Pancreatic Cancer: The Role of Adjuvant Therapies

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Introduction

This year's "How I Do It" session of the Pancreas Club dealt with the topic of pancreatic adenocarcinoma, and specifically the role of adjuvant therapies. Undoubtedly, the role of adjuvant therapy in these tumors remains controversial, and this remains one of several "hot issues" in the field of pancreatic cancer (Table I).

The session featured three speakers: Douglas B. Evans, M.D., who discussed the role of preoperative chemoradiation therapy, focusing on its attempt to downstage marginally resectable patients; Ross A. Abrams, M.D., who discussed the current trials dealing with postoperative chemoradiation therapy; and Vincent J. Picozzi, M.D., who discussed novel approaches to postoperative chemoradiation therapy. Brief synopses of their work are presented herein.

Table I. Hot issues in pancreatic cancer

Early detection
Appropriate screening practices
Search for tumor markers
Familial inheritance patterns
Better understanding of heritable cancer
Possible role of chemoprevention
Appropriate screening techniques
Extent of preoperative workup
CT scan
Magnetic resonance imaging
Magnetic resonance cholangiopancreatography
Endoscopic ultrasound
Staging laparoscopy
Peritoneal cytology
Type of surgical resection
Pylorus-preserving pancreaticoduodenectomy vs. classic pancreaticoduodenectomy
Extent of lymph node dissection
Role of adjuvant therapies
Preoperative chemoradiation
Postoperative chemoradiation
Immunotherapy
Other novel approaches

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Preoperative Chemoradiation for Resectable and Locally Advanced Adenocarcinoma of the Pancreas

Douglas B. Evans, M.D.

TREATMENT VARIABLES IN THE STUDY OF ADJUVANT/NEOADJUVANT CHEMORADIATION

Protocol-based clinical trials directed at determining the survival benefit of chemotherapy, external-beam radiation therapy (EBRT), or both (chemoradiation) must include the following: (1) accurate pretreatment staging that uses an objective, reproducible definition for resectable pancreatic cancer; (2) a standardized technique for surgical resection (pancreaticoduodenectomy); and (3) uniform criteria for the pathologic assessment of the pancreaticoduodenectomy specimen. These three potential variables must be standardized to allow accurate analysis of the impact of adjuvant or neoadjuvant therapy on local-regional tumor control and survival duration.

Assessment of Resectability

Accurate preoperative assessment of resectability is the most critical aspect of the diagnostic and treatment sequence for patients with pancreatic cancer. If the primary tumor cannot be resected completely, pancreaticoduodenectomy offers no survival advantage. Patients whose tumors are resected with positive margins have a survival duration of less than 1 year, which is no different from the survival duration achieved with palliative chemotherapy and irradiation in patients who have locally advanced, unresectable disease.[1] Therefore the use of standardized, objective radiologic criteria for preoperative tumor staging is critical for the development of multi-institution clinical trials examining the use of preoperative or postoperative chemoradiation. We advocate a clinical staging system based on high-quality CT images. The CT criteria defining a potentially resectable pancreatic cancer are (1) the absence of extrapancreatic disease, (2) a patent superior mesenteric-portal vein (SMPV) confluence (assuming the technical ability to resect isolated involvement of the superior mesenteric vein [SMV] or SMPV confluence), and (3) no direct tumor extension to the celiac axis or superior mesenteric artery (SMA). A patient is deemed to have locally advanced, unresectable disease when there is clear evidence on CT scans of tumor extension to the SMA or celiac axis or occlusion of the SMPV confluence. The accuracy of CT in predicting unresectability is well established, and laparotomy is not necessary to assess local tumor resectability.[2,3]

Pancreaticoduodenectomy

Our recommended technique for pancreaticoduodenectomy divides the operation into six clearly defined steps.[4] The most oncologically important and difficult part of the operation is step 6, which involves division of the pancreas, mobilization of the SMPV confluence, and removal of the specimen from the right lateral border of the SMA. Failure to fully mobilize the SMPV confluence usually results in a positive margin of resection due to incomplete removal of the uncinate process and the mesenteric soft tissue adjacent to the SMA. Segmental resection of the SMPV confluence is necessary when the tumor is inseparable from the lateral wall of the SMV or portal vein.[5,6] However, such isolated venous resection should be performed only in carefully selected patients who have tumor adherence to the SMV or SMPV confluence but no evidence of tumor extension to the SMA or celiac axis. Invasion of the SMV or portal vein is not associated with histopathologic variables (margin and lymph node positivity) that suggest a poor prognosis, and patient survival after pancreaticoduodenectomy is not affected by the need for venous resection.[5,6]

Pathologic Assessment of the Pancreaticoduodenectomy Specimen

The detailed description of the system in use at our institution for the pathologic evaluation of pancreaticoduodenectomy specimens has been previously pub-

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lished.[7] The most important part of this evaluation is assessment of the retroperitoneal margin (also referred to as the mesenteric margin) of resection along the right lateral border of the SMA. The only way to assess the completeness of resection is to evaluate the retroperitoneal margin by gross and microscopic examination at the time of tumor resection. It is critical that this margin be identified for the pathologist and assessed histologically; this margin cannot be evaluated retrospectively after the gross examination of the specimen is complete.

PREOPERATIVE (NEOADJUVANT) THERAPY

Neoadjuvant Therapy in Patients With Resectable Pancreatic Cancer

Despite the selection bias involved in the enrollment of patients into postoperative adjuvant therapy studies, prospective and retrospective data suggest that the addition of postoperative adjuvant chemoradiation following pancreaticoduodenectomy improves survival duration.[8] However, a minimum of 30% of patients who undergo pancreaticoduodenectomy will not receive postoperative adjuvant therapy because of perioperative complications, slow recovery of performance status, or patient refusal. Because of the risk that planned postoperative adjuvant therapy will be delayed or not delivered, and published experiences of successful pancreatic resection following EBRT, many institutions initiated studies of chemoradiation given preoperatively. The results suggest that preoperative chemoradiation has the following advantages over postoperative chemoradiation: (1) because chemotherapy and radiation are given first, delayed postoperative recovery has no effect on the delivery of multimodality therapy; (2) the reported high frequency of positive-margin resections supports the concern that the retroperitoneal margin of excision, even when negative, may be only a few millimeters—thus surgery alone is inadequate local therapy for most patients; and (3) patients found to have disseminated disease on restaging studies after chemoradiation will not be subjected to laparotomy. In trials at our institution, patients who had disease progression evident at restaging had a median survival of only 7 months.[1] A distinct advantage of preoperative over postoperative chemoradiation is that preoperative therapy allows the avoidance of a lengthy recovery period and the potential morbidity of pancreaticoduodenectomy in patients with such a short expected survival duration.

Despite its potential benefits, preoperative chemoradiation is associated with toxicities. In one study, even though all patients received intended therapy with a preoperative treatment approach involving standard-fractionation (5.5 weeks) chemoradiation, the gastrointestinal toxic effects (nausea, vomiting, and dehydration) were severe enough to require hospital admission in one third of the patients.[9] Furthermore, the recently reported Eastern Cooperative Oncology Group (ECOG) multicenter trial documented the need for hospital admission in 51% of patients during or within 4 weeks of completing chemoradiation.[10] These findings led to a change in the delivery of radiation therapy and 5-fluorouracil (5-FU) at our institution. The revised regimen consisted of rapid-fractionation chemoradiation delivered over a 2-week period to a total dose of 30 Gy (3 Gy/fraction [10 fractions], 5 days/week). 5-FU was given concurrently by continuous infusion at a dosage of 300 mg/m^2/day for 5 days/week. Restaging with abdominal CT and chest radiography was performed 4 weeks after the completion of chemoradiation, in preparation for pancreaticoduodenectomy. Of the 35 patients who received this treatment, 27 were taken to surgery, and 20 (74%) underwent successful pancreaticoduodenectomy. Local tumor control and patient survival were equal to the results with standard-fractionation (5.5 weeks) chemoradiation; only 2 (10%) of the 20 patients who underwent resection developed local-regional recurrence, and the median survival for all 20 patients was 25 months.[11]

The small survival advantage seen with chemoradiation and surgery compared with surgery alone likely results from improved local-regional tumor control. Because the rates of response to 5-FU–based systemic therapy are poor in patients with measurable metastatic disease, 5-FU–based chemoradiation regimens are unlikely to have a significant impact on the development of distant metastases. Therefore more effective systemic agents are needed both to maximize radiation sensitization and to treat microscopic extrapancreatic metastatic disease. One such potential agent is gemcitabine (2′-deoxy-2′,2′-difluorocytidine, Gemzar), a deoxycytidine analogue capable of inhibiting DNA replication and repair. In a randomized trial, systemic gemcitabine was found to be superior to 5-FU in previously untreated patients with advanced pancreatic cancer.[12] In addition, clinically meaningful effects on disease-related symptoms (pain control, performance status, and weight gain) were seen more often with gemcitabine (24% of patients) than with 5-FU (5% of patients).

Gemcitabine is also a potent radiation sensitizer, and phase I studies of this drug-radiation combination are currently ongoing; gemcitabine is being given in escalating doses weekly as a single agent with EBRT, in combination with 5-FU and EBRT, in combination with cisplatin and EBRT, at a fixed dose with escalating doses of EBRT, and as a twice weekly infu-

sion with either standard-fractionation EBRT or split-course EBRT. Researchers from our institution have reported on a phase I study of rapid-fractionation EBRT (30 Gy/2 weeks; 3 Gy/fraction) and concomitant weekly gemcitabine (for 7 weeks) in patients with locally advanced adenocarcinoma of the pancreatic head.[13] These data are the basis of our current phase II study of this drug-radiation combination as preoperative therapy in patients with potentially resectable disease. There is no published experience with adjuvant gemcitabine-based chemoradiation following pancreaticoduodenectomy; acute and late toxic effects of this drug-radiation combination may be more significant when it is used postoperatively than when it is used preoperatively.

Although postoperative chemoradiation appears to confer a survival advantage compared to pancreaticoduodenectomy alone, at least 30% of eligible patients will not receive their intended therapy because of a prolonged postoperative recovery. Therefore future regimens will likely emphasize neoadjuvant therapy and capitalize on our growing understanding of the molecular basis of metastasis, allowing conventional chemoradiation and surgery to be combined with systemic or regional delivery of novel agents that inhibit essential steps in tumor cell growth.

Neoadjuvant Therapy in Patients With Locally Advanced Pancreatic Cancer

Chemoradiation has been applied to patients with locally advanced, unresectable pancreatic cancer in an effort to improve survival. The Gastrointestinal Tumor Study Group (GITSG) randomized patients to receive 40 Gy of radiation plus 5-FU, 60 Gy plus 5-FU, or 60 Gy without chemotherapy.[14] All patients had undergone laparotomy and therefore had been surgically staged; only patients with disease confined to the pancreas and peripancreatic organs, regional lymph nodes, and regional peritoneum were eligible for the study. Thus, although surgical staging made for a more uniform study population, it also introduced significant selection bias in that only rapidly recovering patients were considered for treatment. Any comparison of results of future studies with these data must account for this selection bias. The median survival was 10 months in each of the chemoradiation groups and 6 months for patients who received 60 Gy without 5-FU. In subsequent GITSG studies, neither doxorubicin (Adriamycin) used as a radiation potentiator nor multidrug chemotherapy (SMF; streptozocin, mitomycin, and 5-FU) alone or continued after chemoradiation was found to be superior to 5-FU chemoradiation. In contrast to the GITSG data, an ECOG study suggested no benefit of chemoradiation over 5-FU alone.[15] The median survival was 8.3 months in the group that received chemoradiation and 8.2 months in the group that received 5-FU alone. It was evident in both the GITSG studies and the ECOG trial that patients with locally advanced, unresectable pancreatic cancer who are symptomatic to the point of not being fully ambulatory do not benefit from anticancer therapy.

Because surgical resection of the primary tumor remains the only potentially curative treatment for pancreatic cancer, preoperative chemoradiation has been studied for its ability to convert locally unresectable pancreatic cancer to resectable disease[16-24] (Table I).

Table I. Experience with neoadjuvant chemoradiation to allow eventual surgical resection in patients with locally advanced pancreatic cancer

Reference (year)	No. of patients	EBRT dose (Gy)	Chemotherapy	No. with radiographic response	No. surgically resected
DiPetrillo and Safran[16] (2000)	40	50.4	Paclitaxel	PR, 11/38 (29%)	3
Epelbaum et al.[17] (2000)	20	50.4	Gem	PR, 4	2
White et al.[18] (1999)	25	45	5-FU + Mito (12) or cisplatin (10)	6/22 had decrease of >1 cm	5* (CR,† 1)
Blackstock et al.[19] (1999)	17	50.4	Gem	PR, 3/15	0
Bajetta et al.[20] (1999)	32	50	5-DFUR	PR, 7/32	5
Todd et al.[21] (1998)	38	0	5-FU + LV + Mito + dipyridamole	PR, 14 CR, 1	4 (CR,† 1)
Kamthan et al.[22] (1997)	35	54	5-FU + STZ + cisplatin	PR, 9 CR, 6	5 (CR,† 2)
Safran et al.[23] (1997)	14	50	Paclitaxel	PR, 4/13	1
Jessup et al.[24] (1993)	16	45	5-FU	NA	2

EBRT = external-beam radiation therapy; 5-FU = 5-fluorouracil; 5-DFUR = doxifluridine; Mito = mitomycin C; Gem = gemcitabine; LV = leucovorin; STZ = streptozocin; CR = complete response; PR = partial response; NA = not available.
*Three of five had positive margins of resection.
†Histologic complete response.

In a study from New England Deaconess Hospital, 16 patients who had locally advanced, unresectable pancreatic cancer were treated with 45 Gy of EBRT and infusional 5-FU to enhance resectability.[24] Only two of the patients (13%) were able to undergo resection. Similarly, investigators at Duke University reported that only 2 (8%) of 25 patients with locally advanced pancreatic cancer treated with 45 Gy of EBRT and 5-FU (with or without cisplatin or mitomycin C) subsequently were able to undergo complete resection with negative margins.[18] As indicated by these and other studies, it it unlikely that neoadjuvant chemoradiation can convert unresectable lesions to resectable ones and thereby increase the number of patients who can be cured with combined-modality therapy. It is important to remember that as the definition of locally advanced pancreatic cancer is broadened, results will appear more promising. However, if one maintains a strict CT definition of locally advanced pancreatic cancer that includes only arterial involvement (low-density tumor inseparable from the SMA or celiac axis on contrast-enhanced CT) or SMV or SMPV confluence occlusion, successful downstaging to allow complete surgical resection will be rare with the currently available chemotherapy and chemoradiation techniques.

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Adjuvant Therapy for Pancreatic Adenocarcinoma

Ross A. Abrams, M.D.

Since 1991 we have undertaken and/or participated in a number of studies and initiatives regarding the adjuvant therapy of pancreatic and nonpancreatic periampullary adenocarcinoma. They are listed as follows: (1) A single-institution phase II trial of therapy was begun in October 1991, which was aimed at increasing therapeutic intensity with continuous-infusion 5-fluorouracil (5-FU) and irradiation, and addressed the risk of hepatic metastases using prophylactic hepatic irradiation.[1,2] (2) A retrospective, stratified analysis of outcomes at our institution among pancreatic cancer patients following pancreaticoduodenectomy compared results between those patients electing to receive versus those declining adjuvant therapy.[3] This analysis was carried out in 1996 and has been recently updated.[4] (3) A second phase II single-institution trial, begun in 1996, utilized local-regional but not hepatic irradiation in combination with multiagent chemotherapy consisting of bolus 5-FU, leucovorin, dipyridamole, and mitomycin C.[5] (4) With the assistance and support of Dr. Bill Regine of the University of Kentucky and Dr. John Hoffman of the Fox Chase Cancer Center, in 1998, we participated in the opening of the first North American Cooperative Group Trial for the adjuvant management of pancreatic cancer since the original Gastrointestinal Tumor Study Group Trial was begun in the 1970s (RTOG 97-04).[6] (5) In 1999 we opened our most recent single-institution phase II trial, which was closely based on our 1996 trial, but we modified the sequence of chemotherapy with irradiation and used continuous-infusion 5-FU rather than bolus 5-FU. (6) Finally, with the leadership of Dr. Elizabeth Jaffee, we have begun studying how to integrate active immunotherapy using a genetically modified allogeneic pancreatic cancer vaccine with adjuvant chemoradiation therapy for these patients.[7]

We began these efforts fully aware of the considerable uncertainty and controversy regarding the value and role of adjuvant therapy in the context of pancreatic adenocarcinoma. We were motivated in this regard by several factors, for example, the success of postoperative adjuvant therapy with either irradiation, chemotherapy, or chemotherapy and irradiation in a number of oncologic contexts including rectal cancer,[8] colon cancer,[9] extremity sarcoma,[10] various upper aerodigestive tract sites,[11] and more recently gastric cancer.[12] In addition, the ability of surgery alone to cure most patients who undergo pancreaticoduodenectomy for pancreatic adenocarcinoma, even though the mortality and morbidity rates associated with this operation have significantly declined, has thereby greatly increased both the ability of postoperative patients to withstand adjuvant therapy and the number of patients presenting for adjuvant therapy. The encouraging results of the original Gastrointestinal Tumor Study Group trial in this context suggest the possibility of a meaningful clinical benefit with very acceptable toxicity.[13,14] Other single-institution phase II experiences support the results observed in the Gastrointestinal Tumor Study Group and suggest that the intensity of the irradiation could be increased with the use of more modern planning and delivery techniques with acceptable toxicity.[6]

The approach we have taken has been to use combined-modality chemoradiation therapy in a carefully planned and executed fashion. Patients are not begun on adjuvant therapy until they have clearly demonstrated physiologic recovery from surgery as judged by adequate nutritional intake, weight gain, performance status, and an improved sense of well-being. Patients are carefully screened for adequate hepatic, gastrointestinal, hematologic, and renal function and for the absence of clinically identifiable metastatic disease. Treatment is delivered under the supervision of medical and radiation oncologists closely supported by research nurses who monitor each patient on a regular and frequent basis. Strict criteria for radiation and chemotherapy administration and dosing are delineated and followed. Strict toxicity criteria for dose modification and/or interruption are also used. Radiation therapy treatment volumes and normal organs are carefully delineated to optimize therapeutic advantages during treatment planning.

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Special comment regarding the use of irradiation is required for trials involving multi-institutional participation. In North America all centers participating in cooperative group trials involving radiation are site visited by members of the Radiologic Physics Center to ensure that treatment machines are appropriately calibrated and quality controlled across a substantial list of parameters. Protocols specify radiation treatment criteria in detail including treatment volumes, machines to be used, time-dose-fraction schemes, fields, and treatment-planning parameters for the treated volume and critical normal organs. Quality assurance is an essential part of these protocols, as well as criteria for when radiation may be instituted and when it should be withheld. External review of radiation therapy fields and treatments to confirm their appropriateness is also utilized. In the recently completed RTOG 90-18 (Intergroup 0116) trial of adjuvant therapy for gastric cancer, approximately 30% of fields needed to be modified based on a prospective review. In our own ongoing RTOG 97-04 trial of adjuvant therapy for pancreatic cancer, preliminary data suggest that roughly 15% of fields will show unacceptable variation from protocol requirements (Pajack T. Personal communication, 2000).

To date, we have concluded from our single-institution trials that whole hepatic irradiation is not helpful, that standard adjuvant therapy improves outcomes in our patients, that chemotherapy can be safely intensified with the use of 5-FU and mitomycin C, and that well-designed multi-institutional trials will rapidly accrue patients, even when randomization across two different treatments is involved. Our efforts remain ongoing and final analyses have not yet been performed. Close coordination and support among surgeons, medical oncologists, and radiation oncologists is critical for these studies to be successfully conducted and concluded.

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Novel Approaches to Postoperative Chemoradiation Therapy in Pancreatic Cancer

Vincent J. Picozzi, M.D.

Approximately 30,000 people in the United States are diagnosed annually with cancer of the pancreas. However, only 10% to 20% of those who are diagnosed are candidates for curative resection. Survival statistics are poor when surgery alone is used as a curative modality; median length of survival is commonly reported to be 10 to 15 months and two-year survival is commonly reported to be 15% to 30%.[1-3]

The classic studies of the Gastrointestinal Tumor Study Group (GITSG) provide the basis for initial consideration of chemoradiation therapy in pancreatic cancer.[2,3] Based on earlier encouraging results from the Mayo Clinic, a trial was constructed in which patients were randomized following pancreaticoduodenectomy to either radiation therapy (4000 cGy, split-course design) and simultaneous 5-fluorouracil (5-FU; 500 mg/m^2 given on the first 3 days of each radiation course) or to observation. Patients randomized to chemoradiation therapy also received bolus 5-FU for up to 2 years following surgery. In this trial, which contained approximately 20 patients in each arm, median survival was lengthened from 11 months in the control (surgery alone) arm to 20 months in the treatment (surgery plus chemoradiation therapy) arm (P = 0.03); 2-year survival was increased from 18% in the control arm to 43% in the treatment arm. A subsequent group of 30 patients assigned to the treatment arm duplicated the initial findings in this group. This work, first published in 1985, supported the use of radiation therapy and 5-FU as a standard of care in patients with resected pancreatic cancer.

Despite the fact that the results of the GITSG were confirmed in multiple phase II trials,[1,4,5] concerns about the validity of the superiority of chemoradiation therapy following pancreaticoduodenectomy over surgery only in cancer of the pancreas has persisted. Specifically these concerns include (1) a selection bias favoring patients with good performance status, (2) initiation of therapy after (in some cases) a significant delay following pancreaticoduodenectomy, and (3) small patient numbers, particularly in specific randomized comparisons. To address these concerns, the European Organization for Research and Treatment of Cancer (EORTC) initiated a study in 1987 comparing radiation and 5-FU to observation following curative surgery.[6] However, the method of administering chemoradiation therapy in the EORTC study differed significantly from that used in the GITSG analysis. 5-FU was given by continuous infusion rather than bolus for up to 5 days to achieve a dose intensity approximately double that in the GITSG trial, but no 5-FU was given following radiation. Using data pertaining to adenocarcinoma of the pancreatic head only, median survival was superior in the treatment arm compared to the control arm (17.1 months vs. 12.6 months) but not to a degree of 95% confidence (P = 0.099). Two-year overall survival was 37% in the treatment arm compared to 23% in the control arm, also a difference that was not statistically different.

The findings of the EORTC would seem to refute the role of chemoradiation therapy in the adjuvant treatment of resected pancreatic cancer. However, issues also exist with regard to interpretation of the results of this study. Approximately 20% of patients in the treatment arm failed to receive any additional treatment but were included for analysis in the treatment arm based on "intent to treat." No 5-FU was administered following radiation therapy. Patient numbers remained small, so much so that a false negative study result could not be definitively excluded on statistical grounds. Thus the role of adjuvant 5-FU and radiation therapy following pancreaticoduodenectomy remains a matter of debate.

What is clear is that any adjuvant program for resected pancreas must take both local and systemic recurrences into consideration. Data on patterns of failure in patients with resected pancreatic cancer indicate local failure rates ranging from 20% to 50% and systemic recurrence rates from 60% to 85%.[3-5]

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Table I. Novel approaches to adjuvant therapy in pancreatic cancer

Change mode of delivery of radiation therapy
Intraoperative radiation
Neutron beam therapy
Radiosensitizers
Interstitial radiation therapy
Change systemic chemotherapy
Non–5-FU–based adjuvant therapy—single agents (e.g., gemcitabine, cisplatin, docetaxel, camptothecans)
Non–5-FU–based adjuvant therapy—combination chemotherapy (e.g., cisplatin/gemcitabine)
Enhanced chemoradiation therapy interaction
Intra-arterial chemotherapy
Novel chemoradiosensitizers (e.g., cisplatin/gemcitabine/α-interferonl)
Novel systemic agents
Matrix metalloproteinases
Perrilyl alcohol
Antiangiogenesis factors
Adoptive immunotherapy (e.g., T-lymphocytes, dendritic cells)
Tumor vaccines
Radiolabeled antibodies
Gene therapy

Other factors important in the construction of adjuvant programs include treatment toxicity and provider acceptance.

Novel approaches to adjuvant therapy in pancreatic cancer are shown in Table I. One approach that has shown some promise involves the use of cisplatin, 5-FU, and alpha-interferon as a "combination radiosensitizer" with radiation therapy following pancreaticoduodenectomy.[7] Each drug acts as a radiosensitizer and also synergizes the direct antineoplastic effect of the others. In a phase II series of 30 patients characterized by highly adverse prognostic factors (over 80% node positive, 30% margin positive), the above-mentioned regimen has produced a median survival of 42 months and a 2-year survival of 67%. Twelve of 16 patients followed for more than 2 years were alive at the time of last follow-up; 10 of 16 patients were alive and disease free. A comparison of the initial 17 patients in this series produced statistically superior results compared to a historic control group of patients who received adjuvant therapy according to the GITSG methology.[8] Important differences between this methodology and those mentioned previously include the following: (1) an even greater dose intensity of 5-FU, plus the addition of other agents during radiosensitization; (2) the use of continuous rather than split-course radiation therapy; and (3) the restriction of postradiation 5-FU to a total adjuvant treatment duration of 6 months postoperatively. This method, although promising in its initial results, also lacks the patient accrual and randomized comparison data necessary for a more definitive conclusion.

More questions than answers exist with regard to the adjuvant treatment of resected pancreatic cancer. However, the recent broadening of interest and the creativity being applied to this condition offer significant hope for outcomes better than those previously known.

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Part II: Achalasia

MODERATOR: Carlos A. Pellegrini, M.D., University of Washington, Seattle, Wash.
PANELISTS: Marco G. Patti, M.D., University of California, San Francisco, San Francisco, Calif.; William O. Richards, M.D., Vanderbilt University, Nashville, Tenn.; and Jeffrey H. Peters, M.D., University of Southern California, Los Angeles, Calif.

Introduction

The management of achalasia has changed radically in the past decade. Before 1990, balloon dilatation of the distal esophagus was the primary form of treatment for this disease. The relatively noninvasive nature of the procedure and the expediency with which it could be carried out seemed to outweigh the fact that its efficacy in relieving dysphagia fell just short of that obtained by traditional Heller myotomy. Even the fact that successful dilatation induced abnormal reflux in 20% to 40% of patients and that the procedure carried a small but definite risk of esophageal perforation did not seem to detract from its routine use in these patients. The introduction of minimally invasive techniques to the treatment of achalasia in the early 1990s allowed patients to receive the known benefits of esophagomyotomy with significantly less discomfort and much faster recovery than had been the case with open approaches. Thus, almost overnight the surgical gastroenterologist began to play—once again—a very important role in the treatment of these patients, and by extension, the treatment of all patients with motility disorders of the esophagus. It is, therefore, important for the surgeon to understand the diagnostic and management aspects of this disease.

In the first of these three presentations, Dr. Patti et al. describe the diagnostic workup of patients suspected of having achalasia and emphasize the importance of a positive diagnosis. They also discuss the importance of ruling out diseases that occasionally mimic achalasia and the importance of appropriate follow-up after laparoscopic Heller myotomy to determine the effects of the operation on cardioesophageal competence.

We asked the other two panelists to take opposing sides on the issue of whether an antireflux procedure should be added to the Heller myotomy. Whether or not this is beneficial or even necessary had been a matter of discussion for several decades during the era of "open" surgery. The debate continues. Those who oppose its routine use are quick to point out that the objective of the operation is to decrease resistance to flow through the sphincter to facilitate emptying of the esophagus and that almost any form of antireflux procedure is likely to add some resistance to the flow. Experience also shows that occasionally the surgeon is called on to "undo" an antireflux procedure that is causing substantial esophageal obstruction in a patient who has undergone treatment for achalasia. Those who favor the use of an antireflux procedure note that a complete myotomy of the esophagogastric junction—which provides the best relief for dysphagia—almost always leads to substantial reflux in a large number of patients, and that reflux-induced mucosal damage can lead to scarring and subsequent dysphagia. Dr. Richards et al. and Dr. Peters, in their articles, provide the rationale for and against the routine use of these procedures and through their references allow readers to check for themselves the latest information available on the subject.

Esophageal Achalasia: Preoperative Assessment and Postoperative Follow-Up

Marco G. Patti, M.D., Urs Diener, M.D., Daniela Molena, M.D.

Esophageal achalasia is a primary esophageal motility disorder of unknown etiology, characterized by absence of esophageal peristalsis and increased resting pressure of the lower esophageal sphincter (LES), which fails to relax appropriately in response to swallowing. Treatment is palliative and is directed toward elimination of the outflow resistance caused by the abnormal LES function.[1]

PREOPERATIVE ASSESSMENT

Symptomatic Evaluation

The severity of the symptoms is scored by the patient before and after surgery using a five-point scale ranging from 0 (no symptoms) to 4 (disabling symptoms). The ability to swallow is graded before and after surgery as follows: excellent (no dysphagia), good (dysphagia once a week or less), fair (frequent dysphagia, more than once a week, requiring dietary adjustments), or poor (severe dysphagia, preventing ingestion of solid food).

Dysphagia is the most common symptom, experienced by virtually all patients. Regurgitation is the second most common symptom and is present in approximately 60% of patients. Chest pain and heartburn occur in approximately 40% of patients.

Barium Swallow

A barium swallow should be the first test performed in the evaluation of dysphagia. It usually shows narrowing at the level of the gastroesophageal junction ("bird beak"), and various degree of esophageal dilatation.

Endoscopy

Endoscopy should follow the barium swallow. This test rules out the presence of gastroduodenal abnormalities or a peptic or malignant stricture. This is particularly important in patients with excessive weight loss (>20 pounds), those over 60 years, and those with recent onset of dysphagia inasmuch as secondary or pseudoachalasia may mimic the clinical and manometric presentation of primary achalasia. Because cancer of the gastroesophageal junction is the most common cause of pseudoachalasia, an endoscopic ultrasound examination and a CT scan of the gastroesophageal junction can establish the correct diagnosis.[2]

Esophageal Manometry

The classic manometric findings are (1) absence of esophageal peristalsis and (2) hypertensive LES, which fails to relax completely in response to swallowing. In patients with a dilated esophagus, it is easier to position the manometric catheter under fluoroscopic guidance.[1]

Prolonged Ambulatory pH Monitoring

Abnormal reflux is quite rare in untreated patients with achalasia. In these patients, heartburn is usually due to stasis and fermentation of food in the distal esophagus. Prolonged pH monitoring should be performed in patients who have undergone previous treatment, to determine if abnormal reflux is present. In patients with a positive score, it is essential to examine the tracings to distinguish between true reflux and false reflux due to stasis and fermentation of food.[3]

- *Patients after pneumatic dilatation.* Between 23% and 35% of patients develop abnormal reflux after pneumatic dilatation.[4] The surgeon needs to know if reflux is already present in a patient with residual dysphagia after pneumatic dilatation, as this clearly indicates the need for a partial fundoplication in addition to the myotomy.

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- *Patients after Heller myotomy.* Approximately 50% to 60% of patients after thoracoscopic myotomy and 7% to 15% of patients after laparoscopic myotomy and partial fundoplication develop abnormal reflux postoperatively.[1] Because reflux is asymptomatic in most patients, it can only be detected by pH monitoring. These patients should be treated with acid-reducing medications.

Postoperative Follow-Up

Patients are examined 2 and 6 weeks after surgery. Esophageal manometry and pH monitoring are performed 2 to 3 months after the operation. Subsequently patients are contacted by telephone every 4 months for symptomatic evaluation.

Evaluation of Patients With Persistent Dysphagia After Heller Myotomy

In patients who have ***persistent*** dysphagia postoperatively, we obtain a barium swallow and repeat the esophageal manometry. In our experience, persistent dysphagia is usually due to one of the following technical factors.[1]

- *Myotomy too short distally.* The barium swallow shows persistent narrowing at the level of the gastroesophageal junction with slow emptying of the esophagus. Esophageal manometry identifies the length of the residual high-pressure zone. In these patients, pneumatic dilatation or laparoscopic elongation of the myotomy is indicated.
- *Myotomy too short proximally.* The barium swallow shows the area of narrowing in the distal esophagus (well above the gastroesophageal junction), and esophageal manometry shows a hypotensive LES. The myotomy can be extended proximally by a left thoracoscopic approach.
- *Faulty configuration of Dor fundoplication.* The barium swallow can identify the problem, but in some patients the cause becomes clear only at the time of a second operation.

Finally, in some patients persistent dysphagia is due to a transmural fibrosis and stricture (involving the mucosa as well as the muscle layers) secondary to previous treatment with intrasphincteric injection of botulinum toxin.[5] This lesion often is not amenable to treatment with a Heller myotomy, and esophagectomy may be needed.

Evaluation of Patients With Recurrent Dysphagia After Heller Myotomy

Some patients may have ***recurrent*** dysphagia after a symptom-free interval.[1] Barium swallow, endoscopy, esophageal manometry, and pH monitoring must be performed again to identify the cause of the dysphagia.

- *"Healing" of the distal portion of the myotomy.* Pneumatic dilatation or laparoscopic elongation of the myotomy is indicated.
- *Gastroesophageal reflux with peptic stricture.* Pneumatic dilatation and treatment with proton pump inhibitors are indicated. Some patients may require esophagectomy.
- *Faulty configuration of the fundoplication.* The fundoplication can be taken down laparoscopically.

Conclusions

A complete preoperative evaluation is a key element of a successful operation. With experience, the number of patients with persistent dysphagia due to technical factors decreases. In our experience, the incidence of postoperative dysphagia was 23% among 43 patients operated on between 1991 and 1994 but only 3% among 125 patients who had a laparoscopic Heller myotomy and Dor fundoplication between 1995 and 1998.[1] Close follow-up is of paramount importance to identify and treat appropriately patients with recurrent dysphagia.

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An Antireflux Procedure Should Not Routinely Be Added to a Heller Myotomy

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Although laparoscopic Heller myotomy has won widespread acceptance as being the most effective means of alleviating dysphagia in patients with achalasia, it still remains controversial as to whether an antireflux procedure should be added to the Heller myotomy. The debate over whether or not to add an antireflux procedure revolves around attempts to strike a balance between a myotomy that reduces the obstruction that exists at the lower esophageal sphincter (LES) and fine tuning the LES to allow passage of food easily but not allow acid reflux by adding an antireflux procedure to the myotomy. Our philosophy has been to surgically divide the fibers of the LES lowering its pressure to between 10 and 14 mm Hg without adding an antireflux procedure. We believe that all of the antireflux procedures, particularly the Nissen fundoplication and possibly partial fundoplications such as the Toupet and Dor, add resistance to the flow of food through the lower LES, which reduces the effectiveness of the procedure.

WHEN DO WE ADD A FUNDOPLICATION?

There are certain situations in which a partial fundoplication should be added to the Heller myotomy. Mucosal perforations are repaired laparoscopically with 4-0 or 5-0 absorbable sutures and then with a serosal patch of stomach. The performance of a Dor fundoplication over a repaired perforation increases the likelihood of a successful repair. Patients with a sliding hiatal hernia or in whom 24-hour pH studies indicate pathologic reflux should undergo repair of the hiatal hernia and a partial fundoplication should be added to the Heller myotomy.

A NISSEN FUNDOPLICATION SHOULD NEVER BE ADDED TO A HELLER MYOTOMY

Topart et al.[1] evaluated 17 patients who underwent Heller myotomy plus Nissen fundoplication for achalasia (N = 13) and diffuse esophageal spasm (N = 4). Preoperatively patients had 32% of radionucleotide-tagged water retained in the esophagus at 2 minutes. In the immediate postoperative period, patients showed no significant improvement in esophageal clearance; however, esophageal clearance deteriorated progressively with 68% retention 2 to 4 years after surgery and 75% retention after 6 years. These patients had a 29% reoperation rate for dysphagia in which the Nissen fundoplication had to be taken down. These studies carefully document that Nissen fundoplication impairs esophageal clearance in patients with an aperistaltic esophagus, which leads to a progressive increase in esophageal diameter. Adding the Nissen fundoplication to the Heller myotomy increases resistance across the gastroesophageal junction, which defeats the purpose of the operation.

DO PARTIAL FUNDOPLICATIONS INCREASE RESISTANCE TO FLOW?

Donahue et al.[2] have reported their experience with 58 laparoscopic Heller myotomies plus either a Toupet or Dor fundoplication. Two of the 58 patients were reoperated for recurrent dysphagia and 13 of the 58 underwent pneumatic dilatation to treat postoperative dysphagia. Twenty-five percent of their patients had some level of postoperative obstruction after Heller myotomy plus partial fundoplication. This is much higher than the rate of dysphagia or reoperation for patients undergoing a Heller myotomy alone and indicates there may be some obstruction created by the partial fundoplication. Patti et al.[3] noted that four patients who underwent Heller myotomy plus Dor fundoplication developed dysphagia as a result of technical problems with the Dor fundoplication during their early experience prior to 1997. Although they claim that careful attention to technical details in creating the wrap can alleviate the problem, few surgeons will be able to accumulate the number of cases accumulated by Patti et al. (N = 133). Our simpler approach may lead to fewer technical problems for most surgeons who perform these procedures in fewer cases.

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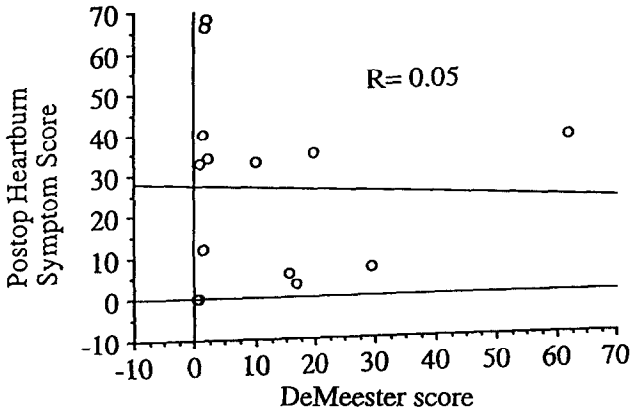

Fig. 1. DeMeester scores for each patient (calculated from 24-hour pH studies) plotted against their gastrointestinal symptom scores for heartburn (range: 0 = no heartburn to 100 = continuous discomfort with only temporary relief afforded by antacids). There is a poor correlation between the symptoms of heartburn reported by the patient and the objective measurement of acid reflux on 24-hour pH testing. (From Richards WO, Clemments RH, Wang PC, et al. Prevalence of gastroesophageal reflux after laparoscopic Heller myotomy. Surg Endosc 13:1010-1014, 1999.)

VANDERBILT EXPERIENCE

Our approach has been to perform a very limited dissection of the anterior esophagus in order to preserve the lateral and posterior attachments of the phrenoesophageal membrane, and it has previously been described in detail.[4] The myotomy is started 5 to 7 cm above the gastroesophageal junction to the left of the anterior vagal trunk and is carried down 1 to 2 cm on the stomach wall. The extent of the myotomy onto the stomach wall is guided by simultaneous endoscopy to gauge the adequacy of the myotomy.

Seventy-six Heller myotomies in 75 patients have been performed at Vanderbilt since 1992. One patient underwent reoperation at 3 months for inadequate relief of dysphagia. During his redo procedure the myotomy was extended distally and proximally, and the patient has achieved excellent relief of his dysphagia. Eight Heller myotomies plus Dor fundoplication have been performed. Six of these were performed to cover a mucosal perforation.

Patients have had a significant reduction in LES pressure from a mean of 41.4 ± 4.2 mm Hg before myotomy to 14.2 ± 1.3 mm Hg after myotomy.[5] Postoperative manometry shows that the LES remains nonrelaxing and remains within the high-pressure zone of the abdomen, which indicates that the LES still provides a significant barrier to reflux even when the pressure is lowered to 10 mm Hg.

Symptoms of gastroesophageal reflux were assessed pre- and postoperatively using a previously validated gastrointestinal questionnaire that rates gastroesophageal reflux disease (GERD), dysphagia, abdominal pain, and irritable bowel symptoms. The 19 questions are scored on a Likert scale with zero indicating no symptoms and 100 indicating continuous discomfort with only temporary relief with medication. Preoperatively patients with achalasia have moderate symptoms of gastroesophageal reflux as marked by a mean score of 51. There was a significant decrease in symptoms of gastroesophageal reflux after Heller myotomy down to a mean score of 20.[5] It is likely that many achalasia patients who have chest pain misinterpret their symptoms as "heartburn" when they are actually having stasis or spasms. When patients' subjective symptoms of gastroesophageal reflux are plotted against the objective DeMeester score taken from the distal pH sensor, there is very poor correlation as shown in Fig. 1.[5] These findings indicate that heartburn symptoms are not a reliable measurement of gastroesophageal reflux in patients with achalasia.

POST-HELLER pH STUDIES

We have previously reported our results of esophageal manometry (n = 16) and 24-hour pH studies (N = 14) in patients who have undergone Heller myotomy alone.[5] We have now studied 24 patients with manometry and 24-hour pH studies after Heller myotomy. Three (13%) of the 24 have pathologic acid reflux (total time of reflux above 4.2%), whereas 58% of our patients have less than 2% total time of reflux as shown in Fig. 2. Typically the reflux events last much longer and are more infrequent in these individuals than in patients who present with typical GERD. Fig. 3 shows the 24-hour pH study in one such individual indicating that there are very few events of reflux in this person, but the few that are present last a considerable length of time. In panel B, the 3-hour recording of one reflux event reveals a nearly hour-long acid exposure in which the patient is unable to clear the refluxate while in the supine position.[5] The prolonged acid exposure times, particularly in the supine position, suggest that much of the pathologic exposure to acid in the distal esophagus is caused by inadequate clearance of refluxed acid from the esophagus. Two of the three patients with pathologic acid reflux in our series have LES pressures of 22 and 24 mm Hg. These pressures are much higher than can be expected to reliably reduce resistance across the gastroesophageal junction. In these two pa-

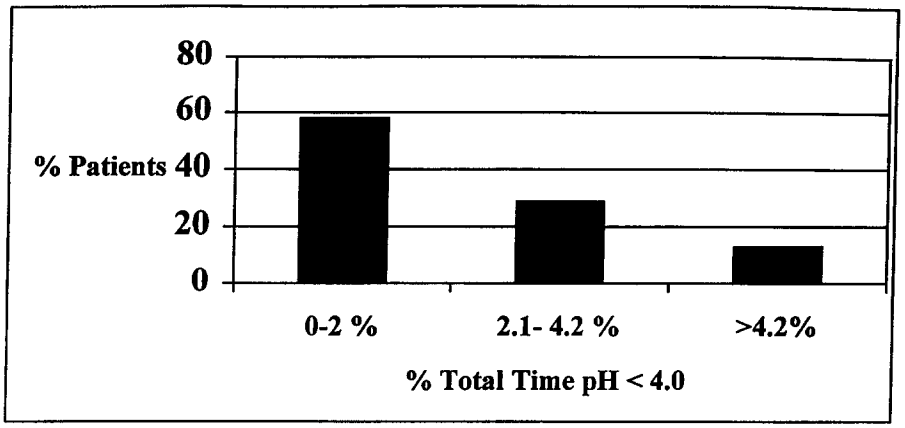

Fig. 2. Number of patients with acid exposure in the distal esophagus expressed as the total time of pH <4.0. Pathologic reflux, defined as total time of acid exposure more than 4.2% of the total time, occurred in only 13% of patients after Heller myotomy without an added antireflux procedure. The majority of patients (58%) had no acid exposure or exposure less than 2% of the time in the distal esophagus.

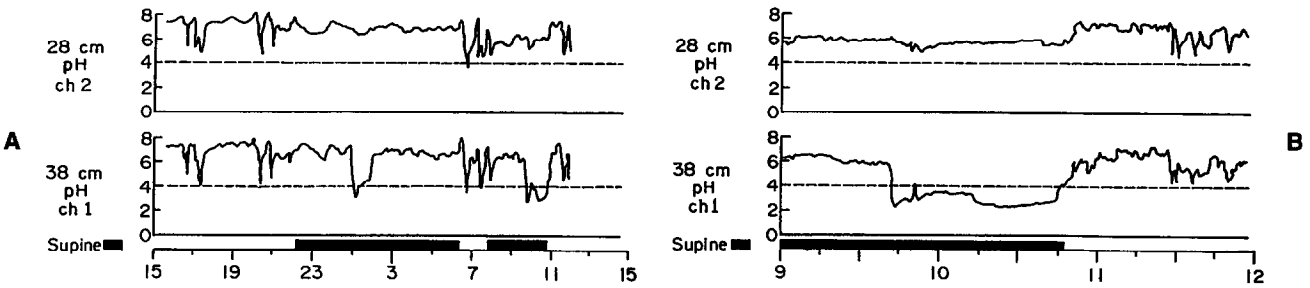

Fig. 3. Dual pH probe recording of esophageal acid exposure in a patient after Heller myotomy. **A,** Twenty-four–hour pH study in a patient with an elevated time of total reflux (8%). There are infrequent episodes of reflux that last for long periods of time. **B,** A 3-hour segment from the patient in the supine position demonstrating a pattern of slow clearance of acid from the esophagus, which can be attributed to the aperistaltic esophagus and high LES pressure (22 mm Hg). Longer episodes of reflux occurred while the patient was supine rather than in the upright position, which again is consistent with the concept that clearance of acid refluxate is impaired when gravity does not assist in the clearance of acid. (From Richards WO, Clemments RH, Wang PC, et al. Prevalence of gastroesophageal reflux after laparoscopic Heller myotomy. Surg Endosc 13:1010-1014, 1999.)

tients, repeat myotomy and reduction of their LES pressures may paradoxically reduce their acid exposure through increased clearance of refluxed acid. Because Heller myotomy does not improve esophageal aperistalsis, esophageal clearance can only be improved through reduction in resistance across the gastroesophageal junction.

DOES A PARTIAL FUNDOPLICATION REDUCE REFLUX?

Patti et al.[3] have reported their experience in 133 patients undergoing laparoscopic Heller myotomy plus Dor fundoplication. Six (17%) of 35 patients who underwent 24-hour pH testing were found to have pathologic acid exposure in the esophagus defined by a DeMeester score of ≥ 15. Patients with achalasia do have pathologic reflux after Heller myotomy with partial fundoplication. Since Dor fundoplications are not done for routine reflux disease, it is not known how effective they are in preventing reflux or by what degree they increase resistance at the LES.

