Editorial

Surgical Therapy for Pancreatic Pseudocysts

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The article by Dr. Howard and his colleagues, in this issue of the JOURNAL, reviews their experience with resection of pancreatic pseudocysts, as opposed to the more commonly employed surgical technique of internal drainage. The significant operative blood loss in their patients and the 59% postoperative morbidity that ensued is evidence of the severity of the disease that must have been present. The various indications for resection as proposed by Howard et al. are listed below, and although we agree with some, others are more controversial.

Multiple pseudocysts
Cysts in the uncinate process or head of the pancreas in which internal drainage is not technically feasible
Cysts associated with common bile duct or duodenal obstruction
Cysts that cannot be differentiated from malignant neoplasms
Cysts that recur after prior internal or external drainage

Multiple pseudocysts, especially those that occur in the body or tail of the pancreas, certainly may be managed effectively by distal pancreatic resection. This is an attractive approach when that part of the gland is the site of a great deal of inflammation, and the patient has symptoms that may reasonably be attributed to those findings. On the other hand, we have managed some patients with two or three pancreatic cysts by means of internal drainage, either with separate anastomoses of each cyst to the Roux-en-Y jejunal limb or by converting two cysts into one larger one before a single anastomosis to both is performed. The procedure is quite effective and the morbidity is low.

Some cysts in the head of the pancreas may require a pancreaticoduodenectomy. However, it is important to recognize that several newer procedures in which a small amount of pancreatic head is resected with the cysts, while preserving gastroduodenal continuity, appear to be less morbid alternatives to the Whipple procedure. These operations developed by Beger et al.[1] and Frey and Amikura[2] are mentioned by Howard and his colleagues, and they should be considered as options in patients with cysts in the head of the gland. They appear to be safe, are associated with low operative blood loss and low short- and long-term morbidity, and are technically easier to perform than the Whipple resection in these circumstances.

In our experience, cysts that cause common bile duct or duodenal obstruction almost never require resection. If the cyst is amenable to internal drainage, the obstruction usually is relieved. If it is not (e.g., because of fibrous or inflammatory changes that continue to compress the bile duct or duodenum), then biliary bypass or a gastrojejunostomy are safer and simpler alternatives to resection. The biliary obstruction can be relieved with either a choledochoduodenostomy, if the duct is large, or a Roux-en-Y hepaticojejunostomy, if it is not. In those patients with common duct obstruction from a cystic mass in the head of the pancreas who undergo some form of duodenum-preserving resection, the bile duct compression is also relieved in approximately 75%.

The management of patients who have a recurrence of a pseudocyst after prior external or internal drainage should be individualized. One must first consider whether the presumed "pseudocyst" is instead a cystic neoplasm. If this is a concern, resection of the cystic lesion is mandatory. (Certain characteristics of neoplastic cysts that should be a clue to their true nature are listed in Table I.) However, recurrence of pseudocysts after external drainage usually is the result of communication of the cyst cavity with the pancreatic ductal system. The cyst refills with pancreatic juice after the external drain is removed. This is usually an indication for internal drainage rather than resection. The recurrence or persistence of a cyst

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Table I. Features of pancreatic "cysts" that may suggest a pancreatic cystic neoplasm

Information from	Features
Clinical presentation	No history of pancreatitis
CT scan	Internal septa
	Solid intracystic components
	Calcification within the cyst or its wall
	Hypervascularity
Percutaneous drainage	Abnormal cytologic findings
	Normal amylase levels
	Recurrence of cyst after drainage
Cyst wall biopsy	Presence of an epithelial lining

that already has been internally drained may be due to the premature closure of a small anastomosis between the cyst cavity and the stomach or intestinal limb. If possible, we try to create an opening that is at least 3 to 4 cm in diameter. Discussion with the original surgeon may be helpful in making this determination, and the creation of a larger anastomotic opening can eliminate the cyst.

It is important to point out that the overall management of pseudocysts has changed greatly over the past 10 years. Howard and his colleagues have indicated that endoscopic and/or percutaneous drainage of some pseudocysts is being done in certain cases. We agree with them that the eventual role of these techniques is still undefined. Percutaneous drainage procedures are associated with a higher risk of cyst infection, and a substantial number of cysts managed in this fashion will persist as a pancreatic fistula once the drain is removed. Endoscopic internal drainage appears to be an attractive therapeutic approach in selected patients, but more experience and longer follow-up are needed.

We now know that small, asymptomatic cysts may require no treatment at all and can be safely observed indefinitely. We only treat those cysts that are larger than 5 to 6 cm in diameter because of the greater chance that a complication will occur once they have reached that size. We also treat other cysts, regardless of size, that have produced symptoms (e.g., pain, biliary obstruction, or gastric outlet obstruction). Rapidly enlarging cysts may also require treatment. Because pseudocysts are often found in patients with painful chronic pancreatitis, it may be impossible to determine whether the cyst or the underlying pancreatic disease is responsible for the pain. If surgery is indicated in those patients, both the pseudocyst and the chronic pancreatitis should be treated. This may require either internal drainage or resection.

REFERENCES

1. Beger HG, Büchler M, Bittner RR, Oettinger W, Roscher R. Duodenum-preserving resection of the head of the pancreas in severe chronic pancreatitis: Early and late results. Ann Surg 1989;209:273-278.
2. Frey CF, Amikura K. Local resection of the head of the pancreas combined with longitudinal pancreaticojejunostomy in the management of patients with chronic pancreatitis. Ann Surg 1994;220:492-507.

The Role of Pancreatic Resection in the Treatment of Pancreatic Pseudocysts

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Complicated pancreatic pseudocysts, including multiple pseudocysts, those that have failed prior internal or external drainage, those with associated biliary or pancreatic duct strictures, and those where the diagnosis of cystic neoplasm cannot be excluded, pose unique problems in terms of treatment by standard internal or external drainage techniques. In the series reported herein, pancreatic resection (pylorus-sparing pancreaticoduodenectomy or distal pancreatectomy) was used to treat patients with these complicated pseudocysts resulting in a 59% morbidity rate, 3% mortality rate, and 6% recurrence rate. Results from a collective series of 152 patients from the literature support these findings. Although pancreatic resection has a limited role in the management of patients with uncomplicated pancreatic pseudocysts, it is the treatment of choice in patients with complicated pancreatic pseudocysts. (J GASTROINTEST SURG 1997;1:205-212.)

Pancreatic pseudocysts are a heterogeneous group of fluid collections that occur in association with either acute or chronic pancreatitis.[1] Successful management of pseudocysts is predicated on choosing the correct therapeutic option to both drain the abnormal fluid collection and correct any underlying pancreatic structural abnormalities (i.e., dilated pancreatic duct, pancreatic duct stricture, or pancreaticolithiasis) that might contribute to a recurrence.[2-4] Endoscopic retrograde cholangiopancreatography has proved invaluable in distinguishing between acute and chronic pseudocysts, identifying patients with concomitant pancreatic or bile duct abnormalities, and planning a definitive surgical procedure.[3,5,6] Surgical management of pancreatic pseudocysts consists of three distinct types of procedures: (1) external drainage, (2) internal drainage, or (3) pancreatic resection. External drainage is used for infected pseudocysts or those with an immature fibrous wall that will not hold sutures. Internal drainage is preferred for chronic pseudocysts that are either adherent to the posterior wall of the stomach (cystogastrostomy) or capable of being drained into a limb of jejunum (cystojejunostomy). Despite its use in up to one third of patients in several large surgical series,[7,8] the exact role of pancreatic resection in the treatment of pancreatic pseudocysts has yet to be defined.[1,2] The purpose of this study was to examine our recent experience with the use of pancreatic resection to treat pancreatic pseudocysts, focusing specifically on the clinical indications for its use, operative factors, morbidity and mortality rates, recurrence rates, and patient outcome during long-term follow-up.

MATERIAL AND METHODS

Over a 10-year period (1984 to 1994), 873 patients were hospitalized at Indiana University Medical Center with acute pancreatitis and 290 patients were evaluated with the diagnosis of pancreatic pseudocyst. All patients with cystic degeneration of pancreatic carcinoma, cystic neoplasms, infected or sterile peripancreatic fluid collections, and postnecrotic pancreatic sequestrum were excluded from study. Patients who underwent pancreatic resection or pancreatic duct drainage specifically to treat complications of chronic pancreatitis (i.e., pain, recurrent pancreatitis, or biliary tract obstruction) and who in addition had small associated pseudocysts drained at the time of the definitive operative procedure were also excluded. This left 122 patients with true pancreatic pseudocysts. Of these, 36 (29%) were successfully managed nonoper-

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atively, 32 (26%) were treated by pancreatic resection, 18 (15%) by internal surgical drainage, 15 (12%) by endoscopic internal drainage, 15 (12%) by percutaneous external drainage, and six (5%) by a combination of endoscopic and percutaneous drainage (Table I). Based on our criteria, 38 (32%) qualified as complicated pseudocysts all of which were managed operatively: 32 (84%) were treated by pancreatic resection and six (16%) by internal surgical drainage. The 32 patients treated by pancreatic resection form the basis of this report.

Indications for resection included multiple (more than one) pseudocysts (n = 13), failure of prior internal or external drainage (n = 12), inability to exclude the diagnosis of cystic neoplasm (n = 5), and an associated high-grade pancreatic duct stricture (n = 2). Hospital records from these patients were carefully analyzed for preoperative evidence of pancreatic endocrine or exocrine insufficiency, pseudocyst size, location, and type. Operative reports were reviewed for pseudocyst size, type, and location as well as operative time and blood loss. When the radiographic report and the operative report conflicted in terms of the number, location, or size of the pseudocysts, the operative report was taken as the final arbiter. All seven pancreaticoduodenectomies were pyloric-sparing modifications as described by Traverso and Longmire.[9] Twenty-two (88%) of the 25 distal pancreatectomies were standard 50% to 60% resections of the gland accomplished by dividing the pancreas over the superior mesenteric and portal vein confluence. Three (12%) of the 25 patients required an 80% to 95% distal pancreatectomy because of pseudocyst involvement of the pancreatic genu. The pancreatic duct was directly suture ligated when possible. No patient in this series underwent a spleen-preserving distal pancreatectomy because the splenic vessels in patients with pseudocysts are generally encased in inflammatory scar tissue, compressed, and often thrombosed, making splenic conservation virtually impossible.[2] Somatostatin was not used perioperatively as surgical prophylaxis for pancreatic fistulas in any patient in this series.

Table I. Treatment options used in 122 patients with pancreatic pseudocysts at Indiana University Medical Center

Treatment	No. of patients	% of total
Nonoperative	36	29
Pancreatic resection	32	26
Surgical drainage	18	15
Endoscopic drainage	15	12
Percutaneous drainage	15	12
Endoscopic + percutaneous drainage	6	5

The mean age of the 21 men and 11 women in this series was 42 ± 12 years. Eight patients (25%) had acute pancreatitis and 24 patients (75%) had chronic pancreatitis. The etiology of pancreatitis in these patients was consistent with that in the majority of patients admitted with pancreatic diseases to our institution (Table II). Four patients (13%) had preoperative diabetes and nine (28%) had preoperative exocrine insufficiency requiring enzyme supplementation.

For operative and postoperative analysis, the 32 patients who underwent resection were divided by type

Table II. Patient demographics and etiology of pancreatitis in 32 patients with pancreatic pseudocysts treated primarily by pancreatic resection

	Total (n = 32)	PSP (n = 7)	DP (n = 25)
Age (yr)	45 ± 12	44 ± 12	46 ± 11
Sex (% male)	66	57	68
Preoperative diabetes mellitus	4 (13)	3 (43)	1 (4)
Preoperative exocrine insufficiency	9 (28)	3 (43)	6 (24)
Etiology of pancreatitis			
Alcohol abuse	12 (38)	4 (57)	8 (32)
Indeterminate	10 (31)	1 (14)	9 (36)
Biliary	3 (9)	1 (14)	2 (8)
Trauma	2 (6)	1 (14)	1 (14)
Post-ERCP	2 (6)	0	2 (8)
Pancreas divisum	2 (6)	0	2 (8)
Postoperative	1 (2)	0	1 (4)

PSP = pylorus-sparing pancreaticoduodenectomy; DP = distal pancreatectomy; ERCP = endoscopic retrograde cholangiopancreatography. Numbers in parentheses are percentages.

of operative procedure as follows: seven (22%) who underwent pylorus-sparing pancreaticoduodenectomy and 25 (78%) who underwent distal pancreatic resection (Table III). In the seven patients who underwent pancreaticoduodenectomy, the average cyst size was 3.4 ± 1.7 cm in diameter and all of these cysts were located in the pancreatic head. In the 25 patients treated by distal pancreatectomy and splenectomy, the average cyst size was 4.9 ± 2.4 cm in diameter. There were 22 patients (69%) with primary pseudocysts and 10 (31%) with recurrent pseudocysts. Among the patients with primary pseudocysts, 11 had multiple pseudocysts, six had pancreatic duct strictures or cutoff, and in five patients the diagnosis of cystic neoplasm could not be excluded. All patients with recurrent pseudocysts had failed a prior drainage procedure (six failed prior internal drainage and four failed prior external drainage). Four pseudocysts were located in the body, 14 in the tail, and seven in both the body and tail of the pancreas.

Postoperative courses were evaluated for length of stay, complications, and reoperations. Follow-up was assessed through the last outpatient clinic appointment with special reference to the incidence of recurrent pancreatitis, recurrent pseudocysts, or the postoperative development of pancreatic endocrine or exocrine insufficiency. Pancreatic endocrine insufficiency was defined as the need for exogenous insulin to control the blood glucose level. Pancreatic exocrine insufficiency was defined as steatorrhea and weight loss despite adequate oral intake, which responded to exogenous pancreatic enzyme replacement.

A collective series of patients with pancreatic pseudocysts who were treated primarily by pancreatic resection was developed from a collection of previously published series. A review of the literature over the past 10 years (1986 to 1996) identified 326 articles on the operative treatment of pseudocysts of which 190 were in the English language. Of these 190 articles, eight were chosen based on the following criteria: they involved large series of patients (n >35), identifiable patients were treated by pancreatic resection, and morbidity and mortality data were presented for those patients who were treated by pancreatic resection. Included with these eight papers are the current series and a large series by Frey[7] published in 1978.

Statistical analyses of these data are not indicated because the groups presented were not comparable either to one another or to historical controls.

RESULTS

The average operative time for the Whipple pancreaticoduodenectomy group was 500 ± 186 minutes, which was a great deal longer than the 266 ± 137 minutes required for distal pancreatectomy (Table IV). The operative blood loss averaged 1675 ± 519 cc in the Whipple pancreaticoduodenectomy group and 2010 ± 1108 cc in the distal pancreatectomy group. Patients undergoing pancreaticoduodenectomy remained in the hospital longer, on average (21.0 ± 10.6 days), than those undergoing distal pancreatectomy (12.5 ± 7.1 days). There were no perioperative deaths in the Whipple pancreaticoduodenectomy group and one postoperative death (4%) in the distal pancreatectomy group. The postoperative death in the distal pancreatectomy group occurred in a 53-year-old patient who developed renal failure after an acute upper gastrointestinal hemorrhage from both an anastomotic ulcer (prior Billroth II gastrectomy) and a Mallory-Weiss tear. This patient died of a malignant ventricular arrhythmia induced by hypovolemic shock 18 days following distal

Table III. Measurable characteristics of pancreatic pseudocysts in 32 patients treated by pancreatic resection

	Total (n = 32)	PSP (n = 7)	DP (n = 25)
Average pseudocyst size (cm)	4.6 ± 2.2	3.4 ± 1.7	4.9 ± 2.4
Indication for resection			
Multiple pseudocysts	13 (41)	4 (57)	9 (36)
Failed prior drainage	12 (37)	2 (29)	10 (40)
Question of cystic neoplasm	5 (16)	1 (14)	4 (16)
Pancreatic duct obstruction	2 (6)	0	2 (8)
Location in pancreas			
Head	7 (22)	7 (100)	0
Body	4 (12)		4 (16)
Tail	14 (44)		14 (56)
Body/tail	7 (22)		7 (28)

Abbreviations as in Table II.
Numbers in parentheses are percentages.

Table IV. Operative factors, length of stay, and morbidity and mortality rates for patients undergoing pancreatic resection for complicated pancreatic pseudocysts

	Total	PSP (n = 7)	DP (n = 25)
Operative time (minutes)		500 ± 186	266 ± 137
Operative blood loss (cc)		1675 ± 519	2010 ± 1108
Postoperative length of stay (days)		21.0 ± 10.6	12.5 ± 7.1
Total complications	19 (59)	5 (71)	14 (56)
Intra-abdominal abscess	1 (3)	0	1 (4)
Pancreatic fistula	3 (9)	0	3 (12)
ARDS	1 (3)	0	1 (4)
Pneumonia	1 (3)	0	1 (4)
Wound infection	2 (6)	0	2 (8)
Upper gastrointestinal hemorrhage	1 (3)	0	1 (4)
Delayed gastric emptying	4 (13)	3 (43)	1 (4)
Reoperation	2 (6)	0	2 (8)
Stroke	1 (3)	1 (14)	0
Seizure	1 (3)	1 (14)	0
SVC thrombosis	1 (3)	0	1 (4)
Death	1 (3)	0	1 (4)

ARDS = adult respiratory distress syndrome; SVC = superior vena cava; other abbreviations as in Table II.
Numbers in parentheses are percentages.

Table V. Long-term follow-up of patients undergoing pancreatic resection for complicated pancreatic pseudocysts

	Total (n = 28)	PSP (n = 7)	DP (n = 21)
Average follow-up (mo)	20.3 ± 12	17.5 ± 20.6	20.6 ± 11.9
Pseudocyst recurrence	2 (6)	0	2 (10)
Recurrent pancreatitis	8 (25)	1 (14)	7 (33)
Postoperative diabetes mellitus	4 (13)	0	4 (19)
Postoperative exocrine insufficiency	7 (22)	2 (29)	5 (23)

Abbreviations as in Table II.
Numbers in parentheses are percentages.

pancreatectomy. Postmortem examination confirmed the presence of an anastomotic ulcer and Mallory-Weiss tear but showed no evidence of intra-abdominal sepsis or abdominal pseudoaneurysms.

Complications in the group undergoing pylorus-sparing pancreaticoduodenectomy included delayed gastric emptying in three patients (43%), pseudomembranous colitis in one patient (14%), and a cerebral vascular accident and subsequent seizure in one patient (14%). There were no pancreatic fistulas, intra-abdominal fluid collections, or reoperations in this group. The overall morbidity rate for distal pancreatectomy was 40% and included intra-abdominal abscesses in four patients, pancreatic fistulas in three, and adult respiratory distress syndrome in one. Reoperation was required in two patients in the distal pancreatectomy group—one in the early postoperative period, 3 days after resection, for omental necrosis. The other patient underwent reoperation late in the postoperative period, 63 days following distal pancreatic resection, for takedown of a persistent pancreaticocutaneous fistula.

Twenty-eight (88%) of 32 patients were available for follow-up that averaged 20.3 ± 12 months (Table V). No patient in the Whipple pancreaticoduodenectomy group developed a recurrent pseudocyst, although one patient had a subsequent episode of pancreatitis requiring hospitalization. Three patients developed exocrine insufficiency postoperatively and required exogenous pancreatic enzyme replacement.

No patient developed diabetes during the postoperative follow-up period. Of the 21 patients in the distal pancreatectomy group who were able to be evaluated, the average follow-up was 21 ± 12 months. During this period seven patients developed recurrent pancreatitis, two of whom developed recurrent pseudocysts. One patient was an alcoholic with chronic pancreatitis and a type III pseudocyst in the tail of the gland associated with a pancreatic duct cutoff at the genu. He underwent distal pancreatectomy and splenectomy. An episode of recidivism occurred 22 months following surgery, which led to recurrent pancreatitis and a pseudocyst in the head of the gland. This was later drained by surgical cystojejunostomy. He is currently 8 months out from his second surgery and he remains well. The second patient had idiopathic pancreatitis and multiple pseudocysts in the body and tail of the pancreas; this was treated by distal pancreatectomy and splenectomy after he had failed a prior attempt at internal surgical drainage. Thirteen months later he had another episode of pancreatitis and developed a pseudocyst in the uncinate process of the pancreas. This recurrent pseudocyst was asymptomatic and resolved completely with conservative medical management.

DISCUSSION

Differentiation between acute and chronic pseudocysts, the presence or absence of necrosis and infection, and associated pancreatic and bile duct abnormalities all influence the specific type of therapy selected for patients with pseudocysts.[2-4] Acute asymptomatic pancreatic pseudocysts in glands without underlying pancreatic duct abnormalities generally resolve spontaneously.[10,11] Patients with acute symptomatic pancreatic pseudocysts or those with asymptomatic pseudocysts who fail nonoperative management should be treated either by CT-directed percutaneous drainage,[12,13] or by internal or external surgical drainage.[1,2] New minimally invasive techniques for internal pseudocyst drainage, such as percutaneous transgastric cystogastrostomy,[14] endoscopic cystoduodenostomy,[15] or transpapillary endoscopic pseudocyst drainage,[16] have been advocated for acute symptomatic pseudocysts, but their use remains confined to small groups of patients in select centers.

Chronic pancreatic pseudocysts, on the other hand, invariably have a communication to the pancreatic duct or are associated with structural abnormalities of the pancreatic duct or distal common bile duct that are best treated surgically.[3,5,12] Internal surgical drainage (cystogastrostomy, cystojejunostomy, or cystoduodenostomy) with concomitant drainage of the diseased or obstructed pancreatic duct or bile duct is the preferred therapeutic option because of the low operative morbidity and mortality rates.[3,4] Endoscopic drainage in this setting, although feasible, carries a high procedure-related morbidity rate and its role in the treatment of chronic pancreatic pseudocysts remains to be established.[2,15] Duodenum-preserving resection of the head of the pancreas and local resection of the head of the pancreas combined with longitudinal pancreaticojejunostomy are two new procedures developed to resect, decompress, and drain an enlarged fibrotic pancreatic head in patients with chronic pancreatitis.[17,18] Although 50% of the patients in the series of Frey and Amikura[18] had associated pancreatic pseudocysts, the use of these two procedures specifically to treat patients with pancreatic pseudocysts has not been defined. Pancreatic resections, either proximal pancreaticoduodenectomy or distal pancreatectomy, have been used sporadically but persistently in most large surgical series dealing with the treatment of pancreatic pseudocysts. Presumably the high morbidity and mortality associated with pancreatic resection has led to uncertainty over the clinical indications for its use in patients with pancreatic pseudocysts.

Frey[7] articulated the indications for pancreatic resection in the treatment of pancreatic pseudocysts in 1978. His indications included multiple pseudocysts, cysts located in the uncinate process or head of the pancreas in which internal drainage is not technically feasible, and pseudocysts associated with common bile duct or duodenal obstruction. We agree with these indications and, based on our experience, we would add pseudocysts that cannot be reliably differentiated from a cystic neoplasm and pseudocysts that recur after prior internal or external drainage. All of these situations pose unique problems in terms of treatment by either internal or external surgical drainage techniques.

Although CT-directed percutaneous drainage is an excellent treatment for symptomatic, single acute pseudocysts, preliminary data on its use in patients with multiple pseudocysts have been disappointing.[19] Similarly, small multiple pseudocysts located in the body and tail of the gland, or small pseudocysts situated deep within the head of the pancreas, are notoriously difficult to treat by means of internal drainage procedures such as cystogastrostomy or cystojejunostomy[7] (Fig. 1). Furthermore, inability to adequately identify and drain all the components of multiple pseudocysts can lead to early recurrence.[19] Resection obviates many of these difficulties by removing the entire portion of the pancreas involved with these multiple pseudocysts.

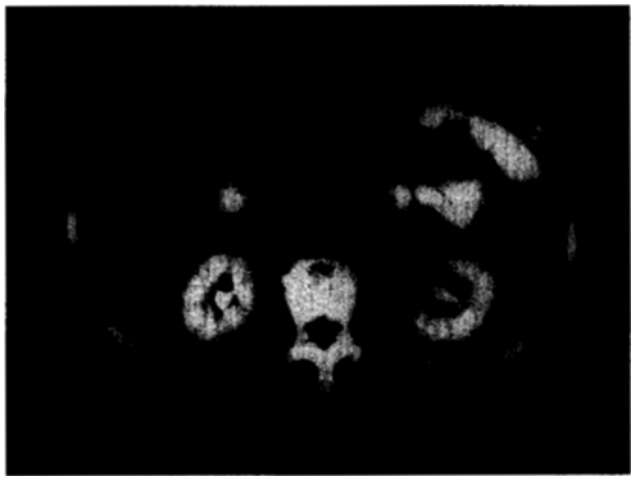

Fig. 1. Complicated chronic pseudocyst located in the head of the pancreas (arrow) in a patient with chronic calcific pancreatitis and high-grade strictures of both the pancreatic and distal common bile ducts. This patient was treated with a pylorus-sparing pancreaticoduodenectomy.

Obstruction of the distal common bile duct in patients with chronic pseudocysts implies scarring and fibrosis of the pancreatic head from underlying chronic pancreatitis that rarely resolves after simple pseudocyst drainage and frequently requires biliary-enteric bypass.[20] In fact, operative drainage procedures for pancreatic pseudocysts in the setting of chronic pancreatitis frequently require simultaneous drainage of a dilated or obstructed pancreatic (pancreaticojejunostomy) or distal common bile duct (choledochoduodenostomy or choledochojejunostomy).[3,5,21] Although not a specific indication for resection in our series, five (71%) of seven patients who underwent pylorus-sparing pancreaticoduodenectomy had preoperative evidence of significant functional biliary obstruction based on an elevated serum alkaline phosphatase level.[22] Pancreatic resection in this setting not only removes the pseudocyst but is a well-established treatment for these specific complications of chronic pancreatitis.[23-25]

Pancreatic pseudocysts that cannot be differentiated from cystic neoplasms on the basis of clinical, radiographic, and/or cyst fluid analysis should be treated as malignant neoplasms. This implies that aggressive pancreatic resection should be performed rather than a mistaken internal drainage procedure inasmuch as the consequences of this error in judgment can be profound.[26]

For all of these clinical situations associated with pancreatic pseudocysts, pancreatic resection offers distinct advantages over the more conventional internal surgical drainage, but at what cost? Considering the data from this current series, pancreatic resection for the preceding indications can be done with a 59% morbidity rate, a 3% mortality rate, and a 6% recurrence rate at a mean of 20 months' follow-up. The mortality and recurrence rates for this series compare quite favorably with those reported for internal surgical drainage of pseudocysts.[7,8,27-29] A marked disparity is found in the 59% morbidity rate reported for the current series as compared to the 15% to 30% morbidity reported for series of internal surgical drainage.[7,8,27-29] There are several reasons for our high morbidity rate. First, we included both major and minor occurrences in our definition of morbidity including delayed gastric emptying, which accounted for 21% (4 of 19) of our complications. This complication is particularly common after pylorus-sparing pancreaticoduodenectomy for all indications[30] and is uniquely absent from most series of internal surgical drainage.[7,8,27-29] Furthermore, 37% (12 of 32) of the patients in this series had failed prior attempts at internal or external pseudocyst drainage. It has been shown that this particular subgroup of patients has an operative morbidity rate for reintervention that is twice as high as that in patients who have had no prior attempts at drainage.[31]

Perhaps it is more appropriate to compare other series of patients who have undergone pancreatic resection. In this context our overall 40% morbidity rate for distal pancreatectomy is similar to that for the series reported by Richardson and Scott-Conner[23] but slightly higher than the 24% and 27% reported by others.[32,33] The discrepancies between these series can again be attributed to the authors' definition of morbidity. If one uses a consistent criterion of major morbidity, all studies have a comparable 24% to 27% morbidity rate for distal pancreatic resection regardless of the indication. Similarly, the morbidity rate of 43% for pancreaticoduodenectomy in this study is similar to the morbidity rates of 36% and 32% in other reported series of patients with chronic pancreatitis.[24,25] One patient in our series who underwent distal pancreatectomy died of postoperative hemorrhage from an anastomotic ulcer and simultaneous Mallory-Weiss tear for an overall mortality rate of 4%. This is similar to the 2% mortality rate reported by Dalton et al.[33] in a series of 44 patients undergoing distal pancreatectomy for carcinoma.

The average operative time for patients undergoing distal pancreatectomy was 4.43 hours in this series, which is longer than the 3.74 hours reported by Richardson and Scott-Conner.[23] Our mean length of hospital stay for these patients (12.5 days), although similar to that for the Mayo group,[33] is significantly shorter that the 18 days reported by Richardson and Scott-Conner.[23] Our operative time of 8.33 hours for

Table VI. Collective series of patients undergoing pancreatic resection for pancreatic pseudocysts

Author	No.	% of series	Proximal	Distal	Morbidity	Mortality	Recurrence
Frey[7]	31	26	5	26	5 (16)	2 (6)	5 (16)
Shatney and Lillehei[28]	4	3	0	4	1 (25)	0	0
Aranha et al.[27]	11	14	0	11	4 (36)	2 (18)	0
Sanfey et al.[29]	10	10	0	10	2 (20)	0	0
Nealon et al.[3]	7	17	0	7	0	0	—
Vitas and Sarr[11]	8	17	1	7	2 (20)	0	0
Kiviluoto et al.[8]	41	40	6	35	19 (46)	5 (12)	4 (10)
Andersson et al.[37]	2	5	0	2	0	0	—
O'Connor et al.[6]	6	15	0	6	2 (30)	0	—
Howard et al. (present series)	32	26	7	25	18 (56)	1 (3)	2 (6)
TOTAL	152	23	19	133	53 (35)	10 (7)	11 (8)

Numbers in parentheses are percentages.

a pylorus-sparing pancreaticoduodenectomy and the average blood loss of 1675 cc is comparable to the operative time of 9.6 hours and blood loss of 1507 cc reported by Traverso and Kozarek[25] for a similar group of challenging patients with chronic inflammation of the pancreas. This is considerably greater than the 5.75 hours and the 900 cc blood loss reported in patients undergoing pancreaticoduodenectomy for carcinoma.[34] These findings underscore the dramatic differences in technical difficulty between pancreatic resection in patients with periampullary malignancies and near-normal glands and the intense peripancreatic inflammation, fibrosis, and obfuscation of anatomic planes found in patients with chronic pancreatitis and pancreatic pseudocysts. These data stress that pancreatic resection in this setting can be a challenging and difficult exercise.

A collective series of patients who underwent pancreatic resection as primary treatment for pancreatic pseudocysts is presented in Table VI. There are a total of 133 distal pancreatectomies and 19 Whipple pancreaticoduodenectomies (152 resections) in this series. These patients represent 23% of the total patient population with pseudocysts treated by surgery in these reports. Overall, there was a 35% morbidity rate, a 7% mortality rate, and an 8% recurrence rate for pancreatic resection in the treatment of pancreatic pseudocysts. The vast majority of these resections were performed for complicated pseudocysts. These results are particularly satisfying considering the negative selection bias that occurs for most patients treated by resection who have either multiple pseudocysts, recurrent pseudocysts, or pseudocysts associated with structural abnormalities of the pancreas making internal surgical drainage difficult or unappealing.

Caution should be used in attempting to extrapolate from the results of resection for the specific indications cited herein to the treatment of *all* patients with chronic pancreatitis and a pancreatic pseudocyst. As we have stated, the vast majority of patients with uncomplicated pseudocysts can be managed by means of a variety of drainage techniques with low morbidity and mortality and excellent long-term results. Resection in this population is unnecessary. Similarly, this study focuses specifically on pseudocyst outcome (resolution or recurrence) and does not address the more complicated issue of pain relief in chronic pancreatitis. An adequate evaluation of pain relief in this series would require both a pre- and postoperative pain scale or quality-of-life assessment as well as an average follow-up of at least 3 years and preferably 5 years.[35] This study as designed is unable to answer this question. Despite these limitations, when performed for the specific indications previously set forth, we believe that pancreatic resection offers distinct advantages over internal drainage and can be performed with acceptable morbidity, mortality, and recurrence rates.

CONCLUSION

Over the past 10 years, the morbidity and mortality rates for pancreatic resection have declined dramatically when this procedure is performed in a high-volume setting.[36] Patients with complicated pseudocysts including those with multiple pseudocysts, those who have failed prior internal or external drainage, those with associated biliary or pancreatic duct strictures, and those in whom the diagnosis of cystic neoplasm cannot be excluded should be considered for pancreatic resection as definitive surgical treatment. Results from this series indicate that pancreatic resec-

tion, both pylorus-sparing pancreaticoduodenectomy and distal pancreatectomy, can be used to treat patients with these complicated pseudocysts with an overall 59% morbidity rate, 3% mortality rate, and 6% recurrence rate. Results from a collective series of 152 patients from the literature support these findings. Based on these data we consider pancreatic resection to be the treatment of choice for patients with complicated pancreatic pseudocysts.

REFERENCES

1. Yeo CJ, Sarr MG. Cystic and pseudocystic diseases of the pancreas. Curr Probl Surg 1994;31:167-243.
2. Grace PA, Williamson RCN. Modern management of pancreatic pseudocysts. Br J Surg 1993;80:573-581.
3. Nealon WH, Townsend CM, Thompson JC. Preoperative endoscopic retrograde cholangiopancreatography (ERCP) in patients with pancreatic pseudocyst associated with resolving acute and chronic pancreatitis. Ann Surg 1989;209:532-540.
4. Munn JS, Aranha GV, Greenlee HB, Prinz RA. Simultaneous treatment of chronic pancreatitis and pancreatic pseudocyst. Arch Surg 1987;122:662-667.
5. Ahearne PM, Baillie JM, Cotton PB, Baker ME, Meyers WC, Pappas TN. An endoscopic retrograde cholangiopancreatography (ERCP)-based algorithm for the management of pancreatic pseudocysts. Am J Surg 1992;163:111-116.
6. O'Connor M, Kolars J, Ansel H, Silvis S, Vennes J. Preoperative endoscopic retrograde cholangiopancreatography in the surgical management of pancreatic pseudocysts. Am J Surg 1986;151:18-24.
7. Frey CF. Pancreatic pseudocyst: Operative strategy. Ann Surg 1978;188:652-662.
8. Kiviluoto T, Kivisaari L, Kivilaakso E, Lempinen M. Pseudocysts in chronic pancreatitis: Surgical results in 102 consecutive patients. Arch Surg 1989;124:240-243.
9. Traverso LW, Longmire WP. Preservation of the pylorus during pancreaticoduodenectomy. Surg Gynecol Obstet 1978; 146:959-962.
10. Yeo CJ, Bastidas JA, Lynch-Nyhan A, Fishman EK, Zinner MJ, Cameron JL. The natural history of pancreatic pseudocysts documented by computed tomography. Surg Gynecol Obstet 1990;170:411-417.
11. Vitas GJ, Sarr MG. Selected management of pancreatic pseudocysts: Operative versus expectant management. Surgery 1992;111:123-130.
12. D'Egidio A, Schein M. Percutaneous drainage of pancreatic pseudocysts: A prospective study. World J Surg 1991;16:141-146.
13. Adams DB, Anderson MC. Percutaneous catheter drainage compared with internal drainage in the management of pancreatic pseudocyst. Ann Surg 1992;215:571-578.
14. Hanke S, Henriksen FW. Percutaneous pancreatic cystogastrostomy by ultrasound scanning and gastroscopy. Br J Surg 1985;72:916-917.
15. Sahel J, Bastid C, Pellat B, Schurgers P, Sarles H. Endoscopic cystoduodenostomy of cysts of chronic calcifying pancreatitis. A report of 20 cases. Pancreas 1987;2:447-453.
16. Kozarek RA, Ball TJ, Patterson DF, Freeny PC, Ryan JA, Traverso WL. Endoscopic transpapillary therapy for disrupted pancreatic duct and peripancreatic fluid collections. Gastroenterology 1991;100:1362-1370.
17. Beger HG, Büchler M, Bittner RR, Oettinger W, Roscher R. Duodenum-preserving resection of the head of the pancreas in severe chronic pancreatitis: Early and late results. Ann Surg 1989;209:273-278.
18. Frey CF, Amikura K. Local resection of the head of the pancreas combined with longitudinal pancreaticojejunostomy in the management of patients with chronic pancreatitis. Ann Surg 1994;220:492-507.
19. Fedorak IJ, Rao R, Prinz RA. The clinical challenge of multiple pancreatic pseudocysts. Am J Surg 1993;168:22-28.
20. Warshaw AL, Rattner DW. Facts and fallacies of common bile duct obstruction by pancreatic pseudocysts. Ann Surg 1980; 192:33-37.
21. Adams DB, Anderson MC. Changing concepts in the surgical management of pancreatic pseudocysts. Am Surg 1992; 58:173-180.
22. Stabile BE, Calabria R, Wilson SE, Passaro E Jr. Stricture of the common bile duct from chronic pancreatitis. Surg Gynecol Obstet 1987;165:121-126.
23. Richardson DQ, Scott-Conner CEH. Distal pancreatectomy with and without splenectomy. Am Surg 1989;55:21-25.
24. Rossi RL, Rothschild J, Braasch JW, Munson JL, ReMine SG. Pancreaticoduodenectomy in the management of chronic pancreatitis. Arch Surg 1987;122:416-420.
25. Traverso LW, Kozarek RA. The Whipple procedure for severe complications of chronic pancreatitis. Arch Surg 1993; 128:1047-1053.
26. Warshaw A, Compton C, Lewandrowski K, Cardenosa G, Mueller PR. Cystic tumors of the pancreas: New clinical, radiologic, and pathologic observations in 67 patients. Ann Surg 1990;212:432-445.
27. Aranha GV, Prinz RA, Freeark RJ, Kruss DM, Greenlee HB. Evaluation of therapeutic options for pancreatic pseudocysts. Arch Surg 1982.;117:717-721.
28. Shatney CH, Lillehei RC. Surgical treatment of pancreatic pseudocysts: Analysis of 119 cases. Ann Surg 1979;189:386-394.
29. Sanfey H, Aguilar M, Jones RS. Pseudocysts of the pancreas, a review of 97 cases. Am Surg 1994;60:661-668.
30. Yeo CJ. Management of complications following pancreaticoduodenectomy. Surg Clin North Am 1995;75:913-924.
31. Rao R, Fedorak I, Prinz RA. Effect of failed computed tomography-guided and endoscopic drainage on pancreatic pseudocyst management. Surgery 1993;114:843-849.
32. Aldridge MD, Williamson RCN. Distal pancreatectomy with and without splenectomy. Br J Surg 1991;78:976-979.
33. Dalton RR, Sarr MG, van Heerden JA, Colby TV. Carcinoma of the body and tail of the pancreas: Is curative resection justified? Surgery 1992;11:489-494.
34. Trede M, Schwall F, Saeger HD. Survival after pancreaticoduodenectomy: 118 consecutive resections without an operative mortality. Ann Surg 1990;211:447-458.
35. Frey CF, Pitt HA, Yeo CJ, Prinz RA. A plea for uniform reporting of patient outcome in chronic pancreatitis. Arch Surg 1996;131:233-234.
36. Fernandez-del Castillo C, Rattner DW, Warshaw AL. Standards for pancreatic resection in the 1990s. Arch Surg 1995;130:295-300.
37. Andersson R, Janzon M, Sundberg I, Bengmark S. Management of pancreatic pseudocysts. Br J Surg 1989;766:550-552.

