstimulation (50 μg/kg intraperitoneally). Animals were killed at 24, 48, 72, and 96 hours. Control animals underwent sham operation and received no cerulein. Pancreata were prepared for hematoxylin and eosin and sirius red (collagen-specific) staining and for hydroxyproline assay (measure of total collagen content). We found moderate amounts of edema and inflammation but minimal parenchymal necrosis. Significant loss of acinar cell mass was noted by 48 hours, and normal acinar cells were essentially absent by 96 hours. Tissue collagen content increased with time and large amounts of interstitial collagen were detected by 72 hours. In conclusion, SHOP is a novel model of early pancreatic fibrosis associated with minimal necrosis and a significant decrease in acinar cell mass, making it an ideal model to study the early cellular mechanisms of pancreatic fibrogenesis. (J GASTROINTEST SURG 1999;3:418-425.)

KEY WORDS: Pancreatic fibrosis, pancreatic atrophy

Acute pancreatitis results in destruction of pancreatic parenchyma and deposition of interstitial collagen with complete or near-complete resolution once the etiologic events are removed.[1-3] Conversely, chronic pancreatitis is characterized by persistent irreversible pancreatic fibrosis and loss of acinar cell mass. Whether or not this fibrosis results from repeated episodes of acute pancreatitis or as a de novo process is largely unknown.[1-5] Kloppel and Maillet[3] proposed a "necrosis-fibrosis sequence" to explain the progression of acute pancreatitis to the chronic state, essentially proposing that repeated acute pancreatic injury leads to periacinar fat necrosis, fibrosis, and abnormalities in pancreatic duct morphology. To date, little is known about the pathophysiology of pancreatic fibrogenesis, and hence its prevention is not possible and treatment is directed at symptom relief.

Studies of the fibrogenic mechanisms involved in human chronic pancreatitis are severely limited by a lack of available tissue, and thus several animal models have been used with limited success. Animal models of chronic pancreatitis caused by long-term alcohol intake and administration of nutritionally deficient diets have been associated with inconsistent results.[6-8] Intravenous injection of dibutylin chloride[9] and intraductal administration of oleic acid[10] or sulfonic acid[11] result in pancreatic fibrosis in rats. Obstruction of the pancreatic duct will result in loss of normal acinar architecture, with an apparent proliferation of duct-like elements, and evidence of enhanced stromal cell proliferation, but these changes may take weeks to develop.[12] Interestingly, although administration of supramaximal doses of cerulein is well documented to cause edematous acute pancreatitis, administration of

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multiple doses over an extended period of time does not induce pancreatic fibrosis in rats.[1]

Regardless of the etiology of pancreatic fibrosis, a severe acute model is best suited for mechanistic studies of the early phases of the fibrogenesis resulting from pancreatic injury. In an effort to develop a better understanding of pancreatic fibrogenesis, our goal was to develop a reliable and reproducible model of acute pancreatitis characterized by significant loss of acinar cell mass and a substantial increase in interstitial fibrosis.

MATERIAL AND METHODS

Surgical Procedures

Male Sprague-Dawley rats weighing 250 to 300 grams were used for all procedures, with adherence to standard institutional animal welfare guidelines. Rats were anesthetized by inhalation of methoxyflurane and laparotomy was performed via midline abdominal incision. Acute pancreatitis was induced by combined pancreatic duct obstruction and cerulein (Sigma Chemical, St. Louis, Mo.) hyperstimulation (50 μg/kg intraperitoneally). Pancreatic duct obstruction was accomplished by application of titanium surgical clips across the common bile–pancreatic duct just prior to its entrance into the duodenum and across the common bile duct prior to its entrance into the pancreas (Fig. 1). Cerulein was administered into the peritoneal cavity before closure of the incision and once every 24 hours intraperitoneally thereafter until the animals were killed. Sham-operated control rats underwent manipulation of the duodenum and pancreas with no ductal ligation and received only saline solution (0.5 ml) intraperitoneally prior to closure of the laparotomy. The abdominal incision was closed using surgical clips; the animals were then allowed to recover in a warmed incubator and subsequently placed in housing cages with access to standard chow and water ad libitum.

Rats in the experimental groups were killed after 24 hours (n = 4), 48 hours (n = 4), 72 hours (n = 3) and 96 hours (n = 4). Sham-operated animals were killed after 24 hours (n = 4) and 96 hours (n = 4). Untreated control rats were killed to obtain normal tissue and blood samples. At the time of death, the laparotomy incision was reopened and pancreata were harvested, weighed, and divided. Two pieces (approximately 3 mm^3/piece) of each pancreas were separated, and one was fixed in 10% buffered formalin for histologic evaluation and the second was placed in optimal cutting temperature (OCT) compound (Tissue-Tek, Fisher Scientific Co., Pittsburgh, Pa.) for immunohistochemical analysis. The remainder of the gland was weighed to determine its wet weight, placed in liquid nitrogen, and stored at −80° C for quantification of hydroxyproline content. Animals were exsanguinated via aortic puncture, and blood was permitted to clot in preparation for amylase measurements.

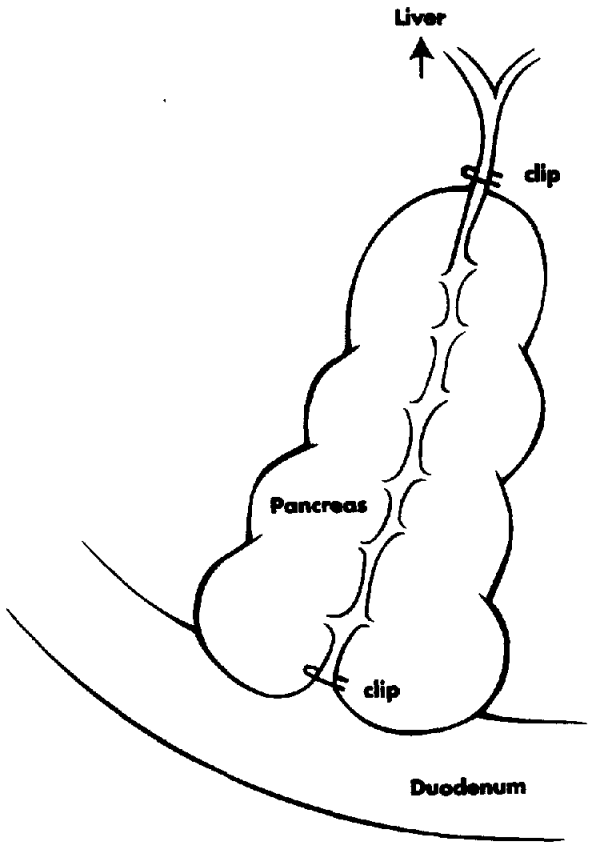

Fig. 1. Schematic model demonstrating placement of surgical clips to induce SHOP in rats.

Evaluation of Pancreatitis

Amylase Determination. Blood was allowed to clot at room temperature and was then centrifuged at 1250 × *g* to separate the sera. The serum was divided into aliquots, frozen, and stored at −80° C. Serum amylase was determined spectrophotometrically using a commercial amyloclastic assay (Pharmacia, Kalamazoo, Mich.).

Histologic Evaluation. Formalin-fixed pancreata were embedded in paraffin, sectioned at 5 μm thickness, and evaluated under light microscopy after hematoxylin and eosin staining. A modified severity assessment grading scale was used to quantify the degree of pancreatic injury (Table I). Slides were randomized, coded, and graded by an unbiased pathologist.

Table I. Pancreatitis severity assessment scale

Edema	0-3
Inflammation	0-3
Acinar necrosis	0-6
Acinar atrophy	0-6
Hemorrhage	0-5

A modified severity assessment score utilized to grade pancreatic injury using light microscopy. All samples were fixed for 24 hours and stained with hematoxylin and eosin.

Table II. Sirius red stain assessment scale

	Grade
Normal amounts of collagen	1
Moderate amounts of interstitial collagen	2
Prominent collagen in periacinar and perivascular areas	3

A histologic assessment score utilized to quantify the extent of sirius red staining under light microscopy. Sirius red staining is specific for quantifying collagen deposition.

Collagen Determination

Hydroxyproline Measurement. Total tissue collagen was quantitated by using a hydroxyproline assay of lyophilized pancreatic samples, as previously described.[2,10,13] Briefly, lyophilized pancreata were homogenized using a freezer mill, and 5 mg of each specimen was hydrolyzed in 6N hydrogen chloride at 110° C for 16 to 18 hours. Hydrolyzed samples were then relyophilized and suspended in deionized water. Oxidation of hydroxyproline residues to pyrrole was performed in acetate/citrate buffer (38.5% isopropanol, 400 mmol/L sodium acetate, 127 mmol/L sodium citrate, and 24 mmol/L citric acid monohydrate) and chloramine T (7%). Formation of a chromophore with the oxidized hydroxyproline was obtained by exposing samples to p-dimethylaminobenzaldehyde (357 mmol/L, Sigma Chemical). Absorbance was measured spectrophotometrically at 562 nm, and hydroxyproline was quantitated by extrapolation to a standard curve using purified hydroxyproline (Sigma Chemical). The amount of collagen within each sample was quantified by converting derived hydroxyproline values to collagen mass based on the 10% hydroxyproline content of collagen.[2]

Sirius Red Staining. Formalin-fixed pancreata were embedded in paraffin, sectioned at 5 μm thickness, and stained with sirius red. A scale (Table II) was developed to grade the amount of collagen present in all specimens. Slides were reviewed and scored by an unbiased pathologist.

Statistical Analysis

All data are presented as mean ± standard error of the mean. Data across all groups were analyzed by analysis of variance with a Scheffe post hoc test. A P value <0.05 was taken to represent statistical significance.

RESULTS

Serum Amylase

Serum amylase values are presented in Fig. 2. Serum amylase levels from control rats were consistently low (12,000 U/L). Twenty-four hours after the induction of severe hyperstimulation and obstruction pancreatitis (SHOP), however, serum amylase levels rose nearly threefold (30,000 U/L). Interestingly, serum amylase levels from animals subjected to longer durations of SHOP (48 to 96 hours) were low and statistically comparable to those detected in control animals (9000 to 10,000 U/L).

Histologic Evaluation

Evaluation of hematoxylin and eosin–stained specimens from control animals demonstrated normal pancreatic histoarchitecture denoted by the presence of well-defined acini with little interstitial tissue (Fig. 3, *A*). Furthermore, the apical cytoplasm of the acinar cells contained an abundance of zymogen granules. Evaluation of pancreatic specimens from animals subjected to SHOP, however, demonstrated marked differences in morphology. Significant edema and inflammation were evident as were patchy areas of acinar cell necrosis. The most significant finding was the rapid and progressive loss of acinar cell mass with the formation of numerous "duct-like" complexes with widely dilated lumens and concomitant increases in interstitial tissue by 96 hours (Fig. 3, *B*). Additionally, zymogen granules became difficult to identify in the acinar cells under these conditions. Frank hemorrhage was not a major component of the histologic changes detected in this model of pancreatitis.

Hydroxyproline Measurement

Measurements of collagen content are presented in Fig. 4. The collagen content of control pancreata averaged 15 μg/mg of dried tissue. Similar values were detected in pancreatic samples harvested from animals subjected to SHOP for 24 or 48 hours. Statistically significant increases in pancreatic collagen content were first evident in animals subjected to 72 hours of SHOP. Furthermore, after 96 hours of SHOP the collagen content of pancreatic samples had increased more than twofold. Interestingly, linear regression

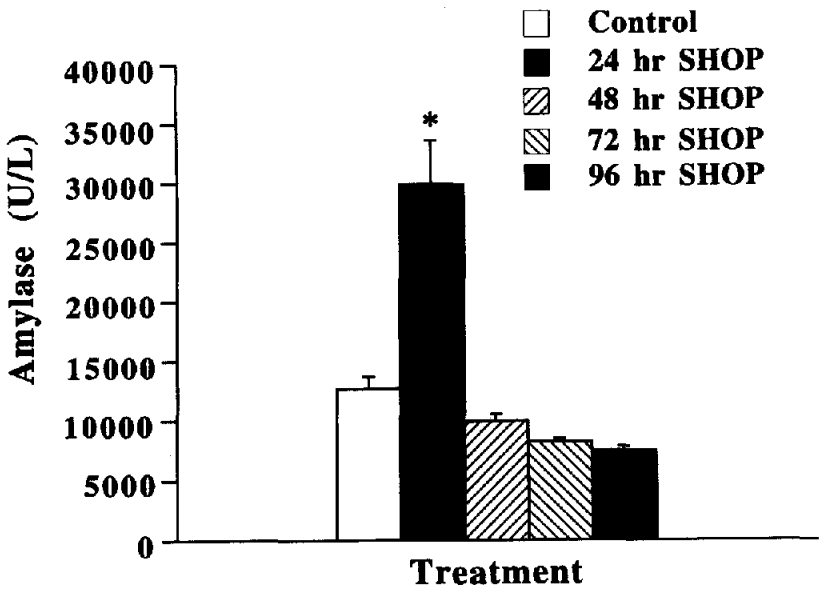

Fig. 2. Serum amylase levels in control rats and rats subjected to 24 to 96 hours of SHOP (n = 8 to 9 per group; * = $P < 0.05$ vs. control rats).

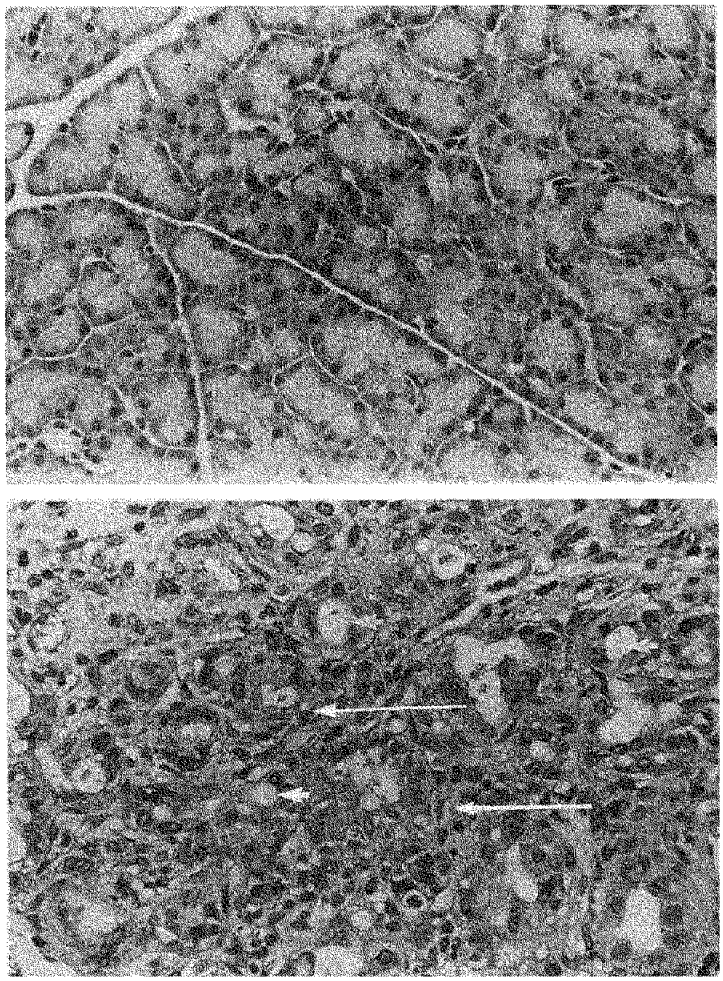

Fig. 3. Light photomicrographs of hematoxylin and eosin–stained pancreata harvested from control rats **(A)** or after 96 hours of SHOP **(B).** Control pancreata revealed normal pancreatic histoarchitecture with well-defined acini and little interstial tissue. In contrast, pancreata harvested 96 hours after the induction of SHOP revealed the appearance of numerous duct-like complexes with dilated lumens (small arrows) and marked increases in interstitial tissue (large arrows). (×400.)