SUMMARY

Achalasia is a disease that can only be palliated, not corrected, by surgery. The philosophy at Vanderbilt has been to maximize the relief of dysphagia through

myotomy that is measured using intraoperative endoscopy while minimizing the mechanical factors that may increase gastroesophageal reflux. Only a few of our patients (3 [13%] of 24) have developed pathologic reflux after Heller myotomy without an antireflux procedure, and all have been treated medically with excellent results. The addition of an antireflux procedure would inappropriately treat the 87% of patients who have no objective measurement of gastroesophageal reflux. Because gastroesophageal reflux does occur in patients who have undergone Heller myotomy and Dor fundoplication, we have chosen not to add a procedure that may increase dysphagia. Our argument against the routine use of fundoplication rests on the concept that a fundoplication, either total or partial, increases resistance to flow across the LES and therefore decreases symptom relief. Our studies, as well as others, indicate that esophageal clearance is an important aspect of reflux after Heller myotomy, and postoperatively patients with achalasia are more prone to long periods of acid exposure caused by inadequate clearance. Symptoms of GERD in patients with achalasia do not correlate with objective measurements of acid exposure in the esophagus; therefore they cannot be used to follow up patients after Heller myotomy. Gastroesophageal reflux can be a significant problem in patients whether they have undergone Heller myotomy alone or Heller myotomy plus fundoplication. We recommend 24-hour pH studies to monitor acid exposure in the distal esophagus postoperatively to identify pathologic GERD after Heller myotomy. Patients found to have pathologic reflux after Heller myotomy with or without fundoplication should be treated medically. In short, acid reflux after a myotomy can be controlled simply with medication, but dysphagia requires more drastic and potentially hazardous treatment such as pneumatic dilatation or reoperation.

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An Antireflux Procedure Is Critical to the Long-Term Outcome of Esophageal Myotomy for Achalasia

Jeffrey H. Peters, M.D.

Secondary to reflux disease, achalasia is the most common functional disorder of the esophagus to require surgical intervention. Both surgical and endoscopic treatment involves a delicate balance between relief of esophageal outflow obstruction and destruction of the normal mechanisms preventing gastroesophageal reflux. The question of whether a limited myotomy without an antireflux procedure provides acceptable long-term results rests on whether, in practice, it is possible to successfully negotiate this balance.

The necessity for the addition of an antireflux procedure to a surgical myotomy has been debated for decades. There are insufficient data to come to a definitive conclusion. Although acceptable results have been reported following a meticulously performed myotomy without an antireflux component, most published studies support the need for antireflux protection, particularly if there is extensive dissection of the hiatus. This becomes even more relevant to this discussion if one considers the fact that the development of a reflux-induced stricture after an esophageal myotomy is a major problem and usually requires esophagectomy for relief of symptoms. If an antireflux procedure is used as an adjunct to esophageal myotomy, a complete 360-degree fundoplication should be avoided. Rather, a 270-degree Belsey fundoplication or a Dor hemifundoplication should be used to avoid long-term esophageal dysfunction secondary to the outflow obstruction afforded by the fundoplication itself.[1]

LONG-TERM DATA EMPHASIZE THE NEED FOR ANTIREFLUX PROTECTION

Malthaner et al.[2] reported long-term clinical results in 35 patients with achalasia having a minimum follow-up of 10 years. Twenty-two of these patients underwent primary esophageal myotomy and Belsey hemifundoplication at Toronto General Hospital. Excellent or good results were achieved in 95% of patients at 1 year, declining to 68%, 69%, and 67% at 10, 15, and 20 years, respectively. Two patients underwent early reoperation for an incomplete myotomy and three underwent an esophagectomy for progressive disease. It was concluded that there was a deterioration of the initially good results after surgical myotomy and hiatal repair for achalasia and that most of the deterioration was due to late complications of gastroesophageal reflux. This occurred despite the fact that an antireflux procedure had been performed.

Ellis[3] reported his lifetime experience with transthoracic short esophageal myotomy without an antireflux procedure. One hundred seventy-nine patients were analyzed at a mean follow-up of 9 years ranging from 6 months to 20 years. Overall, 89% of patients were improved at the 9-year mark. Ellis also observed that the level of improvement deteriorated over time, with excellent results (asymptomatic patients) decreasing from 54% at 10 years to 32% at 20 years. Although he did not emphasize the occurrence of reflux complications, the fact that his clinical data were similar to the findings in the Toronto study, that fewer patients underwent upper endoscopy after myotomy, and that he has reported 24-hour pH testing in 19 of his patients following limited myotomy, eight of whom had pH patterns indicative of reflux, suggest the likelihood that reflux played a significant role in his findings as well. Interestingly, Ellis found that abnormal esophageal acid exposure tended to occur in patients with the highest rather than the lowest resting lower esophageal sphincter (LES) pressures.

WHAT IS THE PROPER END POINT OF TREATMENT?

The goal of treatment in patients with achalasia is to relieve the functional outflow obstruction secondary to the loss of relaxation and compliance of the LES. Although the relief of dysphagia is the ultimate clinical goal, it should not be the only one. First, the propensity for patients to unconsciously modify their diet to avoid difficulty swallowing is underestimated,

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Table I. Laparoscopic myotomy for achalasia: Results of selected series

Reference	Year	No. of patients	Mean follow-up (mo.)	Wrap	Pretreatment LESP (mm Hg)	Post-treatment LESP (mm Hg)	Excellent/Good outcome
Swanstrom and Pennings[7]	1994	12	16	Toupet	33.4	19.3	12/12
Rosati et al.[8]	1995	25	12	Dor	30.8	11.8	24/25
Mitchell et al.[9]	1995	14	12	Dor	45	6	12/14
Anselmino et al.[10]	1997	43	12	Dor	28.6	8.8	38/43
Holzman et al.[11]	1997	10	7-39	None	39		9/10
Hunter et al.[12]	1997	45	12.5	Toupet			41/45
TOTAL		149			35.4	11.4	136/149 (91%)

LESP = lower esophageal sphincter pressure.

and second, recurrence of dysphagia in the long run correlates with the degree of LES pressure reduction at the initial treatment. Several recent studies have shown that a post-treatment sphincter pressure of less than 10 mm Hg is required for long-term relief of dysphagia.[4,5] This fact is relevant to the decision to perform an antireflux procedure as it documents that near-complete disruption of the sphincter is required to relieve dysphagia long term.

CLINICAL AND PHYSIOLOGIC OUTCOME OF ABDOMINAL MYOTOMY AND HEMIFUNDOPLICATION

The majority of surgeons worldwide presently favor an abdominal approach. Thus much of the data regarding outcome of esophageal myotomy have been generated following abdominal myotomy and partial fundoplication including the only prospective randomized study of myotomy versus pneumatic dilation.

Transabdominal Open Myotomy

In one of the largest series reported to date, Bonavina et al.[6] report good to excellent results with transabdominal myotomy and Dor fundoplication. Ninety-four percent of 198 patients had excellent/good outcomes after a mean follow-up of 5.4 years. Minimal dissection of the cardia was used. A remarkable 81 patients returned for postoperative 24-hour pH studies, of which only seven (8.6%) were positive. Esophageal diameter was significantly reduced post myotomy as was LES pressure (40.5 ± 9.7 to 11.7 ± 4.7 mm Hg).

Laparoscopic Myotomy and Partial Fundoplication

Several excellent series of laparoscopic esophageal myotomy combined with an antireflux procedure have now been published[7-12] (Table I). These reports document the relief of dysphagia in more than 90% of patients and compare favorably to those of the "modern" era of open myotomy. The average length of follow-up is approaching 2 years. LES pressures are reduced, on average, from 35.4 mm Hg to 11.4 mm Hg. Zaninotto et al.[13] reported results in 100 patients (Table II). Seventy percent of patients reported no dysphagia and 22% complained of only occasional difficulty swallowing. Seven patients were "salvaged" by postoperative pneumatic dilatation. Of note, 24-hour esophageal pH monitoring showed abnormal reflux in only 5 (6.5%) of 63 patients tested. They concluded that laparoscopic Heller-Dor fundoplication achieves excellent medium-term results.

Table II. Results of 100 laparoscopic Heller-Dor procedures

Parameter	Outcome (mean follow-up 20.5 mo)
Conversion (%)	6
Morbidity (%)	5.2
Hospital stay (days)	4
Free of dysphagia	70
Occasional dysphagia	20
Postoperative dilation	7
Mean weight increase (kg)	5
Positive postoperative pH	5/63 (6.3%)

Data from Zaninotto G, Costantini M, Molena D, Buin F, Carta A, Nicoletti L, Ancona E. Treatment of esophageal achalasia with laparoscopic Heller myotomy and Dor partial fundoplication: Prospective evaluation of 100 consecutive patietns. J GASTROINTEST SURG 4:282-289, 2000.

There are two published comparisons of laparoscopic and thoracoscopic myotomy. In both, an antireflux procedure accompanied the laparoscopic but not the thoracoscopic approach. In the first study, Patti et al.[14] compared the outcome of 30 patients who had undergone laparoscopic myotomy with a

Table III. Comparison of thoracoscopic and laparoscopic Heller myotomy for achalasia

Result	Thoracoscopic (N = 30)	Laparoscopic (N = 30)
Excellent (no dysphagia)	70%	77%
Good (dysphagia <1/wk)	17%	13%
Operating room time (min)	150 ± 16	166 ± 10
Hospital stay (hr)	84	42
Abnormal gastroesophageal reflux	6/10 (60%)	1/10 (10%)

Data from Patti MG, Arcerito M, De Pinto M, Feo CV, Tong J, Gantert W, Way LW. Comparison of thoracoscopic and laparoscopic Heller myotomy for achalasia. J GASTROINTEST SURG 2:561-566, 1998.

Table IV. Postoperative pH studies following transthoracic limited myotomy for achalasia: Collected series

Reference	Year	No. of patients	Wrap	Postoperative pH+
Streitz et al.[18]	1984	103	None	8/19 (42%)
Shoenut and Duerksen[19]	1997	15	None	6/15 (40%)
Patti et al.[14]	1997	10	None	6/10 (60%)
TOTAL				20/44 (45%)

Table V. Postoperative pH studies following laparoscopic myotomy for achalasia: Collected series

Reference	Year	No. of patients	Wrap	Post-treatment LESP (mm Hg)	Postoperative pH+
Bonavina et al.[6]	1995	193	Dor	11.7	7/81 (8.6%)
Swanstrom and Pennings[7]	1994	12	Toupet	19.3	0/9
Mitchell et al.[9]	1995	14	Dor	6	0/5
Anselmino et al.[10]	1997	43	Dor	8.8	2/35 (5.7%)
Patti et al.[14]	1998	30	Dor	NA	1/10 (10%)
TOTAL					10/140 (7.1%)

LESP = lower esophageal sphincter pressure; NA = not applicable.

Dor anterior hemifundoplication to that of 30 patients who had undergone thoracoscopic myotomy without an antireflux repair. Dysphagia was completely relieved in 77% of the laparoscopic group and 70% of the thoracoscopic group. Patients were more comfortable and left the hospital earlier after laparoscopy (42 hours vs. 84 hours). Twenty percent of the patients in the thoracoscopic group had positive postoperative 24-hour pH studies compared to only 3% after the laparoscopic Heller-Dor procedure (Table III). The second study by Stewart et al.[15] evaluated outcomes in 24 patients treated by thoracoscopic myotomy and 63 patients treated by laparoscopic myotomy. None of the former patients had a fundoplication performed, whereas 55 (87%) of 63 treated laparoscopically did. Both the operative time (3 hours vs. 4.3 hours) and the hospital stay (4 days vs. 5 days) were in favor of laparoscopic treatment. Relief of dysphagia was achieved in 90% of those treated laparoscopically and 31% of those treated thoracoscopically. Both of these reports indicate that laparoscopic myotomy is the superior approach.

OBJECTIVE ASSESSMENT OF POSTOPERATIVE GASTROESOPHAGEAL REFLUX

The problem of iatrogenic gastroesophageal reflux disease is a problem that has only recently been given proper consideration and recognition.[16,17] There are a limited number of studies objectively documenting esophageal acid exposure after treatment for achalasia. Those that are available reveal interesting trends[14,18,19] (Tables IV and V). In one of the few studies available investigating acid exposure following pneumatic dilatation, Shoenut and Duerksen[19] found a 35% prevalence of positive pH studies, similar to that found following open transthoracic limited myotomy. The data also suggested that symptomatic reflux occurs in less than half of patients with pathologic esophageal pH

studies following surgery, and thus is a poor guide to the presence of gastroesophageal reflux.

CONCLUSIONS

Based on these findings, the following conclusions may be drawn:

1. Abdominal myotomy combined with fundoplication provides excellent symptomatic outcome both short and long term in patients with achalasia.
2. Gastroesophageal reflux disease and its sequelae are significant long-term problems in postmyotomy patients with achalasia.
3. The preponderance of evidence suggests that pH-proved reflux is minimized by the addition of a partial fundoplication.
4. Approximately one third of patients treated with dilatation or myotomy without fundoplication will have pH-proved reflux.
5. Laparoscopic myotomy plus fundoplication results in less reflux (<10%) than myotomy without an antireflux procedure (30%+).
6. Symptoms are not a reliable guide for detecting reflux.
7. Postoperative pH studies are necessary to detect patients with pathologic gastroesophageal reflux.
8. Reflux is uncommon in patients who have not undergone dilatation or surgery.

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Frequency With Which Surgeons Undertake Pancreaticoduodenectomy Determines Length of Stay, Hospital Charges, and In-Hospital Mortality

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Others have suggested that in certain technically challenging operations, outcome and experience are related. Because pancreaticoduodenectomy is a technically complex procedure, this study was undertaken to evaluate mortality, length of hospital stay, and hospital charges when compared to volume of experience. The database of the State of Florida Agency for Health Care Administration was queried for pancreaticoduodenectomies undertaken during a recent 33-month period. Length of stay, hospital charges, and in-hospital mortality were stratified by the frequency of pancreaticoduodenectomy. A total of 282 surgeons performed 698 pancreaticoduodenectomies over 33 months. Eighty-nine percent of surgeons performed one pancreaticoduodenectomy per year or less and accounted for 52% of the procedures. Overall mortality rate was 5.1%. Average hospital charges were $72,171.64. The more frequently pancreaticoduodenectomy was undertaken, the shorter the hospital stay ($P = 0.025$, regression analysis) and the lower the hospital charges ($P = 0.008$, regression analysis) and in-hospital mortality ($P = 0.036$, log likelihood ratio test). Surgeons who undertake pancreaticoduodenectomy more frequently have patients with shorter hospital stays, lower hospital charges, and lower in-hospital mortality rates, independent of hospital volume. Variations exist among surgeons and among different areas of the state. Data regarding cost and mortality are available for use in programs of cost and quality improvement. (J GASTROINTEST SURG 2001;5:21-26.)

KEY WORDS: Pancreatic cancers, pancreaticoduodenectomy, perioperative outcome

Patients with a variety of major surgical diagnoses have the best outcomes when they undergo treatment at "high-volume" referral centers. This statement has been put forth for more than 20 years and is supported by a still growing number of studies. For example, focused studies on the treatment of a host of disorders, including hepatocellular carcinoma[1] and liver tumors,[2,3] pancreatic cancer,[3,4] esophageal cancer,[3,5] carotid disease,[6] and hip degeneration,[7] show a direct correlation between volume and outcome. As well, the best results with major operations have been found to correlate with hospital volume.[8,9] Regardless of how and why these correlations exist, these studies support the concentration of care and regionalization of high-risk or major surgical procedures to hospitals that exceed a yearly minimum experience.

The role of "specialists" in major operations has been studied, but less so than institutional volume. Specialist training and yearly operative volume have been found to have an impact on the mortality of lung cancer resections,[10] cardiothoracic trauma,[11] and the complication rate and length of stay after thyroidectomy.[12] Although the relationship between high-volume centers and high-volume surgeons seems inextricable, there are a few studies that support "regionalization" of major surgical procedures to high-volume surgeons or specialists for given operations.[6,10,12] With this in mind, we sought to correlate the frequency with which individual surgeons undertook pancreaticoduodenectomy with length of stay, hospital charges, and mortality following resection. Our hypothesis in undertaking this study was that operative volumes for individual surgeons would correlate inversely with length of stay, hospital charges, and mortality after pancreaticoduodenectomy.

METHODS

The database for the State of Florida Agency for Health Care Administration was queried to identify

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all pancreaticoduodenectomies undertaken from January 1, 1995 through September 30, 1997. Surgeons undertaking pancreaticoduodenectomy during this period were identified, as were the hospitals in which their work was undertaken. Patient age, length of hospital stay, perioperative mortality, and gross hospital charges were obtained and grouped by year of operation and perioperative comorbidities. Comorbidities, rather than being described, were categorized by hospital coders at the time of discharge as follows: none or minor, moderate, major, or extreme.

The data were stratified by the number of pancreaticoduodenectomies undertaken per surgeon over the 33-month period. For the surgeons, the frequency with which pancreaticoduodenectomy was undertaken was defined rather arbitrarily. High-volume was defined as one pancreaticoduodenectomy undertaken every other month over the life of the database. Seventeen or more pancreaticoduodenectomies over the 33-month period covered by the database averages out to be more than one pancreaticoduodenectomy every other month and is thus considered high volume. For illustrative purposes only, the frequency of pancreaticoduodenectomy was divided into groups of 1, 2, 3, 4 to 6, 7 to 9, 10 to 16, and 17 or more pancreaticoduodenectomies over 33 months.

The data from the Agency for Health Care Administration was entered into Microsoft Excel. Statistical analysis was undertaken using True Epistat (Epistat Services, Richardson, Tex.). Because the Agency for Health Care Administration groups the data by year of pancreaticoduodenectomy and by perioperative comorbidities, statistical analyses were, in some circumstances, impossible. Some data were summary data rather than individual data, and were not amenable to definition by standard deviations.

RESULTS

Statewide Results

A total of 282 Florida surgeons undertook pancreaticoduodenectomy in 698 patients over a 33-month period beginning January 1, 1995 and ending September 30, 1997, for an average of 2.48 pancreaticoduodenectomies per surgeon. Three hundred thirty-three of the resections (48%) were performed by 31 surgeons doing four or more pancreaticoduodenectomies. The remaining 251 surgeons (89%) undertaking pancreaticoduodenectomy averaged one pancreaticoduodenectomy per year or less.

The average age of the 698 patients was 62.5 years. The average length of hospital stay was 20.6 days. Thirty-five of 681 patients died during their hospitalization, for an in-hospital mortality rate of 5.1%. The average hospital charge was $72,171.64.

Results Stratified by Surgeon Volume

Average length of stay, hospital charges, and in-hospital mortality are stratified by the frequency of pancreaticoduodenectomy in Table I. As shown by regression analyses, the more frequently pancreaticoduodenectomy was undertaken by a given surgeon, the lower the average length of say ($P = 0.025$) and the lower the hospital charges ($P = 0.008$). As well, the more frequently pancreaticoduodenectomy was undertaken, the lower the in-hospital mortality (logistic likelihood ratio rest, $P = 0.036$).

Results Among High-Volume Surgeons

Six surgeons undertook more than 17 pancreaticoduodenectomies during this time period (see Table I). Five of these surgeons came from medical schools in Florida—three from one and two from another. Comparing these five surgeons from these two schools documents disparity among the high-volume surgeons undertaking at least one pancreaticoduodenectomy every other month (Table II). If surgeons from medical school A are eliminated from the data in Table I, this analysis of hospital charges concludes very differently and the favorable significance of high volume is lost. This is similarly true for length of hospital stay (see Table II), if the surgeons from medical school B are removed from the data in Table I.

Results Among High-Volume Centers

Further analysis of the three medical schools in the state of Florida allows comparison of high-volume centers beyond that of high-volume surgeons, as surgeons other than the high-volume surgeons at these schools and their hospitals undertook pancreaticoduodenectomies (Table III).

The pancreaticoduodenectomies undertaken at the three medical schools can be stratified by severity of perioperative comorbidities (Table IV). Among the three schools, the differences in severity of perioperative comorbidities are significant (log likelihood ratio test, $P < 0.001$). Differences between the two schools doing the greater volumes (school A and school B) are also significant (log likelihood ratio test, $P = 0.0025$) (see Table IV). Patients undergoing pancreaticoduodenectomies at medical school A were more likely to have major perioperative comorbidities than patients undergoing resections at school B. Consistent with the distribution of comorbidities, the patients undergoing pancreaticoduodenectomy at school A were older than those at school B (Table V). The ages of the patients at medical school C were quite consistent with their advanced perioperative comorbidities, although the

Table I. All pancreaticoduodenectomies performed in Florida over 33 months stratified by frequency with which surgeons undertook pancreaticoduodenectomy

No. of PDs per surgeon (over 33 mo.)	No. of surgeons	No. of PDs	ALOS (days)	Hospital charges (mean)	In-hospital mortality (%)
1	166	166	27.0	$91,975	6.6
2	56	112	19.4	$71,744	5.4
3	29	87	19.7	$81,844	3.4
4-6	18	88	19.7	$69,768	10.2
7-9	3	23	19.6	$73,198	8.7
10-16	4	52	17.6	$67,193	0.0
≥17	6	170	16.8	$48,419	2.6
TOTAL	282	698	20.6	$72,171	5.1

PDs = pancreaticoduodenectomies; ALOS = average length of hospital stay.

Table II. Number of pancreatectomies performed by high-volume surgeons at two medical schools in Florida with length of stay, charges, and mortality

Medical school	No. of high-volume surgeons	No. of PDs (by surgeons at each school)	ALOS (mean)	Hospital charges (mean)	In-hospital mortality (%)
A	3	86	19.8	$33,697	3.5
B	2	64	13.1	$66,748	0.0

Abbreviations as in Table I.

Table III. Number of pancreaticoduodenectomies undertaken by surgeons at each of the three medical schools in Florida

Medical school	Surgeon	No. of pancreaticoduodenectomies over 33 mo
A	1	46
	2	23
	3	17
	4	5
	5	4
	6	3
B	1	47
	2	17
	3	14
	4	10
C	1	12
	2	7

Table IV. Severity of perioperative comorbidity in patients undergoing pancreaticoduodenectomy at each of the three medical schools in Florida

	Severity of comorbidity				
Medical school	Minor	Moderate	Major	Extreme	Total pancreaticoduodenectomies
A	7	9	55	26*	97
B	18	16	43	11†	88
C	0	3	6	10	19

*Three deaths.
†One death.

volumes at school C were considerably less than those at schools A and B (see Tables IV and V).

Among the three schools, there were considerable differences in average length of hospital stay after pancreaticoduodenectomy (Table VI). Notably, for each school, average length of stay correlated with the severity of preoperative comorbidities (see Table VI). The average length of stay was shortest at medical school B.

Hospital charges correlated with severity of perioperative comorbidities, although they differed among the schools (Table VII). The more severe the comorbidities, the greater the charges. Pancreaticoduodenectomy at medical school C was, on average, most expensive, although their volume was the lowest. The costs associated with pancreaticoduodenectomy at medical school A were 51% of the other high-volume regional provider, school B.

Table V. Average age of patients undergoing pancreaticoduodenectomy at each of the three medical schools in Florida

Medical school	Average age (yr)
A	64.6
B	59.8
C	63.6

Results Within One High-Volume Provider

At one high-volume center (medical school A), there is a range in the number of pancreaticoduodenectomies per surgeon undertaken in the period covered by the database (Table VIII). Notably, the average severity of preoperative comorbidities occurring in patients operated on by each surgeon at that center varied without correlation to the number of pancreaticoduodenectomies that each surgeon undertook (see Table VIII). The busiest surgeons did not operate on the sickest or the healthiest patients.

Table VI. Average length of hospital stay for patients undergoing pancreaticoduodenectomy at each of the three medical schools in Florida stratified by perioperative comorbidity

	Severity of comorbidity				
Medical school	Minor (days)	Moderate (days)	Major (days)	Extreme (days)	ALOS (days)
A	17.4	17.5	17.7	27.4	20.3
B	10.6	10.7	13.5	30.2	14.5
C	–	21.3	18.2	27.3	23.5

ALOS = average length of hospital stay.

Table VII. Cost of hospitalization for patients undergoing pancreaticoduodenectomy at each of the three medical schools in Florida stratified by perioperative comorbidity

	Severity of comorbidity				
Medical school	Minor	Moderate	Major	Extreme	Average cost
A	$27,214	$37,145	$27,458	$67,099	$38,964
B	$53,382	$49,647	$65,160	$128,349	$67,828
C	–	$47,498	$50,111	$98,627	$75,233

Table VIII. Results of pancreaticoduodenectomy for the six surgeons at medical school A who performed pancreaticoduodenectomies during the 33 months defined by the database

Surgeon	No. of pancreaticoduodenectomies	Comorbidity* (%)	ALOS (days)	Hospital charges	In-hospital mortality (%)
1	46	78	14.3	$35,717.00	2.2
2	23	91	16.7	$34,286.00	4.3
3	17	83	16.0	$31,434.00	5.9
4	5	80	22.0	$52,262.00	0.0
5	4	67	21.0	$90,093.00	0.0
6	3	100	25.3	$81,652.00	0.0

ALOS = average length of hospital stay.
*Major or extreme comorbidity.

The in-hospital mortality rate for the center was 3.8%. In-hospital mortality did not correlate with the frequency with which each surgeon undertook pancreaticoduodenectomy. Conversely, average perioperative length of stay and hospital charges correlated significantly ($P < 0.05$, regression analysis) with the frequency of procedures (see Table VIII). In other words, patients undergoing pancreaticoduodenectomy by busier surgeons had shorter hospital stays and lower hospital charges.

DISCUSSION

The medical literature supports regionalization of care for "major" illnesses and operative procedures based on length of stay, mortality, and cost data. These reports do not provide a comprehensive review of how specialists influence these high-volume centers and how high-volume and high-prestige centers within a geographic area differ. In this study we compare the outcomes obtained by surgeons in one state undertaking pancreaticoduodenectomy with varying frequency. The outcomes after pancreaticoduodenectomy among several high-volume centers in the same state, the outcomes among the high-volume surgeons within these high-volume centers, and the outcomes among surgeons at one high-volume center were compared.

The patients in this study underwent pancreaticoduodenectomy in Florida over a recent 33-month period covered by the inclusive State of Florida Agency for Health Care Administration database. Patients undergoing pancreaticoduodenectomy were generally older and spent an average of 3 weeks in the hospital after surgery. Nearly 9 of 10 surgeons undertaking pancreaticoduodenectomy performed, on average, less than one per year. Similarly, the majority of pancreaticoduodenectomies were completed by surgeons who undertook pancreaticoduodenectomy, on average, one or fewer times per year. Overall, the operations were undertaken at substantial cost and a hospital mortality just over 5%, similar to benchmarks often quoted in the literature,[13] but less than optimal.

The frequency with which each surgeon performed pancreaticoduodenectomies had an inverse correlation to average length of stay, in-hospital mortality, and hospital charges. However, for each surgeon, the benefits of frequently undertaking pancreaticoduodenectomy were not universal. The cost advantage associated with pancreaticoduodenectomy undertaken by the state's busiest surgeons would be lost if the high-volume surgeons at medical school A were eliminated. This is similarly true with length of stay if the high-volume surgeons from medical school B were removed from the database. Stated simply, the outcomes of all high-volume surgeons are not alike following pancreaticoduodenectomy.

Stratification of the data by each of the three Florida medical schools shows that these centers did not care for similar groups of patients. The patients undergoing pancreaticoduodenectomy at medical school A were generally older and had more severe comorbidities when compared to medical school B. Although medical school C had a similar case mix to medical school A, their volumes were considerably less. As such, based on perioperative comorbidities, patients operated on at medical school B were in better health. It follows that the average length of stay for patients operated on at medical school B was considerably shorter that that for patients at the other two medical schools.

Some similar conclusions can be drawn with regard to comparisons in charges among the three medical schools. The average hospital charge for pancreaticoduodenectomy was much less at medical school A than at school B or C. This is unexpected, as medical school A cared for a large number of patients with major and extreme comorbidities. Possibly as a result of the high volumes and associated experience of surgeons at school A, the patients were cared for in a cost-effective manner. Possibly, although unlikely, the cost structure for care of the type required for patients following pancreaticoduodenectomy had notable regional differences, even within one state. More likely, cost of care varied among the medical schools because of regional differences in the utilization of preoperative procedures (e.g., endoscopic retrograde cholangiopancreatography, percutaneous transhepatic stenting), operative technology (e.g., laparoscopy, stapling devices, ultrasound), medications, and intensive care unit facilities, including monitoring. In general, the cost of caring for sicker patients weighed heavily on the average patient charges at each of the institutions, although the cost of care was considerably less at medical school A. Unlike differences in charges, there were no notable differences in perioperative mortality among the three medical schools.

It may be thought that the outcomes of a high-volume surgeon reflect the outcomes of the center at which he or she practices. Actually, outcomes among various surgeons within one high-volume center were quite different. At the high-volume and high-prestige centers studied, there were strong inverse correlations between the frequency with which surgeons undertook pancreaticoduodenectomy and average perioperative length of stay and hospital charges. Stated differently, low-volume surgeons at a high-volume center did not achieve the same favorable outcomes as the high-volume surgeons at the same institution.

The use of hospital charges in studying cost of care is open to discussion. Hospital charges do not equal direct hospital costs. But hospital costs do determine, in some manner, hospital charges. Furthermore, the

"true cost" of hospital care is not readily available, and perhaps never really known. Cost of hospital care is often a contrived number, based on cost shifting and broad hospital issues independent of the time and resources expended in the care of specific patients. Short of an independent "blue ribbon" cost accounting by unparalleled accountants, it seems that hospital charges will continue to be our best measure of the cost of hospital care.

Furthermore, hospital charges are the cost of care for patients and their payers. Hospital charges do represent the cost of "doing business," figuring some margin that allows for a return on invested capital to provide for care in the future, as has been done in the past to allow for care today.

Payers of health care and health care providers are becoming more focused on outcomes, especially those that are easily quantifiable and available through large databases. As more data are available documenting length of stay, hospital mortality rates, and cost of care, centers providing care will be scrutinized more closely. As data become available about less quantifiable measures, such as disability and levels of palliation, the scrutiny will intensify. Although there is a self-serving call to regionalize major surgical procedures to high-volume specialty centers, not all high-volume centers are alike. As well, not all surgeons within a given high-volume center can expect favorable outcomes by virtue of where they practice. In essence, a successful high-volume center requires successful high-volume surgeons.

Based on this study, we conclude that surgeons most frequently undertaking pancreaticoduodenectomy provide the most cost-effective care. We believe that high-volume surgeons provide optimal care in planning "which procedure for which patient," being able to undertake curative resections in patients deemed noncurable or unresectable by surgeons for whom pancreaticoduodenectomy is far beyond their scope. In addition, high-volume surgeons are the most valuable in providing optimal palliative care for patients with unresectable cancers of the pancreas.

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Preoperative Chemoradiation for Marginally Resectable Adenocarcinoma of the Pancreas

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Only 10% to 20% of patients with pancreatic cancer are considered candidates for curative resection at the time of diagnosis. We postulated that preoperative chemoradiation therapy might promote tumor regression, eradicate nodal metastases, and allow for definitive surgical resection in marginally resectable patients. The objective of this study was to evaluate the effect of a preoperative chemoradiation therapy regimen on tumor response, resectability, and local control among patients with marginally resectable adenocarcinoma of the pancreas and to report potential treatment-related toxicity. Patients with marginally resectable adenocarcinoma of the pancreas (defined as portal vein, superior mesenteric vein, or artery involvement) were eligible for this protocol. Patients received 50.4 to 56 Gy in 1.8 to 2.0 Gy/day fractions with concurrent protracted venous infusion of 5-fluorouracil (250 mg/m^2/day). Reevaluation for surgical resection occurred 4 to 6 weeks after therapy. Fifteen patients (9 men and 6 women) completed preoperative chemoradiation without interruption. One patient required a reduction in the dosage of 5-fluorouracil because of stomatitis. Acute toxicity from chemoradiation consisted of grade 1 or 2 nausea, vomiting, diarrhea, stomatitis, palmar and plantar erythrodysesthesia, and hematologic suppression. CA 19-9 levels declined in all nine of the patients with elevated pretreatment levels. Nine of the 15 patients underwent a pancreaticoduodenectomy, and all had uninvolved surgical margins. Two of these patients had a complete pathologic response, and two had microscopic involvement of a single lymph node. With a median follow-up of 30 months, the median survival for resected patients was 30 months, whereas in the unresected group median survival was 8 months. Six of the nine patients who underwent resection remain alive and disease free with follow-up of 12, 30, 30, 34, 39, and 72 months, respectively. Preoperative chemoradiation therapy is well tolerated. It may downstage tumors, sterilize regional lymph nodes, and improve resectability in patients with marginally resectable pancreatic cancer. Greater patient accrual and longer follow-up are needed to more accurately assess its future role in therapy. (J GASTROINTEST SURG 2001;5:27-35.)

KEY WORDS: Pancreas, adenocarcinoma, preoperative, chemotherapy, radiation therapy, chemoradiation, neoadjuvant

Cancer of the pancreas is the fifth most prevalent solid malignancy in the United States. The 5-year survival rate is a dismal 3% with more than 28,000 deaths annually in the United States. Surgical resection remains a prerequisite for cure of pancreatic cancer. However, only about 15% of patients at presentation are resectable because of the existence of locally advanced disease or distant dissemination.[1] Of those patients who undergo surgical resection at a major academic center, only 60% to 70% have a complete surgical resection with uninvolved surgical margins.[2] In this most favorable subgroup, the median survival after resection is 12 to 18 months and 2-year survival is 25% to 45% with 5-year survival generally less than

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15%. For the great majority of patients who cannot undergo resection, the median survival is 4 to 6 months and 2-year survival is 5%.

Adjuvant chemotherapy and radiation (chemoradiation) has resulted in modest gains in the treatment of pancreatic cancer. The Gastrointestinal Tumor Study Group (GITSG) randomized patients who had undergone resection with negative margins to adjuvant chemoradiation versus observation. The chemoradiation arm improved the median survival from 11 to 21 months and more than doubled the 2- and 5-year overall survival rates (18% vs. 43% at 2 years, and 8% vs. 18% at 5 years).[3]

There is a strong theoretical rationale for the use of preoperative or "neoadjuvant" chemoradiation in the treatment of pancreatic cancer. Preoperative radiation therapy might result in sufficient downstaging of tumor and decrease the frequency of involved surgical margins. Presurgical radiation therapy may decrease the viability of tumor cells that might disseminate at the time of operation. Preoperative radiation therapy might be better tolerated when there is no recent operation from which to recover and no fixed small bowel in the radiation therapy portal. In addition, neoadjuvant treatment ensures that adjuvant treatment is not delayed or abandoned in the 20% to 30% of patients who experience postoperative complications.[4] Preoperative radiation therapy should also be more effective than postoperative radiation therapy because the cancer cells presumably have an undisturbed vascular supply. Furthermore, because the preoperative treatment course entails a 10- to 12-week observation period, patients with occult metastases who go on to develop clinically apparent distant disease may be spared laparotomy.

Involvement of the superior mesenteric vein (SMV), superior mesenteric artery (SMA), or portal vein (PV) is a relative but not absolute contraindication to surgical resection. PV resection and pancreaticoduodenectomy have been performed with reported short-term survival statistics equivalent to those seen with conventional Whipple resections.[5] Despite these accomplishments, many patients with recognized involvement of the PV or SMV have clinically occult retroperitoneal or mesenteric root extension, and resection is often abandoned after lengthy and meticulous dissection. Furthermore, the high probability of extension of malignant cells beyond the surgically apparent tumor mass must be recognized. The potential for inducing tumor regression and the ability to eradicate microscopic regional metastases provide the rationale for using preoperative chemoradiation therapy in this clinical subgroup.

In the present trial we present the initial results of preoperative chemoradiation for marginally resectable pancreatic cancer. We report on toxicity, resectability, clinical response, local control, and survival.

METHODS

Beginning in 1994, we initiated a treatment protocol that consisted of preoperative chemoradiation therapy for patients with marginally resectable pancreatic cancer. All eligible patients had histologically or cytologically confirmed American Joint Committee on Cancer stage II or III adenocarcinoma of the exocrine pancreas. Patients with tumors of neuroendocrine origin, carcinoid, and carcinomas of the duodenum, distal common bile duct, or ampulla of Vater were excluded. All pathology specimens were formally reviewed at Stanford University Medical Center. Patients had to have a Karnofsky performance status greater than 70, adequate bone marrow function (>3000 white blood cells, >100,000 platelets), adequate renal function, and normal liver function (bilirubin <2). In addition, patients were required to have adequate cardiovascular and pulmonary function to enable them to tolerate pancreaticoduodenectomy. Staging studies included CT scan of the abdomen, chest radiograph, complete blood count, chemistry panel, and liver function tests. All patients were required to have radiographically typical primary tumors of the head or body of the pancreas. Cystic tumors were not eligible for this analysis.

A multidisciplinary gastrointestinal tumor board (consisting of faculty from the departments of radiation oncology, medical oncology, gastroenterology, surgery, radiology, and pathology) evaluated all patients before treatment was initiated. A high-speed, high-resolution, helical CT scan with rapid contrast injection and dual phase imaging was obtained for all patients. "Marginally resectable" lesions were defined as those lesions in which the perivascular fat plane was absent over 180 degrees of the SMA, SMV, or PV and persisted for a length of greater than 1 cm (Fig. 1). Pancreatic lesions with only minimal PV/SMV involvement, with or without splenic vein involvement, were considered resectable and therefore not eligible for this protocol. Similarly, tumors involving the celiac, hepatic, or left gastric artery, or encasing the SMA or invading the root of the colonic mesentery were considered unresectable.

Characteristics of the 15 patients (9 men and 6 women) with marginally unresectable disease are detailed in Table I. Four patients were unresectable based on laparotomy at an outside institution. These patients were reclassified as marginally resectable after reimaging at Stanford Medical Center. The median Karnofsky performance status for the group was 80.

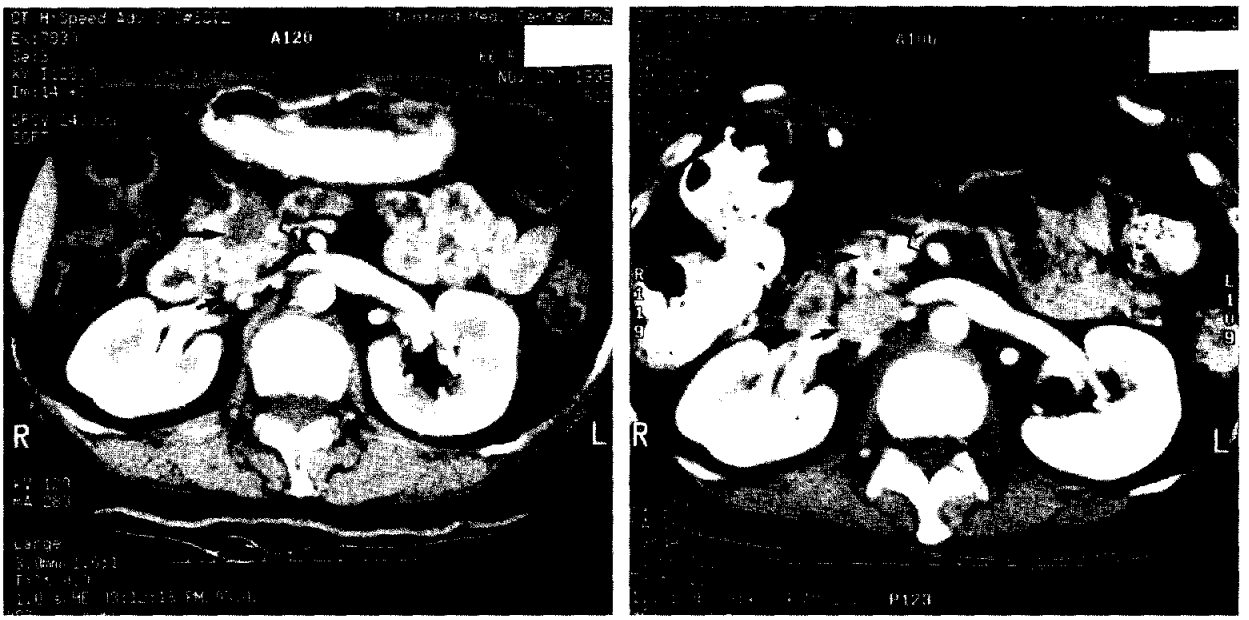

Fig. 1. A, Marginally unresectable, biopsy-confirmed pancreatic adenocarcinoma (long black arrow), with significant narrowing of the superior mesenteric vein (open arrow) and compression of the inferior vena cava (short black arrow), is shown. **B,** One month after neoadjuvant chemoradiation therapy, there is complete regression of the tumor (long black arrow) and normal patency of the superior mesenteric vein (open arrow) and inferior vena cava (short black arrow).

Table I. Patient characteristics

Patient	Age (yr)	Sex	Location	Tumor size (cm)	Stage	Vessels involved	CT response	CA 19-9 level		Surgery
								Pre-treatment	Post-treatment	
1	66	F	H	3.5	T3N0	SMV/PV	CR	1359	95	Yes
2	51	M	H	5.3	T3N0	PV/SMV	PR	30	9	Yes
3	73	M	H	11.0	T3N1	SMV	Stable	41,000	17	Yes
4	62	F	H	4.0	T4N1	SMV	Stable	10	9	Yes
5	48	F	H	4.7	T3N0	SMV	Stable	750	186	Yes
6	54	M	H	5.0	T3N1	SMA	Stable	200	50	Yes
7	71	F	H	6.0	T3N0	SMA	MR	145	21	Yes
8	60	M	T	3.5	T2N1	SMA	Stable	18	11	Yes
9	52	M	H	4.0	T3N0	SMA	Stable	33	14	Yes
10	51	F	H	2.8	T3N0	SMV	MR	69	41	No
11	54	M	H	4.0	T3N1	SMA	Stable	760	82	No
12	81	M	H	4.0	T2N0	SMV, SMA	Stable, liver metastases	450	198	No
13	46	F	B/T	4.6	T4N0	SpV, PV	Stable	230	104	No
14	53	M	H	4.5	T3N0	SMA, SMV	Stable, liver metastases	NA	NA	No
15	52	M	H	4.2	T3N0	SMA, SMV	Stable	NA	NA	No

SMV = superior mesenteric vein; SMA = superior mesenteric artery; PV = portal vein; SpV = splenic vein; MR = minor response radiographically; PR = partial response radiographically; CR = complete response radiographically.

Preoperative chemoradiation consisted of 50.4 to 56.0 Gy to the target volume with concurrent protracted venous infusion of 5-fluorouracil (5-FU) (250 mg/m^2/day). Two patients received bolus administration of 5-FU instead of the protracted venous infusion because of oncologist preference. Reimaging studies were obtained 4 to 6 weeks after completion of therapy to reevaluate for surgical resection.

Radiation treatment consisted of megavoltage irradiation (generally 15 MV photons) of 50.4 Gy in 28 fractions of 1.8 Gy per fraction, five fractions per week. The initial target volume included gross tumor and regional pancreatic draining lymph nodes plus a 2 to 3 cm margin to a dose of 45 Gy. The cone-down volume included gross tumor with a 1 to 2 cm margin for an additional 5.4 to 10.8 Gy. Treatment field design was individualized based on the volume and location of the disease. Field arrangements were generally four field (anterior, posterior, and two lateral fields). Since 1997 all neoadjuvant treatment fields were planned in our department using three-dimensional conformal radiotherapy techniques. All patients underwent CT scanning for radiation treatment planning 1 week before treatment was initiated. Customized cerrobend blocks were used in all cases.

Radiologic assessment used high-speed, high-resolution helical CT scans with rapid contrast injection and dual phase imaging. Serial sections of 3.0 to 5.0 mm were obtained. The tumor size was recorded as the largest tumor diameter. The median pretreatment size was 4.8 cm (range 2.8 to 11 cm). The degree of radiographic response was recorded based on the change in the size of the primary lesion (stable disease less than 25% reduction, minor response less than 50% reduction, partial response greater than 50% reduction, and disease progression defined as increase in size greater than 25%).

Surgical resection was scheduled 4 to 6 weeks after completion of chemoradiation if there was no evidence of disease progression or metastases. Lymphadenectomy included all tissue to the right side of the SMA, extending just above the superior aspect of the left renal vein, and up to and including the portal lymph nodes. The pathologists inked all pancreatic, bile duct, and retroperitoneal margins of the resected tissue. All pre- and postoperative histopathologic specimens were reviewed by Stanford pathologists and collectively by the multidisciplinary gastrointestinal tumor board.

Toxicity was scored using the Radiation Therapy Oncology Group/European Organization for Research and Treatment of Cancer criteria. Survival and disease-free survival were evaluated from the time of tissue diagnosis of cancer. All patients who entered the study were analyzed. All end points were reviewed in June 2000.

RESULTS

Patient characteristics are summarized in Table I. All patients completed the prescribed treatment without interruptions in either chemotherapy or radiation therapy. Acute toxicity from chemoradiation consisted of nausea, vomiting, diarrhea, stomatitis, palmar-plantar erythrodysesthesia, and hematologic suppression. No grade 3 or 4 toxicity was noted. One patient required a reduction in the dosage of 5-FU for grade 2 stomatitis. One patient experienced bile duct obstruction, was hospitalized for stent placement, and then completed therapy without any further complications.

Before initiating treatment, 9 of 12 patients had an elevated CA 19-9 level (range 69 to 41,000; normal range 0 to 37). Six weeks after they finished the neoadjuvant treatment, all nine patients with an elevated CA 19-9 level had a decrease in CA 19-9 (range 17 to 198), but only two patients had values that returned to the normal range.

One patient demonstrated a complete radiographic response at the primary site, one patient demonstrated a partial radiographic response (>50% reduction), two patients demonstrated a minor response, nine patients demonstrated stable disease, and two patients developed liver metastases on the postchemoradiation CT scan. No patient had radiographic progression of disease within the treatment volume.

Nine of 15 patients were potentially resectable based on the post-treatment CT scan and underwent laparotomy following chemoradiation. Six patients were not taken to surgery (2 patients had new liver metastases, 3 patients remained "marginally resectable," and 1 declined). The median time from completion of chemoradiation to surgery was 42 days (range 18 to 92 days) and from diagnosis to surgery 120 days (range 67 to 300 days). All patients underwent a standard pancreaticoduodenectomy. In addition, one patient also underwent solitary liver resection for a small isolated liver metastases discovered intraoperatively. The pathologic findings are summarized in Table II. All tumor specimens revealed evidence of treatment effect with significant fibrosis, hyalinization, and necrosis. The median resected tumor size was 3.3 cm (range 0 to 7 cm). The average number of lymph nodes dissected was eight (range 5 to 13). Two patients had microscopic involvement in one of the dissected lymph nodes; all other patients had uninvolved lymph nodes. All patients had negative surgical margins. Two patients had no evidence of residual tumor cells (pathologic complete responses). One patient had only sporadic microscopic foci of presumably viable tumor cells in the pathology specimen.