The Gastric Bypass Operation Reduces the Progression and Mortality of Non–Insulin-Dependent Diabetes Mellitus

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Of 232 morbidly obese patients with non–insulin-dependent diabetes mellitus referred to East Carolina University between March 5, 1979, and January 1, 1994, 154 had a Roux-en-Y gastric bypass operation and 78 did not undergo surgery because of personal preference or their insurance company's refusal to pay for the procedure. The surgical and the nonoperative (control) groups were comparable in terms of age, weight, body mass index, sex, and percentage with hypertension. The two groups were compared retrospectively to determine differences in survival and the need for medical management of their diabetes. Mean length of follow-up was 9 years in the surgical group and 6.2 years in the control group. The mean glucose levels in the surgical group fell from 187 mg/dl preoperatively and remained less than 140 mg/dl for up to 10 years of follow-up. The percentage of control subjects being treated with oral hypoglycemics or insulin increased from 56.4% at initial contact to 87.5% at last contact ($P = 0.0003$), whereas the percentage of surgical patients requiring medical management fell from 31.8% preoperatively to 8.6% at last contact ($P = 0.0001$). The mortality rate in the control group was 28% compared to 9% in the surgical group (including perioperative deaths). For every year of follow-up, patients in the control group had a 4.5% chance of dying vs. a 1.0% chance for those in the surgical group. The improvement in the mortality rate in the surgical group was primarily due to a decrease in the number of cardiovascular deaths.
(J GASTROINTEST SURG 1997;1:213-220.)

Morbid obesity is a serious health problem that is associated with significant morbidity and mortality,[1-3] much of which is secondary to conditions either caused by or exacerbated by the obesity; this is commonly referred to as "comorbidity." These comorbid conditions include non–insulin-dependent diabetes mellitus (NIDDM), hypertension,[4,5] obstructive sleep apnea and obesity hypoventilation syndrome,[6] hyperlipidemia, degenerative joint disease, cardiovascular disease,[7] cholelithiasis, and increased incidence of certain cancers. Psychosocial impairments, most frequently depression and feelings of insecurity and inadequacy, occur often and may be disabling.[7]

NIDDM, the subject of this report, is a devastating disease and a major cause of atherosclerotic cardiovascular disease, renal disease, retinopathy, and neuropathy. Of the estimated 10 million Americans with diabetes, approximately 95% have NIDDM,[8] much of which can be attributed to obesity. Previous reports from our studies at East Carolina University have shown that 82.9% to 88.7% of obese patients with diabetes became euglycemic following Roux-en-Y gastric bypass.[8,9] Patients whose glucose metabolism failed to return to normal were older and had had diabetes for longer than those who became euglycemic. We have also demonstrated amelioration of hyperinsulinemia, which is characteristic of NIDDM, following gastric bypass.[8] We have previously published data showing that gastric bypass prevents the progression of subclinical diabetes, or impaired glucose tolerance, to overt diabetes.[9,10]

Despite this documented control of NIDDM after gastric bypass, there is still little documentation of improved morbidity and mortality in comparison to

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morbidly obese patients with diabetes who do not undergo surgery. In a recent review, Benotti and Forse [11] concluded that "future longitudinal studies of patients who have undergone surgical therapy for obesity should be conducted in order to assess whether long-term weight control in these patients will result in improved health risks." This article represents our ongoing attempt to provide long-term follow-up data that could answer this question.

MATERIAL AND METHODS

Design

From March 5, 1979, to January 1, 1994, a total of 1309 patients were referred to the Obesity Research Program at East Carolina University where they were evaluated for possible gastric restrictive surgery for obesity. Of this group, 603 patients ultimately had an initial gastric bypass procedure performed at East Carolina University. Five hundred sixty-nine of these patients met the strict criteria for morbid obesity; that is, they weighed more than 100 pounds over their ideal body weight, as defined by the median weight of the 1983 Metropolitan Life Insurance Tables, at the time of their surgery. One hundred fifty-four of these morbidly obese patients were diagnosed with NIDDM either before or during their preoperative evaluation for gastric bypass; these patients form the surgical group for this study. Seven hundred six patients seen by project investigators for initial consultation ultimately did not undergo gastric bypass. Among this group, 127 patients considered possible candidates for surgery were diagnosed with NIDDM either before or during their preoperative evaluations. Forty-nine of these 127 patients were excluded from this analysis for the following reasons: weight inadequate for a diagnosis of morbid obesity (22 patients), surgery denied for medical reasons (15patients), age greater than 64 years (2 patients), going elsewhere for surgery (8 patients), or lost to follow-up (2 patients). The remaining 78 patients were termed the control group for the purposes of this study.

Data on the patients in the surgical group were collected by reviewing the charts from the Obesity Research Clinic, where patients are followed at regular intervals after surgery. Data on the control group at the time of their initial presentation were obtained from their clinic records. Current follow-up data on survival and medical management of diabetes were obtained by phone interviews with the patients, their families, or their physicians for all 78 patients in this group. When patients were found to be deceased, the cause of death was obtained from the death certificate in all but two cases.

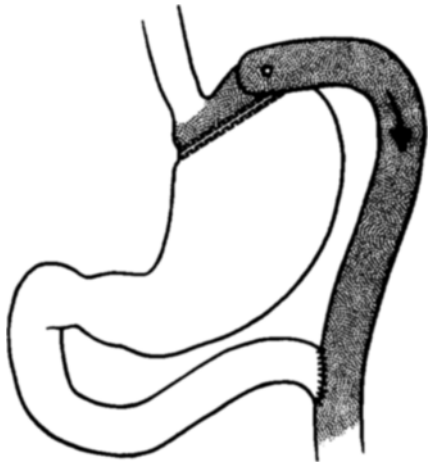

Fig. 1. Roux-en-Y gastric bypass.

Statistical Analysis

Statistical analysis was conducted using the SAS software with graphics supplied by the Plot-It program (Plot-It Scientific Programming Enterprises, Haslett, Mich.). Descriptive statistics such as means, standard deviations, counts of events, and totals were calculated for both the control and surgical groups. Student's t test for independent samples was used to test for differences between continuous variables, and the chi-square test was used to test for differences between proportions. The incidence densities (events divided by time of exposure to risk), incidence density ratios, and tests of hypothesis were also calculated. Confidence intervals (CI of 95%) were conducted under an exponential model, following a previously described approach.

The Gastric Bypass

Between March 5, 1979 and July 1, 1990, a standardized Roux-en-Y gastric bypass, as described by Pories et al.,[12] was performed in all patients (Fig. 1). A small proximal gastric pouch of approximately 30 ml was created by placing four rows of staples using a (double-armed PI-90 stapler, 3M Company, St. Paul, Minn.) from the angle of His to a point on the lesser curvature approximately 3 cm distal to the cardia. Much of the volume of the pouch was created by pulling up the anterior wall of the stomach. No vessels to the stomach were routinely divided during construction of the pouch. The jejunum was then divided at its apex, approximately 30 cm from the ligament of Treitz, with a GIA stapling instrument (Ethicon, Inc., Somerville, N.J.). The distal jejunal limb was then brought through the transverse meso-

Table I. Characteristics of surgical and control groups

Characteristics	Group		*P* value
	Surgical (n = 154)	Control (n = 78)	
Mean baseline age (yr)	41.9	43.5	NS
Mean BMI	50.6	48.8	NS
Mean weight (pounds)	313.9	303.3	NS
Mean length of follow-up (yr)	9.0	6.2	<0.0001
% with hypertension	80.5	76.9	NS
Race (% white)	76.6	50.0	<0.001
Sex (% female)	76.6	73.1	NS

BMI = body mass index; NS = not significant.

colon and around the greater curvature to lie adjacent to the proximal gastric pouch. A side-to-side gastrojejunostomy was then performed in two layers with a running 3-0 polypropylene suture around an 18 F sump nasogastric tube, providing an internal diameter of approximately 0.8 cm. The Roux anastomosis, or jejunojejunostomy, was then constructed approximately 40 cm from the gastrojejunostomy.

With the use of this technique of partitioning the pouch with four parallel staple lines, we found an 11.8% incidence of staple line dehiscence over 11 years of follow-up.[13] The larger breakdowns usually were associated with a regain of lost weight and, in the diabetic patients, a return to abnormal glucose metabolism. In response to these findings, in July 1990, we modified our technique and began to perform a divided gastric bypass for primary as well as revision procedures. By dividing the proximal gastric pouch from the distal stomach, we hoped to eliminate the problem of staple line breakdowns; the gastrojejunostomy was constructed in a manner identical to the original procedure. In 100 consecutive divided gastric bypass operations, both primary and revision procedures, we encountered a 6% incidence of gastrogastric fistulas, or a fistulous communication between the proximal pouch and the distal stomach.[14] In five of the six patients these were revision operations. Although it appeared that minor variations in technique could lower the incidence of this complication, we wished to find a simpler, more reliable alternative. Since February 25, 1994, we have partitioned the stomach in our last 100 procedures with three superimposed applications of the PI-90 stapler without division. Only one of these 100 patients has thus far demonstrated a staple line breakdown on routine barium upper gastrointestinal studies obtained 6 months postoperatively. Except for the above-mentioned differences in construction of the partition, the operations have been identical and have been evaluated as one cohort in this study.

RESULTS

Group Characteristics

Table I lists the characteristics of the surgical and control groups, which were not significantly different in terms of age, weight, body mass index, sex, or the percentage with hypertension (80.5% of the surgical group vs. 76.9% of the control group). FIfty-six percent of the control group were being treated with antihypertensive drugs vs. 47% of the surgical group, again not a significant difference. The mean length of follow-up was significantly higher in the surgical group (9 years) compared to the control group (6.2 years). Follow-up for the surgical patients began at the time of the gastric bypass operation, whereas follow-up for the control subjects began at the time of the initial evaluation or when the diabetes was first diagnosed. There was a significantly higher percentage of white patients in the surgical group (76.6%) compared to the control group (50%). Fifty-six percent of the control group were on either oral hypoglycemic medication or insulin for treatment of their diabetes at the begining of the study, which was significantly greater than the 32% of the surgical patients whose diabetes was medically treated prior to gastric bypass surgery (Fig. 2).

Weight Loss and Glucose Levels After Gastric Bypass

Fig. 3 shows the mean percentage of excess body weight lost in the surgical group. After reaching a maximum 62.4% loss of excess body weight at 1 year after gastric bypass, the group demonstrated a slight regaining of weight over time with the mean loss re-

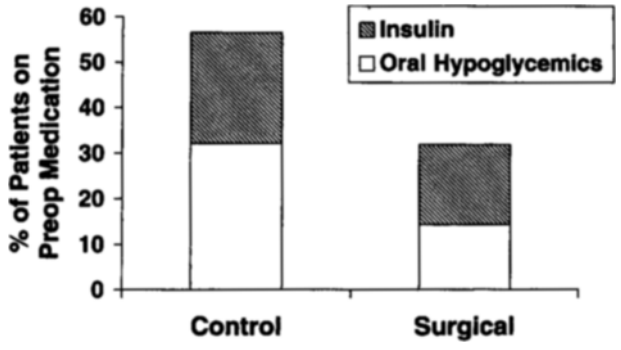

Fig. 2. Fifty-six percent of the control patients were treated with either insulin or oral hypoglycemics at the beginning of the study period, whereas only 32% of those in the surgical group were medically managed preoperatively.

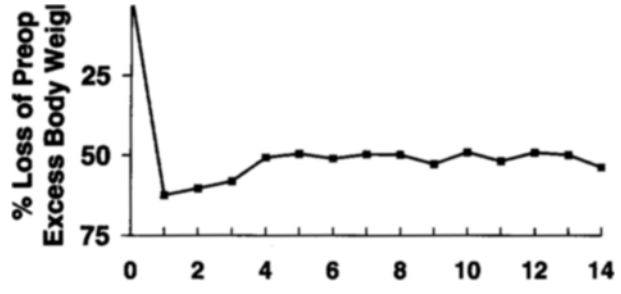

Fig. 3. Percentage of excess body weight lost in the surgical group (n = 154). The mean percentage loss of excess body weight reached a maximum of 62.4% 1 year after gastric bypass and remained at approximately 50% out to 14 years.

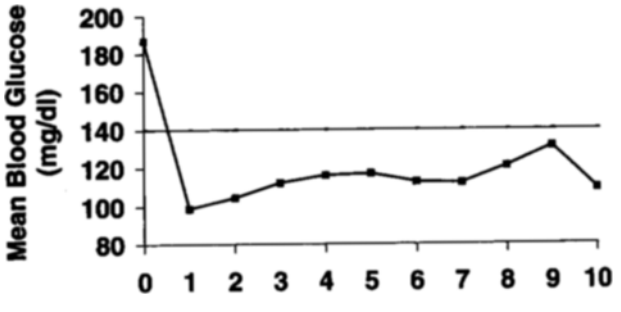

Fig. 4. The mean blood glucose level fell from 187 mg/dl (all fasting) to a minimum of 98.9 mg/dl (combination of random and fasting) at 1 year after surgery, and then remained less than 140 mg/dl out to 10 years.

maining at approximately 50% for up to 14 years of follow-up. In terms of actual weight, the group ranged from a preoperative mean weight of 314 pounds to a minimum of 206 pounds at 1 year after surgery. The mean weight then remained within a stable range of 199 to 224 pounds for up to 14 years.

Fig. 4 shows the mean blood glucose levels in the surgical group after gastric bypass. The preoperative glucose levels were all fasting values, whereas the mean values from the postoperative period were a combination of fasting and random glucose levels. Therefore these postoperative means should be skewed toward higher levels. Regardless, the mean blood glucose level fell from 187 mg/dl preoperatively to a low of 98.9 mg/dl at 1 year after surgery. The mean values then remained less than 140 mg/dl for up to 10 years of follow-up. Unfortunately, similar data were not available for a sufficient number of the control patients to allow a significant comparison.

Surgical vs. Control Groups

Diabetes. One method of comparing control of diabetes in the two groups with the data that were available to us was to compare the percentage of patients being treated with either oral hypoglycemics or insulin at the initiation of the study with the percentage of those requiring medical management at the time of our last contact with them. As shown in Fig. 5, the percentage of patients in the control group who were taking either oral hypoglycemics or insulin increased from 56.4% at the initiation of the study to 87.5% at the time of last contact, a significant increase ($P = 0.0003$). Conversely, the percentage of surgical

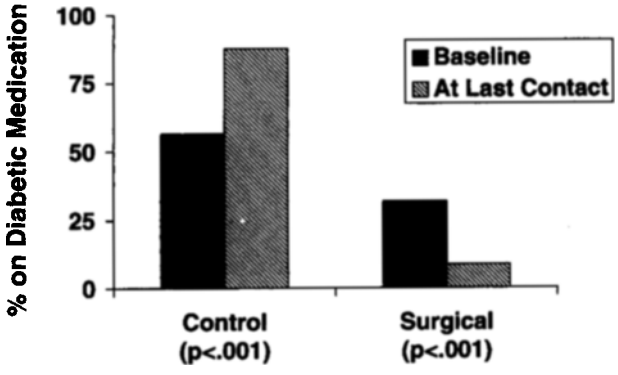

Fig. 5. The percentage of control patients being treated with insulin or oral hypoglycemics increased from 56.4% at first contact to 87.5% at last contact. The percentage of surgical patients requiring medical management fell from 31.8% preoperatively to 8.6% at last contact.

patients requiring medical management of diabetes fell from 31.8% preoperatively to only 8.6% at time of last contact, a significant decrease ($P = 0.0001$).

Death. Analysis of the number of deaths in each group (Fig. 6) revealed that 22 of the 78 control patients had died, compared to only 14 of the 154 surgical patients. This difference in mortality rates—28% for the control group vs. 9% for the surgical group—was highly significant ($P < 0.0003$) and was further emphasized by the fact that the mean follow-up in the surgical group was longer at 9 years than the 6.2 years in the control group. When this difference in length of follow-up was included in the equation to calculate the incidence of death per patient-year of follow-up (Fig. 7), the incidence in the control group was 4.5 times that of the surgical group ($P < 0.0001$). Based on the 95% confidence interval of the control group, we would expect to have observed between 46 and 58 deaths in the surgical group rather than the 14 deaths actually recorded.

Causes of Death

Table II lists the causes of death in the surgical and control groups. Most notable was the markedly increased incidence of cardiovascular death in the control group, which was responsible for 54.5% of the deaths among control subjects compared to only 14.3% of the deaths in the surgical group. The 12 cardiovascular deaths in the control group included eight myocardial infarctions, one sudden cardiac death, one case of congestive heart failure, one dissecting aneurysm, and one cerebrovascular accident. The single most common cause of death in the surgical group (4 patients) was perioperative mortality, which was responsible for 28% of the deaths in this group. These deaths included two cases of pulmonary emboli, one case of sepsis secondary to a leak, and one patient who died of unknown causes after being discharged from the hospital. The overall perioperative mortality rate for the entire group of 154 diabetic patients was 2.6%.

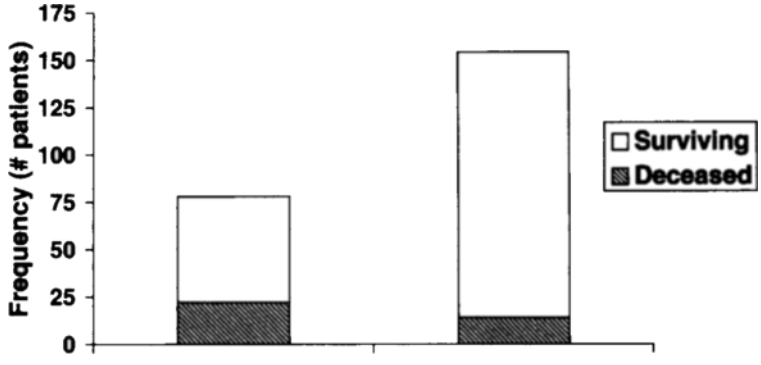

Fig. 6. The mortality rate in the control group was 28% (22 of 78 patients) compared to 9% (14 of 154 patients) in the surgical group ($P < 0.0003$).

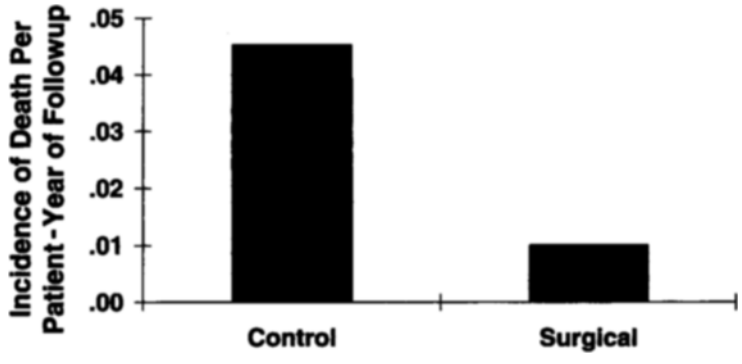

Fig. 7. Patients in the control group had 4.5 times the incidence of death of patients in the surgical group ($P < 0.0001$).

Table II. Causes of death in control and surgical groups

Cause of death	No.
Control group	
Cardiovascular	12
Pulmonary embolism	1
Cancer	2
Sepsis	2
Acute respiratory distress	1
End-stage renal disease	1
Trauma	1
Suicide	1
Unknown	1
Total deaths	22
Surgical group	
Perioperative	4
Pulmonary embolism (subsequent revision)	1
Non–gastric bypass-related sepsis	1
Anemia	1
Asphyxia	1
Cardiovascular	2
Malnutrition	1
Auto accident	2
Suicide	1
Total deaths	14

Effect of Race on Mortality

Characteristics of the surgical and control groups that differed significantly were examined to determine those that were possibly responsible for the difference in mortality rates between the two groups. As shown earlier, black patients comprised 50% of the control group compared to only 23% of the surgical group, a significant difference. Fig. 8 shows that the statistically significant improvement in the mortality rate in the surgical group compared to the control group was maintained for both black and white patients. Although there was no difference in the incidence of death per patient-year of follow-up between black and white patients in the control group, there was a significant difference in the surgical group (0.024 in blacks vs. 0.006 in whites).

Effect of Diabetes Treatment on Mortality

Inasmuch as there was also a significant difference in the percentage of patients being treated with oral hypoglycemics or insulin at the beginning of the study period between the control and surgical groups (56% vs. 32%, respectively), we looked for a possible association with the difference in mortality rates between the two groups. It could be assumed, for example, that those requiring medical management had diabetes of longer duration or greater severity. We found, however, that there was a significant improvement in the mortality rate in the surgical group regardless of the initial treatment status of the patients (Fig. 9).

DISCUSSION

Although we have long had evidence that gastric bypass effectively controls NIDDM in most morbidly obese patients,[1,2] these are the first data from our group to demonstrate a decrease in the number of deaths after surgery as compared to a similar group of obese patients with diabetes who did not have surgery for nonmedical reasons. This improvement in the mortality rate was unrelated to differences in race or in the percentage of patients requiring oral hypoglycemics or insulin between the two groups.

We realize there are significant limitations to this retrospective study; although we were able to adequately determine the cause of death and type of medical management of diabetes in all control patients, we did not have adequate follow-up data regarding weight, blood glucose levels, or other comorbid conditions such as cardiac, pulmonary, or renal disease, which may have had an impact on survival. We do not believe a randomized, prospective trial of surgical vs. medical management would be possible, because most patients who are referred for surgery have exhausted all other options for weight loss and surgery represents the last resort. To suggest alternatives known not to be effective would raise ethical questions.

Acknowledging these limitations, we still believe this report strongly infers that gastric bypass surgery significantly improves the long-term mortality rate in morbidly obese diabetic patients, largely by reducing the number of deaths from cardiovascular causes. This improvement in cardiovascular disease could be due to other benefits of the surgery, in addition to control of diabetes, such as improvement in pulmonary artery hypertension due to obstructive sleep apnea and improvement in hypertension. Inasmuch as the hyperinsulinemia associated with NIDDM has been associated with the progression of coronary artery disease,[15] the greater reduction in insulin levels achieved with gastric bypass compared to operations that do not bypass the foregut, such as vertical banded gastroplasty,[16] may confer an additional benefit.

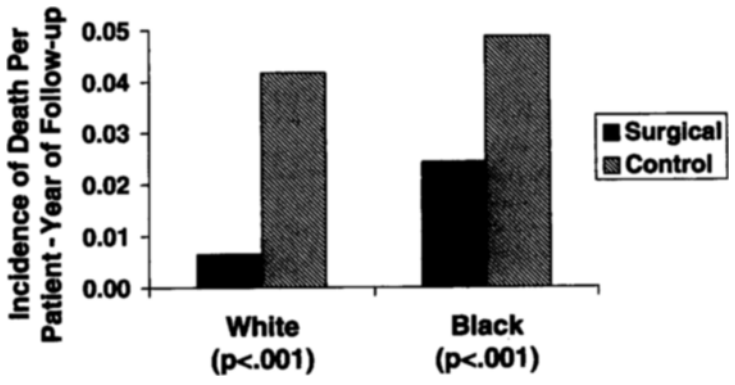

Fig. 8. The decrease in the mortality rate in the surgical group vs. the control group was significant for both white and black patients, although within the surgical group, black patients had a significantly higher mortality rate compared to white patients.

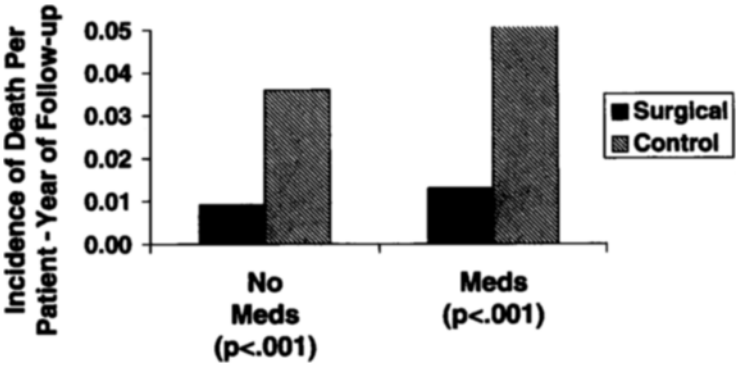

Fig. 9. The improvement in the mortality rate in the surgical group as compared to the control group was maintained whether or not diabetes was medically managed preoperatively *(Meds)*.

The percentage of control patients requiring medical management of their diabetes increases significantly over the follow-up period, from 56.4% to 87.5%, whereas among surgical patients on medication for diabetes the percentage droppped from 31.8% preoperatively to 8.6% at the time of the last contact. These results demonstrate the progressive nature of the disease when obesity is not treated and reconfirm the effective control of diabetes with gastric bypass in the majority of patients. In a previous study[9] we reported that 9% of 298 patients with either NIDDM or impaired glucose tolerance failed to become euglycemic after gastric bypass. Thirty-seven percent of this group were found to have mechanical failure of the gastric bypass, such as breakdown of the staple line or dilatation of the gastrojejunostomy. The remaining patients with intact gastric bypasses were found to be on average 7.3 years older and had had diabetes for 3 years longer than those patients who became euglycemic, again suggesting that NIDDM is a progressive disease that becomes more irreversible with time. Fortunately gastric bypass can prevent this progression in the majority of patients if it is performed in a timely fashion, before irreversible destruction of the function of the islets.

REFERENCES

1. Sjostrom L. Morbidity of severely obese subjects. Am J Clin Nutr 1992;55:508S-515S.
2. Sjostrom L. Mortality of severly obese subjects. Am J Clin Nutr 1992;55:516S-523S.
3. Drenick FJ, Gurunanjappa SB, Seltzer FSA, et al. Excessive mortality and causes of death in morbidly obese men. JAMA 1980;243:443-445.
4. Carson JL, Ruddy ME, Duff AE, et al. The effect of gastric bypass surgery on hypertension in morbidly obese patients. Arch Intern Med 1994;154:193-200.

5. Foley EF, Benotti PN, Borlase BC, et al. Impact of gastric restrictive surgery on hypertension in the morbidly obese. Am J Surg 1992;163:294-297.
6. Surgerman HJ, Fairman RP, Baron PL, et al. Gastric surgery for respiratory insufficiency of obesity. Chest 1986;90:81-86.
7. Manson JE, Colditz GA, Stampfer MJ, et al. A prospective study of obesity and risk of coronary heart disease in women. N Engl J Med 1990;322:882-889.
8. Pories WJ, MacDonald KG, Flickinger EG, et al. Is type II diabetes mellitus (NIDDM) a surgical disease? Ann Surg 1992;215:633-643.
9. Pories WJ, Swanson MS, MacDonald KG, et al. Who would have thought it? An operation proves to be the most effective therapy for adult-onset diabetes mellitus. Ann Surg 1995;222:339-352.
10. Long S, O'Brien K, MacDonald K, et al. Weight loss in severely obese subjects prevents progression of impaired glucose tolerance to type II diabetes. Diabetes Care 1994;17:372-375.
11. Benotti PN, Forse RA. The role of gastric surgery in the multidisciplinary management of severe obesity. Am J Surg 1995; 169:361-367.
12. Pories WJ, Flickinger EG, Meelheim D, et al. The effectiveness of gastric bypass over gastric partitioning in morbid obesity. Ann Surg 1982;196:389-399.
13. Jordan CP, MacDonald KG, Pories WJ, et al. Staple line failure: An avoidable complication of the gastric bypass (submitted for publication).
14. Cucchi SDG, Pories WJ, MacDonald KG, Morgan EJ. Gastrogastric fistulas—A complication of divided gastric bypass surgery. Ann Surg 1995;221:387-391.
15. Krolewski AS, Warram JH. Epidemiology of late complications of diabetes. In Kahn CR, Weir GC, eds. Joslyn's Diabetes Mellitus, 13th ed. Philadelphia: Lea & Febiger, 1994, pp 605-619.
16. Kellum JM, Kuemmerly JF, Oderisio PM, et al. Gastrointestinal hormone responses to meals before and after gastric bypass and vertical banded gastroplasty. Ann Surg 1990; 211:763-770.

Discussion

Dr. J. Kral (Brooklyn, N.Y.). From a public health perspective, this is one of the most important papers ever written by surgeons, given the enormity of the epidemic of obesity not only in industrialized nations but increasingly in third world countries as well. It is a credit to the authors and to the SSAT.

This report presents a whole new concept in surgery — that is, the notion that surgery can be used as a preventive measure. With early intervention in identifiable at-risk individuals, substantial morbidity and mortality can be prevented. Within a relatively short period of time, given the young age of these patients (mean of 42 to 44 years), it is extraordinary to achieve a fivefold decrease in mortality just by operating.

Equally spectacular is the 28% mortality rate in the control group, which is sufficient evidence of how devastating this disease is without effective treatment. There are two scientific flaws in this important work, and the authors are aware of both: the difference in race and the difference in severity of disease between the two groups. That is part of the problem of a retrospective study. Larger numbers are naturally needed. A prospective study, or at least a case-control or matching study, is necessary to be more conclusive. Such data are being gathered in the Swedish Obese Subject study. The major weakness in this present report is the source of information; that is, telephone interviews with patients or family do not provide the precision needed for this type of study. Finally, did you notice any gender difference in the deaths? I would predict that there might be an increased prevalence of death and poor outcome among men.

Dr. K.G. MacDonald. Unfortunately we do not know the sex of the patients who died. We were aware of the limitations of this study, but believed it was the best we could do. We tried to make the best possible use of the telephone interviews. The most accurate information we were able to obtain was whether or not the patients were taking medication for their diabetes and whether or not they had died. For the patients who died, we obtained the cause of death from the death certificate in all but two patients, so we thought it reasonable to at least try to use these data.

Complications of Laparoscopic Paraesophageal Hernia Repair

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The complications of laparoscopic paraesophageal hernia repair at two institutions were reviewed to determine the rate and type of complications. A total of 76 patients underwent laparoscopic paraesophageal hernia repair between December 1992 and April 1996. Seventy-one of them had fundoplication (6 required a Collis-Nissen procedure). Five patients underwent hernia reduction and gastropexy only. There was one conversion to laparotomy. Traumatic visceral injury occurred in eight patients (11%) (gastric lacerations in 3, esophageal lacerations in 2, and bougie dilator perforations in 3). All lacerations were repaired intraoperatively except for one that was not recognized until postoperative day 2. Vagus nerve injuries occurred in at least three patients. Three delayed perforations occurred in the postoperative period (4%) (2 gastric and 1 esophageal). Two patients had pulmonary complications, two had gastroparesis, and one had fever of unknown origin. Seven patients required reoperation for gastroparesis (n = 2), dysphagia after mesh hiatal closure of the hiatus (n = 1), or recurrent herniation (n = 4). There were two deaths (3%): one from septic complications and one from myocardial infarction. Paraesophageal hernia repair took significantly longer (3.7 hours) than standard fundoplication (2.5 hours) in a concurrent series ($P < 0.05$). Laparoscopic paraesophageal hernia repair is feasible but challenging. The overall complication rate, although significant, is lower than that for nonsurgically managed paraesophageal hernia. (J GASTROINTEST SURG 1997;1:221-228.)

Sliding hiatal hernia (type I), in which there is a migration of the gastroesophageal junction above the diaphragm into the thorax, is common. True paraesophageal hernia (type II), in which the stomach herniates into the thorax but the gastroesophageal junction remains fixed in its normal or near-normal position below the diaphragm, is rare. More commonly, a mixed (type III) hernia (sliding hernia with some or all of the stomach above the gastroesophageal junction) is seen (Figs. 1 to 3). When these hernias become extremely large, the stomach can assume a nonanatomic orientation within the hernia sac (Fig. 4) (gastric volvulus). Paraesophageal herniations (types II and III) account for only 3% to 10% of hiatal hernias. They are more common in the elderly and probably evolve from type I hernias.[1] Uncommonly seen are parahiatal hernias, where gastric herniation occurs through a diaphragmatic defect separate from the hiatus (Fig. 5), or type IV hernias, which include abdominal viscera and/or solid organs within the hernia sac.

Type I hernia is commonly associated with gastroesophageal reflux but rarely requires emergency treatment. On the other hand, as many as 30% of patients with type II or type III hernias will present with serious, even fatal, complications. Because of this notable complication rate, the presence of a paraesophageal hernia, regardless of size or symptoms, is an indication for repair. Laparoscopic paraesophageal hernia repair is now feasible,[2-5] although it is technically far more challenging than type I hiatal hernia repair. It also seems to us that there is a higher incidence of attendant complications with laparoscopic repair of paraesophageal hernias than with repair of type I hernias. The purpose of this study was to review the collective experience of laparoscopic paraesophageal hernia repair at two institutions to determine the nature and rate of complications.

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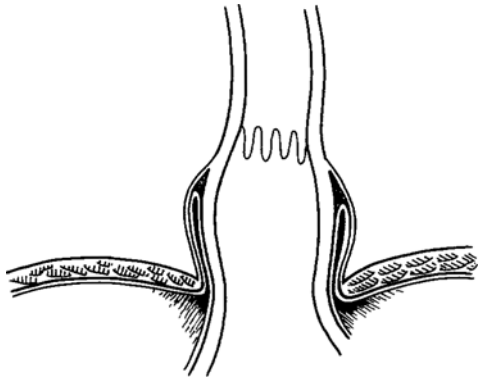

Fig. 1. Type I hernia. The gastroesophageal junction is most cephalad.

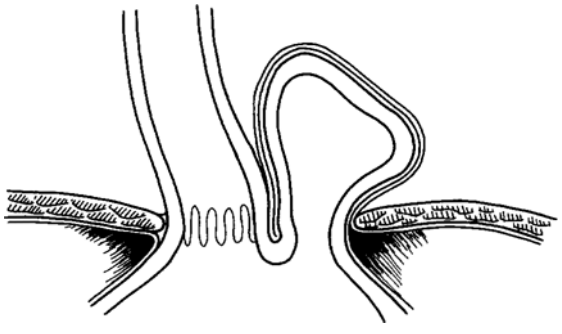

Fig. 2. Type II (true paraesophageal) hernia. The gastroesophageal junction is in the abdomen; the fundus is in the chest.

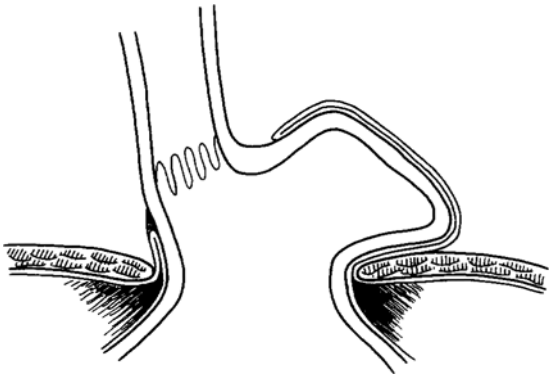

Fig. 3. Type III (mixed paraesophageal) hernia. Both the gastroesophageal junction and the fundus are in the chest with the stomach cephalad to the gastroesophageal junction.

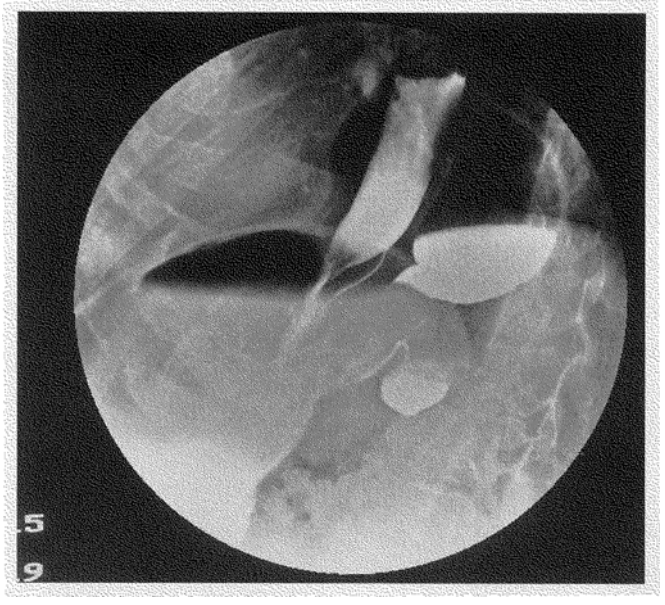

Fig. 4. Giant paraesophageal hernia containing most of the stomach within the chest.

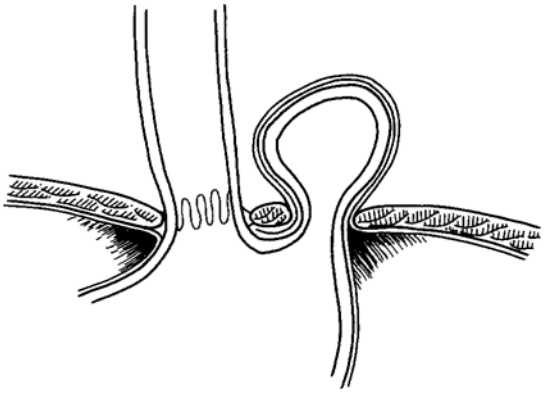

Fig. 5. Parahiatal hernia. The stomach herniates through a diaphragmatic defect distinct from the hiatus.

PATIENTS AND METHODS

From December 1992 through April 1996, a total of 76 patients had paraesophageal hernias repaired laparoscopically at our two institutions. There were 30 men and 46 women. The mean age of the patients was 65 years (range 38 to 91 years). Presenting symptoms included dysphagia, chest pain, postprandial pain, nausea, vomiting, and excessive belching. Thirty-one patients (41%) had symptoms of gastroesophageal reflux. Six patients were treated on an emergency basis for gastric volvulus, and one presented with bleeding from a gastric ulcer in the herniated portion of the stomach. Eight patients (11%) were asymptomatic. All patients underwent a preoperative barium swallow, which confirmed the diagnosis of paraesophageal hernia. All but three patients also underwent preoperative esophagogastroduodenoscopy. The majority of patients (n = 45) were evaluated by esophageal manometry, whereas 24-hour ambulatory esophageal pH monitoring was performed selectively in 22 patients. The mean physical status classification of the American Society of Anesthesiologists (ASA) score

Table I. Comparison of type II and III hiatal hernia (paraesophageal) with type I hiatal hernia

	Paraesophageal hernia	Hiatal hernia	*P* value*
Mean age in years (range)	65 (38-91)	46 (14-79)	<0.005
Male:Female ratio	0.7:1	1.4:1	
Mean physical status classification of the American Society of Anesthesiologists (SD)	2.3 (0.6)	2.1 (0.6)	<0.005

*Mann-Whitney U test.

was 2.3 ± 0.6. Four patients were scored as ASA I, 43 patients as ASA II, 28 as ASA III, and one as ASA IV. Most patients were operated on electively at the earliest possible time after completion of their preoperative investigations. Patient demographics were compared with those in a group of patients who underwent laparoscopic fundoplication for gastroesophageal reflux disease (GERD) in a concurrent series (Table I).[6]

Operative Approach

All operations were performed with the patient under general anesthesia with an orogastric tube, Foley catheter, and deep venous thrombosis prophylaxis. Patients were placed in a reverse Trendelenburg position with their legs abducted 45 degrees at the hip. The operating surgeon stood between the patient's legs, the first assistant at the patient's left side and, when necessary, a second assistant stood at the patient's right side. The abdomen was insufflated with carbon dioxide by means of a Veress needle inserted through the umbilicus. Trocar placement was identical to that used for antireflux procedures.[7] A forward oblique telescope (30 or 45 degrees) was used in all cases.