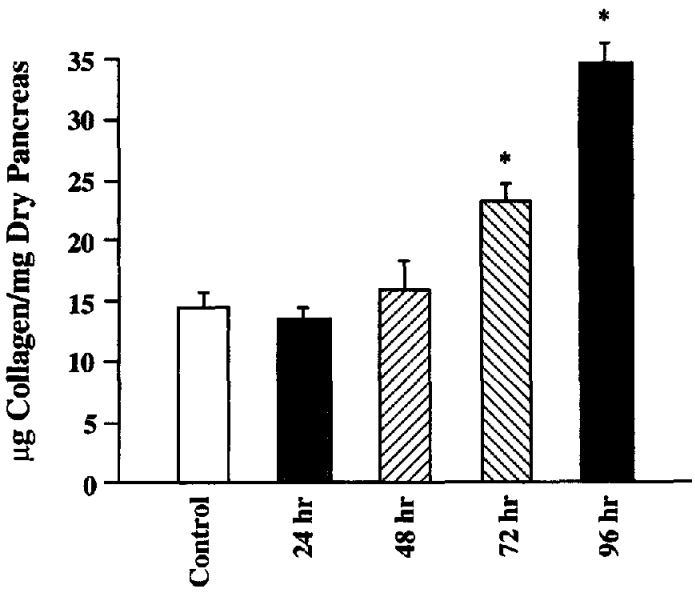

Fig. 4. Collagen content in control pancreata and pancreata subjected to 24 to 96 hours of SHOP (n = 3 to 4 per group; * = $P < 0.05$ vs. control rats).

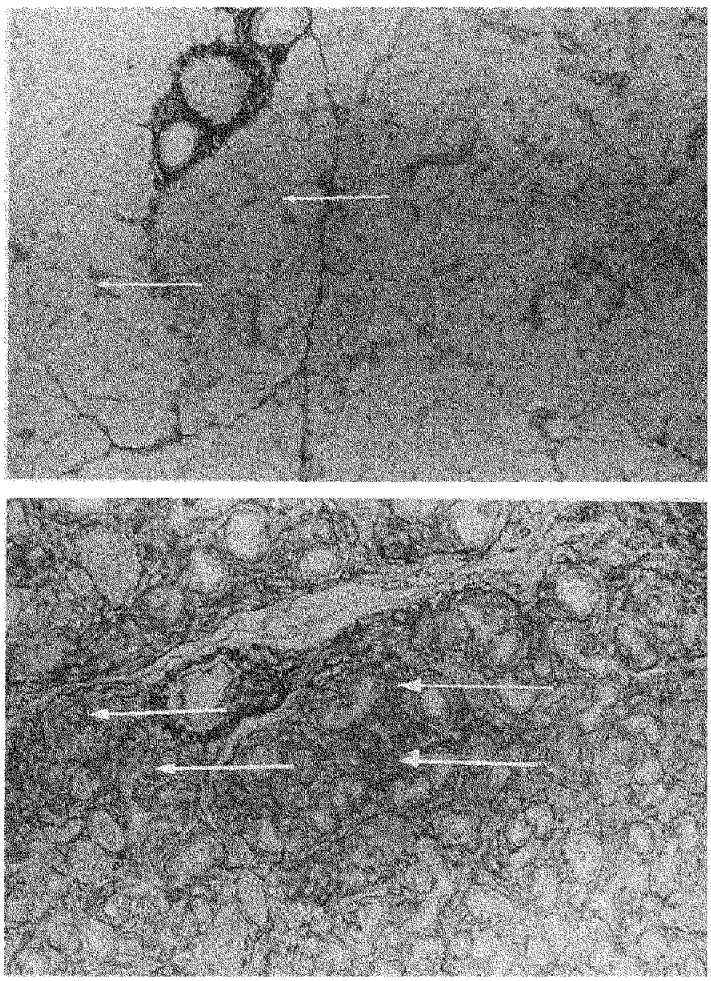

Fig. 5. Light photomicrographs of sirius red–stained pancreata harvested from control animals **(A)** or animals subjected to 96 hours of SHOP **(B).** Control pancreata revealed minimal sirius red staining in periacinar areas indicating low levels of collagen content (small arrows). In contrast, pancreata harvested after 96 hours of SHOP revealed enhanced sirius red staining in identical areas (large arrows) indicating enhanced collagen deposition in the extracellular matrix. (×400.)

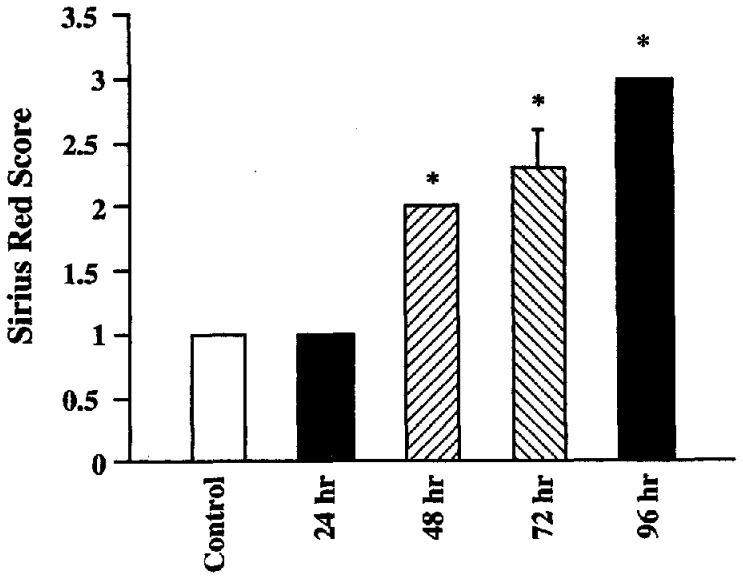

Fig. 6. Histologic assessment of sirius red staining in control pancreata and pancreata subjected to 24 to 96 hours of SHOP (n = 3 to 4 per group; * = $P < 0.05$ vs. control and 24 hour groups).

analysis of these data yielded a high degree of correlation ($r^2 = 0.96248$) between the amount of total collagen present in tissue and the duration of SHOP.

Sirius Red Staining

Sirius red staining of control pancreatic samples revealed diffuse and thin strands of collagen localized mainly to periacinar areas (Fig. 5, *A*). Significantly more sirius red staining was detected in pancreata from rats subjected to graded durations of SHOP. These results indicate a temporal increase in the amount of collagen deposited in the periacinar interstitial regions, consistent with worsening interstitial pancreatic fibrosis over time (Fig. 5, *B*). Fig. 6 depicts the morphologic studies quantifying sirius red staining under these conditions. Control pancreata had a sirius red staining score of 1, which is indicative of low levels of interstitial collagen. A similar score was noted in pancreata harvested from rats subjected to 24 hours of SHOP. Thereafter, sirius red staining scores increased with time and were noted to be maximal at 96 hours. At this time point, sirius red staining was noted to have increased threefold over control levels. Thus these morphologic assessments support the contention that SHOP induces profound changes in the collagen content of the pancreas.

DISCUSSION

Chronic pancreatitis in humans is characterized by significant fibrosis and loss of acinar cell mass resulting in exocrine pancreatic insufficiency.[10,14] Unfortunately, studies of pancreatic fibrogenesis are limited by the paucity of available human tissue and relative lack of reproducible and reliable animal models. We have described a novel model of severe pancreatitis resulting from hyperstimulation with cerulein and obstruction of the main pancreatic duct in rats (SHOP). This model is associated with early evidence of pancreatic fibrosis and loss of acinar cell mass, similar to that seen with human chronic pancreatitis, within 72 to 96 hours after the induction of injury.

Pancreatic duct occlusion in larger animals such as cats,[15] dogs,[16] and pigs[17] results in atrophy of the pancreas and fibrosis, but such models require longer periods of injury to elicit these effects. Other animal models of chronic pancreatitis have been reported, but most reproduce only some histologic aspects of the human disease.[11] More recently a model of chronic pancreatitis has been described in rats where retrograde pancreatic duct instillation of trinitrobenzene sulfonic acid (TNBS) was used; however, fibrosis took 3 weeks to develop and 20% of animals did not develop morphology that resembled human chronic pancreatitis.[11]

In an extensive evaluation, Walker et al.[12] characterized the histologic changes in the pancreas after ductal ligation in rats. Within 5 days, glandular atrophy and densely packed small ducts were evident. Stromal fibroblasts increased in size within the first 3 days and began to undergo enhanced mitosis between 24 and 48 hours. The authors demonstrated some collagen deposition in the interstitial spaces, but ex-

tensive fibrosis was not reported. Interestingly, it appeared that fibroblasts underwent changes indicative of myofibroblast differentiation between days 7 and 14 after the induction of injury.

Cerulein hyperstimulation is a well-documented model of nonfatal edematous pancreatitis, but repetitive injections of supramaximal doses of cerulein at 20-day intervals have not been shown to cause pancreatic fibrosis.[1] Although cerulein-induced pancreatitis has been shown to result in an upregulation of collagen messengerRNA and increased protein production, the enhanced collagen production apparently does not result in pancreatic fibrosis.[18,19]

SHOP combines these two recognized and well-described models of pancreatitis: cerulein hyperstimulation and pancreatic duct obstruction. Interestingly, the severity of pancreatic destruction and the early fibrosis evident in SHOP are not reported in animals that underwent either model alone. Hematoxylin and eosin–stained sections from SHOP specimens demonstrate edema and inflammation. These changes, however, peaked at and did not increase in severity after 48 hours. Additionally, intraparenchymal hemorrhage was not a significant histologic finding in SHOP pancreata. Edema and inflammatory cell infiltrates confirm pancreatic injury, and the persistent presence of these findings would suggest ongoing injury resulting from duct obstruction and repeated cerulein administration.

One of the most striking features of SHOP is the early loss of acinar cell mass. Although Walker et al.[12] did demonstrate a similar finding with duct obstruction alone, it was not evident until 5 days after injury. We found significant acinar atrophy within 48 hours that worsened over the ensuing 2 days to the point where normal acini were virtually absent by 96 hours after the induction of initial injury. Interestingly, although parenchymal necrosis was present, it was minimal and did not worsen after 48 hours. Previously the loss of acinar cell mass with concomitant minimal necrosis has been shown in rats to be the result of apoptotic cell death.[20-23]

In parallel to this apparent loss of acinar cell mass, we found significant proliferation of "duct-like" complexes by 72 hours after the initial injury. The origin of these "duct-like" complexes has been argued. Walker[20] theorized a proliferation of centroacinar and intercalated duct cells as the origin of these complexes. Subsequent studies, however, have supported the hypothesis that acinar cells actually redifferentiate and assume the morphology of duct cells.[23,24] We propose that the loss of acinar cell mass is, in part, due to both apoptotic cell death with the remaining acinar cells reverting to the morphology of duct cells. From a potential future therapeutic perspective, understanding the pathophysiology of this finding may provide insight. Specifically, can exocrine function be improved in patients with chronic pancreatitis by inducing these "duct-like" cells to reassume their acinar appearance and function?

As previously stated, the pathophysiology of pancreatic fibrosis is poorly understood, and efforts to further identify the etiologic factors contributing to upregulation of collagen production as well as irreversible fibrosis are hampered by the lack of an appropriate animal model. Since most existing models of pancreatic fibrosis take weeks or more to develop, the objective of the present study was to develop a reliable and reproducible model of early pancreatic fibrogenesis. In support of this, increases in tissue collagen were verified using two different methodologies. SHOP was associated with significant elevations of pancreatic hydroxyproline content (a measure of tissue collagen) within 48 hours, progressing to more than twice normal levels by 96 hours. This early increase in tissue collagen has been associated with cerulein-induced pancreatitis, but without persistent insult it resolves within 10 to 14 days.[1] Although the present study only followed animals to 96 hours, the significant increase in hydroxyproline content between 48 and 96 hours is contrary to the findings of Elsasser et al.[1] of rapid degradation of newly synthesized collagen in cerulein pancreatitis.

Sirius red stains specifically for collagen, but is not type specific, and therefore the type of collagen cannot be positively identified. Grading of sirius red–stained slides by an independent observer demonstrated moderate increases in interstitial collagen by 48 hours after the initial injury. Collagen deposition appeared to increase to the most significant levels at 96 hours, at which time it was noted to be relatively ubiquitous throughout all specimens.

Microscopic findings further support SHOP as a model of progressive pancreatic fibrosis as evidenced by the increase in collagen density with time. In addition, with worsening injury, it appeared that collagen was not merely structurally supportive in its periacinar location, but became diffuse and increasingly distributed in interstitial spaces. The normal arrangement of the pancreatic acini is progressively disrupted with continued injury in SHOP resulting in a disorganized appearance of the pancreatic parenchyma.

In conclusion, both methods of collagen determination (hydroxyproline assay and sirius red collagen staining) demonstrated significant increases in pancreatic interstitial collagen with the induction of SHOP. This increase began between 48 and 72 hours after the initial injury and worsened with time. Early, progressive collagen deposition makes SHOP an ideal model to study the pathophysiology of pancreatic fi-

brosis. Although duct obstruction alone leads to pancreatic fibrosis, the desired result takes weeks. Administration of multiple cerulein doses results in the upregulation of collagen messengerRNA and production of protein but no fibrosis. The combination of the two in the SHOP model causes an apparently persistent and progressive fibrosis that is evident as early as 48 hours after the initial injury, making the SHOP model well suited to study the early phases of pancreatic fibrogenesis.