The median postoperative hospital stay for the nine resected patients was 18 days. There was no perioperative mortality. Morbidity was minimal. One

Table II. Pathologic characteristics of patients who underwent surgical resection after neoadjuvant chemoradiation

Patient	Histology	Primary tumor (cm)	Nodes	Margins	Perineural invasion	Survival (mo)	Status
1	AC	CR	Negative	Negative	None	14	DOD*
2	AC	CR	Negative	Negative	None	34	NED
3	ACmuc	7.0	1/8	Negative	None	30	NED
4	ACmuc	3.7	1/5	Negative	None	30	NED
5	AC	5.5 (liver metastasis, resected)	Negative	Negative	None	11	DOD*
6	AC	3.0	Negative	Negative	None	39	NED
7	AC	4.0	Negative	Negative	None	7	DOD†
8	AC	2.5	Negative	Negative	None	72	NED
9	AC	No discrete mass, only few islands of tumor cells	Negative	Negative	Present	12	NED

NED = no evidence of disease at time of last follow-up; DOD = died of disease; AC = adenocarcinoma; ACmuc = adenocarcinoma mucinous component; CR = complete response (no evidence of viable cells).
*Died of liver failure.
†Died of pulmonary embolus.

patient remained hospitalized for 32 days secondary to high Jackson-Pratt drain output (pancreatic fistula).

The median follow-up was 30 months. The median survival for the entire cohort was 12 months. The median survival for the patients who did not undergo surgical resection was 8 months. The patients who underwent surgical resection had a median survival of 30 months (range 7 to 72 months). Six of the nine patients are alive and free of disease after 12, 30, 30, 34, 39, and 72 months, respectively.

DISCUSSION

Pancreatic cancer is a challenging malignancy that, despite intensive multimodality therapy, rarely yields 5-year survivors. Although chemotherapy and radiation therapy can improve survival, complete surgical resection of the tumor is required if long-term survival is to be achieved. The observation that most of the long-term survivors have negative surgical margins and negative lymph nodes has led surgeons to aggressively attempt resection of pancreatic tumors. Consequently many series report positive margin rates of 30% to 40% in patients undergoing pancreaticoduodenectomy.[2] Involvement of the SMV or PV is a relative but not an absolute contraindication to surgical resection. Many patients with recognized PV or SMV involvement have clinically occult retroperitoneal or mesenteric root extension, and resection is often abandoned after lengthy and meticulous dissection. Although some patients appeared to benefit from regional "extended" pancreatectomy (resection of the PV or some type of arterial resection), increased perioperative morbidity and mortality have discouraged some centers.[6] We initiated a preoperative chemoradiation regimen in an attempt to improve on the resectability of this clinical subgroup.

Although our series includes only a small number of patients, the results are encouraging. Our experience demonstrates that preoperative chemoradiation for locally advanced marginally resectable pancreatic cancer is a viable treatment approach. All patients completed the prescribed therapy with no interruption in treatment. There was evidence of a tumor marker response in all patients who presented with an elevated CA 19-9 level before chemoradiation. Nine (60%) of 15 patients underwent a Whipple resection, and surgical margins were uninvolved grossly and microscopically in all specimens. Only two patients had involvement of lymph nodes (in each case a single microscopically involved lymph node was noted), and one had a resection of a radiographically occult solitary liver lesion. All resected tumors demonstrated histologic evidence of a tumor response to chemoradiation. Two patients had complete pathologic responses (no viable tumor cells present), and one patient had nonmeasurable disease with only sporadic islands of tumor cells separated by fibrosis and reaction. Finally, there was no increase in perioperative mortality or morbidity following preoperative chemoradiation. The overall survival for patients undergoing surgical resection was 30 months (range 7 to 72 months) with six patients remaining alive and disease free at 12, 30, 30, 34, 39, and 72 months, respectively.

Table III. Recent studies of preoperative chemoradiation for pancreatic adenocarcinoma

Institution (type of treatment)	Patients	Resected/ Total	Surgical margin positive	Pathologic complete response	Median survival
Eastern Cooperative Oncology Group[7] (RT/FU/MMC)	53 LA	24/53	13/24	0	15.7 mo resected
M.D. Anderson Cancer Center[4] (RT/FU)	27 R	20/27	2/20	0	23% (3 yr) resected
De Chirurgie General, France[8] (RT/FU/CDDP)	7 UR	2/7	NS	0	
Istituto di Radiologia, Rome, Italy[9] (RT/FU)	12 UR 8 R	9/20	NS	0	18.5 mo resected
Duke University[10] (RT/FU)	25 LA	5/25	4/5	1	
New England Deaconess Hospital[11] (RT/FU)	16 UR	2/16	0/2	0	
University of Osaka, Japan[12] (RT alone)	23 R	17/23	NS	0	
Fox Chase Cancer Center[13] (RT/FU/MMC)	27 R	13/27	0/13	0	
Mt. Sinai[14] (RT/FU/STZ/CDDP)	35 UR	5/35	4/9	2	31 mo resected
Roger Williams Cancer Center[15] (RT/Paclitaxel)	18 UR	1/18	0/1	0	
Boston University[16] (RT/FU/CDDP)	6 UR* 8 R	9/14	0/9	2	19 mo resected
Stanford University (current) (RT/FU/PVI)	15 MR	9/15	0/9	2	30 mo resected

NOTE: The definitions of locally advanced (LA) and unresectable (U) varied slightly between published series. Resectable tumors (R) included AJCC stage I tumors.
MR = marginally resectable; RT = radiation therapy; FU = 5-fluorouracil; CDDP = cisplatin; STZ = streptozocin; MMC = mitomycin C; PVI = protracted venous infusion; NS = not stated.
*Defined at initial laparotomy.

Numerous recent trials of preoperative chemoradiation have been conducted (Table III). Although inclusion criteria differed, it is clear that significant histologic responses to chemoradiation have been observed, pathologic complete responses have been rare, and reductions in radiographic tumor size have been modest.

Obtaining negative microscopic retroperitoneal margins during pancreaticoduodenectomy is a critical factor for both local control and survival. A positive retroperitoneal margin of resection arises as a consequence of (1) poor patient selection (attempting resection of tumors extending to the SMA), (2) failure to separate the tumor from the retroperitoneum in the immediate periadventitial plane of the SMA (this maximizes the margin between the posteromedial edge of the tumor and the plane of retroperitoneal resection), or (3) the infiltrative nature of pancreatic adenocarcinoma. Patients left with a positive margin after pancreaticoduodenectomy have a median survival of 11 months,[17] and this poor survival is not significantly different from that achieved by treatment of locally advanced pancreatic adenocarcinoma using chemoradiation without surgery.[18] It was encouraging that in our series all patients who underwent surgical resection had grossly and microscopically uninvolved surgical margins. In our institution, where all patients are evaluated for resectability by the same radiographic and surgical criteria, the incidence of positive surgical margins after pancreaticoduodenectomy is 35% (somewhat higher than the reported 29% by Johns Hopkins University).[19] In general, the patients at our institution who are selected for initial surgical resection have a more favorable disease presentation than those patients with marginally resectable disease who received neoadjuvant treatment. Our experience suggests that preoperative chemoradiation therapy may improve resectability and sterilize surgical margins as was reported in the M.D. Anderson Cancer Center experience.[4]

In the present series we noted considerable reduction in tumor extent in the resected operative speci-

mens. Much of the tumor specimen revealed necrosis, hyalinization, and fibrosis. Defining the extent of residual cancer in resected specimens after neoadjuvant chemoradiation therapy is challenging and dependent on the institution. Pathologic complete responses in the resected pancreas after preoperative chemoradiation are rare for adenocarcinoma of the pancreas. Collective analysis of the trials listed in Table III reveals that the pathologic complete response rate is 4% (5 of 112 patients resected after neoadjuvant therapy). Our pathologic complete response rate of 22% (2 of 9 resected patients) is most encouraging, although a greater number of similarly selected patients is required for confirmation. Interestingly, Coia et al.[13] at Fox Chase Cancer Center treated four patients with primary duodenal/ampullary carcinomas on a neoadjuvant chemoradiation therapy protocol and all four patients had a pathologic complete response at the time of surgical resection.

We suspect that neoadjuvant chemoradiation sterilizes microscopic disease in regional lymph nodes. In a large surgical series from the Johns Hopkins Medical Institutions, the incidence of involved lymph nodes was 74% in spite of the fact that the mean size of the tumor resected was only 3 cm.[19] Both our series and the series from the Fox Chase Cancer Center report a less than expected incidence of nodal involvement after resection further suggesting tumor downstaging. In a retrospective comparison, Spitz et al.[4] reported a decrease in the expected incidence of involved lymph nodes (46% vs. 63%) after preoperative therapy. In the Boston University experience,[15] two of three patients with pathologically proved involvement of lymph nodes (based on exploratory laparotomy) who then received neoadjuvant chemoradiation therapy were subsequently lymph node negative on "second-look" laparotomy.

Radiographic responses after neoadjuvant chemoradiation therapy are uncommon. Table IV summarizes the reported radiographic responses from other series. Comparison of these results is problematic because of the lack of uniformity in defining radiographic responses. Some series defined radiographic response according to a reduction in the size of the greatest diameter. Other series reported responses as a decrease in the bidimensional product. Finally, one series defined a reduction in any dimension of greater than 1 cm as a response. The lack of a radiographic response may reflect the need for a longer interval between completion of treatment and reimaging to detect subtle differences in tumor size. However, it is also clear that CT scans fail to delineate the difference between active cancer and reactive fibrosis after neoadjuvant therapy. Given the clear antitumor effect noted on pathologic examination, classic definitions of radiographic response may not be useful in determining resectability after chemoradiation therapy.

Table IV. Radiographic response after neoadjuvant chemoradiation

Institution (type of treatment)	Tumor response
Eastern Cooperative Oncology Group[7] (RT/FU/MMC)	4/51 (8%) radiographic
De Chirurgie General, France[8] (RT/FU/CDDP)	2/7 (minor)
Istituto di Radiologia, Rome, Italy[9] (RT/FU)	4/20 (minor)
Duke University[10] (RT/FU)	3/22 regression 5/22 stable 14/22 progression
New England Deaconess Hospital[11] (RT/FU)	2/16 (minor)
Fox Chase Cancer Center[13] (RT/FU/MMC)	1/27 partial 26/27 stable
Mt. Sinai[14] (RT/FU/STZ/CDDP)	15/35 (43%) radiographic
Roger Williams Cancer Center[15] (RT/FU/CDDP)	4/13 partial 4/13 stable 5/13 progression

Abbreviations as in Table III.

When we initiated this treatment approach, we were less aggressive about performing surgical exploration in patients who had minimal responses radiographically. We now recognize that radiographic imaging after chemoradiation therapy underestimates the effectiveness of therapy and may be a poor predictor of resectability. So unless there is evidence of metastatic disease at the time of restaging, all patients who receive neoadjuvant chemoradiation therapy should undergo surgical exploration. Formal laparotomy, as opposed to assessing "resection potential" laparoscopically, is necessary in all these patients. At Stanford University Medical Center, we additionally use intraoperative ultrasound to reevaluate the liver and to image the mesenteric vessels. In our experience, intraoperative ultrasound, however, is also not always reliable in predicting tumor invasion into the SMV. At our institution, patients with locally advanced/marginally resectable pancreatic cancer who are considered poor surgical candidates receive definitive chemoradiation to at least 60 Gy (rather than the shorter neoadjuvant treatment regimen).

A potential disadvantage of preoperative chemoradiation would be increased perioperative morbidity and mortality. This concern does not appear to be the case in our series or in the published literature. We reviewed the last 86 pancreaticoduodenectomies for

pancreatic cancer performed at Stanford University Medical Center. When we compared the patients who underwent "upfront" surgery (n = 86) with the patients who received neoadjuvant chemoradiation therapy followed by surgery (n = 9), we found no differences in the time of surgery (7 hours 18 minutes vs. 7 hours 0 minutes), estimated blood loss (720 ml vs. 600 ml), and postoperative length of stay (18 days vs. 14 days). Recent trials of preoperative chemoradiation (employing different regimens of chemotherapy and radiation) have reported surgical complications and mortality rates similar to those in patients who did not receive preoperative therapy.

Of course, survival is the most meaningful end point. Although our results are preliminary, the patients who underwent surgical resection have a median survival of 30 months (with 6 patients still alive and disease free). These results compare favorably to those reported by M.D. Anderson Cancer Center (19.2 months), Fox Chase Cancer Center (18 months), Boston University (19 months), and Eastern Cooperative Oncology Group (15.7 months) for median survival after preoperative chemoradiation therapy and surgical resection. These results are comparable to published reports of survival after Whipple resection, and adjuvant therapy in favorable patients. In a small analysis, Ishikawa et al.[12] reviewed their experience with 50 Gy preoperative radiation therapy alone for pancreatic cancer. At laparotomy, pancreaticoduodenectomy was performed in 17 of 23 patients. Compared to a similar group that received postoperative radiation therapy, there was an improved 1-year survival but the 3- and 5-year survival rates were nearly identical.

Perhaps, 5-FU administered via protracted venous infusion with concurrent radiation therapy may have contributed to the strong antitumor effect (tumor downstaging, nodal sterilization, and improved resectability). Protracted venous infusion of 5-FU compared with bolus 5-FU results in increased temporal exposure to therapeutic serum levels and increased total dose administered over a given treatment interval. Although the relative importance of these two factors is not known, the theoretical advantages have been realized in a number of prospective studies comparing protracted venous infusion and bolus 5-FU administration as adjuvant treatment in early-stage rectal cancer and primary treatment in advanced colorectal cancer.[20] Our group has previously reported that protracted venous infusion of 5-FU given concurrently with radiation therapy to treat pancreatic cancer allows for significantly greater chemotherapy and radiation therapy dose intensity with reduced acute toxicity compared with bolus 5-FU.[21] Our effort to achieve dose intensity may explain our relatively high negative margin, negative lymph node, and pathologic complete response rates.

CONCLUSION

Chemoradiation therapy was given to 15 patients with "marginally resectable" pancreatic cancer, nine of whom subsequently underwent surgical resection. The treatment was well tolerated and without any excess morbidity. We found a high rate of uninvolved lymph nodes and surgical margins compared with our historical experience in less advanced, "resectable" pancreatic cancer. Six of nine patients remain alive and disease free with a median follow-up of 30 months. Greater patient accrual and longer follow-up are needed to more accurately assess its proper role in therapy. A large multicenter randomized trial might be able to answer the question of whether neoadjuvant chemoradiation therapy would enable a greater number of patients to ultimately undergo a margin-negative surgical resection and have improved survival.

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Complete Fundoplication Is Not Associated With Increased Dysphagia in Patients With Abnormal Esophageal Motility

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Abnormal esophageal motility is a relative contraindication to complete (360-degree) fundoplication because of a purported risk of postoperative dysphagia. Partial fundoplication, however, may be associated with increased postoperative esophageal acid exposure. Our aim was to determine if complete fundoplication is associated with increased postoperative dysphagia in patients with abnormal esophageal motor function. Medical records of 140 patients (79 females; mean age 48 ± 1.1 years) who underwent fundoplication for gastroesophageal reflux disease (GERD) were reviewed retrospectively to document demographic data, symptoms, and diagnostic test results. Of the 126 patients who underwent complete fundoplication, 25 met manometric criteria for abnormal esophageal motility (≤30 mm Hg mean distal esophageal body pressure or ≤80% peristalsis), 68 had normal esophageal function, and 33 had incomplete manometric data and were therefore excluded from analysis. Of the 11 patients who underwent partial fundoplication, eight met criteria for abnormal esophageal motility, two had normal esophageal function, and one had incomplete data and was therefore excluded. After a median follow-up of 2 years (range 0.5 to 5 years), patients were asked to report heartburn, difficulty swallowing, and overall satisfaction using a standardized scoring scale. Complete responses were obtained in 72%. Sixty-five patients who underwent complete fundoplication and had manometric data available responded (46 normal manometry; 19 abnormal manometry). Outcomes were compared using the Mann-Whitney U test. After complete fundoplication, similar postoperative heartburn, swallowing, and overall satisfaction were reported by patients with normal and abnormal esophageal motility. Likewise, similar outcomes were reported after partial fundoplication. This retrospective study found equally low dysphagia rates regardless of baseline esophageal motility; therefore a randomized trial comparing complete versus partial fundoplication in patients with abnormal esophageal motility is warranted. (J GASTROINTEST SURG 2001;5:36-41.)

KEY WORDS: Gastroesophageal reflux, GERD, esophageal motility, esophageal manometry, fundoplication, dysphagia, Nissen fundoplication, Toupet fundoplication

The diagnosis and management of gastroesophageal reflux disease (GERD) has undergone significant scrutiny since the advent of proton pump inhibitors and laparoscopic fundoplication. A major focus of investigation has been the stratification of patients to either medical or surgical therapy. Patients with GERD and concomitant esophageal dysmotility represent the most heavily analyzed subgroup because these patients may benefit significantly from fundoplication, but also may be at increased risk for postoperative dysphagia. Surprisingly, few data establish a casual relationship between GERD and esophageal dysmotility.[1-3] Furthermore, evidence that decreased esophageal acid exposure improves esophageal motor function is controversial.[1,4,5]

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Proving an association between GERD and esophageal dysmotility is difficult. Recent physiologic studies have been unable to ascertain whether esophageal motor abnormalities are the cause of GERD, or if a proportion of esophageal dysmotility results from acid-induced injury.[6] If esophageal dysmotility were caused by GERD, then acid suppression by medical therapy would be expected to improve esophageal motor function.[7] To date, however, no conclusive medical data support the expectation that deacidification of esophageal refluxate improves esophageal motility.[3,8-11] Conversely, several surgical series have suggested that fundoplication improves esophageal motor function.[1,2,5,7,11-15] Despite this, both patients and physicians still fear the possibility of postfundoplication dysphagia. Therefore some surgeons advocate complete fundoplication for patients with normal esophageal motility and partial fundoplication for patients with disordered esophageal motility.[15] This approach assumes partial fundoplication augments the lower esophageal sphincter (LES) to a lesser degree than complete fundoplication, thereby reducing the risk of postoperative dysphagia in those patients with esophageal dysmotility while accepting the possibility of increased esophageal acid exposure postoperatively.[16,17] The spectrum of opinion varies widely, with some authors recommending partial fundoplication in all patients[18] and others advocating complete fundoplication regardless of baseline esophageal function.[5]

Our aim was to determine if complete fundoplication is associated with increased postoperative dysphagia in patients with esophageal dysmotility.

METHODS

Patient Consent

This study was reviewed and approved by, and performed in accordance with, the guidelines of the University of North Carolina at Chapel Hill Committee on the Protection of Rights of Human Subjects.

Patients

The medical records of 140 consecutive patients who underwent fundoplication for GERD (79 females; mean age 48 ± 1.1 years) were reviewed retrospectively to document demographic data, symptoms, indications, and diagnostic test results. Operative candidacy required indisputable proof of pathologic acid reflux, such as esophagitis, ulceration, peptic stricture, or Barrett's metaplasia on esophagogastroduodenoscopy (EGD), or an abnormal ambulatory 24-hour pH study. All patients underwent esophageal manometry. In selected patients, a barium esophagogram was obtained. During the study period there was an initial tendency toward complete fundoplication, then a short-lived enthusiasm for partial fundoplication because of concerns over postoperative dysphagia, followed by a return to preferential 360-degree fundoplication. Of the 140 antireflux procedures, 125 were completed laparoscopically. Overall, 126 patients underwent complete (360-degree; Nissen) fundoplication, 11 patients had a partial fundoplication, and three patients had other antireflux operations and were excluded from analysis. Of the patients undergoing complete fundoplication, 25 met manometric criteria for abnormal esophageal motility (≤30 mm Hg mean distal esophageal body pressure and/or ≤80% peristalsis) and 68 had normal esophageal motility. Thirty-three patients had incomplete manometric data and were therefore excluded from the final analysis (Fig. 1). Of the 11 patients who underwent partial fundoplication, eight met criteria for abnormal esophageal motility, two had normal esophageal function, and one had incomplete manometric data. Mean peristaltic amplitude and percentage of peristalsis were similar among those with abnormal esophageal motility who underwent complete fundoplication and those undergoing partial fundoplication.

Intraoperative Management

All procedures were performed with wide mobilization of the gastric fundus and creation of a ≤2 cm, 270-degree or 360-degree fundoplication around a 56 to 60 F bougie. All procedures were performed by three of us (K.E.B., C.C., and M.J.K.).

Postoperative Follow-Up

A previously validated, modified, GERD-specific symptom survey was mailed to all patients.[19] Nonresponders had follow-up by telephone. Patients were asked to report *heartburn*, *difficulty swallowing*, and *overall satisfaction* using a discontinuous scoring scale (Table I). Survey responses were obtained in 72% of patients. Sixty-five patients who underwent complete fundoplication and had manometric data available responded (46 normal manometry; 19 abnormal manometry). Median follow-up was 2 years (range 6 months to 5 years).

Statistical Analysis

Outcomes were compared using the Mann-Whitney U test (ordinal, unmatched data) using a 0.05 level of significance.

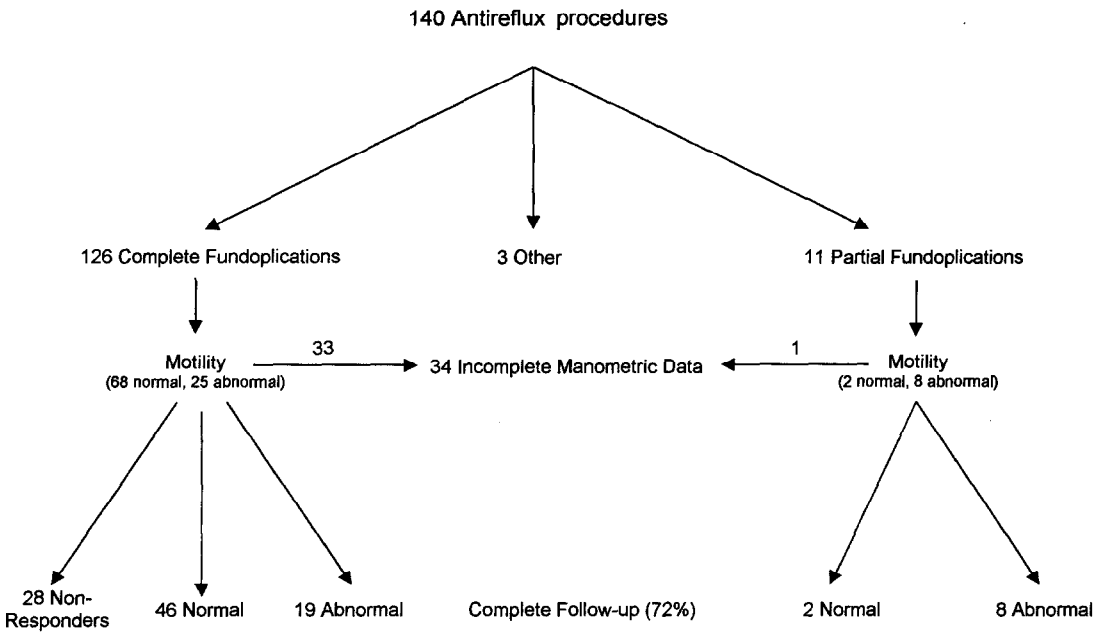

Fig. 1. Number of patients with normal and abnormal esophageal motility undergoing antireflux surgery.

Table I. Postoperative symptom survey

Question category	Scoring scale
Heartburn	0 = No symptoms
	1 = Symptoms noticeable/not bothersome
	2 = Symptoms noticeable/bothersome but not every day
	3 = Symptoms bothersome every day
	4 = Symptoms affect daily activities
	5 = Symptoms incapacitating
Difficulty swallowing	0 = Normal diet
	1 = Eat some solid food
	2 = Eat semisolids only
	3 = Swallow liquids only
	4 = Complete dysphagia
Overall satisfaction	Satisfied
	Neutral
	Dissatisfied

RESULTS

In the complete fundoplication group, 65 patients had full manometric data and survey responses available. There were no significant differences in postoperative heartburn ($P > 0.05$; Fig. 2, *A*), dysphagia ($P > 0.05$; Fig. 2, *B*), or overall satisfaction ($P > 0.05$; Fig. 2, *C*) between patients with normal (N = 46) and abnormal (N = 19) esophageal motility. Similarly, of the 10 patients with a partial fundoplication who had full manometric data available and responded to our survey, no significant differences among esophageal motility groups were noted with regard to postoperative heartburn ($P > 0.05$), dysphagia ($P > 0.05$), or overall satisfaction ($P > 0.05$). The small number of patients in this subset, however, precludes meaningful analysis.

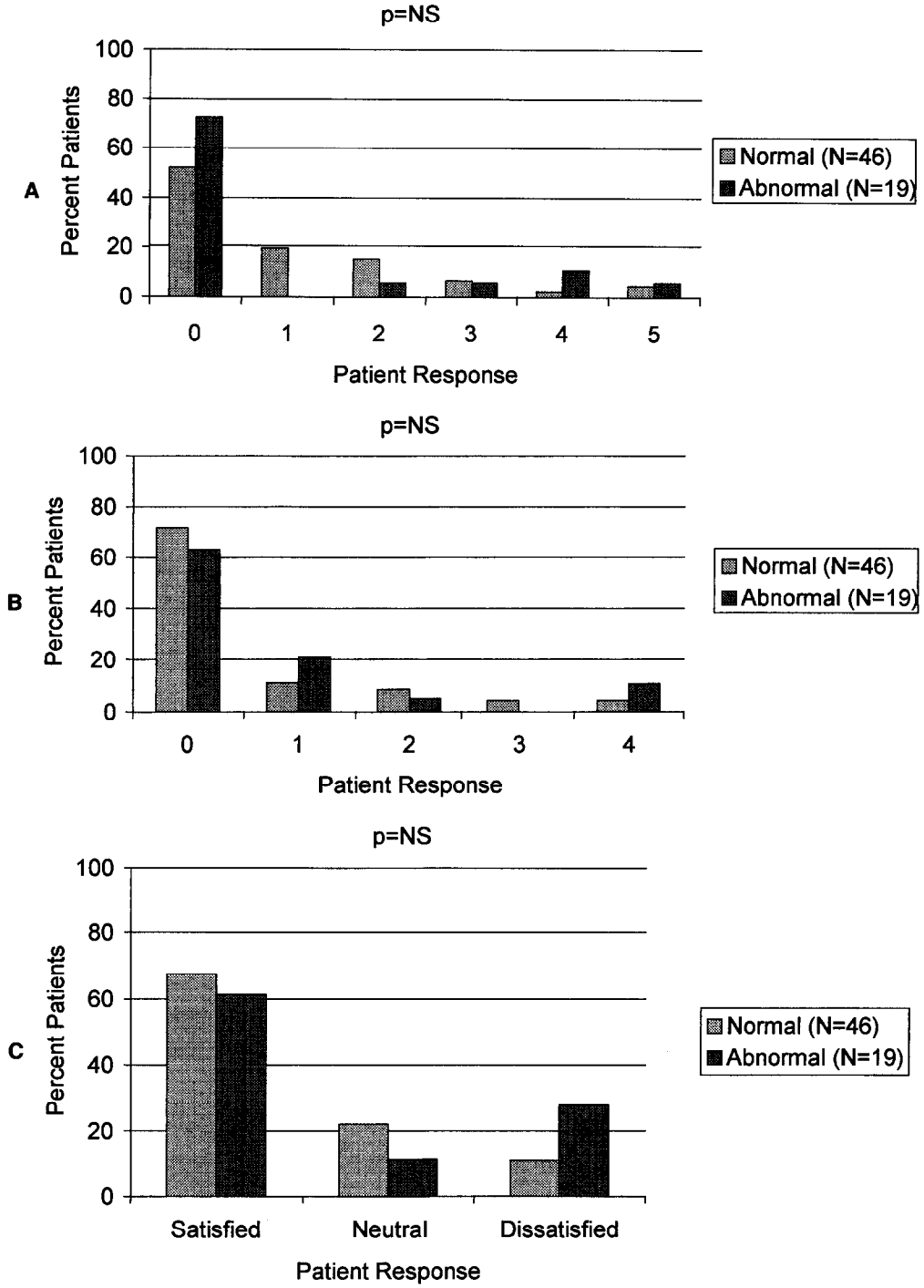

Fig. 2. Patient response to postoperative symptom survey after complete fundoplication. Variables assessed included heartburn **(A)**, dysphagia **(B)**, and overall satisfaction **(C)**. No statistical difference was evident between patients with normal or abnormal esophageal motility for the variables analyzed.

DISCUSSION

The aim of this study was to determine if complete fundoplication is associated with increased postoperative dysphagia in patients with preoperative esophageal dysmotility. Our data demonstrate no significant difference in dysphagia after complete fundoplication irrespective of preoperative esophageal motility. In addition, postoperative symptoms for heartburn and overall satisfaction did not differ between patients with normal and abnormal esophageal motility. Similarly, examination of these outcomes in a limited number of patients with a partial fundoplication demonstrated no differences in postoperative symptoms. These findings are in agreement with other studies that demonstrated a convergence over time of dysphagia rates after complete and partial fundoplication.[20,21]

The genesis of dysphagia following fundoplication may be of a mechanical origin or intrinsic to esophageal motor function. Mechanical failure of the operation must be considered because an excessively tight crural repair, a poorly located ("slipped") fundoplication, a herniated fundoplication, a long/tight esophageal wrap, a torsion-inducing fundoplication, or a gastric emptying abnormality all may contribute to postoperative dysphagia. A thorough anatomic and physiologic evaluation, including selective utilization of EGD, barium swallow, 24-hour pH study, esophageal manometry, and gastric emptying study, is necessary to identify possible causes of persistent dysphagia. Assuming appropriate patient selection and a technically sound fundoplication, recurrent postoperative esophageal acid exposure may be the most important variable to address.[7]

Our results demonstrate that complete fundoplication is tolerated equally well by patients with normal and abnormal esophageal motility at median 2-year follow-up. Whereas other investigators have reported similar early and intermediate outcomes after complete and partial fundoplication,[5,13,15,22,23] more recent reports suggest partial fundoplication is associated with less durable acid protection at longer follow-up, especially in patients with poor esophageal acid clearance.[16,17] Some authors have suggested that the optimal fundoplication is "tailored" to the preoperative esophageal physiologic findings,[24-26] yet this approach has failed to gain acceptance universally.[27] We hypothesize that the emerging superiority of complete fundoplication for acid protection will become evident with less long-term dysphagia as partial fundoplications fail over time and result in recurrent acid reflux.

The assumption that complete fundoplication creates greater resistance to the aboral passage of an esophageal bolus than partial fundoplication has not been well studied. Indirect evidence suggests this assumption may be incorrect. In porcine and human explants, both complete and partial fundoplication provided similar augmentation of the LES irrespective of in vivo anatomic relationships.[28,29] Manometric data support these findings, with LES resting pressures ranging from 10 to 15 mm Hg after both complete and partial fundoplications in human patients.[26,30]

The antireflux capacity of fundoplications appears to be more complex than simple augmentation of the LES resting pressure. Measurement of coincident pressures within the body of the stomach and at the fundoplication during gastric distention demonstrates a well-maintained pressure differential over physiologic and supraphysiologic pressures that serve to resist reflux.[28] These findings suggest that the high-pressure zone may not be vital for preventing reflux, and its association with postoperative dysphagia is unclear. Additional manometric studies in patients with complete and partial fundoplications are needed to improve our understanding of this relationship.

This retrospective study demonstrates that postoperative esophageal symptoms in patients with normal and abnormal esophageal motility do not differ after complete fundoplication. These findings suggest that a prospective, randomized trail of complete versus partial fundoplication in patients with abnormal esophageal motility would not increase substantially the risk of postoperative dysphagia in patients undergoing complete fundoplication. This study, however, has the limitations of a relatively small group of patients with only intermediate follow-up. In addition, 34 patients were excluded from the analysis because of incomplete manometric data. These patients were generally referred with esophageal manometry performed, but not quantified adequately for statistical analysis. In those patients with complete manometric data, the postoperative survey was completed in 72%. This study also does not address postoperative esophageal function determined by manometry or 24-hour pH analysis, both of which should be performed in a prospective study.

Our findings suggest that postoperative heartburn and dysphagia after complete fundoplication do not differ in patients with normal and abnormal esophageal motility. Furthermore, emerging data showing that partial fundoplication may result in increased GERD recurrence compared to complete fundoplication suggest that a prospective, randomized trial of complete versus partial fundoplication is safe and warranted.

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Five- to Eight-Year Outcome of the First Laparoscopic Nissen Fundoplications

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The operative mortality and morbidity of laparoscopic fundoplication are lower than for the open procedure. Questions have been raised regarding its long-term durability. One hundred seventy-one patients who had undergone laparoscopic Nissen fundoplication at least 5 years previously answered a questionnaire. During this period, 291 patients underwent a laparoscopic Nissen fundoplication. Surveillance data were available for 171 patients at a mean of 6.4 years after surgery. Overall, 96.5% were satisfied and 3.5% were not satisfied with the result of the procedure. Persistent symptoms included abdominal bloating (20.5%), diarrhea (12.3%), regurgitation (6.4%), heartburn (5.8%) and chest pain (4.1%); 27.5% reported dysphagia, and 7% had required dilatation. Fourteen percent were on continuous proton pump inhibitor therapy, but 79% of these patients were treated for vague abdominal or chest symptoms unrelated to reflux, which calls into question the indications for this therapy. Ninety-three percent of all patients were satisfied with their decision to have surgery. The overall well-being score increased significantly from 2.2 ± 1.6 before surgery to 8.8 ± 2 ($P > 0.0001$) at more than 5 years after surgery. Twenty-one percent had undergone additional diagnostic procedures after surgery such as endoscopy and/or barium swallow. Laparoscopic Nissen fundoplication is an excellent long-term treatment for gastroesophageal reflux disease with persistent success for more than 5 years. Some patients have continuing symptoms and remain on therapy, but more than 90% of all patients undergoing laparoscopic Nissen fundoplication remain satisfied with their decision to have surgery. These results are at least as good as those achieved with open fundoplication and prove the long-term worth of this procedure. (J GASTROINTEST SURG 2001;5:42-48.)

KEY WORDS: Long-term outcome, laparoscopic Nissen fundoplication, gastroesophageal reflux disease

In 1956 Nissen described "a simple surgical technique to influence reflux esophagitis."[1] In 1977 Donahue et al.[2] developed the "floppy Nissen," which offered an effective surgical treatment for gastroesophageal reflux disease (GERD). Reflux symptoms are immediately improved with 70% to 90% good results reported at up to 20 years after surgery.[3-6] Because of a mortality rate of up to 1.4% and morbidity of 12% for the open procedure, H2R antagonists or proton pump inhibitors remained the preferred treatment.[7-9]

In 1991 Dallemagne et al.[10] published their initial experience with the laparoscopic Nissen fundoplication. The initial operative outcome of the laparoscopic approach was similar to that of the open procedure, but mortality and morbidity were less than 0.2% and 5%, respectively.[11] The question remained as to whether the laparoscopic technique achieves the same long-term result as the open procedure.

Our experience with this procedure began in 1991 allowing us now to examine our 5- to 8-year follow-up results.

METHODS

We searched our computerized log for all 291 patients who underwent laparoscopic antireflux surgery from the time of our first laparoscopic procedure (July 1991) until September 1994. All procedures were done at least 5 years before the outcomes were evaluated. A total of 171 patients who had undergone lap-

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aroscopic Nissen fundoplication for persistent gastroesophageal reflux disease (GERD) and answered a questionnaire were included in this study.

All 291 patients were sent a specifically developed outcome questionnaire. Patients who did not reply were called and interviewed by a member of the Mayo Clinic Jacksonville clinical studies unit who entered the answers onto the questionnaire.

The questionnaire consisted of 10 groups of questions written in simple English that were easily understood by all patients. Satisfaction with the surgical procedure was collated using a score of very satisfied, satisfied, acceptable, or unacceptable. Patients were asked to rank current GERD-related symptoms such as heartburn, regurgitation, cough, chest pain, abdominal bloating, and diarrhea according to the following five grades of severity: none, minimal, mild, significant, or severe. Persistent symptoms were recorded as present if they were significant or severe. Difficulty swallowing solids, liquids, or both and whether patients had required dilatation since surgery were recorded. Patients were asked to report on their need for medication to counteract acid, names of the drugs, dosages, and whether these drugs were for continuous or intermittent use. Patients on continuous proton pump inhibitors were called and personally reevaluated as to the reason for the use of proton pump inhibitors. A range of well-being score (10 = excellent and 1 = poor) after laparoscopic Nissen fundoplication was compared with patients' retrospective feelings of well-being before surgery. Satisfaction with the decision to have surgery as a treatment for GERD was collated.

Preoperative symptoms were compared with persistent postoperative symptoms. Data concerning preoperative symptoms were available in a database and patient charts. Outcomes in the first 100 patients who underwent laparoscopic antireflux surgery were compared with outcomes in the remaining patients.

Statistics

Symptoms and well-being before and after surgery were compared using the paired t test or McNemar test. Comparison between the first 100 patients and the remainder was done using the Mann-Whitney U test. Statistical significance was indicated by a P value ≤ 0.05.

RESULTS

A total of 291 patients underwent laparoscopic Nissen fundoplication by us during this period. One hundred seventy-one patients were available to answer the questionnaire. Mean surveillance was 6.4 years (range 5 to 8.2 years) after surgery. Demographics are shown in Table I.

Table I. Demographics, duration of symptoms, manometry and esophageal pH testing before surgery (n = 171)

Sex ratio (M/F)	1.6:1
Age (yr)	52 ± 14
Duration of symptoms (mo)	119 ± 122
Lower esophageal sphincter pressure (mm Hg)	4.4 ± 3.0 mm Hg
Overall length (cm)	4.1 ± 1.8
Intra-abdominal length (cm)	1.5 ± 0.7
DeMeester score	46.7 ± 48

Ninety-six percent of the patients were satisfied with the results of the procedure (69% very satisfied, 19.3% satisfied, and 8.2% moderately satisfied) and 3.5% found it was not acceptable (poor). All patients with poor satisfaction had continuing symptoms of gastroesophageal reflux. Five of the six patients had severe abdominal pain, and one of them also had heartburn, reflux, and chest pain. Another one of these patients suffered from dysphagia. The sixth patient required repeated esophageal dilatation for dysphagia. In comparison to the first 100 patients who underwent laparoscopic Nissen fundoplication (follow-up data available from 58), a significantly better satisfaction rate was found in subsequent patients (follow-up data available from 113) ($P < 0.02$) (Fig. 1). The problems reported persisted over several years suggesting that this difference is due to the learning curve rather than deterioration of the procedure over time.

Persistent GERD symptoms at follow-up were regurgitation in 6.4%, heartburn in 5.8%, chest pain in 4.1%, and cough in 3.6%. Some patients had more than one symptom. All of these symptoms had improved significantly after laparoscopic antireflux surgery (Fig. 2). Other symptoms reported at follow-up were difficulty swallowing in 27.5% of the patients, either with solids, liquids, or both (Fig. 3). Fifteen percent of these patients had an esophageal stricture before surgery. During the follow-up period, 7% required dilatation for dysphagia. However, the overall incidence of dysphagia after surgery was less than before surgery (Fig. 4). Two thirds of patients with dysphagia before surgery did not have dysphagia after surgery. But approximately half of the patients who had dysphagia after surgery did not have dysphagia before surgery.

Furthermore, 20.5% of patients reported abdominal bloating and 12.3% had diarrhea. Continuous proton pump inhibitor therapy was used in 14% of

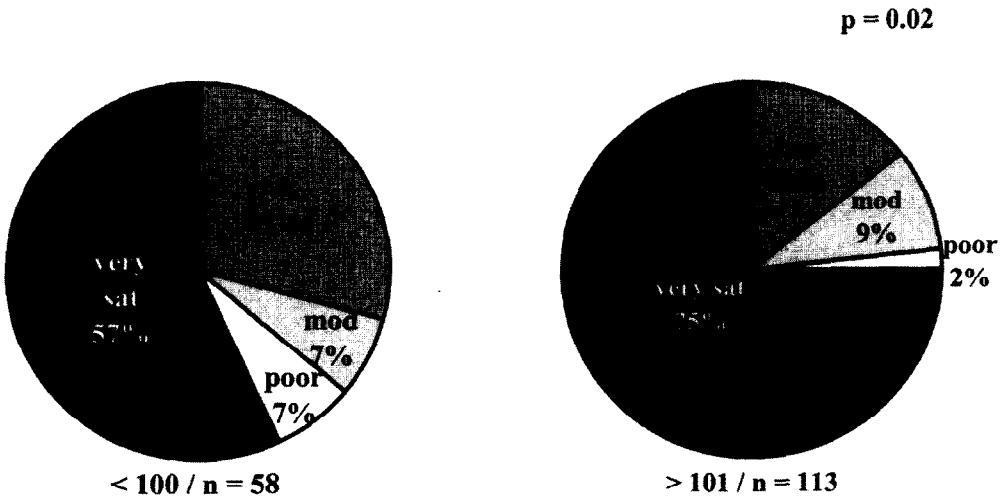

Fig. 1. Satisfaction with results of laparoscopic Nissen fundoplication. Comparison of the first 100 patients who underwent surgery with the remaining patients (very sat = very satisfied; sat = satisfied; mod = moderate).

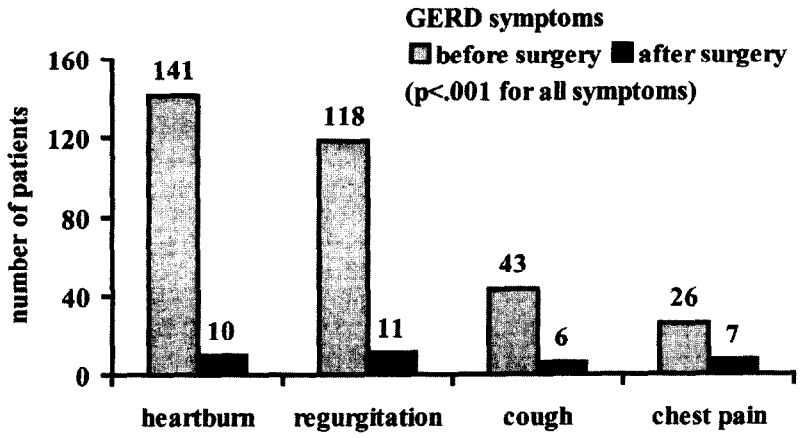

Fig. 2. Five to 8 years after laparoscopic Nissen fundoplication, GERD symptoms had improved significantly and remained stable.

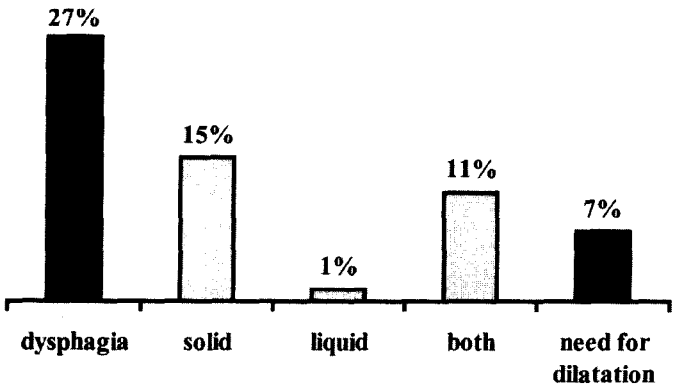

Fig. 3. Dysphagia after laparoscopic Nissen fundoplication and need for dilatation.

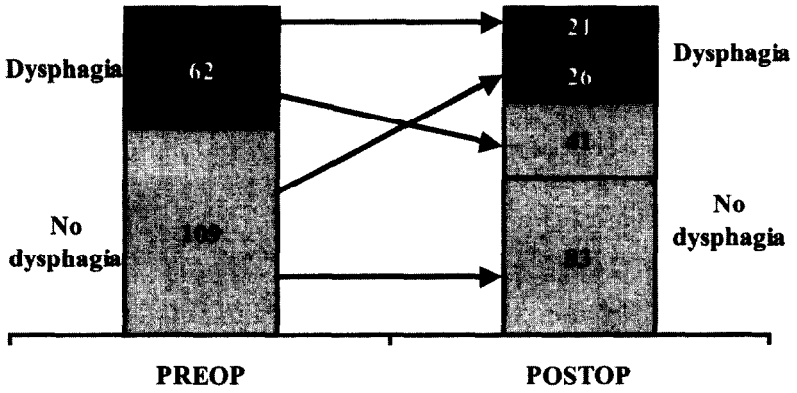

Fig. 4. Change in the presence or absence of dysphagia for individual patients before and after fundoplication.

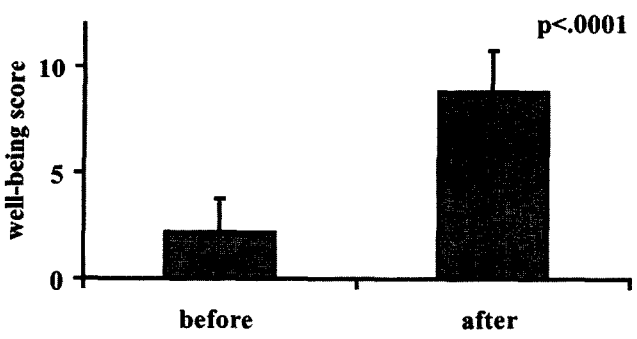

Fig. 5. Well-being score before laparoscopic Nissen fundoplication and 5 to 8 years after surgery.

patients. Seventy-nine percent of them were on this therapy for abdominal or chest symptoms thought to be unrelated to reflux.

During the follow-up period, five patients needed further abdominal surgery after laparoscopic Nissen fundoplication. Two patients had adhesions (one was converted to the open procedure during the original Nissen fundoplication), two had a redo Nissen procedure for GERD, and one patient had an incarcerated stomach and colon in the chest. The latter patient underwent repair of a large Morgagni hernia during the original laparoscopic Nissen fundoplication. Minor problems with the trocar wounds or scars were reported in 6% of the patients.

Ninety-three percent were satisfied, 2% were unsure, and 5% were not satisfied with their decision to have surgery. At more than 5 years, overall well-being showed significant improvement compared to before surgery ($P < 0.0001$) (Fig. 5).

Thirty-seven patients (12.7%) undergoing surgery had Barrett's esophagus. None reported the development of dysplasia or adenocarcinoma of the esophagus after surgery.

DISCUSSION

Laparoscopic general surgery was popularized at the end of the preceding millennium; however, the overall result of any procedure should not be compromised by the technique employed. Many studies have reported excellent short-term outcome for laparoscopic antireflex surgery similar to that achieved with open procedure.[11,12] In our study we have shown that the long-term results of laparoscopic antireflux surgery appear to be as good as those previously reported for open antireflux surgery.[4-6]

These results need to be compared to the best obtainable in patients on medical therapy. Prospective randomized studies have shown that surgery for GERD is superior to medical treatment. In the 1970s the open Nissen procedure was found to be superior to antacids,[13] in the 1990s better results were achieved with open antireflux surgery than with H_2 receptor antagonists,[14] and in 1999 it was shown that laparoscopic antireflux surgery is superior to proton pump inhibitor therapy.[15] Surgery is clinically more effective and less expensive than medical therapy. Spechler[16] showed that for men under 48 years of age and women over 55 years of age, there is a cost advantage for surgery. Nevertheless, it was argued that no long-term data were available after laparoscopic antireflux surgery. We now have data extending the good short-term results.