The left lobe of the liver was elevated by means of a flexible retractor placed through the far right subcostal port and fixed to a mechanical arm. The herniated stomach was reduced as much as possible by applying gentle traction using atraumatic graspers. Undue effort at total reduction was not attempted because this only leads to gastric laceration. Complete or near-complete hernia sac removal was attempted in all but four cases. Dissection was usually begun along the left crus, separating the sac from the mediastinal structures and pleura using sharp and blunt dissection. Care was taken to identify and preserve the vagal nerves whenever possible. The gastroesophageal junction was encircled by a Penrose drain to allow for further esophageal mobilization and posterior crural dissection. The hernia defect was closed posterior to the esophagus with interrupted nonabsorbable sutures in all but one case where mesh was used. Pledgets were used for closure of large hernia defects under significant tension.

Table II. Surgical procedures performed

Procedure	No.
Nissen fundoplication	60
Toupet fundoplication	4
Collis-Nissen fundoplication	6
Gastropexy only	5
Gastrostomy	4
Open Nissen fundoplication (conversion)	1
Gastric wedge resection for large polyp	1

An antireflux procedure was performed in all but five patients. A loose, 2 cm Nissen fundoplication was performed over a large bougie (52 to 60 Fr) in 61 patients. Four patients underwent Toupet (270 degree) fundoplication because of evidence of esophageal dysmotility on preoperative esophageal manometry. Six patients required a Collis-Nissen fundoplication because of a short esophagus. Esophageal lengthening was achieved by thoracoscopically guided linear stapling in four patients and laparoscopic stapling in two. Four patients also underwent gastrostomy tube placement. Five patients underwent hernia reduction and gastropexy only because of poor general health and poor prognosis (Table II).

Postoperatively, routine nasogastric tube placement was not used. Patients with very large hernia repairs requiring extensive dissection and those who required a Collis procedure underwent a Gastrografin swallow on postoperative day 1. Otherwise patients were started on clear liquids on the same day as their surgery and advanced to a modified solid diet on postoperative day 1. If the diet was well tolerated, the patients were discharged on postoperative day 2.

RESULTS

The majority of patients (n = 71) had a mixed paraesophageal (type III) hernia. Four patients had a true paraesophageal hernia (type II) and one patient had a

parahiatal herniation of the stomach through a defect distinct from the esophageal hiatus. Paraesophageal hernia repair took significantly longer (3.7 hours; range 1.0 to 7.1 hours) than standard laparoscopic fundoplication (2.5 hours) in a concurrent series[6] ($P < 0.05$). Length of hospital stay was 4.2 days (range 1 to 127 days) in the group with paraesophageal hernias, which was significantly longer than that for patients undergoing standard fundoplication[6] (2.2 days, range 1 to 32 days; $P < 0.05$). The longest hospital stay was 127 days. This was recorded in a frail 68-year-old female patient on long-term corticosteroid therapy. She was the only patient in this series who required conversion to open surgery (for bleeding and excessively friable tissue). She suffered an esophageal laceration during open surgery and developed adult respiratory distress syndrome postoperatively, which required prolonged ventilation. The next longest hospital stay was significantly shorter (33 days) in a patient who had an unrecognized intraoperative bougie dilator perforation (see below).

The most frequent major intraoperative complication was traumatic visceral injury, which occurred in eight patients (11%) (gastric lacerations in 3, esophageal lacerations in 2, and esophageal bougie dilator perforations in 3). All lacerations were repaired intraoperatively except for one esophageal bougie perforation at the level of the thoracic inlet, which was not recognized until postoperative day 2. This patient subsequently underwent closure of the esophageal perforation and mediastinal drainage through a right thoracotomy. He also required a third operation through the right side of the neck to drain and close an esophageal fistula.

Three delayed perforations (4%) occurred in the early postoperative period (two gastric and one esophageal perforation on postoperative days 3, 7, and 3, respectively). The gastric perforations appeared to be secondary to ischemic necrosis and both were repaired at laparotomy by excision of the perforated segment. The esophageal perforation was repaired through a left thoracotomy.

Recognized vagus nerve injury occurred in three patients. Two of these patients had significant gastric atony in the first few weeks postoperatively and developed bezoars. One of these patients underwent partial gastrectomy and Billroth II reconstruction, and the other patient underwent laparoscopic pyloroplasty. One patient developed postoperative gastric dilatation, which resolved with nasogastric tube drainage. One patient developed pneumonia, two developed adult respiratory distress syndrome (see above), and one patient had fever of unknown origin. One patient was readmitted with nausea on postoperative day 28 and suffered a myocardial infarction on postoperative day 30. In total, postoperative complications occurred in 13 patients (17%) and required operative repair in six (8%).

Five patients required reoperation more than a month after the original operation for dysphagia (n = 1) or recurrent hernia (n = 4). One patient developed refractory dysphagia after mesh closure of the hiatus. At laparotomy a cicatrix of scar was found encircling the distal esophagus related to the mesh used for closure of the hiatus. The mesh was excised and myotomy of the distal esophagus was performed before a primary hiatal closure was accomplished. A second patient with a fundoplication stricture (perhaps ischemic) has responded to multiple (more than six) dilations.

Five patients had recurrent paraesophageal herniation at 2, 4, 6, 12, and 16 months postoperatively. Three patients presented with new-onset dysphagia and one patient had heartburn secondary to reflux. One patient complained of only mild occasional chest pain and did not require reoperation. The other four patients were reoperated laparoscopically; one patient underwent revision of gastropexy, one had revision of a Nissen fundoplication to Collis-Nissen fundoplication, and the remaining two underwent revision of a Nissen fundoplication.

There were two deaths (3%), one from septic complications following a delayed gastric perforation and one caused by intracranial hemorrhage following administration of tissue plasminogen activator for treatment of an acute myocardial infarction on postoperative day 30. This patient had been at home and well for 3 weeks prior to this event. The complications are summarized in Table III.

DISCUSSION

Paraesophageal hernia is an exceptional defect of the diaphragmatic hiatus that is associated with significant complications (e.g., obstruction, bleeding, strangulation, and perforation) compared to the more common sliding hiatal hernia. Patients may be asymptomatic but more often tolerate a variety of vague, nondescript symptoms for many years prior to diagnosis. There are no safe, effective nonoperative management options for these patients. In fact, patients treated in this manner will frequently present with catastrophic complications that may prove fatal in up to 27% of cases.[8]

Although general agreement exists regarding the indications for surgery, a number of procedures have been advocated ranging from simple hiatal closure to a wide variety of gastropexy procedures. Despite these differences, most would agree that the hernia sac should be excised to reduce the risk of recurrence and

Table III. Complications of laparoscopic paraesophageal hernia repair (n = 76)

	No. (%)	Reoperation No. (%)
Intraoperative difficulties		
Gastric laceration	3 (4)	
Esophageal laceration	2 (3)	
Bougie dilator perforation	3 (4)	
	8 (11)	
Early postoperative complications (<30 days postop)		
Delayed gastric perforation	2 (3)*	2 (3)
Delayed esophageal perforation	1 (1)	1 (1)
Mediastinal abscess (from bougie perforation)	1 (1)	1 (1)
Acute gastric dilatation	1 (1)	
Gastric atony	2 (3)	2 (3)
Congestive heart failure (no infarction)	1 (1)	
Pneumonia	1 (1)	
Fever of unknown origin	1 (1)	
Adult respiratory distress syndrome	2 (3)	
Myocardial infarction (day 30)	1 (1)*	
	13 (17)	
Late postoperative complications (>30 days postop)		
Recurrent paraesophageal herniation	5 (7)	4 (5)
Distal esophageal stricture	2 (3)	1 (1)
	7 (9)	11 (14)

*There were two deaths (3%): one from septic complications following delayed gastric perforation and one following treatment of a myocardial infarction.

the formation of mediastinal serous cysts. There are cases where sac removal is particularly challenging because of the presence of adhesions, and it is better to leave portions of the sac behind rather than create an iatrogenic injury. In these cases the sac must be disconnected at the crural ring at a minimum.

In the literature considerable debate continues as to whether it is necessary to perform an antireflux procedure as part of the repair. By definition the gastroesophageal junction in type II hernias is in its proper anatomic location and should function as an antireflux mechanism alleviating the need for an antireflux procedure. It has been shown, however, that 60% of patients with a paraesophageal hernia will have objective evidence of reflux (i.e., abnormal findings on 24 hour pH monitoring) associated with a hypotensive lower esophageal sphincter.[9] Many of our patients had a change in symptomatology from heartburn to dysphagia and substernal distress when the body of the stomach herniated into the chest. For this reason it appears that preoperative symptoms are an unreliable predictor of postoperative reflux in these patients, as the distorted gastroesophageal junction of the intrathoracic stomach may protect against reflux. Under these conditions, reflux will recur after liberation and reorientation of the stomach. As a hernia enlarges, sliding (type I) hernias may progress to type III hernias, and true paraesophageal hernias may also evolve into type III hernias by loss of fixation of the gastroesophageal junction.[10] To obtain proper closure of the crura behind the esophagus, disruption of some fixation of the gastroesophageal junction must occur, which may contribute to postoperative reflux if a fundoplication is not performed. Failure to perform an antireflux procedure has been shown to result in postoperative reflux in 20% of patients.

Innovations and experience in minimally invasive surgery have allowed us to approach paraesophageal hernia repair laparoscopically in a wide range of patients. These patients were older and more debilitated than those undergoing standard antireflux procedures. Although their mean ASA scores were only modestly different from those of patients undergoing laparoscopic antireflux surgery, their comorbid conditions were so severe that the referring physician frequently advised against laparotomy.

Our operative time for paraesophageal hernia repair was significantly longer than the time needed for

the standard laparoscopic antireflux procedure.[6] Paraesophageal hernia repair, because of its size and the distorted anatomy, requires more dissection for sac removal, hernia reduction, and hiatal repair. These challenges have prompted many surgeons to perform inferior repairs, including mesh closure of the hernia, or simple reduction of the stomach and anterior gastropexy. Although we believe a simplified technique may be appropriate for a few patients with limited life expectancy, the majority of patients deserve a proper repair. Despite the complexity of proper laparoscopic repair, these patients have a brief hospital stay (mean 4.2 days) for a major foregut procedure in an elderly population.

The complications of laparoscopic paraesophageal hernia repair were significantly greater than those seen with laparoscopic fundoplication for GERD. In a large (n = 300) concurrent series of laparoscopic fundoplications, the major complication rate was 2%, the 30-day reoperation rate was 0.7%, and mortality was nil.[6] In this series of laparoscopic paraesophageal hernia repairs, the major complication rate was 17%, the 30-day reoperation rate was 8%, and the mortality rate was 3%. We expected the complication rate for a more extensive procedure in an elderly patient group to be higher, but we did not expect a difference of such magnitude.

Large paraesophageal hernias present some unique challenges for the skilled laparoscopic surgeon. Early in our experience, intraoperative difficulties in hernia reduction and manipulation of attenuated, friable tissue led to a much higher rate of intraoperative esophageal and gastric perforations relative to standard laparoscopic antireflux surgery. The delayed esophageal perforation in this series was also an error of inexperience, as this complication resulted from excessive use of electrosurgery on a large esophageal vessel, leading to subsequent perforation from conduction into or direct burn of the esophageal wall. A complication not seen in the standard antireflux surgery series, and not related to inexperience, was delayed gastric perforation. Two of these occurred and both were thought to be a result of ischemic necrosis along the greater curvature of the stomach. One patient was known to have a bleeding ulcer in the incarcerated stomach, which was, in retrospect, probably ischemic in etiology. It is not clear how this problem can be avoided; however, it may be wise to leave the short gastric vessels attached to the stomach if gastric ischemia is suggested by preoperative symptoms or endoscopic findings.

Late side effects and hernia recurrence were also seen more frequently in patients operated on for paraesophageal hernia than in patients operated on for GERD and sliding hiatal hernia. The esophagus is frequently foreshortened in large type III hernias, requiring a rather extensive dissection to restore the gastroesophageal junction to its normal subdiaphragmatic location. An extensive dissection increases the risk of esophageal perforation and ischemic stricture. Both of these complications were seen in this series. In addition, esophageal foreshortening may be a contributing factor in the development of recurrent herniation. The esophagus will stretch to reach the abdomen during the operation, but it may have a tendency to shorten again postoperatively, pulling an intact fundoplication into the mediastinum or stripping the fundoplication down onto the stomach. Esophageal lengthening with a Collis gastroplasty may decrease this late complication.[11] Reoperation was also required for the one patient in whom prosthetic mesh was applied to a large defect. The movement of the diaphragm relative to the esophagus 22,000 times a day exposes the esophagus to a significant risk of erosion or stricture, as occurred in this case.

Table IV. Results of open paraesophageal hernia repair

Series	No.	Morbidity	Mortality
Menguy[12]	30	2 (7%)	0
Harriss et al.[13]	29	3 (13%)	3 (13%)
Williamson et al.[14]	119	27 (23%)	2 (1.7%)
Myers et al.[15]	37	14 (37%)	0

Once we became aware of this rather alarming complication rate, in comparison to laparoscopic fundoplication for GERD, we questioned the wisdom of laparoscopic paraesophageal hernia repair. When compared to four reports of paraesophageal hernia repair performed through a laparotomy or thoracotomy, our complication rate was equivalent to a combined complication rate from these four centers (Table IV).[12-15] Coupling an equivalent complication rate with the extremely good results achieved in 75% of the patients undergoing laparoscopic repair (e.g., hospitalization 1 to 3 days) and a substantial cost savings per procedure ($10,000 at Emory, $6,400 in Portland), we believe that laparoscopic paraesophageal hernia repair is the preferred procedure for the majority of patients with this disorder. Additionally, there is little doubt that the lessons learned from this experience will translate into a decrease in complications down the road. In fact, the only complication of paraesophageal hernia repair at Emory University

Hospital in the last 135 fundoplications was the death of one patient resulting from a myocardial infarction 30 days postoperatively, following an unremarkable operation, early discharge, and rapid recovery at home. Unfortunately the nature of the abnormality and the advanced age of patients with the disease make it extremely unlikely that the complication rate will ever be reduced to levels seen with laparoscopic fundoplication for GERD. There appeared to be a remarkable correlation between intraoperative difficulties and postoperative complications at the two centers involved with this study. The only differences we noticed were that the late reoperation rate was slightly higher in Portland than at Emory (13% and 2%, respectively) and that both deaths occurred in Atlanta.

This is the largest series of laparoscopic paraesophageal hernia repairs thus far reported. Although it would be easy to focus on the superb results in the majority of our patients, we chose to point out the pronounced difference between this operation and laparoscopic fundoplication for GERD. To escape the avoidable complications, we have learned to strictly adhere to the following critical guidelines:

1. The incarcerated stomach is often very friable; therefore gentle tissue handling should be used and one should not attempt to reduce the stomach with force.
2. Start the dissection along the left crus; it is easier to identify than the right crus once the fundus is reduced.
3. Stay cephalad to the fat along the lesser curvature. There are several large vessels in the gastrohepatic omentum including the left gastric artery.
4. Treat the esophagus with respect; gain esophageal length with a Collis gastroplasty as opposed to extensive mobilization.
5. Close the hiatal defect behind the esophagus. It will gain additional intra-abdominal esophageal length. Do not use mesh.
6. Dilators are a necessary evil. Develop a strategy (graded dilatation and/or guidewire-assisted dilatation) with the anesthesia team.
7. Insufflation pressures must occasionally be lowered to 10 mm Hg to avoid hypercarbia and hypotension when dissecting in the mediastinum of elderly, frail patients.
8. Beware the vagus nerves. They often lie well off the esophagus and are therefore more prone to injury.

Finally, we believe that one should acquire sufficient experience with laparoscopic type I hernia repair (a minimum of 35 cases) before attempting to repair a large paraesophageal hernia.

REFERENCES

1. Wo JM, Branum GD, Hunter JG, et al. Clinical features of type III (mixed) paraesophageal hernia. Am J Gastroenterol 1996;91:914-916.
2. Oddsdottir M, Franco AL, Laycock WS, et al. Laparoscopic repair of paraesophageal hernia: New access, old technique. Surg Endosc 1995;9:164-168.
3. Kroger KE, Stone JM. Laparoscopic reduction of acute gastric volvulus. Am Surg 1993;59:325-328.
4. Congreve DP. Laparoscopic paraesophageal hernia repair. J Laparoendosc Surg 1992;2:45-48.
5. Kuster GGR, Gilray PA-C. Laparoscopic technique of repair of paraesophageal hiatal hernias. J Laparoendosc Surg 1993; 3:331-338.
6. Hunter JG, Trus TL, Branum GD, et al. A physiologic approach to laparoscopic fundoplication for gastroesophageal reflux disease. Ann Surg 1996;223:673-687.
7. Trus TL, Hunter JG. Laparoscopic surgery of the esophagus and stomach. Am J Surg (in press).
8. Skinner DB, Belsey RHR. Surgical management of esophageal reflux and hiatus hernia: Long-term results with 1,030 patients. J Thorac Cardiovasc Surg 1967;53:33-54.
9. Walther B, DeMeester TR, Lafontaine E, et al. Effect of paraesophageal hernia on sphincter function and its implication in surgical therapy. Am J Surg 1984;147:111-116.
10. Patti MG, Goldberg HI, Arcerito M, et al. Hiatal hernia size affects lower esophageal sphincter function, esophageal acid exposure, and the degree of mucosal injury. Am J Surg 1996; 171:182-186.
11. Swanstrom L, Marcus D, Galloway G. Laparoscopic Collis gastroplasty is the treatment of choice for the shortened esophagus. Am J Surg 1996;171:477-481.
12. Menguy R. Surgical management of large paraesophageal hernia with complete intrathoracic stomach. World J Surg 1988;12:415-422.
13. Harriss DR, Graham TR, Galea M, et al. Paraoesophageal hiatal hernias, when to operate. J R Coll Surg Edinb 1992;37: 97-98.
14. Williamson WA, Ellis FH, Streitz JM. Paraesophageal hiatal hernia: Is an antireflux procedure necessary? Ann Thorac Surg 1993;56:447-452.
15. Myers GA, Horms BA, Starling JR. Management of paraesophageal hernia with a selective approach to antireflux surgery. Am J Surg 1995;170:375-380.

Discussion

Dr. G. Fried (Montreal, Quebec, Canada). The authors have clearly shown that this is an operation that is feasible. I think it is appropriate to question whether it is something that should be performed widely. You have a group of surgeons who are very experienced in laparoscopic surgery around the esophagogastric junction, and you report considerable morbidity and mortality and high reoperation rate.

Is this operation superior to no surgery at all? Is it superior to a concurrent series of patients in whom "gold standard" open surgical repair is used? I do not think we should rely on comparisons of morbidity and mortality with historical series.

Dr. T. Trus. I agree. We have asked ourselves the same question. A number of patients referred for laparoscopic repair were actually patients whose gastroenterologists or internists believed they would not tolerate an open repair, so I think the patient population may be slightly different. I think some of the complications resulting from technical difficulties that we experienced early in the procedure can now be avoided. I am not recommending that all patients undergo laparoscopic paraesophageal hernia repair, but I think it is still worthy of critical evaluation.

Dr. L. Way (San Francisco, Calif.). There were 11 perforations, a substantial number. Some were made by the operating surgeons and others by whoever was passing the bougie. In my experience, events such as these are largely avoidable; somehow one learns to prevent them. I wonder, therefore, whether those who were actually handling the instruments were thoroughly experienced, or if they were relatively inexperienced individuals (e.g., residents) who were acting under the supervision of more experienced people? I suspect this is a systems problem that can be corrected by making changes in the delegation of responsibilities in the operating room.

Dr. Trus. With regard to the bougie perforations, we have actually changed our approach in passing the bougie dilators. We insist on having a staff anesthesiologist present in the room who passes the dilators, or else we pass them ourselves. Also, instead of immediately selecting a large dilator, we progressively increase our dilator size from a 36 Fr dilator to facilitate passage of a large dilator.

Dr. N. Soper (St. Louis, Mo.). There has been controversy regarding whether wraps need to be attached to the abdominal wall. I think some of your problems were related to a shortened esophagus. What type of preoperative tests do you use to measure how high the gastroesophageal junction is and whether or not the procedure can be carried out through the abdomen? You did use mesh in one patient. Would you recommend that?

Dr. Trus. I would not recommend placing mesh in the hiatus to close it. In terms of our preoperative workup, approximately six or seven patients presented acutely and were referred for surgery before a full workup could be completed. In general, we try to perform a barium swallow test and endoscopic examination on all patients, as well as manometry. We do not routinely perform pH studies on these patients. Unfortunately it is sometimes very difficult to obtain manometric data because the catheters cannot be passed. The preoperative barium swallow is only a guide to esophageal length. We do not make our final decision about whether to perform a Collis procedure until we have fully mobilized the esophagus and are not able to obtain adequate length. We perform all of our lengthening procedures laparoscopically at Emory. I believe the same criteria are used in Portland but they have been using a thoracoscopic approach for the Collis procedure. We use fundoplication to prevent reflux, which has been shown to be present in up to 60% of these patients. Gastropexy was used only in patients who were quite frail, when there was concern that the patient would not tolerate a more extensive procedure.

Portal Hypertension Triggers Local Activation of Inducible Nitric Oxide Synthase Gene in Colonic Mucosa

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Recently a new clinical entity "portal hypertensive colopathy" has been reported. It involves vascular abnormalities and bleeding. Because nitric oxide may mediate these changes, we studied whether portal hypertension affects nitric oxide synthase in portal hypertensive colonic mucosa. In portal hypertensive and sham-operated rats the following studies were done: (1) colonic mucosal blood flow, (2) quantitative histologic examination, (3) reverse transcription–polymerase chain reaction for nitric oxide synthase mRNA, (4) nitric oxide synthase activity assay, and (5) immunostaining for nitric oxide synthase. In portal hypertensive rats, colonic mucosal blood flow and the number of submucosal veins were significantly increased in comparison to sham-operated rats. The mRNA expression and enzyme activity for inducible nitric oxide synthase (but not constitutive nitric oxide synthase) were significantly increased in portal hypertensive rats. Fluorescence signal intensity for inducible nitric oxide synthase in endothelia of mucosal and submucosal veins was significantly higher in portal hypertensive rats than in sham-operated rats. Portal hypertension activates inducible nitric oxide synthase gene and protein in colonic mucosal vessels. The excess of nitric oxide generated by overexpressed inducible nitric oxide synthase may play an important role in the development of vascular and hemodynamic abnormalities characterizing portal hypertensive colopathy. (J GASTROINTEST SURG 1997;1:229-235.)

Recent studies suggest that in addition to portal hypertensive (PHT) gastropathy,[1,2] portal hypertension may also affect the colonic mucosa, causing hemorrhage in PHT patients.[3,4] This new clinical entity has been named "portal hypertensive colopathy," and it is characterized by lesions including colonic vascular ectasias and rectal varices.[5,6] However, the pathophysiology of the PHT colon has not been clearly defined.

Nitric oxide is considered to be a mediator of the hyperdynamic circulation of portal hypertension.[7] The nitric oxide synthase (NOS) enzyme exists in two forms: a constitutive moiety (c-NOS), which is calcium dependent, and an inducible moiety (i-NOS), which is calcium independent.[8] The i-NOS is induced in response to bacterial endotoxins and cytokines in a variety of cell types including endothelial cells, hepatocytes, and macrophages.[8] Recently we demonstrated overexpression of c- and i-NOS genes and proteins in the PHT esophagus,[9] suggesting that development of vascular abnormalities in the PHT esophagus can be related to overproduction of nitric oxide. However, it is not known whether the NOS gene and protein may also be activated in PHT colonic mucosa, which has a different structure and function from esophageal mucosa. Furthermore, lipopolysaccharide has varying effects on induction of i-NOS expression in different tissues,[10] and the degree of NOS expression may also

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Presented in part at the Thirty-Seventh Annual Meeting of The Society for Surgery of the Alimentary Tract, San Francisco, Calif., May 19-22, 1996. An abstract of this work was published in Gastroenterology 1996;110:1408A.
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be different in various segments of the gastrointestinal tract in PHT rats. Therefore this study was performed as a first step to characterize functional and morphologic abnormalities in the PHT colonic mucosa, focusing our investigations on expression of NOS genes and proteins in the PHT colonic mucosa.

MATERIAL AND METHODS

Experimental Design

This study was approved by the Subcommittee for Animal Studies of the Long Beach (Calif.) Department of Veterans Affairs Medical Center. Thirty Sprague-Dawley rats (weight 250 to 300 g) were used for the experiments. Portal hypertension was produced by staged portal vein occlusion as previously described.[11] Sham-operated rats underwent similar procedures but with no occlusion of the portal and splenic veins. After 2 weeks, the rats were fasted for 24 hours and each rat underwent measurement of portal venous pressure and colonic mucosal blood flow. Following the hemodynamic studies, a 5 cm segment of the colon, 1 cm proximal to the anal verge, was excised and rapidly frozen in liquid nitrogen or fixed in 10% formalin or 4% paraformaldehyde.

Measurements of Portal Venous Pressure and Colonic Mucosal Blood Flow

Portal venous pressure was measured in each rat by means of a PE-50 catheter inserted through a peripheral mesenteric vein. The zero reference point was the inferior vena cava. Colonic mucosal blood flow was measured using the laser Doppler flowmeter (BLF-21, Transonic Systems Inc., Ithica, N.Y.). The fiberoptic probe (HL-P1002) was inserted into the colonic lumen through the anus and applied gently to the colonic mucosa. Blood flow measurements were recorded on a computer equipped with a Windaq/200 program (Dataq Instruments, Inc., Akron, Ohio). A measurement was considered satisfactory when it was stable for at least 10 seconds, free of motion artifacts, and the reading was reproducible. The mucosal blood flow was expressed as tissue perfusion units (tpu), which approximately correspond to milliliters per minute per 100 g of tissue.

Quantitative Histologic Examination

Colonic specimens fixed in 10% formalin were embedded in paraffin. Mucosal sections were stained with hematoxylin and eosin. Coded sections were evaluated quantitatively using a microscope (Olympus BH-2, Olympus Corp., Tokyo, Japan) connected to a computer software program (SigmaScan/Image, Jandel Scientific, San Rafael, Calif.). The thickness of the epithelium, lamina propria, and muscularis mucosae was measured under 200× magnification in 10 randomly selected fields. In addition, we measured the total number and area of submucosal veins in 5 cm mucosal strips.

RNA Isolation and Reverse Transcription–Polymerase Chain Reaction for NOS

Frozen specimens were homogenized with a Polytron (Kinematica AG, Littau, Switzerland) in 4 mol/L guanidinium isothiocyanate, and total RNA was prepared after the guanidinium isothiocyanate-phenol-chloroform procedure.[12] Reverse transcription–polymerase chain reactions (RT-PCR) for NOS were carried out using a GeneAmp RNA PCR kit and a DNA thermal cycler (Perkin-Elmer Corp., Norwalk, Conn.) as previously described.[9] The specific primer set used for c-NOS was 5′-TACGGAGCAG-CAAATCCAC-3′ (forward) and 5′-CAGGCT-GCAGTCCTTTGATC-3′ (reverse).[13] The specific primer set used for i-NOS was 5′-CACAAGGCCA-CATCGGATTTC-3′ (forward) and 5′-TGCATAC-CACTTCAACCCGAG-3′ (reverse).[14] PCR for β-actin was used as a positive control and an internal standard. The specific primer set for rat β-actin was 5′-TTGTAACCAACTGGGACGATATGG-3′ (forward) and 5-′GATCTTGATCTTCATGGT-GCTAGG-3′ (reverse.)[15] The PCR products were subjected to electrophoresis on 1.5% agarose gel and visualized by ethidium bromide staining. For quantitation we determined the intensity of the PCR products on the negative film of the gel photographs using a video image analysis system (Image-1/FL, Universal Imaging Corp., Westchester, Pa.).[9,14] The NOS signal was standardized against the β-actin signal obtained from the same RNA sample and expressed as the NOS/β-actin ratio.

Assay of NOS Activity

Nitric oxide synthase activity was measured in frozen colonic tissues by determining the conversion of L-[^{3}H] arginine to [^{3}H] citrulline according to the method described by Fernandez et al.[10] Briefly, after homogenization of colonic samples, the supernate was added to a control buffer consisting of 40 mmol/L potassium phosphate, pH 7.4; 8 mmol/L L-valine; 1

mmol/L reduced nicotinamide adenine dinucleotide phosphate; 1 mmol/L $MgCl_2$; 2 mmol/L $CaCl_2$; 40 μmol/L L-arginine; 10 μg/ml calmodulin; and 0.16 μmol/L L-[^{3}H] arginine monohydrochloride (specific activity 64 Ci/mmol, Amersham Corp., Arlington Heights, Ill.). Assays were also performed in the presence of 10 mmol/L N^{ω}-nitro-L-arginine methyl ester (L-NAME buffer) or in the presence of 10 mmol/L ethyleneglycoltetraacetic acid (EGTA buffer). Samples were incubated for 10 minutes at 37° C and then treated with the Na^+ form of Dowex 50W-X8 (Bio-Rad Laboratories, Life Science Group, Hercules, Calif.) for 15 minutes. The protein content was determined by the bicinchoninic acid protein assay.[16] Results were expressed as picomoles per milligrams of protein per minute. The c-NOS activity was determined from the difference between activities obtained in control and EGTA buffer, and the i-NOS activity was determined from the difference between activities obtained in EGTA and L-NAME buffer.

Immunofluorescence Staining for NOS

Colonic specimens were fixed in 4% paraformaldehyde for 4 hours and subsequently transferred to 0.5 mol/L sucrose in phosphate-buffered saline solution for 24 hours. Then they were frozen at −80° C until cutting. Cryostat sections (10 μm thick; Jung CRYOCUT 1800, Leica, Inc./North America, Deerfield, Ill.) were digested with 0.1% trypsin (Sigma Diagnostics, St. Louis, Mo.) at 37° C for 10 minutes and incubated overnight with specific polyclonal antibodies against c- or i-NOS (anti-rabbit; Affinity BioReagents, Neshanic Station, N.J.) diluted 1:100. After washing with phosphate-buffered saline solution, colonic specimens were incubated for 30 minutes with fluorescein-conjugated anti-rabbit immunoglobulin (Sigma Diagnostics) diluted 1:100. Immunofluorescence was evaluated qualitatively on coded slides using a Nikon Optiphot epifluorescence microscope with B filter composition (Nikon Inc., Garden City, N.Y.). For the quantitative assessment of fluorescence intensity, we used a Nikon TMD Diaphot microscope connected to a video analysis system (Image-1/FL, Universal Imaging Corp.).[9] Fluorescence intensity in the endothelia of mucosal veins (collecting veins) and submucosal veins, muscularis mucosae, and muscularis propria was measured on a pixel-per-pixel basis under 400× magnification in 10 randomly selected fields of each section. All samples for c- or i-NOS were processed and immunostained at the same time, and fluorescence intensity was measured on coded sections during the same session and under the same conditions.

Statistical Analysis

The results were expressed as the mean ± standard deviation. Student's *t* test was used to determine statistical significance between PHT and sham-operated rats.

RESULTS

Portal Venous Pressure and Colonic Mucosal Blood Flow

Portal venous pressure and colonic mucosal blood flow were significantly increased in PHT rats compared to sham-operated control rats (portal venous pressure 25.2 ± 3.4 vs. 16.0 ± 1.9 cm H_2O; colonic mucosal blood flow 5.9 ± 1.4 vs. 3.8 ± 0.6 tpu, respectively; $P < 0.01$).

Quantitative Histologic Findings

There was no significant difference between PHT and sham-operated rats in the thickness of the epithelium, lamina propria, and muscularis mucosae. The number of submucosal veins in PHT rats was significantly increased in comparison to sham-operated rats (29.6 ± 9.7 vs. 18.6 ± 6.4/cm of mucosal strip length; $P < 0.05$), whereas there was no significant difference in the total area of the submucosal veins (0.051 ± 0.016 vs. 0.037 ± 0.024 mm^2/cm of mucosal strip length).

RT-PCR for NOS

The i-NOS mRNA expression in the colonic mucosa of PHT rats was significantly increased compared to sham-operated control rats (i-NOS/β-actin ratio 0.53 ± 0.19 vs. 0.26 ± 0.04, $P < 0.01$; Figs. 1 and 2). However, there was no significant difference in expression of c-NOS mRNA between PHT and sham-operated colonic mucosa (c-NOS/β-actin ratio 0.68 ± 0.06 vs. 0.65 ± 0.12).

NOS Activity Assay

The i-NOS activity in the colonic mucosa of PHT rats was significantly increased compared to sham-operated control rats (0.82 ± 0.53 vs. 0.32 ± 0.30 pmol/mg/min, $P < 0.05$; Fig. 3). However, there was no significant difference in c-NOS activity between

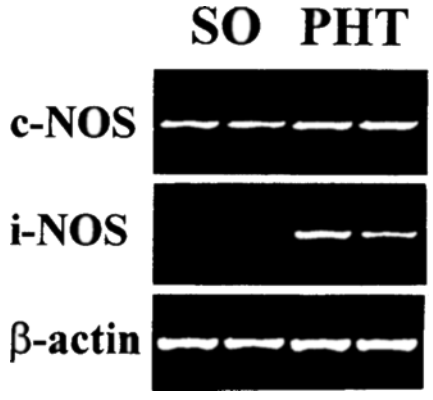

Fig. 1. Expression of c- and i-NOS mRNAs determined with reverse transcription–polymerase chain reaction in colonic specimens of portal hypertensive *(PHT)* and sham-operated *(SO)* rats. The products for β-actin are used as internal standards.

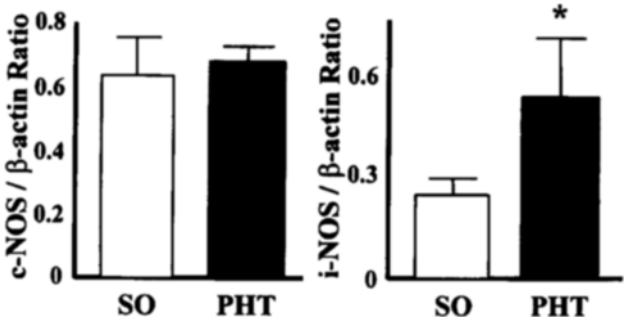

Fig. 2. Relative amounts of c- and i-NOS mRNAs quantified by the Image-1 system and expressed as the NOS/β-actin ratio. Values are mean ± standard deviation. * = P <0.01 compared to sham-operated controls; SO = sham-operated rats (n = 6); PHT = portal hypertensive rats (n = 6).

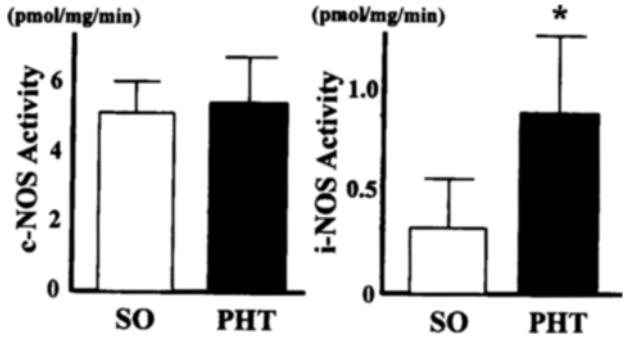

Fig. 3. Enzyme activity for c- and i-NOS in colonic specimens of sham-operated and portal hypertensive rats. Values are mean ± standard deviation (in pmol/mg/min). * = P <0.05 compared to sham-operated controls; SO = sham-operated rats (n = 8); PHT = portal hypertensive rats (n = 8).

PHT and sham-operated colonic mucosa (5.25 ± 1.72 vs. 5.10 ± 1.06 pmol/mg/min).

Immunofluorescence Staining for NOS

The distribution of c- and i-NOS was localized to endothelia of mucosal and submucosal vessels and muscularis mucosae and propria (Figs. 4 and 5).

The intensity of c-NOS immunostaining in endothelia of mucosal and submucosal veins was more than fourfold higher than that in muscularis mucosae and propria in both groups (sham-operated rats: mucosal veins 86.5 ± 12.5, submucosal veins 91.3 ± 11.8, muscularis mucosae 18.2 ± 5.1, and muscularis propria 19.8 ± 12.2; PHT rats: mucosal veins 85.2 ± 15.6, submucosal veins 96.9 ± 10.4, muscularis mucosae 19.2 ± 2.4, and muscularis propria 16.5 ± 5.4 units). Comparison and analysis of these data showed that there was no significant difference in c-NOS fluorescence intensity in endothelia of mucosal and submucosal veins between PHT and sham-operated rats (Fig. 6).

The intensity of the i-NOS fluorescence signal in endothelia of mucosal and submucosal veins was markedly increased by twofold or more compared to that in the muscularis mucosae and propria in PHT colonic mucosa (mucosal veins 35.9 ± 9.4, submucosal veins 47.9 ± 7.7, muscularis mucosae 19.0 ± 6.4, and muscularis propria 18.2 ± 4.6 units). However, there was no difference in i-NOS staining intensity among these four locations in the sham-operated colonic mucosa (mucosal veins 17.1 ± 5.2, submucosal veins 20.8 ± 5.6, muscularis mucosae 16.0 ± 2.9, and muscularis propria 21.2 ± 5.6 units). In PHT rats the intensity of the i-NOS fluorescence signal in endothelia of mucosal and submucosal veins was significantly increased compared to sham-operated controls (respectively, P <0.01; Fig. 6).