REFERENCES

1. Elsasser H, Haake T, Grimmig M, Adler G, Kern HF. Repetitive cerulein-induced pancreatitis and pancreatic fibrosis in the rat. Pancreas 1992;7:385-390.
2. Imamura T, Iguchi H, Manabe T, Ohshio G, Yoshimura T, Wang Z, Suwa H, Ishigami S, Imamura M. Quantitative analysis of collagen and collagen subtypes I, III, and V in human pancreatic cancer, tumor-associated chronic pancreatitis, and alcoholic chronic pancreatitis. Pancreas 1995;11:357-364.
3. Kloppel G, Maillet B. The morphological basis for the evolution of acute pancreatitis into chronic pancreatitis. Virchows Arch A Pathol Anat 1992;420:1-4.
4. Riaz C, Ochi K, Tanaka J, Harada H, Ichimura M, Miki H. Does recurrent acute pancreatitis lead to chronic pancreatitis? Sequential morphological and biochemical studies. Pancreas 1997;14:334-341.
5. Suda K, Mogaki M, Oyama T, Matsumoto Y. Histopathologic and immunohistochemical studies on alcoholic pancreatitis and chronic obstructive pancreatitis: Special emphasis on ductal obstruction and genesis of pancreatitis. Am J Gastroenterol 1990;85:271-276.
6. Tiscornia O, Palasciano G, Sarles H. Effects of chronic ethanol administration on canine exocrine pancreatic secretion. Digestion 1974;11:172-182.
7. Singh M, LaSure MM, Bockman DE. Pancreatic acinar cell function and morphology in rats chronically fed an ethanol diet. Gastroenterology 1982;82:425-434.
8. Fell B, King TP, Davies NT. Pancreatic atrophy in copper-deficient rats: Histochemical and ultrastructural evidence of a selective effect on acinar cell. Histochem J 1982;14:665-680.
9. Sparmann G, Merkord J, Jaschke A, Nizze H, Jonas L, Lohr M, Liebe S, Emmrich J. Pancreatic fibrosis in experimental pancreatitis induced by dibutylin dichloride. Gastroenterology 1997;112:1664-1672.
10. Kakugawa Y, Paraskevas S, Metrakos P, Giaid A, Qi SJ, Duguid WP, Rosenberg L. Alterations in pancreatic microcirculation and expression of endothelin-1 in a model of chronic pancreatitis. Pancreas 1996;13:89-95.
11. Puig-Divi V, Molero X, Salas A, et al. Induction of chronic pancreatic disease by trinitrobenzene sulfonic acid infusion into rat pancreatic ducts. Pancreas 1996;13:417-424.
12. Walker NI, Winterford CM, Kerr JFR. Ultrastructure of the rat pancreas after experimental duct ligation. II. Duct and stromal cell proliferation, differentiation, and deletion. Pancreas 1992;7:420-434.
13. Bergman I, Losley R. Two improved and simplified methods for the spectrophotometric determination of hydroxyproline. Anal Chem 1962;35:1961-1965.
14. Murayama KM, Joehl RJ. Chronic pancreatitis. In Cameron J, ed. Current Surgical Therapy, 6th ed. St. Louis: Mosby, 1989, p 514-519.
15. Austin JL, Roberts C, Rosenholtz MJ, Reber HA. Effect of partial duct obstruction and drainage on pancreatic function. J Surg Res 1980;28:426-433.
16. Churg A, Richter WR. Early changes in the exocrine pancreas of the dog and rat after ligation of the pancreatic duct. Am J Pathol 1971;63:521-546.
17. Pitkaranta P, Kivisaari L, Nordling S, Saari A, Schroder T. Experimental chronic pancreatitis in the pig. Scand J Gastroenterol 1989;24:987-992.
18. Gress TM, Muller-Pillasch F, Elsasser HP, Bachem M, Ferrara C, Weidenbach H, Lerch M, Adler G. Enhancement of transforming growth factor β1 expression in the rat pancreas during regeneration from caerulein-induced pancreatitis. Eur J Clin Invest 1994;24:679-685.
19. Elsasser HP, Adler G, Kern HF. Fibroblast structure and function during regeneration from hormone-induced pancreatitis in the rat. Pancreas 1989;4:169-178.
20. Walker NI. Ultrastructure of the rat pancreas after experimental duct ligation: The role of apoptosis and intraepithelial macrophages in acinar cell deletion. Am J Pathol 1987;126:439-451.
21. Cummings MC, Winterford CM, Walker NI. Apoptosis. Am J Surg Pathol 1997;21:88-101.
22. Gukovskaya AS, Perkins P, Zaninovic V, Sandoval D, Rutherford R, Fitzsimmons T, Pandol SJ, Poucell-Hatton S. Mechanisms of cell death after pancreatic duct obstruction in the opossum and the rat. Gastroenterology 1996;110:875-884.
23. Doi R, Wada M, Hosotani R, Lee JU, Koshiba T, Fujimoto K, Mori C, Nakamura N, Shiota K, Imamura M. Role of apoptosis in duct obstruction-induced pancreatic involution in rats. Pancreas 1997;14:39-46.
24. Bockman DE. Morphology of the exocrine pancreas related to pancreatitis. Microsc Res Tech 1997;37:509-519.

Palliative Superselective Intra-Arterial Chemotherapy for Advanced Nonresectable Gastric Cancer

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From November 1988 to May 1996, a prospective randomized study was undertaken to assess the efficacy of superselective intra-arterial chemotherapy for surgically proved unresectable gastric carcinoma. Each patient had undergone endoscopy as well as abdominal and pelvic CT scanning for staging. Patients with evidence of liver metastasis, peritoneal carcinomatosis, enlarged retroperitoneal lymph nodes, or locally advanced disease beyond curative resection were excluded from the study. A total of 386 patients with potentially curable disease were randomized to one of three treatment groups: (1) control; (2) systemic intravenous chemotherapy; or (3) superselective intra-arterial chemotherapy. On completion of preoperative chemotherapy, all patients underwent operative exploration with curative intent. A total of 74 consecutive patients were found to be unresectable, as evidenced by the presence of liver metastasis, peritoneal carcinomatosis, enlarged retroperitoneal lymph nodes, or locally extensive disease not detected by preoperative CT scanning. The median survival time in the control group and after intravenous chemotherapy was only 91 and 96 days, respectively, as compared to 401 days in the patients receiving intra-arterial chemotherapy. The results confirmed that superselective intra-arterial chemotherapy conferred a highly significant survival advantage compared to control or systemic intravenous chemotherapy adjusted for all patient characteristics ($P < 0.0001$). (J GASTROINTEST SURG 1999;3:426-431.)

KEY WORDS: Intra-arterial chemotherapy, gastric cancer, palliative treatment

A recent study of gastric cancer mortality rates in six geographic areas (United States, Eastern Europe, Western Europe, East Asia, Oceania, and Scandinavia) demonstrated a substantial decline in the number of deaths from stomach cancer, resulting primarily from a decreasing incidence of this type of cancer.[1] Despite the lower incidence, gastric adenocarcinoma remains a highly malignant neoplasm with a poor prognosis, especially in patients with unresectable disease. Nearly all patients who cannot undergo curative surgery die within a few months after exploration with or without palliative surgery. Different modalities of conservative treatment including radiation and chemotherapy do not demonstrate any survival benefits. Single-agent chemotherapy with drugs such as 5-fluorouracil (5-FU), mitomycin, doxorubicin, and cisplatin has achieved overall response rates ranging from 19% to 30%.[2] However, randomized controlled trials, with rare exception, fail to show a survival advantage for combination therapy in cases of advanced gastric cancer.[2] These circumstances underscore the need for new, more effective strategies for the treatment of gastric cancer. We propose a method for treatment of patients with advanced nonresectable gastric cancer that employs preoperative superselective catheterization of the gastric vessels supplying these tumors for the administration of intra-arterial chemotherapy.

PATIENTS AND METHODS

Patient Population

All patients with biopsy-proved gastric cancer diagnosed between November 1988 and May 1996 were admitted to the Department of Abdominal Tumors at the Ukrainian Institute of Oncology and Radiology in Kiev. Each patient had undergone upper endoscopy with biopsy, in addition to abdominal and

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pelvic CT scans for staging. Patients with evidence of liver metastasis, peritoneal carcinomatosis, enlarged retroperitoneal lymph nodes, or locally advanced disease beyond curative resection as seen on CT were excluded from the study. A total of 386 patients with potentially curable disease were randomized to one of three treatment groups: (1) control; (2) systemic intravenous chemotherapy; or (3) superselective intra-arterial chemotherapy. Patients were assigned to treatment groups by random selection of sealed envelopes. Intra-arterial chemotherapy was administered after approval by a local human investigations committee. Informed consent was obtained from all patients before randomization.

On completion of preoperative chemotherapy, all patients underwent operative exploration with curative intent. A total of 74 consecutive patients underwent exploration and were found to be unresectable, as evidenced by the presence of liver metastasis, peritoneal carcinomatosis, enlarged retroperitoneal lymph nodes, or locally extensive disease not detected by preoperative CT scanning. The study group was comprised of 51 men and 23 women ranging in age from 31 to 72 years (mean 48 years). These patients were then closely followed postoperatively to determine whether systemic intranvenous chemotherapy or superselective intra-arterial chemotherapy had any impact on survival in unresectable patients, and they are the focus of this report.

Twenty-six (35%) of the unresectable patients received no treatment and they represent the control group, 23 (31%) received preoperative systemic intravenous chemotherapy, and 25 (34%) received preoperative superselective intra-arterial chemotherapy. Excluded from the analysis were two patients who underwent gastrectomy 2.5 to 3 months after the initial course of intra-arterial chemotherapy and exploration because of significant improvement in their clinical status and a decrease in the size of their tumors. Three patients who lacked follow-up data were also excluded from the study. All five of the excluded patients were from the intra-arterial chemotherapy group.

Fig. 1. Catheterization of the left gastric artery for preoperative superselective intra-arterial chemotherapy.

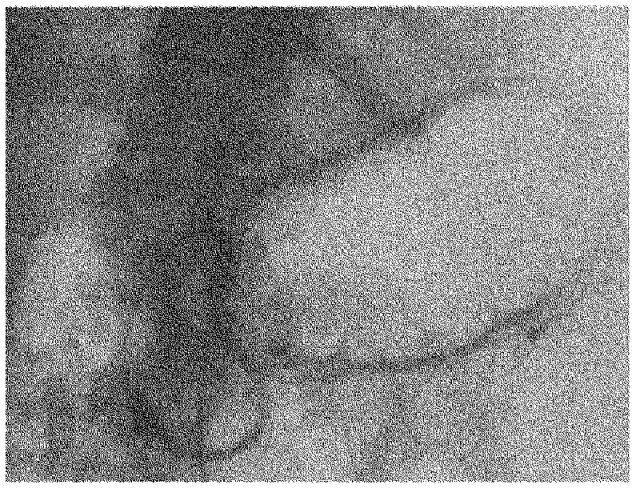

Fig. 2. Catheterization of the right gastroepiploic artery for preoperative superselective intra-arterial chemotherapy.

Catheterization Technique

Under local anesthesia, percutaneous femoral artery puncture was accomplished by the Seldinger technique. The catheter was directed to a superselective location by manipulation through the abdominal aorta and celiac axis and into the left gastric artery, or through the common hepatic and then the gastroduodenal artery into the right gastroepiploic artery. Distal placement in the arterial supply to the involved organ distinguishes superselective intra-arterial chemotherapy from regional infusions delivered through a more proximal vessel. The choice of which vessel to use for this selective catherization was dependent on the location of the tumor. In 60% (15/25) of patients whose tumors were located on the lesser curvature, the left gastric artery was catheterized (Fig. 1). In 40% (10/25) of patients whose tumors were located on the pylorus, greater curvature, or fundus, the right gastroepiploic artery was catheterized (Fig. 2). The catheter was positioned in the artery

as close to the neoplasm as possible so that the infusion bathed the bulk of the tumor but spared normal tissue to the extent possible. Angiographic checks of catheter position and arterial distribution were performed before each chemotherapy infusion to ensure satisfactory positioning of the catheter. The catheter was removed under fluoroscopic guidance immediately after the second infusion, and surgery was performed 7 to 10 days after the completion of chemotherapy.

Chemotherapy Regimen

5-FU and doxorubicin were selected as the chemotherapeutic agents for both intra-arterial and intravenous chemotherapy. Dosages were determined using an actual body surface area calculation and a standard height and weight nomogram. The intra-arterial chemotherapy cycle was as follows: 1000 mg of 5-FU per square meter of body surface area and 30 mg of doxorubicin per square meter of body surface area given simultaneously on day 1. Each drug was separately diluted in two 500 ml flasks. The contents of the flasks were administered over a period of 5 to 6 hours through the appropriate artery. A high-pressure slow infusion rate was achieved using an infusion pump. The drug infusion was administered in two cycles with a 6-day interval between them approximately 2 weeks before surgery. The intravenous regimen was given at the same dosage and timing as the intra-arterial regimen. On noninfusion days, heparinized saline solution alone was infused slowly to maintain the patency of the catheter.

Statistical Methods

Proportions were compared using the exact finite-sample distribution of the Pearson chi-square test. Mean age was compared using the two-sample t test. Survival curves were plotted using the Kaplan-Meier method[3] and compared using the log-rank test.[4] Time was measured from the first day of treatment. Multivariate analysis of prognostic factors was conducted via Cox proportional-hazards regression.[5] The proportional-hazards assumption was checked using the diagnostic procedures of Grambsch and Therneau.[6] Diagnostic plots based on the Grambsch-Therneau method were used to investigate whether the effect of any prognostic factor varied over time.[6] All tests were two sided and P values less than 0.05 were considered significant. Surgery, intra-arterial manipulation, administration of chemotherapy, endoscopy, pathologic examination, and statistical analysis were performed by a single team.

RESULTS

Patient distribution based on the different criteria that influence the prognosis of the disease is shown in Table 1. There were no significant differences among the three treatment groups in terms of age, sex, or TNM stage.

No significant side effects were seen during superselective intra-arterial chemotherapy. During infusions, 48% (12/25) of patients had experienced a slight pain in the epigastric region, 44% (11/25) had nausea, and 12% (3/25) had vomiting secondary to the treatment. Symptoms of systemic chemotherapy drug toxicity were also minimal and were detected in 20% (5/25) of patients after intra-arterial infusions and in 26% (6/23) of patients after intravenous injections. Regular hematologic assessment was performed every 3 to 4 days during treatment. A significant decrease in the white blood cell count (less than 3.0×10^9) was not detected in any of the patients.

Because of the small number of patients in each group, we could not stratify patients into subgroups based on similar patterns of tumor spread in the abdominal cavity nor could we separately determine results of treatment in patients with locally advanced tumors, liver metastases, or peritoneal carcinomatosis. Survival of patients was determined in only three different treatment groups. Sixty-eight deaths were observed among the 69 available patients. The median survival in the control group and after intravenous chemotherapy was only 91 and 96 days, respectively, as compared to 401 days after intra-arterial chemotherapy (Fig. 3). The survival benefit of superselective intra-arterial chemotherapy was highly significant in comparison with the two other groups ($P < 0.0001$).