Antireflux surgery, especially the laparoscopic technique, has a definite learning curve. An institutional learning curve of 50 procedures and an individual learning curve of 20 operations were identified.[17] Our study confirms that the first 100 patients had a less satisfactory outcome than subsequent patients, thus indicating the effect of the learning curve on outcome.

Heartburn, regurgitation, cough, and chest pain at 5 to 8 years after laparoscopic surgery were significantly improved compared to before surgery. This

proves that the laparoscopic Nissen fundoplication provides an excellent antireflux barrier. However, patients reported a high incidence of gas bloat and diarrhea. This is similar to what was found in a prospective study of 100 patients who underwent laparoscopic Nissen fundoplication where gas bloat and diarrhea did not change in frequency and severity when these symptoms were compared before and after surgery.[18] This suggests that these may often be preexisting symptoms that are not changed by antireflux surgery. The failure rate of laparoscopic antireflux surgery requiring redo surgery was only 2%.

The small number of patients with Barrett's esophagus does not allow for conclusions regarding the protective effect of fundoplication on the progression of disease.

One of the main problems after Nissen fundoplication is dysphagia. Even if the wrap is very "floppy," some patients will develop dysphagia after surgery. The incidence of dysphagia after open fundoplication ranges from 4% to 43%.[19-21] This wide variation reflects the difference in definition of dysphagia by various authors. Our reported rate of 27% is high, which is a reflection of our strict definition of dysphagia. None required continued dilatation and all patients manage well with slight dietary modification. Many patients who have dysphagia before surgery are improved after surgery. This is particularly true of those with a stricture.[22] However, some patients are unchanged or have worse dysphagia after surgery. Individual responses generally cannot be predicted. A prospective study found that dysphagia can be predicted according to the personality of the patient,[23] and postoperative dysphagia is more common in patients with endoscopy-negative GERD.[24] A lower pain threshold in the esophagus in patients without esophagitis may be the reason for this dysphagia.[25] Elevated lower esophageal sphincter resting pressure and decreased relaxation of the sphincter after laparoscopic antireflux surgery may cause outflow resistance during a swallow, and this may be enough to elicit dysphagia.[26]

One seventh of our patients were on continuous proton pump inhibitor therapy 5 to 8 years after laparoscopic antireflux surgery. Another study found 39% of patients 2 years after laparoscopic antireflux surgery to be on acid suppressive or promotility agents. Eighty-four percent of these subjects reported a good surgical outcome despite continuing on medication.[27] This is an unexpectedly high need for antireflux medication, but an evaluation of postoperative use of medication showed that the indication for proton pump inhibitors is often for vague, nonspecific symptoms. Only 6% had evidence of GERD requiring therapy; therefore the high postoperative use of proton pump inhibitors is questionable. It is important for surgeons and gastroenterologists to avoid improper use of these drugs.

In conclusion, laparoscopic Nissen fundoplication is an excellent long-term treatment for GERD with good success for at least 5 to 8 years. There is low morbidity and mortality similar to medical treatment, and it is cost-effective. Some patients have continuing symptoms and remain on therapy, but more than 90% of patients remain satisfied with their decision to undergo surgery. These results are at least as good as those achieved with open fundoplication and prove the long-term worth of this procedure.

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Discussion

Dr. C. Pellegrini (Seattle, Wash.). Can you expand on what you mean by dysphagia? You reported that 27% of patients had it, a number that seems too high to me. Was this early after operation? Do you have a severity index? I just want to make sure that we do not present data to the gastroenterology community that exaggerates a problem. Since satisfaction appears to be so high at the end of 6 or 8 years, do you have any objective data on these patients that suggested acid reflux control, for example, 24-hour pH monitoring?

Dr. R.A. Hinder. The dysphagia rate is high because we asked patients whether they ever experienced any difficulty swallowing liquids, solids, or both. Unfortunately, the degree was not ascertained so I cannot give you any details on that. In the vast majority of patients it was not a problem, as indicated by the fact that only 7% of the patients required a dilatation. One in four of our patients tell us that food does occasionally get stuck, but in the vast majority this is not a problem. With regard to objective evidence of reflux, no we did not measure pH.

Dr. K.H. Fuchs (Wurzburg, Germany). In your study, patients had a poor quality of life preoperatively and were taking proton pump inhibitors (PPIs). After operation, as was shown very nicely in your study, quality of life improved, even though some of these patients are back on medication. What is your explanation for why the medication seemed to work better after the operation than before?

Dr. Hinder. This population of patients on PPIs who now report satisfaction with the results of surgery were patients who had surgery because they achieved poor results with PPIs. Failure of medical therapy was the most frequently cited reason for surgery. So these patients who have previously failed PPI therapy are now satisfied with PPIs plus surgery. Some patients require both PPIs and surgery to obtain relief, but most patients are taking these drugs for unconventional reasons.

Dr. L.W. Way (San Francisco, Calif.). I would like you to further clarify the dysphagia question. Are the patients who had dysphagia severe enough to require dilatation still undergoing dilatation at the 5- to 8-year mark or were they largely clustered in the early postoperative period? Second, did all of the patients who were less than fully satisfied have abnormal reflux scores preoperatively and was pH monitoring done in all these patients? How did that correlate with the results?

Dr. Hinder. More than 90% of the dilatations occurred in the first months after surgery. The mean number of dilatations was 1.5 per patient, and most of the patients required only one dilatation to obtain relief. The maximum number of dilatations was six. These patients did not require frequent, prolonged dilatation. All patients who were not satisfied had a positive preoperative pH score.

Dr. J.G. Hunter (Atlanta, Ga.). How many patients had strictures preoperatively that required late dilatation? Were all of the dilatations purely as the result of the fundoplication? Our number for patients who are back on medication is identical to yours, but when we ask our patients whether the medication is doing any good, the answer is, "I don't notice any difference." Did you ask that question of your patients who are back on PPIs, and did you try taking those who said that PPIs did not make a difference off of their medication to see what would happen?

Dr. Hinder. We found that about half of our patients with strictures required dilatations postoperatively, and have previously reported that. However, most of our patients who required dilatation did not have strictures before the operation. You asked whether our patients thought the medication had helped. When patients had typical GERD symptoms, their response to medication was good. If they had atypical symptoms, the response was poor, and I suppose predictably so.

Dr. V.H. Finch (Chicago, Ill.). The results of Nissen fundoplication and PPIs therapy have been roughly the same—91% or 93%, in that range. Many of the English groups offer their patients a choice—that is, they sit down and talk to them—but in the United States most gastroenterologists use the Nissen procedure only when medical treatment fails. We have used PPIs for 10 years or more, with rather satisfactory results. Do you have any data on the Nissen procedure 10 years or more after the initial procedure?

Dr. Hinder. The Nissen fundoplication seems to be holding up well over the longer term. We have data from Scandinavia that extends to 20 years, and it seems as if the procedure is lasting well into the second decade after it is performed. When we looked at our failure rate in another study, we found that most of the failures occurred in the first few years after surgery and the number then tapered off over the next 10 or 20 years.

Thyroid Hormone and the D-Type Cyclins Interact in Regulating Enterocyte Gene Transcription

Shufen Meng, M.D., Jason Badrinarain, Eric Sibley, M.D., Ph.D., Rixun Fang, Ph.D., Richard Hodin, M.D.

Thyroid hormone (T3) is an important regulator of gut mucosal development and differentiation, inducing intestinal alkaline phosphatase (IAP) and repressing lactase gene transcription. In contrast, cyclin D1 (CD1) appears to be a growth promoter in the gut, functioning to maintain the undifferentiated state. The present studies were designed to examine the effects of CD1 on T3 action within intestinal epithelia. Caco-2 cells were maintained in hypothyroid medium and transiently transfected with either rat lactase (3.0 kb) or human IAP (2.4 kb) luciferase (Luc) reporter plasmids. Cotransfections were carried out using two T3 receptor (TR) isoforms, TRα-1 and TRβ-1, as well as plasmids expressing CD1, CD3, CA, or CB1. Cells were then treated ± 10 nmol/L T3 for 24 hours and luciferase activity was determined. With T3 treatment, IAP-Luc activity was induced (TRα-1 = eightfold, TRβ-1 = ninefold), but these effects were dramatically inhibited (>50%) by CD1 and CD3. In contrast, CA and CB1 did not alter T3-mediated IAP gene activation. The ability of CD1 and CD3 to inhibit T3 action was also tested in the context of the lactase gene, which is negatively regulated by T3. As expected, lactase reporter gene activity was repressed by T3 treatment in the case of both receptor isoforms, TRα-1 = 30% and TRβ-1 = 40%. In contrast to its effects on the IAP gene, CD1 did not inhibit T3-mediated changes in lactase reporter gene activity. The D-type cyclins (CD1 and CD3), but not CA or CB1, specifically inhibit T3-mediated activation of the IAP gene. In contrast, the D-type cyclins do not inhibit T3-mediated repression of the lactase gene. These studies have identified a novel molecular interaction that exists between the pathways of growth and differentiation within intestinal epithelia. (J GASTROINTEST SURG 2001; 5:49-55.)

KEY WORDS: Thyroid hormone, intestine, cyclin, lactase, intestinal alkaline phosphatase

The mammalian small intestine is lined by a simple columnar epithelium in which pluripotent, proliferating crypt cells give rise to four distinct lineages of differentiated cells, including enterocytes (95%), goblet and enteroendocrine cells on the villi, and paneth cells, which reside at the crypt base. The processes of gut epithelial growth and differentiation are tightly regulated along crypt-villus, longitudinal (duodenum to colon), and developmental axes, and also respond to dietary and hormonal factors.[1,2]

Among the most important regulators of intestinal epithelial differentiation is thyroid hormone (T3). Numerous studies in experimental animals have demonstrated that T3 is a critical regulator of both the structural and functional maturation of the mammalian small intestine.[3-5] Although most of the work on thyroid hormone and the gut has focused on the developmental period, T3 also exerts marked influences on the adult small intestine. For example, in regard to the brush-border enzymes, lactase activity is dramatically decreased in adult rats treated with thyroxine[6] and in hyperthyroid patients,[7] whereas intestinal alkaline phosphatase (IAP) activity increases markedly in response to T3.[8]

As in other tissues, gut epithelial differentiation is closely linked to the cell cycle, such that withdrawal from the cell cycle occurs as cells leave the crypts and enter the differentiated villus compartment. The

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mammalian cell cycle is controlled by a variety of protein cyclins and their associated kinases (cdk's), interactions that are required for progression through the M, G1, S, and G2 phases.[9] In addition to these cell cycle promoters, a number of inhibitors of the cell cycle have been identified and include p15, p16, p21, p27, p57, and others.[10] Of particular interest in gut biology is cyclin D1 (CD1), since it is highly expressed within intestinal crypt cells, but is absent in the villi, a pattern documented in both in vivo and in vitro model systems.[11,12] In general, CD1 levels correlate with the cellular proliferative rate and, as such, it has been suggested that a function of CD1 is to "maintain the undifferentiated state."

Given the role of T3 in intestinal maturation and differentiation, and the fact that CD1 generally exerts "antidifferentiating" effects, we wondered whether CD1 functions to inhibit T3 action within the gut. This hypothesis was supported by the observation that CD1 inhibits androgen-mediated gene transcription in prostate cells,[13] and the androgen and T3 receptors are both part of the nuclear receptor superfamily, sharing significant structural and functional homology. Furthermore, CD1 appears to interact with and inhibit the function of the P300/CBP coactivator, a protein involved in T3-mediated transcriptional activation (Leiter A, personal communication).

The present studies were designed to examine the possible role that CD1 may play in T3-mediated gene activation and repression within intestinal epithelia. We have used the human colon cancer–derived Caco-2 cells, which have the advantage of containing few, if any, endogenous T3 receptors. In addition, we have examined two different enterocyte differentiation marker genes, IAP and lactase, which are differentially regulated by T3.

MATERIAL AND METHODS

Cell Culture

Caco-2 cells (purchased from American Type Culture Collection [ATCC], Rockville, Md.) were grown in 160 cm^2 plastic flasks at 37° C/5%CO_2 in Dulbecco's modified Eagle medium (Gibco, Grand Island, N.Y.) supplemented with 10% fetal bovine serum (Sigma, St. Louis, Mo.), 2 mmol/L L-glutamine, and penicillin/streptomycin (100 U/ml). The medium was changed every 3 days and the cells were split via trypsinization when they reached 80% to 90% confluence.

Transient Transfections

Cells were transferred to 60 mm dishes and grown to 80% confluence. The DNA was transfected using the Superfect system (Qiagen, Valencia, California) and the cells were then incubated for 24 hours in medium "stripped" of thyroid hormone by the charcoal/resin method.[14] At 24 hours, the medium was changed, keeping the cells in the stripped serum, and treatments were carried out ± 10 nanomoles/L triiodothyronine for another 24 hours. The cells were washed with phosphate-buffered saline, extracts prepared using a freeze-thaw method, and protein assays done by the Bradford method.[15] Luciferase assays were performed using standard luminometry.[16]

The lactase reporter plasmid contains 3.0 kb of the 5′ flanking region from the rat lactase gene.[17] The IAP plasmid contains 2.4 kb of the 5′ flanking region from the human IAP gene.[18] Co transfections were carried out using either of two rat T3 receptor (TR) isoforms, α-1 or β-1,[19] as well as plasmids expressing CD1, CD3, CA, or CB1 (obtained from ATCC). A cytomegalovirus–β-galactosidase plasmid was used to control for transfection efficiency. For each transfection the amount of total DNA was kept constant: 3 μg of the luciferase reporter, 2 μg of the TR-expressing plasmid, 1.5 μg of the cyclin-expressing plasmid, and 1 μg of the β-gal control plasmid. All transfection results are based on repeated experiments, n = 5 or greater. Statistical significance ($P < 0.05$) was determined using Student's unpaired t test.

RESULTS

We first examined the effects of CD1 on T3-mediated IAP gene activation. The results with the TRα-1 receptor are shown in Fig. 1. T3 treatment induced a dramatic increase in IAP reporter gene activity, approximately eightfold ($P < 0.001$). This increase in IAP gene expression in response to T3 is consistent with previous in vivo data.[20] Cotransfection of the CD1-expressing plasmid markedly inhibited (approximate 60% decrease) the magnitude of IAP activation by T3 mediated by the TRα-1 receptor.

Fig. 2 depicts the results with the other gut T3 receptor, TRβ-1. Similar to what was seen with TRα-1, a large increase in IAP reporter gene activity occurred in response to T3 treatment of these TRβ-1–transfected cells. Furthermore, CD1 cotransfection inhibited the T3-mediated effects on IAP reporter gene activity, similar to that seen in the case of TRα-1.

We next examined the T3 and CD1 effects on the lactase gene. In contrast to the IAP activation by T3, lactase reporter gene activity was repressed by T3 treatment, consistent with our previous in vivo observations.[20] Both the TRβ-1 and TRα-1 receptor isoforms were able to mediate the repression by T3, approximate 40% decreases being seen in both cases (Fig. 3). Unlike its inhibitory effects on the IAP gene,

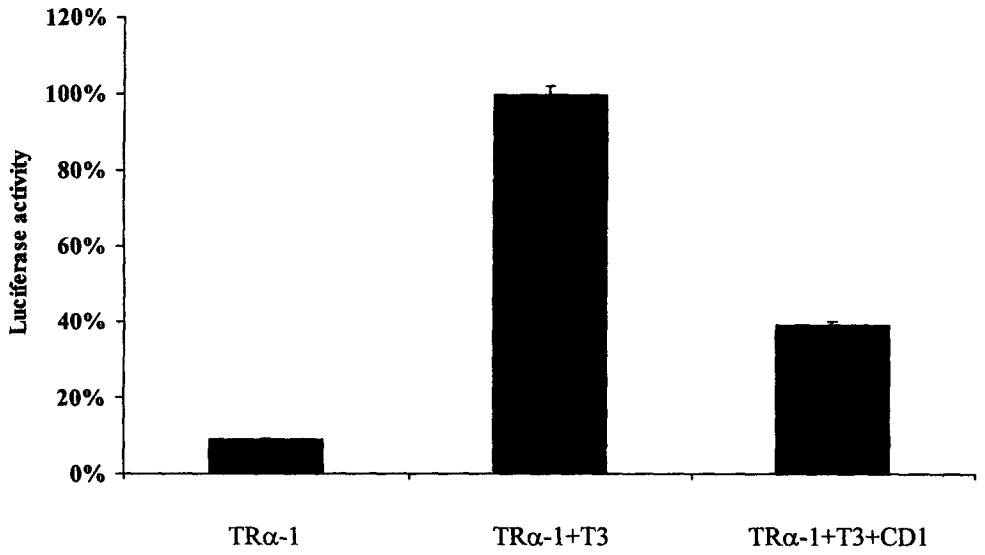

Fig. 1. CD1 effects on TRα-1–mediated T3 activation of the IAP gene. Transient transfections were performed in Caco-2 cells with the human IAP-Luc reporter plasmid along with an expression plasmid encoding the TRα-1 receptor. Cells were treated ± 10 nmol/L T3. A CD1-expression plasmid was cotransfected, as indicated. Transfections were repeated at least five times for each group and luciferase activity was normalized using a β-gal plasmid to control for transfection efficiency.

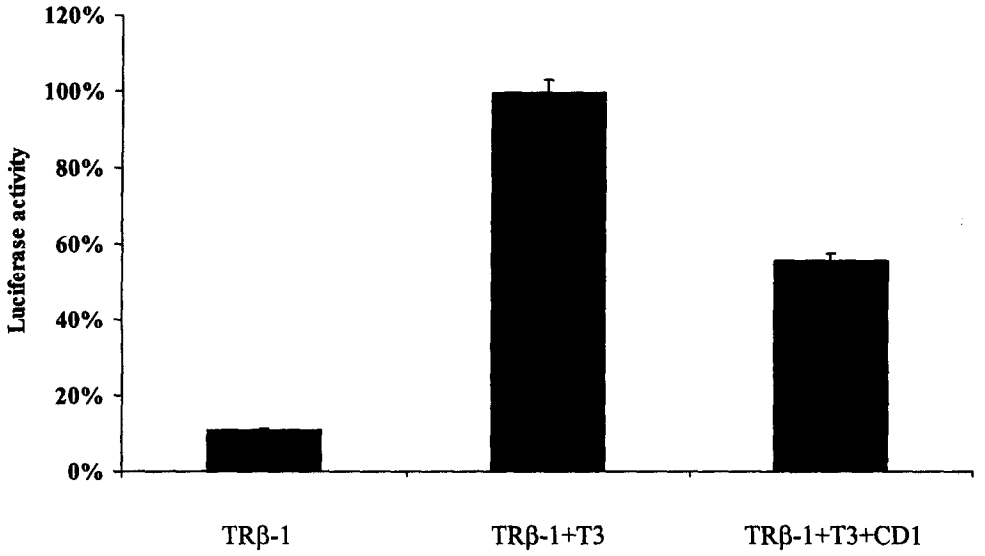

Fig. 2. CD1 effects on TRβ-1–mediated T3 activation of the IAP gene. Transient transfections were performed in Caco-2 cells with the human IAP-Luc reporter plasmid along with an expression plasmid encoding the TRβ-1 receptor. Cells were treated ± 10 nmol/L T3. A CD1-expression plasmid was cotransfected, as indicated. Transfections were repeated at least five times for each group and luciferase activity was normalized using a β-gal plasmid to control for transfection efficiency.

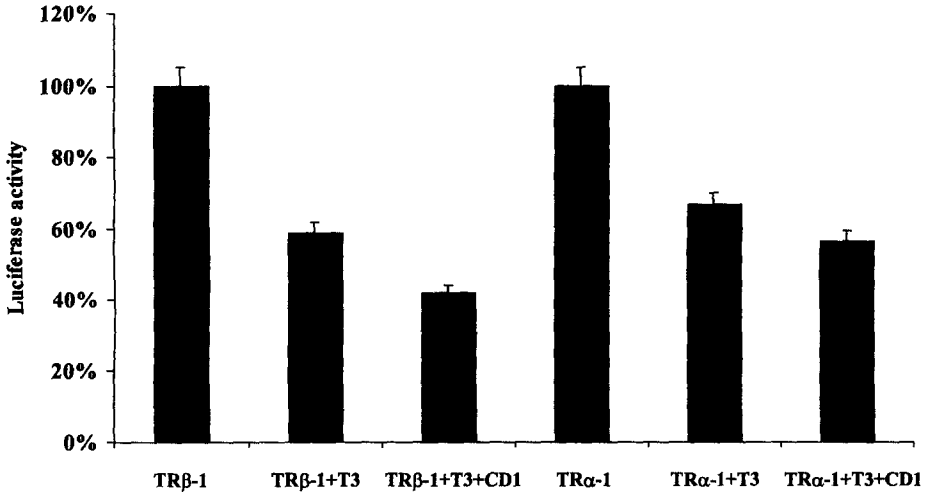

Fig. 3. CD1 effects on TRβ-1–mediated T3 repression of the lactase gene. Transient transfections were performed in Caco-2 cells with the rat lactase-Luc reporter plasmid along with an expression plasmids encoding either the TRβ-1 or TRβ-1 receptor. Cells were treated ± 10 nmol/L T3. A CD1-expression plasmid was cotransfected, as indicated. Transfections were repeated at least five times for each group and luciferase activity was normalized using a β-gal plasmid to control for transfection efficiency.

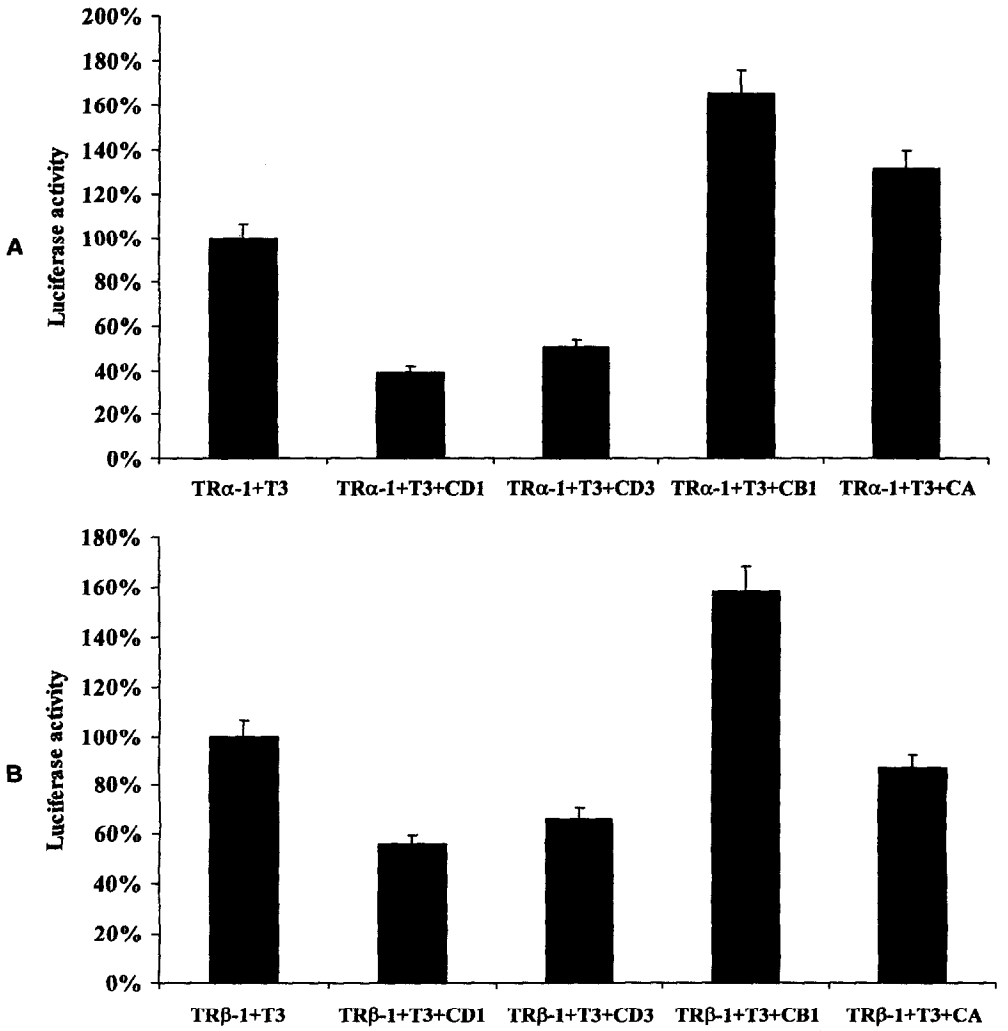

Fig. 4. Effects of the various cyclins on TR-mediated T3 activation of the IAP gene. Transient transfections were performed in Caco-2 cells with the human IAP-Luc reporter plasmid along with expression plasmids encoding either the **(A)** TRα-1 or **(B)** TRβ-1 receptor. Cells were treated ± 10 nmol/L T3. CD1-, CD3-, CA-, and CB1-expression plasmids were cotransfected, as indicated. Transfections were repeated at least five times for each group and luciferase activity was normalized using a β-gal plasmid to control for transfection efficiency.

cotransfection of CD1 had little effect on lactase reporter gene repression by T3. With either of the T3 receptors, the T3-mediated repression was essentially unaltered by CD1. These results suggest that the ability of CD1 to inhibit T3 action was limited to the positively regulated IAP gene.

We next examined a variety of other cyclins to determine the specificity of the CD1 effects. Fig. 4, *A* depicts the results seen with the TRα-1 receptor. Once again the inhibitory effects of CD1 in regard to the IAP gene were evident, and similar inhibition was seen in the case of the other D-type cyclin, CD3. In contrast, cotransfection of CB1 or CA did not inhibit IAP activation by T3. In fact, CB1 and CA cotransfection appeared to augment T3-mediated IAP gene activation. Fig. 4, *B* depicts the results with the TRβ-1 receptor form and again shows that CD1 and CD3 were able to inhibit T3-mediated IAP gene activation. As was seen with the TRα-1 receptor, CB1 and CA did not inhibit T3 action in terms of the IAP gene, and, in fact, there was superinduction evident with CB1.

DISCUSSION

Thyroid hormone is among the most important regulators of intestinal epithelial development and also exerts profound homeostatic influences on the adult gut, affecting the fundamental processes of growth and differentiation. The importance of T3 on gut development was first suspected with the observation that endogenous thyroid hormone levels increase just prior to weaning, a period marked by a dramatic growth spurt within the gut, as well as a shift in the expression of a number of intestinal gene products. Indeed, T3 has been shown to be a critical regulator of both the structural and functional maturation of the mammalian small intestine. We have focused the present work on two T3 target genes, IAP and lactase, both of which are well-characterized markers of enterocyte differentiation. The mRNA expression of these enterocyte genes is differentially regulated by thyroid hormone in vivo.[20] Since IAP is activated whereas lactase is repressed by T3, these target genes provide the unique opportunity to examine the "dual" effects of thyroid hormone within a single (enterocyte) cell.

IAP is a brush-border protein that hydrolyses monophosphate esters and may play an important role in the absorption of dietary fat, comprising a major portion of the surfactant-like particle seen with fat ingestion.[21] Lactase-phlorizin hydrolase (lactase) is a brush-border enzyme that cleaves dietary lactose into its components, glucose and galactose. Deficiency of lactase is a common disorder in Western societies, resulting in the clinical syndrome of abdominal cramps and diarrhea. The precise molecular mechanisms responsible for regulating the complex patterns of IAP and lactase gene expression are not well known.

The mechanism by which T3 exerts its effects on the gastrointestinal tract is not well understood. There have been few in vitro studies on thyroid hormone action in intestinal cells, probably because the widely used intestinal epithelial cell lines are derived from human colon cancers and express few, if any, T3 receptors. In general, T3 is known to exert its cellular effects through binding to a receptor protein (TR) located within the nucleus of target cells.[22] The TR is part of a large superfamily of structurally related, ligand-inducible transcription factors, which also includes the receptors for estrogen, glucocorticoids, vitamin D_3, retinoic acid, androgens, and others. These receptors all contain distinct DNA and ligand-binding domains, and mediate ligand-dependent transcriptional control of target genes, either activation or repression.[8,23] Multiple forms of the TR exist in humans, rats, and other species, and are encoded by either the α or β c-erbA genes.[19] Through alternative splicing, the c-erbAα gene gives rise to TRα-1, a bonafide receptor, and a non–hormone-binding variant called TRα-2. Interestingly, TRα-2 is known to inhibit T3 action, and we have previously shown that this may be a factor in the gut unresponsiveness to thyroid hormone during the suckling stage.[24] The c-erbAβ gene gives rise to two bonafide receptors, TRβ-1 and TRβ-2. The various TRs are expressed in a tissue-specific manner. For example, TRβ-2 is found almost exclusively in the pituitary and to a lesser extent in central nervous system tissues.[25] The other three TR gene products are more widely expressed and, importantly, have all been documented to be present within the gut epithelia.[26]

Recently much has been learned regarding the T3 effects on the gut through the study of TR knockout mice. The TRα knockout mice died within 5 weeks after birth as a result of progressive hypothyroidism, and, interestingly, these mice were found to have markedly impaired development in two major tissues, bone and intestine.[26,27] These TRα knockout animals provide convincing evidence for the critical role that thyroid hormone plays in gut development and homeostasis, especially since many other organs, for example, lung, heart, kidneys, and so forth, were not grossly affected.

The present work has demonstrated a previously unknown link between the cell cycle and T3 action. The D-type cyclins appear to specifically inhibit T3-mediated activation of the IAP gene. The other cyclins tested, CB1 and CA, did not block T3 action in this context. Interestingly, the inhibitory effects of the D-type cyclins were not seen in the case of T3-mediated repression of the lactase gene. Further work will

be needed to determine whether these effects of the D-type cyclins are seen with other T3-responsive genes and/or in other cell types, and, if so, if the inhibition is only seen in the case of positively regulated genes.

The D-type cyclins (D1, D2, and D3) promote cell cycle progression via an interaction with cdk4 and cdk6, and in contrast to cyclins A and B, the D-type cyclins are regulated by extracellular signals.[10] CD1 appears to generally play a role in maintaining the proliferative capacity of undifferentiated cells, inhibiting the differentiation process.[28] This association with rapidly dividing cells is consistent with the role that CD1 appears to play in neoplasia. It is overexpressed in non–small cell lung cancer and has been used as a marker of poor prognosis in hypopharyngeal carcinoma.[29,30] In colon cancers, CD1 levels are abnormally high and, interestingly, transcription of CD1 is induced by β-catenin.[31,32] As such, the neoplastic transformation related to mutations in the adenomatous polyposis coli (APC) gene may be mediated at least partly through CD1. In addition, the antiproliferative effects of transforming growth factor-β in intestinal epithelial cells has been shown to be due to CD1 inhibition.[33] Since CD1 is present in crypt cells, but not villus cells, the results of the present studies are consistent with CD1 functioning in a more general way to maintain the undifferentiated state, perhaps by blocking certain transcription factors (such as the TR) from regulating the expression of cell type–specific genes.

Taken together, our studies have identified a novel pathway by which a cell cycle–related protein appears to interact with the differentiation program. The precise physiologic significance of this link between CD1 and T3 action will need to be elucidated by future in vivo studies. It will be of particular interest to determine whether CD1 exerts its inhibitory effects on other aspects of gut epithelial differentiation.

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Sensitization of Human Colon Cancer Cells to TRAIL-Mediated Apoptosis

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TNF-related apoptosis-inducing ligand (TRAIL), a novel member of the tumor necrosis factor (TNF) family, is thought to induce apoptosis preferentially in cancer cells; however, increasing evidence suggests that a number of cancers are resistant to TRAIL treatment. FLICE-like inhibitory protein (FLIP), which structurally resembles caspase-8, can act as an inhibitor of apoptosis when expressed at high levels in certain cancer cells. The purpose of our present study was to determine whether human colon cancer cells are sensitive to TRAIL treatment and, if not, to identify potential mechanisms of resistance. Colon cancer cells of different metastatic potential (KM12C, KML4A, and KM20) were found to be resistant to the effects of TRAIL when used as a single agent. FLIP expression levels were increased in all three KM cell lines. Treatment with either actinomycin D (Act D;10 μg/ml) or cycloheximide (CHX; 10 μg/ml) decreased FLIP expression levels in all three cell lines. The decrease in cellular levels of FLIP was associated with sensitization to TRAIL-mediated apoptosis, as demonstrated by enhanced cell death and caspase-3 activity compared with either Act D or CHX alone. Our findings suggest that reduction of FLIP levels by Act D or CHX renders TRAIL-resistant human colon cancer cells sensitive to TRAIL-mediated apoptosis. The combination of TRAIL along with agents such as Act D or CHX, which target proteins that prevent cell death, may provide a more effective and less toxic regimen for treatment of resistant colon cancers. (J GASTROINTEST SURG 2001;5:56-65.)

KEY WORDS: Colon cancers, apoptosis, TRAIL, FLIP, actinomycin D, cycloheximide

Programmed cell death (i.e., apoptosis) is a vital and fundamental biologic process that plays a critical role in the normal development of multicellular organisms and in maintaining tissue homeostasis.[1] However, dysregulation of apoptosis may contribute to the development of diseases such as cancer.[2] Some of the well-known regulators of apoptosis include the tumor necrosis factor (TNF) family of cytokines and receptors, which can function as either inhibitors or activators of apoptosis. Activators of apoptosis include Fas ligand (FasL) and TNF, which induce apoptosis by activation of their corresponding receptors, Fas and TNFR-1.[3] Both FasL and TNF contain an extracellular ligand-binding domain and an intracellular region, which has been designated the cytoplasmic "death domain," that is responsible for transducing the death signal. This group of proteins has been implicated in diverse cellular processes including autoimmunity, neurodegenerative diseases, activation-induced cell death and "immune escape"of normal tissues (e.g., testis and the anterior chamber of the eye), and cancer metastases.[1,2,4-7]

TRAIL, a novel member of the TNF family, is a type II membrane protein that interacts with its cell receptors (DR4 and DR5) to induce apoptosis of surrounding immune cells. TRAIL has been suggested as a potential anticancer agent since initial reports indicated that TRAIL preferentially kills cancer cells.[8,9] Recent studies, however, have demonstrated that an increasing number of cancers are resistant to TRAIL.[10-14] One potential mechanism for this resistance may be the presence of "decoy" receptors (e.g., DcR1, DcR2, and

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osteoprotegerin), which bind TRAIL but are not linked to the apoptotic cascade. Another possibility is the presence of cellular proteins, which act as intracellular inhibitors of the apoptotic process.

FLICE-Like Inhibitory Protein (FLIP) was first described as a viral product that can inhibit Fas- or TNF-mediated apoptosis.[15-17] Stimulation of Fas enables the adapter molecule FADD and the death protease caspase-8 to bind to the receptor forming the death-inducing signaling complex (DISC).[15-17] Recruitment of caspase-8 to the DISC leads to its proteolytic activation, which initiates a cascade of caspases, leading to apoptosis.[15-17] FLIPs structurally resemble caspase-8, except they lack proteolytic activity.[15-17] FLIP contains a FADD binding domain but lacks the active-center cysteine residue and therefore may function as a dominant negative for caspase-8 (FLICE), thus blocking Fas-mediated apoptosis.[15-17] Several investigators have evaluated FLIP expression in normal and neoplastic melanoma cells.[14,18] For example, quiescent immune cells express FLIP protein and are resistant to TNF/FasL-induced cell death, but, on activation, FLIP levels decrease rendering these cells sensitive to TNF/FasL-induced cell death.[19,20] Furthermore, human melanoma cell lines, resistant to TRAIL-mediated cell death, were noted to have increased FLIP protein levels, which were reduced by addition of either actinomycin D (Act D) or cycloheximide (CHX).[13] This reduction was associated with an increased sensitivity to TRAIL-mediated cell death.

We have recently demonstrated increased TRAIL mRNA and protein expression in the more metastatic colon cancer cells (KML4A and KM20) compared with the KM12C cell line (derived from a Dukes' B cancer).[21] The increase in TRAIL expression correlates with increased Jurkat T-cell death in coculture experiments (Hernandez A, unpublished results). It is not known, however, whether these KM colon cancer cells are sensitive to TRAIL treatment. Therefore the purpose of this study was to determine whether these human colon cancer cells are sensitive to TRAIL and, if not, to identify potential mechanisms of resistance.

MATERIAL AND METHODS

Materials

Tissue culture media and reagents were obtained from Gibco BRL (Grand Island, N.Y.). Act D, CHX, and the monotetrazolium (MTT) assay were purchased from Sigma Chemical (St. Louis, Mo.). Total RNA was isolated using RNAzol (Biotex, Houston, Tex.) and digested with RNAse-free DNase I (Clontech, Inc., Palo Alto, Calif.). The concentrated protein assay dye was purchased from Bio-Rad Laboratories (Hercules, Calif.). Immobilon-P nylon membranes for Western blots were purchased from Millipore (Bedford, Mass.) and x-ray film was purchased from Eastman Kodak (Rochester, N.Y.). The enhanced chemiluminescence system for Western immunoblot analysis was obtained from Amersham (Arlington, Heights, Ill.). All antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, Calif.). The TRAIL apoptosis kit (17-226) was purchased from Upstate Biotechnology (Lake Placid, N.Y.). Caspase-3 assay substrate, Ac-Val-Ala-Asp-AFC, was purchased from Enzyme Systems Products (Livermore, Calif.).

Cell Culture

The human colon cancer cell lines KM12C, KML4A, and KM20 were provided by Dr. Isaiah J. Fidler (University of Texas M.D. Anderson Cancer Center, Houston, Tex.). The KM12C cell line was derived from a patient with a Dukes' B colon cancer. KML4A was derived from KM12C and has been "trained" to metastasize to the liver by multiple rounds of injection into the spleens of athymic nude mice. The KM20 cell line was derived from a patient with Dukes' D (metastatic to the liver) colon cancer. The cells were cultured in minimum essential medium (MEM) supplemented with 10% fetal calf serum, 1% sodium pyruvate, 1% nonessential amino acids, and 1% MEM essential vitamin mixture (complete media). Jurkat T cells, obtained from American Type Culture Collection (ATCC; Rockville, Md.), are grown in RPMI 1640 medium supplemented with 10% fetal calf serum (complete medium).

RNA Isolation and RT-PCR

Total RNA was extracted from cultured cell lines with RNAzol following the recommendations of the manufacturer. RNA was digested with RNase-free DNase I for 30 minutes at 37° C in 50 mmol/L Tris buffer, pH 6.5, with 10 mmol/L $MgCl_2$ and 10 mmol/L dithiothreitol (DTT). The reaction was terminated with 10× termination mix (0.1 mol/L EDTA, pH 8.0, and 1 mg/ml of glycogen), and RNA extraction was performed using the phenol method.[14] The quality of total RNA was assessed by agarose gel electrophoresis, as demonstrated by the presence of intact ribosomal RNA (28S and 18S).

Two micrograms of total RNA was first used in the synthesis of first-strand cDNA, following the manufacturer's directions. Briefly, a 50 μl reaction comprised of 2 μg total RNA, 1 μl oligo (dT), and RNase–free water was incubated for 10 minutes at 70° C. The reaction mixture was then mixed with 5× first-strand

Table I. Polymerase chain reaction

FLIP
Forward-5′-GCTCTAGAGGCCCGGAGCTGTACTGCAA-3′
Reverse-5′-GCGAATTCTATCCAGAAGGTCCCTGACA-3′
DR4
Forward-5′-CTGAGCAACGCAGACTCGCTGTCCAC-3′
Reverse-5′-TCAAAGGACACGGCAGAGCCTGTGCCAT-3′
DR5
Forward-5′-ATGGAACAACGGGGACAGAAC-3′
Reverse-5′-TTAGGACATGGCAGAGTCTGCATTAC-3′
DcR1
Forward-5′-ACCCTAAAGTTCGTCGTCGTCATC-3′
Reverse-5′-TCAAACAAACACAATCAGAAGCAC-3′
DcR2
Forward-5′-CTTTTCCGGCGGCGTTCATGTCCTTC-3′
Reverse-5′-GTTTCTTCCAGGCTGCTTCCCTTTGTA G-3′
GAPDH
Forward-5′-TCCACCACCCTGTTGCTGTA-3′
Reverse-5′-ACCACAGTCCATGCCATCAC-3′

buffer, 0.1 mol/L DTT and 10 mmol/L dNTP mix, and incubated for 2 minutes at 42° C. This was followed by the addition of Superscript II (200 U/μl) and incubation for 50 minutes at 42° C. The reaction was terminated by increasing the reaction mixture temperature to 70° C for 15 minutes. The cDNA generated was then used for polymerase chain reactions using a reverse transcription-polymerase chain reaction (RT-PCR) kit. Specific sequence primers (forward and reverse) for FLIP, DR4, DR5, DcR1, DcR2, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) are shown in Table I.[8,22-25]

Protein Preparation and Western Immunoblot

Cells were lysed with lysis buffer A (50 mmol/L Tris HCl [pH 7.5], 150 mmol/L NaCl, 0.5 mmol/L Nonidet P-40, 50 mmol/L NaF, 1 mmol/L sodium orthovanadate, 1 mmol/L dithiothreitol DTT, and 1 mmol/L phenylmethanesulfonyl fluoride, and 25 μg/ml each of aprotinin, leupeptin, and pepstatin A) at 4° C for 30 minutes. Lysates were clarified by centrifugation (10,000 *g* for 30 minutes at 4° C) and protein concentrations determined using the method of Bradford.[26] Western immunoblot analysis was performed as previously described.[27] Briefly, total protein (50 μg) was resolved on a 10% polyacrylamide gel and transferred to Immobilon-P nylon membranes. Filters were incubated overnight at 4° C in blotting solution (Tris-buffered saline containing 5% nonfat dried milk and 0.1% Tween 20) and then for 3 hours with the primary antibody to human FLIP or β-actin. Filters were incubated with a horseradish peroxidase–conjugated antirabbit or antigoat antibody as a secondary antibody for 1 hour. After four final washes, the immune complexes were visualized using enhanced chemiluminescence detection.

Monotetrazolium Assay

To determine whether TRAIL induces cell death of the KM cell lines, cells were added to 96-well Falcon plates (Becton Dickinson, Franklin Lakes, N.J.) at a concentration of 5×10^5 cells/well. Human recombinant TRAIL (hrTRAIL) was added at various concentrations (10 ng/ml, 50 ng/ml, and 100 ng/ml) and cell viability was determined by the MTT assay. In this assay, a tetrazolium salt, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide, is used as a colorimetric substrate for measuring cell viability. When cells are injured, there is an alteration of cellular redox activity so that cells are not able to reduce the dye. At the indicated time points, the MTT reagent (5 mg/ml) in phosphate-buffered saline solution was added to each well and incubated for 2 hours at 37° C in 5% CO_2. Plates were then centrifuged at 2500 rpm for 5 minutes to form cell pellets. Medium was removed and the resulting formazan was dissolved in 100 μl of acidic (0.1N HCl) isopropanol for 5 minutes at room temperature. Sample absorbance at 570 nm was measured using a spectrophotometer. All experiments were performed in duplicate.

JAM Assay–Isotope Incorporation assay with ^{3}H-thymidine (^{3}H-TdR)

To determine cell killing, we used a JAM assay, which measures the percentage of specific apoptosis.[28,29] The assay was essentially performed as previously described by Matzinger et al.[30] All experiments

were performed in triplicate. Tumor cells (i.e., as target cells) were labeled by incubation with 1 μCi of ^{3}H-TdR (ICN Pharmaceuticals, Costa Mesa, Calif.) for 15 hours at a density of 0.5×10^6 cells/ml. After labeling, the target cells were washed twice with complete medium, trypsinized, and seeded at a density of 2×10^5 cells per well into 96-well flat-bottom microtiter plates (Nunc, Wiesbaden, Germany). Cells were incubated in 250 μl of their respective growth media for 24 hours at 37° C. Medium was then removed and either new complete medium, Act D (10 μg/ml), CHX (10 μg/ml), hrTRAIL (100 ng/ml), or a combination of these agents was added in a final volume of 200 μl. Cells were then placed in a tissue culture incubator and allowed to grow for 24 and 48 hours at 37° C. Cells were then harvested using the cell harvester LKB/Wallac 1295-001 (LKB/Wallac, Freiburg, Germany). During this procedure, cells are lysed osmotically and the transferred macromolecular DNA is bound onto a glass fiber filter mat (Wallac). Apoptotic, fragmented DNA is too small to bind to the filter and is therefore washed through. The filter mat was air dried for 30 minutes and placed in 5 ml of scintillation liquid (Wallac). Bound radioactivity was measured by scintillation counting in a 1205 betaplate counter (LKB/Wallac). Percentage of apoptosis was calculated using the following formula:

$$\% \text{ Specific apoptosis} = \left(\frac{\text{cpm}_{\text{spontaneous}} - \text{cpm}_{\text{experimental}}}{\text{cpm}_{\text{spontaneous}}}\right) \times 100$$

The value for $\text{cpm}_{\text{spontaneous}}$ was determined by incubating target cells with medium only. The proportion of cells undergoing spontaneous apoptosis, calculated from the formula: $1\text{-}(\text{cpm}_{\text{spontaneous}}/\text{cpm}_{\text{total}})$, was usually less than 15%. The total amount of incorporated ^{3}H-thymidine ($\text{cpm}_{\text{total}}$) was determined by harvesting a cell aliquot at the beginning of the test. To assure complete collection of the radioactivity from the wells, a second and a third harvesting round was performed. Each harvesting round included two washing cycles. Then cumulative apoptosis was calculated—that is, both the $\text{cpm}_{\text{experimental}}$ and the $\text{cpm}_{\text{spontaneous}}$ were sums of the respective cpm obtained after two or three harvesting rounds.

Caspase-3 Assay

Caspase-3 assays were performed following the manufacture's protocol. Briefly, 10 μl of protein lysate was added to 490 μl of phosphate-buffered saline and incubated with 20 μl of Ac-Val-Ala-Asp-AFC substrate at 30° C for 30 minutes. Fluorescence recordings were obtained with fluorometer adjusted to 400 nm excitation and 505 nm emission. Fluorescence units per microgram of protein (enzyme activity) was plotted as relative caspase-3–like activity per microgram of protein.

Statistical Analysis

Results are expressed as the mean ± standard error of the mean (SEM). Killing rates were compared using a two-tailed Student's *t* test and considered significant if *P* values were <0.05.

RESULTS

Human Colon Cancer Cells (KM12C, KML4A, and KM20) Are Resistant to TRAIL-Mediated Cell Death

We have previously demonstrated that the KM colon cancer cells express TRAIL mRNA and protein.[21] To determine whether these cells are resistant to the killing effects of TRAIL, the three KM colon cancer cell lines were treated with increasing concentrations of hrTRAIL (10 ng/ml, 50 ng/ml, and 100 ng/ml) and assessed for cell viability by MTT assays (Fig. 1). TRAIL treatment had no effect on KM cell viability. In contrast, TRAIL treatment of Jurkat cells resulted in a significant decrease in cell viability, as previously reported.[31-33] Therefore these studies demonstrate that all three of the KM colon cancers are resistant to the effects of TRAIL.