DISCUSSION

Naveau et al.[6] showed that the number of intramucosal vascular channels and the cross-sectional vascular area in the colonic mucosa in cirrhotic patients with PHT colopathy were significantly increased in comparison to control patients.[6] Munakata et al.[17] reported that portal hypertension alters the colonic mucosal venous structure by increasing the number and caliber of veins. Our study, which was performed in a standardized experimental model of portal hypertension, confirmed some of these clinical observations. Inasmuch as frequency of rectal varices in patients with extrahepatic portal vein obstruction is much higher than in patients with cirrhosis and the frequency of colonic vasculopathy in patients with

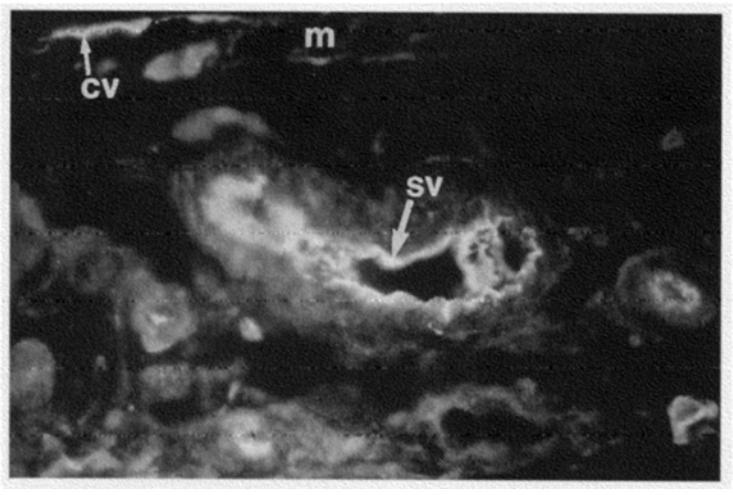

Fig. 4. Photomicrograph of immunofluorescence staining for c-NOS in portal hypertensive colonic mucosa. Staining is localized to endothelia of mucosal and submucosal vessels, muscularis mucosae, and muscularis propria (×400). cv = endothelia of collecting vein (mucosal vein); sv = endothelia of submucosal vein, m = muscularis mucosae.

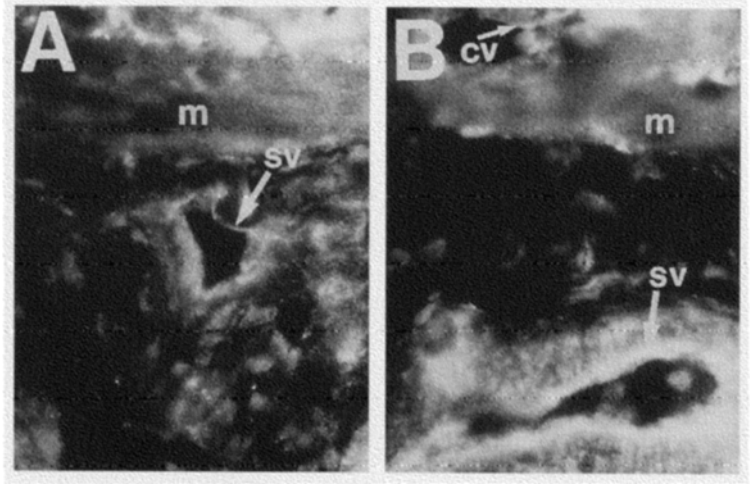

Fig. 5. Photomicrographs of immunofluorescence staining for i-NOS in colonic specimens. The i-NOS localization is similar to that of c-NOS. **A,** Sham-operated rat (×400). **B,** Portal hypertensive rat (×400). cv = endothelia of collecting vein (mucosal vein); sv = endothelia of submucosal vein; m = muscularis mucosae.

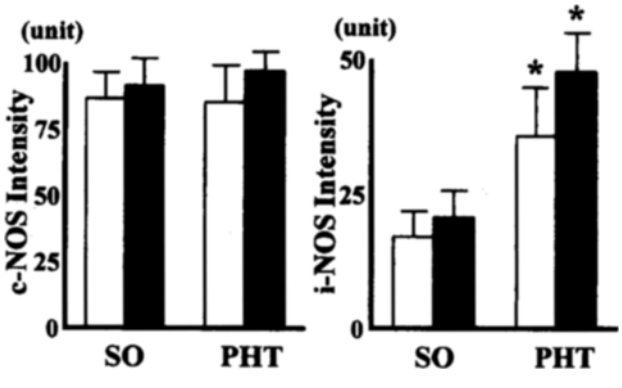

Fig. 6. Immunofluorescence intensity of c- and i-NOS in endothelia of colonic mucosal veins (□) and submucosal veins (■). Values are mean ± standard deviation in image unit. * = $P < 0.01$ compared to sham-operated controls; SO = sham-operated rats (n = 7); PHT = portal hypertensive rats (n = 7).

extrahepatic portal vein obstruction is the same as in patients with cirrhosis,[4] the prehepatic model for portal hypertension in the rat, which we used in the present study, may be valid for investigating PHT colopathy.

Our current study showed that the PHT colonic mucosa has increased blood flow. In addition, we found that portal hypertension increased the i-NOS mRNA expression, resulting in overproduction of i-NOS protein in endothelia of the colonic mucosal and submucosal veins. These findings suggest that overproduction of nitric oxide could contribute to the development of abnormal hemodynamics and microvasculature in PHT colonic mucosa. We also demonstrated that i-NOS enzyme activity in PHT rats was significantly increased compared to sham-operated control rats. However, a direct comparison demonstrated that c-NOS enzyme activity predominates over i-NOS activity in the PHT colon (more than sixfold greater than for i-NOS activity). Thus the total activity of NOS in the PHT colon is increased by only 12% in comparison to sham-operated controls. Since overexpression of i-NOS in the PHT colon, assessed immunohistochemically, is localized to relatively small but strategic areas (predominantly mucosal and submucosal vessels), total NOS activity in these limited areas in the PHT colon is most likely much greater than that in sham-operated control rats. This contention is supported by a significant 55% increase in colonic mucosal blood flow in PHT rats compared to sham-operated rats.

The precise mechanism of i-NOS gene activation by portal hypertension in the colonic mucosa is not clear. Recent evidence suggests that one major mechanism may be based on tumor necrosis factor-alpha (TNF-α). In portal hypertension, plasma levels of TNF-α are significantly increased.[18] This cytokine and interferon gamma increase i-NOS mRNA and NOS activities.[19] Lopez-Talavera et al.[20] demonstrated that TNF-α may be a major contributor to the hyperdynamic circulation of portal hypertension. Since TNF-α does not have a vasodilative effect and there is no correlation between TNF-α blood levels and hemodynamic changes in portal hypertension, TNF-α may be one contributor to a cascade of events that are mediated by other cytokines and metabolic products.[20] The important role of TNF-α is demonstrated by the observation that thalidomide, a selective inhibitor of TNF production, decreases TNF-α plasma levels, nitric oxide production, and the systemic hyperdynamic circulation of portal hypertension.[21] Thus TNF-α may be the key to understanding the regulation of i-NOS in portal hypertension.

We have previously demonstrated that both c- and i-NOS are overexpressed in the PHT esophagus compared to sham-operated controls,[9] whereas in the PHT colon only i-NOS expression is significantly increased, as was found in the present study. These findings demonstrate that portal hypertension produces different changes in NOS expression in different segments of the gastrointestinal tract. These differences in NOS expression between the PHT esophagus and colon may be related to the variability in development of collateral vessels in the PHT esophagus and colon. In the PHT esophagus the total area of submucosal veins is markedly increased by 185% compared to sham-operated controls,[9] but in the PHT colonic mucosa we found no significant differences from sham-operated controls. Thus the PHT esophagus, because of the overabundance of venous architecture, may necessarily overexpress c-NOS, which is a component of the endothelial cell.[8]

CONCLUSION

As demonstrated in this rat model, portal hypertension activates the i-NOS gene in the colonic mucosa with overexpression of i-NOS protein in endothelia of mucosal and submucosal veins. The excess of nitric oxide generated by overexpression of i-NOS may play an important role in the development of the microvascular and hemodynamic abnormalities that characterize PHT colopathy.

We thank Mr. Fred C. Sander for his technical assistance in NOS enzyme activity assay.

REFERENCES

1. Sarfeh IJ, Juler GL, Stemmer EA, Mason GR. Results of surgical management of hemorrhagic gastritis in patients with gastroesophageal varices. Surg Gynecol Obstet 1982;155:167-170.
2. Ohta M, Hashizume M, Higashi H, Ueno K, Tomikawa M, Kishihara F, Kawanaka H, Tanoue K, Sugimachi K. Portal and gastric mucosal hemodynamics in cirrhotic patients with portal-hypertensive gastropathy. Hepatology 1994;20:1432-1436.
3. Ohta M, Hashizume M, Kishihara F, Kawanaka H, Tanoue K, Sugimachi K. Recurrent rectal bleeding from portal hypertensive colopathy in a patient with hemorrhoids. Am J Gastroenterol 1995;90:1531-1533.
4. Ganguly S, Sarin SK, Bhatia V, Lahoti D. The prevalence and spectrum of colonic lesions in patients with cirrhotic and noncirrhotic portal hypertension. Hepatology 1995;21:1226-1231.
5. Kozarek RA, Botoman VA, Bredfeldt JE, Roach JM, Patterson DJ, Ball TJ. Portal colopathy: Prospective study of colonoscopy in patients with portal hypertension. Gastroenterology 1991;101:1192-1197.
6. Naveau S, Bedossa P, Poynard T, Mory B, Chaput JC. Portal hypertensive colopathy: A new entity. Dig Dis Sci 1991;36:1774-1781.

7. Pizcueta MP, Pique JM, Bosch J, Whittle BJR, Moncada S. Effects of inhibiting nitric oxide biosynthesis on the systemic and splanchnic circulation of rats with portal hypertension. Br J Pharmacol 1992;105:184-190.
8. Moncada S, Palmer RMJ, Higgs EA. Nitric oxide: Physiology, pathophysiology and pharmacology. Pharmacol Rev 1991; 43:109-142.
9. Tanoue K, Ohta M, Tarnawski AS, Wahlstrom KJ, Sugimachi K, Sarfeh IJ. Portal hypertension activates the nitric oxide synthase genes in esophageal mucosa of rats. Gastroenterology 1996;110:549-557.
10. Fernandez M, Garcia-Pagan JC, Casadevall M, Bernadich C, Piera C, Whittle BJR, Pique JM, Bosch J, Rodes J. Evidence against a role for inducible nitric oxide synthase in the hyperdynamic circulation of portal hypertensive rats. Gastroenterology 1995;108:1487-1495.
11. Sarfeh IJ, Tarnawski A, Malki A, Mason GR, Mach T, Ivey KJ. Portal hypertension and gastric mucosal injury in rats. Effects of alcohol. Gastroenterology 1983;84:987-993.
12. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidiniumthiocyanate-phenol-chloroform extraction. Anal Biochem 1987;162:156-159.
13. Ujiie K, Yuen J, Hogarth L, Danziger R, Star RA. Localization and regulation of endothelial nitric oxide synthase mRNA expression in the rat kidney. Am J Physiol 1994;267:F296-F302.
14. Griffiths MJD, Liu S, Curzen NP, Messent M, Evans TW. In vivo treatment with endotoxin induces nitric oxide synthase in rat main pulmonary artery. Am J Physiol 1995;268:L509-L518.
15. Nudel U, Zakut R, Shani M, Neuman S, Levy Z, Yaffe D. The nucleotide sequence of the rat cytoplasmic b-actin gene. Nucleic Acids Res 1983;11:1759-1771.
16. Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH. Measurement of protein using bicinchoninic acid. Anal Biochem 1985;150:76-85.
17. Munakata A, Nakajima H, Sasaki Y, Hada R. Does portal hypertension modify colonic mucosal vasculature? Quantification of alteration by image processing and topology. Am J Gastroenterol 1995;90:1997-2001.
18. Panes J, Perry MA, Anderson DC, Muzykantov VR, Carden DL, Miyasaka M, Granger DN. Portal hypertension enhances endotoxin-induced intercellular adhesion molecule 1 up-regulation in the rat. Gastroenterology 1996;110:866-874.
19. Lin JY, Seguin R, Keller K, Chadee K. Tumor necrosis factor alpha augments nitric oxide–dependent macrophage cytotoxicity against *Entamoeba histolytica* by enhanced expression of the nitric oxide synthase gene. Infect Immun 1994;62:1534-1541.
20. Lopez-Talavera JC, Merrill WW, Groszmann RJ. Tumor necrosis factor α: A major contributor to the hyperdynamic circulation in prehepatic portal-hypertensive rats. Gastroenterology 1995;108:761-767.
21. Lopez-Talavera JC, Cadelina G, Olchowski J, Merrill W, Groszmann RJ. Thalidomide inhibits tumor necrosis factor α, decreases nitric oxide synthesis, and ameliorates the hyperdynamic circulatory syndrome in portal-hypertensive rats. Hepatology 1996;23:1616-1621.

The Role of Laparoscopy in the Management of Suspected Pancreatic and Periampullary Malignancies

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Laparoscopic evaluation of patients with suspected periampullary malignancies has been utilized more frequently in recent years. Its exact role with regard to staging and surgical bypass for palliation have yet to be clearly defined. To better define the role of laparoscopy in the evaluation and palliation of periampullary malignancy, a retrospective review of the Duke experience was carried out. Fifty-three patients with suspected pancreatic or periampullary malignancies were referred for surgical evaluation at Duke University Medical Center between 1993 and 1995. All patients underwent CT scanning and lesions were classified as resectable or unresectable based on previously established criteria. Patients either underwent laparoscopic evaluation (n = 30; 11 with laparoscopic palliation) or proceeded directly to celiotomy (n = 23). Charts were reviewed for postoperative course including complications, length of stay, and hospital costs. Although laparoscopy had a sensitivity of 93.3% for metastatic disease, CT scans accurately staged 86.8% of patients missing only one patient with peritoneal/hepatic disease. Based on these results, laparoscopy may not be beneficial for every patient with a suspected pancreatic malignancy. Retrospectively an attempt was made to determine which patients benefited from laparoscopy and which patients are best served by proceeding directly to open exploration. From these data we devised an algorithm that outlines an efficient and cost-effective approach for this patient population. (J GASTROINTEST SURG 1997;1:236-244.)

Every year more than 100,000 Americans die of gastrointestinal malignancies, and carcinoma of the pancreas accounts for almost one fourth of these deaths.[1] Despite advances in diagnosis and surgical treatment, pancreatic cancer continues to have the worst prognosis of all gastrointestinal malignancies with only 3% of patients expected to be alive 5 years after diagnosis.[2] Furthermore, less than 10% to 15% of patients have tumors that are considered surgically resectable at the time of initial presentation and diagnosis.[3,4] Not long ago surgical exploration was the "gold standard" for diagnosis and treatment of patients with presumed pancreatic cancer.[5,6] Surgical intervention was the only way to obtain tissue for accurate diagnosis and at the same time provide palliation for biliary and gastrointestinal obstructions. In recent years more accurate preoperative staging methods have become available, optimizing treatment resources and avoiding surgical exploration in patients with unresectable disease.[7]

The goal of preoperative staging is to ascertain the optimal treatment for each patient by differentiating potentially resectable tumors from locally advanced or metastatic disease. The addition of computerized tomography (CT) has dramatically changed our ability to stage many of these patients preoperatively.[8-12] Recently there has been increasing interest in adding laparoscopy to the methods available to the general surgeon for staging of periampullary malignancies.[7,12-17] Although advances in laparoscopy have been demonstrated in many areas of general surgery within the past several years, the impact of these advances in the management of periampullary malignancy remains unclear. We therefore decided to evaluate our experiences to determine the role of laparoscopy in patients with pancreatic and periampullary malignancies in

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hopes of developing an algorithm that would incorporate laparoscopy into the diagnosis and palliation of this patient population.

PATIENTS AND METHODS

Patients were seen over a 2-year period from October 1993 to October 1995. The study group comprised all patients referred to the surgical service with pancreatic or ampullary masses suspected of being malignant. Previous surgery was not considered cause for exclusion. Patients with biopsy-proved metastatic disease who did not require surgical intervention for palliative purposes were excluded. The final group consisted of 53 patients with localized or locally advanced disease. Forty masses were located in the head of the pancreas, seven lesions were in the ampulla, and six lesions were in the body or tail of the pancreas.

All patients were evaluated by means of routine laboratory tests, chest roentgenography, and CT. Most of the CT scans were obtained by means of dedicated pancreatic protocol. This consisted of a dynamic contrast-enhanced study with 3 to 5 mm scans at 5 mm intervals from the third portion of the duodenum to just above the porta hepatis, with 10 mm contiguous scans through the remainder of the liver and from the third portion of the duodenum to the iliac crests, as previously described by our institution.[10] CT criteria for unresectability, as previously published,[10] included the following: hepatic or distant metastasis; lymphadenopathy (short-axis dimension greater than 7 mm for portal nodes, 10 mm for portacaval nodes, 9 mm for para-aortica nodes, and 8 mm for gastrohepatic ligament nodes[18]); involvement of perivascular fat planes or encasement or occlusion of the superior mesenteric artery or vein, celiac artery, or portal vein; invasion of adjacent organs or peripancreatic fat planes (excluding perivascular fat); ascites; and peritoneal metastasis.

The laparoscopic evaluation was performed starting with a direct entry into the peritoneal cavity at the umbilicus using an open Hasson technique in which a 10 mm port is inserted, the abdominal cavity is insufflated with carbon dioxide, and the laparoscope is inserted. The peritoneal surfaces of liver, intestines, anterior abdominal wall, and pelvis are then inspected. This is assisted by placement of two 5 mm ports at the level of the umbilicus in both the right and left midaxillary lines. The liver is elevated for evaluation of the undersurfaces of both the right and left lobes. The region of the porta hepatis is inspected for enlarged lymph nodes. The transverse colon is elevated and the root of the mesentery is inspected at the level of the superior mesenteric artery and vein, looking specifically for mass effect or frank invasion in this region. The patient is placed in the Trendelenburg position and the pelvis is inspected for "drop metastasis." Any suspicious lesions are biopsied for frozen-section analysis.

Laparoscopic palliative procedures were performed using techniques previously described by our group and others.[19-22] No strict algorithm was prospectively employed. The decision as to whether to proceed directly to celiotomy or to first perform laparoscopy was left to the surgeon's discretion. Factors influencing this decision included resectability of the lesion based on CT scan, previous abdominal operations, need for palliation, and patient age.

All patient charts were reviewed retrospectively for demographic data, preoperative evaluation, operative findings and procedures, and postoperative course. Preoperative CT scans were reviewed for the preoperative interpretation of resectability. The presence of or ability to place an endoscopic or percutaneous biliary stent was noted. The operative and pathologic findings were reviewed. Information related to metastatic spread and vascular or local invasion, as well as the reason for conversion if a laparoscopic examination was performed, was noted. Hospital costs, determined as microcost analysis by the cost accounting specialist at Duke University Hospital, were obtained for each patient including operative costs and total hospitalization.

The information was then retrospectively evaluated in an attempt to determine the most efficient and cost-effective algorithm for the management of patients with suspected pancreatic and periampullary malignancies. Statistical analysis was performed using paired Student's *t* tests of unequal variance.

RESULTS

The study group consisted of 53 patients who had a mean age of 59.1 ± 10.3 years (range 29 to 82 years). Preoperative localization by CT revealed 40 lesions arising in the head, seven in the ampulla, and six in the body or tail of the pancreas.

Preoperative evaluation of the CT scans determined that lesions in 30 patients met the criteria for unresectability and 23 were considered resectable. Operatively 35 patients were considered unresectable and 18 underwent resection with intent for cure. Two of the 30 patients with unresectable "tumors" based on CT were found to be resectable at the time of surgery. Of the 23 patients considered resectable by CT criteria, only 18 had an operation with intent to cure. Of the five patients whose disease was "understaged" by CT, three were found to have lymph nodes with evidence of metastatic disease, one patient had vascular invasion, and one patient had peritoneal dis-

Table I. Relative contraindications*

Previous biliary or pancreatic surgery with or without upper abdominal surgery
Obstructed cystic duct
Tumor <1 cm from hepatocystic junction
Portal hypertension

*These represent what we consider to be the relative contraindications to minimally invasive biliary and gastrointestinal bypass. The current technique for biliary bypass is a cholecystojejunostomy; therefore patients with surgically absent gallbladders are not candidates. An obstructed cystic duct or tumor within 1 cm of the hepatocystic junction is a contraindication to cholecystojejunostomy.[37] Similarly, patients with previous pancreatic, biliary and, to a lesser extent, gastric or upper abdominal procedures have an increased likelihood of adhesions, which might make laparoscopic palliation difficult. In our experience patients with portal hypertension are best treated using an open technique because of bleeding complications.

ease. It was thought that only the latter patient could have benefited from our current technique of staging laparoscopy prior to celiotomy. The overall accuracy of the CT findings was 86.8% with a sensitivity of 78.3% and a specificity of 93.3%. The positive predictive value of a resectable lesion by CT was 90% with a negative predictive value of 84.8%.

Twenty-five patients proceeded directly to celiotomy. Of these, 18 had lesions that were resectable by CT criteria and seven had unresectable lesions. Those with unresectable tumors required surgical palliation and had relative contraindications to laparoscopic palliation (Table I). The five patients with lesions thought to be resectable were mentioned earlier.

Twenty-eight patients underwent a staging laparoscopy. This group consisted of the remaining 23 patients with unresectable tumors and five whose lesions were resectable based on CT. Of the five with resectable disease, two had tail or body lesions, two were elderly, and one was taking part in a neoadjuvant protocol. Both patients with tail/body lesions proceeded to curative resection as did the patient in the neoadjuvant protocol. The other two were found at subsequent celiotomy to have vascular invasion. Two patients with unresectable lesions according to CT criteria (both because of extensive lymphadenopathy) were found at operation: one had chronic pancreatitis and the other had Brunner's gland hamartoma. Both underwent definitive operations. Therefore a total of five patients who ultimately underwent curative resection were in the laparoscopic group. Of the remaining 23 patients who underwent laparoscopy, five had laparoscopy with biopsy alone, six had laparoscopy with laparoscopic palliation, and 12 proceeded to celiotomy and palliation. All 11 patients who underwent laparoscopy, either alone or in conjunction with laparoscopic palliation, had peritoneal, liver, or extensive local disease. All of them had lesions that were unresectable according to the CT criteria. Seventeen patients proceeded to celiotomy after laparoscopy. As mentioned earlier, five underwent resection with intent to cure. Four patients were found to have vascular invasion preventing curative resection; three had positive lymph nodes and one had liver metastasis that was missed at laparoscopy because of adhesions. Of the remaining four patients, all were deemed unresectable at laparoscopy (three with peritoneal/hepatic disease and one with locally extensive disease) but required conversion for palliative purposes: one because of portal hypertension and three because of unfavorable anatomy for laparoscopic palliation.

Overall, when it was used, the laparoscope identified 14 of the 15 patients with either peritoneal or hepatic disease for a sensitivity of 93.3%.

Final pathologic evaluation of the specimens revealed 50 malignancies (41 adenocarcinomas of the pancreas, 7 adenocarcinomas of the ampulla, and 2 neuroendocrine tumors) and three benign lesions (Brunner's gland hamartoma, papillary adenoma, and chronic pancreatitis).

Patients were divided into five subgroups according to the type of treatment they received: (1) celiotomy with intent to cure; (2) celiotomy and palliation; (3) laparoscopy followed by celiotomy with intent to cure; (4) laparoscopy, celiotomy, and palliation; and (5) laparoscopy alone or with laparoscopic palliation. Each of these groups was evaluated with regard to length of postoperative hospital stay, postoperative complications, operating room costs, and total hospital costs (Table II).

All patient groups had similar demographics. However, there were differences in postoperative length of stay and hospital costs that were statistically significant. The patients treated with laparoscopy only and biopsy or laparoscopy and laparoscopic palliation had a 3.7 ± 1.5 day length of stay with a mean hospitalization cost of $9687 ± $4141. In comparison, patients who proceeded directly to celiotomy and had only a palliative procedure had a 10.3 ± 2.6 day hospitalization ($P < 0.00005$) with a mean cost of $14,769 ± $4756 ($P < 0.003$). The group that underwent laparoscopy prior to celiotomy for a palliative procedure had a hospital stay of 11.1 ± 5.9 days and incurred a total cost of $20,145 ± $6639. Although there was no statistical difference in postoperative length of stay between the patients who proceeded directly to celiotomy and those who were converted after laparoscopy ($P < 0.3$), the difference in hospital costs approached statistical significance ($P < 0.13$) with those in the converted group being more expensive to

Table II. Hospitalization statistics*

Procedure	Length of stay (days)	vs. Laparoscopy	vs. Open	Cost (dollars)	vs. Laparoscopy	vs. Open
Laparoscopic	3.7 ± 1.5	—		9687 ± 4171		
Converted	11.1 ± 5.9	$P < 0.0003$	$P < 0.33$	20,145 ± 6639	$P < 0.005$	$P < 0.13$
Celiotomy	10.3 ± 2.6	$P < 0.00005$		14,769 ± 4756	$P < 0.003$	
Whipple	17.5 ± 9.3			24,070 ± 8760		

*Postoperative length of stay and hospital costs are presented as the mean ± standard error of the mean. Patients palliated laparoscopically had the shortest hospital stay and the lowest cost of all groups. Patients who required conversion from laparoscopic to open procedure for palliation had a longer length of stay, costs were higher in this group, and the difference neared statistical significance. Paired Student's *t* tests were used for statistical analysis.

Table III. Postoperative complications by groups*

	Palliation			Cure	
	Laparoscopic	Converted	Celiotomy	Converted	Celiotomy
Postoperative bleeding		2 (17%)			1 (8%)
Ileus/gastroparesis	2 (18%)	1 (8%)	1 (8%)	1 (20%)	3 (23%)
Pulmonary		3 (25%)	1 (8%)	1 (20%)	3 (23%)
Abdominal abscess			1 (8%)		2 (15%)
Wound complication					1 (8%)
Pancreatic leak					2 (15%)
Urinary tract infection					2 (15%)

*A total of 35 patients underwent palliative procedures (11 laparoscopic, 12 converted, and 12 celiotomy). Eighteen patients underwent resection with intent to cure (13 celiotomy and 5 converted).

treat. Laparoscopy at our institution adds approximately 25 to 30 minutes to the operating time at an additional cost of approximately $1200 (for operating room time and supplies).

There was only one operative death in the entire study group. The patient, a 74-year-old man, was found at laparoscopy to have miliary disease and significant portal hypertension. Because of bleeding, he was converted to an open palliative procedure and ultimately died on postoperative day 20. The remaining postoperative complications are listed in Table III.

DISCUSSION

Pancreatic malignancies continue to carry a dismal prognosis despite advances in imaging techniques, more aggressive surgical approaches, and systemic chemotherapy. The 5-year survival averages 1% to 2% with 85% of the patients dying within 12 months of presentation.[14] The main reason for the poor prognosis in exocrine pancreatic cancer is the advanced nature of the disease when it becomes symptomatic. It is estimated that 78% to 95% of all patients have incurable cancer at the time of diagnosis or surgical exploration.[23] Currently the majority of patients are subjected to celiotomy, which is still the most commonly used method for establishing a tissue diagnosis and assessing operability at most centers. Patients with advanced/incurable disease gain little benefit from this surgical exploration except in instances where palliation is necessary. Although we have not been able to markedly increase the survival of patients with pancreatic malignancies, several advances have been made in the past decade that can affect the unfortunate patient with incurable disease.

CT has had a significant impact on the diagnosis and staging of pancreatic malignancies in recent years.[8-10,12,15,24] Intravenous contrast enhancement, dynamic, and now spiral CT imaging have improved the accuracy of this radiologic modality. The accuracy of CT in predicting unresectability of malignancies in the pancreas and periampullary region has been reported to range from 79% to 100%.[10-12,24-26] However, there has been much less agreement on the ability of CT to accurately predict resectability of these malignancies with positive predictive values ranging from 15% to 80%.[9-12,24,26,27] Gulliver et al.[10] previously published results from our institution of a ret-

rospective analysis of 360 consecutive cases of malignant biliary obstruction. That study demonstrated the accuracy of a thin-slice pancreatic protocol for suspected pancreatic and periampullary malignancies, which had a positive predictive value of 89% for unresectable and 80% for resectable tumors. Our current review yielded similar findings with CT accuracy for resectability reaching 86.8%. The sensitivity and specificity of tumor resectability was 78.3% and 93.3%, respectively.

Because of our high level of confidence in the radiologic interpretation of these scans, we believed that the added advantage of staging laparoscopy would not benefit the majority of these patients and would add to the operative time and expense. This is reflected in the fact that only one (4.4%) of the 23 patients with a "resectable" lesion on CT had peritoneal metastasis that could have been detected by laparoscopy. The four patients who had "resectable" lesions on CT scans but who were found to have unresectable lesions at celiotomy had vascular invasion or lymph node involvement. These patients in all likelihood would not have benefited from our current routine of staging laparoscopy. By avoiding unnecessary laparoscopy, not only are the operative time and costs reduced but the patients are spared the as yet unknown effects of laparoscopy on potentially curable malignancies.[28-30]

There were two patients in the retrospective review whose lesions appeared unresectable on CT scans but who went on to resection with an intent to cure. Both patients had enlarged lymph nodes suggestive of metastatic disease. At laparoscopy neither patient was found to have evidence of malignant spread and both were converted to celiotomy. One patient was found to have a Brunner's gland hamartoma and the other had chronic pancreatitis. Neither patient was precluded from definitive procedures as a result of the inaccurate CT or negative laparoscopic findings.

The advent of percutaneous and endoscopic biliary stenting has dramatically altered our approach to patients with clearly unresectable disease. Patients with metastatic disease that is confirmed by percutaneous biopsy can now be treated nonoperatively with no adverse effect on survival.[19,31-33] This patient population was not included in our retrospective study because these patients do not require surgical intervention for either diagnosis or palliation. However, there are a number of patients in whom the diagnosis is not so certain, either because of nondiagnostic percutaneous biopsies or questionable interpretation of CT scans. This is the group of patients that we believe can gain the greatest benefit from diagnostic and therapeutic laparoscopy. Many studies have shown that laparoscopy and guided biopsy can provide a tissue diagnosis of malignant disease affecting the liver and peritoneum with an accuracy of 80% to 98%.[12,34-36] Our results were similar with laparoscopy identifying 14 (93.3%) of 15 patients with peritoneal or hepatic metastases. Therefore those patients in whom a more accurate assessment is needed can then undergo laparoscopy for the definitive diagnosis. Similarly, a small subset of these patients may require biliary or gastrointestinal bypass for palliation. With advancing technique, experience, and instrumentation, these patients can now be treated by means of minimally invasive surgical procedures,[19-22] thus markedly reducing their postoperative stay and hospital costs ($P < 0.000005$).

Our optimal algorithm would be to direct the unresectable patients to the laparoscopic group when possible. However, we have identified a subset of unresectable patients who did not benefit from laparoscopy. This group included the following patients: (1) those who because of previous biliary (excluding cholecystectomy) and pancreatic surgery had adhesions, which precluded adequate visual examination of the peritoneal cavity; (2) those in whom stenting was unsuccessful and who had previously undergone cholecystectomy or had obstructed cystic ducts precluding them from a cholecystojejunostomy[37]; and (3) those with portal hypertension. These patients all required a celiotomy for palliative purposes and therefore did not benefit from laparoscopy. In these cases length of hospitalization is not shortened and there is the added expense of the laparoscopy. If these patients can be identified preoperatively, we favor treating them initially with celiotomy.

We propose the following algorithm to maximize the role of laparoscopy to benefit the patient with a suspected pancreatic or periampullary malignancy (Fig. 1). All patients should undergo a contrast-enhanced dynamic or spiral CT examination with interpretation by an experienced radiologist. Those patients with resectable disease should proceed straight to celiotomy with exploration and attempts at curative resection. Conversely, patients with unfavorable anatomy (because of previous pancreatic or biliary operations, obstruction or absence of a cystic duct in patients in whom endoscopic stenting is unsuccessful, or portal hypertension) should proceed directly to celiotomy. Patients with unresectable disease by CT and in whom a diagnosis cannot be documented by percutaneous biopsy, or who cannot have stents placed endoscopically or percutaneously, should undergo laparoscopy for diagnosis and/or palliation. Those patients in whom the presence of unresectable disease cannot be established at laparoscopy and those in whom surgical palliation cannot be satisfactorily achieved by laparoscopic means, can then undergo ex-

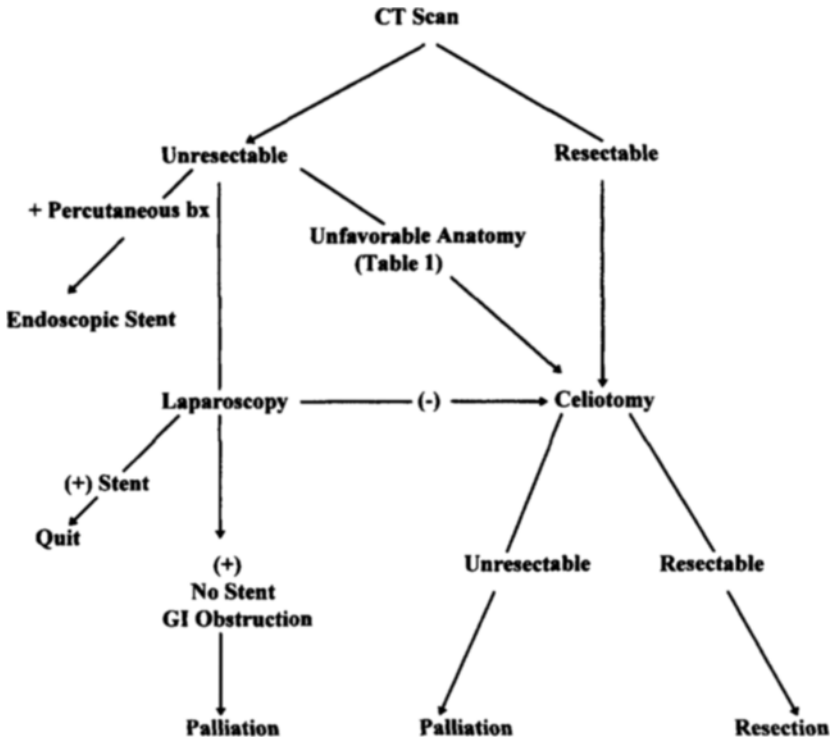

Fig. 1. Proposed algorithm for the use of laparoscopy in the management of patients with suspected pancreatic or periampullary malignancies. This algorithm requires a highly accurate CT scan with intravenous contrast and thin slices (3 mm) through the pancreatic tissue. Advanced laparoscopic skills are required if surgical palliative procedures become necessary.

ploratory celiotomy and possible curative resection.

By following this algorithm for patients with suspected pancreatic or periampullary malignancies, a potential cost savings of up to 13% could be realized. Based on a CT accuracy of 86.8% and the fact that laparoscopy identified peritoneal or hepatic disease in only 4.4% of the patients with "resectable" lesions on CT scans, theoretically 95.6% of the patients would gain no benefit from a laparoscopic examination. Furthermore, these potentially curable patients would not be subjected to the as yet unknown effects of laparoscopy on neoplastic processes.

It is important to note that this review includes all patients who preoperatively were suspected of having pancreatic or periampullary malignancies. The accuracy of the CT scan in determining whether or not a mass is resectable is similar to findings in several previously published series.[9-11,38] Our findings of peritoneal disease in patients thought to be resectable differ from some reports[7,12,16,17] but are similar to those of Fuhrman et al.[38] who used a thin-section CT to determine staging. Some of this difference can be attributed to several factors. This is a small retrospective review. The group of patients reviewed included all patients with *suspected* pancreatic and periampullary malignancies. The final pathologic findings in the group of patients who underwent resection with intent to cure yielded a large number of ampullary carcinomas (n = 7), neuroendocrine tumors (n = 2), and benign processes (n = 3). Taking into account only those patients with pancreatic adenocarcinomas and lesions shown to be resectable by CT scans, the incidence of peritoneal and/or hepatic disease was 17%, which is closer to other reports.

The approach to patients with pancreatic and periampullary malignancies continues to evolve as new advances are being made with increasing frequency. Future factors that could have an impact on this algorithm include (1) laparoscopic ultrasonography,[16,39,40] (2) advanced laparoscopic procedures allowing common bile duct palliation, and (3) the role of neoadjuvant radiation and chemotherapy.

By following this algorithm for patients with suspected pancreatic or periampullary malignancies, we believe that we can optimize the role of laparoscopic evaluation and treatment in this patient population. If patients with unresectable disease can be spared a celiotomy, they have a shorter hospital stay, their costs

are reduced, and they can return to normal activities more rapidly. Similarly, patients with resectable disease do not incur the added expense of laparoscopy and they avoid the possible consequences of laparoscopy, which are still unknown, as they relate to pancreatic and biliary malignancies.

CONCLUSION

Physicians may currently choose from an ever-increasing array of studies and interventions for diagnostic evaluation. Laparoscopy for diagnostic and potential therapeutic interventions is finding an increasing role in the general surgeon's armamentarium. The situation in which the use of laparoscopy will be of most benefit to the patient is still in the evolutionary stage. We propose an algorithm that maximizes the benefits of diagnostic and therapeutic laparoscopy for patients with pancreatic or periampullary malignancies. As technology and experience increase, we must continue to reevaluate our approach to these patients. However, currently we believe that this approach will provide cost-effective care without jeopardizing patient outcome.