To adjust the treatment comparison for potential confounding factors, we fitted a series of Cox proportional-hazards regression models that included as explanatory variables the treatment assignment as well as the patient characteristics shown in Table I. The first model included all patient characteristics; however, no solution was possible because of overspecification (i.e., the sample size was too small to accommodate the fitting of such a large model). In the second model we separated TNM stage into two main effects (tumor [T] status and node [N] status, the latter defined as a continuous variable) and were able to obtain a model fit. The results confirmed that superselective intra-arterial chemotherapy conferred a highly significant survival advantage compared to surgery alone and to systemic intravenous chemotherapy, adjusted for all patient characteristics ($P < 0.0001$). The small sample size precluded a detailed study of other prognostic factors.

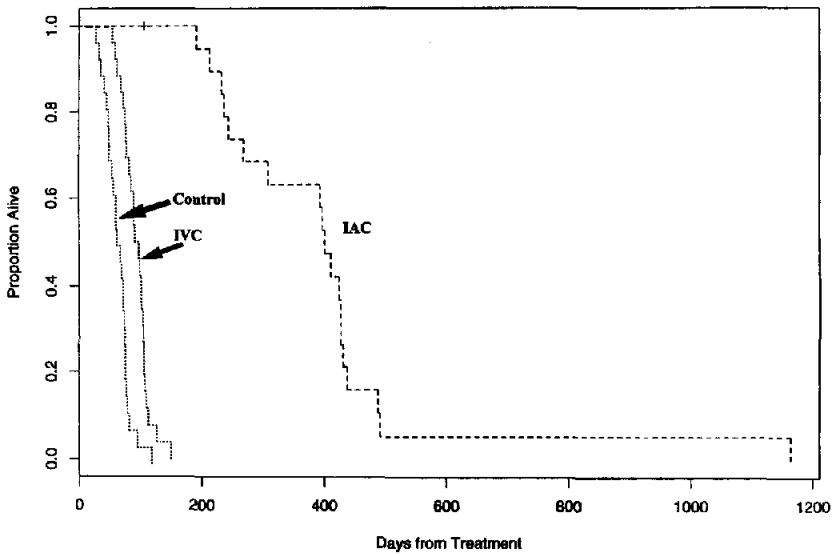

Fig. 3. Overall survival in the various treatment groups. Median survival in the control group (n = 26) and after systemic intravenous chemotherapy (*IVC*; n = 23) was only 91 and 96 days, respectively, as compared to 401 days after superselective intra-arterial chemotherapy (*IAC*; n = 20). The results showed that superselective intra-arterial chemotherapy conferred a highly significant survival advantage compared to control or systemic intravenous chemotherapy adjusted for all patient characteristics ($P < 0.0001$).

Table I. Distribution of patient characteristics in three different treatment groups

Characteristics	Control (n = 26)	IVC (n = 23)	IAC (n = 20)	*P*-value*
Sex				
Male	65%	63%	60%	0.76
Female	35%	37%	40%	
Age (yr)				
Mean	51	50	46	0.11
Median	51	49	43	
Standard deviation	9.1	8.5	8.9	
Range	35-68	36-66	33-59	
Differentiation				
Poor	38%	34%	30%	0.53
Undifferentiated	62%	58%	45%	
Unknown	0%	8%	25%	
TNM stage				
T3N0M1	8%	7%	5%	0.47
T3N1M1	8%	9%	5%	
T3N2M1	4%	5%	0%	
N3N3M1	8%	4%	5%	
N3NxM1	4%	6%	5%	
T4N0M1	0%	9%	15%	
T4N1M1	38%	32%	15%	
T4N2M1	23%	22%	30%	
T4N3M1	4%	3%	5%	
T4NxM1	4%	3%	15%	
Type of operation				
Exploration	46%	53%	55%	0.77
Gastrointestinal anastomosis	54%	47%	45%	

*Test of no association between trial arm and patient characteristic.
IVC = systemic intravenous chemotherapy; IAC = superselective intra-arterial chemotherapy.

DISCUSSION

The development of more effective and less toxic treatment options for gastric cancer is the goal of many researchers. Recent data clearly indicate the benefit of preoperative neoadjuvant chemotherapy for patients with gastric cancer,[7,8] whereas traditional postoperative adjuvant treatment shows no such advantage.[2] Wilke et al.[7] summarized the most recent experience in the administration of neoadjuvant chemotherapy for gastric cancer. Preoperative chemotherapy is easy to administer and is not accompanied by additional operative morbidity or mortality compared to surgery alone. Wilke et al.[7] also described a regimen of etoposide, doxorubicin, and cisplatin that induces significant tumor regression, allowing for curative resection in 40% to 50% of patients with previously unresectable cancers.

Preoperative intra-arterial chemotherapy is a somewhat more sophisticated neoadjuvant method of cancer treatment. As we reported previously, preoperative superselective intra-arterial chemotherapy followed by radical gastrectomy provides a substantial survival benefit when used as a component of a combined-modality gastric cancer treatment.[9] After intra-arterial chemotherapy, a measurable tumor response was registered in 87.1% of the patients; in 61.6% no residual tumor was found in the resected stomach. Systemic intravenous chemotherapy produced no such survival benefit relative to surgery alone. Intra-arterial chemotherapy followed by surgery improved 2-year survival relative to surgery alone (95.6 ± 4.3% vs. 49.5 ± 5%; $P < 0.01$).[9] The theoretical advantages of intra-arterial chemotherapy over intravenous chemotherapy include the following: preoperative chemotherapy provides increased drug concentrations at the tumor site and decreased systemic drug levels and toxicity, and allows for continuous tumor exposure to chemotherapeutic agents with the possibility of systemic rescue. The lymph nodes draining the stomach (which are also supplied by the celiac axis) receive cytotoxic perfusion, and the liver is also infused directly by higher concentrations of cytotoxic agents from both the hepatic artery and the portal venous circulation.[10] One could hypothesize that preoperative superselective intra-arterial chemotherapy would only benefit unresectable patients where tumors are isolated to the region of perfusion (i.e., locally advanced gastric cancers). Patients with liver metastases would also benefit because venous drainage of the chemotherapy perfusion is via the portal circulation. It is difficult to believe that patients with evidence of retroperitoneal nodal disease would benefit from this modality. Because the number of patients in the superselective intra-arterial chemotherapy arm of the study was small, it is difficult to determine which subset of patients benefited from this treatment modality.

The clinical efficacy of adjuvant therapy for gastric cancer clearly depends on the type of chemotherapy drug, the mode of administration, and the number of cycles. The present study did not show any survival benefit for patients with unresectable gastric cancer after one course of systemic intravenous administration of 5-FU and doxorubicin. However, Ajani et al.[11] demonstrated that administration of two to three cycles of etoposide, 5-FU, and cisplatin induces clinically complete or partial responses in 24% and is feasible for gastric cancer patients despite substantial toxicity.[12]

Administration of the same regimen of chemotherapy drugs for preoperative superselective intra-arterial chemotherapy in our study showed a significant survival benefit for gastric cancer patients in comparison to the control group or those receiving traditional intravenous neoadjuvant chemotherapy. We did not achieve the same dramatic results as Wilke et al.[7] who achieved downstaging of the primary tumor in 40% to 50% of patients with unresectable cancer, which thus increased the chances of complete macroscopic and microscopic tumor resection on subsequent surgery. The design of our study did not allow us to check this hypothesis because all patients treated with preoperative superselective intra-arterial chemotherapy followed by curative or potentially curative surgery were not surgically staged prior to chemotherapy.[9] However, two patients who received superselective intra-arterial chemotherapy followed by exploratory laparotomy underwent potentially curative gastrectomy within 2.5 to 3 months after the initial treatment because of significant improvement in clinical status and a decrease in tumor size. Long-term survival in one patient was 2.3 years and in a second patient was 4.2 years. Taking into consideration the significant improvement in patient survival after superselective intra-arterial chemotherapy, we can only suggest that curative or potentially curative surgery may be possible to perform in a larger number of patients.

CONCLUSION

Preoperative superselective intra-arterial chemotherapy significantly improves survival in patients with unresectable gastric cancer and may be effective in downstaging the disease, which makes a secondary curative or potentially curative resection possible. Further investigation of the efficacy of preoperative superselective intra-arterial chemotherapy in differ-

ent stratified subgroups of patients will help to identify those patients who may benefit from neoadjuvant superselective intra-arterial chemotherapy.

REFERENCES

1. Noel DG, Davis DL, Miller AB. Trends in cancer mortality in 15 industrialized countries, 1969-1986. J Natl Cancer Inst 1992;84:313-320.
2. Kelsen D. The use of chemotherapy in the treatment of advanced gastric and pancreatic cancers. Semin Oncol 1994;21 (Suppl 7):58-66.
3. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J Am Statist Assoc 1958;53:457-481.
4. Mantel N. Evaluation of survival data and two new rank order statistics arising in its consideration. Cancer Chemother Rep 1966;50:163-170.
5. Cox DR. Regression models and life tables (with discussion). J R Statist Soc, Series B 1972;34:187-220.
6. Grambsch P, Therneau TM. Proportional hazards tests and diagnostics based on weighted residuals. Biometrika 1994; 81:515-526.
7. Wilke H, Stahl M, Fink U, Meyer HJ, Siewert JR. Preoperative chemotherapy for unresectable gastric cancer. World J Surg 1995;19:210-215.
8. Fink U, Stein HJ, Schuhmacher C, Wilke HJ. Neoadjuvant chemotherapy for gastric cancer: Update. World J Surg 1995;19:509-516.
9. Shchepotin IB, Evans STR, Chorny V, Ugrinov O, Osinsky S, Galakhin K, Troitsky I, Buras RR, Shabahang M, Nauta RJ. Pre-operative superselective intra-arterial chemotherapy in the combined treatment of gastric carcinoma. Oncol Rep 1995;2:473-479.
10. Stephens FO, Johnson AW, Crea P. Pre-operative basal chemotherapy in the management of cancer of the stomach. Med J Aust 1984;140:143-147.
11. Ajani JA, Ota DM, Jessup JM, Ames FC, McBride C, Boddie A, Levin B, Jackson DE, Roh M, Hohn D. Resectable gastric carcinoma: An evaluation of preoperative and postoperative chemotherapy. Cancer 1991;68:1501-1506.
12. Ajani JA, Mayer RJ, Ota DM, Steel GD, Evans D, Roh M, Sugarbaker DJ, Dumas P, Gray C, Vena DA, Stablein DM. Preoperative and postoperative combined chemotherapy for potentially resectable gastric carcinoma. J Natl Cancer Inst 1993;85:1839-1844.

Enterotrophic Effects of Glucagon-Like Peptide 2 Are Enhanced By Neurotensin

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Combination therapy with enterotrophic agents may be useful in patients with the short bowel syndrome. The gut hormones neurotensin (NT) and glucagon-like peptide 2 (GLP-2) are potent enterotrophic factors when administered alone; however, their combined effects are not known. Using a GLP-2–producing tumor (STC-1), we determined whether administration of NT enhances the effect of GLP-2 on intestinal growth. Athymic mice were injected with STC-1 cells (6×10^6) subcutaneously. Twenty-three days after STC-1 implantation, mice received either NT (300 μg/kg or 600 μg/kg) or saline solution (control) subcutaneously three times a day for 6 days. Two groups of tumor-free mice received either saline or NT for 6 days. At sacrifice, jejunum and ileum were collected, weighed, and analyzed for DNA and protein content. In the jejunum, NT combined with GLP-2 (from STC-1) increased weight, protein content (markers of mucosal hypertrophy), and DNA content (a marker of mucosal hyperplasia), compared to either NT or GLP-2 alone. In the ileum, the combination of NT and GLP-2 significantly increased weight and/or protein content compared to NT or GLP-2 alone. Administration of NT enhances the enterotrophic effects of GLP-2, augmenting hypertrophy of the entire small bowel and hyperplasia of the jejunum. The combination of NT and GLP-2 may be useful to enhance intestinal growth in patients with the short bowel syndrome. (J GASTROINTEST SURG 1999;3:432-440.)

KEY WORDS: Intestinal growth factors, short bowel syndrome, glucagon-like peptide 2, neurotensin

Intestinal regeneration and adaptation following disease or injury is a highly organized process that results in growth (i.e., hyperplasia and hypertrophy) of the remnant gut mucosa and, in most cases, can effectively compensate for the loss of mucosal mass.[1-3] Intestinal growth is a complex process involving the effects of multiple factors including intraluminal pancreatobiliary secretions and nutrients[1,2] and selected humoral intestinotrophic factors such as epidermal growth factor,[4] insulin-like growth factor-1,[5] and growth hormone.[6] In addition, certain regulatory gut peptides or hormones, such as bombesin,[7,8] neurotensin (NT),[9-12] and glucagon-like peptide 2 (GLP-2),[13,14] contribute to gut growth. In particular, NT has been shown to stimulate gut regeneration in rats after massive small bowel resection,[11] and both NT and GLP-2, a 33-amino acid peptide secreted from L-type enteroendocrine cells of the intestine,[13,14] have been shown to reduce elemental diet– or total parenteral nutrition (TPN)–induced gut atrophy in rats.[12,15] Although the exact roles of these gut hormones in intestinal regeneration and adaptation have not been entirely defined, our laboratory has been interested in the potential clinical use of these agents to maintain or restore gut function during periods of disease or injury.

Deficiencies in intestinal regeneration and adaptation become clinically significant when the remaining functional intestine is unable to compensate completely for massive mucosal loss (e.g., massive intesti-

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nal resection).[1-3] In addition, pathologic conditions such as Crohn's disease and chemotherapy-induced enterocolitis may cause malabsorption secondary to a loss of mucosal surface area. When there has been a loss of a critical mass of intestinal mucosa, severe malabsorption characterized by incapacitating diarrhea, dehydration, and malnutrition may ensue and is often referred to as the short bowel syndrome. The short bowel syndrome is a devastating medical condition that usually requires lifelong TPN and severely disrupts the affected individual's productivity. Operative strategies to treat the short bowel syndrome by reversal of intestinal segments to slow intestinal transit or by small bowel transplantation have not been effective.[1-3,16] Therefore the administration of enterotrophic factors for patients with the short bowel syndrome may provide an attractive strategy to augment gut growth and alleviate the need for TPN[17,18]; however, single-agent therapy in these patients has proved largely ineffective.[17] Combined therapy with different intestinotrophic factors may be more useful than agents given separately. Support of this hypothesis is provided by a recent study suggesting that the combination of the amino acid glutamine, which is an essential energy source for enterocytes, and growth hormone may be more efficacious, compared to either agent alone, in reducing the requirement of TPN in certain patients with the short bowel syndrome.[18] However, growth hormone is diabetogenic and, moreover, exerts a nonspecific growth response in a number of different tissues with effects that are not limited to the intestinal mucosa.[6] Therefore these effects of growth hormone may potentially limit its clinical usefulness. Novel enterotrophic agents are needed to more effectively and selectively treat patients with the short bowel syndrome.