One possible mechanism for this resistance could be the lack of functional TRAIL receptors (i.e., DR4 and DR5) or the presence of decoy receptors. RT-PCR was used to determine the status of TRAIL receptor expression in these cells. Both DR4 and DR5 as well as one of the decoy receptors (i.e., DcR2) were noted to be expressed in all three lines (data not shown). Although the presence of this one decoy receptor may partly explain the resistance of these cells to TRAIL, it is unlikely that this provides the sole mechanism, particularly since both of the functional receptors are present.

Expression of FLIP in KM Colon Cancer Cell Lines

A more likely explanation for the marked resistance of these cells to high doses of TRAIL is the possibility of the expression of intracellular antiapoptotic proteins (e.g., FLIP), which have been shown to render certain cancer cells resistant to FasL or TRAIL treatment. We assessed FLIP mRNA and protein expression in the KM cells by RT-PCR and Western immunoblot, respectively (Fig. 2). FLIP mRNA is expressed in all three cancer lines (see Fig. 2, *A*). Consistent with these results, similar levels of FLIP protein expression were noted in the three KM cell lines (see Fig. 2, *B*). The expression of FLIP in all three hu-

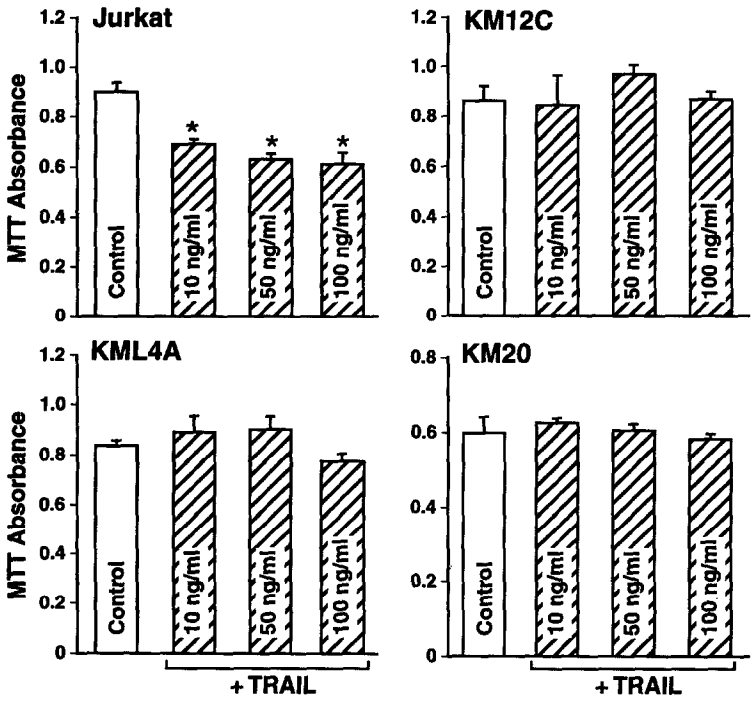

Fig. 1. MTT assays. Jurkat or KM cell lines (1×10^5 cells/well) were plated in 96-well plates and treated with increasing concentrations (10, 50, and 100 ng/ml) of soluble human recombinant TRAIL or vehicle (control). Cell viability was analyzed by MTT assays at 24 hours after treatment (all experiments were performed in duplicate). (Data are expressed as mean ± SEM; * = $P < 0.05$ compared with control values.)

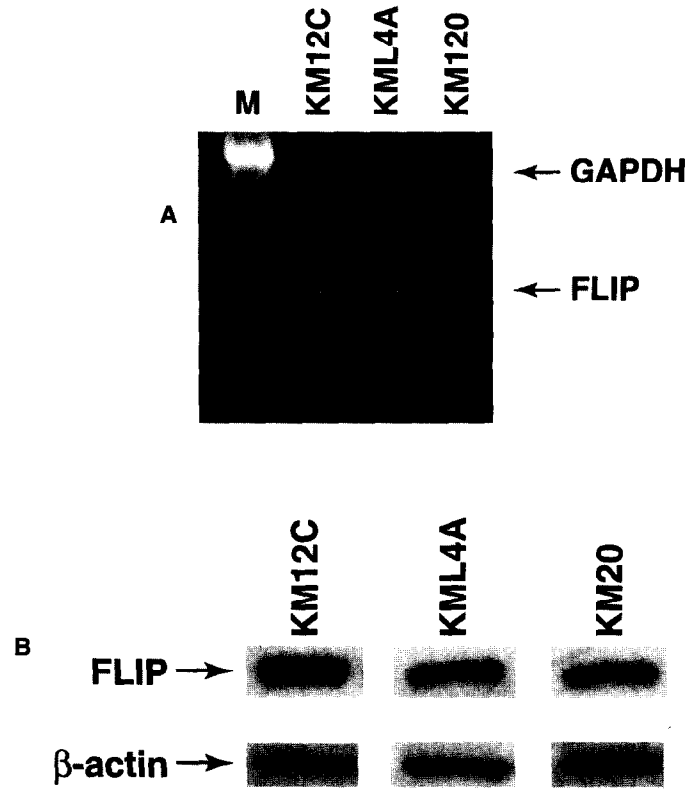

Fig. 2. FLIP mRNA and protein expression. **A,** RNA was extracted from the KM colon cancer cell lines and analyzed by RT-PCR using primers for human FLIP and GAPDH in the same reaction vessel as described in Material and Methods. M = molecular weight marker. **B,** Western immunoblot analysis of protein extracted from the KM colon cancer cells and probed with a human anti-FLIP antibody (Santa Cruz). The blot was then stripped and reprobed with a polyclonal antibody to β-actin.

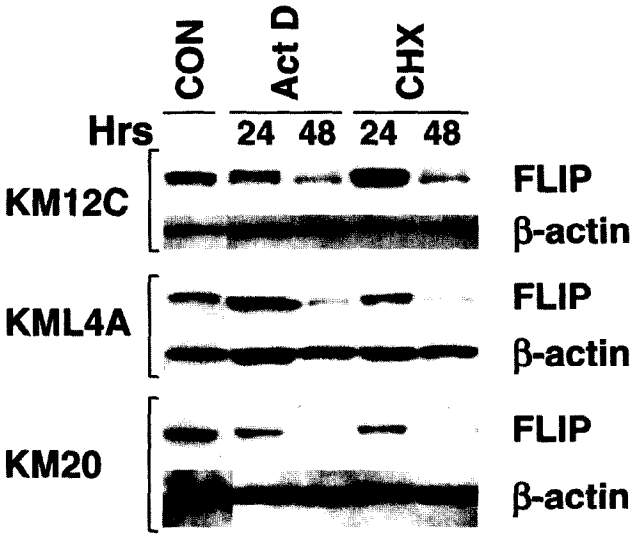

Fig. 3. Western blot analysis of FLIP expression after Act D or CHX treatment. Western immunoblot analysis of protein extracts (50 μg) from KM colon cancer cells treated with either Act D (10 μg/ml) or CHX (10 μg/ml) for 24 and 48 hours. Blots were probed for FLIP expression using a human anti-FLIP antibody (Santa Cruz). The blot was then stripped and reprobed with a polyclonal antibody to β-actin.

man colon cancer cell lines suggests the possibility that FLIP may contribute to the TRAIL resistance noted in these cells.

Modulation of FLIP Protein Levels by Act D or CHX

It has previously been shown that either Act D or CHX can decrease FLIP expression levels in melanoma cells, thus making these cells sensitive to treatment with TRAIL.[13,34,35] We next determined whether these agents could decrease the high levels of FLIP expression in the KM colon cancer cells. Cells were treated with either Act D (10 μg/ml) or CHX (10 μg/ml), and FLIP expression was assessed by Western blot at 24 and 48 hours after treatment (Fig. 3). FLIP expression was decreased at 24 hours in all three cell lines by either treatment; FLIP expression was absent at 48 hours. Therefore, similar to melanoma cells, either Act D or CHX can decrease cellular levels of FLIP in the KM human colon cancer cells.

Treatment With Either Act D or CHX Sensitizes KM cells to TRAIL-Mediated Cell Death

We next determined whether the decrease in FLIP levels by either Act D or CHX sensitizes the KM cells to TRAIL-mediated cell death using JAM assays, which quantify the percentage of cellular apoptosis (Fig. 4). After labeling the KM cell lines with ^{3}H-TdR, cells were cultured in complete medium and treated with either Act D (10 μg/ml) or CHX (10 μg/ml), alone or in combination with hrTRAIL (100 ng/ml), for 24 and 48 hours, respectively. As previously shown by MTT assay, all three KM cell lines are resistant to TRAIL-mediated cell death. An increase in cell death was demonstrated in the presence of either Act D or CHX alone. Furthermore, the combination of either Act D or CHX with TRAIL significantly enhanced cell death in the KM cell lines compared to either agent alone. Taken together, our data suggest a critical role for FLIP as an intracellular death inhibitor, thus conferring resistance to the effects of TRAIL and promoting cell survival in KM colon cancer cell lines.

To confirm that the enhanced cell death is mediated via the caspase cascade, we measured the relative caspase-3–like activity of KM cell lysates after treatment (Fig. 5). Caspase-3 activity was increased in the presence of either Act D or CHX alone compared to untreated cells. Similar to the enhanced cell death noted by the JAM assays, caspase-3 increased with the combination of TRAIL and either Act D or CHX compared with Act D or CHX alone. Therefore these findings further support a protective role for FLIP in these human colon cancer cell lines. Reduction in FLIP levels by either Act D or CHX enhances TRAIL-mediated apoptosis and caspase-3–like activity.

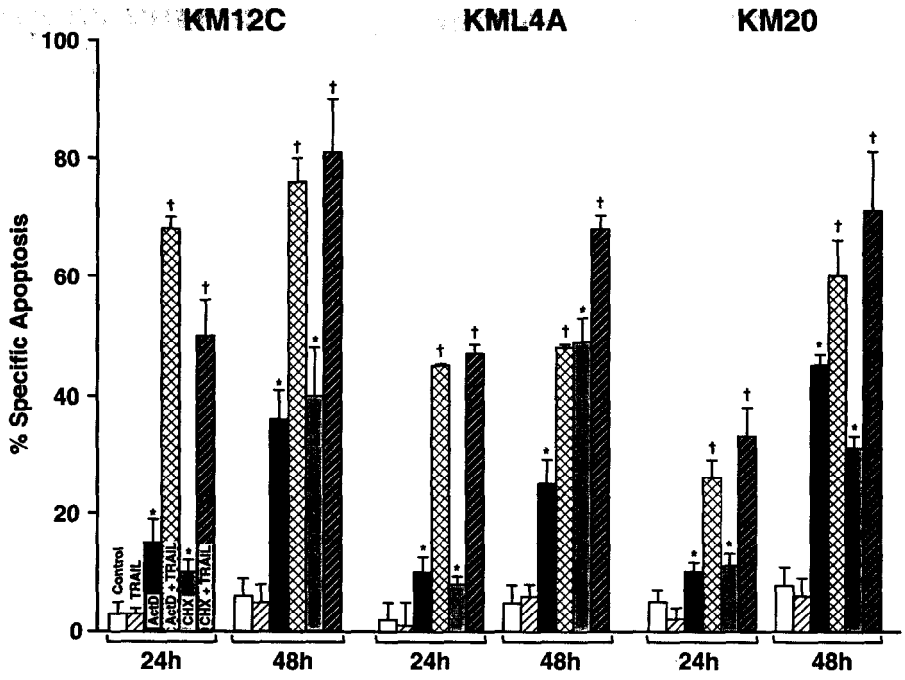

Fig. 4. JAM assays. The KM colon cancer cells were plated in 96-well plates, labeled with ^{3}H-thymidine, and treated with either Act D (10 μg/ml) or CHX (100 μg/ml), alone or combined with human recombinant TRAIL (100 ng/ml), for 24 and 48 hours. Apoptosis (percentage of specific apoptosis) was determined by JAM assays and compared to cells treated with vehicle (control) or TRAIL alone. (Data expressed as mean ± SEM; * = P <0.05 vs. control values; † = P <0.05 vs. Act D or CHX alone.)

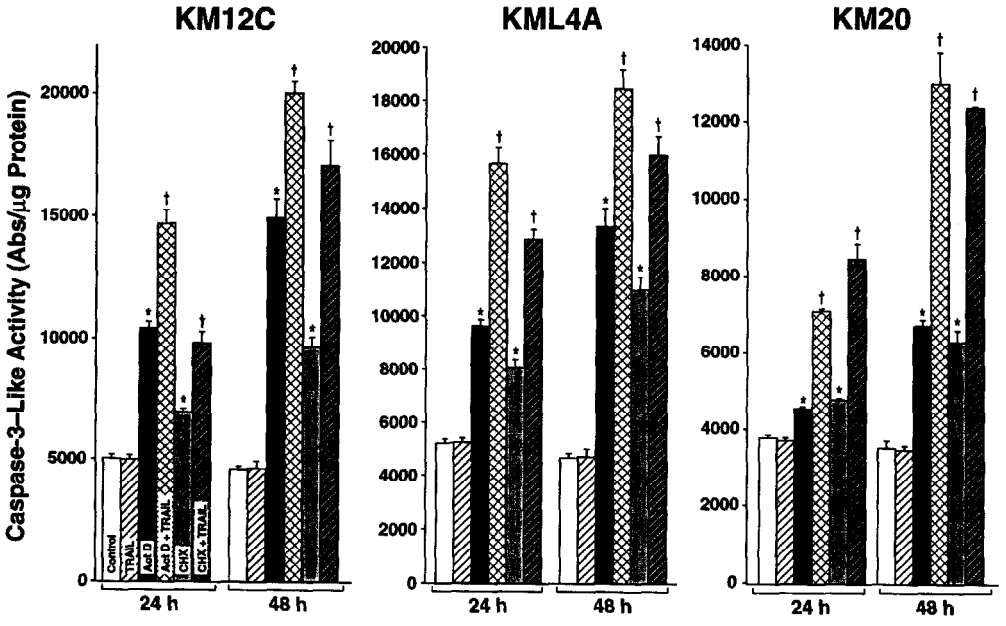

Fig. 5. Caspase-3–like activity. The KM cells were treated as described in Fig. 4. Caspase-3–like activity was measured using the chromogenic substrate Ac-Val-Ala-Asp-AFC and normalized to 1 μg of protein. (Data are expressed as mean ± SEM; * = P <0.05 vs. control; † = P <0.05 vs. Act D or CHX alone.)

DISCUSSION

The mechanisms responsible for the resistance of certain cancers to the effects of chemotherapeutic agents are not completely understood. TRAIL, a unique member of the TNF family that, like FasL, is a type II membrane protein capable of inducing cell death in a variety of cell types.[8,9,36] Initial preclinical reports suggested that normal tissues were resistant to TRAIL-mediated apoptosis, whereas cancer cells were preferentially killed by TRAIL.[8,9] In fact, clinical trials incorporating TRAIL treatment have been suggested. However, it has been recently shown that human hepatocytes may be sensitive to the effects of TRAIL treatment.[36] Furthermore, it is becoming increasingly apparent that a number of cancers, including breast, lung, prostate, and bladder cancer cells, are resistant to TRAIL-mediated apoptosis.[10-14,37-41] In our present study we have shown that human colon cancer cell lines (KM12C, KML4A, and KM20) are resistant to the effects of TRAIL treatment despite the expression of both DR4 and DR5 receptors. Similar to our findings, other colon cancer cell lines (e.g., HT-29 and SW620) have demonstrated resistance to TRAIL.[42] Therefore these findings suggest that, contrary to what was initially thought, a number of cancers are resistant to TRAIL treatment.

A possible explanation for the resistance of cells to TRAIL treatment is the existence of decoy receptors that bind TRAIL but are not linked to the apoptotic cascade.[43] Decoy receptors have been identified on normal cells as well as some cancer cells. We identified one decoy receptor (DcR2) in the KM cell lines. However, the decoy hypothesis cannot entirely explain the resistance of these cells to TRAIL, given the fact that both DR4 and DR5 are present, but no effect of TRAIL was noted even at high concentrations. Furthermore, certain cancer cells are resistant to TRAIL and also lack decoy receptors.[14,44] Therefore other mechanisms for cellular resistance are more likely.

An alternative hypothesis involves the differential expression of intracellular inhibitors of the apoptotic process. Support of this hypothesis is provided by the finding of high levels of FLIP, a recently identified protein homologue to caspase-8 that lacks catalytic activity, in TRAIL-resistant melanoma cell lines.[14] FLIP levels decreased when cells were cultured with either Act D or CHX paralleled by an increase in their sensitivity to the effects of TRAIL. Similarly we found increased FLIP levels in the KM cell lines. Treatment with either Act D or CHX resulted in decreased FLIP levels in these colon cancer cells. Therefore these are the first findings to demonstrate modulation of FLIP levels in colon cancers by either Act D or CHX. In addition, treatment with either Act D or CHX sensitizes all three KM colon cancer cell lines to TRAIL-mediated apoptosis. These findings suggest that, similar to results in TRAIL-resistant melanoma cells, modulation of FLIP levels may alter the sensitivity of these cells to TRAIL treatment.

Although our data implicate FLIP as a potential inhibitor of TRAIL-mediated cancer cell death, other cellular mechanisms may play a role in this process. For example, an increase in the levels of NF-κB, Bcl-2, and survivin has been described in cancer cells resistant to apoptosis by chemotherapeutic agents.[45-50] Future studies are required to delineate the specific roles of these proteins in colon cancer survival.

In conclusion, reduction of cellular levels of FLIP by either Act D or CHX results in a "priming" effect, thus rendering the KM cells sensitive to the effects of TRAIL. The combination of TRAIL along with agents such as Act D or CHX, which target proteins that prevent cell death, may provide a more effective and less toxic regimen for the adjuvant treatment of resistant colon cancers.

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Laparoscopic-Assisted vs. Open Colectomy for Colorectal Cancer: Influence on Neoplastic Cell Mobilization

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Laparoscopic surgery for treatment of colorectal cancer has been suggested to enhance tumor dissemination. Recently, molecular techniques have been developed to detect micrometastatic disease in patients with solid tumors, with a higher accuracy than cytologic or immunohistochemical approaches. This study was undertaken to investigate the potential harmful effects of laparoscopic-assisted colectomy on neoplastic cell mobilization in patients with resectable colorectal cancer. Fifty patients with nonmetastatic colorectal cancer were randomly assigned to laparoscopic-assisted (LAC, n = 26) or open (OC, n = 24) colectomy. Peripheral venous blood samples were obtained preoperatively, immediately after tumor removal, and 24 hours later. In 10 patients from each treatment group, portal blood and peritoneal fluid samples were also obtained before and after resection. Neoplastic cells were detected by means of reverse transcriptase–polymerase chain reaction targeted to carcinoembryonic antigen (CEA) transcription. CEA mRNA was detected in peripheral venous blood samples from 35 of 50 colorectal cancer patients preoperatively. Among those 15 baseline-negative patients, four experienced conversion 24 hours after tumor resection (2 [33%] of 6 in the LAC group vs. 2 [22%] of 9 in the OC group; NS). At that time point, clearance of CEA mRNA expression was observed in 14 of the 35 baseline-positive patients (9 [45%] of 20 in the LAC group vs. 5 [33%] of 15 in the OC group; NS). In addition, only one patient in the LAC group with baseline-negative CEA mRNA expression experienced portal blood conversion after tumor removal, although his peripheral blood level remained negative. Finally, baseline peritoneal fluid CEA mRNA expression was never detected, but one patient in each group became positive postoperatively. These results confirm that preoperative and perioperative mobilization of neoplastic cells is a frequent occurrence in patients with colorectal cancer, but the surgical approach (LAC vs. OC) does not seem to be a determining factor. (J GASTROINTEST SURG 2001;5:66-73.)

KEY WORDS: Laparoscopic colectomy, circulating neoplastic cells, colorectal cancer, carcinoembryonic antigen

Colorectal cancer is exceedingly common, and is the second most common cause of cancer-related death in most Western countries despite increasing investment in both diagnostic and therapeutic resources. The prognosis of colorectal cancer is largely dependent on distant spread of the disease, a process caused by preoperative or perioperative dissemination of tumor cells. In fact, up to 30% to 40% of patients with colorectal cancer operated on for cure will have a recurrence during follow-up.[1] In that sense, there is a large body of experimental and clinical evidence showing that surgical manipulation of primary tumors promotes malignant cell shedding into the bloodstream, probably aiding cancer spread.[2-11]

In the past decade, laparoscopy has become the standard surgical procedure for several gastrointesti-

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nal disorders. However, its role in the treatment of intra-abdominal neoplasms has not yet been defined, and controversy remains regarding its potential harmful effects on tumor dissemination. Port-site metastases have been reported in some clinical observations and open studies, although their precise incidence and pathophysiology are still unknown.[12-14] Experimental studies have suggested that laparoscopic approaches might further enhance tumor cell detachment and mobilization,[15-18] calling into question the appropriateness of minimally invasive surgery for gastrointestinal neoplasms. Although the definitive answer will come from long-term results of ongoing randomized controlled trials, the critical relevance of this issue justifies the exploration of any alternative methodologic approach.

Recently, molecular techniques have been developed to detect micrometastatic disease in patients with solid tumors. These methods, based on the reverse transcriptase–polymerase chain reaction (RT-PCR) targeting tissue-specific gene transcription, allow a more confident assessment of tumor cell dissemination than cytologic or immunohistochemical techniques. Using this approach, we and others have been able to identify tumor cells circulating in the blood of patients with colorectal cancer,[19-23] as well as other neoplastic processes,[24-27] with a high degree of sensitivity (one tumor cell per 10^7 white blood cells) and specificity (no positivity among healthy control subjects). Moreover, this molecular technique has also been used to evaluate whether surgery induces tumor cell mobilization in certain neoplasms,[3-7] including colorectal cancer.[8-11]

The present study was aimed at investigating the potential harmful effects of laparoscopic surgery on neoplastic cell mobilization in patients with colorectal cancer. The presence of tumor cells was evaluated in both peripheral and portal venous blood, as well as in peritoneal fluid samples, from a subset of patients enrolled in a randomized controlled trial comparing laparoscopic-assisted (LAC) vs. open colectomy (OC) for treatment of colorectal cancer.

PATIENTS AND METHODS

Fifty consecutive patients with nonmetastatic colorectal cancer who were referred for radical resection were included in this study. These 32 men and 18 women had a mean age of 69 ± 11 years (range 43 to 87). According to the TNM staging of the system International Union Against Cancer, 14 patients were classified as stage I, 22 were stage II, and 14 were stage III. These patients were enrolled in a randomized controlled trial comparing LAC vs. OC for treatment of colorectal cancer.[28,29] Patients were assigned to either the LAC (n = 26) or the OC (n = 24) group in a random fashion. Patients were fully informed of the investigational nature of the procedure and written informed consent was obtained before they were included. The study protocol was approved by the Clinical Investigation Ethics Committee of the Hospital Clínic of Barcelona.

Study Design

All patients included in the study underwent serial peripheral venous blood extractions through standard venipuncture techniques according to the following schedule: a first sample was obtained preoperatively (PE1); a second sample at the end of surgery, immediately after closure of the abdominal incision (PE2); and the last sample was collected 24 hours later (PE3). Blood (20 ml) was collected in heparinized tubes and immediately processed to avoid RNA degradation.

A subset of 20 patients (10 from each treatment group) was also evaluated for the presence of neoplastic cells in portal venous blood and peritoneal fluid samples. Baseline determinations (PO1 and PF1, respectively) were performed at the initiation of surgery prior to any tumor manipulation, just before the high-pressure pneumoperitoneum was established. In addition, a second pair of samples was obtained at the end of surgery, immediately before the abdomen was closed (PO2 and PF2, respectively). Portal venous blood was obtained through repermeabilization of the umbilical vein. When this approach was not feasible, samples were obtained by catheterization of a branch of the inferior mesenteric vein. Peritoneal fluid samples were drawn following instillation and dispersion of 200 ml of isotonic sodium chloride solution at a temperature of 28° C to 29° C. Washes were then aspirated into sterile containers. Both portal venous blood and peritoneal fluid samples were immediately processed to avoid RNA degradation.

Finally, the potential pernicious effects of preoperative anterograde intestinal cleansing with an osmotic solution on neoplastic cell mobilization were also evaluated. To this end, peripheral venous blood samples were obtained from 20 additional patients with resectable colorectal cancer immediately before and after oral administration of a solution of polyethylene glycol (Solución Evacuante Bohm, Madrid, Spain).

Surgical Procedures

All operations were performed by staff members of the same surgical team who had extensive experience in advanced laparoscopic techniques. For LAC, a pneumoperitoneum was established after insertion of a Veress needle, and intra-abdominal pressure was

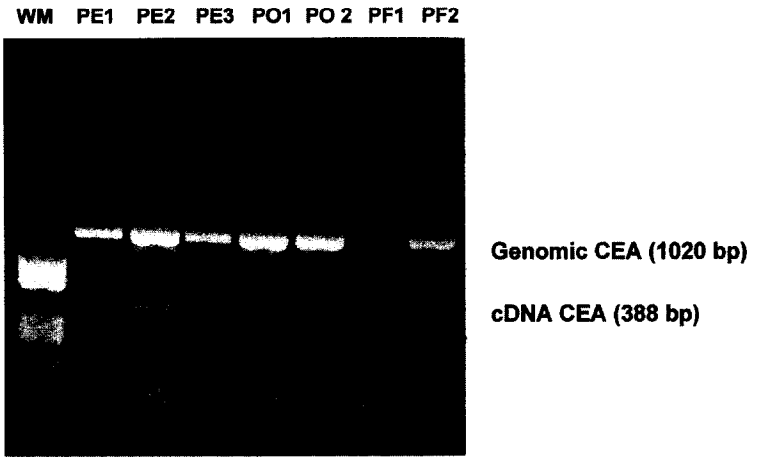

Fig. 1. Detection of CEA mRNA in peripheral and portal venous blood and in peritoneal fluid samples. Electrophoresis on 2% agarose gel, followed by ethidium bromide staining. WM = molecular weight marker V; PE1, PE2, and PE3 = peripheral blood samples obtained preoperatively, at the end of surgery, and 24 hours later, respectively; PO1 and PO2 = portal venous blood samples obtained before tumor manipulation and after tumor removal, respectively; PF1 and PF2 = peritoneal fluid obtained before tumor manipulation and after tumor removal, respectively.

maintained between 12 and 14 mm Hg. A detailed description of the operative procedures has been previously reported.[29]

In both groups of patients, a "no-touch" technique[30] with initial vascular ligation was used.

Mononuclear Cell Isolation and RNA Extraction

Mononuclear cells from blood and peritoneal fluid samples were isolated using a Ficoll gradient. Samples were diluted with 30 ml of phosphate-buffered saline, layered on 15 ml of Ficoll gradient solution, and centrifuged at 700 *g* for 30 minutes. Mononuclear cells, localized in the interphase between plasma and Ficoll, were collected and precipitated by centrifugation at 1000 *g* for 10 minutes. Pellets obtained were washed twice with phosphate-buffered saline.[23] The specimens were stored at −80° C.

Total RNA extraction was performed according to the method of Chirgwin. Because of the lack of significant differences between groups with regard to white blood cell count (data not shown), this parameter was not taken into account in extracting total RNA.

Reverse Transcriptase and Polymerase Chain Reaction

Detection of circulating neoplastic cells was assessed by means of RT-PCR targeted to carcinoembryonic antigen (CEA) mRNA, as previously described.[23] The reverse transcriptase reaction was prepared in a final volume of 20 μl, containing 4 μl of total RNA, and incubated at 42° C for 45 minutes followed by a 5-minute period at 95° C to deactivate the reverse transcriptase.

CEA-specific oligonucleotide primers were synthesized according to published sequence information.[31] Primers were selected to span an intron, in order to synthesize different-sized amplification products from the CEA mRNA (388 bp) and any contaminating genomic DNA (1020 bp) (Fig. 1).

Reverse transcriptase product (2.5 μl) was diluted in a final volume of 50 μl of polymerase chain reaction mixture, overlaid with mineral oil, and denatured at 94° C for 3 minutes. Cycle conditions were as follows: 94° C for 30 seconds, 62° C for 1 minute, and 72° C for 1 minute, for 35 cycles. Polymerase chain reaction was completed within a period of 5 minutes at 72° C.[23] Samples were electrophoresed on 2% agarose gels and visualized by ethidium bromide staining. According to our previous study, the sensitivity limit for this technique is approximately one tumor cell per 10^7 white blood cells.

The integrity of the RNA was confirmed by determining the presence of glyceraldehyde phosphate dehydrogenase (GAPDH) mRNA in the same samples.

The investigator performing the RT-PCR analysis was blinded with respect to patients' clinical characteristics. Positive and negative control samples were included in each experiment.

For the purpose of this study, patients were considered to experience conversion when CEA mRNA expression was detected in the latest available sample (either at the end of surgery for portal venous blood

Table I. Baseline characteristics of patients with colorectal cancer included in the present study*

	Peripheral blood analysis		Portal blood and peritoneal analysis	
	LAC (n = 26)	OC (n = 24)	LAC (n = 10)	OC (n = 10)
Age (yr)	69 ± 10	70 ± 12	66 ± 9	69 ± 15
Sex (males/females)	16/10	16/8	7/3	4/6
CEA level (ng/ml)	4.4 ± 6.3	5.2 ± 6.1	4.4 ± 5.2	5.2 ± 7.4
Tumor location (proximal/distal)†	8/18	7/17	0/10	2/8
TNM stage				
I	7 (30%)	7 (29%)	2 (20%)	1 (10%)
II	10 (37%)	12 (50%)	4 (40%)	7 (70%)
III	9 (33%)	5 (21%)	4 (40%)	2 (20%)
Degree of differentiation				
Good	1 (4%)	3 (12%)	1 (10%)	1 (10%)
Moderate	23 (89%)	19 (79%)	9 (90%)	8 (80%)
Poor	2 (7%)	2 (8%)	—	1 (10%)

LAC = laparoscopic-assisted colectomy; OC = open colectomy; CEA = carcinoembryonic antigen.
*There were no differences between treatment groups with regard to any of the variables.
†Proximal and distal location was considered with respect to splenic flexure.

and peritoneal fluid samples, or 24 hours after surgery for peripheral venous blood samples) in subjects whose corresponding baseline sample was negative. In addition, transient conversion was defined as the detection of CEA mRNA transcripts in peripheral venous blood in the immediate postoperative period, but not 24 hours later, in patients with baseline negative expression (see Fig. 1).

Statistical Methods

Continuous variables are expressed as mean ± standard deviation and were compared by means of Student's *t* test. Differences in the relative frequency of detected neoplastic colorectal cells and correlations between qualitative variables were evaluated by means of the chi-square test, applying the Yates correction when needed. Statistical significance was set at $P < 0.05$.

RESULTS

CEA mRNA Expression in Peripheral Venous Blood

CEA mRNA was detected in peripheral venous blood samples from 35 of 50 patients with colorectal cancer preoperatively. According to treatment assignment, baseline CEA mRNA expression was observed in 20 (77%) of 26 patients in the LAC group and 15 (63%) of 24 patients in the OC group (not significant [NS]). There were no differences between treatment groups with regard to demographic, clinical, or tumor-related characteristics at entry into the study (Table I).

Table II. Correlation between neoplastic cell detection in peripheral venous blood and timing of sample collection in patients undergoing resection of primary colorectal cancer

Baseline	After resection	24 hr after surgery	No. of patients
Laparoscopic-assisted colectomy (n = 26)			
Negative	Negative	Negative	3
Negative	Positive	Negative	1
Negative	Negative	Positive	0
Negative	Positive	Positive	2
Positive	Negative	Negative	4
Positive	Positive	Negative	5
Positive	Negative	Positive	2
Positive	Positive	Positive	9
Open colectomy (n = 24)			
Negative	Negative	Negative	7
Negative	Positive	Negative	0
Negative	Negative	Positive	0
Negative	Positive	Positive	2
Positive	Negative	Negative	3
Positive	Positive	Negative	2
Positive	Negative	Positive	2
Positive	Positive	Positive	8

Twenty-four hours postoperatively, CEA mRNA expression was detected in peripheral venous blood samples from 25 of 50 patients (13 [50%] of 26 in the LAC group vs. 12 (50%) of 24 in the OC group; NS) (Table II). Among the 15 baseline-negative patients, four experienced conversion 24 hours after tumor re-

Table III. Correlation between detection of neoplastic cells in portal venous blood and timing of sample collection in patients undergoing resection of primary colorectal cancer

Baseline	After resection	No. of patients
Laparoscopic-assisted colectomy (n = 10)		
Negative	Negative	2
Negative	Positive	1
Positive	Positive	6
Positive	Negative	1
Open colectomy (n = 10)		
Negative	Negative	2
Negative	Positive	0
Positive	Positive	5
Positive	Negative	3

Table IV. Correlation between detection of neoplastic cells in peritoneal fluid and timing of sample collection in patients undergoing resection of primary colorectal cancer

Baseline	After resection	No. of patients
Laparoscopic-assisted colectomy (n = 10)		
Negative	Negative	9
Negative	Positive	1
Positive	Positive	0
Positive	Negative	0
Open colectomy (n = 10)		
Negative	Negative	9
Negative	Positive	1
Positive	Positive	0
Positive	Negative	0

section (two patients [33%] in the LAC group vs. two patients [22%] in the OC group; NS). Clearance of CEA mRNA expression at that time point was observed in 14 of 35 baseline-positive patients, there being no differences between the two treatment groups (9 [45%] of 20 in the LAC group vs. 5 [33%] of 15 in the OC group; NS). Finally, a transient conversion (positivity immediately after surgery but not 24 hours later) was detected in one patient in the LAC group (see Table II).

CEA mRNA Expression in Portal Venous Blood

Before any tumor manipulation, CEA mRNA expression was detected in portal venous blood samples from 15 of 20 patients with colorectal cancer (7 [70%] of 10 LAC patients vs. 8 [80%] of 10 OC patients; NS). There were no differences between treatment groups with regard to demographic, clinical, or tumor-related characteristics at entry (see Table I).

At the end of surgery, only one LAC patient with baseline-negative CEA mRNA expression experienced portal blood conversion, although his peripheral blood level remained negative (Table III). In addition, after tumor excision, clearance of CEA mRNA transcripts was observed in 4 of 15 baseline-positive patients (1 [14%] of 7 LAC patients vs. 3 [37%] of 8 OC patients; NS) (see Table III).

Correlation Between CEA mRNA Expression in Peripheral and Portal Blood

Correlation between CEA mRNA expression in both vascular regions was evaluated before (PE1 and PO1) and after (PE2 and PO2) tumor removal. Concordance between peripheral and portal venous blood levels was observed in 26 (65%) of 40 samples. In contrast, in 7 patients (17%) CEA transcripts were only detected at the peripheral level, whereas in the remaining seven patients (17%) this only occurred in portal blood.

When determinations at two different time points were analyzed separately, the highest concordance rate corresponded to baseline samples, obtained prior to tumor removal (PE1 and PO1). In that setting, coincidental results between the two vascular regions were observed in 16 (80%) of 20 patients, whereas this concordance rate was limited to 10 (50%) of 20 patients after surgical resection (PE2 and PO2).

CEA mRNA Expression in Peritoneal Fluid

Before tumor manipulation, all patients were negative for CEA mRNA expression in peritoneal fluid. At the end of surgery, the peritoneal fluid sample from only one patient (10%) in each treatment group became positive (Table IV). The patient from the LAC group had a stage III lesion (T2 N1 M0), whereas the patient from the OC group had a stage II lesion (T4 N0 M0).

Effect of Preoperative Intestinal Cleansing on CEA mRNA Expression

The effect of preoperative anterograde intestinal cleansing was evaluated in peripheral venous blood samples from 20 patients with resectable colorectal cancer. At baseline, CEA mRNA expression was detected in 12 (60%) of 20 patients. After oral administration of a solution of polyethylene glycol, none of the eight baseline-negative patients experienced con-

version with regard to their expression status in peripheral venous blood. In addition, 10 (83%) of the 12 patients with baseline CEA mRNA expression remained positive after bowel conditioning. Globally, coincidental results before and after intestinal cleansing were observed in 18 patients (90%).

DISCUSSION

This pilot study represents the first investigation evaluating the effect of laparoscopic colectomy on neoplastic cell mobilization in patients with colorectal cancer who underwent surgery with intent to cure. Based on an RT-PCR–based technique, our data confirm that tumor cells can often be detected in peripheral and portal venous blood samples from patients with nonmetastatic lesions before, during, and after surgical procedures. Moreover, these results indicate that tumor manipulation could induce neoplastic colorectal cell mobilization in up to one third of patients, but this effect seems to be unrelated to the surgical approach. Finally, we provide substantial evidence to indicate that peritoneal tumor spread during surgery is an infrequent occurrence and, again, not influenced by the surgical technique. Taken altogether, these results seem to argue against laparoscopic surgery having any effect on neoplastic cell mobilization in patients with resectable colorectal cancer.

The application of laparoscopy to the field of general surgery has brought a revolutionary approach to the treatment of preexisting gastrointestinal diseases. Minimal access surgery, indeed, has been extended to a wide variety of disorders, including colorectal malignancies. Although the feasibility of laparoscopic colectomy has been extensively demonstrated,[12,13,28,32,33] controversy remains regarding its oncologic safety profile. In a preliminary analysis of an ongoing randomized controlled trial, we demonstrated that tumor resection and staging performed laparoscopically could be as accurate as open surgery.[29] In fact, laparoscopic colectomy does not compromise the number of lymph nodes removed or the margins of resection, as compared with the standard open procedure.[29] Nevertheless, in spite of these findings, concern persists in regard to long-term results.

The recent development of molecular techniques for detecting neoplastic cells in bone marrow, lymph nodes, and blood provides a valuable tool to study the complex mechanisms involved in metastatic spread. This is a highly inefficient multistep process in which cell detachment and mobilization are the first necessary, but not sufficient, stages. According to this postulate, detection of circulating tumor cells by RT-PCR should not necessarily imply the development of distant metastases. Although its prognostic value remains to be determined and is currently under investigation at our institution, the presence of tumor/tissue-specific transcripts in regions different from their origin must be considered as a marker of neoplastic cell mobilization.

An increase in peripheral blood detection of neoplastic cells during surgery has been described for breast[3] and prostate[4] cancer, situations in which up to 35% of patients may exhibit this phenomenon. There is also a large body of evidence indicating that surgical manipulation may promote colorectal cancer cell detachment and mobilization.[8-11] Weitz et al.,[8] by means of an RT-PCR assay targeting citokeratin 20 transcripts, demonstrated the presence of circulating cancer cells in up to 25% of baseline-negative patients during standard open colectomy. In most cases, this was a transient phenomenon since neoplastic cells became undetectable once the surgery had ended. Sales et al.,[9] using a no-touch technique, obtained a lower rate of CEA mRNA detection in samples from tumor drainage veins. The protective effect of no-touch surgery on the spread of colorectal cancer cells has been further evaluated by directly comparing no-touch vs. conventional surgery.[10] Using a mutant allele–specific amplification technique, Hayashi et al.[10] showed a significantly lower rate of intraoperative cancer cell mobilization in portal venous blood from patients operated on using a no-touch approach, and this fact seemed to be associated with a decreased probability of developing liver metastases during follow-up. In the present study, in which the no-touch technique was used in all patients in both treatment groups, surgical neoplastic cell mobilization was observed in only four patients, there being no differences with regard to the surgical approach (see Table II). Although the sample size in the present investigation precludes drawing definitive conclusions, our data suggest that laparoscopic colectomy did not promote a higher rate of tumor cell mobilization and that the disappearance of blood-circulating neoplastic cells early after surgery is a common occurrence and independent of the surgical procedure employed.

It has been suggested that surgery-induced cancer cell mobilization in gastrointestinal malignancies would be better assessed in the portal vein than in the peripheral region because of the possibility that circulating neoplastic cells could become entrapped in the liver. This hypothesis is supported by a study performed in patients with biliary-pancreatic cancer in whom the detection of circulating cancer cells in portal blood was not associated with parallel rates in peripheral venous blood.[6] However, a larger study did not confirm this observation, since the rate of circulating neoplastic cells was the same in the portal vein as in a peripheral artery or the superior vena cava.[7] In

our study, overall concordance between CEA mRNA expression in peripheral and portal venous blood was 65%. This lack of correlation between the two regions was not due to an excess detection of circulating neoplastic cells in portal blood, a result that would be expected if tumor cells were arrested in the hepatic microcirculation. This apparent discrepancy would instead depend on the sampling variability of neoplastic cell detection. Because tumor cell detachment is an intermittent process, cancer cells circulate in nonhomogeneously distributed clumps, making their detection a stochastic event.[34,35] In that sense, one study evaluating the reproducibility of repeated RT-PCR assessments showed a coincidental rate of 66%, in spite of the fact that only patients with advanced colorectal cancer were included.[20]

One of the most controversial aspects of laparoscopic colectomy is the suspected harmful effect on peritoneal cell spread. It has been suggested that the induced high-pressure pneumoperitoneum may increase cancer cell exfoliation, which may promote the development of port-site metastases (chimney or spray effect). By means of cytologic or immunocytologic techniques, two prospective trials comparing the two surgical approaches suggested that laparoscopic colectomy does not increase intraperitoneal cell spillage as compared with conventional open surgery.[36,37] In the present study, which used a highly sensitive RT-PCR method, CEA mRNA expression in peritoneal fluid was only detected in one patient from each treatment group after tumor removal. These results confirm that tumor cell exfoliation during surgery is not increased by the use of laparoscopic approaches and argue against the suggested spray effect induced by the pneumoperitoneum.

The high proportion of patients with baseline CEA mRNA expression observed in the present investigation warrants some comments, in that there are several possible explanations. First, this figure could be attributed to the false positive results that can occur with this technique, which usually range from 0% to 26%.[11,20,34,38] However, an identical RT-PCR protocol was used in our previous study[23] in which a high specificity (no positivity among healthy control subjects) was observed. In fact, to minimize false positive results, nucleic acid extraction, polymerase chain reaction amplification, and amplicon manipulation were performed in separate rooms. Second, the unexpectedly high rate of baseline circulating cancer cells could be related to the preoperative intestinal cleansing. This fact prompted us to evaluate whether oral administration of polyethylene glycol could increase cancer cell mobilization, a possibility that was ruled out by our data. Finally, the high proportion of baseline-positive determinations may be attributed to the illegitimate transcription favored by a highly sensitive polymerase chain reaction technique,[34,35] as well as amplification of CEA pseudogenes or transcripts from cross-reacting antigens, which have been identified in white blood and skin cells.[39] However, it is important to note that the pairwise design of the study overshoots this limitation.

In summary, our data suggest that laparoscopic colectomy does not favor mobilization of colorectal cancer cells. Since detection of circulating neoplastic cells has not been definitely identified as a predictor of tumor recurrence or subsequent metastasis, confirmation of the safety profile of laparoscopic surgery in patients with colorectal cancer still requires long-term results from ongoing randomized controlled clinical trials.

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Laparoscopic Endobiliary Stenting: A Simplified Approach to the Management of Occult Common Bile Duct Stones

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Three years ago we described laparoscopic placement of biliary stents as an adjunct to laparoscopic common bile duct exploration (LCBDE) in 16 patients. We now present a modification of our technique and experience with 48 additional patients. Laparoscopic cholecystectomy with intraoperative fluorocholangiography (LC/IOC) performed in 372 consecutive patients during a 36-month period revealed common bile duct stones (CBDS) in 48 patients (12.9%). In this series, LCBDE was not performed and no attempt was made to clear CBDS prior to transcystic stent placement. Stent placement added 9 to 26 minutes of operative time to LC/IOC alone. Forty-four patients (92%) were discharged after surgery and four (8%) were observed overnight. Outpatient endoscopic retrograde cholangiopancreatography 1 to 4 weeks later succeeded in clearing CBDS in all patients. All stents were retrieved without difficulty and 3- to 36-month follow-up demonstrates no surgical, endoscopic, or stent-related complications to date. Laparoscopic biliary stent placement for the treatment of CBDS is a safe, rapid, technically less challenging alternative to existing methods of LCBDE. It preserves the benefits of minimally invasive surgery for patients, and virtually assures success of postoperative endoscopic retrograde cholangiopancreatography with complete stone clearance. (J GASTROINTEST SURG 2001;5:74-80).

KEY WORDS: Laparoscopic cholecystectomy, laparoscopic common bile duct exploration, common bile duct stones, biliary stents, postoperative ERCP, fluorocholangiography

Surgeons agree that common bile duct stones (CBDS) discovered preoperatively are most effectively treated with preoperative endoscopic retrograde cholangiopancreatography (ERCP). The management of occult CBDS discovered during laparoscopic cholecystectomy with intraoperative fluorocholangiography (LC/IOC) remains challenging and has evolved to include several techniques, including postoperative ERCP, intraoperative biliary lithotripsy, laparoscopic common bile duct exploration (LCBDE), and conversion to laparotomy with open common bile duct exploration.[1-8] Novel techniques such as intraoperative ERCP and antegrade sphincterotomy are advocated by some, but remain largely unavailable to most surgeons.[9,10]

Routine reliance on postoperative ERCP exposes up to 20% of patients with occult CBDS to the risk of a second operative procedure for stone extraction if endoscopic clearance fails.[11-13] Intraoperative biliary lithotripsy requires expensive, infrequently used equipment that is unavailable in many hospitals. LCBDE has become more popular and can be performed either by the transcystic route or by choledochotomy, but requires advanced laparoscopic surgical skills for stone extraction and suturing that might escape some surgeons in practice. Despite several advanced options for treatment of patients with CBDS, many surgeons still rely on conversion to laparotomy for open common bile duct exploration with placement of drains and T-tubes. Patients treated by means of these techniques are deprived of the lower morbidity and quicker recovery and return to productivity that has become the hallmark of minimally invasive biliary surgery. Intraoperative ERCP is promising and has been highly success-

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ful in our hands, yet requires immediate availability of specialty equipment and a second physician skilled in therapeutic ERCP. These limitations have made this technique slow to be adopted in most practice settings.

Procedures that rely on the placement of T-tubes and drains require that patients remain hospitalized for several days postoperatively. Even when placed laparoscopically, indwelling T-tubes and drains are uncomfortable, require continuous management, and significantly limit patient activity and return to work because of fear of dislodgment. Conversion to open surgery increases the length of stay in the hospital, prolongs convalescence, and is associated with greater morbidity than laparoscopic surgery.[14-17]

We have previously described a method of LCBDE with placement of an endobiliary stent that obviates the need for T-tube placement, eliminates the morbidity of open common bile duct exploration, and allows patients to return to unrestricted activity as quickly as those undergoing LC/IOC alone.[18] We present herein a modification of our original successful technique that extends the capability of this procedure to all surgeons regardless of their familiarity with advanced laparoscopic techniques, hospital size, equipment limitations, and surgical setting that is useful in treating all patients with occult CBDS.