REFERENCES

1. American Cancer Society. Cancer Facts and Figures 1991. Atlanta: American Cancer Society, 1991.
2. National Cancer Institute. Annual Cancer Statistics Review 1973-1989. National Institutes of Health Publication No. 92-2789. Bethesda: US Department of Health and Human Services, 1992.
3. Edis AJ, Kiernan PD, Taylor WF. Attempted Curative Resection of Ductal Carcinoma of the Pancreas: Review of Mayo Clinic Experience, 1951-1975. Mayo Clinic Proc 1981;55: 531-536.
4. Longmire WF Jr, Traverso LW. The Whipple procedure and other standard operative approaches to pancreatic cancer. Cancer 1981;47(Suppl):1706-1711.
5. Rosenberg JM, Welch JP, Macaulay WP. Cancer of the head of the pancreas: An institutional review with emphasis on surgical therapy. J Surg Oncol 1985;28:217-221.
6. Connolly MM, Dawson PJ, Michelassi F, Moosa AR, Lowenstein F. Survival in 1001 patients with carcinoma of the pancreas. Ann Surg 1978;206:366-373.
7. Fernandez-del Castillo C, Rattner DW, Warshaw AL. Further experience with laparoscopy and peritoneal cytology in the staging of pancreatic cancer. Br J Surg 1995;82:1127-1129.
8. Bluemke DA, Cameron JL, Hruban RH, Pitt HA, Siegelman SS, Soyer P, Fishman EK. Potentially resectable pancreatic adenocarcinoma: Spiral CT assessment with surgical and pathologic correlation. Radiology 1995;197:381-385.
9. Freeny PC, Marks WM, Ryan JA, Traverso LW. Pancreatic ductal adenocarcinoma: Diagnosis and staging with dynamic CT. Radiology 1988;166:125-133.
10. Gulliver DJ, Baker ME, Cheng CA, Meyers WC, Pappas TN. Malignant biliary obstruction: Efficacy of thin-slice dynamic CT in determining resectability. Am J Radiol 1992; 159:503-507.
11. Freeny PC, Traverso LW, Ryan JA. Diagnosis and staging of pancreatic adenocarcinoma with dynamic computed tomography. Am J Surg 1993;165:600-606.
12. Warshaw AL, Gu Z, Wittenberg J, Waltman AL. Preoperative staging and assessment of resectability of pancreatic cancer. Arch Surg 1990;125:230-237.
13. Cuschieri A, Hall AW, Clark J. Value of laparoscopy in the diagnosis and management of pancreatic carcinoma. Gut 1978;19:672-677.
14. Cuschieri A. Laparoscopy for pancreatic cancer: Does it benefit the patient? Eur J Surg Oncol 1988;14:41-44.
15. Warshaw AL, Tepper JE, Shipley WU. Laparoscopy in the staging and planning of therapy for pancreatic cancer. Am J Surg 1986;151:76-80.
16. John TG, Greig JD, Carter DC, Garden OJ. Carcinoma of the pancreatic head and periampullary region. Tumor staging with laparoscopy and laparoscopic ultrasound. Ann Surg 1995;221:156-164.
17. Cuschieri A. Laparoscopic surgery of the pancreas. J R Coll Surg Edinb 1994;39:178-184.
18. Dorfman RE, Alpern MB, Gross BH, Sandler MA. Upper abdominal lymph nodes: Criteria for normal size determination with CT. Radiology 1991;180:319-322.
19. Collins BH, Pappas TN. Laparoscopic gastrojejunostomy. In Pappas TN, Schwartz LB, Eubanks S, eds. Atlas of Laparoscopic Surgery. Philadelphia: Churchill Livingstone, 1996, pp 5.3-5.13.
20. Chari RS, Pappas TN. Laparoscopic cholecystojejunostomy. In Pappas TN, Schwartz LB, Eubanks S, eds. Atlas of Laparoscopic Surgery. Philadelphia: Current Medicine, 1996, pp 10.2-10.8.
21. Brune IB, Schonleben K. Laparoskopische Seit-Zu-Seit Gastro-Jejunostomie. Chirurg 1992;63:577-580.
22. Fletcher DR, Jones RM. Laparoscopic cholecystojejunostomy as palliation for obstructive jaundice in inoperable cancer of the pancreas. Surg Endosc 1992;6:147-149.
23. Warshaw AL, Fernandez-del Castillo C. Pancreatic cancer. N Engl J Med 1992;326:455-465.
24. Dooley WC, Cameron JL, Pitt HA, Lillemoe KD, Yue NC, Venbrux AC. Is preoperative angiography useful in patients with periampullary tumors? Ann Surg 1990;211:649-655.
25. Roos WK, Welvaart K, Bloem JL, Hermans J. Assessment of resectability of carcinoma of the pancreatic head by ultrasound and computed tomography: A retrospective analysis. Eur J Surg Oncol 1990;16:411-416.
26. Ross CB, Kaufman AJ, Sharp KW, Andrews T, Williams LF. Efficacy of computerized tomography in the preoperative staging of pancreatic carcinoma. Am Surg 1988;54:221-226.
27. Nesbit GM, Johnson CA, James EM, MacCarty RL, Nagorney DM, Bender CE. Cholangiocarcinoma: Diagnosis and evaluation of resectability by CT and sonography as procedures complementary to cholangiography. AJR 1988;151:933-938.
28. Easter DW. Potential for abdominal wall implantation after laparoscopic procedures of the hepatobiliary tract. Semin Laparosc Surg 1995;2:163-166.
29. Nduka CC, Monson JRT, Menzies-Gow N, Darzi A. Abdominal wall metastases following laparoscopy. Br J Surg 1994;81:648-652.
30. Mouiel J, Gugenheim J, Toouli J, Crafa F, Cursio R, Chastanet S. Port-site recurrence of cancer associated with laparoscopic diagnosis and resection: The European experience. Semin Laparosc Surg 1995;2:167-175.
31. Cotton PB. Endoscopic methods for relief of malignant obstructive jaundice. World J Surg 1984;8:854-861.

32. Huibregtse K, Katon RM, Coene PP, Tytgat GNJ. Endoscopic palliative treatment in pancreatic cancer. Gastrointest Endosc 1986;32:334-338.
33. Seigel JH, Snady H. The significance of endoscopically placed prosthesis in the management of biliary obstruction due to carcinoma of the pancreas: Results of nonoperative decompression in 277 patients. Am J Gastroenterol 1986;81:634-641.
34. Kuster G, Biel F. Accuracy of laparoscopic diagnosis. Am J Med 1966;42:388-393.
35. Sauer R, Fahrlander H, Friedreich R. Comparison of the accuracy of liver scan and peritoneoscopy in benign and malignant primary metastatic tumors of the liver. Scand J Gastroenterol 1973;8:389-394.
36. Lightdale CJ. Laparoscopy for cancer staging. Endoscopy 1992;24:682-686.
37. Tarnasky PR, England RE, Lail LM, Pappas TN, Cotton PB. Cystic duct patency in malignant obstructive jaundice. An ERCP-based study relevant to the role of laparoscopic cholecystojejunostomy. Ann Surg 1995;221:265-271.
38. Fuhrman GM, Charnsangaveji C, Abbruzzese JL, Cleary KR, Martin RG, Fenoglio CJ, Evans DB. Thin-section contrast-enhanced computed tomography accurately predicts the resectability of malignant pancreatic neoplasms. Am J Surg 1994;167:104-113.
39. Murugiah M, Paterson-Brown S, Windsor JA, Miles WFA. Early experience of laparoscopic ultrasonography in the management of pancreatic carcinoma. Surg Endosc 1993;7:177-181.
40. Machi J, Sigel B, Zaren HA, Kurohiji T, Yamashita Y. Operative ultrasonography during hepatobiliary and pancreatic surgery. World J Surg 1993;17:640-646.

Discussion

Dr. A.L. Warshaw (Boston, Mass.). The role of laparoscopy in the preoperative staging of pancreatic cancer appears to be earning a valid niche; the exact role is still being defined. I have several questions regarding your data and conclusions. First, you included patients with ampullary carcinoma and neuroendocrine tumors, both of which can be expected to have a very low prevalence of hepatic or peritoneal metastases. I wonder how much you have diluted your positive findings of metastases by including those lesions as opposed to restricting your evaluation to true ductal adenocarcinoma.

Second, you have used lymphadenopathy by CT criteria as an index of unresectability. Many studies, including our own, have found that index to be highly unreliable; that is, neither the presence nor the absence of enlarged lymph nodes is an accurate marker for metastases in nodes. Why did you include that criterion?

Third, the prevalence of metastatic disease found by laparoscopy, even adjusting for your patient mix, is considerably lower than other studies have found, both our own and those of Alfred Cuschieri in Scotland. I find it difficult to accept that even thin-slice CT with a good pancreatic protocol makes the difference, because these metastases are so often only a few millimeters in size. We continue to find liver and peritoneal metastases in more than 15% of cases of ductal adenocarcinoma of the head of the pancreas, despite a normal CT scan, and we find an additional 6% with positive peritoneal cytologic findings. Did you look at cytologic washings? Can you comment on the significance or staging utility of occult peritoneal dissemination as manifested by positive cytologic findings?

Dr. M.D. Holzman. To answer your last question first, we have not specifically studied the cytologic findings in this group at this point. With regard to diluting our series, we believe that in most patients with a suspected mass a definitive diagnosis is not made until the pathologic specimen is examined. The group we reviewed was small and, as you point out, there were a large number of ampullary carcinomas, which have a lower anticipated rate of peritoneal disease (10%) as compared to ductal carcinoma (50%). A larger series might well show a higher incidence of peritoneal and/or hepatic disease. However, the incidence of peritoneal disease is similar to that at some other institutions, particularly M.D. Anderson Hospital, as was pointed out by Fuhrman and his group at this meeting in 1993. Their findings in patients with resectable lesions by thin-slice CT showed about a 4% incidence of peritoneal or hepatic disease, which is similar to our findings. We did find that if you selected out the resectable periampullary and neuroendocrine tumors, there was a 1 in 8, or 12%, incidence of peritoneal or hepatic disease, which once again is lower than what you have reported in your series. Some of this difference might be due to a higher degree of accuracy for thin-slice CT and pancreatic protocol. Radiographic examination of lymph nodes, I would agree, is overkill and potentially inaccurate; in fact, in two cases that were deemed unresectable by CT scan, the lesions were ultimately found to be resectable. We do not place a tremendous amount of emphasis on that. However, if anything, it favors the patient going into the laparoscopic arm of the algorithm. If at laparoscopy no metastatic disease is found, patients would undergo surgical exploration; therefore this does not preclude them from a potentially curative resection, but I agree with you that this is not a sensitive indicator of resectability of the specimen.

Dr. S. Strasberg (St. Louis, Mo.). I think it is important to refer to the previously published work of Garden from Edinburgh, who showed that it is possible to virtually eliminate laparotomies in which resections cannot be performed by combining laparoscopy with laparoscopic ultrasound. Laparoscopic ultrasound appears to be an important component of staging by means of laparoscopy, particularly since it allows a good evaluation of the portal vein. That has been our experience as well.

Dr. Holzman. We did include that in the manuscript. Laparoscopic ultrasound was not used at our institution at

the time that I reviewed this material, and I agree with you that it will only increase the ability of laparoscopic evaluation to determine the resectability of a lesion.

Dr. D. Evans (Houston, Tex.). As you mentioned, our results are very similar to yours. The confusing aspect of all of this literature for me is that most authors define resectability based on the relationship of a low-density tumor to the superior mesenteric artery–celiac axis and/or superior mesenteric vein–portal vein confluence. Then the end point that everyone uses in their studies is whether the tumor, either in whole or in part, is removed by the surgeon. Should not the end point really be the histologic assessment of the margins?

Dr. Holzman We agree that if you leave histologically positive margins, the chance of a curative resection is very small. I would agree that patients with positive histologic margins should be deemed as having unresectable disease for the purpose of the study and those patients should be considered as having undergone a "palliative Whipple procedure."

The Role of Laparoscopy in the Preoperative Staging of Pancreatic Carcinoma

B. Rumstadt, M. Schwab, K. Schuster, E. Hagmüller, M. Trede

Between January 1990 and December 1995, a total of 398 patients underwent laparotomy for pancreatic or periampullary carcinoma at the Surgical Clinic of Mannheim. The tumor was located in the pancreatic head in 290 patients (72.9%), in the body of the pancreas in 42 patients (10.6%), and in the pancreatic tail in 19 patients (4.7%). Forty-seven patients (11.8%) presented with periampullary carcinoma. The preoperative diagnostic workup included abdominal ultrasound, CT scan, endoscopic retrograde cholangiopancreatography, and angiography. One hundred seventy-two patients (43.2%) underwent a tumor resection, 150 (37.7%) had a palliative bypass operation, and 76 (19.1%) underwent only an exploratory laparotomy. Preoperative diagnosis had predicted unresectability in 66 (87%) of the patients who underwent exploratory laparotomy. In 76 patients the intraoperative findings showed an unresectable tumor, which was located in the head of the pancreas in 54 cases (71%), in the body of the pancreas in 17 (22.4%), in the tail region in four (5.3%), and in the periampullary region in one (1.3%). Local signs of unresectability were found in 47 patients (62%) and peritoneal or hepatic metastases in 29 (28.2%). Given that local inoperability can be reliably assessed only at laparotomy, this leaves just 29 (7%) of 398 patients who did not require palliation and whose signs of unresectability could possibly have been discovered by means of the laparoscopic approach. Laparoscopy (including laparoscopic ultrasound) should be used selectively in patients considered probably unresectable who do not require a palliative procedure immediately before the planned operation. (J GASTROINTEST SURG 1997;1:245-250.)

Despite some recent progress, the surgical treatment of pancreatic cancer is still regarded as dangerous (high operative mortality), largely ineffective (poor long-term survival rates), and costly (estimated cost per pancreatic cancer resected in the United States in 1995 = $150,000.[1] These exaggerated and pessimistic conclusions drawn by Gudjonsson[1] must be reconciled in view of the results reported within the past 5 years. Recent advances have led to a decrease in morbidity and mortality, at least in referral centers.[2-9]

Progress has also resulted in improved staging methods (if not earlier diagnosis) for this particular cancer. The aim of these staging procedures is to separate the operable from the inoperable patients early on to avoid unnecessary exploratory laparotomies. However, the inadequacies of common noninvasive staging modalities (e.g., ultrasound imaging including endosonography, endoscopic retrograde cholangiopancreatography [ERCP], CT scan, MRI, or angiography) in the detection of small peritoneal or hepatic metastases have led to the call for laparoscopy as an additional procedure before laparotomy is undertaken. This analysis was undertaken to clarify whether laparoscopy might have contributed to the decrease in unnecessary exploratory laparotomies.

PATIENTS AND METHODS

We prospectively analyzed the medical records from a group of 398 patients who underwent laparotomy for pancreatic or periampullary carcinoma at the Surgical University Clinic Mannheim between January 1990 and December 1995. In 290 patients (72.9%) the tumor was located in the pancreatic head, in 42 patients (10.6%) in the body of the pancreas, and in 19 patients (4.7%) in the pancreatic tail. In 47 patients (11.8%) the lesion was in the periampullary

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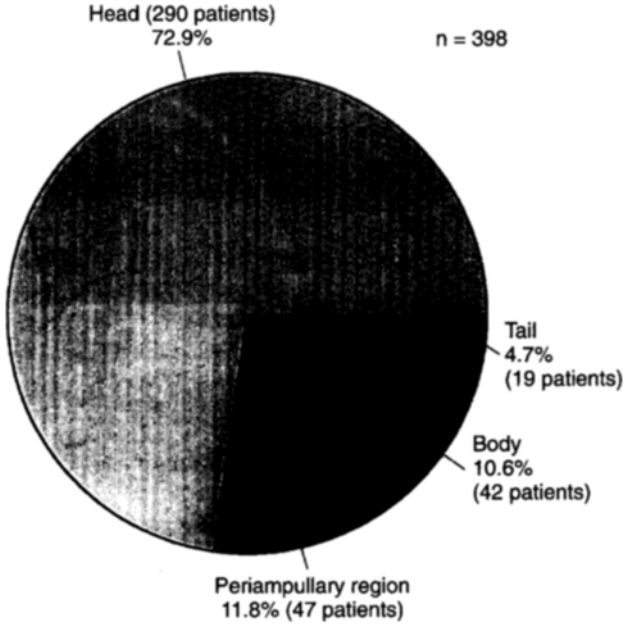

Fig. 1. Localization of pancreatic tumors at the Surgical University Clinic Mannheim, 1990 to 1995.

region (Fig. 1). The diagnostic workup included ultrasound, CT scan, ERCP, and in selected cases mesenteric angiography. Histologic confirmation of malignancy was obtained in all cases.

Patients who underwent only an exploratory laparotomy were divided into the following three groups according to their preoperatively determined likelihood of inoperability:

Group 1 = Inoperability very likely. In these cases infiltration into adjacent tissue or infiltration of the large retroperitoneal vessels or the presence of liver or peritoneal metastases was diagnosed.

Group 2 = Inoperability likely. In these cases staging raised the possibility of infiltration into adjacent tissues, or the presence of suspicious adjacent lymph nodes or a questionable vascular appearance with no signs of vessel infiltration, or the presence of liver or peritoneal metastases.

Group 3 = Inoperability unlikely. In these cases preoperative examination yielded no signs of infiltration into adjacent tissue or distant metastases.

To determine whether an exploratory laparotomy could have been avoided by using diagnostic laparoscopy, we compared the results of preoperative assessment of resectability with the operative findings. Statistical analysis was performed using Fisher's exact test. A P value of ≤ 0.05 was considered significant.

RESULTS

In 172 patients (43.2%) the tumor could be resected curatively (i.e., resected tissue margins were normal and distant metastases were absent) with an operative mortality rate of 2.9%. This left 226 patients (56.8%) for whom a curative resection was not possible because of local unresectability or metastasis. One hundred fifty patients (37.7%) required a bypass operation either for obstruction of the upper gastrointestinal tract or for inadequate endoscopic drainage of the bile duct. One hundred twenty-eight patients (85.3%) had a combination biliary bypass and gastrojejunostomy, 17 patients (11.3%) had a biliary bypass, and five patients (3.3%) underwent gastrojejunostomy alone.

Seventy-six patients (19.1%) had only an exploratory laparotomy (Fig. 2). In this group the tumor was located in the pancreatic head in 54 patients (71%), in the body of the pancreas in 17 (22.4%), and in the pancreatic tail in four (5.3%), and one patient (1.3%) presented with periampullary carcinoma (Fig. 3). The number of patients requiring exploratory laparotomy whose cancer was located in the body of the pancreas is significantly ($P < 0.0001$) higher in comparison to the total patient group.

Of these 76 patients, 48 (63.1%) were classified as group 1 (inoperability very likely), 18 patients (23.7%) as group 2 (inoperability likely), and 10 patients (13.2%) as group 3 (inoperability unlikely). Table I lists the reasons for unresectability. Forty-seven patients (61.8%) showed signs of local unresectability and 29 patients (28.2%) had hepatic or peritoneal metastases. Among these 29 patients, the tumor was located in the head of the pancreas in 21 patients (72.4%), in the body of the pancreas in five (17.2%), and in the tail of the pancreas in two (6.9%), and one patient (3.5%) presented with periampullary carcinoma (Fig. 4). There was no statistically significant difference between the individual tumor locations with regard to the presence of liver or peritoneal metastases. In relation to the total patient group, the prevalence of distant metastases in cases of cancer of the pancreatic body/tail vs. the pancreatic head or periampullary region was 2:1.

Tables II and III demonstrate the effectiveness of preoperative staging with regard to the diagnosis of inoperability in the groups undergoing palliative bypass surgery and complete tumor resection, respectively.

The postoperative morbidity rate after exploratory laparotomy was 2.9% (n = 2); one patient had a minor wound infection and one had prolonged bowel atony. Both patients were managed conservatively. No patient died as a result of the exploratory laparotomy.

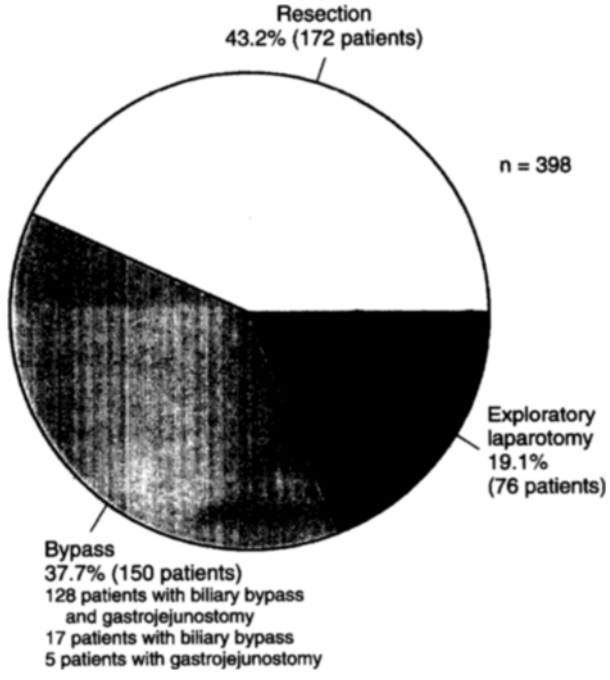

Fig. 2. Surgical therapy for pancreatic malignancies at the Surgical University Clinic Mannheim, 1990-1995.

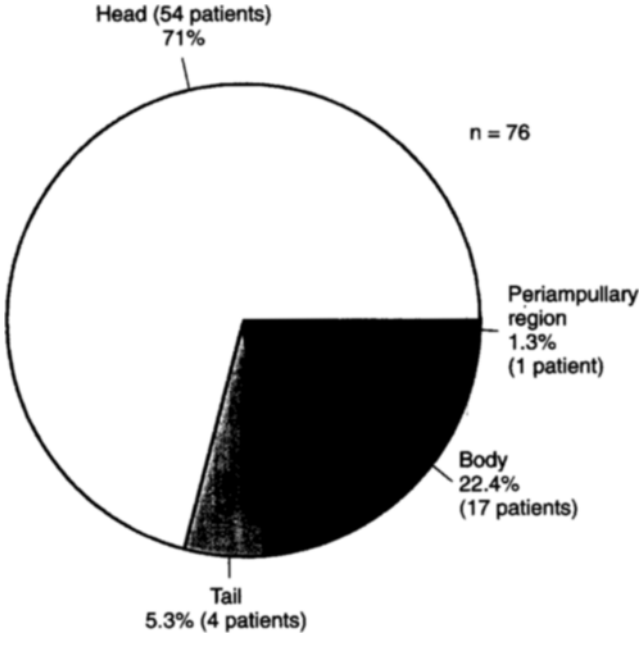

Fig. 3. Localization of pancreatic tumors in patients undergoing exploratory laparotomy for pancreatic carcinoma at the Surgical University Clinic Mannheim, 1990-1995.

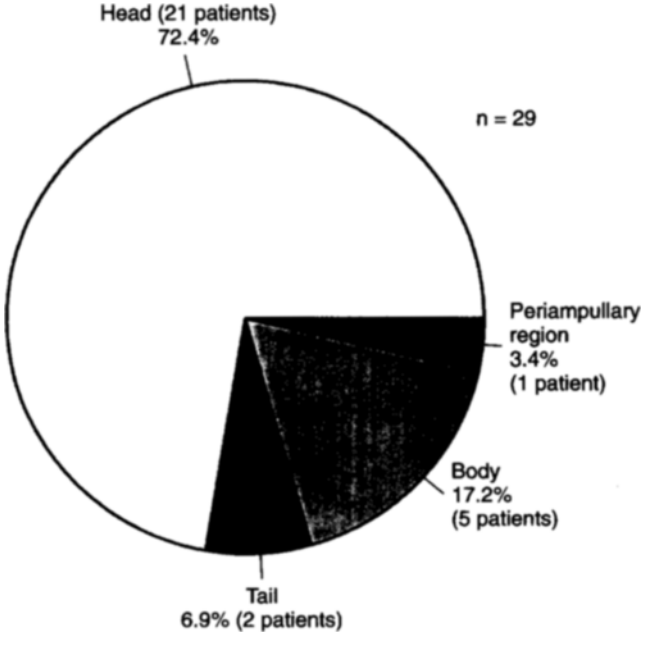

Fig. 4. Localization of pancreatic tumors in patients undergoing exploratory laparotomy because of peritoneal carcinomatosis or hepatic metastases at the Surgical University Clinic Mannheim, 1990-1995.

Table I. Effectiveness of preoperative staging (assessment of unresectability) in 76 patients in whom exploratory laparotomy did confirm unresectability

Preoperative assessment of unresectability*	Total No. of patients (%)	Locally unresectable	Liver metastases	Peritoneal carcinomatosis
Very likely	48 (63.1)	28	12	8
Likely	18 (23.7)	12	6	—
Unlikely	10 (13.2)	7	1	2
TOTAL	76	47	19	10

*Preoperative assessment included ultrasound, CT scan, ERCP, and mesenteric angiography.

Table II. Effectiveness of preoperative staging (assessment of unresectability) in 150 patients in whom a bypass operation was performed

Preoperative assessment of unresectability*	Total No. of patients (%)	Locally unresectable	Liver metastases	Peritoneal carcinomatosis
Very likely	93 (62.0)	46	31	16
Likely	38 (25.3)	13	24	1
Unlikely	19 (12.7)	13	3	3
TOTAL	150	72	58	20

*Preoperative assessment included ultrasound, CT scan, ERCP, and mesenteric angiography.

Table III. Effectiveness of preoperative staging (assessment of unresectability) in 172 patients in whom a curative resection was possible

Preoperative assessment of unresectability*	Total No. of patients (%)	Locally unresectable	Liver metastases	Peritoneal carcinomatosis
Very likely	1 (0.6)	—	—	—
Likely	6 (3.5)	—	—	—
Unlikely	165 (95.9)	—	—	—
TOTAL	172			

*Preoperative assessment included ultrasound, CT scan, ERCP, and mesenteric angiography.

DISCUSSION

Surgery still offers the only prospect of cure for patients with pancreatic carcinoma. Despite substantially improved diagnostic methods, the early diagnosis of pancreatic carcinoma is still more or less a matter of chance, and the surgeon will be confronted predominantly with patients in the advanced stages of the disease. In view of the effective "noninvasive" palliation techniques available[10,11] and the considerable impact on the quality of life for patients undergoing unnecessary exploratory laparotomy, accurate staging is of major importance. The shortcomings of traditional staging modalities, with margins of error as high as 40%,[12-15] have led to the introduction of laparoscopy into the staging process.[16-18] The decisive advantage of laparoscopy lies in its ability to detect most (if not all) small but visible peritoneal or hepatic metastases, nodules that would be missed by all other staging procedures currently available. Its shortcoming lies in its inability to predict local unresectability, a factor that proved decisive given the 62% rate of unresectability for all patients who underwent only exploratory laparotomy.

John et al.[19] were able to show that laparoscopic ultrasound could provide valuable information in addition to the knowledge gained from conventional laparoscopy. Laparoscopic ultrasound can be very valuable in the assessment of local unresectability, particularly in the difficult anatomic area of the pancreatic head.[20,21] A critical review of the study pre-

sented by John et al. also shows, however, that the combination of laparoscopy and laparoscopic ultrasound yielded false negative results in 16 of 40 patients and a false positive result in one. Also, there was a 2.5% morbidity rate (asymptomatic port site hemorrhage in one patient).

Conlon et al.,[22] in their prospective study of 115 patients, reported a positive predictive value of 100% and a false negative rate of 9%. In the assessment of local resectability, they examined the foramen of Winslow, the ligament of Treitz, and the mesocolon; in addition, after making an incision in the gastrohepatic omentum, they inspected the celiac axis and the subhepatic vena cava. This major operation, performed through four ports (3 × 10 mm, 1 × 5 mm), was possible in 94% of the patients studied. Twelve patients had to undergo laparotomy for a bypass procedure after the exploratory laparoscopy and seven patients were subjected to an additional exploratory laparotomy. There was no postoperative morbidity or mortality. The authors did not state the time required for this surgery. Despite the positive results reported by Conlon et al., we would question whether laparoscopic inspection of a portion of the portal vein through the foramen of Winslow or laparoscopic preparation of the gastrohepatic omentum and the celiac axis can effectively replace localized staging procedures including a Kocher maneuver performed by an experienced surgeon. The same applies to the detection of lymph node involvement between the vena cava and the aorta.

If one assumes that truly accurate assessment of local resectability is possible only by means of the traditional surgical approach of open exploration, then only the 29 patients with peritoneal carcinomatosis or hepatic metastases would have benefited from laparoscopy. Thus, in a cohort of 398 patients, "unnecessary" laparotomy could have been avoided in only 29 (7%)!

In our series the rate of unresectability detected by laparoscopy would have been increased to 27% (107 of 398 patients) if patients with bypass procedures and liver/peritoneal metastases were included (n = 78). The question of palliation solely by means of interventional therapy for the 78 patients who required palliation is difficult to analyze retrospectively. Without a doubt the nonoperative biliary stenting procedure is an effective option for palliative management of biliary obstruction.[11,23-25] Considering the high rate of recurrent jaundice—up to 38%—following stent implantation,[8,11,23] with the need for rehospitalization and gastrojejunostomy in 89% of our patients, we favor surgical palliation. In patients treated with interventional therapy, the initially higher cost of surgical palliation should be compared to the cost of repeated stent changes. Of course primary management does not exclude interventional therapy for those patients in whom an exploratory laparotomy would result in a diminished quality of life.

One may wonder why 66 patients were subjected to surgical exploration in view of the fact that conventional staging procedures had indicated that the tumors were "likely" or even "very likely" unresectable. In our opinion a laparotomy is always justified if there is the slightest doubt about local resectability (particularly in a fit and motivated patient). Often patients are encountered who have pinned all their hopes on an operation. The operation should, of course, never be performed solely to comply with a patient's request, but to refuse the last chance of definitive confirmation of the diagnosis may do more psychological harm than physical good.

The morbidity after explorative laparotomy in our series (2.9%) was no greater than that after extensive laparoscopic surgery (2.5%).[19] The potential risk posed by laparoscopic surgery is not negligible and increases with the scope of the intra-abdominal preparation and the number of ports used.[26] In terms of economic considerations, the introduction of laparoscopy as an independent staging modality must also be viewed critically. The personnel involved and the technical equipment required for the procedure are considerable, and the use of general anesthesia is unavoidable. Laparoscopy, especially in combination with laparoscopic ultrasound,[19] is a useful addition in the staging of pancreatic carcinoma. Although this modality has significant advantages, we believe that it should be used selectively in view of its inherent limitations and risks. For these reasons, at the Surgical Clinic of Mannheim, diagnostic laparoscopy is being used selectively in patients deemed "likely unresectable" who require no palliative procedures. The number of patients with carcinoma of the body of the pancreas who have undergone exploratory laparotomies is significantly higher when compared to the total number of patients. The ratio of prevalence of liver/peritoneal metastases in patients with cancer of the pancreatic body and tail to patients with cancer of the pancreatic head or periampullary region was 2:1. This increase in distant metastases signifies that patients with tumors in the body or tail region benefit from a diagnostic laparoscopy, since palliative operative bypass procedures are usually not warranted.

Laparoscopy (including laparoscopic ultrasound) is performed during the same session (and under the same general anesthesia) immediately before possible exploratory laparotomy.

Continuing advances in noninvasive staging

modalities, such as ultrafast magnetic resonance imaging,[27] will probably result in a further reduction in the use of laparoscopy in the staging of pancreatic tumors. This development could represent real progress and might lead to an effective minimization of preoperative staging as well as avoidance of exploratory laparotomies.

REFERENCES

1. Gudjonsson B. Carcinoma of the pancreas: Critical analysis of costs, results of resections, and the need for standardized reporting. J Am Coll Surg 1995;181:483-495.
2. Trede M, Scwall G, Saeger HD. Survival after pancreaticoduodenectomy. Ann Surg 1990;211:447-458.
3. Cameron JL, Crist DW, Sitmann JV, et al. Factors influencing survival after pancreaticoduodenectomy for pancreatic cancer. Am J Surg 1991;161:120-125.
4. Gordon TA, Burleyson GP, Teilsch JM, et al. The effects of regionalization on cost and outcome for one general high-risk surgical procedure. Ann Surg 1995;221:43-49.
5. Conlon KC, Klimstra DS, Brennan MF. Long-term survival after curative resection for pancreatic ductal adenocarcinoma. Ann Surg 1996;223:273-279.
6. del Castillo CF, Rattner DW, Warshaw AL. Standards for pancreatic resection in the 1990s. Arch Surg 1995;130:295-300.
7. Nitecki SS, Sarr MG, Colby TV, et al. Long-term survival after resection for ductal adenocarcinoma of the pancreas. Ann Surg 1995;221:59-66.
8. Lillemoe KD. Current management of pancreatic carcinoma. Ann Surg 1995;221:133-148.
9. Yeo CJ, Cameron JL, Lillemoe KD, et al. Pancreaticoduodenectomy for cancer of the head of the pancreas. Ann Surg 1995;221:721-733.
10. Watanapa P, Williamson RCN. Surgical palliation for pancreatic cancer: Developments during the past two decades. Br J Surg 1991;78:1053-1058.
11. Shepard HA, Royle G, Ross APR, et al. Endoscopic biliary endoprosthesis in the palliation of malignant obstruction of the distal common bile duct: A randomized trial. Br J Surg 1988;75:1166-1168.
12. Roder JD, Rösch T, Bautz W, et al. Pankreaskarzinom—präoperative Diagnostik und Indikationsstellung. Chirurg 1994;65:225-231.
13. Ishikawa O, Imaoka S, Ohigashi H, et al. A new method of intraoperative cytodiagnosis for more precisely locating the occult neoplasm of the pancreas. Surgery 1992;111:294-300.
14. Murughia M, Windsor JA, Redhead DN, et al. The role of selective visceral angiography in the management of pancreatic and periampullary cancer. World J Surg 1993;17:796-800.
15. Fuhrmann GM, Charnsangavej C, Abbruzzese JL, et al. Thin-section contrast-enhanced computed tomography accurately predicts the resectability of malignant pancreatic neoplasms. Am J Surg 1994;167:104-113.
16. Cuschieri A, Hall AW, Clark J. Value of laparoscopy in the diagnosis and management of pancreatic carcinoma. Gut 1978;672-677.
17. Warshaw AL, Tepper JE, Shipley WU. Laparoscopy in the staging and planning of therapy for pancreatic cancer. Am J Surg 1986;151:76-80.
18. Del Castillo CF, Warshaw L. Peritoneal metastases in pancreatic carcinoma. Hepatogastroenterology 1993;40:430-432.
19. John TG, Greig JD, Carter DC, et al. Carcinoma of the pancreatic head and periampullary region. Tumor staging with laparoscopy and laparoscopic ultrasonography. Ann Surg 1995;221:156-164.
20. Serio G, Fugazzola C, Iacono C, et al. Intraoperative ultrasonography in pancreatic cancer. Int J Pancreatol 1992;11:31-41.
21. Cuesta MA, Meijer S, Borgstein PJ, et al. Laparoscopic ultrasonography for hepatobiliary and pancreatic malignancy. Br J Surg 1993;80:1571-1574.
22. Conlon KC, Dougherty E, Klimstra D, et al. The value of minimal access surgery in the staging of patients with potentially resectable peripancreatic malignancy. Ann Surg 1996;223:134-140.
23. Bornman PC, Harries-Jones EP, Tobias R, et al. Prospective controlled trial of transhepatic biliary endoprosthesis versus bypass surgery for incurable carcinoma of head of pancreas. Lancet 1986;1:69-71.
24. Andersen JR, Sorensen SM, Kruse A, et al. Randomized trial of endoscopic endoprosthesis versus operative bypass in malignant obstructive jaundice. Gut 1989;30:1132-1135.
25. Dowsett JF, Russell RCG, Hatfield ARW, et al. Malignant obstructive jaundice: A prospective randomized trial of by-pass surgery versus endoscopic stenting [abst]. Gastroenterology 1989;96:128A.
26. Oshinsky GS, Smith AD. Laparoscopic needles and trocars: An overview of designs and complications. J Laparosc Surg 1992;2:117-125.
27. Edelmann RR, Wielopolski P, Schmitt F. Echo-planar MR imaging. Radiology 1994;192:600-612.

Intraislet Somatostatin Inhibits Insulin (Via a Subtype-2 Somatostatin Receptor) But Not Islet Amyloid Polypeptide Secretion in the Isolated Perfused Human Pancreas

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It is our hypothesis that intraislet somatostatin regulates beta cell secretion in the isolated perfused human pancreas. The present study was designed to determine the relative influence of intraislet somatostatin on the regulation of islet amyloid polypeptide (IAPP) and insulin secretion, and to determine the effect of specific somatostatin receptor (SSTR) agonists on beta cell secretion during immunoneutralization of endogenous somatostatin in the isolated perfused human pancreas. Single-pass perfusion was performed in pancreata obtained from seven cadaveric organ donors using a modified Krebs medium with 3.9 mmol/L glucose. Sequential test periods were separated by basal periods and experiments were performed by infusion of any of the following: (1) somatostatin monoclonal antibody (S-Ab); (2) S-Ab + SSTR2 agonist (DC32-87); or (3) S-Ab + SSTR5 agonist (DC32-92). The changes in insulin and IAPP secretion from basal levels during each stimulation were calculated. Infusion of S-Ab resulted in a significant increase in insulin secretion (2033 ± 429 pmol/L; $P < 0.05$) but not IAPP. In the presence of S-Ab, infusion of the SSTR2 agonist resulted in a significant inhibition of insulin secretion (−1128 ± 457 pmol/L; $P < 0.05$) but not IAPP. In the presence of S-Ab, infusion of the SSTR5 agonist had no significant effect on insulin or IAPP secretion. We conclude that intraislet somatostatin inhibits insulin secretion via SSTR2, but not IAPP secretion, in the isolated perfused human pancreas model and that this effect occurs via SSTR2. These results also suggest that insulin and IAPP secretion are regulated by different mechanisms despite being co-localized to the beta cell.(J GASTROINTEST SURG 1997;1:251-256.)

The inhibitory biologic activities of somatostatin are mediated by five high-affinity receptors that have been identified in pancreatic islet cells, pituitary cells, pituitary plasma membranes, and brain synaptosome membranes.[1-6] Data from our laboratory suggest that there exists an endocrine axis within the human islet in which somatostatin secreted from the delta cell inhibits secretion of insulin from the beta cell, thus the axis is delta to beta.[7,8] Furthermore, the data suggest that somatostatin inhibits insulin secretion in human islet via a somatostatin receptor subtype-2 (SSTR2).[9]

Islet amyloid polypeptide (IAPP) and insulin are costored in beta cell secretory granules and are co-secreted in response to various stimuli.[10-12] Although the two hormones are co-localized within the secretory granules, studies have demonstrated that IAPP and insulin secretion may be differentially regulated, resulting in changes in the molar ratio of IAPP/insulin secreted under certain circumstances.[13,14]

The purpose of the present study was to determine whether intraislet somatostatin inhibits both insulin and IAPP secretion from the beta cell. Furthermore, the effect of specific somatostatin receptor agonism during immunoneutralization of endogenous intraislet somatostatin on IAPP and insulin secretion was investigated in the isolated perfused human pancreas.

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MATERIALS AND METHODS

Isolated Perfused Human Pancreas

Pancreata were obtained from seven cadaveric organ donors following brain death resulting from subarachnoid hemorrhage (n = 5) or trauma (n = 2). The donors ranged in age from 11 to 56 years. There were five males and two females. There was no history of pancreatic disease in any of the donors; however, they all received a multitude of preprocurement medications.

During organ procurement the pancreas was perfused with chilled Viaspan solution (University of Wisconsin organ preservation solution, Du Pont Pharmaceuticals, Wilmington, Del.) and transported back to the laboratory on ice in preparation for perfusion as previously described.[15] On the perfusion apparatus the splenic artery was cannulated with a 14-gauge catheter and single-pass perfusion was performed. All leaking vessels were ligated and the pancreatic duct was cannulated with an 18-gauge catheter. The splenic vein was cannulated with silicone rubber tubing with multiple side holes. The perfusate was a Krebs-bicarbonate buffer that contained 0.5% human albumin (Alpha Therapeutics, Torrance, Calif.), 4% T-70 dextran (Sigma Chemical, St. Louis, Mo.), and 3.9 mmol/L glucose. The pH of the buffer was 7.4 and it was oxygenated with a mixture of 95% oxygen/5% carbon dioxide. The temperature was maintained at 37° C. The perfusion rate was kept constant at 0.4 ± 0.01 ml/min/g and the perfusion pressure was 30 ± 2 cm H_2O.