The purpose of our study was to assess the combined effects of the potent enterotrophic factors NT and GLP-2 on small bowel growth in mice. We used the transplantable rat glucagonoma cell line, STC-1, which secretes GLP-2[19,20] and induces significant small bowel growth in athymic nude mice,[13] as a delivery system of "endogenous" GLP-2.

MATERIAL AND METHODS

Cell Culture

STC-1 cells were a generous gift from Dr. Andrew B. Leiter (New England Medical Center, Boston, Mass.). As has been described in detail elsewhere,[19,20] STC-1 cells were derived from an intestinal glucagonoma in transgenic mice and secrete significantly greater concentrations of proglucagon-derived peptides, such as GLP-2, compared to the concentrations of cholecystokinin and secretin, which are also secreted. STC-1 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) with penicillin (100 U/ml) and streptomycin (100 μg/ml), supplemented with 15% (volume/volume) horse serum and 2.5% fetal bovine serum. The human pancreatic adenocarcinoma cell line, MIA PaCa-2,[21] obtained from the American Type Culture Collection (Rockville, Md.), was used as a control for tumor implantation on small bowel growth. MIA PaCa-2 cells were maintained in DMEM with 10% fetal bovine serum. Both STC-1 and MIA PaCa-2 cell lines were cultured in a 95% humidified incubator with 5% carbon dioxide at 37° C.

Animals

Male athymic nude mice (Balb/c, 22 to 27 g, 5 weeks old) (Life Science, St. Petersburg, Fla.) were housed under specific pathogen-free conditions in a temperature-controlled (22° C) isolation unit with 12-hour light/dark cycles, in accordance with the National Research Council recommendations.[22] The mice were fed a standard chow (Autoclavable Rodent Chow No. 5010, Ralston Purina, St. Louis, Mo.) and sterile water, both given ad libitum.

Peptide Preparation

A stock solution of NT (Bachem California, Torrance, Calif.) was prepared by first dissolving the amount needed for the study in 1 ml of sterile water with 1% (weight/volume) bovine serum albumin (Sigma Chemical Co., St. Louis, Mo.) and then diluted to the required concentration with saline containing 1% bovine serum albumin. Equal portions of this solution, sufficient for a single subcutaneous injection of all animals of a given group, were stored in plastic vials at −20° C. Saline solution containing 1% bovine serum albumin (control) was divided into aliquots and stored at −20° C. To prolong absorption, control or NT solutions were mixed 1:4 (volume:volume) with 16% (weight/volume) hydrolyzed gelatin (Sigma Chemical Co.).

Experimental Protocol

Two independent experiments were performed to evaluate the effects of two different dosages of NT (300 μg/kg and 600 μg/kg) either alone or combined with GLP-2 (produced from the STC-1 cells). In each experiment, STC-1 cells were gently trypsinized with 0.25% trypsin, and then the STC-1 cell suspension (6×10^6 cells/0.15 ml) was injected subcutaneously into athymic nude mice. Tumors were allowed to grow for 23 days. After 23 days, tumors were

measured and tumor-bearing mice (n = 6 per group) were weighed and randomized to two groups to receive subcutaneous injections of either NT (at a dosage of 300 μg/kg three times a day for the first experiment or 600 μg/kg three times a day for the second experiment) or saline with bovine serum albumin (control) three times a day for 6 days. In addition, two groups of tumor-free mice also received either NT (300 μg/kg subcutaneously three times a day for the first experiment or 600 μg/kg subcutaneously three times a day for the second experiment) or saline solution (control) subcutaneously three times a day. The dosages of NT used in this study previously have been shown to be the most effective in stimulating bowel growth in mice and rats, with the larger dose representing the maximal effective dosage.[9-12] At sacrifice (day 30), mice were weighed and small intestine (from ligament of Treitz to cecum) was collected. The small intestine was bisected with the proximal half designated jejunum and the distal half designated ileum. The intestinal segments were opened longitudinally and luminal contents were removed by flushing with cold saline solution and gentle blotting. All segments were blotted dry, weighed, and immediately frozen at −70° C until assayed for DNA and protein content. A small portion of each intestinal segment was preserved in 10% buffered formaldehyde for histologic evaluation.

As a control for the potential effects of tumor implantation on small bowel growth, MIA PaCa-2 cells (6×10^6), a nonendocrine pancreatic adenocarcinoma, were similarly injected subcutaneously into a separate group of mice (n = 6). After 30 days, these mice were killed, and intestine was removed and analyzed as for the other groups of mice.

DNA and Protein Determination

Full-thickness tissues were thawed and homogenized (Polytron, Kinematica GmbH, Kriens-Luzem, Switzerland). DNA content was measured by the Burton[25] modification of the diphenylamine procedure with calf thymus DNA used as the standard. Protein content was determined by the method of Lowry et al.,[26] with bovine serum albumin as the standard.

Statistical Analysis

Intestinal weight, DNA, and protein content were expressed as mean ± standard error of the mean (SEM) and analyzed using one-way classification analysis of variance with Fisher's least significant difference for multiple comparisons. A P value <0.05 was considered significant.

RESULTS

The tumor-bearing mice lost a significant amount of body weight compared to tumor-free mice (data not shown); therefore results were normalized for body weight. The implantation of the nonendocrine pancreatic cancer, MIA PaCa-2, did not affect intestinal growth, compared to tumor-free mice (data not shown). Therefore, as previously noted by others,[13] these results confirm that the trophic effects in mice bearing the GLP-2–producing STC-1 tumor are specific for this tumor and not a nonspecific effect related to tumor placement.

Effects of NT and GLP-2 on the Jejunum

NT, at the dosage of 300 μg/kg, significantly augmented the effects of GLP-2 on jejunal growth (Fig. 1). NT by itself (in tumor-free mice) increased bowel weight by 21% and protein content by 17%, compared to the control group. In addition, GLP-2 by itself (in STC-1–bearing mice treated with saline) resulted in a 32% increase in jejunal weight, a 100% increase in DNA content, and a 27% increase in protein content compared to control mice. Compared to NT, GLP-2 also significantly increased DNA content by 45%. The combination of NT (300 μg/kg) and GLP-2 significantly increased jejunal weight and protein content, but not DNA content, compared to all the other groups.

Increasing the dosage of NT to 600 μg/kg significantly enhanced the effects of GLP-2 on jejunal DNA content, as well as both weight and protein content (Fig. 2). NT (600 μg/kg), by itself, induced significant increases in jejunal weight and protein content, as was noted for NT at the dosage of 300 μg/kg. In addition, NT (600 μg/kg) produced a 36% increase in DNA content compared to control. The combination of NT (600 μg/kg) with GLP-2 produced a significant increase in DNA content compared to either NT or GLP-2 alone (53% and 16% increase, respectively). Histologic examination of sections of jejunum for each of the four treatment groups demonstrated increased crypt-to-villus height (an indicator of mucosal growth) in the STC-1–bearing mice (Fig. 3); truncated villi noted in these sections were secondary to mechanical shearing forces during processing.

Effects of NT and GLP-2 on the Ileum

NT at the 300 μg/kg dosage significantly enhanced the trophic effects of GLP-2 on the ileum (Fig. 4). As we have shown previously, NT (300 μg/kg) alone preferentially affected the jejunum,[10,27] and produced minimal growth of the ileum of athymic nude mice.[27]

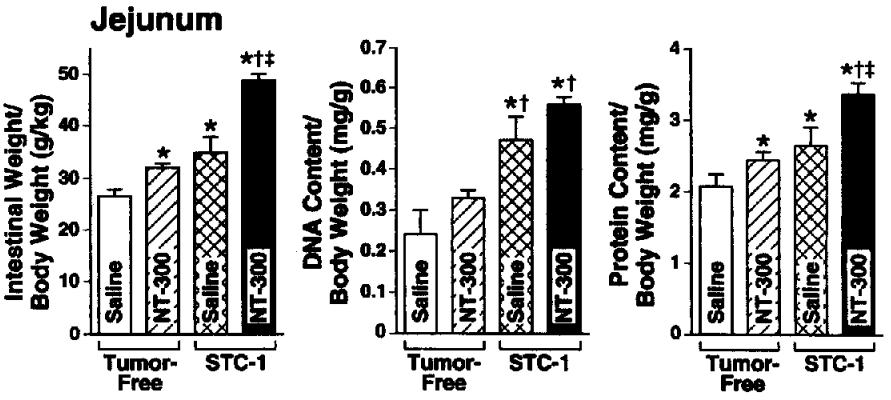

Fig. 1. Combined effects of 300 μg/kg neurotensin (NT) and GLP-2 (from STC-1 tumors) on jejunal weight, DNA, and protein content. To evaluate the effects of NT alone, tumor-free mice were treated with either saline *(open bar)* or NT *(NT-300; single-hatched bar)*. To evaluate the effects of GLP-2, either alone or in combination with NT, STC-1–bearing mice were treated with either saline *(double-hatched bar)* or NT *(NT-300; closed bar)*, respectively. Data represent mean ± SEM; * = $P < 0.05$ vs. tumor-free mice treated with saline; † = $P < 0.05$ vs. tumor-free mice treated with NT; ‡ = $P < 0.05$ vs. STC-1–bearing mice treated with saline.

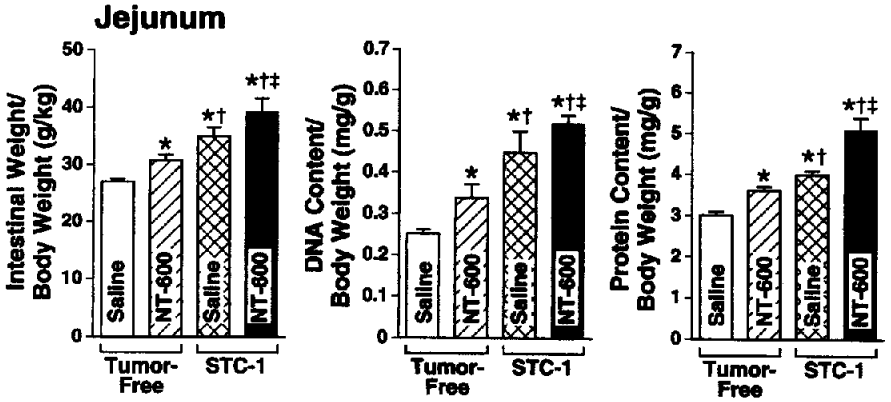

Fig. 2. Combined effects of 600 μg/kg neurotensin (NT) and GLP-2 (from STC-1 tumors) on jejunal weight, DNA, and protein content. To evaluate the effects of NT alone, tumor-free mice were treated with either saline *(open bar)* or NT *(NT-600; single-hatched bar)*. To evaluate the effects of GLP-2, either alone or in combination with NT, STC-1–bearing mice were treated with either saline *(double-hatched bar)* or NT *(NT-600; closed bar)*, respectively. Data represent mean ± SEM; * = $P < 0.05$ vs. tumor-free mice treated with saline; † = $P < 0.05$ vs. tumor-free mice treated with NT; ‡ = $P < 0.05$ vs. STC-1–bearing mice treated with saline.

GLP-2 by itself resulted in significant increases in weight (21% increase) and protein content (25% increase) of the ileum compared to control. However, NT, in combination with GLP-2 (in STC-1 mice), resulted in a 29% increase in weight compared to GLP-2 alone and a 46% increase compared to NT alone. The combination of these two peptides also significantly increased ileal protein content by 34% compared to NT alone.

When the dosage of NT was increased to 600 μg/kg, there was a significant increase in ileal protein content (Fig. 5), which was not evident when the smaller dosage of NT was used. As in the first experiment, we noted that GLP-2 alone increased all indices of ileal growth compared to control and to NT by itself. Similar to our results with NT (300 μg/kg), the combination of NT (600 μg/kg) in STC-1–bearing mice resulted in significantly greater ileal hy-

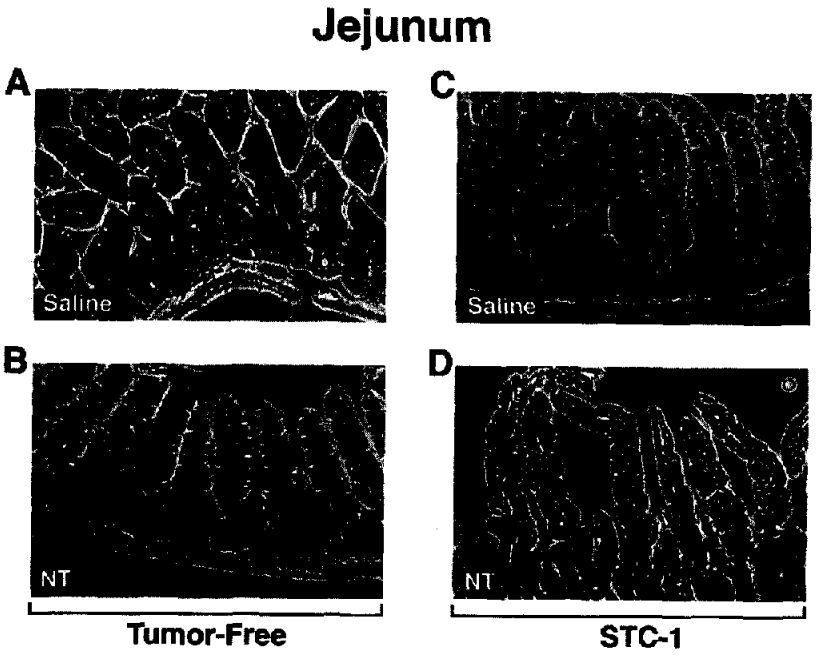

Fig. 3. Representative histologic sections of jejunum from tumor-free mice treated with either **(A)** saline or **(B)** neurotensin (NT) or from STC-1–bearing mice treated with either **(C)** saline or **(D)** NT. (Original magnification × 200.)