MATERIAL AND METHODS

Standardized four-port LC/IOC was used to treat 372 consecutive patients presenting for elective or urgent surgery during a 36-month period ending in July 1999. Our operative approach included open infraumbilical placement of a Hasson cannula and three upper abdominal 5 mm blunt-tipped ports. General anesthesia, carbon dioxide insufflation, and fluorocholangiography were used in all cases; 48 patients (12.9%) were found to have CBDS (Fig. 1). All patients with occult CBDS were considered eligible for stent placement without exception, regardless of cystic duct size, common bile duct diameter, and stone size, number, and location. Patients with CBDS diagnosed preoperatively who refused ERCP, those requiring LCBDE because of failed preoperative ERCP, and patients without occult CBDS were not eligible for stent placement.

Routine fluorocholangiography was performed through the epigastric port using a flexible-tip catheter with a three-way adapter introduced through a cholangiogram clamp (Cook Surgical, Inc., Bloomington, Ind.). The configuration of this cholangiogram catheter allows smooth passage of a guidewire through the central port while preserving the ability to inject additional contrast medium without introducing air bubbles (Fig. 2). No attempt was made to clear CBDS

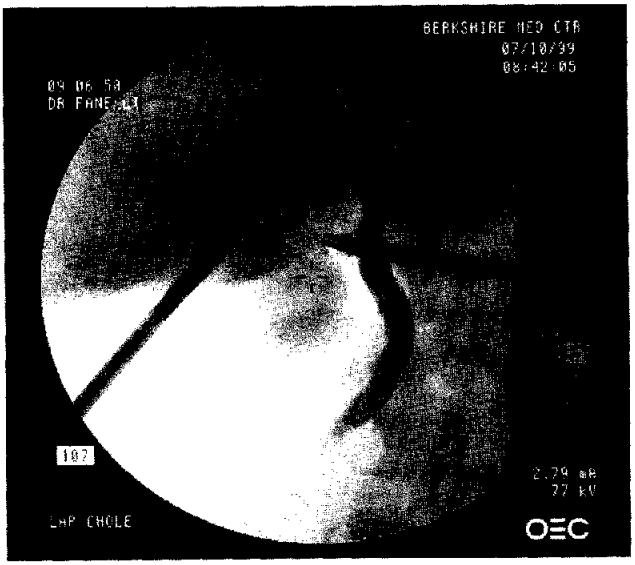

Fig. 1. Intraoperative fluorocholangiogram demonstrating multiple common bile duct stones (solid white arrow) with limited flow of contrast medium into duodenum. Note starting time of 8:42 AM.

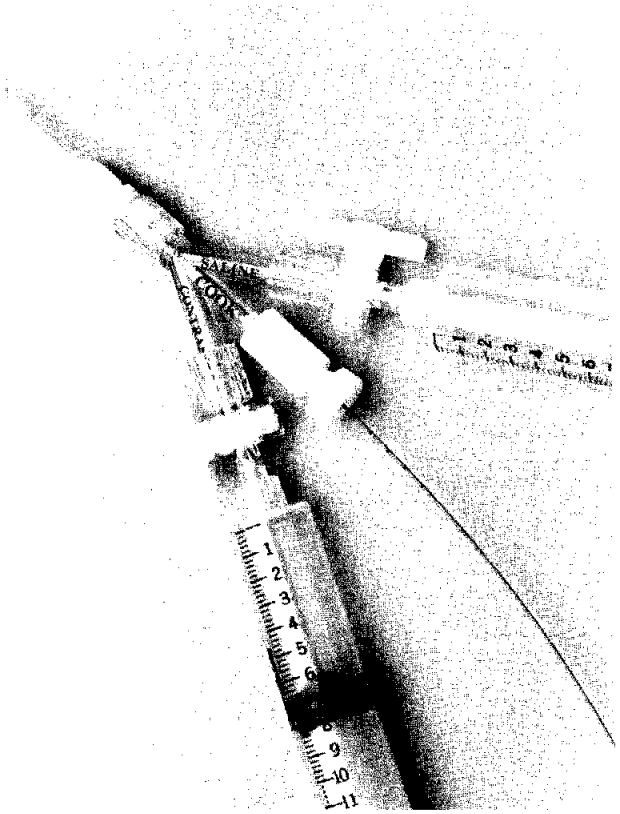

Fig. 2. Flexible atraumatic tip and unique three-way injection adapter ease insertion of cholangiogram catheter and permit saline flush, contrast injection, and rapid guidewire placement without changing syringes or introducing air (Cook Surgical Inc.).

identified during fluorocholangiography. A standard hydrophilic 0.035 inch 480 cm ERCP guidewire was advanced through the wire port of the cholangiogram catheter, and directed fluoroscopically through the common bile duct across the ampulla to coil gently within the duodenum. Later in this series, a 150 cm long modified Tracer Hybrid guidewire was used (Wilson-Cook Medical, Inc., Winston-Salem, N.C.) (Fig. 3).

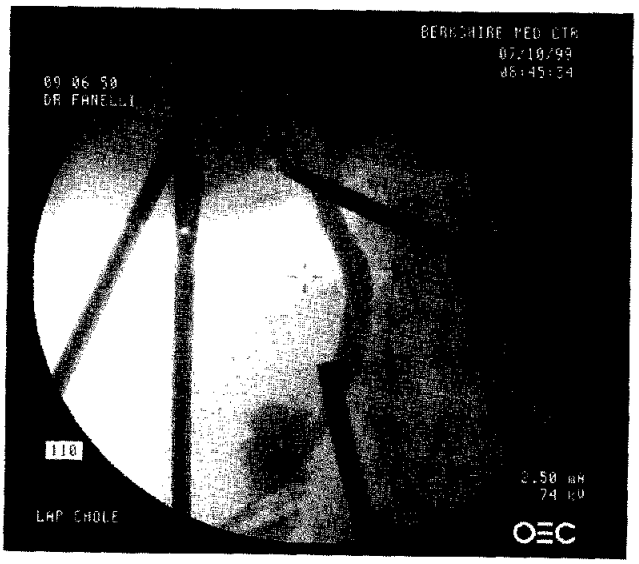

Fig. 3. Tracer Hybrid guidewire (Wilson-Cook Medical, Inc.) has been inserted through the cholangiography catheter, and directed through the common bile duct, across the ampulla, and is gently coiled within the duodenum.

With 15 to 20 cm of the guidewire positioned securely within the duodenum to prevent inadvertent removal, the cholangiogram clamp and catheter are withdrawn over the guidewire. Frequent intermittent fluoroscopic imaging is helpful in maintaining appropriate guidewire positioning within the duodenum. Finger occlusion of the laparoscopic port can be used to prevent excessive loss of insufflation, although this is not necessary when using a 5 mm port or the sealed introducer included in the kit.

Under continuous fluoroscopic guidance, the biliary stent is advanced over the guidewire until its distal tip enters the duodenum and the distal flange has cleared the ampulla. Contrast medium injected through the stent delivery mechanism provides excellent visualization of stent location, ensuring appropriate positioning across the ampulla. Early in this series, a standard 7 F, 5 cm endobiliary stent from the ERCP set was used. Later in this series, a custom 7 F, 6 cm laparoscopic stent loaded onto a 50 cm long delivery system with specialized radiographic markers was used (Cook Surgical, Inc.) (Fig. 4). The radiographic markers incorporated into this delivery mechanism are extremely helpful in the accurate placement of the stent across the ampulla prior to deployment (Fig. 5).

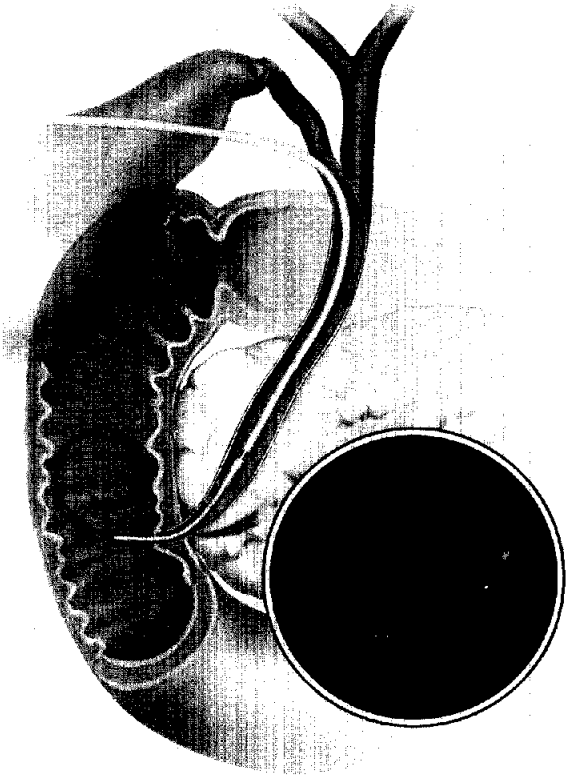

Fig. 4. Stent delivery mechanism in place across the ampulla, after advancement over the guidewire. Note the radiographic markers, and compare them to the actual fluoroscopic image shown in Fig. 5, *A*.

When the native size of the cystic duct was insufficient to accommodate the 8.5 F carrier mechanism used for stent placement, balloon dilation of the cystic duct to 12 F was performed.

After successful deployment of the stent, cystic duct stump ligation was accomplished with the 5 mm Endo Clip (United States Surgical Corp., Norwalk, Conn.), and LC/IOC was completed. Choledochotomy was not used during this series, and no drains were placed in any of the patients.

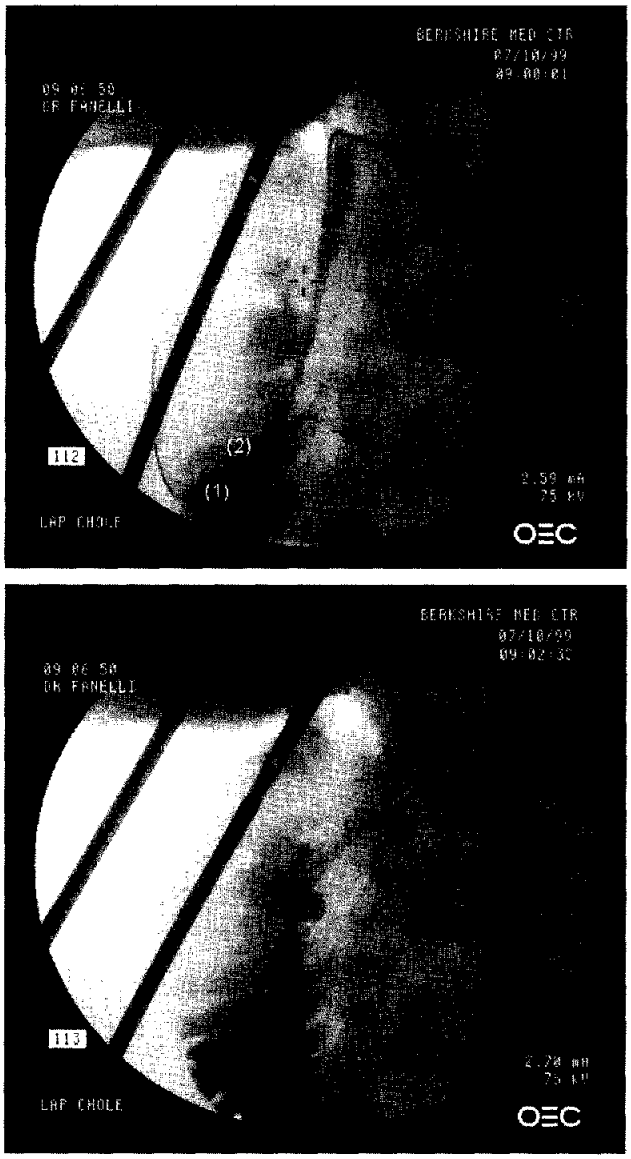

Fig. 5. A, Stent is in place and ready for deployment. Radiographic markers identify the following parts of the stent: *(1)* distal tip, *(2)* distal anchor, *(3)* proximal anchor, and *(4)* proximal end against the backstop. The secure release mechanism is withdrawn from the stent during deployment. **B,** Final fluoroscopic image demonstrates appropriate stent placement and complete drainage of contrast medium from common bile duct into duodenum. Note ending time of 9:02 AM. Stent placement added 20 minutes to this patient's operative time.

RESULTS

LC/IOC was successful in all 372 patients, and intraoperative fluorocholangiograms were obtained during all procedures. CBDS were discovered in 48 patients (12.9%) and all were treated by transcystic stent placement. Cystic duct balloon dilatation was necessary in 14 patients (29.2%) prior to stent placement. Laparoscopic stent placement added 9 to 26 minutes of operating time over that required for LC/IOC alone. Hemorrhage requiring blood transfusion, bile duct injuries, duodenal perforations and other operative complications did not occur. Suboptimal stent deployment, that is, stent entirely within the common bile duct or duodenum, did not occur, and choledochotomy, laparoscopic suturing, LCBDE, and other advanced laparoscopic skills were not used during this series.

Forty-four patients (92%) were treated as outpatients and four (8%) were admitted overnight for observation and discharged within 24 hours of surgery. Indications for admission were postoperative nausea (two patients), surgery completed too late for discharge (one patient), and weather too severe to permit safe discharge (one patient).

Stent retrieval, selective common bile duct cannulation, and complete CBDS clearance were accomplished in all patients during outpatient ERCP 1 to 4 weeks later. All patients were treated with endoscopic sphincterotomy during ERCP to aid stone extraction. No false positive cholangiograms were obtained as CBDS were found during ERCP in all patients. No patients developed ERCP- or stent-related pancreatitis. No stents passed spontaneously in this series, and stent migration did not occur.

Three- to 36-month follow-up demonstrates no surgical, endoscopic, or stent-related complications to date in this series of patients, and none have occurred in our 16 original patients.

DISCUSSION

Laparoscopic endobiliary stent placement has been shown previously to eliminate the need for T-tubes and drains in patients undergoing LCBDE by either choledochotomy or transcystic methods.[18,19] Patient comfort was improved during our original series, and T-tube–related complications such as bile leaks, common bile duct obstruction, duodenal erosions, and retained foreign bodies were eliminated.[20-23] Early discharge and early return to unrestricted activity resulted from laparoscopic placement of biliary stents

after LCBDE. Reliable selective cannulation of the common bile duct during postoperative ERCP and lack of complications from our original technique stimulated interest in our current approach where no attempt is made to clear CBDS laparoscopically. Our current work demonstrates that stent placement eliminates the need for LCBDE, which remains challenging for many surgeons, and provides an easily employed, rapid technique for the laparoscopic treatment of all patients with occult CBDS.

Surgeons skilled in LCBDE have reported a CBDS clearance rate ranging from 81% to 100% after LCBDE.[10,24,25] In cases of incomplete stone removal, our stenting technique will provide effective biliary decompression, eliminating concern for postoperative gallstone pancreatitis, cholangitis, obstructive jaundice, and cystic duct stump leaks. Since the use of endobiliary stents has been shown to be effective in the treatment of postoperative bile leaks, their use in protecting the primary closure of a choledochotomy limits the risk of postoperative bile leak.[26]

Surgeons commonly offered two criticisms after our earlier presentations detailing laparoscopic endobiliary stenting following LCBDE. Surgeons skilled in performing LCBDE objected to the routine performance of postoperative ERCP for stent retrieval, since many believe this is unnecessary after CBDS are cleared laparoscopically, and it is not cost-effective. Our experience with LCBDE suggests that intraoperative choledochoscopy is often required to ensure complete clearance of CBDS, which can add substantially to the operative time, and therefore the treatment cost, for patients undergoing LCBDE. This valuable technique also requires specialized equipment and skills that might not be available uniformly. Additionally, since it has been shown that LCBDE is successful in clearing CBDS only 81% of the time in some cases, there is a substantial risk of retained CBDS that justifies postoperative ERCP in many of these patients.[10,24,25]

The second criticism, heard much more frequently, came from surgeons who were not skilled in performing LCBDE. Surgeons cited their lack of familiarity with stone extraction techniques, unavailability of LCBDE equipment, and lack of confidence in their endoscopic suturing abilities, as limiting factors. Others indicated that they simply found it more desirable to leave CBDS in place and refer patients for postoperative ERCP than to attempt LCBDE. These surgeons sought an easy to use method that did not require special equipment, training, or skills, which could be employed rapidly when CBDS were identified.

Our experience suggests that most surgeons who discover occult CBDS during LC/IOC opt to leave the stones in situ, and arrange for postoperative ERCP. Since selective cannulation of the common bile duct during ERCP is achieved in only 80% of procedures in many community hospitals and low-volume medical centers,[11-13] up to 20% of patients with CBDS in these centers will require repeated attempts at ERCP, radiographic biliary drainage, or additional surgery. Surgery in this situation often involves laparotomy with open common bile duct exploration.

Some surgeons may avoid performing routine cholangiography during laparoscopic cholecystectomy because they believe they have nothing to offer patients with CBDS. Because our technique provides all surgeons with a method for treating patients who have CBDS, it might further benefit patients undergoing laparoscopic cholecystectomy by encouraging all surgeons to perform intraoperative fluorocholangiography routinely, which has been shown to limit bile duct injuries and improve operative outcomes.[27,28]

Laparoscopic endobiliary stenting eliminates all concern that postoperative ERCP might fail because of inability to cannulate the common bile duct and clear CBDS. Because the stent guides the endoscopist directly into the common bile duct, selective biliary cannulation is virtually guaranteed during postoperative ERCP. The stent can be removed using a snare prior to selective cannulation with a sphincterotome and performance of sphincterotomy to clear CBDS, or a precut sphincterotomy can be fashioned over the indwelling stent prior to snare removal and subsequent stone clearance. We have cannulated the common bile duct alongside the stent for creation of the sphincterotomy prior to stent removal and stone clearance, and have also investigated retrograde placement of a guidewire through the stent prior to removal of the stent during postoperative ERCP, which maintains biliary access as instrument exchanges are performed.

We did not study the specific costs of this procedure, but propose that cost-effectiveness is inferred from our data. Since equipment like choledochoscopes, stone baskets, lithotriptors, and laparoscopic suturing instruments that can be expensive to purchase and maintain are not required, there are no hidden acquisition costs associated with this method. The only hospital expense over that normally associated with routine LC/IOC is procurement of the laparoscopic endobiliary stent placement kit (Cook Surgical, Inc.). However, the reduced patient morbidity from early recognition and treatment of CBDS and the reduction in operating room time utilization compared with LCBDE more than offset the expense of the kit. The all-inclusive nature of this kit will likely reduce required hospital inventory of separate components, representing additional savings (Fig. 6). Improved patient satisfaction and elimination of future biliary complications related to choledochotomy are

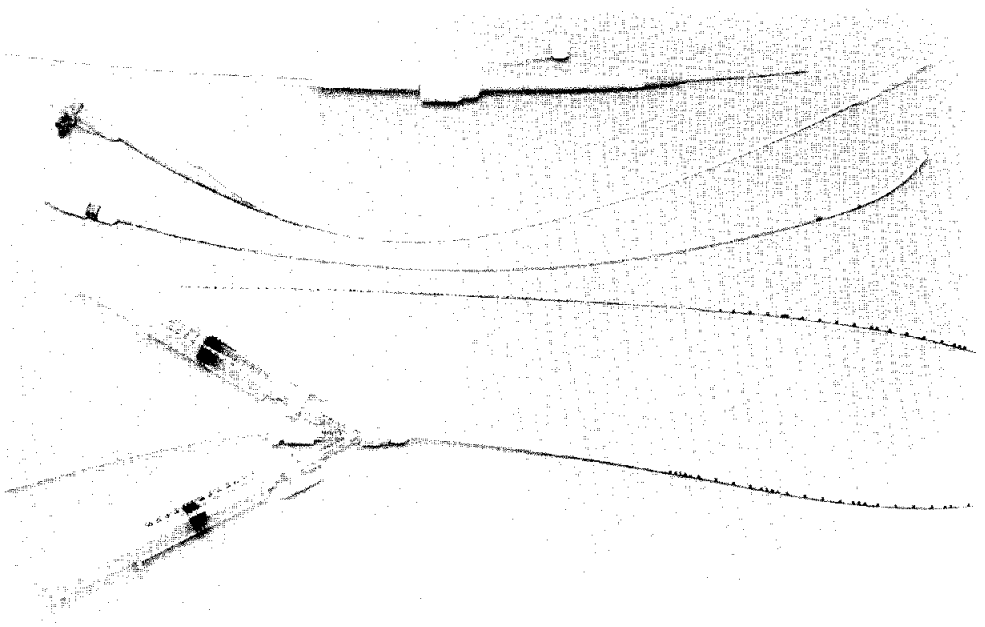

Fig. 6. Components of the laparoscopic common bile duct stent kit (Cook Surgical, Inc.). Cholangiography catheter, three-way injection adapter, guidewires, cystic duct dilatation balloon, sealed introducer sheath set, stent, and delivery mechanism are included.

benefits that are less easily measured, but important nonetheless.

Since the majority of surgeons polled routinely refer their patients with CBDS discovered during LC/IOC for postoperative ERCP, there is no additional expense associated with stent placement since endoscopic management is similar. Further savings may be realized by laparoscopic endobiliary stent placement since these patients incur no postoperative complications from retained CBDS and are virtually assured a successful postoperative ERCP even in low-volume centers, obviating the costs of repeated procedures and multiple referrals.

Preserving the outpatient nature of LC/IOC, even after stent placement, further reduces hospital costs. Surgeons who use ambulatory surgery centers for LC/IOC will find our technique appealing because it allows them to definitively treat their patients with CBDS, yet it eliminates the need for transfer to an inpatient facility. Our results compare favorably with a review by Ferzli et al.[29] in which the length of stay following LCBDE ranged from 1.7 to 12.0 days.

CONCLUSION

The discovery of CBDS demands a rational treatment approach that does not increase patient morbidity, hospital expenses, or treatment costs, or negate the primary benefits of LC/IOC. Many laparoscopic surgeons have mastered LCBDE, but many of their patients must still endure the limitations of indwelling T-tubes and drains. Our method obviates the use of T-tubes and external drains, eliminates complications related to these devices, improves patient comfort, allows same-day discharge, and accelerates recovery for patients with CBDS. Laparoscopic endobiliary stents also protect the closure of the cystic duct stump or choledochotomy after LCBDE and eliminate the risks associated with retained CBDS.

Most surgeons have not learned LCBDE and routinely refer their patients with CBDS for postoperative ERCP, which has a failure rate as high as 20% in some settings. Our method is significantly easier to perform than LCBDE, is not dependent on the laparoscopic suturing skills of the surgeon, and is a most reliable method for the treatment of CBDS. Laparoscopic stent placement virtually ensures successful common bile duct cannulation and stone clearance during postoperative ERCP, even in low-volume centers, eliminating the need for repeated endoscopic or radiographic procedures or additional surgery for treatment of retained CBDS. Laparoscopic endobiliary stent placement may improve the overall safety of laparoscopic cholecystectomy by encouraging routine intraoperative fluorocholangiography since it provides all surgeons with a technically feasible method for treating CBDS.

Laparoscopic endobiliary stent placement has low acquisition costs for hospitals, efficiently utilizes operating room time, is appropriate in the hospital and

ambulatory surgery center setting, and can be instituted in any facility with minimal capital expense. Our experience demonstrates this to be a safe and effective procedure useful for treating all patients with CBDS that is uniformly successful and preserves the minimally invasive benefits of LC/IOC.

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Functional Interleukin-4 Receptor and Interleukin-2 Receptor Common Gamma Chain in Human Gastric Carcinoma: A Possible Mechanism for Cytokine-Based Therapy

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Interleukin (IL)-2 and IL-4 play a critical role in the regulation of the immune response. Yet both of the receptors for these cytokines have been found on nonhematopoietic cells, including human gastric carcinoma cell lines and tissue specimens. IL-4 causes G1 phase cell cycle arrest of gastric carcinoma; the effect directly correlates with the expression of IL-4 receptor (IL-4R) and is seen within 48 hours after treatment. Cells lacking IL-4R are unaffected by IL-4. We examined signal transduction pathways employed by IL-4 that may account for cell cycle arrest of an established human gastric carcinoma cell line, CRL 1739. Western blot analysis was performed on CRL 1739 cultured in the presence of IL-4 (500 U/ml). Cells were lysed, protein extracted, and electroblotted; blots were then probed with murine monoclonal antibodies to specific intracellular proteins. Western blotting of CRL 1739 with antiphosphotyrosine antibody (4G10) demonstrated multiple (140 kDa and 65 kDa) phosphoproteins seen only in IL-4–treated CRL 1739. Immunoprecipitation and blotting of CRL 1739 with specific secondary antibodies demonstrated that the 140 kDa phosphoprotein was IL-4Rα, the 65kDa phosphoprotein was IL-2Rγc, the 130 kDa phosphoprotein was Janus kinase (JAK1), and the 116 kDa phosphoprotein was JAK3. Reverse transcription–polymerase chain reaction with specific primers demonstrated that multiple human gastric tumor specimens expressed IL-4Rα and IL-2Rγc but did not express the leukocyte marker CD45. These results suggest that human gastric carcinomas may express functional cytokine receptors, including the IL-2Rγc commonly found in association with the lymphocyte IL-2R. These receptors may represent novel targets for directing cytokine-based therapy. (J GASTROINTEST SURG 2001;5:81-90.)

KEY WORDS: Gastric cancer, cytokines, signal transduction

Interleukin-4 (IL-4) is a 20 kDa glycoprotein product of activated T lymphocytes and mast cells known to possess a variety of biologic functions. Originally described for its ability to stimulate B cells, IL-4 has been shown to activate cytotoxic T lymphocytes and dendritic cells, modulate macrophage function, and cause B-cell immunoglobulin class switching.[1,2] Our results and the work of other investigators demonstrate that IL-4 can inhibit growth of a variety of nonhematopoietic malignancies, including gastric,[3,4] lung,[5] and colorectal carcinomas.[6] We have previously reported that the effect of IL-4 is through its receptor, and growth inhibition is induced by G0/G1 cell cycle arrest of IL-4 receptor (IL-4R)–positive cell lines.[4] Yet the molecular mechanisms that regulate the downstream process of the IL-4 signal in nonhematopoietic cells and in particular gastric cancer cell lines are not well understood.

The cDNA for human IL-4Rα has been cloned, is well characterized, and is part of the hematopoietic superfamily of receptors.[7] Cross-linking studies demonstrate that IL-4 binds to the high-affinity 140 kDa IL-4Rα and 65 kDa IL-2Rγc in hematopoietic cells,[8] although this process has not been demonstrated in nonhematopoietic malignancies. The intracellular domains of the IL-4Rα have no consensus sequences for tyrosine or serine/threonine kinases, although tyrosine phosphorylation (170 kDa, 140 kDa,

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and 110 kDa proteins) has been observed after IL-4 activation of murine lymphoid cell lines. These studies suggest the 140 kDa protein is IL-4Rα and this protein activates both the 170 kDa insulin receptor substrate 1 (IRS-1), the Janus kinase (JAK) family of transcription factors, and the signal transducers and activators of transcription STAT6.[9,10] In the current investigation we examined the mechanisms of IL-4–induced growth inhibition on the human gastric carcinoma cell line CRL 1739. Our results demonstrate that IL-2Rγc along with JAK1 and JAK3 participate in IL-4–induced growth inhibition of CRL 1739. Whereas IL–4 alone (although untested) may not represent an effective therapy for gastric cancer, understanding the pathways that result in cell cycle arrest by this agent may lead to the design of novel therapies for gastric cancer.

MATERIAL AND METHODS

Cell Lines

Human gastric carcinoma cell lines CRL 1739[11] and HTB 135, colon carcinoma Colo 320, pancreatic adenocarcinomas HTB 134 and Panc-2, and non–small cell lung carcinoma SK-MES-1 were obtained from American Type Culture Collection (Rockville, Md.). Human non–small cell lung carcinoma line LUst (established at the University of California, Los Angeles, gift of Dr. G. Juillard) and colon carcinoma line Spiro (or CoSp), established at our laboratory, served as positive control specimens in some studies, as they have shown growth inhibition in the presence of IL-4. Melanoma cell line Att was established in our laboratory. Cell lines were cultured in RPMI 1640 culture medium (JRH Biosciences, Lenexa, Kan.) or Dulbecco's modified Eagle medium (Life Technologies, Inc., Grand Island, N.Y.) supplemented with 10% heat-inactivated fetal calf serum (Gemini Bioproducts, Calabasas, Calif.) and antibiotics (100 U/ml each of penicillin and streptomycin) in 75 cm^2 flasks. All cell cultures were maintained in continuous exponential growth by weekly passage as previously described.[3]

Cytokines and Reagents

Recombinant human IL-4 (1.8×10^7 U/mg) was the generous gift of Schering-Plough (Kenilsworth, N.J.). Mouse monoclonal immunoglobulin (Ig) G2b antiphosphotyrosine (clone 4G10) and IgG anti-JAK1 antibodies were obtained from Upstate Biotechnology (Lake Placid, N.Y.). Rabbit polyclonal IgG anti–IL-4Rα, anti–IL-4 Stat (STAT6), anti–IL-2Rγc, and anti-JAK3 antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, Calif.). Anti–IL-2Rγc monoclonal antibody was a gift of Dr. K. Sugamura (Tohoku University School of Medicine, Sendai, Japan).

RNA Extraction

Total cellular RNA was extracted using the UltraSpec isolation system (Biotecx Laboratories, Inc., Houston, Tex.) as described by the manufacturer. Briefly, cells were trypsinized, washed in 1× phosphate-buffered saline, lysed in 2 ml of UltraSpec reagent by repetitive pipetting, and placed on ice for 5 minutes in an RNAse-free Eppendorf tube. Four hundred microliters of chloroform was added, after vigorous mixing for 15 minutes, the solution was placed on ice for 5 minutes, and then centrifuged at 12,000 *g* at 4° C for 15 minutes. The aqueous phase was transferred into another RNAse-free Eppendorf tube; one volume of isopropanol was added, and the solution was precipitated at 4° C for 30 minutes. The tube was centifuged at 12,000 *g* at 4° C for 20 minutes to obtain an RNA pellet. The sample was washed with 70% ethanol, dried, and resuspended in 50 μl of diethyl pyrocarbonate (DEPC)–treated Tris-ethylenediaminetetraacetic (TE) buffer. All RNA extraction procedures were performed in a designated laminar flow hood under sterile conditions. Polymerase chain reaction (PCR) reagent setup and gel electrophoresis were performed in separate rooms to avoid potential RNA contamination.

Reverse Transcription

Total sample RNA was reverse transcribed by using a reverse transcription (RT) mixture consisting of Moloney murine leukemia virus reverse transcriptase with oligo (dT) primer as previously described.[4] Three micrograms of sample RNA was used in the reactions. All reagents were obtained from Promega (Madison, Wis.). The reaction was incubated at 37° C for 2 hours, at 99° C for 5 minutes, and on ice for 5 minutes.

Polymerase Chain Reaction Amplification

Oligonucleotide primers were synthesized and purified by Gibco BRL (Gaithersburg, Md.). Oligonucleotide 5′ and 3′ primers for individual genes were designed as follows: IL-2Rγc—5′-GGCCACACAGATGCTAAAACT-3′ and 3′-GAACAATGACTTATGGTGCCC-5′; IL-4Rα—5′-ATGGGGTGGCTTTGCTCTGGG-3′ and 3′-ACCTTCCCGAGGAAGTTCGGG-5′; CD45—5′-AGCCCTGCTTGTTGTTCTCT-3′and 3′-CTATTTGCCTCTACGTCCA-5′; and β-actin—5′-CCTTCCTGGGCATGGAGTCCTG-3′ and 3′-CTTCT-

AGTTCTAGTAACGAGG-5′. The PCR cDNA products of IL-2Rγc, IL-4Rα, CD45, and β-actin were 492 bp, 345 bp, 338 bp, and 202 bp, respectively.[12,13] The PCR was performed as previously described.[4] The annealing temperature for primers was designed by using OLIGO Primer Analysis Software 5.0 (Plymouth, Minn.), and the PCR conditions were set up as follows: 95° C for 5 minutes, followed by the cycle of 95° C for 1 minute, 67° C for IL-2Rγc and CD45 (64° C for IL-4Rα and 55° C for β-actin) for 1 minute, 72° C for 1 minute with repeat of the three 1-minute phases for 35 cycles, and a final 72° C for 10 minutes extension time and final soaking at 4° C. The PCR reaction was performed in an OmniGene temperature cycler (Hybaid, Middlesex, England). The preparation of PCR mixture for the temperature cycler was performed in a designated PCR room in a sterile laminar flow hood.

The PCR cDNA product was detected by electrophoresis on a 2% agarose gel (Life Technologies Inc.) and visualized by ethidium bromide staining under ultraviolet light. A 100 bp DNA ladder (Life Technologies Inc.) was used as a reference marker for all assays.

Immunoprecipitation and Immunoblot Analysis

Cell lines were washed and incubated in serum-free medium one night before cytokine was added. After stimulation with IL-4 (500 U/ml) for approximately 10 minutes at 37° C, the cells were lysed in lysis buffer (10 mmol/L Tris-HCl [pH 7.2], 150 mmol/L sodium chloride, 1% sodium deoxycholate, 5 mmol/L EDTA, 1 mmol/L phenylmethylsulfonyl fluoride, 20 mmol/L sodium fluoride, 100 μmol/L sodium vanadate, and 10 μg/ml leupetin and aprotinin [Sigma Chemical, St. Louis, Mo.]). The lysates were centrifuged at 12,000 *g* for 20 minutes at 4° C to remove insoluble material. The total protein content of the lysates was determined by Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, Calif.). Equal amounts of clarified cell lysates (approximately 2 mg) were immunoprecipitated with 3 μg anti–IL-2Rγc or anti–IL-4Rα antibodies (Santa Cruz Biotechnology) using 20 μl protein A rendered insoluble by means of sepharose 4B fast flow (Sigma Chemical Co.). The immunoprecipitates were washed twice in dilution buffer (0.1% Triton X-100 and bovine hemoglobin in trisodium azide (TSA) solution (0.01 mol/L Tris-HCl [pH 8.0], 0.14 mol/L sodium chloride, 0.025% sodium azide), one time in TSA solution and another in 0.05 mol/L Tris-HCl [pH 6.8] solution, solubilized with Laemmli buffer, boiled, and resolved by Tris-glycine 4-12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Novex, San Diego, Calif.). In some experiments, proteins (75 μg) were directly resolved by SDS-PAGE without prior immunoprecipitation.

Western blot analyses were performed by transferring proteins from polyacrylamide gels onto Hybond enhanced chemiluminescence (ECL) nitrocellulose membranes (Amersham Corp., Arlington Heights, Ill.) at 25 V for 2 hours in Tris-glycine buffer containing 25 mmol/L Tris, 192 mmol/L glycine, 0.1% SDS, 100 μmol/L sodium vanadate, and 20% methanol. The blots were treated for 1 hour with blocking buffer (2.5% nonfat dry milk, 10 mmol/L Tris-HCl [pH 7.5], 100 mmol/L sodium chloride, 0.1% Tween 20), and then incubated with 2 μg/ml antibody in blocking buffer for another hour. Antibody binding was detected by incubating the blots for 1 hour with sheep antirabbit immunoglobulin conjugated with horseradish peroxidase, followed by a 1-minute incubation with iodinated substrate and then ECL detection. The blots were exposed to autoradiography film (Hyperfilm-ECL). Some blots were reprobed after being stripped with 2% SDS, 6.25 mmol/L Tris [pH 6.7], and 100 mmol/L 2-mercaptoethanol at 55° C for 30 minutes with gentle agitation. Relative differences in protein expression were determined through densitometry using a dual-wavelength flying-spot scanner (Shimadzu Corp., Kyoto, Japan).

RESULTS

Expression of IL-4Rα

Our previous studies have demonstrated that IL-4 induced growth inhibition of the gastric carcinoma cell line CRL 1739.[3] We performed Western blot analyses on CRL 1739 using specific antibodies to IL-4Rα and demonstrated evidence of the 140 kDa IL-4Rα. Colon carcinoma cell line Spiro (CoSp) and non–small cell lung cancer cell line LUst both served as positive control specimens. The IL-4 nonresponsive cell line SK-MES-1 expressed faint evidence of the IL-4R (Fig. 1).

Tyrosine Phosphorylation in Response to IL-4

Although the IL-4R has no intrinsic tyrosine kinase activity, we sought to determine if tyrosine phosphorylation occurred in the IL-4–sensitive cell line CRL 1739 in response to IL-4. Four cell lines were cultured in serum-free medium overnight, and IL-4 (500 U/ml) was added to the medium for 10 minutes and cells were harvested (Fig. 2). By Western blot analyses, CRL 1739, LUst, and Spiro demonstrated 140 kDa and 65 kDa phosphoproteins in response to stimulation with IL-4. In the absence of IL-4, only Spiro demonstrated faint expression of a 65 kDa pro-

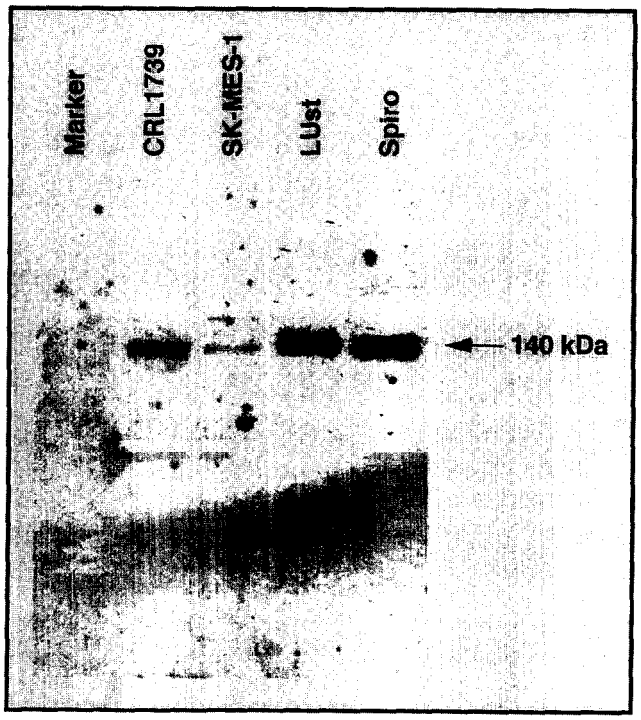

Fig. 1. Western blot analysis demonstrating specific binding of the 140 kDa IL-4Rα. Western blots were performed from cell lysates using antibody to IL-4Rα (see Material and Methods). The human colon carcinoma cell lines Spiro (CoSp), established in our laboratory, and LUst have previously been found to express a high level of IL-4Rα and served as positive control specimens for this experiment.[29]

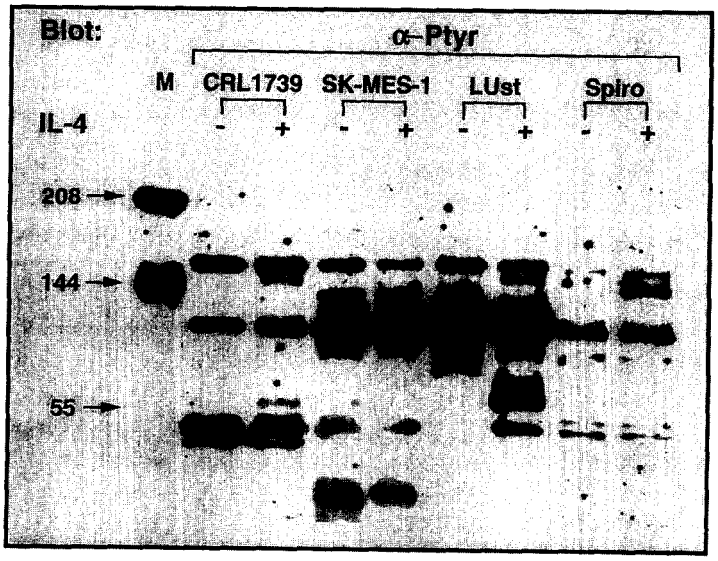

Fig. 2. Tyrosine phosphorylation of IL-4–treated and untreated CRL 1739. Human nonhematopoietic cell lines CRL 1739, SK-MES-1 (lung), LUst (lung), and Spiro (colon) were cultured in serum-free medium overnight and then treated with IL-4, 500 U/ml, for 10 minutes. Western blotting with antiphosphotyrosine antibody (4G10) demonstrated 140 kDa and 65 kDa phosphoproteins from IL-4–treated CRL 1739 (+) (and LUst and Spiro) but not from SK-MES-1 or other cell lines in the absence of IL-4 (−).

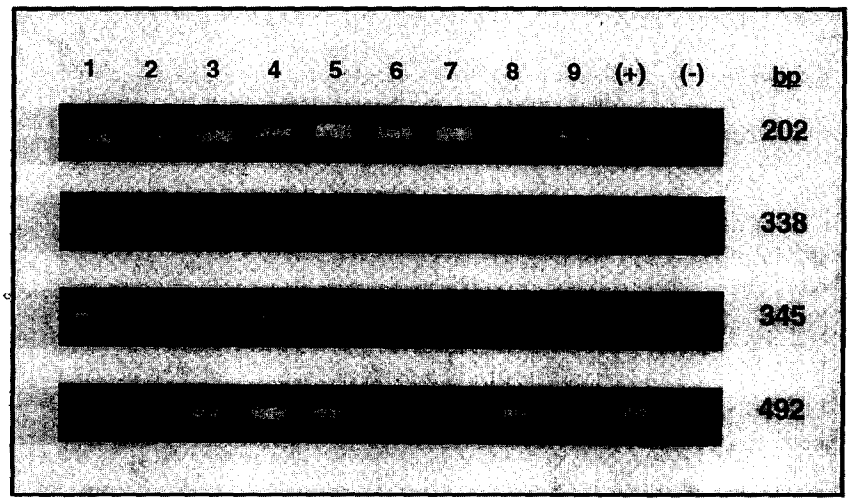

Fig. 3. IL-4Rα and IL-2Rγc mRNA expression from human carcinoma cell lines. Nine human carcinoma cell lines were chosen based on their expression of the 345 bp product of the IL-4Rα. Seven of the nine cell lines also expressed the 492 bp product of IL-2Rγc (lanes 2 and 7 lacked expression). Human peripheral lymphocytes (PBL) served as a positive control specimen (CD45, common leukocyte marker; bp 338). Integrity of mRNA for RT-PCR was confirmed by amplification of the β-actin gene (bp 202). Lane 1, LUst (lung); lane 2, SK-MES-1 (lung); lane 3, CRL 1739 (gastric); lane 4, HTB-135 (gastric); lane 5, Spiro (colon); lane 6, colo320 (colon); lane 7, Panc II (pancreas); lane 8, HTB-134 (pancreas); lane 9, Att (JWCI melanoma); (+) = PBL; (−) = mock.

tein. The IL-4 nonresponsive cell line SK-MES-1 did not express the 140 kDa or 65 kDa phosphoproteins.

Expression of IL-2 Rγc

IL-2Rγc has been found to be an important component of IL-2 binding in hematopoietic cells and recently has been shown to be essential for signal transduction with a variety of cytokines, including IL-4.[14,15] We assessed IL-2Rγc gene expression by RT-PCR to determine the presence of IL-2Rγc in nonhematopoietic malignancies including CRL 1739. Nine human carcinoma cell lines were selected for their expression of IL-4Rα (Fig. 3; CRL 1739, lane 3). All of these cell lines have been shown to express the IL-4Rα. Seven (78%) of the nine expressed the transcribed IL-2Rγc. The 492 bp IL-2Rγc was found in untreated CRL 1739 and human peripheral blood lymphocyte (+) control cells but was absent in the IL-4 nonresponsive cell line SK-MES-1 (see Fig. 3, lane 2) and the pancreatic cell line Panc-2 (lane 7). These results suggest that the expression of IL-4Rα and IL-2Rγc are conserved through multiple human carcinomas and not unique to CRL 1739.

Because the CRL 1739 cell line expressed both IL-4Rα and IL-2Rγc, we sought to determine whether the 65 kDa phosphoprotein identified by Western blot analyses in response to IL-4 was IL-2Rγc. We performed immunoprecipitations and blotting with anti–IL-4Rα and anti–IL-2Rγc antibodies, respectively (Fig. 4). CRL 1739 (along with LUst and Spiro) expressed the 65 kDa IL-2Rγc in response to IL-4 stimulation. The IL-4 nonresponsive cell line SK-MES-1 did not express IL-2Rγc either in the presence or absence of IL-4.

Phosphorylation of Janus Kinases

The Janus kinases JAK3 and JAK1 have been shown to be an important part of IL-4 signal transduction in hematopoietic cells.[15-17] The 116 kDa JAK3 protein has been found to be associated with IL-2Rγc in nonhematopoietic cells and serves as an activator of the JAK-STAT pathway. We performed immunoprecipitations of cell lysates with anti–IL-2Rγc antibody and probed with anti-JAK3 antibodies. Western blotting with anti-JAK3 antibody demonstrated enhancement in quantitative binding (determined by densitometry of the blot films) between IL-4–treated and untreated CRL 1739 (Fig. 5). Similar results were observed when blots were reprobed for the 130 kDa JAK1. JAK1 binds to the IL-4 receptor and complexes with IL-2Rγc (Fig. 6). IL-4–responsive CRL 1739 demonstrated activation of JAK1. We attempted to examine the response of the STAT6 protein in IL-4–sensitive CRL 1739. Immunoprecipitations with anti–IL-2Rγc antibodies and blotting with anti-STAT6 or blotting alone demon-

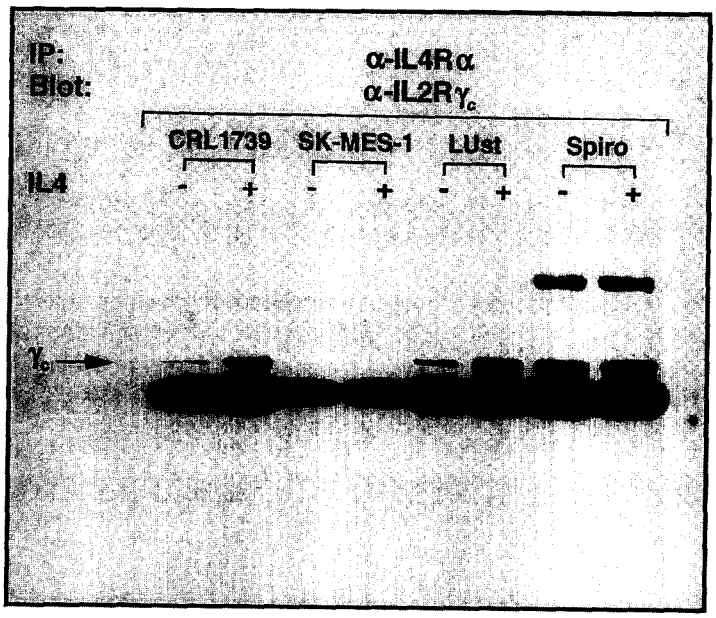

Fig. 4. Activation of IL-4Rα and IL-2Rγc in the gastric carcinoma cell line CRL 1739 after treatment with IL-4. Four cell lines were cultured overnight in serum-free medium and treated with IL-4 for the final 10 minutes. Immunoprecipitation and blotting of the cell lysates demonstrated the 65 kDa IL-2Rγc present in the IL-4–responsive lines (CRL 1739, LUst, and Spiro) and not from the nonresponsive SK-MES-1.