Experimental Design

Infusates were added to the perfusion medium via sidearm infusion at a rate of 0.1 ml/min (Harvard Pump, Harvard Apparatus, Inc., Natick, Mass.). After a 60-minute equilibration period, 10-minute stimulation periods separated by 10-minute basal periods were used to perform experiments over a perfusion time of 280 ± 72 minutes. The following infusates were used: somatostatin monoclonal antibody (S-Ab) (2.0 mg/ml) alone, S-Ab + SSTR2 agonist (DC 32-87) (5 ng/ml), and S-Ab + SSTR5 agonist (DC-32-92) (5 ng/ml). Antibody dosages were calculated to allow for immunoneutralization of a 100-fold excess of estimated levels of intraislet somatostatin, and SSTR receptor agonists were used at a dosage in the range of the estimated intraislet level of endogenous somatostatin.[7,9] Each preparation also received a 10-minute infusion of 16.7 mmol/L glucose at the end of each experiment. Aliquots of venous effluent were collected every 2 minutes from the splenic vein catheter, mixed with 500 KIU/ml Trasylol (Miles Laboratories, New York, N.Y.), and frozen at −20° C for subsequent radioimmunoassay.

Immunoreactive insulin (IRI) and immunoreactive islet amyloid polypeptide (IR-IAPP) levels in the splenic vein effluent were measured by radioimmunoassay using standard single-antibody technique as described previously.[16,17]

Data Analysis

Hormone levels in the splenic vein effluent were measured to assess the effects of S-Ab and the SSTR agonists on basal IRI and IR-IAPP. Hormone secretion in response to test infusions is represented as the delta change from the basal level in picomoles per liter. The integrated hormonal response seen during an infusion period is calculated as the weighted mean decrease (or increase) in hormone secretion below (or above) basal levels using the trapezoidal rule. The hormone secretory rate immediately preceding a test infusion was taken as the baseline secretory rate for that infusion period. The influence of each infusate on the delta change of hormone secretion from the basal level was analyzed by performing a paired Student's t test. A P value <0.05 was considered to be significant in all cases.

This study was reviewed and approved by the Institutional Review Board of the VAMC–West Los Angeles, Los Angeles, California. The human pancreata were procured in coordination with the Regional Organ Procurement Agency in Southern California and with the Liver Transplantation Service at UCLA School of Medicine. Informed consent was obtained from the next-of-kin of each donor subject.

RESULTS

Insulin and IAPP Secretion

The effect of each infusate on insulin and IAPP secretion is presented in Figs. 1 and 2, respectively.

S-Ab Infusion. Infusion of S-Ab resulted in a significant increase in insulin secretion. In response to immunoneutralization of intraislet somatostatin by the infusion of S-Ab, the average change from basal secretion for insulin was 2033 ± 429 pmol/L (n = 11; $P<0.05$). Infusion of S-Ab had no significant effect on IAPP secretion. In response to immunoneutralization of intraislet somatostatin by the infusion of S-Ab, the average change from basal secretion for IAPP was 10 ± 14 pmol/L (n = 11; P = NS).

S-Ab + SSTR2 Agonist (DC32-87) Infusion. In the presence of S-Ab, infusion of the SSTR2 agonist (DC32-87) resulted in a significant suppression of insulin secretion. The average change from basal secretion during combined antibody and agonist infusion for insulin was −1128 ± 457 pmol/L (n = 7; $P<0.05$). In the presence of S-Ab, infusion of the SSTR2 agonist had no significant effect on IAPP secretion. The

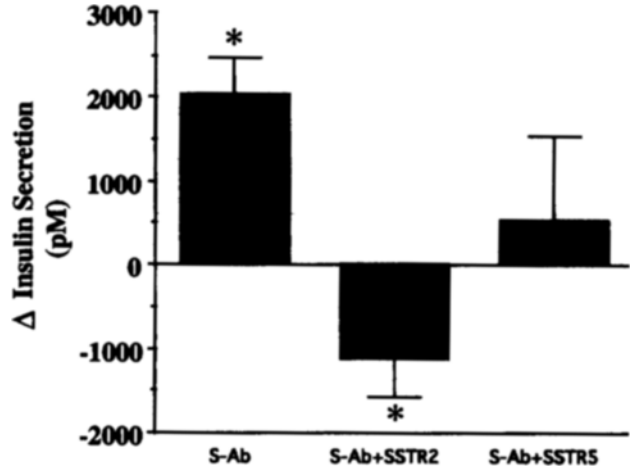

Fig. 1. Response of insulin secretion to infusion of S-Ab alone and S-Ab + SSTR agonist. Infusion of S-Ab resulted in a significant increase in insulin secretion. The average change from basal secretion during antibody infusion for insulin was 2033 ± 429 pmol/L (n = 11; $P < 0.05$). In the presence of S-Ab, infusion of the SSTR2 agonist resulted in significant suppression of insulin secretion. The average change from basal secretion during antibody and agonist infusion was −1128 ± 457 pmol/L (n = 7; $P < 0.05$). In the presence of S-Ab, infusion of the SSTR5 agonist had no significant effect on insulin secretion. The average change from basal secretion during antibody and agonist infusion for insulin was 583 ± 1000 pmol/L (n = 5; P = NS).

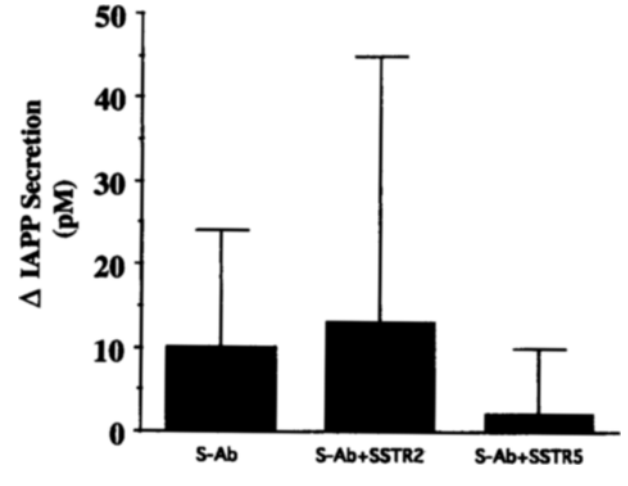

Fig. 2. Response of IAPP secretion to infusion of S-Ab alone and S-Ab + SSTR agonist. Infusion of S-Ab had no significant effect on IAPP secretion. The average change from basal secretion during antibody infusion for IAPP was 10 ± 14 pmol/L (n = 11; P = NS). In the presence of S-Ab, infusion of the SSTR2 agonist had no significant effect on IAPP secretion. The average change from basal secretion during antibody and agonist infusion for IAPP was 13 ± 32 pmol/L (n = 7; P = NS). In the presence of S-Ab, infusion of the SSTR5 agonist had no significant effect on IAPP secretion. The average change from basal secretion during antibody and agonist infusion for IAPP was 2 ± 8 pmol/L (n = 5; P = NS).

average change from basal secretion during combined antibody and agonist infusion for IAPP was 13 ± 32 pmol/L (n = 7; P = NS).

S-Ab + SSTR5 Agonist (DC32-92) Infusion. In the presence of S-Ab, infusion of the SSTR5 (DC32-92) agonist had no significant effect on insulin secretion. The average change from basal secretion during combined antibody and agonist infusion for insulin was 583 ± 1000 pmol/L (n = 5; P = NS). In the presence of S-Ab, infusion of the SSTR5 agonist had no significant effect on IAPP secretion. The average change from basal secretion during combined antibody and agonist infusion for IAPP was 2 ± 8 pmol/L (n = 5; P = NS).

DISCUSSION

In the present study, infusion of a potent somatostatin monoclonal antibody into the human pancreas resulted in a significant increase in insulin secretion but not IAPP secretion. During immunoneutralization of endogenous somatostatin with S-Ab, the SSTR2 agonist significantly inhibited insulin secretion but not IAPP. The potency of this SSTR agonist is demonstrated by the ability of the agonist to completely override the stimulatory effect of somatostatin immunoneutralization and inhibit insulin secretion. These data confirm our earlier finding that immunoneutralization of intraislet somatostatin results in increased insulin secretion and that this effect occurs via a somatostatin receptor subtype-2.[7-9] Furthermore, the data suggest that insulin and IAPP are regulated by different mechanisms in the isolated perfused human pancreas.

The role of the somatostatin-secreting D cell in the islet of Langerhans remains unresolved. One potential role for islet somatostatin is the regulation of glucagon, insulin, and pancreatic polypeptide secretion by the A, B, and PP cells, respectively; however, local regulatory effects have not been conclusively demonstrated. It remains unclear whether A and B cells are physiologically exposed or responsive in vivo to the high concentrations of somatostatin-14 (SS-14) that are likely to be present within the islet. Several studies have supported a role for somatostatin within the islet. Low doses of a somatostatin analogue ([D-Ala^5-D-Trp^8]SS) indirectly stimulated glucagon and insulin secretion by suppressing pancreatic somatostatin release in dogs.[18] In isolated rat islets, insulin and glucagon secretion was stimulated in the presence of a polyclonal somatostatin antibody.[19] Other studies have demonstrated no effect of somatostatin within the islet. In an elegant series of studies using perfused pancreata of different species, antegrade infusion of a polyclonal somatostatin antibody had no effect on insulin secretion.[20,21] It was hy-

pothesized from these studies that SS-14 is secreted from the delta cell and exits the islet without affecting the secretion of the other cell types within the islet. The conclusion drawn from these studies was that intraislet somatostatin had no role within the islet. We have consistently demonstrated a significant increase in insulin secretion during the immunoneutralization of somatostatin in the isolated perfused human pancreas. In previous studies, we demonstrated that infusions of either the S-Ab or the Fab fragment to S-Ab resulted in a significant increase in insulin secretion, suggesting that intraislet somatostatin inhibits insulin secretion.[7,8] These results, and the observations in the current study, suggest that intraislet somatostatin has an inhibitory effect on insulin secretion in the human islet. It is difficult to state why the results from our model would differ from the above-mentioned studies in which perfused pancreas models were used; possibilities include differences in the technique or the antibodies used for immunoneutralization. The observations in the current study provide support for the existence of an intraislet delta-to-beta cell axis in which intraislet somatostatin secreted from the delta cell inhibits insulin secreted from the beta cell.

There have recently been five somatostatin receptor subtypes cloned. To determine the receptor subtype responsible for the effect of somatostatin on insulin secretion, receptor agonists were infused into the isolated perfused human pancreas.[9] Infusions of SS-14, octreotide, or an SSTR2 agonist into the isolated perfused human pancreas resulted in a significant inhibition of insulin secretion.[7-9] Infusion of the SSTR5 agonist also appeared to inhibit insulin secretion, but the response narrowly missed significance. An SSTR3 had no significant effect on insulin secretion. Agonists to SSTR1 and SSTR4 have not yet been synthesized.

To determine whether immunoneutralizing endogenous intraislet somatostatin would alter the insulin secretory response to the receptor agonists, the SSTR2 and SSTR5 agonists were infused together with the S-Ab. In a previous study, infusions of SS-14 with S-Ab resulted in stimulation of insulin secretion, indicating the potency of the S-Ab for immunoneutralizing both endogenous and exgenous SS-14.[7,8] In this study the combined infusion of S-Ab with the SSTR2 agonist resulted in a significant inhibition of insulin secretion. This surprising result indicates the potency of this receptor agonist, DC32-87, which was able to completely counteract the stimulatory effect on beta cell secretion of somatostatin immunoneutralization. Although it is known that the S-Ab does not cross-react with the SSTR agonists, the result was nevertheless surprising. It should be pointed out that the SSTR5 agonist, DC32-92, appeared to inhibit the stimulatory response of insulin to S-Ab; however, the difference did not achieve statistical significance. These data support the concept that somatostatin inhibits insulin secretion via SSTR2 in the human islet.

IAPP is a 37–amino acid polypeptide for which a single-copy gene exists on human chromosome 12.[22] IAPP is produced predominantly in the pancreas where it co-localizes with insulin in beta cell secretory granules,[12] and scattered IAPP immunoreactivity is seen in cells in the stomach, duodenum, and rectum.[23] The increased incidence of amyloid deposits in patients with non–insulin-dependent diabetes mellitus,[24] the association of elevated plasma IAPP levels with some states of glucose intolerance,[17,25] and the ability of IAPP to cause peripheral insulin resistance in vitro suggest that IAPP may have some diabetogenic role. However, IAPP is found in the pancreata of healthy individuals as well and may have a normal physiologic role.[26]

Results of previous studies suggest that although IAPP and insulin are co-localized within the beta cell, the two hormones have different secretory patterns and appear to be regulated by differing mechanisms.[10-12, 14, 27-32] Exogenous somatostatin infusion results in a proportional decrease in IAPP and insulin secretion from the isolated perfused rat pancreas,[33,34] and infusion of the somatostatin analogue SMS 201-995 resulted in decreased IAPP and serum insulin levels in normal human volunteers.[27] The findings of a relatively greater increase in IAPP messenger RNA versus insulin messenger RNA in human islets exposed to high levels of glucose or induction of diabetes via pretreatment with dexamethasone or streptozotocin supports the existence of differential regulation of IAPP and insulin gene expression.[28] An increase in the ratio of circulating IAPP to insulin is seen in patients with pancreatic cancer.[17] In the isolated perfused rat pancreas, significant differences were seen in the response of IAPP and insulin secretion to glucose stimulation.[14,29] Estimates from studies of IAPP and serum levels of insulin in normal humans in response to glucose stimulation indicate an early decrease from the baseline IAPP/insulin ratio in response to oral or intravenous glucose tolerance tests.[27,30] Other studies differ with regard to the differential regulation of secretion of IAPP and insulin; in both neonatal rat monolayer cell cultures and isolated rat islets, equivalent responses of IAPP and insulin to glucose were demonstrated.[10,11] However, the data in this study suggest that IAPP and insulin are not secreted in constant molar ratios, although they

are copackaged in beta cell secretory granules. Possible explanations for these differences in secretory patterns include the presence of a consecutive pathway for IAPP secretion, possible production of IAPP by a non–beta cell source as potential routes, and the presence of granules with differing relative concentrations of these two hormones.[31,32] Although IAPP and insulin are copackaged in beta cell secretory granules and are generally secreted in parallel, this study and others present evidence that IAPP and insulin are not secreted in a constant molar proportion and may have differing responses to beta cell stimulants. Relative differences in the inhibitory tone of intraislet somatostatin on the pathways of IAPP and insulin secretion may account for the differential regulation of these hormones.

In the isolated perfused human pancreas, infusion of S-Ab had no effect on IAPP secretion. Infusions of the SSTR2 and SSTR5 agonists during immunoneutralization of intraislet somatostatin by S-Ab also had no significant effect on IAPP secretion. These observations suggest that although intraislet somatostatin and the SSTR2 agonist have potent inhibitory effects on insulin secretion, they have no effect on IAPP secretion in this model. It is possible that exogenous somatostatin could suppress IAPP secretion via a different SSTR, for example, SSTR1 or SSTR4. These data confirm results from previous studies demonstrating that regulation of IAPP and insulin secretion occurs via differing mechanisms despite their colocalization within the secretory granules of the human beta cell.

In the present study we demonstrated that immunoneutralization of intraislet somatostatin with S-Ab resulted in increased insulin secretion, but not IAPP secretion, supporting the concept that intraislet somatostatin inhibits insulin secretion but not IAPP secretion. The inhibitory effect on insulin secretion appears to occur via SSTR2. We conclude that intraislet somatostatin inhibits insulin secretion, but not IAPP secretion, via SSTR2 in the isolated perfused human pancreas model and that insulin and IAPP are regulated by differing mechanisms despite being colocalized to the beta cell. This study supports the proposal that there exists a delta-to-beta cell endocrine axis within the human islet in which the delta cell secretory product inhibits insulin secretion from the beta cell, and that the effect occurs via SSTR2.

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REFERENCES

1. Mehler PS, Sussman AL, Maman A, et al. Role of insulin secretagogues in the regulation of somatostatin binding by isolated rat islet. J Clin Invest 1980;66:1334-1338.
2. Schonbrunn A, Tasjian AH Jr. Characterization of functional receptors for somatostatin in rat pituitary cells in culture. J Biol Chem 1978;253:6473-6483.
3. Schonbrunn A, Tasjian AH Jr. Modulation of somatostatin receptors by thyrotropin-releasing hormone in a clonal pituitary cell strain. J Biol Chem 1980;255:190-198.
4. Srikant CB, Patel YC. Somatostatin analogs: Dissociation of brain receptor binding affinities and pituitary actions in the rat. Endocrinology 1981;108:341-343.
5. Leitner JW, Rifkin RM, Maman A, et al. Somatostatin binding to pituitary plasma membranes. Biochem Biophys Res Commun 1979;87:919-927.
6. Srikant CB, Patel YC. Somatostatin receptors: Identification and characterization in rat brain membranes. Proc Natl Acad Sci USA 1981;78:3930-3934.
7. Kleinman R, Ohning G, Wong H, et al. The regulatory role of intraislet somatostatin on insulin secretion in the isolated perfused human pancreas. Pancreas 1994;9:172-178.
8. Kleinman R, Gingerich R, Wong H, et al. Use of the Fab fragment for immunoneutralization of somatostatin in the isolated perfused human pancreas. Am J Surg 1994;167:114-119.
9. Moldovan S, Atiya A, Adrian TE, et al. Somatostatin inhibits B-cell secretion via a subtype-2 somatostatin receptor in the isolated perfused human pancreas. J Surg Res 1995;59:85-90.
10. Kahn SE, D'Alessio DA, Schwartz MW, et al. Evidence of cosecretion of islet amyloid polypeptide and insulin by B-cells. Diabetes 1990;39:634-638.
11. Stridsberg M, Sandler S, Wilander E. Cosecretion of islet amyloid polypeptide (IAPP) and insulin from isolated rat pancreatic islets following stimulation or inhibition of B-cell function. Regul Pept 1993;45:363-370.
12. Lukinius A, Wilander E, Westermark GT, et al. Co-localization of islet amyloid polypeptide and insulin in the B-cell secretory granules of the human pancreatic islets. Diabetologia 1989;32:240-244.
13. Inoue K, Hisatome A, Umeda F, et al. Relative hypersecretion of amylin to insulin from rat pancreas after neonatal STZ treatment. Diabetes 1992;41:723-727.
14. Gedulin B, Cooper GJS, Young AA. Amylin secretion from the perfused pancreas: Dissociation from insulin and abnormal elevation in insulin-resistant diabetic rats. Biochem Biophys Res Commun 1991;180:782-789.
15. Brunicardi FC, Druck P, Seymour NE, et al. Selective neurohormonal interactions in islet cell secretion in the isolated perfused human pancreas. J Surg Res 1991;48:273-278.
16. El Shami AS, Durham AP. Solid phase insulin radioimmunoassay with human serum based calibrators: Clinical data and comparison with four other methodologies [abstr]. Clin Chem 1983;29:1170-1171.
17. Permert J, Larsson J, Westermark GT, et al. Islet amyloid polypeptide in patients with pancreatic cancer and diabetes. N Engl J Med 1994;330:313-318.
18. Taborsky GJ. Evidence of a paracrine role for pancreatic somatostatin in vivo. Am J Physiol 1983;245:E598-E603.
19. Itoh M, Mandarino L, Gerich JE. Antisomatostatin gamma globulin augments secretion of both insulin and glucagon in vitro: Evidence for a physiologic role for endogenous somatostatin in the regulation of pancreatic A- and B-cell function. Diabetes 1980;29:693-696.

20. Samols E, Stagner JI, Ewart RBL, et al. The order of islet microvascular cellular perfusion is B to A to D in the perfused rat pancreas. J Clin Invest 1988;82:350-353.
21. Stagner JI, Samols E. The vascular order of islet cellular perfusion in the human pancreas. Diabetes 1992;41:93-97.
22. Stridsberg M, Wilander E. Islet amyloid polypeptide (IAPP): A short review. Acta Oncol 1991;30:451-456.
23. Toshimori H, Narita R, Nakazuto M, et al. Islet amyloid polypeptide (IAPP) in the gastrointestinal tract and pancreas of man and rat. Cell Tissue Res 1990;262:401-406.
24. O'Brien TD, Butler PC, Westermark P, et al. Islet amyloid polypeptide: A review of its biology and potential roles in the pathogenesis of diabetes mellitus. Vet Pathol 1993;30:317-332.
25. Koopmans SJ, Radde JK, Krans HM, et al. Biologic action of pancreatic amylin: Relationship with glucose metabolism, diabetes, obesity and calcium metabolism. Neth J Med 1992; 41:82-90.
26. Novials A, Sarri Y, Casamitjana R, et al. Regulation of islet amyloid polypeptide in human pancreatic islets. Diabetes 1993;42:1514-1519.
27. Mitsukawa T, Takemura J, Asai J, et al. Islet amyloid polypeptide response to glucose, insulin and somatostatin analogue administration. Diabetes 1990;39:639-642.
28. Bretherton-Watt D, Ghatei MA, Bloom SR. Altered islet amyloid polypeptide (amylin) gene expression in rat models of diabetes. Diabetologia 1989;32:881-883.
29. O'Brien TD, Westermark P, Johnson KH. Islet amyloid polypeptide (IAPP) does not inhibit glucose-stimulated insulin secretion from isolated perfused rat pancreas. Biochem Biophys Res Commun 1990;170:1223-1228.
30. Hartter E, Svoboda T, Ludvik B, et al. Basal and stimulated plasma levels of pancreatic amylin indicate its co-secretion with insulin in humans. Diabetalogia 1991;34:52-54.
31. Kahn SE, Verchere CB, D'Alessio DA, et al. Evidence for selective release of rodent islet amyloid polypeptide through the constitutive secretory pathway. Diabetologia 1993;36:570-573.
32. De Vroede M, Foriers A, Van De Winkel M, et al. Presence of islet amyloid polypeptide in rat islet B and D cells determines parallelism and dissociation between rat pancreatic islet amyloid polypeptide and insulin content. Biochem Biophys Res Commun 1992;182:886-889.
33. Inoue K, Hisatomi A, Umeda F, et al. Effects of exogenous somatostatin and insulin on islet amyloid polypeptide (Amylin) release from perfused rat pancreas. Horm Metab Res 1992; 24:251-253.
34. Mandarino L, Stenner D, Blanchard W, et al. Selective effects of somatostatin-14, -25 and -28 on in vitro insulin and glucagon secretion. Nature 1981;291:76-77.

Discussion

Dr. D. Anderson (New Haven, Conn.). As I understand the design of your study, you are examining basal secretion of insulin and IAPP. In other words, secretion is not being stimulated by any secretagogue, which calls into question whether this is really a study of regulated secretion or whether it might be a study of either constitutive secretion or leakage of the products from these cells. IAPP and insulin have been colocalized to vacuoles within specific beta cells, so one possible conclusion that could be drawn from your data is that there may be different populations of beta cells, some of which have somatostatin subtype receptors on them and others which do not. Have you conducted any localization studies to examine the cellular distribution of these somatostatin receptor subtypes in populations of beta cells?

Dr. A. Atiya. We have not determined whether there are different subtypes of islet cells that may or may not have somatostatin receptors on them. We assume that all beta cells have somatostatin receptors. It is our belief that intraislet somatostatin secreted from the delta cell regulates beta cell secretion with a tonic inhibition of insulin secretion. Previous data from our laboratory demonstrated that this tonic inhibition occurs during steady-state insulin secretion, whether glucose levels are at 3.9 mmol/L (basal) or 12.1 mmol/L (high glucose). It appears that intraislet somatostatin has no effect on the insulin response to glucose stimulation, that is, to acute stimulation in beta cell secretion. These data support the disinhibition theory. It is interesting that although intraislet somatostatin inhibits insulin secretion, it appears to have no effect on IAPP secretion.

Dr. M. Sarr (Rochester, Minn.). You used seven pancreata, yet on a number of your slides I observed "n = 11." I do not understand that.

Dr. Atiya. The "n" for each study is calculated by the total number of infusions of the agonist or the monoclonal antibody. Each pancreas is subjected to a number of randomized infusions; therefore the "n" can exceed the number of pancreata used. We were concerned about using more than one infusion of a given substance per pancreas for data analysis and we performed a complex intrapancreatic and interpancreatic statistical analysis in conjunction with a biostatistician. He assured us that our technique for statistical analysis of the data was valid.

Dr. Sarr. How does somatostatin work? Is it a hormone, a paracrine agent, or a neurocrine agent.

Dr. Atiya. We believe that intraislet somatostatin is secreted from the delta cell and reaches the beta cell via the microcirculation, not through the interstitium; therefore somatostatin is an endocrine mediator of beta cell secretion within the human islet. Data from our laboratory suggest that there exists a delta-to-beta cell endocrine axis within the islet. No one to date, however, has been able to demonstrate paracrine effects, so it is possible that somatostatin is acting via the interstitium. It could also be acting as a neuropeptide. These issues need to be clarified.

Effects of Dopamine and Alpha-2 Adrenoreceptor Blockade on L-Dopa and Cholecystokinin-Induced Gastroprotection

James M. Cross, M.D., David W. Mercer, M.D., Jeffrey Gunter, B.S., Thomas A. Miller, M.D.

Dopamine and cholecystokinin have been colocalized in neurons and represent endogenous enteric neurotransmitters. Both peptides possess potent protective actions against gastric injury when given exogenously. This study was undertaken in conscious female rats to test the hypothesis that cholecystokinin may exert its protective actions via release of dopamine. Experiments were designed to ascertain whether L-dopa, a dopamine precursor, could prevent gastric injury with the same degree of efficacy as cholecystokinin and to determine what role alpha-2 adrenoreceptors and dopamine receptors play in mediating the protective actions of these peptides. Intraperitoneal administration of L-dopa (1 to 25 mg/kg) in a dose-dependent manner prevented the type of macroscopic injury to the acid-secreting portion of the stomach that is caused by 1 ml of orogastric acidified ethanol (150 mmol/L hydrochloric acid/50% ethanol), an effect corroborated by histologic examination. Administration of either the alpha-2 adrenoreceptor antagonist yohimbine (0.1 to 1.0 mg/kg) or the dopamine receptor antagonist haloperidol (1 to 5 mg/kg) caused a partial reversal of L-dopa–induced protection but not the protective actions of subcutaneous cholecystokinin (100 μg/kg). Simultaneous administration of both receptor antagonists had an additive effect and completely reversed the protective actions of L-dopa. The dopamine precursor L-dopa was just as effective in maintaining the integrity of the gastric epithelium in the face of a damaging insult as the gut peptide cholecystokinin. However, the data indicate that L-dopa initiates its protective actions through activation of both alpha-2 adrenoreceptors and dopamine receptors, whereas the protective effects of cholecystokinin are elicited by means of a different mechanism. (J GASTROINTEST SURG 1997;1:257-265.)

The role of dopamine in gastrointestinal disease was first observed by Strang[1] in 1965. In that report it was noted that there was an association between gastroduodenal ulceration and Parkinson's disease, a disease process characterized by central dopaminergic deficiency. In contrast, duodenal ulcers were, and still are, rarely observed in patients with schizophrenia, a psychiatric disorder characterized by excess dopamine.[2] Since these observations were made, numerous studies have demonstrated the role of dopaminergic involvement in experimental models of gastroduodenal ulceration.[3-7] For example, dopamine agonists have been shown to prevent gastric injury resulting from indomethacin,[5] stress,[6] and ethanol.[8] In addition, dopamine agonists prevent, whereas dopamine antagonists enhance, cysteamine-induced duodenal ulcers.[7] Furthermore, some dopamine antagonists have been shown to induce gastric lesions,[9] which further suggests that endogenous dopamine may play a role in the intrinsic gastric mucosal defense system. However, the signal transduction mechanism(s) by which dopamine renders the stomach less susceptible to damage is not fully understood.

Dopamine is present in large concentrations in the stomach[10] and is a known enteric neurotransmitter.[11] Once released it can bind to a variety of receptors,[12] although by convention it has been proposed that only two dopamine receptors exist.[13] Blockade of these dopamine receptors has been shown to negate the gastroprotective actions of dopamine.[4,14] How-

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ever, it is noteworthy that alpha-2 adrenoreceptor blockade also inhibits dopamine-induced gastroprotection[8] as well as other actions of dopamine.[15]

Cholecystokinin (CCK), on the other hand, is an endogenous gut peptide and is a potent protective agent against acidified ethanol-induced gastric injury when given exogenously.[16] Interestingly, CCK has been colocalized to dopaminergic neurons in the rat ventral mesencephalon, suggesting that CCK may modulate dopaminergic neurotransmission.[17] Since CCK is also a known enteric neurotransmitter,[18] we hypothesized that CCK may exert its protective actions via release of endogenous dopamine. This being the case, we undertook a series of experiments in a conscious rat model of gastric injury.[16] The first study was designed to ascertain whether L-dopa, a dopamine precursor, could prevent gastric injury with the same degree of efficiency as exogenous CCK octapeptide (CCK-8). The second set of experiments examined the effect of haloperidol, a dopamine receptor antagonist, on L-dopa– and CCK-8–induced gastric protection. The third study assessed whether alpha-2 adrenoreceptor blockade could reverse or attenuate the protective actions of these peptides by utilizing the selective antagonist yohimbine.

MATERIAL AND METHODS

Chemicals

L-Dopa, CCK-8, yohimbine, and haloperidol were obtained from Sigma Chemical Company, St. Louis, Missouri. L-dopa, CCK-8, and yohimbine were dissolved in saline solution (0.9%) and haloperidol was dissolved in 100% ethanol.

Animals

Female Sprague-Dawley rats weighing approximately 200 g were used in all experiments and were housed in wire bottom cages at a constant temperature with 12-hour light/dark cycles. The rats were fasted for 18 to 24 hours but allowed free access to water up to the beginning of the studies. On the day of experimentation, all animals were randomly assigned to one of several groups. All experimental protocols were approved by the University of Texas Animal Welfare Committee before any of these studies were conducted.

Effects of L-Dopa on Gastric Injury from Acidified Ethanol

The first set of experiments was designed to ascertain whether the dopamine precursor L-dopa could prevent gastric injury caused by acidified ethanol. Experiments were performed on conscious animals and L-dopa was given intraperitoneally in doses of 1 (N = 6), 5 (N = 6), or 25 (N = 8) mg/kg. The 25 mg/kg dose of L-dopa has been previously shown to prevent gastric injury from absolute ethanol in conscious rats when given 30 minutes before challenging the stomach with this damaging agent.[19] Thus 30 minutes after each pretreatment, all animals were given a 1 ml orogastric bolus of acidified ethanol (150 mmol/L HCl/50% ethanol). The concentration of alcohol used to elicit gastric injury (50%) was chosen because humans consume liquor that contains as much as 40% to 50% alcohol (i.e., 80 to 100 proof whiskey). In addition, this concentration of alcohol in combination with hydrochloric acid has been previously demonstrated to reproducibly induce visible lesion formation in the glandular portion of stomach within 5 minutes of exposure that is easy to quantify by means of macroscopic examinations.[16] Accordingly, rats were killed 5 minutes after being exposed to this damaging agent. Immediately after the animals were killed, all of their stomachs were removed and the total area of macroscopic injury to the acid-secreting portion of the stomach, where damage routinely occurs, was quantified. This was accomplished by measuring the length and width of each lesion with calipers to determine the surface area involved with each lesion and then summating the individual surface areas to gauge the overall surface area injured for each stomach. Because gross damage was confined to the glandular or acid-secreting portion of the stomach, results were recorded as mean damage to this region in $mm^2 \pm$ standard error of the mean for each experimental group.

Morphologic Analysis

In a separate set of experiments using a similar protocol, the morphologic correlates of the resultant injury or protection were determined in animals receiving a 30-minute pretreatment with either intraperitoneal saline solution or L-dopa (25 mg/kg) followed by exposure of the stomach to acidified ethanol. Five minutes later, the rats were killed and a midline laparotomy was performed. The gastroesophageal junction and pylorus were ligated, and 1.5 ml of one-half strength Karnofsky's fixative[20] was injected through the nonglandular forestomach into the gastric lumen using a 27-gauge needle and syringe. Each stomach was rapidly removed and immersed in the same fixative for at least 24 hours prior to processing for light microscopy. After fixation, each stomach was opened along the lesser curvature and sections (2 mm × 10 mm) of the glandular epithelium were excised from identical regions of each stomach. No attempt was made to include or exclude any lesions. Sections were stained with hematoxylin and eosin and then

processed for routine microscopic examination using standard techniques. All slides were prepared in such a fashion that the observer assessing the extent of the resultant injury or protection was kept blinded to the experimental protocol. Only after the degree of damage had been assessed was the specimen decoded and the results collated. Tissue sections were evaluated according to previously published criteria[16,21] for assessing gastric mucosal damage categorized as follows: type I damage = involvement of luminal surface mucous cells only; type II damage = involvement of luminal surface and gastric pit mucous cells; type III damage = involvement of surface and gastric pit mucous cells as well as upper gland cells; and type IV damage = severe injury to all surface and all or most of the glandular epithelium.

Haloperidol Studies

The role of dopamine receptors in L-dopa– and CCK-8–induced gastroprotection was examined by assessing the effect of the dopamine receptor antagonist haloperidol during pretreatment with these agents. For these studies the experimental protocol was identical to that previously described, with the exception of the subcutaneous administration of the dopamine receptor antagonist 30 minutes prior to pretreatment with intraperitoneal saline, L-dopa, or CCK-8. The doses of haloperidol chosen were similar to those used by MacNaughton and Wallace[4] and were shown to prevent adaptive cytoprotection in a dose-dependent manner. Accordingly, haloperidol was given subcutaneously in doses ranging from 1 to 5 mg/kg, whereas control rats received an equal volume (0.2 ml) of 100% ethanol. L-Dopa was given in a dose of 25 mg/kg. CCK-8 was given in a dose of 100 μg/kg, a dosage that was previously shown to prevent acidified ethanol-induced gastric injury.[16] Following the 30-minute pretreatment with saline, L-dopa, or CCK-8, gastric mucosal injury was induced with acidified ethanol and macroscopic injury determined as previously described. A sample size of six or more animals per group was used.

Yohimbine Studies

To ascertain whether the protective actions of L-dopa or CCK-8 against acidified ethanol-induced gastric injury were mediated by activation of alpha-2 adrenoreceptors, studies were undertaken in which the naturally occurring indole alkaloid, yohimbine, was used to block the alpha-2 adrenoreceptor.[15] For these studies the experimental protocol was again virtually identical to that previously cited, except that a single 1 ml intraperitoneal injection of yohimbine (0.1 to 1 mg/kg) was administered 30 minutes prior to saline, L-dopa (25 mg/kg), or CCK-8 (100 μg/kg) pretreatment, whereas control rats received an equal volume of saline solution. Following the 30-minute pretreatment with saline, L-dopa, or CCK-8, gastric mucosal injury was induced and macroscopic damage quantified. The 0.5 mg/kg dose of yohimbine has been previously shown to inhibit the protective actions of L-dopa against concentrated ethanol[19] and is highly selective in blocking only the alpha-2 adrenoreceptor when used in this dosage range.[15] A sample size of six or more animals per group was used.

Haloperidol and Yohimbine Combination Studies

Since it was shown that the protective actions of L-dopa were partially reversed by haloperidol and yohimbine, an additional set of experiments was performed to ascertain whether the effects of these two receptor antagonists were additive. Using a similar experimental protocol, haloperidol (5 mg/kg) and yohimbine (0.5 mg/kg) were given subcutaneously 30 minutes prior to pretreatment with saline (N = 6) or L-dopa (25 mg/kg; N = 6). Following the 30-minute pretreatment with saline or L-dopa, gastric mucosal injury was induced and injury quantified.

Statistics

All data were expressed as mean ± standard error the mean (SEM). For all experiments, differences among the various groups were determined by means of analysis of variance followed by a Scheffe post hoc test. Differences in mean values were considered significant at a P value of <0.05 for a type 1 error.

RESULTS

L-Dopa Dose Dependently Prevents Gastric Injury

Macroscopic Findings. As shown in Fig. 1, control animals pretreated with saline solution had extensive damage to the gastric mucosa. This damage was confined to the acid-secreting portion of the stomach and was characterized by the presence of hemorrhagic lesions oriented parallel to the gastric folds. Despite the extensive nature of the damage caused by acidified ethanol, L-dopa was capable of dose dependently preventing the development of macroscopic lesions. In animals pretreated with the highest dose of L-dopa (25 mg/kg), less than 1% of the mucosal surface exhibited any signs of overt damage.

Morphologic Findings. Results of light microscopic evaluation of the animals pretreated with saline and L-dopa (25 mg/kg) are shown in Figs. 2 and 3. Animals pretreated with saline solution followed by

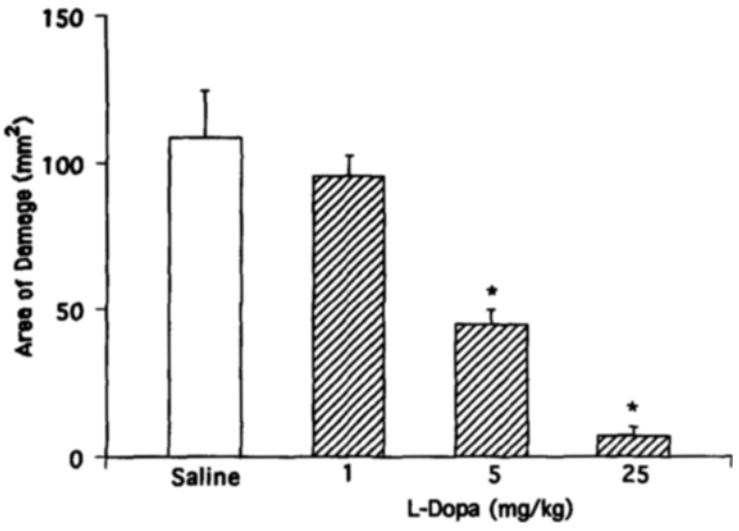

Fig. 1. Effects of intraperitoneal L-dopa (1 to 25 mg/kg) and saline solution on total area of macroscopic injury to rat gastric mucosa exposed to acidified ethanol. Values are expressed as mean ± SEM. N ≥6 for all groups. *$P \leq 0.007$ vs. saline solution.

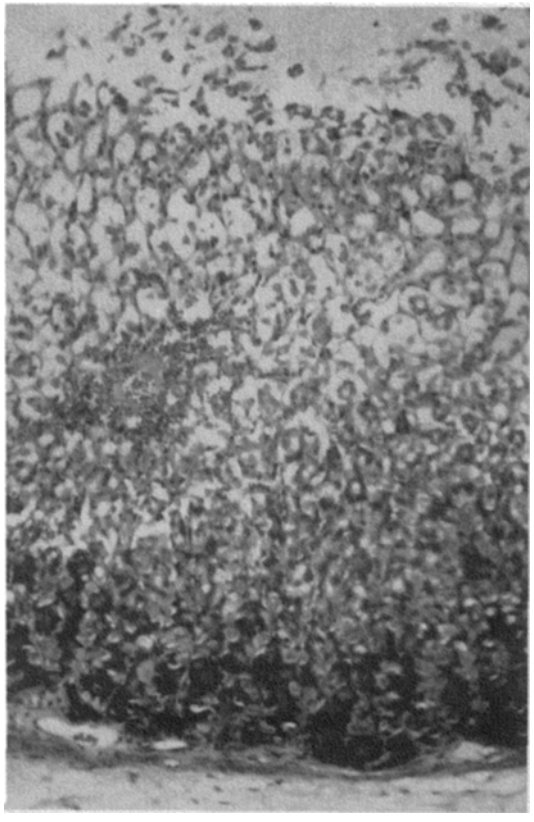

Fig. 2. Exposure of stomach to 1 ml of orogastric acidified ethanol (150 mmol/L HCl/50% ethanol) typically results in areas of type III injury characterized by loss of gastric pit cells and damage to the upper gastric glands as depicted in this micrograph. (×250.)