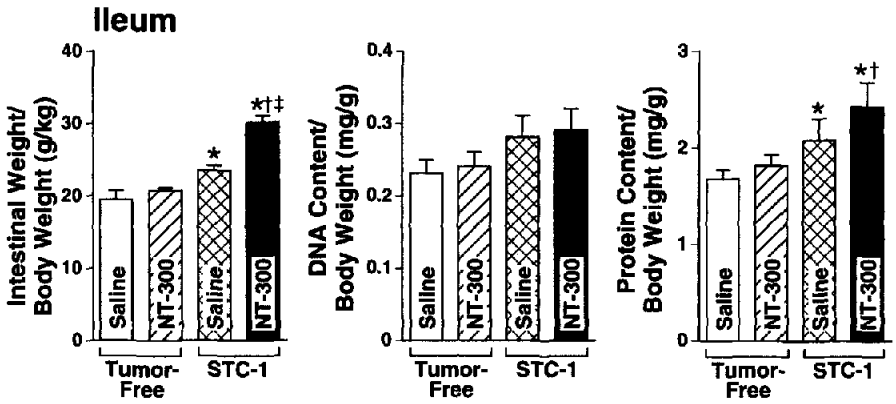

Fig. 4. Combined effects of 300 μg/kg neurotensin (NT) and GLP-2 (from STC-1 tumors) on ileal weight, DNA, and protein content. To evaluate the effects of NT alone, tumor-free mice were treated with either saline *(open bar)* or NT *(NT-300; single-hatched bar)*. To evaluate the effects of GLP-2, either alone or in combination with NT, STC-1–bearing mice were treated with either saline *(double-hatched bar)* or NT *(NT-300; closed bar)*, respectively. Data represent mean ± SEM; * = $P < 0.05$ vs. tumor-free mice treated with saline; † = $P < 0.05$ vs. tumor-free mice treated with NT; ‡ = $P < 0.05$ vs. STC-1–bearing mice treated with saline.

pertrophy, reflected by the 24% increase in protein content, compared to NT alone and the 14% increase, compared to GLP-2 by itself. As for the jejunum, our quantitative results for the ileum were confirmed by examination of histologic sections of ileum for the four groups, which demonstrated increased crypt-villus height and mucosal thickness in STC-1–bearing mice treated with NT (Fig. 6).

Collectively, our findings demonstrate that the combination of NT (300 μg/kg) and GLP-2 results in greater jejunal weight and protein content and ileal weight compared to either agent alone. Furthermore, when the maximal effective dosage of NT (600 μg/kg) was used in combination with GLP-2, there also was an increase in jejunal DNA content compared to either agent by itself.

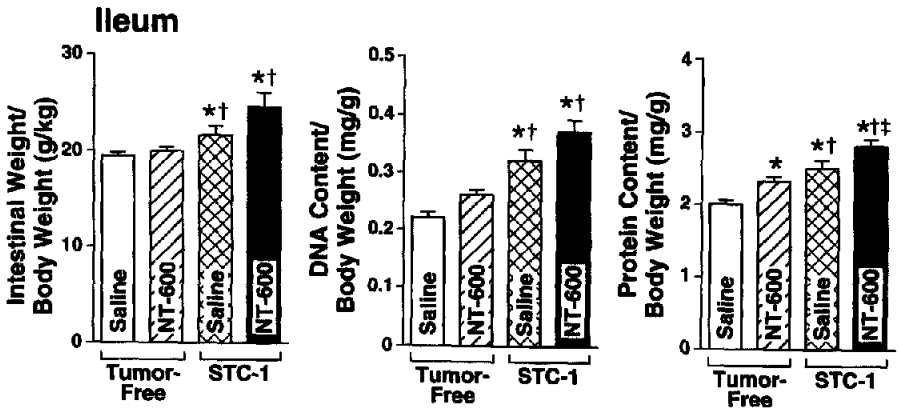

Fig. 5. Combined effects of 600 μg/kg neurotensin (NT) and GLP-2 (from STC-1 tumors) on ileal weight, DNA, and protein content. To evaluate the effects of NT alone, tumor-free mice were treated with either saline *(open bar)* or NT *(NT-600; single-hatched bar)*. To evaluate the effects of either GLP-2 alone or in combination with NT, STC-1–bearing mice were treated with either saline *(double-hatched bar)* or NT *(NT-600; closed bar)*, respectively. Data represent mean ± SEM; * = $P < 0.05$ vs. tumor-free mice treated with saline; † = $P < 0.05$ vs. tumor free mice treated with NT; ‡ = $P < 0.05$ vs. STC-1–bearing mice treated with saline.

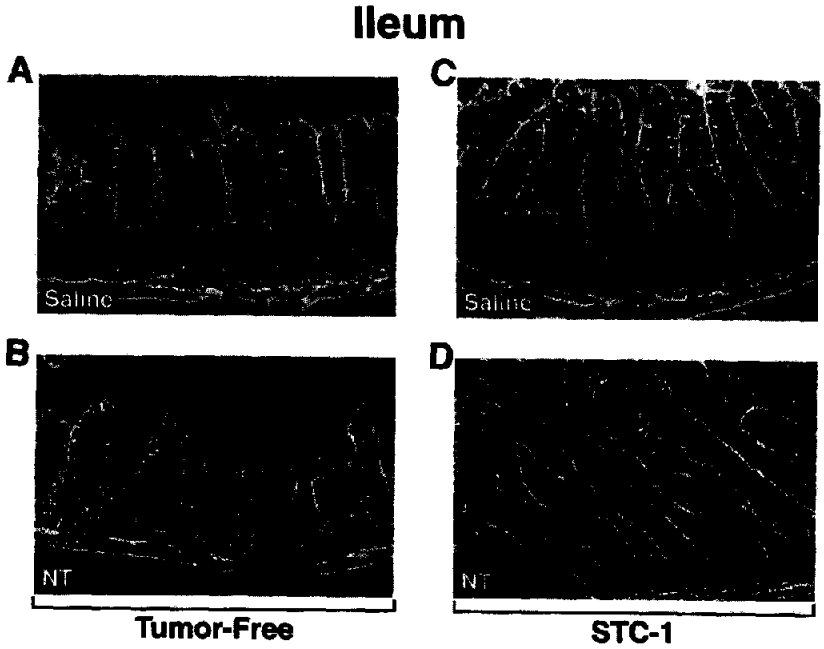

Fig. 6. Representative histologic sections of ileum from tumor-free mice treated with either **(A)** saline or **(B)** neurotensin (NT) or from STC-1–bearing mice treated with either **(C)** saline or **(D)** NT. (Original magnification ×200.)

DISCUSSION

Using GLP-2–secreting STC-1 tumors in athymic nude mice, we have demonstrated that NT augments the enterotrophic effects of GLP-2. We have confirmed previous findings demonstrating that both NT and GLP-2 are potent intestinal trophic factors when used as single agents[9-14] and that GLP-2 is more effective than NT in stimulating bowel growth in mice.[27] In addition, we have shown that NT (300 μg/kg), in combination with GLP-2 (from STC-1), induces greater small bowel hypertrophy (i.e., weight or protein content) than either agent alone. Further-

more, the maximal effective dosage of NT (600 μg/kg) combined with GLP-2 resulted in increased jejunal hyperplasia, in addition to small bowel hypertrophy.

The combined effects of NT and GLP-2 resulted in small bowel mucosal hypertrophy and hyperplasia. Similar to our previous findings,[10-12,27] we confirmed that NT, as a single agent, has a dose-related preferential effect on growth of the jejunum. When the dosage of NT was increased to 600 μg/kg, we noted jejunal hyperplasia, as well as ileal hypertrophy. Previously NT has been shown to produce a maximal trophic effect in rats at a dosage of 600 μg/kg.[9-12] Similar to our findings for NT, we also found that GLP-2 (secreted from STC-1 tumors) exerted a preferential trophic effect on the jejunum, inducing both hyperplasia and hypertrophy, whereas in the ileum GLP-2 affected only ileal hypertrophy. Our findings of gut growth for "endogenous" GLP-2 from STC-1 tumors are similar to our previous findings for exogenously administered GLP-2 in athymic nude mice.[27] Furthermore, our results are consistent with the findings of Drucker et al.[13] demonstrating that small bowel growth from STC-1–bearing athymic nude mice was comparable to that noted after GLP-2 administration in CD1 mice. Moreover, in our present study we noted that the combination of NT and GLP-2 increased bowel growth more than either agent alone. When we treated STC-1–bearing mice with NT at the dosage of 300 μg/kg, an increase in both jejunal and ileal hypertrophy was demonstrated, compared to tumor-free mice treated with NT or STC-1–bearing mice treated with saline solution. Increasing the dosage of NT to 600 μg/kg produced more pronounced jejunal hyperplasia compared to either agent (NT or GLP-2) alone. Although our findings suggest that the effects of GLP-2 and NT are additive, the exact biologic response and cellular mechanisms induced by each agent have not been completely characterized. Similar to our findings, Drucker et al.[28] have recently demonstrated that administration of either growth hormone or insulin-like growth factor-1 with GLP-2 increases small bowel growth in mice more than either of the three agents alone. Collectively the results of our present study suggest that the combination of NT and GLP-2 may be more effective than either agent alone in augmenting small bowel growth.

Attempting to increase intestinal mucosal growth during the reparative and compensatory processes of intestinal regeneration and adaptation is an attractive strategy to decrease or prevent the severe sequelae of the short bowel syndrome. Following massive loss of overall mucosal surface area, intestinal regeneration and adaptation occur as three phases over weeks to years: the first phase, which takes place in the first 1 to 3 months, marks the beginning of mucosal hyperplasia and is the most critical to the patient; the second phase, which lasts 1 to 2 years, represents the majority of intestinal adaptation; and the third phase marks the finality of adaptation.[1-3] It has been postulated that the administration of enterotrophic factors to patients during these phases potentially may ameliorate or prevent the devastating clinical consequences of the short bowel syndrome.[11,17,18] Previously investigators from our laboratory have demonstrated the efficacy of the regulatory gut peptide, bombesin, in reducing deaths from methotrexate-induced enterocolitis in rats[8] and the gut hormone NT in preventing elemental diet–induced gut atrophy in rats.[12] In addition, Chance et al.[15] have shown that GLP-2 prevents TPN-induced gut atrophy in rats. However, previous pilot studies have shown that single-agent therapy is largely ineffective for patients with the short bowel syndrome.[17] In contrast to the disappointing results reported for single-agent therapy, a recent clinical study by Byrne et al.[18] suggests that combined therapy with glutamine and growth hormone may be helpful in weaning selected patients with massive intestinal mucosal loss from continuous TPN. However, the clinical usefulness of administering growth hormone to patients may be limited by its diabetogenic and nonselective trophic effects.[6] Therefore novel enterotrophic agents that selectively target growth of the intestine are needed. Both NT and GLP-2 appear to have a more specific trophic effect on the intestine. Although NT has been shown to produce hyperplasia of the pancreas and growth of the gastric antrum in rats,[29] investigators from our laboratory have noted minimal effects on other organ systems outside the gastrointestinal tract.[9-12] In addition, GLP-2 appears to have primarily intestinotrophic effects.[13]

Based on the findings of our present study, as well as previous studies from our laboratory,[10-12,27] we conclude that NT and GLP-2 are more selective trophic factors for the gut than other agents (such as growth hormone) and, in combination, are more effective in stimulating intestinal growth than either agent alone. Furthermore, the combination of NT and GLP-2 may provide a more effective treatment strategy to augment gut growth in patients during periods of disease (e.g., Crohn's disease, chemotherapy-induced enterocolitis) and the short bowel syndrome.

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REFERENCES

1. Williamson RC. Intestinal adaptation. N Engl J Med 1978; 298:1393-1402, 1444-1450.
2. Shanbhogue LKR, Molenaar JC. Short bowel syndrome: Metabolic and surgical management. Br J Surg 1994;81:486-499.
3. Dowling RH, Booth CC. Functional compensation after small-bowel resection in man. Lancet 1966;1:146-147.
4. Goodland RA, Wilson TJG, Lenton W, Gregory H, McCullagh KG, Wright NA. Proliferative effects of urogastrone-EGF on the intestinal epithelium. Gut 1987;28:37-43.
5. Ohneda K, Ulshen MH, Fuller CR, D'Ercole AJ, Lund PK. Enhanced growth of small bowel in transgenic mice expressing human insulin-like growth factor I. Gastroenterology 1997;112:444-454.
6. Hart MH, Phares CK, Erdman SH. Augmentation of postresection mucosal hyperplasia by plerocercoid growth factor (PGF)-analog of human growth hormone. Dig Dis Sci 1987;32:1275-1280.
7. Chu KU, Evers BM, Ishizuka J, Townsend CM Jr, Thompson JC. Role of bombesin on gut mucosal growth. Ann Surg 1995; 222:94-100.
8. Chu KU, Higashide S, Evers BM, Rajaraman S, Ishizuka J, Townsend CM Jr, Thompson JC. Bombesin improves survival from methotrexate-induced enterocolitis. Ann Surg 1994;220: 570-577.
9. Wood JG, Hoang HD, Bussjaeger LJ, Solomon TE. Neurotensin stimulates growth of small intestine in rats. Am J Physiol 1988;255:G813-G817.
10. Chung DH, Evers BM, Shimoda I, Townsend CM Jr, Rajaraman S, Thompson JC. Effect of neurotensin on gut mucosal growth in rats with jejunal and ileal Thiry-Vella fistulas. Gastroenterology 1992;103:1254-1259.
11. Izukura M, Evers BM, Parekh D, Yoshinaga K, Uchida T, Townsend CM Jr, Thompson JC. Neurotensin augments intestinal regeneration after small bowel resection in rats. Ann Surg 1992;215:520-527.
12. Evers BM, Izukura M, Townsend CM Jr, Uchida T, Thompson JC. Neurotensin prevents intestinal mucosal hypoplasia in rats fed an elemental diet. Dig Dis Sci 1992;37:426-431.
13. Drucker DJ, Ehrlich P, Asa SL, Brubaker PL. Induction of intestinal epithelial proliferation by glucagon-like peptide 2. Proc Natl Acad Sci USA 1996;93:7911-7916.
14. Drucker DJ. Epithelial cell growth and differentiation I. Am J Physiol 1997;273:G3-G6.
15. Chance WT, Foley-Nelson T, Thomas I, Balasubramaniam A. prevention of parenteral nutrition-induced gut hypoplasia by coinfusion of glucagon-like peptide-2. Am J Physiol 1997; 273:G559-G563.
16. Berger DL, Malt RA. Management of the short gut syndrome. Adv Surg 1996;29:43-57.
17. Byrne TA, Morrissey TB, Nattakom TV, Ziegler TR, Wilmore DW. Growth hormone, glutamine, and a modified diet enhance nutrient absorption in patients with severe short bowel syndrome. JPEN 1995;19:296-302.
18. Byrne TA, Persinger RL, Young LS, Ziegler TR, Wilmore DW. A new treatment for patients with short-bowel syndrome. Growth hormone, glutamine, and a modified diet. Ann Surg 1995;222:243-255.
19. Rindi G, Grant SGN, Yiangou Y, Ghatei MA, Bloom SR, Bautch VL, Solcia E, Polak JM. Development of neuroendocrine tumors in the gastrointestinal tract of transgenic mice. Am J Pathol 1990;136:1349-1363.
20. Ehrlich P, Tucker D, Asa SL, Brubaker PL, Drucker DJ. Inhibition of pancreatic proglucagon expression in mice bearing subcutaneous endocrine tumors. Am J Physiol 1994;267: E662-E671.
21. Yunis AA, Arimura GK, Russin DJ. Human pancreatic carcinoma (MIA PaCa-2) in continuous culture: Sensitivity to asparaginase. Int J Cancer 1997;19:218-235.
22. Committee on Care and Use of the "Nude" Mouse. Guide for the care and use of the nude (thymus-deficient) mouse in biomedical research. ILAR News 1976;19:M1-20.
23. Wang HL. A site-directed mutagenesis study on the conserved alanine residue in the distal third intracellular loops of cholecystokinin B and neurotensin receptors. Br J Pharmacol 1997;121:310-316.
24. Bold RJ, Lowry PS, Ishizuka J, Battey JF, Townsend CM Jr, Thompson JC. Bombesin stimulates the in vitro growth of a human gastric cancer cell line. J Cell Physiol 1994;161:519-525.
25. Burton K. A study of the conditions and mechanisms of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochemistry 1956;162:315-323.
26. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265-275.
27. Litvak DA, Hellmich MR, Evers BM, Banker NA, Townsend CM Jr. Glucagon-like peptide 2 is a potent growth factor for small intestine and colon. J GASTROINTEST SURG 1998;2:146-150.
28. Drucker DJ, DeForest L, Brubaker PL. Intestinal response to growth factors administered alone or in combination with human [Gly2]glucagon-like peptide 2. Am J Physiol 1997;273: G1252-G1262.
29. Feurle GE, Muller B, Rix E. Neurotensin induces hyperplasia of the pancreas and growth of the gastric antrum in rats. Gut 1987;28(Suppl 1):19-23.