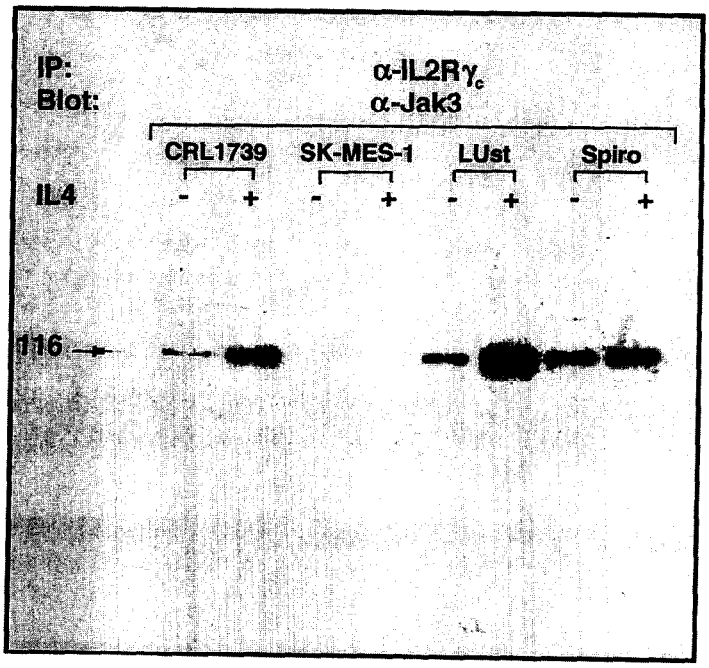

Fig. 5. Expression of JAK3 via IL-2Rγc pathways. The four cell lines were treated with IL-4, 500 U/ml, for 10 minutes, and cell lysates were harvested. Immunoprecipitation and blotting demonstrated enhanced expression of the 116 kDa JAK3 in the presence of IL-4 (+). The nonresponsive cell line SK-MES-1 did not express JAK3 before or after treatment with IL-4. The three cell lines that expressed both IL-4Rα and IL-2Rγc demonstrated enhanced expression of JAK3 after the specimens were immunoprecipitated with anti-IL-2Rγc antibody.

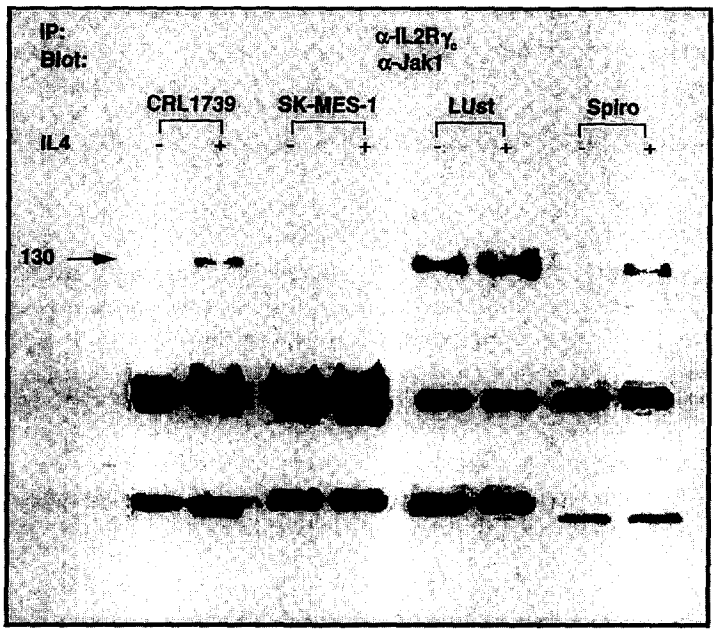

Fig. 6. Activation of JAK1 through IL-2Rγc pathways. Cell lysates from the four cell lines were immunoprecipitated and blotted with anti-JAK1 antibodies. The IL-4–responsive cell lines (CRL 1739, LUst, and Spiro) demonstrated enhanced expression of JAK1 after treatment with IL-4.

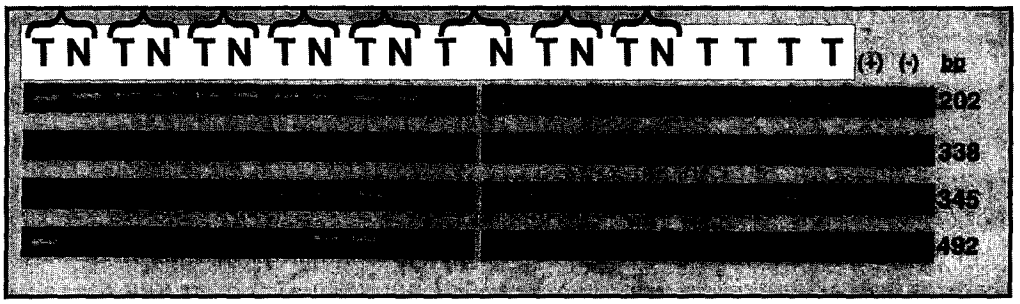

Fig. 7. Detection of IL-4Rα and IL-2Rγc from biopsy specimens of gastric carcinomas *(T)* and normal adjacent gastric tissue *(N)*. Eight surgically resected gastric carcinoma specimens were microdissected for analysis of the tumor and adjacent normal tissue for expression of IL-4Rα and IL-2Rγc.[12] In four other cases, only tumor tissue was available for analysis. Nine (75%) of 12 tumor specimens and seven (87%) of eight normal stomach tissue specimens expressed the gene products of both IL-4Rα (345 bp) and IL-2Rγc (492 bp). In five (67%) of eight cases where tumor and normal tissue were available, both receptors were present in the matched specimens. None of the biopsied specimens expressed the 338 bp gene product of CD45. Integrity of mRNA for RT-PCR was confirmed by amplification of the β-actin gene (bp 202). Normal human PBL (+) and mock samples (−) served as control specimens.

strated no quantitative difference in expression of the 100 kDa STAT6 (IL-4 Stat) in the presence or absence of IL-4 (data not shown). These results suggest that the IL-4Rα/IL-2Rγc complex binds to JAK-dependent pathways after activation with IL-4.[18] The process of STAT6 activation in IL-4–induced growth inhibition is unknown.

We obtained gastric carcinoma biopsy specimens and matched normal gastric mucosa from eight patients undergoing surgical resection of their cancers.[12] In four other patients only tumor specimens were available for analysis (Fig. 7). Our intention was to determine if the IL-4Rα and IL-2Rγc were present in vivo. In 9 (75%) of 12 tumor specimens and seven (87%) of eight normal gastric mucosa specimens, both IL-4Rα and IL-2Rγc were expressed. In only half of the cases where normal and tumor tissues were available were both genes expressed in the matched specimens.

DISCUSSION

A variety of cytokine receptors have been identified in human nonhematopoietic malignancies including gastric carcinomas, yet in general their function is unknown.[19] We have identified IL-4Rα and IL-2Rγc in human cancer cell lines. Biopsy specimens from resected gastric cancer specimens demonstrated a high level of expression both in tumor and adjacent normal tissue (see Fig. 7), suggesting that these cytokine receptors are present in vivo and are not just a reflection of in vitro culture conditions. Our results suggest that IL-4–induced cell cycle arrest of these cells occurs via a process that involves both IL-4Rα and IL-2Rγc.

IL-4Rα has been well characterized and has been found in a number of human nonhematopoietic cell lines. Although we did not quantitate the number of IL-4Rα molecules present in the cell line CRL 1739, other investigators have found anywhere from 2000 to 6000 receptor IL-4Rα molecules per cell in responsive cell lines.[4,6] The minimum number of IL-4Rα molecules for IL-4 to inhibit cell growth is unknown.

We identified a 65 kDa phosphoprotein that is present from rhIL-4–treated CRL 1739. Our data suggest that this 65 kDa species is the IL-2Rγc. Although the IL-2Rγc is more commonly associated with IL-4 (and IL-2, IL-7, and IL-13) binding in hematopoietic cells, these results suggest that the IL-2Rγc is present in an established human gastric cancer cell line. Our studies and the report from another group of investigators[12] have also observed the presence of IL-2Rγc in other human nonhematopoietic cell lines (gastric and colon cancers) and tumor biopsies. Other investigators have observed a 70 kDa product from IL-4–treated nonhematopoietic cells and have suggested that this protein may represent degradation products of the 140 kDa IL-4Rα receptor[20] or may represent an alternative binding protein as seen in IL-13 binding.[21,22] Our data suggest that the 65 kDa protein in CRL 1739 is the IL-2Rγc. The lack of expression of the IL-2Rγc from the IL-4–nonresponsive cell line SK-MES-1 may explain its insensitivity to IL-4.

To understand the mechanisms of IL-4–induced inhibition of tumor cell growth, we investigated the signal transduction pathways employed by IL-4 in the human gastric cancer cell line CRL 1739. IL-4 signaling events appear to act through the IL-4Rα and JAK-STAT pathways used by hematopoietic cells. Several studies have shown that an alternative pathway exists in which the secondary messenger IRS-1 is phosphorylated in response to IL-4.[23] Yet it has been suggested that the phosphorylation of IRS-1 may occur only in the presence of IL-2Rγc.[24] Our previous results suggest that although IRS-1 was expressed in both IL-4–responsive and nonresponsive cell lines, the level of expression was no different and likely excludes the possibility of IRS-1 contributing to IL-4–induced growth arrest.[20] Since JAK and IRS-1 have been found to be associated with the 140 kDa IL-4Rα in murine T cells, it is possible that JAK phosphorylates IRS-1 without involving IL-2Rγc.[25]

Following complexing of IL-4Rα and IL-2Rγc in hematopoietic cells, several investigators have demonstrated activation of both JAK1 and JAK3.[17,18] Ligand binding to IL-4Rα has been shown to activate associated JAK tyrosine kinases, which phosphorylate each other as well as receptor tyrosine phosphoproteins. Several investigators have demonstrated that IL-2Rγc forms complexes with JAK3 after activation with IL-2 or IL-4.[15,16,18] Our results confirm these results, and the lack of growth inhibition observed in other nonhematopoietic cell lines may result from the absence of IL-2Rγc and JAK interactions. Recent studies demonstrate that STAT6 is recruited to the activated IL-4Rα by docking of the SH2 domain where they are phosphorylated by JAK kinases, then dimerize and translocate to the cell nucleus where gene activation occurs.[17,18,20] To our knowledge, our work is the first to describe the expression and activation of IL-4Rα and IL-2Rγc in a human gastric carcinoma cell line along with expression of JAK1 and JAK3.

It is clear that IL-4 has contrasting effects on different tissue types. In immune cells, IL-4 has both potent growth-stimulating and inhibitory effects; however, in nonhematopoietic cells, IL-4 has only growth inhibitory effects. The reason for the dichotomy is unclear. Some of the differential effects may relate to the signaling pathways employed by IL-4Rα in different cell types. It is possible that the presence of IL-2Rγc may be responsible for growth inhibition in some nonhematopoietic cell lines by activity through JAK-1 and JAK-3/STAT (type I IL-4 receptor) pathways, whereas other cells appear to respond even in the absence of IL-2Rγc (type II IL-4 receptor).[16] Still it is possible that the IL-2Rγc is present in normal nonhematopoietic cells,[12] and its expression is lost during dedifferentiation of malignant cells resulting in loss of growth control by IL-4.

Although the function of IL-4Rα and IL-2Rγc on human nonhematopoietic malignancies, and in particular on gastric carcinomas, is unknown, the information gained from dissecting out the signal transduction pathways employed by IL-4 may be critical for the design of new therapies for gastric cancer. IL-4 has been used for adjuvant therapy for non–small

cell lung cancers. Early studies demonstrated the safety of this agent in human subjects.[26,27] Both the phase II and III clinical trials for lung cancer demonstrated only minimal efficacy for IL-4 alone, but the investigators did not examine the expression of IL-4Rα or IL-2Rγc on tissue specimens to determine if the presence of receptors correlated with clinical responses.[26,27] Although it may be that IL-4 (or IL-2) would not be a useful agent for treatment of gastric cancer, the development of drugs aimed at activation of IL-4Rα, IL-2Rγc, or the JAK-STAT pathway may provide an alternative approach for cytostatic therapy.[28,29] Further understanding of the mechanism employed by IL-4 to induce cell cycle arrest of nonhematopoietic cells may be beneficial for the design of better biologic therapies.[30]

CONCLUSION

Human IL-4 induces cell cycle arrest of a variety of nonhematopoietic malignancies including the gastric carcinoma cell line CRL 1739. Our results demonstrate that both IL-4Rα and IL-2Rγc are activated alone with the Janus kinases JAK1 and JAK3 in response to IL-4 stimulation. These results have important implications in regard to the regulation of cell cycle events and may provide useful sites for aiming directed cytostatic therapy.

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The Antioxidant N-Acetylcysteine Increases 5-Fluorouracil Activity Against Colorectal Cancer Xenografts in Nude Mice

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The antioxidant pyrrolidinedithiocarbamate improves the therapeutic efficacy of 5-fluorouracil (5-FU) against HCT-15 colorectal cancer cell line xenografts in nude mice without increasing toxicity to normal intestinal or hematopoietic tissues. In the current study we have shown that a similar clinically licensed antioxidant, N-acetylcysteine (200 mg/kg), can modulate the activity of 5-FU (120 mg/kg) against HCT-15 tumor xenografts in nude mice. We demonstrate that this effect is accompanied by a sustained elevation in p53-independent apoptosis without accompanying alterations in cell cycle kinetics. Extensive tumor necrosis is also a prominent feature of treatment; however, no significant impairment of neovascularization as assessed by intratumor microvessel density occurred. We believe that the clinical efficacy of N-acetylcysteine as an adjunct to 5-FU in advanced colorectal cancer should be investigated further. (J GASTROINTEST SURG 2001;5:91-97.)

KEY WORDS: 5-Fluorouracil, antioxidants, colorectal cancer, apoptosis, N-acetylcysteine

5-Fluorouracil (5-FU) is the most commonly used chemotherapeutic agent in advanced colorectal cancer. Response rates of 22% are currently achieved with infusional protocols that incorporate leucovorin.[1] High-dose therapy with the antioxidant pyrrolidinedithiocarbamate (PDTC) is reported to increase the efficacy of 5-FU, producing complete resolution of colorectal cancer cell line xenografts in nude mice.[2] Further support for the therapeutic use of this compound was obtained when we reported that PDTC did not potentiate either the gastrointestinal or hematopoietic side effects of 5-FU.[3] In fact, PDTC therapy was protective to colonic stem cells following exposure to toxic schedules of 5-FU. An analogue of PDTC, sodium diethylcarbamate (DTC), has previously been administered to humans suffering from acquired immunodeficiency syndrome (AIDS), and although it was initially considered safe,[4,5] this agent was later withdrawn following reports of occasional neurotoxicity. This compound has since been shown to augment the neurotoxicity inherent in certain other compounds.[6] The adverse clinical experience with DTC has thrown into doubt the clinical safety of PDTC and stimulated a search for alternative antioxidants to be used in humans.

We have explored the impact of another thiol-containing antioxidant, N-acetylcysteine (NAC), on 5-FU activity against colorectal cancer cell lines, both in vitro and in vivo. NAC is clinically licensed for use in high doses to treat acetaminophen (paracetamol) poisoning and is generally considered safe, even though it does cause occasional anaphylactic reactions. Although not previously used in colorectal cancer, NAC has been reported to reduce the number of lung metastases in a mouse model of malignant melanoma when administered as an adjunct to doxo-

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rubicin.[7] The mechanism of this response is not currently known, although several relevant observations have been made. NAC induces apoptosis in vascular smooth muscle cells in vitro[8] while inhibiting endothelial cell invasion in tumor tissue consequently reducing tumor angiogenesis.[9] NAC can induce p21 and p16, prolonging the G1 phase of the cell cycle.[10] NAC also reduces the expression of COX-2, an enzyme highly expressed in 85% of colorectal cancers and associated with resistance to apoptosis, increased angiogenesis, and tumor invasiveness.[11,12] However, NAC has other effects that are difficult to reconcile with its proposed anticancer action. For instance, it is cytoprotective toward several transformed cell lines, impairing apoptosis induced by p53,[13] tumor necrosis factor-alpha (TNF-α),[14,15] nuclear factor kappa B (NF-κβ),[16,17] and CD95.[18-21]

In light of these conflicting reports, we have investigated how NAC modulates the activity of 5-FU against colorectal cell line xenografts in nude mice. We determined indices of cellular proliferation, apoptosis, necrosis, and vascularity following treatment in order to establish the pertinent mode of drug action in this model. Our results show that NAC/5-FU therapy significantly attenuates the growth of colorectal cancer cell line xenografts in nude mice compared to single-agent therapy. The principal effect of NAC is to increase cellular apoptosis and necrosis within the tumor without any apparent effect on angiogenesis.

MATERIAL AND METHODS

5-FU was purchased from Roche Pharmaceuticals (Welwyn Garden City, U.K.) and NAC from Sigma (Poole, U.K.). For injection into mice, 5-FU and NAC were diluted in 0.9% (weight/volume) saline solution. All other chemicals were purchased from Sigma unless otherwise indicated.

Cell Culture

HCT-15 human colon adenocarcinoma cells were obtained from American Type Culture Collection (Rockville, Md.). Cells were grown in Dulbecco modified Eagle medium (DMEM) supplemented with 10% (volume/volume) fetal bovine serum, 1 mmol/L sodium pyruvate, penicillin (1000 units/ml), and streptomycin (100 mg/ml) (Gibco, Paisley, U.K.) at 37° C in 7.5% CO_2 in air.

Flow Cytometric Analysis of Cell Cycle and Apoptosis

HCT-15 cells were suspended in 1.5 ml DMEM at 2×10^5 cells/ml, then seeded into six-well plates (Nunc-Life A/S, Roskilde, Denmark) and left to attach overnight. The medium was removed and replaced with 1.5 ml of medium containing NAC and/or 5-FU dissolved in DMEM. Following 24 to 72 hours' incubation, medium from treated cells was removed to a universal tube in order to retain detached cells. Attached cells were then trypsinized and added to this tube. The cells were washed in phosphate-buffered saline and fixed in ice-cold 70% ethanol and suspended in 1 mg/ml RNase (5 minutes) prior to staining with propidium iodide (15 minutes). Flow cytometric analysis was performed with a fluorescence-activated cell sorting FacScan analyzer (Becton-Dickinson, Franklin Lakes, N.J.). Cells were excited at 488 nm, and the emission detected through a 585/42-nm band-pass filter using a minimum of 10,000 cells per sample. Cell cycle analysis was performed using Modfit 5.2 software (Verity, Inc., Maine). Cells were considered apoptotic if they exhibited sub-G_1 DNA fluorescence. Cellular debris was gated out using the electronic threshold prior to analysis. All experiments were performed in triplicate.

Effect of Treatment on Xenograft Growth

Male athymic nude mice (Balb/c nu/nu) bred at the Paterson Institute, aged 10 weeks and weighing 25g, were used. The mice had free access to food and water under an alternate 12-hour light/dark regimen. Mice were injected subcutaneously in each flank with a suspension containing 1×10^6 HCT-15 cells in 0.1 ml DMEM. Animals were examined for evidence of tumor growth on a daily basis. Tumor volume was estimated as the product of three perpendicular measurements made using analogue calipers (i.e., approximated to a cube). Treatment was initiated when mean individual tumor volume exceeded 100 mm^3, which occurred 7 days after tumor implantation. Treatment groups comprised the following: (A) saline, 0.2 ml (n = 5); (B) NAC, 200 mg/kg (n = 5); (C) 5-FU, 120 mg/kg (n = 9); and (D) NAC, 200 mg/kg/5-FU, 120 mg/kg (n = 9) administered by intraperitoneal injection. In mice receiving combined treatment, NAC was administered 10 minutes prior to 5-FU.

Tumor dimensions were subsequently measured every third day by a single observer who was blinded to group treatment. Tumor volume was expressed as the sum of bilateral tumors per animal. In accordance with the Animal (Scientific Procedures) Act of 1986, mice were removed from the study and culled by cervical dislocation when individual tumor volume exceeded 1000 mm^3 or ulceration occurred. Tumors were removed by sharp dissection and fixed using 4% formaldehyde in saline solution for 24 hours prior to dehydration in alcohol and wax embedding.

Table I. In vitro apoptosis of HCT-15 cell line

Treatment	Apoptosis (%)		
	24 hr	48 hr	72 hr
Control	0.4 ± 0.4	0.7 ± 0.3	1.2 ± 0.6
25 mmol/L NAC	3.8 ± 0.2*	2.6 ± 0.4	2.6 ± 2.2
10 μmol/L 5-FU	0.6 ± 1.0	4.9 ± 3.3*	8.7 ± 4.8*
25 mmol/L NAC/10 μmol/L 5-FU	4.9 ± 1.4*	5.7 ± 2.7*	12.5 ± 6.2*

The proportion of apoptoses produced in HCT-15 cell types following incubation with either 10 μmol/L 5-FU, 25 mmol/L NAC, or both was determined by propidium iodide staining and flow cytometry. Each experiment was performed in triplicate to derive the mean ± standard deviation.
*No significant difference between groups.

Quantification of Apoptosis, Mitosis, and Necrosis in Tumor Xenografts

Apoptotic cells were identified by terminal deoxynucleotide transferase labeling using a modification of the ApopTag method (Oncor, Gaithersburg, Md.). After rehydration through an ethanol series, sections were permeabilized with proteinase K (20 μg/ml in phosphate-buffered saline) for 20 minutes. The terminal deoxynucleotide transferase mixture (ApopTag) was prepared as directed, but then diluted 1:2.375 with distilled water to reduce nonspecific background staining. Slides were incubated with the enzyme mixture for 1 hour at 37° C. Digoxigenin-labeled dNTPs were treated with antidigoxigenin-peroxidase for 1 hour at room temperature. Sections were developed with 3,3′-diaminobenzidine tetrahydrochloride (DAB; 0.05 mg/ml in phosphate-buffered saline/0.03% hydrogen peroxide) in phosphate-buffered saline for 5 minutes, followed by a thionine blue counterstain. Apoptosis was quantified by expressing as a percentage the number of TUNEL-positive cells found in a minimum of 1000 tumor cells (from four separate regions in each tumor), identified using a Zeiss microscope (×640). Areas of necrotic tumor were avoided. Similarly the number of mitotic cells in a minimum of 1000 cells from four regions were identified by their characteristic morphology and expressed as a percentage of total cells counted.

To determine the percentage of necrotic tissue per tumor section, the combined area of all necrotic segments was expressed as a fraction of the tumor cross-sectional area. This analysis was performed using a Zeiss AxioHome microscope.

Immunohistochemical Detection of Fas and Fas Ligand and Angiogenesis

For the detection of Fas and Fas ligand (Fas-L), rabbit polyclonal Fas (C-20), and Fas-L (N-20) antibodies (Santa Cruz Biotechnology, Santa Cruz, Calif.) reactive with human epitopes were used (200 μg/ml) in conjunction with Santa Cruz ImmunoCruz staining systems according to the manufacturer's protocol.

Measurement of Intratumor Microvessel Density

Intratumor microvessel density was measured using a monoclonal antibody to CD105 as described previously.[22] Samples were processed using an avidin-biotin complex immunoperoxidase staining system (Santa Cruz Biotechnology), developed using DAB and counterstained using hematoxylin. Using light microscopy, a single observer blinded to group treatment located vascular areas within a tumor at low magnification and counted vessels using a Chalkley point eyepiece graticule at ×400 magnification.[23] Any brown-staining endothelial cell or group of cells in contact with a spot in a grid was counted as an individual vessel. Mean IMD for each tumor was derived from four Chalkley counts.

Statistical Analysis

All statistical analysis was performed using Statistica software (StatSoft, Tulsa, Okla.). Initially the normality of individual data sets was examined using Levene's test for homogeneity of variances. Normally distributed data were subsequently analyzed using analysis of variance (ANOVA) with post hoc comparison of multiple means using the Tukey honest significance difference (HSD) test.[24] Otherwise, non-normally distributed data sets were examined using the Kruskal-Wallis ANOVA by ranks and the Mann-Whitney U test.

RESULTS

5-FU and NAC Induce Apoptosis In Vitro

Flow cytometric analysis demonstrated that the addition of NAC enhanced and accelerated the induction of apoptosis in the p53 mutant colorectal cell line, HCT-15 (Table I). Administration of NAC alone

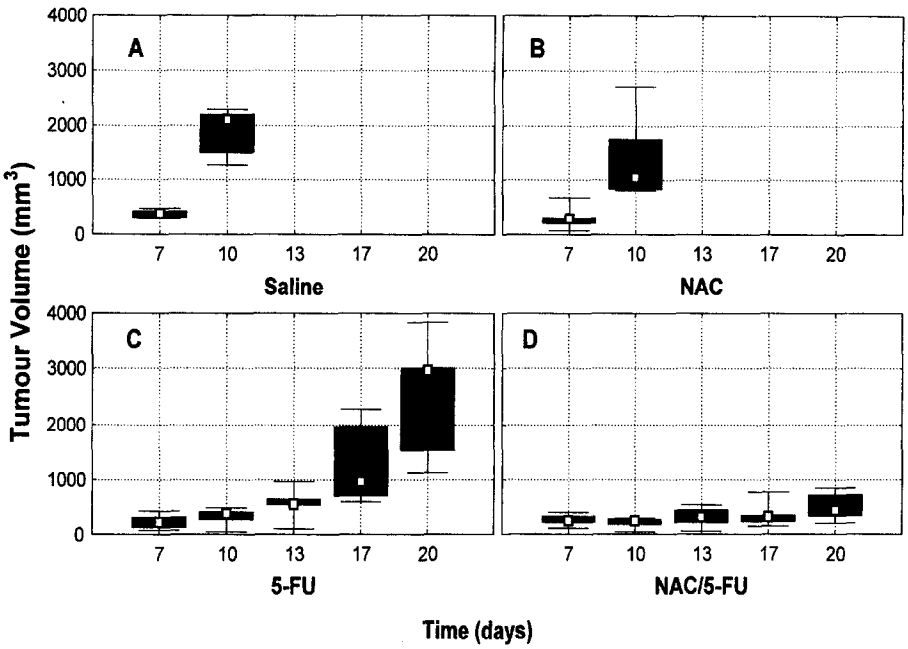

Fig. 1. Box plots of mean xenograft volume per mouse, displayed as the sum of bilateral tumors, following implantation of 1×10^6 HCT-15 colorectal cancer cells at day 0. When mean individual tumor volume reached 100 mm^3 at day 7, treatment was initiated with **(A)** saline, 0.2 ml (n = 5); **(B)** NAC, 200 mg/kg (n = 5); **(C)** 5-FU, 120 mg/kg (n = 9); or **(D)** NAC, 200 mg/kg/5-FU, 120 mg/kg (n = 9). Animals were removed from the study when individual xenograft volume exceeded 1000 mm^3 or tumors became ulcerated. Box plots display median group values, twenty-fifth and seventy-fifth percentiles, and extremes.

had little effect on apoptosis. Cell cycle analysis demonstrated that NAC alone did not significantly alter the proportion of cells in each phase of the cell cycle over the 72-hour period (data not shown). After 72 hours' exposure to 5-FU, the percentage of cells in S-phase was increased to 66 ± 19% from 44 ± 3% in control cells, with a concomitant decrease in cells in G_2/M from 17 ± 4% (control) to 3 ± 5% (5-FU). The presence of NAC did not significantly effect 5-FU–mediated cell cycle alterations (data not shown). Together these data show that the principal effect of NAC was to enhance the induction of apoptosis by 5-FU without significant effects on cell cycle progression.

NAC Enhances 5-FU–Mediated Tumor Regression in Xenografts In Vivo

The effect of NAC and 5-FU on HCT-15 tumor xenograft growth was investigated. We chose to administer NAC at a dosage of 200 mg/kg, as this dosage is close to that used clinically in humans (150 mg/kg).[25] Before treatment at day 7, there was no difference in tumor volume between the experimental groups indicating that initial tumor growth had been equal across the groups ($P = 0.18$, Kruskal-Wallis ANOVA). At day 10, rapid tumor growth was observed in the groups treated with saline and 200 mg/kg NAC, necessitating their removal from the study (Fig. 1, *A* and *B*). In mice treated with 120 mg/kg 5-FU, growth was initially slow; however, between days 13 and 20, tumor size increased rapidly (Fig. 1, *C*). In comparison, combined NAC/5-FU treatment (Fig. 1, *D*) resulted in almost complete inhibition of xenograft growth to day 20 (day 13, $P = 0.03$; days 17 and 20, $P < 0.001$, Mann-Whitney U test). No statistical change in tumor volume occurred in these mice over the 2-week period following treatment ($P = 0.04$, Kruskal-Wallis ANOVA). NAC (200 mg/kg) did not augment the antitumor effect of either 40 or 80 mg/kg 5-FU (data not shown).

NAC and 5-FU Increase Apoptosis and Necrosis, With No Effect on Mitosis, in Tumor Xenografts

Tumor cross sections were stained using the TUNEL technique and apoptotic indices determined (Fig. 2). Apoptosis in NAC-5-FU–treated tumors harvested at day 20 was significantly higher than in

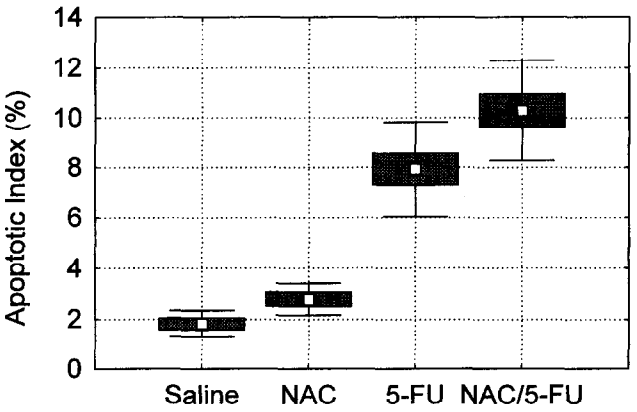

Fig. 2. Box plots showing distribution of apoptotic index within treatment groups, as determined by analysis of more than 1000 cells per tumor following immunohistochemical staining using the TUNEL technique. Mice were culled at day 10 (saline and NAC) or day 20 (5-FU and NAC/5-FU). Box plots represent mean values, standard error of the mean, and standard deviation.

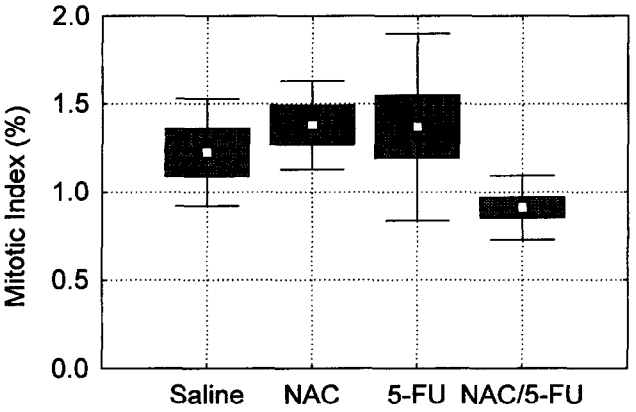

Fig. 4. Box plots showing distribution of mitotic index within treatment groups, as determined by analysis of more than 1000 cells per tumor. Mitoses were determined morphologically by their characteristic appearance. Mice were culled at day 10 (saline and NAC) or day 20 (5-FU and NAC/5-FU). Box plots represent mean values, standard error of the mean, and standard deviation.

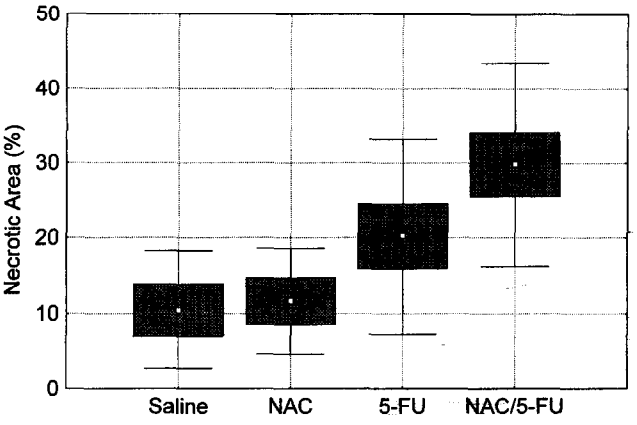

Fig. 3. Box plots showing proportion of necrotic tumor in cross section for each treatment group. A single representative cross section was taken from the midpoint of each tumor. Total area and areas of all necrotic sections were determined using a Zeiss AxioHome microscope. Box plots represent mean values, standard error of the mean, and standard deviation.

tumors treated with 5-FU alone ($P = 0.02$, Tukey HSD test), NAC, or saline ($P < 0.001$). Gross morphologic differences were observed in the hematoxylin and eosin–stained tumor cross sections between treatment groups. Tumors of mice treated with saline or NAC were found to consist of homogeneous cellular sheets interspersed with small scattered necrotic areas. In contrast, tumors of mice treated with NAC/5-FU or 5-FU contained a large localized area of central necrosis located within a cuff of healthy tumor tissue. Fig. 3 illustrates that the proportion of necrotic tissue was increased following NAC/5-FU treatment ($P = 0.01$, Kruskal-Wallis ANOVA) compared to saline control and NAC alone, but not 5-FU alone ($P = 0.31$, Tukey HSD test). This indicates that necrotic cell death is an important mechanism of drug action in this model.

In contrast to apoptosis, no significant difference was observed in mitosis between treatment groups in xenografts harvested at day 20 (Fig. 4).

Angiogenesis and Fas and Fas-L Expression Were Not Significantly Altered by NAC/5-FU Treatment

Previous studies have shown that treatment with NAC can reduce angiogenesis in cell culture models.[8,9] We assessed angiogenesis within tumor xenografts using the anti-CD105 antibody to detect activated endothelial cells. No significant difference was apparent in intratumor microvessel density between xenografts of the individual treatment groups (Fig. 5) ($P = 0.11$, ANOVA).

It has been reported that NAC reduces Fas expression and induction of Fas ligand by cytotoxic drugs.[20,21] However, Houghton et al.[26] have reported that thymineless death in colorectal cancer cell lines is mediated by the Fas system. We therefore determined the expression of Fas and its ligand in tumor xenografts by immunohistochemical analysis. No significant Fas or Fas-L staining of neoplastic epithelial cells occurred within the tumor body (data not shown).

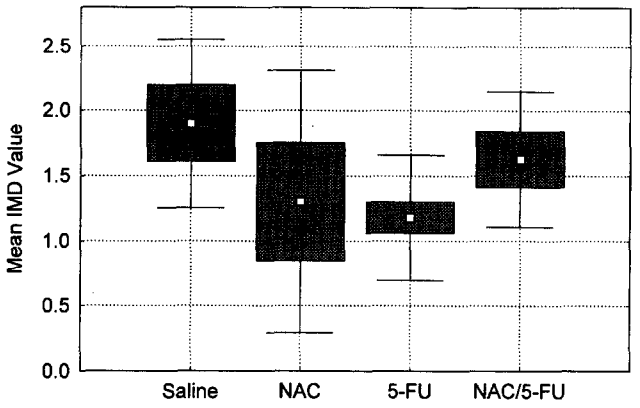

Fig. 5. Box plots showing distribution of mean intratumor microvessel density *(IMD)* for each treatment group. Four Chalkley counts were performed per tumor and averaged to produce the mean value. Box plots represent group means, standard error of the mean, and standard deviation.

DISCUSSION

The objective of this study was to determine whether the thiol-containing antioxidant NAC increased the efficacy of 5-FU therapy against colorectal cancer cells in vitro and in vivo. We measured indices of apoptosis, cellular proliferation, and tumor angiogenesis to resolve how a therapeutic effect might be mediated. Our results show that a single dose of NAC in combination with 5-FU potently increases the efficacy of 5-FU against HCT-15 tumor xenografts (see Fig. 1). Like the majority of human colorectal cancers, this cell line expresses mutant nonfunctional p53 emphasizing the clinical relevance of these results.

The dosages of NAC employed in the xenograft experiments were similar to those used in humans. In standard therapy for acetaminophen poisoning, patients receive a loading dose of NAC, 150 mg/kg. Preliminary experiments showed that our strain of nude mice could tolerate up to 250 mg/kg NAC. We chose to study 200 mg/kg to ensure we would not miss important biological effects. For reasons of animal welfare, we did not undertake dose-response studies of NAC in mice with implanted xenografts.

The principal action of NAC, as determined by this study, was to enhance the apoptosis induced by 5-FU therapy. When administered as a single agent, NAC does not induce sustained apoptosis; however, in combination with 5-FU a significant increase in apoptosis beyond control values occurs both in vitro (at 24 hours) and in vivo (at 2 weeks), without induction of Fas or Fas-L (see Table I and Fig. 2). It is unknown by what mechanism NAC and 5-FU induce this apoptosis, although their oxidative activity may be of importance.[27,28] Indeed a number of studies have shown that NAC antagonizes the induction of apoptosis through pathways mediated by p53,[13] TNF-α,[14,15] NF-κβ,[16,17] and CD95.[18-21]

However, in agreement with our studies, NAC has been shown to induce apoptosis in vascular smooth muscle cells.[8] The capacity of antioxidants such as NAC and PDTC to induce apoptosis in some cell types while preventing its occurrence in others may actually present a therapeutic advantage. This paradox is most clearly seen in colonic epithelium. In normal mouse intestine, antioxidant therapy reduces apoptosis induced by 5-FU within colonic crypts, thereby enhancing stem cell survival and preventing unwanted side effects.[3] The same treatment increases apoptosis among colorectal cancer cell lines both in vitro and in vivo.[2]

NAC has been shown to reduce proliferative activity in human colorectal mucosa following polypectomy[29] and colorectal cancer cell lines in vitro.[11] Increased transcriptional activation of the cyclin-dependent kinase inhibitors $p16^{INK4}$ and $p21^{CIP1/WAF1}$ is one consequence of NAC therapy capable of modulating such an effect.[10] It has also been reported that the antiproliferative effects of NAC are related to inhibition of the mitogen-activated protein kinase pathway.[30] In our studies NAC therapy, whether administered alone or in combination with 5-FU, did not reduce long-term cellular proliferation within xenografts (2 weeks after treatment). In addition, this treatment had no effect on cell cycle distribution of HCT-15 cells in vitro (data not shown), nor did NAC have any significant effect on the mitotic index in tumor xenografts at the time of harvest (see Fig. 4). These results do not exclude the possibility that modulation of proliferative activity had occurred earlier in the experiment prior to tumor harvest.

Tumors of mice treated with NAC/5-FU contained a higher proportion of necrotic tissue than those treated with 5-FU alone, although this result was not significant. We wished to determine whether such necrosis occurred as a result of impaired vascular endothelial cell viability. NAC alone is reported to prevent endothelial cell invasion into tumor tissue[9] and when combined with doxorubicin can reduce metastasis of a melanoma cell line in nude mice.[7] PDTC, by suppressing NF-κβ, can inhibit retinal neovascularization.[31] However, in our studies we did not find that treatment with NAC alone or NAC/5-FU was associated with a reduction in microvessel density within viable sections of tumor tissue (see Fig. 5). However, our study does not completely preclude an effect of NAC/5-FU on tumor angiogenesis as the substantial necrosis observed may obscure localized differences in intratumor microdensity. Alternatively, central necrosis may simply reflect a higher concentration of drug at the tumor core, with apoptosis occurring peripherally, where levels are relatively lower.

CONCLUSION

We have shown that NAC therapy can enhance the inhibitory effect of 5-FU on colorectal cancer tumor xenograft growth. Each agent must be administered at a high dose before the effect is apparent. Treatment results in sustained elevation of apoptosis without reduction in cell proliferation or impairment of angiogenesis. A study of these indices at earlier time points is currently in progress. Future experiments will aim to define an optimal schedule for drug administration, hopefully enabling 5-FU to be administered at a lower dosage.

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Ablation of Liver Metastasis: Is Preoperative Imaging Sufficiently Accurate?

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The recent introduction of cryotherapy and radiofrequency ablation of liver metastasis has expanded the indications for treatment. As technology has advanced, a percutaneous approach has been developed. Percutaneous treatment, however, requires accurate preoperative imaging. From 1993 to 1999, 179 patients underwent operative exploration for treatment of suspected hepatic metastases from colorectal carcinoma. One hundred seventy-seven patients were staged by preoperative CT, two patients were staged by MRI, and complete data were available in 176. Hepatic tumor count by preoperative imaging was compared to intraoperative tumor count obtained by inspection, palpation, ultrasonographic examination using a 3.5/7.5 MHz T probe, and careful gross sectioning of the resected specimen. Post hoc analysis was performed on 35 CT scans by two radiologists who specialize in abdominal CT. These radiologists were blinded to the intraoperative findings. Their interpretations were compared to the intraoperative counts and to each other. Thirty-four (19%) of 179 patients were deemed untreatable at operation because of unsuspected overwhelming liver involvement in 11 (6%) or extrahepatic metastases in 23 (13%). For the group, CT was accurate in 80 patients (45%), showed more lesions than were found in 16 (9%), and showed fewer metastases than were found in 80 (45%). When the preoperative scan predicted a solitary metastasis, it was correct in 45 (65%) of 69 patients and underestimated disease in 24 (35%). In the post hoc analysis, the mean numbers of lesions reported by the two radiologists did not differ from the mean number of tumors found; however, the radiologists' counts agreed on 16 (59%) and disagreed on 11 (41%) of the scans. The accuracy of CT decreased with increasing numbers of lesions. Regardless of the type of preoperative imaging, intraoperative findings altered the course of the operation in 96 (55%) of 176 patients. Preoperative imaging is not sufficiently accurate to permit adequate percutaneous treatment of hepatic metastases from colorectal carcinoma. (J GASTROINTEST SURG 2001;5:98-107.)

KEY WORDS: Liver neoplasm, cryotherapy, radiofrequency, liver imaging, intraoperative ultrasound

Colorectal carcinoma is the second leading cause of all cancer-related deaths in the United States.[1] Liver metastasis will be present in 20% to 25% of patients with colorectal cancer at the time of diagnosis, and up to 50% can expect to develop metachronous lesions during their remaining lifetime.[2] Left untreated, median survival rates for patients with colorectal liver metastases have been reported from 3 to 24 months,[3] and 5-year survival rates are only 1%.[2] Previous studies have suggested improved survival after treatment of multiple hepatic colorectal metastases.[4] Although hepatic resection can be performed with low morbidity and mortality, it remains a formidable operation that is appropriate only in patients without significant medical comorbidity.[5-7] This limitation had led to the search for less invasive means of treating liver metastases. However, a survival advantage is only afforded to those in whom all disease is eradicated.[8,9]

With the continuous advances in imaging technology and the advent of cryosurgical techniques, radiofrequency ablation, and chemoembolization, the desire to treat patients percutaneously has gained momentum. However, successful nonoperative therapy is dependent on the detection of all disease within the liver as well as within the abdominal cavity. This re-

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port was undertaken to compare the estimates of preoperative imaging studies with intraoperative findings in patients undergoing surgical exploration for the resection of hepatic colorectal metastases.

MATERIAL AND METHODS

Patients

All patients who underwent surgery for proposed resection and/or cryotherapy of hepatic metastases from colorectal cancer by two surgeons (E.J.Q. and J.R.W.) at the Medical College of Wisconsin/ Froedtert Memorial Lutheran Hospital from August 1993 to January 1999 were entered into a registry and form the basis of this report. Data were confirmed by direct review of the patients' operative reports, outside radiologists' reports, surgeons' evaluation of outside computerized tomography (CT) scans or magnetic resonance imaging (MRI), in-house radiologists' reports, and direct review of the in-house CT scans by two senior CT radiologists (F.A.Q. and W.D.F.). All patients were staged preoperatively by CT scans with the exception of two patients staged by MRI. Most patients were referred with recent CT scans from other facilities (outside CT). CT was repeated at the Froedtert Memorial Lutheran Hospital if the scans were more than 2 months old or the quality was inadequate to demonstrate the extent of tumors (in-house CT).

Preoperative Imaging

CT performed within our institution between 1993 and 1998 utilized a variety of techniques and machines. From 1993 to 1995, in-house CT was performed with angioportography, 7 mm sections, and pitch 1:1. Overlapping that time and continuing to 1998, CT was performed using either a single-slice helical technique with 5 mm axial sections, pitch 1.5:1, or a multidetector scanner with image thickness 5 mm and pitch 3:1. Since 1998 all CT scans have been obtained using helical technique on a General Electric HiSpeed Advantage CT or a multidetector LightSpeed QX scanner (General Electric Medical Systems, Milwaukee, Wis.). All CT performed in house used intravenous contrast administered by power injector. Forty-two grams of iodinated contrast were injected with timed acquisition of the CT scans. At all times the in-house CT scanners were considered the best and latest available technology.

For the purpose of this study, all in-house CT scans were reviewed in a blinded manner using the digital data on a high-resolution monitor by two senior radiologists whose primary clinical focus is abdominal CT. These post hoc readings noted the number, size and, to the best estimate, Couinaud location of each lesion evident on the preoperative CT scan. These blinded readings were then compared to the written radiology report and to the operative findings.

Operative Exploration/Ultrasound Examination

At the time of operation, the abdomen was explored through a midline incision with a right subcostal extension or a bilateral subcostal incision. A thorough examination of the abdomen was performed to identify extrahepatic cancer. The liver was completely mobilized from its attachments to the diaphragm, and visual inspection and bimanual palpation of the liver were performed. Superficial lesions were identified in this manner and compared intraoperatively to the CT scan. Ultrasound examination of the liver using a Tetrad model 2200 (Tetrad Corporation, Englewood, Colo.) with a variable 3.5/7.5 MHz T probe model 6C was performed by the operating surgeon, occasionally supplemented by the radiologist. The number of lesions and the location of each lesion were noted in the operative report. Lesions lacking the sonographic characteristics of metastases were biopsied by Tru-Cut needle (Tru-Cut, Inc., Sebring, Ohio). In the case of resection, the pathologic specimen was examined and all lesions were noted for their number and location. In the case of overwhelming metastatic disease, that is, more than 25 lesions or lesions too numerous to count, "25" was recorded as the count. In the case of resection in which multiple lesions appeared to coalesce or become continuous, "1" lesion was counted.