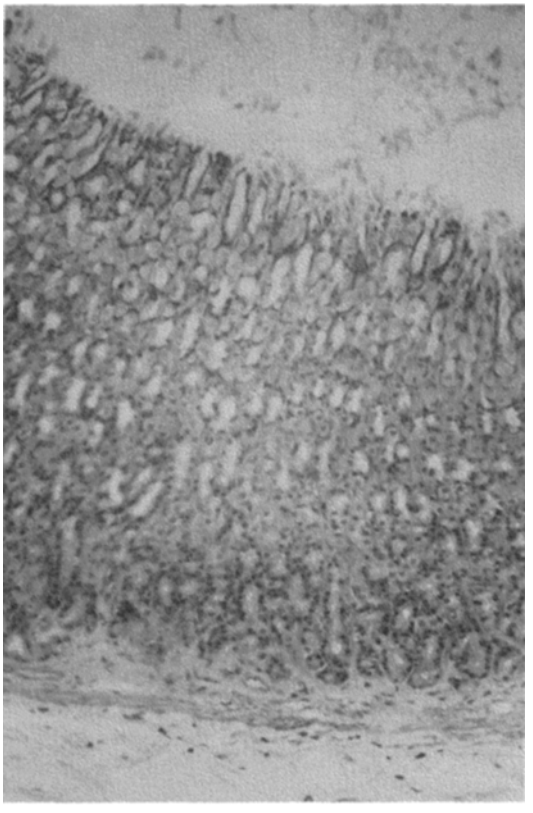

Fig. 3. L-dopa (25 mg/kg) pretreatment provides histologic preservation from acidified ethanol-induced gastric injury, as can be seen on this micrograph showing intact gastric pits and glands. (×250.)

acidified ethanol, generally demonstrated obliteration of the surface epithelium and loss of the mucosa in many areas down through the gastric pits (see Fig. 2). In the areas corresponding to the hemorrhagic lesions seen grossly, disruption of the epithelium could be seen extending for a variable distance down the glands, which is typical of type III and IV injuries. In contrast, L-dopa–pretreated animals exposed to the identical damaging agent demonstrated a distinctly different pattern of morphologic injury (see Fig. 3). In these animals approximately 8% of the gastric mucosa was determined to be normal epithelium. Most of the injury was confined to the surface epithelial cells, although superficial injury to the gastric pits was seen. In contrast to the injury pattern observed in animals pretreated with saline solution, a much larger amount of type I injury and only a small amount of type II injury was present. Taken together these studies demonstrated that pretreatment with L-dopa not only prevented the visible hemorrhagic lesions induced by acidified ethanol but provided a significant degree of histologic preservation as well.

Haloperidol Partially Reverses L-Dopa–Induced Gastroprotection

The effect of dopamine receptor blockade on L-dopa–induced gastroprotection is depicted in Fig. 4. As shown, animals receiving vehicle followed by pretreatment with L-dopa had significantly less gastric injury from acidified ethanol than their saline-pretreated counterparts. Administration of the dopamine receptor antagonist haloperidol brought about a partial reversal of the protective actions of L-dopa in this experimental model. The partial reversal of L-dopa–induced protection was only achieved with the 5 mg/kg dose of haloperidol and was not present with the lower dose of haloperidol.

As shown in Table I, CCK-8 (100 μg/kg) was a potent protective agent against acidified ethanol-induced gastric injury, thus confirming our previous re-

Table I. Effects of haloperidol and yohimbine on CCK-8–induced gastroprotection from acidified ethanol

Pretreatment	Sample size	Injury area (mm^2)
Vehicle		
Saline	6	112 ± 21
CCK-8	6	11 ± 4*
Haloperidol		
1 mg/kg/saline	6	106 ± 14
1 mg/kg/CCK-8	6	22 ± 17†
5 mg/kg/saline	9	102 ± 8
5 mg/kg/CCK-8	10	43 ± 13†
Yohimbine		
0.1 mg/kg/saline	6	120 ± 24
0.1 mg/kg/CCK-8	6	12 ± 6‡
0.5 mg/kg/saline	8	144 ± 26
0.5 mg/kg/CCK-8	6	21 ± 10‡
1 mg/kg/saline	6	140 ± 28
1 mg/kg/CCK-8	6	15 ± 8‡

*P <0.001 vs. vehicle saline.
†P ≤0.02 vs. haloperidol/saline.
‡P <0.001 vs. yohimbine/saline.

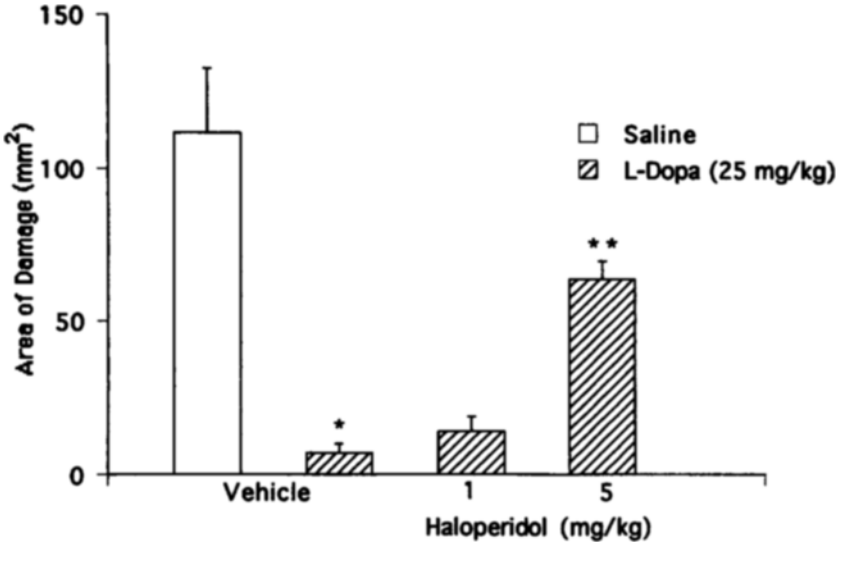

Fig. 4. Effect of subcutaneous haloperidol (1 to 5 mg/kg), a DA-2 dopamine receptor antagonist, on macroscopic protection induced by intraperitoneal L-dopa pretreatment (25 mg/kg) against acidified ethanol. Injury is reported as mean ± SEM. N ≥6 for all groups. *P <0.001 vs. vehicle/saline; **P = 0.001 vs. vehicle/L-dopa.

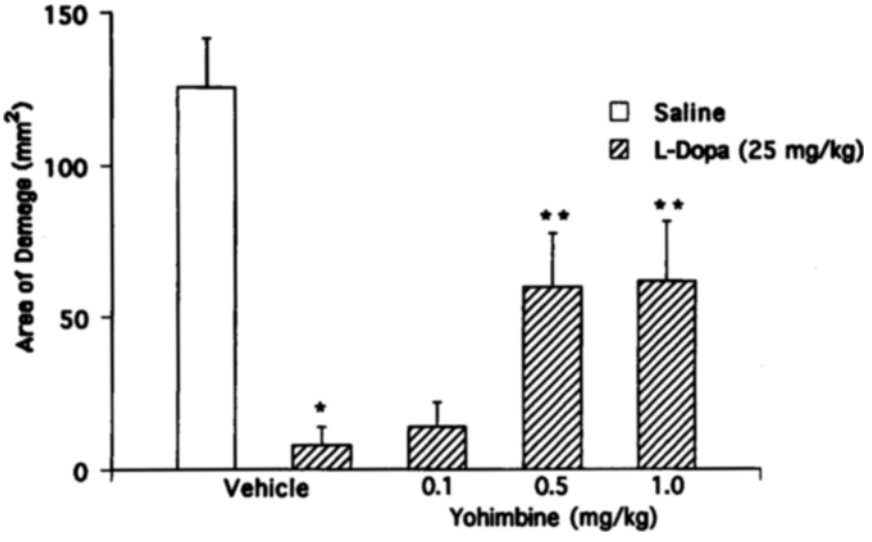

Fig. 5. Effect of intraperitoneal yohimbine (0.1 to 1 mg/kg), an alpha-2 adrenoreceptor antagonist, on macroscopic protection induced by intraperitoneal L-dopa (25 mg/kg) pretreatment against acidified ethanol. Injury is reported as mean ± SEM. N ≥6 for all groups. *P <0.001 vs. vehicle/saline; **$P \leq 0.05$ vs. vehicle/L-dopa.

ports.[16,22] However, despite the ability of haloperidol to attenuate the protective actions of L-dopa, none of the doses of haloperidol reversed the protective actions of CCK-8. In addition, haloperidol did not exacerbate the gastric injury resulting from acidified ethanol (Table I).

Yohimbine Attenuates L-Dopa–Induced Gastroprotection

The effect of the alpha-2 adrenoreceptor antagonist yohimbine on L-dopa– and CCK-8–induced gastroprotection is demonstrated in Fig. 5 and Table I, respectively. Similar to the effects observed with haloperidol, administration of yohimbine partially reversed the protective actions of L-dopa against acidified ethanol. This response was most prominent with the 0.5 and 1 mg/kg doses of yohimbine. However, as shown in Table I, none of the doses of yohimbine prevented the protective actions of CCK-8 in this model.

Dopamine and Alpha-2 Adrenoreceptor Blockade Have Additive Effects

Since only a partial reversal of L-dopa–induced gastroprotection was achieved with individual blockade of either the dopamine receptor or alpha-2 adrenoreceptor, additional studies were undertaken to ascertain whether simultaneous administration of both receptor antagonists would have additive effects on this response. As shown in Fig. 6, simultaneous administration of haloperidol and yohimbine completely reversed the protective actions of L-dopa against acidified ethanol-induced gastric injury. Furthermore, administration of both receptor antagonists did not significantly exacerbate gastric mucosal injury from this damaging agent when given to saline-treated control rats (108 ± 18 vs. 126 ± 16 mm², P = NS).

DISCUSSION

This study demonstrated that L-dopa, a dopamine precursor, was just as effective as CCK-8 at maintaining the integrity of the gastric epithelium in the face of a damaging luminal insult. Administration of L-dopa not only reduced the degree of macroscopic gastric injury from acidified ethanol but also provided significant histologic preservation of the gastric epithelium. In such stomachs the depth of histologic injury was characterized by a shift to the more superficial components of the gastric epithelium with preservation of the underlying glandular architecture, similar to the pattern of morphologic protection reported for CCK-8.[16]

These macroscopic and morphologic findings with the use of L-dopa were consistent with our original hypothesis that CCK decreased the susceptibility of the gastric mucosa to damage by modulating dopaminergic transmission or by eliciting the release of endogenous dopamine. Thus further studies were un-

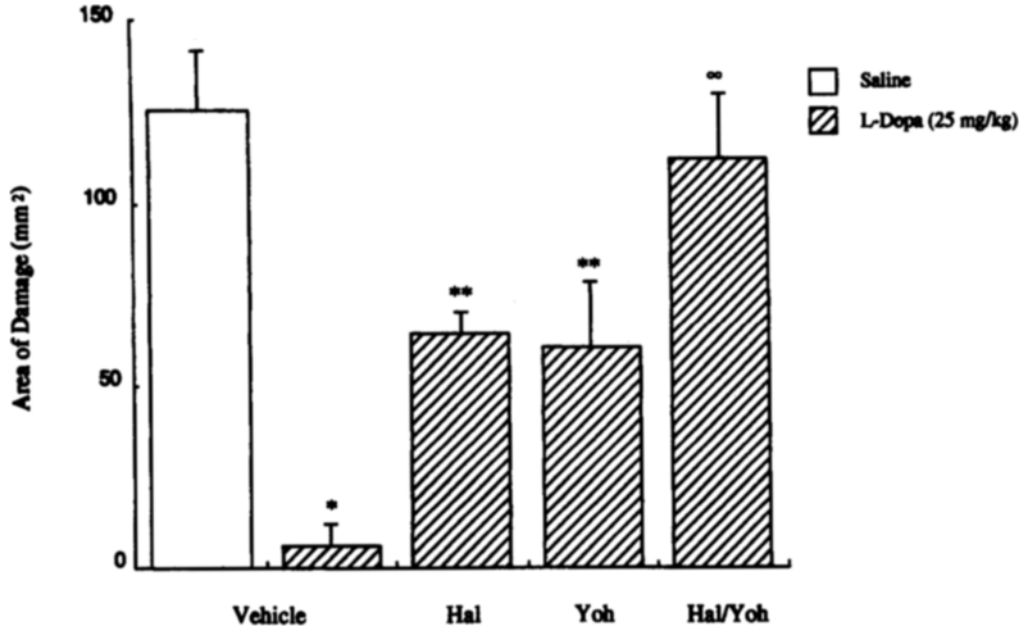

Fig. 6. Effect of subcutaneous haloperidol (5 mg/kg) and intraperitoneal yohimbine (0.5 mg/kg) individually and simultaneously on macroscopic protection induced by intraperitoneal L-dopa (25 mg/kg) pretreatment against acidified ethanol. Injury is reported as mean ± SEM. N ≥6 for all groups. $^{*}P < 0.001$ vs. vehicle/saline; $^{**}P \leq 0.05$ vs. vehicle/L-dopa; $\infty P < 0.001$ vs. vehicle/L-dopa.

dertaken to identify the receptor initiating the signal transduction mechanism responsible for the protective actions of L-dopa in the stomach. For these studies we used haloperidol, a dopamine receptor antagonist, and yohimbine, an alpha-2 adrenoreceptor antagonist. Both receptor antagonists have been shown to inhibit the actions of dopamine in the stomach.[4,8,15] It was our contention that if the protective actions of CCK are mediated via dopamine, then the same antagonists that nullify L-dopa– or dopamine-induced gastroprotection should also reverse or attenuate CCK-8–induced gastroprotection. Our dopamine receptor antagonist studies were conclusive. Haloperidol partially reversed the protective actions of L-dopa at the 5 mg/kg dose. However, none of the doses of haloperidol used were found to diminish the protective actions of CCK-8. Since haloperidol acts primarily at presynaptically located peripheral dopamine receptor subtype (DA-2) rather than postsynaptically located peripheral dopamine receptor subtype (DA-1) receptors,[23] we concluded that the protective actions of L-dopa, but not CCK-8, are mediated, at least in part, by activation of DA-2 receptors.

Our findings with haloperidol suggest that DA-2 dopamine receptors may play a role in gastric mucosal defense. In comparison, Glavin[14] reported that DA-1 dopamine receptors are more important than DA-2 receptors in gastroprotection. Glavin[24] demonstrated that selective DA-1 receptor agonists reduce gastric injury whereas selective antagonists exacerbate it. Furthermore, that same study indicated that DA-2 receptor agonists or antagonists had less dramatic effects on gastric injury caused by either stress or ethanol when compared to their DA-1 counterparts.[24] Nonetheless, haloperidol, given at a dosage of 5 mg/kg, partially negated the protective actions of L-dopa, implicating a role for DA-2 receptors in this response.

Similar to the results obtained in the dopamine receptor antagonist studies, alpha-2 adrenoreceptor blockade was only able to partially reverse the protective actions of L-dopa and failed to prevent CCK-8–induced gastroprotection. These results with yohimbine are, in part, consistent with the findings of Takeuchi et al.[8] Their study demonstrated that a 5 mg/kg dose of yohimbine, as opposed to the 0.1 to 1.0 mg/kg dosage range chosen for our study, was able to inhibit the effect of dopamine on gastric mucosal lesion formation from acidified ethanol. Lower doses, such as those used in our study, were not examined in their study. Consequently it is conceivable that we might have completely negated the protective actions of L-dopa had we administered the 5 mg/kg dose, although we did not detect any significant differences

between the 1.0 and 0.5 mg/kg doses of yohimbine. Nevertheless, our findings with yohimbine, in concert with those of Takeuchi et al.,[8] suggest that alpha-2 adrenoreceptors may also play a role in gastric mucosal defense.

Interestingly, simultaneous administration of both receptor antagonists completely reversed L-dopa–induced gastroprotection, indicating that the inhibitory effects of dopamine receptor and alpha-2 adrenoreceptor blockade are additive. Thus our data suggest that L-dopa renders the stomach less susceptible to damage by activating both dopamine and alpha-2 adrenoreceptors. However, the evidence that L-dopa behaves in such fashion is based on the specificities of the antagonists used. Nonetheless, our findings with L-dopa in the presence of dopamine and alpha-2 adrenoreceptor blockade are not unique. For example, it has been shown that dopamine acts on both of these receptors in the ileum[25] and in the central nervous system.[26] Consequently our data indicate that L-dopa, but not CCK-8, prevents gastric injury from acidified ethanol by activation of DA-2 and alpha-2 adrenoreceptors. However, the location of these receptors and the precise cell type(s) involved in gastroprotection remain to be identified.

As previously stated, deficiencies or excessive amounts of dopamine in the central nervous system have been associated with the presence or absence of gastroduodenal ulcers.[1,2] Furthermore, dopamine receptors have been identified in the brain,[14] and administration of L-dopa via the intracerebral ventricular route results in gastroprotection.[6] Thus it is possible that L-dopa crosses the blood-brain barrier and acts centrally rather than on peripheral dopamine receptors located in the stomach. Apropos of this possibility, it is noteworthy that approximately 95% of L-dopa is decarboxylated in peripheral tissues, leaving only 5% for diffusion across the blood-brain barrier.[27] Nevertheless, the present study did not elucidate whether the protective actions of L-dopa involve dopamine receptors located centrally.

It is interesting that peripheral dopamine receptors have been localized in gastrointestinal smooth muscle[23] where dopamine is postulated to function as a neurotransmitter and induce gastric relaxation.[28] Relaxation of smooth muscle, in turn, could result in a reduction in mucosal folding, which may be important in ethanol injury models, since the damage occurs primarily on the crests of such folds.[29] Alternatively, dopamine has also been shown to activate DA-2 receptors located on rabbit vascular smooth muscle cells in the stomach followed by vasodilatation.[30] The importance of enhanced gastric mucosal blood flow in response to injury has also been well described.[31] Whether or not L-dopa exerts its protective actions by either one of these potential mechanisms remains to be elucidated.

CONCLUSION

L-Dopa and CCK-8 were equally effective in preventing gastric injury from acidified ethanol. The protective actions of L-dopa, but not CCK-8, were partially reversed by individual blockade of either dopamine receptors or alpha-2 adrenoreceptors. Simultaneous administration of both receptor antagonists completely negated L-dopa–induced gastroprotection. This indicates that L-dopa mediates its protective actions through activation of both receptors. However, the failure of these receptor antagonists to reverse or attenuate CCK-8–induced gastroprotection is inconsistent with our hypothesis that CCK mediates its protective actions in the stomach through modulation of dopaminergic neurotransmission or release of endogenous dopamine.

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REFERENCES

1. Strang R. The association of gastro-duodenal ulceration and Parkinson's disease. Med J Aust 1965;1:842-843.
2. Szabo S. Dopamine disorder in duodenal ulceration. Lancet 1979;2:880-882.
3. Glavin GB, Szabo S. Dopamine in gastrointestinal disease. Dig Dis Sci 1990;35:1153-1161.
4. MacNaughton WK, Wallace JL. A role for dopamine as an endogenous protective factor in the rat stomach. Gastroenterology 1989;96:972-980.
5. Sikirić P, Rotkvić, I, Miše S, Križanac Š, Gjuriš V, Jukić J, Suchanek E, Petek M, Udovičić I, Kalogjera L, Geber J, Tućan-Foretić M, Duvnjak M, Philipp M, Balen I, Anić T. The influence of dopamine agonist and antagonist on indomethacin lesions in stomach and small intestine in rats. Eur J Pharmacol 1988;158:61-67.
6. Glavin GB, Dugani AM. Effects of dopamine agonists and antagonists on gastric acid secretion and stress responses in rats. Life Sci 1987;41:1397-1408.
7. Szabo S, Neumeyer J. Dopamine agonists and antagonists in duodenal ulcer disease. In Kaiser C, Kebabian J, eds. Dopamine Receptors. Washington, D.C.: American Chemical Society, 1983, pp 175-196.
8. Takeuchi K, Nishiwaki H, Okabe S. Effects of dopamine on gastric mucosal lesions induced by ethanol in rats. Dig Dis Sci 1988;33:1560-1568.
9. Sikirić P, Geber J, Ivanocić D, Suchanek E, Gjuriš V, Tućan-Foretić M, Miše S, Cvitanović B, Rotkvić I. Dopamine antagonists induce gastric lesions in rats. Eur J Pharmacol 1986; 131:105-109.

10. Lansberg L, Berardino MB, Silva P. Metabolism ^{3}H-L-dopa by the rat gut in vivo; evidence for glucuronide conjugation. Biochem Pharmacol 1975;24:1167-1174.
11. Willems J, Buylaert W, Lefebvre R, Bogaert M. Neuronal dopamine receptors on autonomic ganglia and sympathetic nerves and dopamine receptors in the gastrointestinal system. Pharmacol Rev 1985;37:165-216.
12. Kebabian J, Calne D. Multiple receptors for dopamine. Nature 1979;277:93-96.
13. Stoof J, Kebabian J. Two dopamine receptors: Biochemistry, physiology and pharmacology. Life Sci 1984;35:2281-2296.
14. Glavin GB. Dopamine and gastroprotection: The brain-gut axis. Dig Dis Sci 1991;36:1670-1672.
15. Goldberg MR, Robertson D. Yohimbine: A pharmacological probe for study of the alpha$_2$-adrenoreceptor. Pharmacol Rev 1983;35:143-180.
16. Mercer DW, Cross JM, Barreto JC, Nathaniel HP, Strobel BS, Russell DH, Miller TA. Cholecystokinin is a potent protective agent against alcohol-induced gastric injury in the rat: Role of endogenous prostaglandins. Dig Dis Sci 1995;40: 651-660.
17. Seroogy K, Ceccatelli S, Schalling M, Hökfelt T, Frey P, Walsh J, Dockray G, Brown J, Buchan A, Goldstein M. A subpopulation of dopaminergic neurons in rat ventral mesencephalon contains both neurotensin and cholecystokinin. Brain Res 1988;455:88-98.
18. Keast JR. Muscosal innervation and control of water and ion transport in the intestine. Rev Physiol Biochem Pharmacol 1987;109:1-59.
19. Tornwall MS, Henagan JM, Miller TA. Role of alpha$_2$ adrenoreceptors in the prevention of gastric injury by ethanol. Am Coll Surg 1990;41:140-142.
20. Karnovsky MJ. A formaldehyde gluteraldehyde fixative of high osmolality for use in electron microscopy. J Cell Biol 1965;27:137A-138A.
21. Schmidt KL, Henagan JM, Smith GS, Hilburn PJ, Miller TA. Prostaglandin cytoprotection against ethanol-induced injury in a rat: A histologic and cytologic study. Gastroenterology 1985;88:649-659.
22. Mercer DW, Klem K, Cross JM, Smith GS, Cashman M, Miller TA. Cholecystokinin induced protection against gastric injury is independent of endogenous somatostatin. Am J Physiol 1996;271:6692-6700.
23. Bogaert MG, Buylaert WA, Lefebvre RA, Willems JL. Peripheral dopamine receptors. In Poste G, Crooke ST, eds. Dopamine Receptor Antagonists. New York: Plenum, 1984, pp 139-155.
24. Glavin G. Activity of selective dopamine DA_1 and DA_2 agonists and antagonists on experimental gastric lesions and gastric acid secretion. J Pharmacol Exp Ther 1989;251:726-730.
25. Donowitz M, Cusolito S, Battisti L, Fogel R, Sharp GWG. Dopamine stimulation of active Na and Cl absorption in rabbit ileum: Interaction with alpha$_2$-adrenergic and specific dopamine receptors. J Clin Invest 1982;69:1008-1016.
26. Ruggeri M, Ungerstedt U, Agnati LF, Mutt V, Härfstrand A, Fuxe K. Effects of cholecystokinin peptides and neurotensin on dopamine release and metabolism in the rostral and caudal part of the nucleus accumbes using intracerebral dialysis in the anaesthetized rat. Neurochem Int 1987;10:509-520.
27. AMA Division of Drugs. Drugs used in extrapyramidal movement disorders. AMA Drug Evaluations 1983, 5th ed., pp 329-351.
28. Valenzuela JE. Dopamine as a possible neurotransmitter in gastric relaxation. Gastroenterology 1976;71:1019-1022.
29. Mersereau WA, Hinchey EJ. Role of gastric mucosal folds in formation of focal ulcers in the rat. Surgery 1982;91:150-155.
30. Reisberg J, Kullmann R. Characterization of vascular dopamine receptors in the gastric circulation of the rabbit. J Cardiovasc Pharmacol 1986;8:1067-1073.
31. Ritchie WP, Mercer DW. Mediator of bile acid-induced alterations in gastric mucosal blood flow. Am J Surg 1991;161: 126-130.

Discussion

Dr. B. Schirmer (Charlottesville, Va.). In the physiologic state, do you think that one is more important than the other (dopamine vs. CCK) or do you think that from an evolutionary standpoint there is some advantage to having both as protective mechanisms?

Dr. J.M. Cross. I think the endogenous gastroprotective mechanism probably involves a very redundant series of peptides including CCK, gastrin, and dopamine and L-dopa. I do not really know which one is more significant; I think it is still too early to say.

Dr. G.S. Smith (St. Louis, Mo.). Is L-dopa–induced gastroprotection specific to acidified ethanol or does it affect other luminal irritants such as nosteroidal agents or bile acids?

Dr. Cross. We have not addressed that.

Dr. G. Flemstrom (Sweden). Yohimbine has a peripheral as well as a central neural site of action. What is the site of action in the present context?

Dr. Cross. We did not address that specifically.

Dr. G. Kauffman, Jr. (Hershey, Pa.). Can you just tell us about the effect of CCK-8 and L-dopa on specifics such as acid secretion and blood flow.

Dr. Cross. CCK-8 has been shown to rapidly increase gastric mucosal blood flow; however, this effect usually lasts only about 30 to 45 minutes. The gastroprotective actions of CCK-8 continue, at least in part, for approximately 2 hours. So CCK-8 does increase blood flow; however, it does not seem to be directly correlated with gastroprotection.

Dr. Kauffman. What is the effect of L-dopa on acid secretion?

Dr. Cross. I do not know the specific effect of L-dopa on gastric secretion.

Prognostic Significance of Tumor Markers in Colorectal Cancer Patients: DNA Index, S-Phase Fraction, p53 Expression, and Ki-67 Index

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Risk of colorectal cancer recurrence has traditionally been determined by use of pathologic staging. However, it is apparent that subgroups of patients exist within tumor stages whose clinical behavior differs. This study was undertaken to identify tumor-associated factors that might be predictive of outcome in patients with intermediate stages who will benefit the most from postsurgical adjuvant therapy. Seventy patients with stage II and III colorectal cancer were assessed for DNA index, S-phase fraction, p53 expression, and Ki-67 index. Tumor recurrence was analyzed by means of nonparametric tests and Cox proportional hazard models incorporating standard clinical and pathologic criteria. Of the four prognostic markers evaluated, Ki-67 index was significantly associated with disease recurrence ($P = 0.02$), whereas DNA index, S-phase fraction, and p53 expression were not. After stratification by tumor stage, significant associations between Ki-67 index and disease recurrence were retained in stage II tumors ($P = 0.01$) but not in stage III tumors ($P = 0.23$). Cox proportional hazard regression analysis indicated that among stage II patients, those with a Ki-67 index >45% were associated with 6.5 times greater risk for disease recurrence than those with a Ki-67 index ≤45%. It was concluded that an elevated Ki-67 index is associated with an increased risk of tumor recurrence in stage II colorectal cancer. (J GASTROINTEST SURG 1997;1:266-273.)

Colorectal cancer classification, based predominantly on a system of clinical pathologic staging, is widely used to predict the likelihood of disease recurrence and to define clinical management. For example, patients with stage III disease have a substantial risk of tumor recurrence and are treated with adjuvant therapy, whereas patients with stage II disease have lower recurrence rates and are treated with surgery alone.[1,2] Although the usefulness of clinical pathologic staging has never been in doubt, it has long been apparent that the use of additional staging criteria could lead to better subclassification of tumors, improved predictive power, and optimal selection of therapy for individual patients. Prognostic markers vary widely from histologic evidence of host immune response to molecular evidence of oncogene mutation and loss of tumor suppressor gene function. This study was undertaken in an effort to identify the prognostic capabilities of four tumor-associated biomarkers in colorectal cancer. Markers studied include DNA index, S-phase fraction, p53 overexpression, and cell proliferative activity as measured by the Ki-67 antibody.

MATERIAL AND METHODS

The study population comprised 70 patients who underwent surgical resection of stage II and III colorectal cancer at the University of Minnesota affiliated hospitals between January 1982 and December 1987. Inclusion criteria encompassed the following: (1) no history of cancer except nonmelanoma skin cancer;

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(2) no history of neoadjuvant therapy; (3) no evidence of metastatic disease at initial laparotomy; and (4) complete excision of the primary tumor as determined by histologic examination of the resection margins. Minimum follow-up was set at 24 months.

Traditional clinical pathologic variables regarding patients (age and sex) and tumors (location, size, grade, and stage), as well as treatment and relapse information, were obtained by retrospective review. Assessment of tumor-associated biomarkers was performed by one of us (K.-J.C.) using tissue in paraffin blocks.

Measurement of DNA Ploidy and S-phase Fraction

DNA ploidy and S-phase fraction were measured using flow cytometry. Samples were stained with propidium iodide and were run on an EPICS C flow cytometer (Coulter Corp., Toronto, Canada) equipped with a 2 W argon laser operating at a wavelength of 488 nm. DNA contents were analyzed using a personal computer interfaced to the flow cytometer. A software package (Phoenix Flow Systems, San Diego, Calif.) was used to calculate DNA ploidy and the percentage of cells in S-phase. DNA histograms in the sample were analyzed graphically. DNA index was expressed as a mean value of DNA for the G0/G1 fraction of each histogram divided by control G0/G1 human lymphocytes. A normal cell population with a diploid chromosome complement has a DNA index of 1.0.

Measurement of Ki-67 and p53 Expression

Ki-67 index and p53 expression were measured using immunohistochemical methods. The formalin-fixed, paraffin-embedded tumor specimens were cut into 5 μm sections and mounted on glass slides. Sections were stained with the avidin–biotin peroxidase complex method using the Vectastain mouse immunoglobulin G kit (Vector Laboratories, Inc., Burlingame, Calif.).

Ki-67 nuclear antigen expression was identified using monoclonal MIB-1 antibody (AMAC, Inc., Westbrook, Maine). The Ki-67 index was calculated as the ratio of Ki-67–positive cells to the total number of cells present[3] using the CAS automated cell counter (Cell Analysis Systems, Inc., Elmhurst, Ill.). p53 expression was determined with the monoclonal antibody Pab1801 (Oncogene Science, Inc., Manhassett, N.Y.). This antibody reacts with both wild-type and mutant human p53 protein.[4] Tumors were scored as positive for p53 overexpression if any of the tumor cells showed nuclear immunoreactivity.

Statistical Analysis

Descriptive statistics were tabulated as frequency counts and percentages for discrete data and as the mean and standard deviation for continuous measures. Comparisons of subgroups were carried out using the nonparmetric Wilcoxon signed-rank test for continuous variables and the Kruskal-Wallis test for catagoric or ordinal variables. Cumulative patient survival was estimated by means of the Kaplan-Meier method.[5] Comparisons of equality of survival curves between groups were tested using a log-rank test.[6] Clinicopathologic factors that could potentially influence the association between tumor biomarkers and patient disease-free survival were identified using a stepwise procedure and a Cox proportional hazards linear regression model. Estimates of hazard ratios and 95% confidence intervals were generated from the parameter estimates of regression coefficients and associated standard errors. Statistical significance was assigned at the $P \leq 0.05$ level. All statistical analysis and testing were performed utilizing the SPSS program.[7]

RESULTS

Table I summarizes the clinicopathologic characteristics and tumor-associated biomarkers by disease relapse status. Follow-up ranged from 24 to 156 months with a median follow-up of 67 months. Seventeen patients (24%) had recurrent disease. Recurrence rates were higher in men than in women (38% vs. 13%, $P = 0.03$). The recurrence rate for stage III tumors (36%) was twice that of stage II tumors (18%), but this finding did not reach statistical significance ($P = 0.14$).

Of the four tumor-associated biomarkers examined, only the Ki-67 index was significantly associated with patients' disease relapse status ($P = 0.02$). The mean Ki-67 index was 49.2% for patients with recurrence and 38.2% for patients without. There were no notable differences in S-phase fraction, DNA index, or p53 expression in recurrent vs. nonrecurrent groups (see Table I).

Analyses were further conducted to compare the performance of tumor-associated biomarkers between patients with and without recurrence by tumor stage (Table II). The most dramatic finding was the difference in the Ki-67 index in stage II patients with and without recurrence. The mean Ki-67 index was 54.0% in patients who developed recurrent disease vs. 39.7% in patients who did not have recurrent disease ($P = 0.01$). Similar but less marked differences in the Ki-67 index were observed among stage III patients (44.9% with vs. 34.6% without recurrent disease) but this difference did not reach statistical significance ($P = 0.23$).

Table I. Clinicopathologic characteristics and tumor-associated biomarkers in patients with colorectal cancer by disease relapse status

	Total No.	Recurrence		*P* value*
		Yes No. (%)	No No. (%)	
Total patients	70	17 (24)	53 (76)	
Mean (SD) age (yr)	64.4 (11.6)	66.1 (10.5)	63.9 (12.0)	0.53
Sex				0.03
Male	32	12 (38)	20 (62)	
Female	38	5 (13)	33 (87)	
Stage				0.14
II	45	8 (18)	37 (82)	
III	25	9 (36)	16 (64)	
Mean (SD) tumor size (mm)	48.5 (18.8)	58.2 (22.3)	46.1 (17.8)	0.05
Adjuvant therapy				1.00
Yes	10	2 (20)	8 (80)	
No	60	15 (25)	45 (75)	
Tumor site				0.28
Colon	38	7 (18)	31 (82)	
Rectum	32	10 (31)	22 (69)	
Mean (SD) Ki-67 level	40.8 (16.1)	49.2 (10.4)	38.2 (16.8)	0.02
Mean (SD) S-phase fraction	14.6 (6.1)	15.2 (6.5)	14.4 (6.0)	0.70
DNA index				0.52
1.0	27	6 (22)	21 (78)	
1.1-1.9	38	10 (26)	28 (51)	
≥2.0	4	0 (0)	4 (100)	
p53 expression				0.84
Negative	22	5 (23)	17 (77)	
Positive	48	12 (25)	36 (75)	

*Based on Fisher's exact test for binary variables, Kruskal-Wallis test for ordinal variables, and Wilcoxon signed rank test for continuous variables.

Table II. Tumor-associated biomarkers in patients with colorectal cancer by disease relapse status and tumor stage

	Total No.	Recurrence		*P* value*
		Yes No. (%)	No No. (%)	
Stage II				
Mean (SD) Ki-67 level	42.3 (16.5)	54.0 (8.8)	39.7 (16.8)	0.01
Mean (SD) S-phase fraction	15.6 (6.4)	19.0 (3.4)	15.0 (6.7)	0.21
DNA index				0.89
1.0	20	3 (15)	17 (85)	
1.1-1.9	20	4 (20)	17 (80)	
≥ 2.0	4	0 (0)	4 (100)	
p53 expression				0.21
Negative	14	1 (7)	13 (93)	
Positive	31	7 (23)	24 (77)	
Stage III				
Mean (SD) Ki-67 level	38.3 (15.5)	44.9 (10.2)	34.6 (17.0)	0.23
Mean (SD) S-phase fraction	12.8 (5.1)	12.6 (7.0)	13.0 (4.1)	0.59
DNA index				0.66
1.0	7	3 (43)	4 (57)	
1.1-1.9	18	6 (33)	12 (67)	
≥ 2.0	0	0 (0)	0 (0)	
p53 expression				0.33
Negative	8	4 (50)	4 (50)	
Positive	17	5 (29)	12 (71)	

*Based on Kruskal-Wallis test for ordinal variables and Wilcoxon signed rank test for continuous variables.

To understand the clinical performance of Ki-67 in predicting tumor relapse, cumulative disease-free survival curves were analyzed and compared (Fig. 1). In general, stage II patients had better survival than stage III patients. The 5-year disease-free survival rate was 86% for stage II patients and 72% for stage III patients. Among all patients, a Ki-67 index >45% was associated with decreased survival ($P = 0.04$). The 5-year survival rate for stage III patients was 86% with a Ki-67 index ≤45% and 55% with a Ki-67 index >45% (Fig. 2).

Table III presents the estimated hazard ratios with

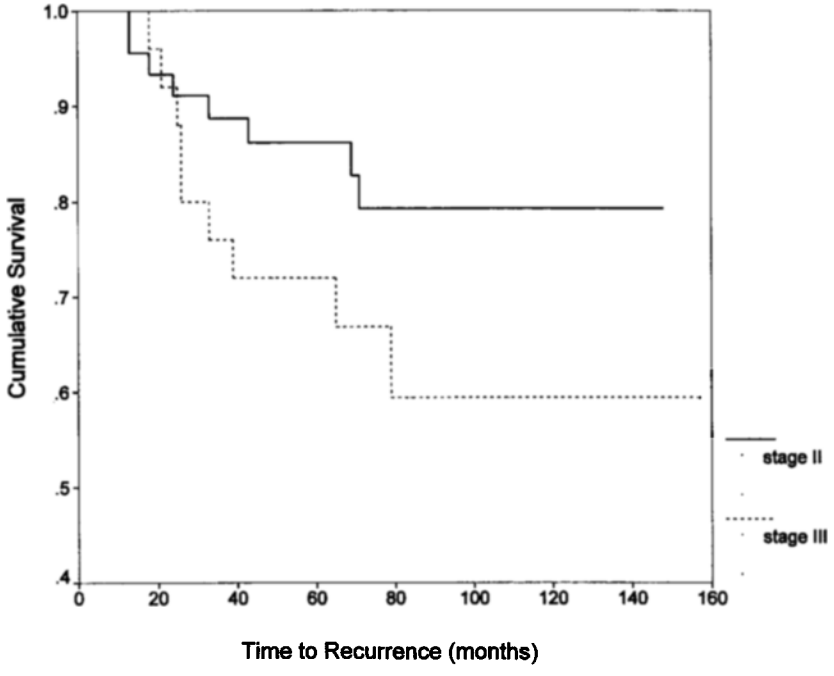

Fig. 1. Kaplan-Meier plot expressing the effect of the tumor stage on disease-free survival.