Discussion

Dr. J. Thompson (Omaha, Neb.). In terms of the clinical implications of this study, both the duration and timing of therapy are important issues. GLP-2 was being produced for 23 days, but NT was given for 7 days. How did you choose this regimen? Do you know anything about the mechanism of these two agents that would suggest that this is the best regimen in terms of sequence and duration?

Dr. D. Litvak. The model was based on a previous study of GLP-2 that compared exogenous GLP-2 to GLP-2 produced by STC-1–bearing mice. We have investigated the model to determine the optimal duration of treatment with GLP-2 from STC-1 and allow at least 7 to 10 days to ensure tumor implantation. The 30-day time course in total, however, was based on a previous study. The mechanisms of

action of both of these peptides are incompletely defined, and we hope that future studies may determine the best way to administer these agents.

Dr. B. Bass (Baltimore, Md.). Have you measured the serum or tissue levels of GLP-2 in these animals to confirm that these tumors are actually generating a reasonable amount of the peptide product? Additionally, have you tried immunoneutralization studies or specific antagonists to determine whether this observed enhanced trophic effect is specific to the GLP-2?

Dr. Litvak. Our laboratory has not established a radioimmunoassay to evaluate the serum levels of GLP-2. We have relied on pervious studies with STC-1 cells, which describe continuously elevated secreted levels of proglucagon-derived peptides, including GLP-2. Our results are consistent with our previous study in which GLP-2 was administered exogenously. The use of neutralizing antibodies has been considered for the future.

Dr. R. Hodin (Boston, Mass.). GLP-2 and NT had a greater effect together than either alone, suggesting that they work via different mechanisms. Do they stimulate different signal transduction pathways?

Dr. Litvak. Whether this is a synergistic or additive effect is really not known. It is a question we are actively investigating in vitro, but we have not been able to identify cell lines that possess a GLP-2 receptor, at least based on the ability to mobilize calcium or cyclic adenosine monophosphate.

Effect of Nutritional Route on Colonic Anastomotic Healing in the Rat

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Although early enteral feeding has been shown to benefit cutaneous healing when compared to parenteral feeding, the effect of the route of nutritional support in gastrointestinal anastomotic healing has not been defined. The aim of the present study was to determine whether the route of nutritional support influences colonic anastomotic healing. Twenty male Sprague-Dawley rats weighing 270 to 290 grams underwent identical surgical manipulation consisting of central venous catheterization, gastrostomy insertion, and distal colonic anastomosis (single-layer, inverted). Identical nutrient infusates composed of 4.25% amino acids, 25% dextrose, and vitamins were administered, with half the animals receiving the infusions via the gastrostomy and the other half via the venous catheter. Animals were killed 5 days after surgery. There were no differences in nutritional parameters between the parenterally and enterally fed groups. Colonic anastomotic bursting pressure was significantly higher in the enterally fed group (180 ± 6 vs. 150 ± 11 mm Hg; $P < 0.01$). The measured insoluble collagen and total protein content in anastomotic tissue were enhanced in the enterally supported group. The fraction of soluble (newly synthesized) collagen did not differ between the two groups. The data demonstrate that the route of nutrient administration influences colonic anastomotic healing. The preservation of colonic structural collagen in the enteral group may improve the ability of the gut to hold sutures and thus enhance anastomotic healing. (J GASTROINTEST SURG 1999;3:441-446.)

KEY WORDS: Colon anastomosis, enteral-enteral nutrition

Dehiscence of colonic anastomoses remains a serious and potentially fatal postoperative complication.[1] Many systemic and local factors contribute to the success or failure of the gastrointestinal healing process.[2] Among these, the nutritional status of the patient is very important and may at times play a pivotal role in the patient's ability to heal. Both prolonged malnutrition and short-term inadequate nutritional intake diminish anastomotic healing.[3]

Nutritional support is most frequently used as short-term adjunctive therapy for patients with protein-calorie malnutrition.[4] Postoperative parenteral nutrition can partially reverse the impaired colonic anastomotic healing observed in protein-malnourished rats.[5,6] Brief and not necessarily full-target nutritional intervention can increase the wound collagen deposition noted with postoperative starvation typified by the dextrose/saline regimen that most patients receive.[7] Although immediate nutritional support has been shown to enhance experimental wound healing,[8] routine postoperative nutritional support does not improve the overall clinical outcome of patients undergoing upper gastrointestinal tract surgery for malignancy.[9]

The route of nutrient administration can affect various functional parameters as well as outcome. Experimental data have demonstrated the superiority of enteral feeding in maintaining local and systemic immune responses, preserving gut structure and function, and improving protein metabolism and survival.[10-12] Trauma patients supported with enteral nutrition demonstrate significantly fewer septic complications when compared to similar patients receiving parenteral nutrition.[13]

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There is little information regarding the effect of the route of nutrient administration on wound healing. We have shown that enteral feeding enhances cutaneous wound-breaking strength and collagen deposition in rats[14]; the effect is significant only during the early postinjury phase. Since there is no information regarding the effect of the feeding route on colonic anastomotic healing, we designed a study to examine this issue in a rat model of colonic anastomotic healing.

MATERIAL AND METHODS

Animals

Twenty male Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, Ind.) weighing 260 to 280 grams were randomized to either enteral or parenteral treatments. All animals were allowed 1 week of acclimatization to our laboratory conditions prior to experimentation. Rats were housed at 21° C, with 12-hour light/dark cycles, and allowed free access to tap water and standard chow-type food (Teklad LM-485, Harlan-Teklad, Madison, Wis.). All experiments were approved by the Sinai Hospital Institutional Animal Care and Utilization Committee and met the National Institutes of Health guidelines for animal experimentation.

Operative Procedure

On day 0 all rats were anesthetized by intraperitoneal injection of sodium pentobarbital at a dose of 50 mg/kg. Under aseptic conditions, jugular vein catheterization to a central venous position was carried out using 0.02 inch inner diameter × 0.037 inch outer diameter silicone tubing (Konigsberg Instruments, Pasadena, Calif.). Next a vertical midline incision was made on the abdomen, the stomach was identified, delivered through the abdominal wound, and punctured with a 19-gauge needle. A second sterile silicone tube was threaded into the stomach through the puncture site and secured with a 5-0 silk pursestring suture. The distal ends of both catheters were tunneled subcutaneously and brought out in the most cephalad portion of the interscapular area. The catheters were fixed to the skin using a Teflon button attached to a swivel assembly.

The distal colon was then divided 2 cm proximal to the peritoneal reflection and a single-layer, inverted, end-to-end colocolostomy was performed with interrupted 6-0 Prolene sutures (Ethicon, Inc., Somerville, N.J.). The fascial layer of the abdominal wall was closed with a continuous 3-0 silk suture and the abdominal skin approximated with stainless steel staples (U.S. Surgical Corp., Norwalk, Conn.). When rats were infused enterally, the intravenous catheters were occluded, whereas in the intravenously infused rats (total parenteral nutrition), the gastrostomy catheters were occluded. Following the operative procedures, animals were housed in individual metabolic cages.

Nutrient Solution

All nutritional solutions containing dextrose, amino acids, electrolytes, minerals, and vitamins were prepared by the clinical pharmacy of Sinai Hospital of Baltimore (Table I). Identical solutions were administered both enterally and parenterally. Continuous infusions via infusion pumps (Bard Medsystems, North Reading, Mass.) were begun immediately after the operation, at 50% target for the first 24 hours, and at full target thereafter. The calculated 100% full energy intake was 216 kcal/kg body weight/day, corresponding to an infusion rate of 9.0 ml/hr/kg body weight.

Nutritional Parameters

Urine was collected daily in acidified containers. After 5 days on the respective regimens, the infusions were stopped. The rats were killed with a lethal intraperitoneal dose of sodium pentobarbital. Cardiac blood was drawn for determination of serum glucose, total protein, albumin, and blood urea nitrogen levels.

Anastomotic Bursting Pressure

After the animals were killed, the abdomen was opened and colonic bursting pressure was measured in situ. A 16-gauge silicone rubber catheter was inserted via a colotomy into the proximal colon and held in position with a 3-0 silk suture. The distal rectum and colon proximal to the anastomosis were ligated with 3-0 silk sutures. Normal saline solution was continuously infused through the catheter via a syringe pump (Harvard Apparatus, Inc., South Natick, Mass.) at a rate of 1.0 ml/min. Intraluminal pressure was monitored continuously via a transducer (Trantec, Baxter Healthcare Corp., Irvine, Calif.) and simultaneously

Table I. Composition of infusates per 1000 ml

Glucose	25%
Amino acids*	4.25%
Tracelyte + Double electrolytes†	20 ml
Ascorbic acid	80 mg/L
Thiamine	1.2 mg/L
Folic acid	0.14 mg/L

*10% FreAmine III (McGaw Inc., Irvine, Calif.).
†Fujisawa USA, Inc., Deerfield, Ill.

recorded on a 120-channel chart recorder (ABB Goerz SE, Fisher Scientific Co., Pittsburgh, Pa.). Bursting pressure was recorded as peak pressure attained before disruption of the anastomosis.

Determination of Anastomotic Collagen and Total Protein Content

Following bursting, the anastomosis was excised along with 0.5 cm of proximal and distal colon, cleared of surrounding fat, and rinsed with saline solution. The 1 cm segments were frozen for subsequent determination of hydroxyproline content (as an index of wound reparative accumulation), α-amino nitrogen (as an index of total protein), and collagen messengerRNA analysis.

Collagen was fractionated into soluble and insoluble fractions by a method adapted from Irvin and Hunt.[15] Briefly, colon segments were weighed (wet) and minced into fragments of less than 1 mm. The salt-soluble fraction was initially extracted by three 24-hour incubations at 4° C in a solution containing 0.15 mol/L NaCl in TRIS buffer (pH 7.2). Tissue fragments were removed by centrifugation and the supernates were frozen at 20° C until assay of hydroxyproline content was performed. Acid-soluble collagen was similarly extracted from the residual colon tissue fragments with a 0.5 mol/L acetic acid solution. The residual colon fragments were designated as the insoluble collagen fraction.

Salt- and acid-soluble extracts were evaporated to dryness at 110° C and hydrolyzed in 6N hydrochloric acid at 130° C for 3 hours. Insoluble colon fragments were similarly hydrolyzed. Hydroxyproline analysis of all samples was performed by the method of Woessner.[16]

Aliquots of the acid hydrolysate for each sample were reserved for α-amino nitrogen determination.[17]

Anastomotic Content of Collagen Type I and III MessengerRNA

Harvested anastomotic segments were minced, homogenized immediately in TRIzol reagent (Life Technologies, Inc., Gaithersburg, Md.), and stored at −70° C until processing. Total RNA was extracted, washed three times in 75% ethanol, and resuspended in DEPEC H_2O (1% diethylpyrocarbonate, prepared by vigorously boiling; Sigma Chemical, St. Louis, Mo.). An aliquot was spectrophotometrically measured at Å260/Å280 to assess for purity and to ensure equal loading. RNA was then subjected to 1.2% agarose-formaldehyde gel electrophoresis and subsequently transferred to a nylon membrane by capillary transfer. The RNA was cross-linked by incubating blots at 80° C for 90 minutes. The blots were prehybridized overnight in buffer containing 50% formamide, 5% Denhardt's solution, 5× concentration SSPE buffer, 1% sodium dodecyl sulfate, and 100 μg/ml salmon sperm DNA, and subsequently hybridized overnight in the same solution as above but with ^{32}P-d(CTP)–labeled collagen I or III probes in place of the salmon sperm DNA. After washing, autoradiography was performed at −70° C. Densitometric analysis was performed using the Stratagene Eagle Eye II video camera and software package (Stratagene, La Jolla, Calif.). Results are expressed as ratio of enteral vs. parenteral groups; the parenteral group value was set at 1.0.

Strict adherence to guidelines outlined by the National Institutes of Health and the Sinai Hospital of Baltimore Radiation Safety Committee was observed in the receipt, storage, handling, and disposal of radioactive material.

Statistical Analysis

All data are expressed as mean ± standard error of the mean. Statistical comparisons were made using the unpaired Student's *t* test (StatView II, Abacus Concepts, Berkeley, Calif.); 95% confidence level was chosen for statistical significance.

RESULTS

Three animals were excluded from analysis within 24 hours of operation. One rat died as a result of anesthesia, one central venous catheter did not function, and there was one pump failure resulting in no nutritional infusion for longer than 8 hours. All three animals were in the parenteral group.

The remaining animals appeared healthy and tolerated the nutritional and operative treatments well. Initial body weight as well as postoperative body weight gains were similar in the two groups. The mean daily energy intake and the mean daily urine volume did not differ between the two groups (Table II). Average daily water balance, calculated as (fluid intake) − (urine volume), was positive in

Table II. Nutritional parameters in the two experimental groups

	ENT	TPN
Body weight (g)	273 ± 2	271 ± 2
Body weight gain (g/5 days)	5.4 ± 3.2	9.6 ± 2.7
Energy intake (kcal/day)	50.7 ± 1	50.8 ± 1.5
Urine volume (ml/day)	29.5 ± 0.7	30.3 ± 1.1

ENT = enterally fed; TPN = total parenteral nutrition.

Table III. Biochemical nutritional parameters in the two groups

	ENT	TPN
Glucose (mg/dl)	104 ± 4	93 ± 5
Blood urea nitrogen (mg/dl)	14.7 ± 1	12.6 ± 0.7
Albumin (g/dl)	2.7 ± 0.4	2.5 ± 0.5
Total protein (g/dl)	3.8 ± 0.5	4.2 ± 0.7

ENT = enterally fed; TPN = total parenteral nutrition.