Statistics

Accuracy was defined as equal total number of tumors found by preoperative and intraoperative evaluation or, in the post hoc analysis, between the two radiologists. Differences between groups were determined using the Wilcoxon signed-rank test and significance was assumed at $P < 0.05$. Data analyses were conducted using Stata, Release 6, 1999 (Stata Press, College Station, Tex.).

RESULTS

Patients

During the 65-month study period, 179 patients with metastatic colorectal cancer underwent exploratory laparotomy for attempted surgical ablation of their disease. The distribution of the types of treatment in these patients is presented in Table I. Thirty-four patients (19%) were deemed unresectable at the time of operation by the finding of unsuspected peri-

Table I. Treatment in 179 patients with suspected hepatic metastases undergoing operation

Treatment	No. of patients	Percent
None	34	19
Cryoablation	28	16
Resection	58	32
Both resection and cryoablation	59	33
TOTAL	179	100

toneal metastases, portal lymph node metastasis, liver metastases too numerous to count, or lesions in a technically unresectable location, and therefore received no treatment. The remaining 145 patients received therapy for their detectable disease consisting of cryotherapy (n = 28), resection (n = 58), or a combination of resection and cryotherapy (n = 59). Of the 179 patients referred for surgery, 142 (79%) had CT scans from referring institutions, two patients (1%) had MRI, three patients had inadequate recorded preoperative data, and 35 (20%) had CT scans from our institution. These latter 35 scans were reviewed post hoc by our radiologists for this study.

The average number of liver metastases detected by CT scanning was 2.9 ± 2.5 (standard deviation) and was significantly less than the 5.1 ± 5.9 found intraoperatively ($P < 0.001$). As shown in Fig. 1, preoperative scans underestimated the number of liver tumors in nearly half of the patients. In 80 patients (45%) the preoperative scan accurately accounted for the disease in the liver. In 16 patients (9%) the preoperative scan predicted more lesions than could be found at operation, and in 80 patients (45%) the preoperative CT scan underestimated the number of lesions within the liver.

The accuracy of the preoperative scan declined with increasing numbers of lesions within the liver (Fig. 2). The preoperative scan accurately predicted one lesion in 45 (65%) of 69 patients, whereas in 24 patients (35%) more than one lesion was found. When the preoperative scan indicated two lesions within the liver, it was accurate in 13 (43%) of 30 patients, overestimated the number of lesions in four patients (13%), and underestimated the number of lesions in 13 patients (43%). The preoperative scan was correct in only 7 (28%) of 25 patients when it predicted three lesions and in 5 (26%) of 19 patients when it predicted four lesions. In patients with five to eight lesions, the preoperative scan accurately predicted the operative findings in only 6 (21%) of 28 patients. Among patients with more than eight lesions, four (50%) of eight patients had accurate preoperative estimation of the number of lesions.

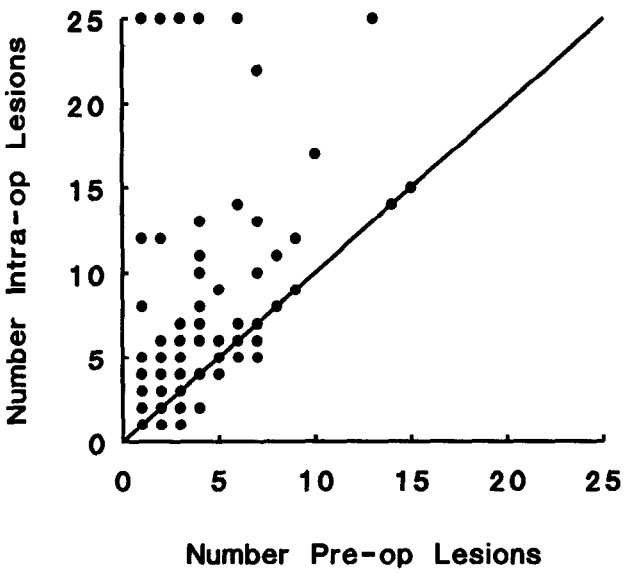

Fig. 1. Comparison of the number of hepatic lesions identified on preoperative CT scans to the number of lesions found intraoperatively. The line of identity has been drawn for clarity. Individual markers may represent multiple cases. Eighty cases lie on the line of identity with 80 above the line and 16 below, the latter two demonstrating underestimation and overestimation of disease, respectively.

Accuracy in Small Lesions

The group of patients most likely to benefit from a percutaneous approach to liver metastases are those in whom the largest tumor is 3 cm or less and with four or fewer tumors. There were 33 patients who met these criteria. Preoperative scans accurately predicted the number of lesions in 17 (52%) of 33 patients, overestimated the disease in three (9%), and underestimated the number of lesions in 13 (39%).

Accuracy Over Time

To assess the potential improvement in the accuracy of scanning with advances in technology over time, patients were divided into two groups—those evaluated prior to January 1, 1998, and those evaluated after January 1, 1998. One hundred seven patients entered the study before January 1998 (Fig. 3). The preoperative CT scan accurately represented the number of lesions within the liver compared to the intraoperative findings in 50 (47%) of 107 patients, predicted more lesions than were found in six (5%),

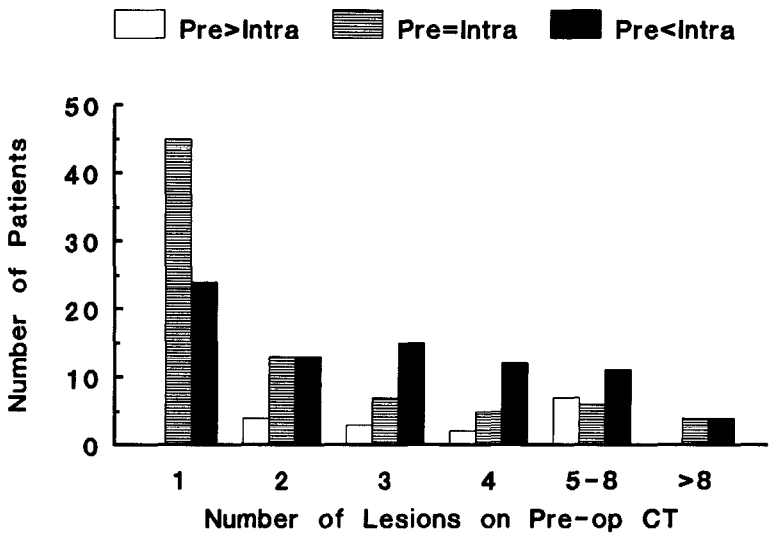

Fig. 2. Accuracy of preoperative CT scans based on the number of lesions. In general, accuracy declines with increasing number of lesions.

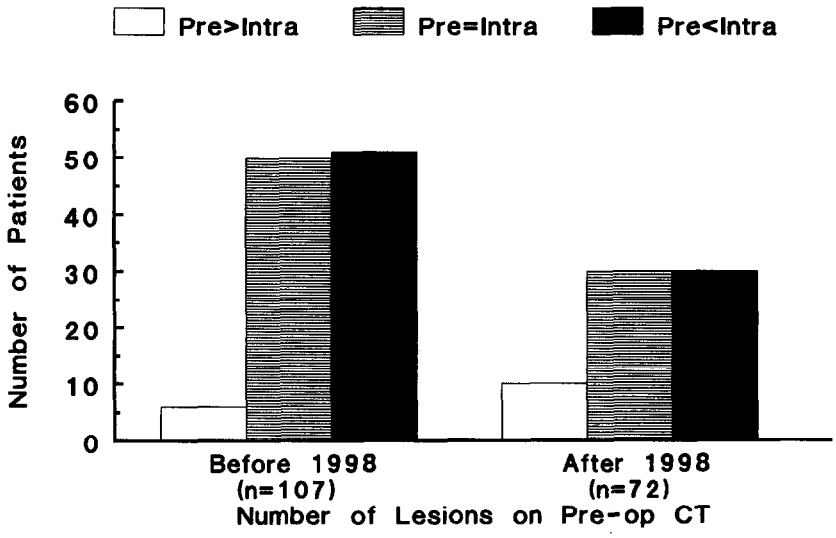

Fig. 3. Comparison of the accuracy of CT prediction of the number of lesions in scans obtained before January 1998 to those obtained after January 1998.

and predicted fewer lesions than were found in 51 (48%). Seventy-two patients were evaluated after January 1998. The CT scan was accurate in 30 (42%) of 72 patients, whereas 10 patients (14%) had fewer lesions than predicted and 32 (44%) had more lesions than predicted by the preoperative CT.

Post Hoc Analysis

To account for inadequacies in film quality, older technology, suboptimal reading, and surgeon bias, the inside CT scans were re-reviewed by two senior radiologists who specialize in abdominal CT. These 35 post hoc CT evaluations by each radiologist were then compared to each other and to the intraoperative findings. Both radiologists were blinded to the intraoperative findings but were aware that there were discrepancies between the initial preoperative report and the intraoperative findings in some patients. The average number of lesions detected by preoperative scan and those found intraoperatively were not significantly different with either radiologist (Table II).

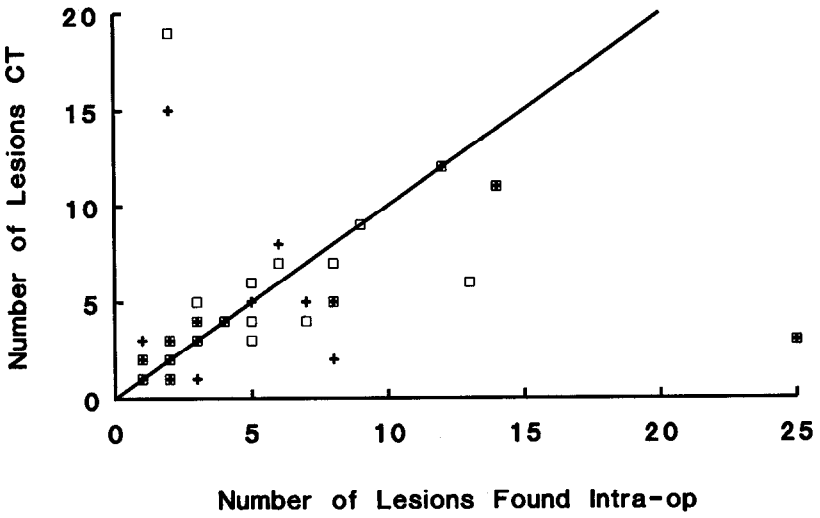

Fig. 4. Comparison of post hoc CT interpretations of radiologist A (+) and radiologist B (⊞) to the intraoperative findings. The line of identity is shown for clarity.

Table II. Average number of lesions on post hoc analysis*

	No. of patients	Average No. of lesions
Radiologist A	35	4.1 ± 3.8
Intraoperative	35	4.7 ± 5.2
Radiologist B	28	3.8 ± 3.6
Intraoperative	28	4.5 ± 5.4

*Comparison of results of post hoc analysis by two radiologists to the intraoperative findings. Groups were compared by Wilcoxon signed-rank test. No significant differences were seen.

However, the radiologists agreed as to the number of lesions when reading the same scans in 16 (59%) and disagreed on 11 (41%). Fig. 4 shows the relationship between the post hoc scan review and the intraoperative findings.

DISCUSSION

This review of our experience was undertaken to assess the accuracy of preoperative scanning techniques in predicting the extent of disease in patients with metastatic colorectal cancer to the liver. The sensitivity of CT scanning has been reported to be 50% to 80% in previous studies.[10-13] Our data confirm the findings in these studies. As shown in Fig. 5, CT can produce either false positive (Fig. 5, *A* and *B*) or false negative (Fig. 5, *C* and *D*) results. In patients with a solitary metastasis in the liver, CT was accurate in 65%; however, this accuracy declined with increasing numbers of lesions. In our series, 23 (13%) of 179 patients had unsuspected peritoneal metastases discovered during exploration. If these patients are excluded, the accuracy of CT is only slightly improved from 65% to 75% in patients with solitary metastasis. Over the past 5 years, CT imaging has changed significantly with improved hardware and software. Helical scanning technique, power contrast injection, and greatly increased image acquisition have resulted in improved resolution of the scans. Reports from 10 years ago are ancient technologically. However, in this series, comparison of scans obtained before January 1998 to those obtained after January 1998 failed to show an increased accuracy in the prediction of numbers of metastases.

MRI has been reported to improve the detection of intrahepatic lesions.[11,14,15] We used MRI in only two patients (1%) and for the analysis included them with the CT group. Hagspiel et al.,[11] in 1995, reported a 99% sensitivity in detecting colorectal metastases in the liver, yet in 4 of 10 patients more lesions were found at operation than were predicted. In other reports, MRI has been shown to be equivalent to CT for a variety of intrahepatic lesions.[14,15] At this time, MRI has not been proved superior to CT for the detection of colorectal metastasis and is not the choice of the authors.

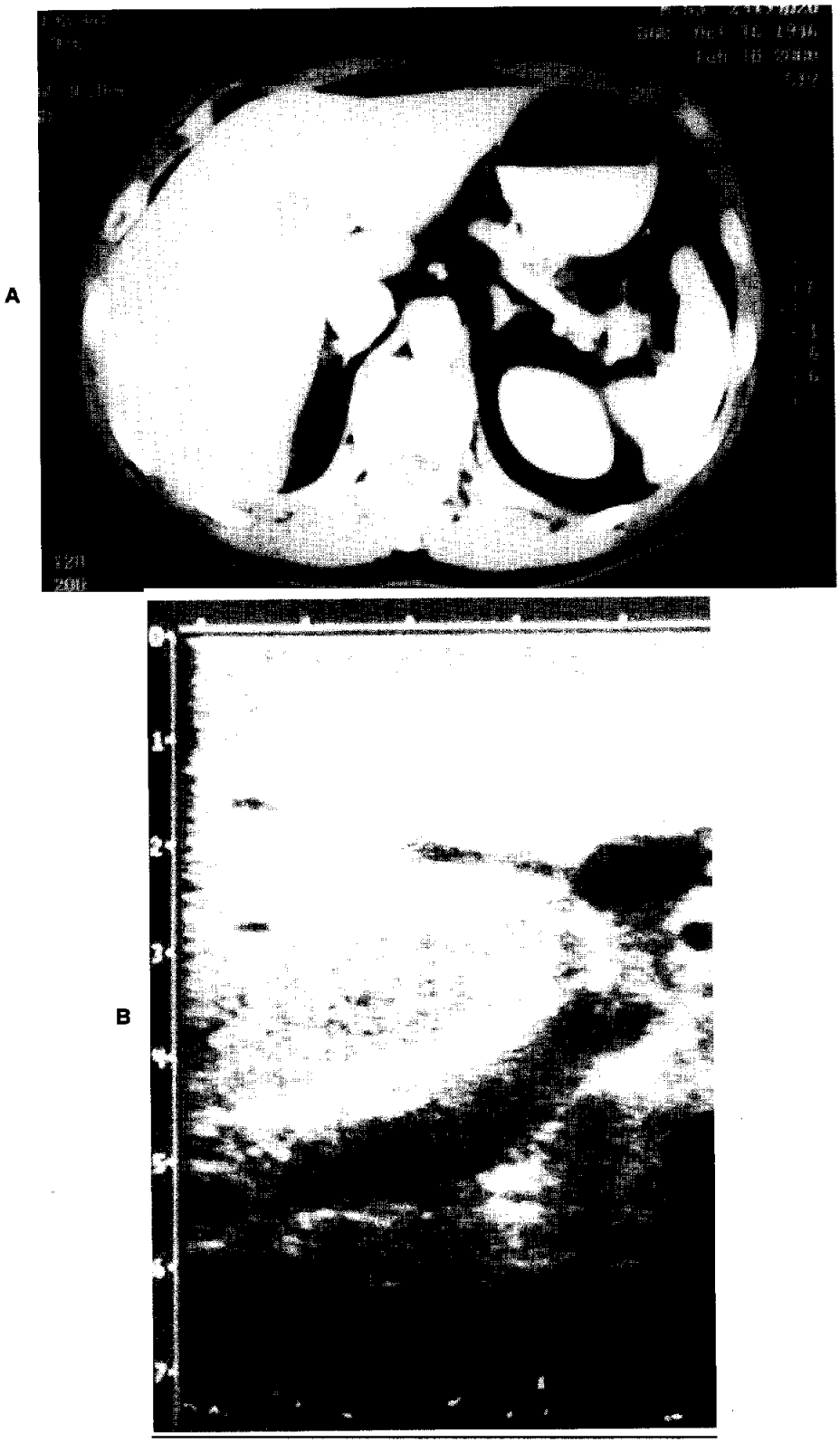

Fig. 5. Examples of false positive and false negative CT scans. **A,** Preoperative CT scan shows a very ominous lesion located near the left main portal vein. **B,** Intraoperative ultrasound of the region reveals no detectable lesion near the left main portal vein. Multiple ultrasound-guided core needle biopsies of the area proved fatty infiltration.

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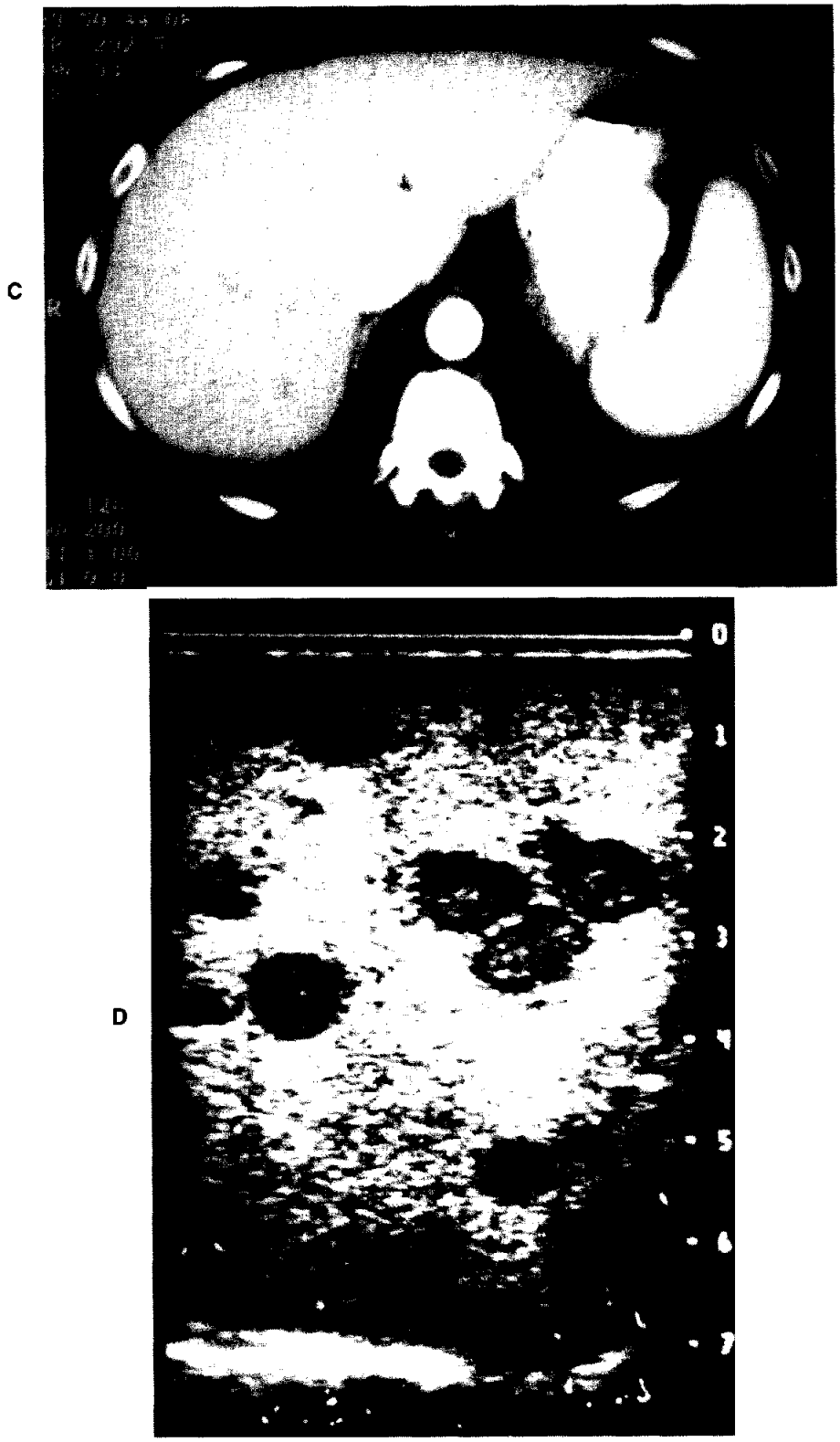

Fig. 5, cont'd. C, Two lesions suspicious for metastases are shown in the central right lobe of the liver. **D,** Four weeks after CT, the patient was found to have metastases too numerous to count within the liver.

Intraoperative ultrasonography (IOUS) is an essential component of hepatic surgery.[4,9] IOUS changes the course of the operation in up to 50% of operative procedures.[12,16] IOUS has been reported to detect additional lesions in 10% to 50% of patients compared with the results of preoperative imaging studies alone.[13,16] Our findings are consistent with those reports. Machi et al.[16] have evaluated the sensitivity of IOUS with postoperative transabdominal ultrasound and CT. In their study, 13 of 188 patients developed hepatic metastases during the 18-month follow-up period. These lesions were assumed to have been present at operation and represented false negative findings for IOUS, thus lowering the sensitivity of IOUS to 82%. IOUS remains significantly superior to either of the preoperative imaging studies. IOUS detects lesions that are not palpable, shows the relationship of lesions to vascular structures within the liver, helps guide resection to ensure an adequate margin, and with ablative therapy ensures that the therapy encompasses the entire lesion. The utility of IOUS has led some to advocate laparoscopic ultrasound in the detection of intrahepatic metastases in planning therapy.[10] IOUS is essential to detect the most metastases and to adequately treat the disease within the liver.

There are a few weaknesses to this report. Our study spans a time period that saw many changes in imaging technology. The majority of the scans used in this report were from outside institutions, some of which used older scanners. However, for scans thought to be inadequate for evaluation CT was repeated; thus in-house and outside scans were considered comparable in this analysis. There is no control of the interpretation of the scans. To attempt to investigate interrater reliability, we re-reviewed our inside CT scans. Although our expert CT radiologists reported a mean number of lesions that was no different from the intraoperative findings, there was wide variation in the interpretation of individual scans between the two radiologists (Fig. 4). Not all lesions were biopsy-proved metastases. Colorectal metastases produce a typical echoic pattern on IOUS that differentiates them from other common solid lesions that may be confused on CT. Hepatic adenomas, fatty infiltration, and hemangiomas may all be distinguished from colorectal metastasis with IOUS. Cystic lesions are also easily identified. Hamartomas or biliary elements can be indistinguishable from metastasis. For lesions in which there was any doubt as to the biology, a biopsy was obtained or the lesion was resected.

Operative cryotherapy and radiofrequency ablation have been used in the treatment of metastatic colorectal cancer.[4,17,18] Both therapies appear to be effective in destroying lesions with radiofrequency ablation perhaps providing greater efficiency in cell death.[19] Long-term follow-up of these modalities has yet to prove equivalent efficacy to resection.[4,9] However, enthusiasm for both cryotherapy and radiofrequency ablation continues to build and, since these modalities are nonresective, a percutaneous approach has been suggested. Our study directly addresses the issue of whether percutaneous therapy will treat all lesions within the liver, since this approach is necessarily based on current preoperative imaging. Based on our findings, under the best of circumstances only 50% of patients will be adequately treated. Approximately 40% of patients will be undertreated, and about 10% of patients will be treated unnecessarily for nonexistent or nonmalignant lesions.

SUMMARY

The estimates of disease by preoperative imaging were compared to intraoperative findings in 179 patients undergoing operation for hepatic metastases from colorectal cancer. The number of hepatic metastases detected by scan equaled the number found by inspection, palpation, and IOUS in 45% of all patients. Intraoperative findings exceeded the preoperative number in 45%, and in 10% the preoperative scans showed lesions that were either not metastatic disease or could not be found. When the preoperative scan showed a solitary lesion, it was correct in 65% but in 35% of patients additional metastases were found. Percutaneous therapies for colorectal metastases will result in inadequate treatment in 25% to 45% of patients because of the inaccuracy of preoperative imaging.

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Discussion

Dr. Y. Fong (New York, N.Y.). In this study only 20% of the scans were obtained at the Medical College of Wisconsin. Were there formal criteria whereby you decided whether or not to perform repeat scanning if a patient arrived with a set of scans from elsewhere? Second, there were 34 patients who actually had no therapy after you performed operative exploration. What were the reasons why patients were not resected? That answer would help us decide what other imaging modality might be useful for staging these patients. Third, in our own experience we found that intraoperative ultrasound plays a very small role in finding more tumors, probably because patients tend to be "overimaged." In how many cases were more tumors found by intraoperative ultrasound, and not just by palpation? Finally, there were 58 patients who were resected. How many were brought to the operating room with the intent to perform a complete resection, and did the yield for CT scans or the yield for intraoperative findings differ in that group from those findings in patients who actually underwent ablation at operation?

Dr. J. Wallace. Whether to obtain a CT scan was based on an evaluation within our institution as to whether we thought a scan was adequate based on the technology at that time. Did the scan adequately show the segments of the liver and the vascular structures? As time has passed, we have become less reliant on preoperative imaging and more reliant on intraoperative imaging. Toward the end of the study, we repeated fewer outside scans.

Of the 34 patients who received no treatment, there were 23 who had extrahepatic disease and 11 who had overwhelming liver disease or disease in critical locations that precluded resection—such as the bifurcation of the portal vein or bifurcation of the bile duct—which was not appreciated on the preoperative scan. Assessing extrahepatic disease is a weakness of CT. If those patients are eliminated, then the sensitivity of the CT scan in the group with a solitary lesion goes from 65% to 75%. That still leaves at least 25% who have intrahepatic disease that is not detected on the preoperative CT scan.

Regarding intent to treat, I have no breakdown as to how many patients we took to the operating room specifically for resection and then changed our mind and performed some other therapy. We do not view our patients this way. We ask whether the disease is removable by any means, whether it is a formal resection, a nonanatomic resection, or some type of ablative procedure. I can tell you that in 90 patients in the database, some finding on the intraoperative evaluation was different from what we expected based on the CT scan.

Dr. A. Bilchek (Los Angeles, Calif.). We too have found that despite excellent preoperative imaging and positron emission tomographic (PET) scanning, approximately 20% of the time we will find more lesions or extrahepatic disease. This is important from the standpoint of radiofrequency ablation, which is being done percutaneously. My question is: Can you avoid laparotomy completely and perform laparoscopy and laparoscopic hepatic ultrasound imaging?

Dr. Wallace. I have very limited experience with laparoscopic ultrasound. The image is good. My reservation is not being able to completely mobilize the liver, which I think is of great assistance when you are imaging the various segments of the liver. But I think you have to do an analysis of how often the laparoscopic ultrasound will actually change the approach; at least based on our findings, that would occur in about half of the 90 patients or approximately one fourth of all patients. Then the cost of laparoscopy is spread out over the other 75% who really did not benefit from it. I do not know if that works out to be cost-effective. But I think it is a valid approach.

Dr. S. Helton (Chicago, Ill.). Our experience at the University of Washington was almost identical to the Memorial Hospital experience. In 5 years, it was rare that we ever identified a lesion in the operating room by ultrasound that was not seen on double spiral CT scan preoperatively. On both of your CT scans showing colorectal metastasis, you demonstrated a delayed portal venous phase, which is notoriously inaccurate for identifying colorectal lesions. If you confine your review to just those scans performed by your own radiologists, how often did you detect lesions when the scanning was correctly done at your own institution?

Dr. Wallace. In the 35 patients who had scans done at our institution, the radiologists were correct as to the mean number of lesions across the entire group, but when they looked at the individual scans they were correct only 60% of the time.

Dr. S. Strasberg (St. Louis, Mo.). There are three reports in the literature indicating that the sensitivity of the preoperative diagnosis of colorectal metastases can be increased from approximately 70% to 95% by PET scanning. Did you use PET scanning in any of these patients, and how would it have influenced your results?

Dr. Wallace. We have no experience with PET scanning. I think the two technologies that are in the wings for preoperative imaging are PET scanning and magnetic resonance imaging (MRI). But the data for MRI have yet to show that it is superior to modern CT technology.

Enhancing Release of Peptide YY After Near-Total Proctocolectomy: Jejunal Pouch vs. Ileal Pouch–Distal Rectal Anastomosis

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Reconstructing the enteric tract after near-total proctocolectomy by interposing a jejunal pouch between the distal ileum and the distal rectum slows small intestinal transit and decreases the number of stools per day compared to a conventional ileal pouch–distal rectal reconstruction. Our hypothesis was that the jejunal pouch operation brings about these results by protecting the ability of the ileal mucosa to secrete peptide YY, thus augmenting the hormonal ileal brake on small intestinal transit and decreasing the stool frequency. In five jejunal pouch dogs and five ileal pouch dogs, more than 6 months after the operation, serum peptide YY concentrations were determined before and at 30-minute intervals for 180 minutes after a standard meal. Fasting serum concentrations of peptide YY, measured by radioimmunoassay, were greater in jejunal pouch dogs (mean ± SEM, 1340 ± 143 pg/ml) than in ileal pouch dogs (804 ± 52 pg/ml; $P < 0.01$). Postprandial peptide YY concentrations in jejunal pouch dogs were also greater at 30 minutes (jejunal pouch = 1524 ± 131 pg/ml, ileal pouch = 913 ± 67 pg/ml; $P = 0.01$) and 60 minutes after the meal (jejunal pouch = 1723 ± 250 pg/ml, ileal pouch = 1001 ± 70 pg/ml; $P = 0.05$) and peaked sooner (jejunal pouch = 81 ± 17 minutes, ileal pouch = 147 ± 12 minutes; $P = 0.01$). We concluded that the jejunal pouch operation results in greater ileal fasting and postprandial secretion of peptide YY than the ileal pouch operation. The greater release may account, in part, for the slower small bowel transit and decreased number of stools after the jejunal pouch operation. (J GASTROINTEST SURG 2001;5:108-112.)

KEY WORDS: Proctocolectomy, ileal pouch–anal canal anastomosis, jejunal pouch–anal canal anastomosis, ileal brake, peptide YY

The ileal pouch–anal canal anastomosis has become the reconstruction of choice for many patients undergoing near-total proctocolectomy for chronic ulcerative colitis.[1] The operation achieves complete excision of the diseased portion of the large intestine, yet transanal defecation and reasonable fecal continence are maintained, and an incontinent ileostomy is avoided. There are drawbacks to the operation, however. After 8 years, patients still have a mean of six stools per 24 hours.[2] Moreover, approximately half of the patients have persistent nocturnal fecal spotting, and almost half have had at least one bout of "pouchitis," inflammation in the pouch. In addition, using the ileum to make a pouch may decrease its ability to absorb electrolytes, vitamin B_{12}, and bile salts, and to secrete hormones such as peptide YY.

We have found in past tests that reconstructing the enteric tract after proctocolectomy by interposing a jejunal pouch between the distal ileum and the distal rectum, rather than an ileal pouch, slows small intestinal transit and decreases the number of postprandial stools compared to a conventional ileal pouch–distal rectal anastomosis.[3] Dogs with jejunal pouches had mouth-to-anus transit times of approximately 4 hours and had only four bowel movements in the 12 hours after a standard meal, whereas ileal pouch dogs had a more rapid transit of only 2 hours and six bowel movements in the first 12 hours after eating.

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Small intestinal transit is regulated, in part, by hormonal controls. When chyme reaches the ileum, especially chyme containing fat, the ileum secretes peptide YY, a hormone that, in turn, acts on the more proximal small bowel to slow transit through it.[4-6] Slower transit delays defecation after meals. We in patients[7] and others in dogs[8] have shown in past tests that the ileum still secretes peptide YY and that the ileal brake is still present after ileal pouch–anal canal anastomosis, but these functions may be impaired in some patients. Some patients have rapid small intestinal transit and frequent bowel movements after the ileal pouch operation.

Our hypothesis in the present tests was that using a jejunal pouch as a rectal substitute after near-total proctocolectomy might better preserve the hormonal ileal brake on small intestinal transit than using an ileal pouch. With a jejunal pouch interposed between the terminal ileum and the anal canal, less stasis and less bacterial overgrowth should occur in the ileal lumen and less impairment of ileal mucosal function might then result. The ileum protected by the jejunal pouch might be able to release greater amounts of peptide YY than when the ileum itself is used to form a pouch. Slower small bowel transit and fewer postprandial bowel movements might then result.

We tested this hypothesis in 10 dogs after near-total proctocolectomy. Five dogs had the jejunal pouch–anal canal anastomosis and five control dogs had the ileal pouch–anal canal anastomosis.

METHODS

Animal Preparation

Ten healthy female mongrel dogs weighing 16 to 26 kg were fasted overnight and given 500 mg of cephazolin intravenously. They underwent general anesthesia with methohexital sodium (12.5 mg/kg) and 1.5% isoflurane. Atropine sulfate (0.04 mg/kg intramuscularly) was given to prevent shortening of the bowel during the operation. Using a sterile operating technique, a midline celiotomy was made, and a colectomy and proximal proctectomy were accomplished. The proctectomy extended distally to a point 2 cm proximal to the pelvic floor.

In 5 of the 10 dogs, a J-shaped pouch was constructed from the distal jejunum. The small bowel was divided at a site one-half the distance between the ligament of Treitz and the ileocolic valve. The 30 cm segment of jejunum immediately proximal to the transection was used to form a J-shaped jejunal pouch. The segment was folded into two 15 cm limbs, and the pouch was constructed using two layers of 3-0 polyglycolic acid sutures. The distal cut end of the proximal jejunum was anastomosed end to end to the proximal cut end of the ileum, whereas the distal cut end of the ileum was anastomosed end to end to the afferent limb of the jejunal pouch. The newly formed jejunal pouch was then anastomosed to the distal rectum (Fig. 1).

In the other five dogs (controls), 30 cm of terminal ileum was used to form a J-shaped ileal pouch, and this pouch was anastomosed to the distal rectum, as is done in a conventional ileal pouch–distal rectal anastomosis (see Fig. 1). Both types of pouch were anastomosed to the distal rectum instead of the anal canal at the dentate line because of the greater ease of distal rectal anastomosis and to preserve completely the anal sphincter and its innervation. This study was designed to explore ileal mucosal function and not the fate of the retained distal rectal and proximal anal canal mucosa. Electrodes were implanted on the small intestine of both types of dogs,[3] but they were not used in the present tests.

The animals were then allowed to recover from the operation. After 6 months, testing was begun. Between 1 and 5 months after the operation, clinical observations and observations of intestinal electrical activity, motility, transit, bacteriology, and anatomy were made on the dogs. These results have been reported previously.[3]

Conduct of Tests

Gastric Emptying. After an overnight fast, the dogs were given an 800 kcal meal (Alpo Petfoods, Lehigh Valley, Pa.—52% protein, 36% fat, 12% carbohydrate, 650 kcal) to which was added 30 ml of a high-calorie solution (Hi-Cal suspension Vedco, Inc., St. Joseph, Mo.—3% protein, 50% fat, 150 kcal) to activate the ileal brake. We measured gastric emptying of solids, rather than liquids, because dogs generally eat solid meals. Moreover, we wanted to measure the mouth-to-anus transit of the residua of the solid meal (see below). Liquids usually leave little residua.

Ninety minutes after they consumed the meal and when, likely, their ileal brake was active, the animals were placed in a Pavlov sling. The animals were then fed a second meal of 60 ml of egg white to which 1.0 mCi of ^{99m}Tc-sulfur colloid (Syncor International Corp., Phoenix, Ariz.) was added. The rate of gastric emptying of the isotope was measured using a gamma camera.[3] The gastric counts per minute were recorded for 1 minute immediately after ingestion of the meal (time 0), 5 minutes after ingestion, 10 minutes after ingestion, and then every 20 minutes until 150 minutes after ingestion. The counts per minute were corrected for decay and expressed as a percentage of isotope remaining in the stomach. Scintigraphic data were stored and later analyzed to determine the $T_{1/4}$

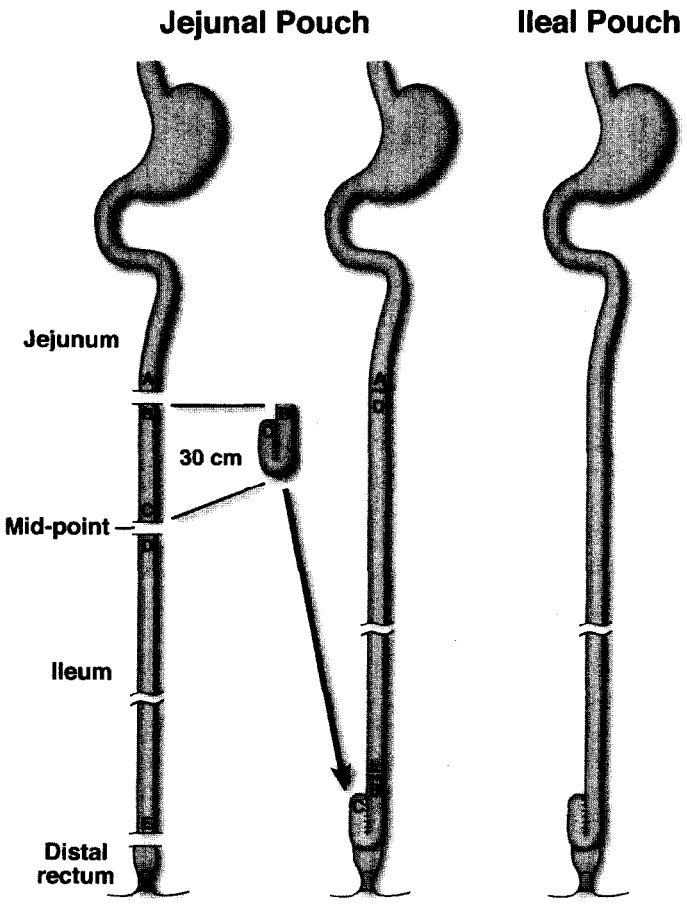

Fig. 1. Canine experimental preparations after near-total proctocolectomy. *Left*, Jejunal pouch–distal rectal reconstruction. *Right*, Ileal pouch–distal rectal reconstruction.

(time for 25% of the isotope to empty from the stomach), $T_{1/2}$ (time for 50% of the isotope to empty), and $T_{3/4}$ (time for 75% of the isotope to empty).[9] The experiment was performed twice in each dog.

Mouth-to-Anus Transit and Defecation. On other days, mouth-to-anus transit time and patterns of defecation were measured as follows. After an overnight fast, the dogs were given a 100 ml saline (154 mmol/L NaCl) enema per anum to cleanse the enteric pouch. The animals were then fed the high-calorie 800 kcal meal. Ninety minutes after the dogs had consumed the meal and when their hormonal ileal brake would likely be active, they were given a second meal of 312 g of canned dog food (CNM, Purina, St. Louis, Mo.) to which was added 2 tablespoons of charcoal (activated carbon 6-14 mesh, Fisher Scientific, Springfield, N.J.). The dogs were placed in the kennel and carefully observed over the ensuing 12 hours to note the time between the eating of the second meal and the passage of the first stool containing charcoal. The number of stools passed during the 12 hours after the second meal was also noted. The experiment was performed twice in each dog.

Serum Peptide YY. On still other days, after an overnight fast, the dogs were placed in a Pavlov stand and an indwelling catheter was inserted into a foreleg vein for drawing blood samples. Blood samples (1.5 ml) were obtained before and at 30-minute intervals for 180 minutes after the high-calorie 800 kcal meal was taken by mouth. Samples were immediately centrifuged, and the serum was frozen at −20° C. Serum concentrations of peptide YY were measured later by radioimmunoassay.[10] The experiment was performed twice in each dog.

Data Analysis

For gastric emptying, the $T_{1/4}$, $T_{1/2}$, and $T_{3/4}$ emptying times were calculated by fitting a power exponential curve to the data from each run using nonlinear regression.[9] For small bowel transit, postprandial defecation, and serum peptide YY, the means of the

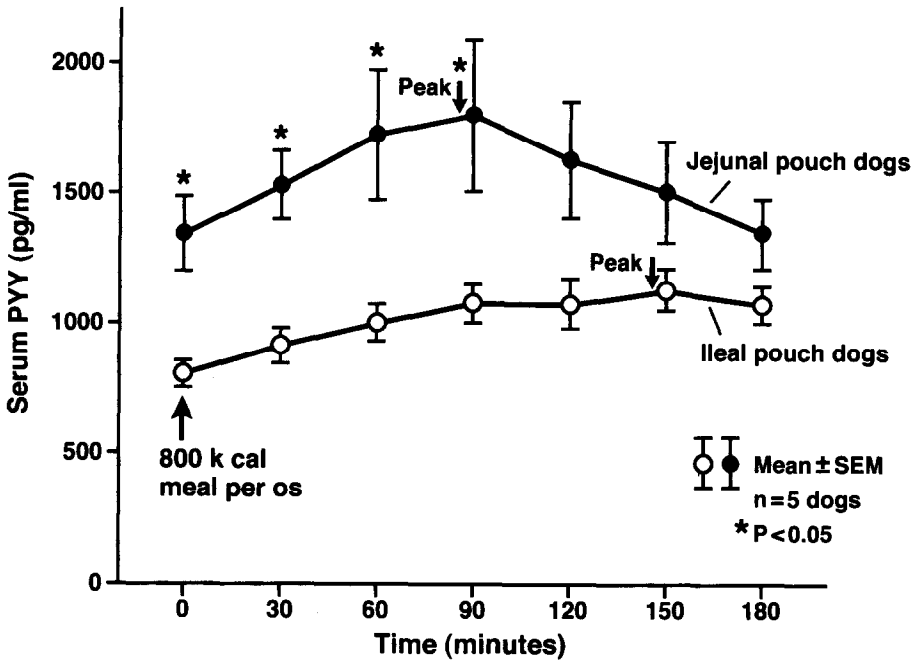

Fig. 2. Canine basal and postprandial serum peptide YY (PYY) concentrations after near-total proctocolectomy and jejunal pouch–distal rectal anastomosis or ileal pouch–distal rectal anastomosis.

values from the two runs on each dog were determined, and overall means and standard errors of the mean (SEM) for each group calculated. Differences between groups were determined using a two-sample Student's *t* test, with Satterthwaite's adjustment for unequal variance. All statistical tests were for two-sided hypotheses. P values ≤0.05 were considered statistically significant.

RESULTS

The dogs were in good health 6 months after their operations at the time of their testing. They ate well and had gained weight, so that they met or exceeded their preoperative weights.[3] Both groups of dogs passed soft, semisolid bowel movements with ease. In between bowel movements, the dogs were continent of feces.

Gastrointestinal Transit and Defecation

Gastric emptying of the ^{99m}Tc-labeled egg white meal was similar in both groups. For example, the means ± SEM $T_{1/4}$, $T_{1/2}$, and $T_{3/4}$ emptying times for the jejunal pouch dogs were 12 ± 3.0 minutes, 80 ± 15 minutes, and 306 ± 33 minutes, whereas those for the ileal pouch dogs were 32 ± 12 minutes, 131 ± 20 minutes, and 253 ± 40 minutes ($P > 0.05$). Proportionate emptying data, computed from estimated parameters of the fitted-power exponential model, also showed no differences at any time period between the two groups. In contrast, the mouth-to-anus transit took longer in the jejunal pouch dogs (247 ± 22 minutes) than in the ileal pouch dogs (160 ± 18 minutes, $P = 0.01$). Moreover, the jejunal pouch dogs had fewer stools in the first 12 hours after eating a second meal (2.4 ± 0.2 stools/12 hours) than the ileal pouch dogs (4.2 ± 0.6 stools/12 hours, $P < 0.05$).

Serum Peptide YY

Concentrations of peptide YY in the serum during fasting were greater in the jejunal pouch dogs (mean ± SEM, 1340 ± 143 pg/ml) than in the ileal pouch dogs (804 ± 52 pg/ml, $P < 0.01$) (Fig. 2). Postprandial peptide YY concentrations in jejunal pouch dogs were also greater at 30 minutes (jejunal pouch = 1524 ± 131 pg/ml, ileal pouch = 913 ± 67 pg/ml; $P = 0.01$) and at 60 minutes after the meal (jejunal pouch = 1723 ± 250 pg/ml, ileal pouch = 1001 ± 70 pg/ml; $P = 0.05$) and peaked sooner (jejunal pouch = 81 ± 17 minutes, ileal pouch = 147 ± 12 minutes; $P = 0.01$).

DISCUSSION

Results of the present tests confirm that the jejunal pouch dogs had similar rates of gastric empyting,

but slower intestinal transit and fewer postprandial bowel movements, compared to ileal pouch dogs, as we have reported in past tests on these dogs.[3] The current tests also show that when a second meal is eaten at a time when the ileal brake is presumably active after the first meal, fewer bowel movements occur in the 12 hours after eating in both groups of dogs compared to when only one meal is eaten, as found in the past tests.[3]

The major new findings in the present tests are that the jejunal pouch dogs had higher concentrations of peptide YY in their serum during fasting and in the first 2 hours after eating than the ileal pouch dogs. Moreover, serum peptide YY values peaked sooner after eating in the jejunal pouch dogs than in the ileal pouch dogs. We suggest that interposing a jejunal pouch between the terminal ileum and the distal rectum enhanced the ability of the ileal mucosa to release peptide YY postprandially compared to when the ileum itself is used to form the pouch. The increased release of peptide YY in the jejunal pouch dogs compared to the ileal pouch dogs likely explains, in part, the slower postprandial small intestinal transit and decreased number of postprandial bowel movements in the jejunal pouch dogs compared to in the ileal pouch dogs.

Other factors may have contributed to the slower transit and decreased bowel movements in the jejunal pouch dogs. The jejunal pouch dogs had two transections of the mid small bowel with a jejunoileal anastomosis there and a second anastomosis of the distal ileum to the transposed jejunal pouch. These transections and anastomoses may have also slowed small bowel transit and contributed to the decrease in postprandial bowel movements in the jejunal pouch dogs. We have found in previous canine tests that jejunal transection followed by a conventional hand-sutured, two-layered anastomosis, as was done here, does slow transit in the jejunum distal to the cut.[11] The slower transit in the jejunal pouch dogs also may have enhanced the release of peptide YY in them, but this is unknown.

We concluded that the jejunal pouch operation enhances basal and postprandial ileal secretion of peptide YY compared to the ileal pouch operation. This may, in part, account for the slower bowel transit and decreased number of stools after the jejunal pouch operation. The use of a jejunal pouch rather than an ileal pouch to replace the rectum may have merit in patients undergoing near-total proctocolectomy for enteric diseases such as chronic ulcerative colitis or familial adenomatous polyposis.

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