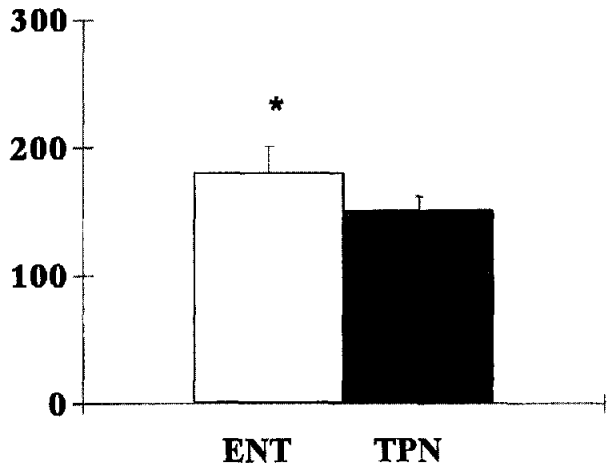

Fig. 1. Anastomotic bursting pressure (mm Hg) in the two experimental groups (ENT = enterally fed; TPN = total parenteral nutrition). Results are mean ± standard error of the mean; * = $P < 0.01$.

both groups and did not differ (21.3 ± 3.2 ml/day and 20.4 ± 3.5 ml/day in the enteral and the parenteral group, respectively). There were no significant differences between the two groups in serum levels of glucose, total protein, albumin, and urea nitrogen (Table III).

There were no perianastomotic abscesses noted in any animals and all tested anastomoses burst at the suture line. Anastomotic bursting pressure was 20% higher in the enterally fed group when compared with the parenterally fed group (180 ± 6 mm Hg vs. 150 mm Hg; $P < 0.01$) (Fig. 1). This higher bursting pressure in the enterally fed rats was accompanied by 17.9% more insoluble collagen measured in the anastomotic tissue (1.91 ± 0.09 μg hydroxyproline/mg wet tissue vs. 1.62 ± 0.13 μg hydroxyproline/mg wet tissue; $P < 0.05$). There was no difference in the amount of soluble collagen between the two groups (0.79 ± 0.04 μg vs. 0.90 ± 0.08 μg hydroxyproline/mg wet tissue) (Fig. 2). Total protein content of the anastomotic tissues, as assessed by the amount of α-amino nitrogen, was 12.4% higher in the enterally fed group than in the parenterally fed group (61.7 ± 1.3 μmol/mg wet tissue vs. 54.9 ± 4.0 μmol/mg wet tissue; $P < 0.05$).

Collagen type III gene transcription at the anastomotic site was greater in the enterally fed group (densitometric value 1.32 vs. 1.00). No difference in type I collagen gene transcription was detected (1.11 vs. 1.00) (Fig. 3).

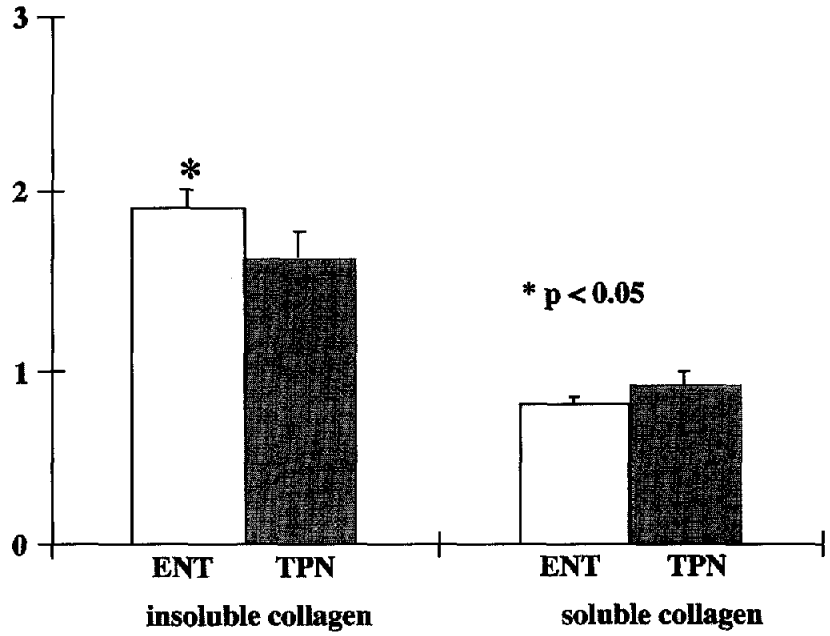

Fig. 2. Content of soluble and insoluble collagen in colonic anastomoses as assessed by the amount of hydroxyproline (μg hydroxyproline/mg wet tissue). ENT = enteral; TPN = total parenteral nutrition; * = $P < 0.05$.

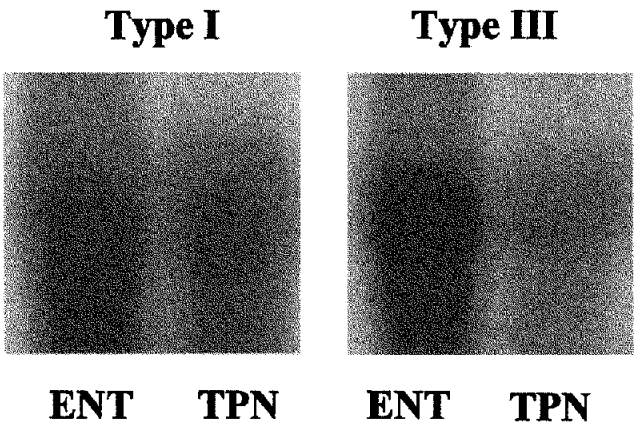

Fig. 3. Collagen gene transcription at the colonic anastomotic site. A representative blot is shown from three similar experiments. ENT = enterally fed; TPN = total parenteral nutrition.

DISCUSSION

The mechanical strength of the intestinal wall lies in the collagenous fibrous network of the submucosa and can be measured experimentally by the bursting strength of an anastomosis.[2] This strength is a reflection of the ability of the bowel to heal after injury. The collagen types that are found in the intestinal wall and contribute to bowel strength are type I, type II, and type V. These make up 68%, 20%, and 12% of the collagen fractions, respectively.[18]

Stabilization of collagen molecules is achieved by lysine-derived covalent bonds as well as the disulfide bond formed as a cross-link between molecules; the more cross-linking that is present, the more mature the collagen.[19] Cross-linking imparts a resistance to solubilization (to both acid and salt), and thus the fraction of insoluble collagen in tissue represents more mature collagen with a higher amount of cross-linking.[20]

Colonic healing is remarkable for early intense collagenolysis that occurs both at and remote from the injured ends of the bowel; this has been implicated as one major reason for anastomotic failure. Bursting pressure reflects the balance between collagen deposition and lysis.[21,22] On postoperative day 5, collagen synthesis reaches maximal transcriptional and translational levels,[23] with type III collagen gene expression preceding that of type I collagen in the colonic anastomosis.[24]

In the present study, enteral feeding resulted in a 20% higher colonic anastomotic bursting pressure when compared to that seen with parenteral feeding. Greater levels of anastomotic tissue hydroxyproline and total protein content accompanied this effect. The enhanced anastomotic healing seen in the enterally fed group was achieved without any apparent differences in nutritional parameters as compared to the parenterally fed animals (see Tables II and III). This suggests that the effect is not a result of nutrient availability but rather due to the route of delivery. Further, the amount of insoluble collagen measured in the anastomoses was 18% greater in the enterally fed group. This increased level of insoluble collagen may be due to elevated collagen synthesis or decreased collagen breakdown during the earliest phases of anastomotic wound healing. Although total protein content in the anastomosis was also 12% greater in the enterally fed group, these data do not allow for clear definition of the mechanism by which enteral feeding increases colonic anastomotic bursting pressure and collagen accumulation. Yet it offers some evidence for additional benefit derived by enteral feeding with regard to cellular function and protein production after injury.

Northern blot analysis of RNA from anastomotic tissue demonstrated enhanced type III collagen gene transcription as a result of enteral nutritional support; type I collagen gene transcription was unaffected. It is unclear whether this represents elevated or sustained production of type III collagen, especially when only a single time point (5 days) is measured. However, the improved healing observed in enterally fed animals appears as a result of enhanced collagen gene transcription and translational levels.

The mechanism by which enteral feeding enhances the colonic anastomotic healing remains to be defined. Enteral feeding has a trophic effect on the mucosa of the gastrointestinal tract. Total parenteral nutrition induces intestinal mucosal atrophy and an increase in cathepsin activity in the small intestine.[25] The enteral feeding of high-calorie, hyperosmolar glucose solution prevents mucosal atrophy and may decrease the activity of proteases.[26]

Although these trophic effects on the mucosa would not contribute to anastomotic strength, it is not known whether similar changes occur following injury in the submucosal layers. These findings suggest that enteral feeding may have a trophic effect on other cells such as smooth muscle cells and fibroblasts, which are the cells most active in collagen synthesis at the anastomotic site.

Two other possible explanations for improved healing with enteral nutrition include the existence of a gut-derived prohealing factor or perhaps an increase in blood flow to the intestines as seen in the postprandial state. Although the former is an attractive hypothesis, there is as yet no evidence for such a factor. The latter, in turn, fails to explain the similar improved healing seen in cutaneous wounds following enteral nutrition.

CONCLUSION

Enteral feeding benefits rat colonic anastomotic healing as compared to parenteral feeding. This benefit is noted at multiple levels of collagen metabolism and is reflected in the measured anastomotic strength. Further investigation is needed to define the mechanism by which the enteral feeding route enhances colonic anastomotic healing.

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REFERENCES

1. Schrock TR, Deveney CW, Dunphy JE. Factors contributing to leakage of colonic anastomoses. Ann Surg 1973;177:513-518.
2. Christensen H, Langfelt S, Laurberg S. Bursting strength of experimental colonic anastomoses. A methodological study. Eur J Surg Res 1993;25:38-45.
3. Barbul A, Purtill WA. Nutrition in wound healing. Clin Dermatol 1994;12:133-140.
4. Souba WW. Nutritional support. N Engl J Med 1997;336: 41-48.
5. Ward MW, Danzi M, Lewin MR, Rennie MJ, Clark CG. The effects of subclinical malnutrition and refeeding on the healing of experimental colonic anastomoses. Br J Surg 1982;69:308-310.
6. Law NW, Ellis H. The effect of parenteral nutrition on the healing of abdominal wall wounds and colonic anastomoses in protein-malnourished rats. Surgery 1990;107:449-454.
7. Haydock DA, Hill GL. Improved wound healing response in surgical patients receiving intravenous nutrition. Br J Surg 1987;74:320-323.
8. Zaloga GP, Bortenschlager L, Black KW, Prielipp R. Immediate postoperative enteral feeding decreases weight loss and improves wound healing after abdominal surgery in rats. Crit Care Med 1992;20:115-118.
9. Heslin MJ, Latkany L, Leung D, Brooks AD, Hochwald SN, Pistersie PW, Shike M, Brennen MF. A prospective, randomized trial of early enteral feeding after resection of upper gastrointestinal malignancy. Ann Surg 1997;226:567-577.
10. Fong YM, Marano MA, Barber A, He W, Moldawer LL, Bushman ED, Coyle SM, Lowry SF. Total parenteral nutrition and bowel rest modify the metabolic response to endotoxin in humans. Ann Surg 1989;210:449-456.
11. Lin MT, Saito H, Fukushima R, Inaba T, Fukatsu K, Inoue T, Furukawa S, Han I, Muto J. Route of nutritional supply influences local, systemic, and remote organ responses to intraperitoneal bacterial challenge. Ann Surg 1996;223:84-93.
12. Delany HM, John J, Teh EL, Li CS, Gliedman ML, Steinberg JJ, Levenson SM. Contrasting effects of identical nutrients given parenterally or enterally after 70% hepatectomy. Am J Surg 1994;167:135-143.
13. Kudsk KA, Croce MA, Fabian TC, Minard G, Tolley EA, Poret HA, Kuhl, MR, Brown RO. Enteral versus parenteral feeding. Effects on septic morbidity after blunt and penetrating abdominal trauma. Ann Surg 1992;215:503-511.
14. Kiyama T, Witte M, Thornton FJ, Barbul A. The route of nutritional support affects the early phase of wound healing. JPEN J Parenter Enteral Nutr 1998;22:276-279.
15. Irvin TT, Hunt TK. Reappraisal of the healing process of anastomosis of the colon. Surg Gynecol Obstet 1974;138:741-746.
16. Woessner J. The determination of hydroxyproline in tissue and protein samples containing small proportions of this amino acid. Ann Biochem Biophys 1961;93:440-446.
17. Moore S, Stein W. Photometric ninhydrin method for use in chromatography of amino acids. J Biol Chem 1948;176:367-369.
18. Graham MF, Blowquist P, Zederfeldt B. The alimentary canal. In Cohen IK, Deigelman RF, Lindblad WJ, eds. Wound Healing: Biochemical and Clinical Aspects. Philadelphia: WB Saunders, 1992, pp 433-449.
19. Miller EJ, Gay S. Collagen structure and function. In Cohen IK, Deigelman RF, Lindblad WJ, eds. Wound Healing: Biochemical and Clinical Aspects. Philadelphia: WB Saunders, 1992, pp 130-151.
20. Ahrendt GM, Gardiner K, Barbul A. Loss of colonic structural collagen impairs healing during intra-abdominal sepsis. Arch Surg 1994;129:1179-1183.
21. Oxlund H, Christensen H, Seyer-Hansen M, Andreassen TT. Collagen deposition and mechanical strength of colon anastomoses and skin incisional wounds of rats. J Surg Res 1996;66:25-30.
22. Chowcat NL, Savage FJ, Lewin MR, Boulos PB. Direct measurement of collagenase in colonic anastomosis. Br J Surg 1990;77:1284-1287.
23. Martens MF, Hendriks T. Postoperative changes in collagen synthesis in intestinal anastomoses of the rat: Differences between small and large bowel. Gut 1991;32:1482-1487.
24. Brasken P, Renvall S, Sandberg M. Fibronectin and collagen gene expression in healing experimental colonic anastomoses. Br J Surg 1991;78:1048-1052.
25. Kishibuchi M, Tsujinaka T, Iijima S, Yano M, Ebisui C, Morimoto, T, Mori T. Interrelation of intracellular proteases with total parenteral nutrition-induced gut mucosal atrophy and increase of mucosal macromolecular transmission in rats. JPEN J Parenter Enteral Nutr 1995;19:187-192.
26. Weser E, Babbitt J, Vandeventer A. Relationship between enteral glucose load and adaptive mucosal growth in the small bowel. Dig Dis Sci 1985;30:675-681.