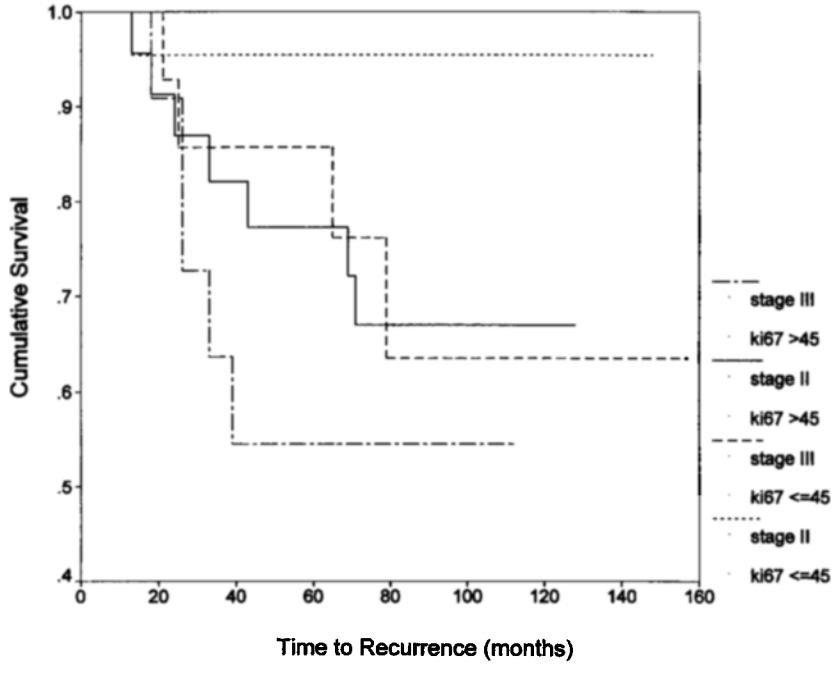

Fig. 2. Kaplan-Meier plot demonstrating the effect of the tumor stage and Ki-67 index on disease-free survival.

Table III. Estimated hazard ratios and 95% confidence intervals for colorectal cancer recurrence by Ki-67 level and tumor stage

Ki-67 level	Recurrence		Hazard ratio (95% CI)	*P* value*
	Yes	No		
All subjects			2.87 (1.00-8.21)	0.05
≤ 45	5	31		
> 45	12	22		
Stage II			6.47 (0.79-52.74)	0.08
≤ 45	1	21		
> 45	7	26		
Stage III			1.83 (0.49-6.86)	0.37
≤ 45	4	10		
> 45	5	6		

*With regard to testing of the hypothesis that the hazard ratio equals 1.0.

95% confidence intervals (CI) comparing patients with Ki-67 indices ≤45% and >45% stratified by tumor stage. When stage II and stage III patients were combined, those with a Ki-67 index >45% were at approximately three times higher risk for subsequent recurrence compared to those with a Ki-67 index <45% (hazard ratio = 2.89; 95% CI = 1.00 to 8.21). Among stage II patients, a Ki-67 index >45% markedly increased the risk of recurrence as compared to those with a Ki-67 index <45% (hazard ratio = 6.47; 95% CI = 0.79 to 52.74). Among stage III patients, a similar but smaller risk of tumor recurrence was associated with a Ki-67 index >45% (hazard ratio = 1.83; 95% CI = 0.49 to 6.86). Multivariate adjustment of important clinical pathologic features showed essentially the same results.

DISCUSSION

Pathologic staging, traditionally based on the system of Dukes and its subsequent modifications, remains the cornerstone of prognostic classification for colorectal cancer. However, although tumor stage does indeed correlate with recurrence and survival rates, prediction of tumor behavior in individual patients based on pathologic criteria alone remains difficult.[8] Prognostic markers of tumor behavior are also important to properly allocate individual patients to appropriate treatment protocols. Thus, although adjuvant chemotherapy has been shown to improve survival in patients with stage III colorectal cancer,[2] it remains quite possible that selected stage II patients with biologically aggressive tumors would benefit from adjuvant chemotherapy. One such poor prognosis subgroup has recently been identified as tumors with deletions in the long arm of chromosome 18, where survival of patients with stage II disease is similar to survival of patients with stage III disease without such chromosomal deletions.[9] Accurate prognostic markers might also prove beneficial in such decisions as the use or nonuse of adjuvant radiation therapy in rectal cancer, the use of localized vs. radical resection in early-stage rectal cancers, and the need for resection vs. observation following colonoscopic removal of a malignant polyp.

In our study node-positive (stage III) disease was associated with twice the recurrence rate of node-negative (stage II) disease (36% vs. 18%), but this result did not reach statistical significance ($P = 0.14$). Male sex was associated with a dramatically increased risk of recurrence (38% vs. 13% for females; $P = 0.03$). Similar gender differences have been observed in some[10-12] but not all[13,14] outcome studies.

Since its development by Gerdes et al.[3] in 1984, immunostaining with the monoclonal antibody Ki-67 has become an increasingly popular method for measuring cell proliferation. The original technique required frozen tissue, which hampered its application to routine and archival pathologic materials. For this reason other antibodies, such as those directed against the proliferating cell nuclear antigen (PCNA),[15] were developed for use in formalin-fixed, paraffin-embedded materials. Unfortunately, studies using anti-PCNA antibodies have yielded conflicting results concerning their applicability as "proliferation markers" because of the different properties of the various antibodies and their dependence on tissue characteristics.[16-18] Recently, the monoclonal antibody that recognizes the Ki-67 antigen in formalin-fixed paraffin-embedded material has finally been pre-

sented.[19,20] Thus it is now possible to perform retrospective studies on archival material using the monoclonal antibody Ki-67.

The current study demonstrates that cell proliferation activity assessed by means of Ki-67 immunostaining is an important prognostic marker in patients with stage II colorectal cancer. The subgroup of stage II patients whose tumors had a Ki-67 index ≤45% had an excellent outcome (5-year disease-free survival of 95%), whereas the stage II patients whose tumors had a Ki-67 index >45% had a poor survival rate, which was similar to that of the stage III patients. In stage III patients, although the differences in survival between tumors with Ki-67 indexes >45% and ≤45% were less distinct, patients with a Ki-67 index ≤45% did have a better prognostic outcome, which was similar to the overall survival of stage II patients.

Previous reports on Ki-67 and prognosis in colorectal cancer based on frozen tissue samples have yielded inconsistent results.[21-23] This study assessed cell proliferative activities using Ki-67 immunostaining in formalin-fixed, paraffin-embedded tissues in patients with colorectal cancer. Although data have suggested a statistically highly significant correlation between immunostaining of the Ki-67 antigen in frozen and paraffin-embedded sections,[17] factors involving tissue fixation and processing could vary the amount of detectable Ki-67 antigen from one study to another.

The utility of flow cytometry in tumor prognostication has been a matter of considerable debate over the past 15 years.[24] A number of studies have shown a correlation between increasing aneuploidy rates and advanced Dukes' stage[25-27] but this finding has not been universal.[28-30] Similarly, although several studies have demonstrated that aneuploid tumors have an increased risk of recurrence and decreased survival rates compared with diploid tumors,[31-34] others have found no such effect.[35-37] We failed to detect any correlation between either ploidy status or S-phase fraction and prognosis in our series of patients. Further work is needed to determine the exact role of these assays in tumor prognostication.

The p53 gene is the most frequently altered gene in solid human malignancies.[38,39] This gene, located on the short arm of chromosome 17 (17p), encodes a 53 kd nuclear phosphoprotein that functions as a tumor suppressor gene. Mutant p53 gene product is characterized by a conformational change of the protein with resultant prolonged half-life and stability.[40] The accumulated mutant protein is detectable in nuclei by immunostaining of the tissue sections, which may be an important surrogate for p53 genetic analysis.[41] However, the relationship of p53 accumulation and chromosome 17p deletion with respect to prognosis remains unclear. For example, Goh et al.[42] found a correlation between p53 mutation and lymph node positivity but not distant metastasis. Conversely, 17p deletions were associated with an increased rate of distant metastases but no increase in nodal metastases. Conflicting results have been reported concerning the effects of p53 accumulation on prognosis.[42] We did not find prognostic significance in p53 expression merged by monoclonal antibody Pab1801 in patients with stage II and III colorectal cancer. However, numerous monoclonal (e.g., Bp53-12, Pab1801, DO7, and Pab240) and polyclonal (e.g., CM1, Signet) antibodies are commercially available, and results vary substantially depending on the antibody used.[46] Definitive clarification of the relationship of p53 mutation, 17p deletion, and tumor prognosis awaits standardization of antibody choice and technique.

Accurate classification of tumor behavior is critical for optimizing therapy and standardizing treatment protocols. Although recent work has heavily emphasized the use of molecular genetic markers such as *ras* mutation and the loss of 17q, 18p, and the nm-23 gene, these assays are costly and require considerable technical expertise. Our results suggest that a relatively simple immunohistochemical test of cellular proliferation using the Ki-67 antibody may provide important independent prognostic information for patients with stage II and III colorectal cancer.

REFERENCES

1. National Institutes of Health Consensus Conference. Adjuvant therapy for patients with colon and rectal cancer: Summary of NIH Consensus Conference. JAMA 1990;264:1444-1450.
2. Moertel CG, Fleming TR, MacDonald JS, et al. Levamisole and fluorouracil for adjuvant therapy of resected colon carcinoma. N Engl J Med 1990;322:352-358.
3. Gerdes J, Kemke H, Baisch H, et al. Cell cycle analysis of cell proliferation associated human nuclear antigen defined by the monoclonal antibody K-67. J Immunol 1984;133:1710-1715.
4. Banks L, Matlashewski G, Crawford L. Isolation of human p53 specific monoclonal antibodies and their use in the studies of human p53 expression. Eur J Biochem 1986;159:529-534.
5. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc 1958;53:457-481.
6. Mantel N, Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. J Natl Cancer Inst 1959;22:719-748.
7. SPSS Advanced Statistics 6.1. Chicago: SPSS, 1994.
8. Jass JR, Love SB, Northover JM. A new prognostic classification of rectal cancer. Lancet 1987;1:1303-1306.
9. Jen J, Kim H, Piantadosi S, et al. Allelic loss of chromosome 18q and prognosis in colorectal cancer. N Engl J Med 1994; 331:213-221.

10. Chapuis PH, Dent OF, Fisher R, et al. A multivariate analysis of clinical and pathological variables in prognosis after resection of large bowel cancer. Br J Surg 1985;72:698-702.
11. Isbister WH, Fraser J. Survival following resection for colorectal cancer: A New Zealand national study. Dis Colon Rectum 1985;28:725-727.
12. Griffin MR, Bergstralh EJ, Coffey RJ, et al. Predictors of survival after curative resection of carcinoma of the colon and rectum. Cancer 1987;60:2318-2324.
13. Fielding LP, Phillips RKS, Fry JS, et al. Prediction of outcome after curative resection for large bowel cancer. Lancet 1986; 2:904-907.
14. Kune GA, Kune S, Field B, et al. Survival in patients with large-bowel cancer. Dis Colon Rectum 1990;33:938-946.
15. Bravo R, Frank R, Blundell PA, et al. Cyclin/PCNA is the auxiliary protein of DNA-polymerase delta. Nature 1987;326: 515-517.
16. Hall PA. Cell proliferation. J Pathol 1991;165:349-354.
17. Diebold J, Dopfer K, Lai M, et al. Comparison of different monoclonal antibodies for the immunohistochemical assessment of cell proliferation in routine colorectal biopsy specimens. Scand J Gastroenterol 1994;29:47-53.
18. Al-Sheneber IF, Shibata HR, Sampalis J, et al. Prognostic significance of proliferating cell nuclear antigen expression in colorectal cancer. Cancer 1993;71:1945-1949.
19. Gerdes J, Li L, Schluter C, et al. Immunobiochemical and molecular biologic characterization of the cell proliferation-associated nuclear antigen that is defined by the monoclonal antibody Ki-67. Am J Pathol 1991;138:867-875.
20. Cattoretti G, Becker MHG, Key G, et al. Monoclonal antibodies against recombinant parts of the Ki-67 antigen (mib1 and mib3) detect proliferating cells in microwave-processed formalin-fixed paraffin sections. J Pathol 1992;168:357-363.
21. Kubota Y, Petras RE, Easley KA, et al. Ki-67 determined growth fraction versus standard staging and grading parameters in colorectal carcinoma. A multivariate analysis. Cancer 1992;70:2602-2609.
22. Porschen R, Kriegel A, Langen C, et al. Assessment of proliferative activity in carcinomas of the human alimentary tract by Ki-67 immunostaining. Int J Cancer 1991;47:686-691.
23. Witzig TE, Loprinzi CL, Gonchoroff NJ, et al. DNA ploidy and cell kinetic measurements as predictors of recurrence and survival in stage B2 and C colorectal adenocarcinoma. Cancer 1991;68:879-888.
24. Dean PA, Vernava AM III. Flow cytometric analysis of DNA content in colorectal carcinoma. Dis Colon Rectum 1992;35: 95-102.
25. Kokal W, Sheibani K, Terz J, et al. Tumor DNA content in the prognosis of colorectal carcinoma. JAMA 1986;255:3123-3127.
26. Scott NA, Rainwater LM, Weigand HS, et al. The relative prognostic value of flow cytometric DNA analysis and conventional clinicopathologic criteria in patients with operable rectal carcinoma. Dis Colon Rectum 1987;30:513-520.
27. Jones DJ, Moore M, Schofield PF. Prognostic significance of DNA ploidy in colorectal cancer: A prospective flow cytometric study. Br J Surg 1988;75:28-33.
28. Quirke P, Dixon MF, Clayden A, et al. Prognostic significance of DNA aneuploidy and cell proliferation in rectal adenocarcinomas. J Pathol 1987;151:285-291.
29. Fischer ER, Siderits RH, Sass R, et al. Value of assessment of ploidy in rectal cancers. Arch Pathol Lab Med 1989;113:525-528.
30. Hood DL, Petras RE, Edinger M, et al. Deoxyribonucleic acid ploidy and cell cycle analysis of colorectal carcinoma by flow cytometry. Am J Clin Pathol 1990;93:615-620.
31. Crissman JD, Zarbo RJ, Ma CK, et al. Histopathologic parameters and DNA analysis in colorectal adenocarcinomas. Pathol Annu 1989;24(part 2):103-147.
32. Emdin SO, Stenling R, Roos G. Prognostic value of DNA content in colorectal carcinoma: A flow cytometric study with some methodologic aspects. Cancer 1987;60:1282-1287.
33. Quirke P, Dixon MF, Clayden A, et al. Prognostic significance of DNA aneuploidy and cell proliferation in rectal adenocarcinomas. J Pathol 1987;151:285-291.
34. Schutte B, Reynders MMJ, Wiggers T, et al. Retrospective analysis of the prognostic significance of DNA content and proliferative activity in large bowel carcinoma. Cancer Res 1987;47:5494-5496.
35. Scott NA, Wiegand HS, Moertel CG, et al. Colorectal cancer: Dukes stage, tumor site, pre-operative plasma CEA level, and patient prognosis related to tumor DNA ploidy pattern. Arch Surg 1987;122:1375-1379.
36. Scott NA, Rainwater LM, Wiegand HS, et al. The relative prognostic value of flow cytometric DNA analysis and conventional clinicopathologic criteria in patients with operable rectal carcinoma. Dis Colon Rectum 1987;30:513-520.
37. Scott NA, Grande JP, Weiland LH, et al. Flow cytometric DNA patterns from colorectal cancers—how reproducible are they? Mayo Clin Proc 1987;62:331-337.
38. Vogelstein B, Kinzler KW. p53 function and dysfunction. Cell 1992;70:523-526.
39. Levine AJ. The p53 tumor supressor gene and product. Cancer Surv 1992;12:59-80.
40. Finlay CA, Hinds PW, Tan TH, et al. Activating mutations for transformation by p53 produce a gene project that forms an hsc700-p53 complex with an altered half-life. Mol Cell Biol 1988;8:531-539.
41. Wynford-Thomas D. p53 in tumour pathology: Can we trust immunocytochemistry? J Pathol 1992;166:329-330.
42. Goh H-S, Chan C-S, Khine K, et al. p53 and behaviour of colorectal cancer. Lancet 1994;344:233-234.
43. Bell SM, Scott N, Cross D, et al. Prognostic value of p53 overexpression and c-Ki-*ras* gene mutations in colorectal cancer. Gastroenterology 1993;104:57-64.
44. Hamelin R, Laurent-Puig P, Olschwang S, et al. Association of p53 mutations with short survival in colorectal cancer. Gastroenterology 1994;106:42-48.
45. Campo E, Miquel R, Jares P, et al. Prognostic significance of the loss of heterozygosity of Nm23-H1 and p53 genes in human colorectal carcinomas. Cancer 1994;73:2913-2921.
46. Baas IO, Mulder JR, Johan G, et al. An evaluation of six antibodies for immunohistochemistry of mutant p53 gene product in archival colorectal neoplasms. J Pathol 1994;172:5-12.

Discussion

Dr. M.E. Zenilman (Bronx, N.Y.). I am relatively new to the field of biomarkers as well, because a protein that I have been following in the pancreas is actually ectopically expressed in the margins or the transition zones of colorectal cancers. I have several questions regarding this oncogene or marker, Ki-67, that you are following. Have you studied its expression on a qualitative basis? Have you examined slides to see where in the tumor this marker is expressed? Is it expressed in all the cancers? Is it expressed at the transition zones? Is it expressed in normal mucosa?

Dr. Y.-T. Chen. The marker seemed to be fairly uniformly expressed in the tumors that we examined. It does not seem to have a particular tendency toward the center or the periphery of the tumors.

Dr. D. Fromm (Detroit, Mich.). Perhaps you could tell us, how confident are you of the statistical power in your series? I know you did not have time to show us those data.

Dr. Chen. I am reasonably confident of the statistical power of the study. The caveat is that if you study the literature on the Ki-67 index and the related literature on PCNA, you can pick out any result that you want. I think this relates, in part, to the fact that the techniques for all of these studies have differed, and we used our own particular technique, which was different from those reported by others. To make some sense of this in the future, in a realistic manner, there will have to be standardization of the techniques and large series.

Does the Presence of a Pre–Ileostomy Closure Asymptomatic Pouch-Anastomotic Sinus Tract Affect the Success of Ileal Pouch-Anal Anastomosis?

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Ileal pouch-anal anastomosis (IPAA) is the procedure of choice for patients with ulcerative colitis and familial adenomatous polyposis. This two-stage procedure with a temporary diverting ileostomy avoids the catastrophic consequences of anastomotic leakage. We set out to determine the incidence and effect of asymptomatic pouch sinuses detected prior to ileostomy closure on the outcome of IPAA. A total 1600 IPAAs performed at the Mayo Clinic were reviewed. Forty-one (2.6%) asymptomatic sinuses were treated expectantly. There were 22 males and 19 females who had a median age of 32 years (range 14 to 58 years). The median time to ileostomy closure was 5.9 months (range 4 to 11 months). Five patients required further surgery following closure of ileostomy. The pouch function in these five patients was similar to that in the remainder of the group. Patients with a persistent sinus at the time of ileostomy closure had the same function as the main cohort. This group had a median of five (range 2 to 12) stools during the day and two (range 0 to 4) at night. The total number of stools per 24 hours was seven (range 2 to 14). Frequent incontinence occurred in 9.7% and 7.3% during the day and at night, respectively. Only 2.4% (1/41) were disappointed with the results of the operation and 80.4% (33/41) found their quality of life improved. Functional outcomes were comparable to those achieved with uncomplicated IPAA. Radiologically detected asymptomatic sinuses can be treated expectantly with a low rate of pouch loss and subsequent surgery. This is not considered a serious setback inasmuch as long-term function and quality of life are comparable to that achieved with IPAA without sinus tracts. (J GASTROINTEST SURG 1997;1:274-277.)

Ileal pouch-anal anastomosis (IPAA) has, over the past 18 years, evolved to become the surgical option of choice in the management of ulcerative colitis and some patients with familial adenomatous polyposis. The procedure is complex and carries a significant risk of complications, of which pelvic sepsis or pouch-anastomotic leakage are among the most feared. These may lead to disastrous complications associated with poor pouch function, which in turn leads to a poor quality of life punctuated by frequent, urgent bowel movements and incontinence.[1] The final outcome would, of course, be pouch loss.

Asymptomatic pouch-anastomotic sinuses are defined as sinuses that are detected only on preileostomy closure pouchograms. Patients with these sinuses are completely asymptomatic. Most of them have had a normal postoperative course after IPAA. They most probably represent one end of the spectrum of pelvic sepsis after IPAA. The incidence and effect of asymptomatic pouch-anastomotic sinuses detected on pouchogram prior to ileostomy closure is not well documented. We set out to determine the effect of these sinuses on the outcome of IPAA with particular reference to complications requiring further surgery, pouch loss, pouch function, and quality of life. An algorithm for the management of these asymptomatic pouch-anastomotic sinuses is presented in Fig. 1.

PATIENTS AND METHODS

Between 1981 and 1995, a total of 1600 patients had IPAAs constructed at the Mayo Clinic as a two-stage procedure. This involved the fabrication of an IPAA and a diverting ileostomy that was closed after

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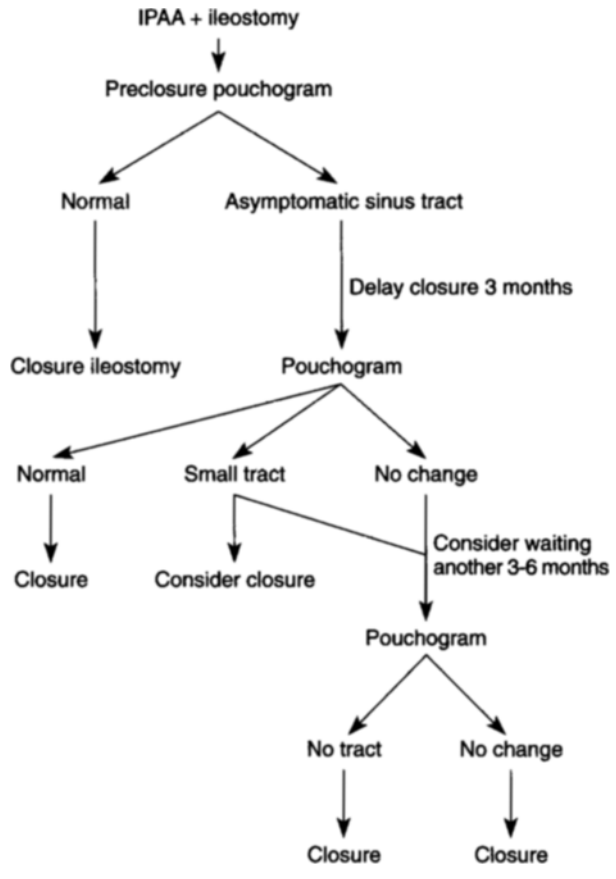

Fig. 1. Algorithm for the management of asymptomatic pouch-anastomotic sinus tract. IPAA = ileal pouch-anal anastomosis.

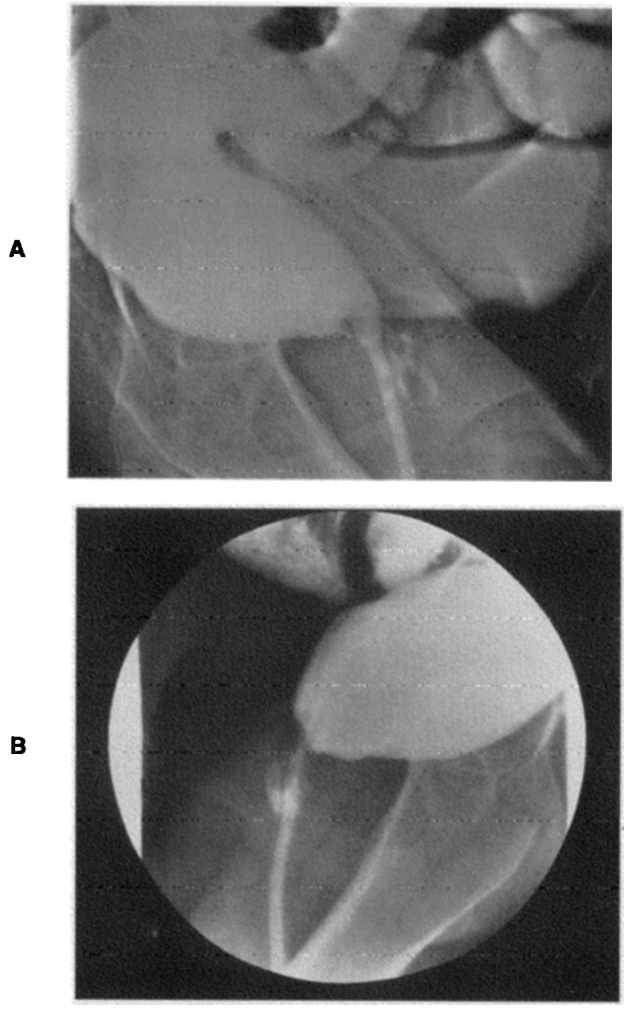

Fig. 2. A, Pouchogram of IPAA at 3 months postoperatively showing a sinus tract at the anastomosis. **B,** Pouchogram of the same patient 6 months postoperatively. The sinus tract documented previously has closed.

approximately 3 months if a pre–ileostomy closure pouchogram was normal. Data on these patients were obtained from a prospective computerized database and a review of patients' charts. Forty-one patients (2.6%) were found to have asymptomatic sinuses on pre–ileostomy closure pouchograms. Patients with symptomatic sinuses who had clinical or radiologic evidence of pelvic or intra-abdominal sepsis were excluded from this study. The ileostomies were closed after a period of expectant treatment when a repeat pouchogram showed that the sinus had closed (Fig. 2). Six of these patients had persistent sinuses despite a prolonged period of expectant management. These patients had their ileostomies closed if there was no increase in the size of the sinus tract on observation.

Chronic ulcerative colitis was the initial diagnosis in 37 of these patients. Subsequently, three patients were diagnosed with Crohn's disease and one with indeterminate colitis. The remaining four patients had familial adenomatous polyposis.

Six patients had prior surgery before IPAA (one had a colectomy for toxic megacolon, four underwent ileorectostomies, and one had a Hartmann's procedure). "J" pouch procedures were performed in all of them.

RESULTS

The overall incidence of asymptomatic pouch-anal sinuses was 2.6% (41/1600). There were 22 males and 19 females who had a median age of 32 years (range 14 to 58 years). The median time to ileostomy closure was 6 months (range 4 to 11 months). Eighty-eight percent (36/41) of these patients required no further surgery after ileostomy closure. Five patients required additional surgery (Table I). The results after further surgery in these patients were good. Six patients who underwent ileostomy closure despite persistent si-

Table I. Results in patients with persistent sinuses who required additional surgery

Patient	Operation performed after ileostomy closure	Results
1	Recurrent pouch sinus; fecal diversion with another ileostomy, observed; closed after a normal pouchogram	Good
2	Recurrent pouch sinus; fecal diversion, drainage of perineal abscess; closed after a normal pouchogram	Good
3	Anastomotic stricture; anoplasty	Good
4	Recurrent pouchitis; diagnosis is changed to Crohn's disease	Pouch excised
5	Drainage of multiple perianal abscesses; diagnosis is changed to Crohn's disease	Good

nuses did not have symptoms after closure of their ileostomies. Their pouch function was similar to that in patients who had normal pouchograms before closure.

Functional Outcome

All patients were able to evacuate spontaneously, and none required intubation. The mean stool frequency in this group of patients was seven per 24 hours (range 2 to 14). There was a mean of five stools during the day (range 2 to 12) and two stools at night (range 0 to 4). Sixty-one percent of the patients were always continent, 28% had occasional fecal spotting, and 11% had frequent soiling (defined as more than twice a week). Frequent incontinence occurred in 9.7% and 7.3% during the day and at night, respectively.

Late Complications and Pouch Loss

In one patient the pouch was excised because of recurrent inflammation. Histologic examination of the pouch revealed evidence of Crohn's disease. No other patient in this series required a permanent ileostomy. One patient has a high residual urinary volume requiring intermittent self-catheterization and one patient was impotent.

Patient Satisfaction and Quality of Life

Based on responses to a questionnaire survey, 97.6% of patients in this group were satisfied with the results of their surgery. Only 2.4% (1/41) were disappointed with the surgical outcome. This was the patient who required excision of the pouch. The quality of life after surgery was assessed with particular reference to sex life, social activities, ability to participate in sports or work around the house, recreational activities, family relationships, and travel. An overall quality-of-life score (1 to 5) was computer based on individual scoring of these aspects of each patient's life and 80.4% had a score higher than four (Table II). These outcomes were comparable to those of the main cohort of uncomplicated IPAAs.

Table II. Quality-of-life scoring

	No.
1 = Severely restricted	0
2 = Moderately restricted	1
3 = Mildly restricted	6
4 = Not affected	16
5 = Improved	18

DISCUSSION

A proctocolectomy "cures" patients with ulcerative colitis and familial adenomatous polyposis by removing all of the disease-bearing mucosa. IPAA restores the "rectal" reservoir and retains the transanal route for defecation. It has over the years been proven safe but carries a morbidity of approximately 29%.[2] Intra-abdominal and pelvis sepsis remain the most feared complications. Fortunately the risk of these appears to decrease according to the level of expertise of the surgeon,[3] occurring in approximately 6% of the total IPAA cohort.[1] However, when these septic complications occurred and required a laparotomy, the result was commonly pouch excision (41%), and among those who kept their pouches, ileostomy closure was infrequent (29%).[1]

The majority of patients who undergo IPAA at our institution have a two-stage procedure, with a pouchogram performed at approximately 3 months postoperatively, prior to ileostomy closure. Pouchograms

were initially used to assess pouch volumes and integrity.[4] Later they were also used to assess postoperative outcome,[5,6] as it was postulated that delaying ileostomy closure in the presence of an abnormal pouchogram prevented more serious complications such as pelvic sepsis and strictures. The current rationale of pouchography is to identify abnormalities that could cause major morbidity so that these can be corrected prior to closure. Tsao et al.[7] reported that abnormal pouchograms prior to ileostomy closure are associated with a high risk of long-term complications. However, the abnormalities included pouch or anastomotic leaks, anastomotic strictures, and mucosal irregularities. These may represent up to 16% of the preileostomy pouchograms.[7]

Patients with asymptomatic sinuses represent one end of the spectrum of pelvic sepsis. These sinuses were found to occur in 2.6% of the total cohort of IPAA patients. Patients who had these sinuses detected on pouchograms had their ileostomy closures delayed. All patients had their ileostomies closed with a median interval of 6 months between IPAA and closure of the ileostomy. The median time interval of the general cohort from the same institution was approximately 2.5 months.[2]

Surgery prior to IPAA did not seem to affect the rate of pouch-anastomotic sinuses. In fact, no patient in this series developed frank sepsis immediately following closure of the ileostomy and 88% required no further surgery.

Two of the patients who underwent further surgery were found to have Crohn's disease. One of them eventually had the pouch excised. In addition to these two patients, one other patient has since been diagnosed with Crohn's disease. Thus Crohn's disease may be a predisposing factor in the formation of a persistent pouch-anal sinus. It may be helpful to reexamine the pathologic findings in patients who develop these persistent sinuses to eliminate the possibility of Crohn's disease.

Three other patients required additional surgery as a result of the sinus. One required anoplasty and the remaining two required a further episode of diversion. The former patient developed a stricture following healing of the pouch-anal sinus, which was resistant to dilatation. Following the anoplasty, this patient has remained asymptomatic. Two of the 35 patients whose sinuses were found to have closed on a later pouchogram subsequently developed recurrent sinuses, which necessitated a further period of "fecal diversion." Both had poor pouch function with frequency and urgency. Both their ileostomies were subsequently closed and follow-up revealed good function in both. It is interesting that six other patients in whom the sinuses were present but stable in size on pouchogram had their ileostomies closed. They did not encounter any problems related to the pouch-anal sinus and they had good pouch function. We could not identify any predisposing factors in the two patients who were subjected to a second period of fecal diversion.

Pouchograms were subsequently obtained from 12 patients during follow-up. Two of these were from among the six patients who had persistent sinuses at the time of closure. In only one was a persistent sinus found. The remaining 10 had normal pouchograms.

As a result of the preceding analysis, the entire group was analyzed together for pouch function. The mean number of stools and incontinence rates in the pouches of patients with a history of pouch-anal sinuses were similar to those in the general cohort of this institution.[2] All patients were able to evacuate spontaneously, and none required a permanent ileostomy. The quality of life in this cohort of patients was good with 80% of them unrestricted in their daily activities. Only 2.4% (1/41) found life to be moderately or severely restrictive after the pouch procedure.

It is therefore reassuring to know that the symptomatic pouch-anal sinuses found on pouchograms before ileostomy closure are relatively benign and can be treated expectantly. The pouch can be preserved in the majority of cases unless some predisposing factor such as Crohn's disease is discovered. The rate of subsequent surgery is low and is not affected if closure is performed in sinuses that are stable over a period of observation. The asymptomatic pouch-anal sinus does not appear to ultimately be a serious setback for patients with the IPAA inasmuch as long-term pouch function and quality of life are comparable to that noted in patients without sinus tracts.

REFERENCES

1. Scott NA, Dozois RR, Beart RW Jr, Pemberton JH, Wolff BG, Ilstrup DM. Postoperative intra-abdominal and pelvic sepsis complicating ileal pouch-anal anastomosis. Int J Colorectal Dis 1988;3:149-152.
2. Pemberton JH, Kelley KA, Beart RW, Dozois RR, Wolff BG, Ilstrup DM. Ileal pouch-anal anastomosis for chronic ulcerative colitis. Long-term results. Ann Surg 1987;206:504-513.
3. Dozois RR, Goldberg SM, Rothenberger DA, Utsunomiya J, Nicholls RJ, Cohen Z, Hulten LAG, Moskowitz RL. Restorative proctocolectomy with ileal reservoir. Int J Colorectal Dis 1986;1:2-19.
4. Parks AG, Nicholls RJ. Proctocolectomy without ileostomy for ulcerative colitis. BMJ 1978;2:85-88.
5. Hillard AE, Mann FA, Becker JM, Nelson JA. The ileo-anal and J-pouch: Radiographic evaluation. Radiology 1985;155: 591-594.
6. Kremers PW, Scholz FJ, Schoetz DJ Jr, Veidenheimer MC, Coller A. Radiology of the ileo-anal reservoir. AJR 1985;145:559-567.
7. Tsao JI, Galandiuk S, Pemberton JH. Pouchogram: Predictor of clinical outcome following ileal pouch-anal anastomosis. Dis Colon Rectum 1992;35:547-551.

Early Postoperative Enteral Feeding Following Major Upper Gastrointestinal Surgery

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For a variety of reasons, enteral feeding is frequently delayed following major abdominal surgery. The purpose of this study was to evaluate prospectively the feasibility and tolerance of early jejunal feeding following major upper gastrointestinal surgery. Beginning on postoperative day 1, patients (n = 167) received a full-strength enteral formula at the rate of 25 ml/hr through a jejunal feeding tube. Diets were advanced to the calculated target rate (25 kcal/kg/day) by postoperative day 4. Complications of tube feeding, calories received, and patient symptoms were recorded daily. There were no major complications or deaths resulting from placement of a jejunal tube or from early enteral feeding. Patients had abdominal symptoms such as cramping, distention, nausea, and diarrhea on 9%, 18%, 4%, and 24% of all feeding days, respectively. The majority of these symptoms, with the exception of diarrhea, were graded as mild. Patients undergoing surgery for pancreatic malignancy had significantly more diarrhea than patients undergoing esophagectomy or gastrectomy. Despite these differences in symptoms, patients received an average of 78% of their targeted caloric goal by postoperative day 4 and maintained this level throughout the study. Early enteral feeding for patients undergoing esophageal, gastric, or pancreatic resections is both safe and feasible despite the occurrence of predominantly mild gastrointestinal symptoms. (J GASTROINTEST SURG 1997; 1:278-285.)

Initiation of enteral feeding following major gastrointestinal surgery is traditionally delayed until the return of overt bowel activity as indicated by the passage of flatus or stool. Despite the current practice, there is little if any evidence to support this approach. In contrast, there is increasing experimental evidence that early enteral feeding has beneficial effects in terms of preventing mucosal atrophy and decreasing the secretion of catabolic hormones.[1,2] Furthermore, there is mounting clinical evidence suggesting that early enteral feeding may be beneficial in reducing the incidence of infections or complications in trauma,[3] burn,[4] cancer,[5] and colon surgery[6] patients. Although some investigators claim that early postoperative jejunal feeding following major abdominal surgery may not be well tolerated,[7] our impression has been that, despite the occurrence of mild symptoms, early postoperative jejunal feedings are generally well tolerated. With this in mind, we used a prospectively gathered database on jejunal tube feeding to conduct an in-depth analysis on the feasibility, safety, and tolerance of early enteral feeding following major esophageal, gastric, and pancreatic surgery.

METHODS

Patients at the University of Pennsylvania undergoing major gastrointestinal surgery for carcinoma of the esophagus, stomach, and pancreas were entered into sequential prospective enteral nutrition studies comparing standard enteral formulas (Osmolite HN [Ross Laboratories, Columbus, Ohio] or TraumaCal [Mead Johnson Nutritionals, Evansville, Ind.]) with a formula supplemented with arginine alone or arginine, fish oil, and ribonucleic acid (Impact [Sandoz Nutrition, Minneapolis, Minn.]).[5,8,9] Table I lists the major components for each of the formulas. The feeding regimen for these studies called for 5% dextrose in water (30 ml/hr) to begin immediately postoperatively via a surgically placed feeding jejunostomy tube. Beginning the morning of postoperative day 1, full-strength nutritional feedings were initiated at a

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rate of 25 ml/hr and increased by 25 ml/hr each day until the target rate was achieved (maximum 100 ml/hr). Each patient's ability to tolerate this tube feeding regimen was recorded daily for the first eight postoperative days noting symptoms associated with tube feeding such as cramping, distention, nausea, and diarrhea. Symptoms were graded on a scale of 1 to 3 as follows: 1 = mild; 2 = moderate; and 3 = severe. Patients with grade 3 symptoms had their tube feedings withheld for 8 hours and then resumed at the previously tolerated rate. Tube feedings were then increased as before to the target rate as tolerated. Formula volume and calories received were recorded daily as were any interruptions in the tube feeding schedule. In addition, both minor mechanical complications of feeding tube (e.g., tube occlusion or dislodgment) and major complications (e.g., intra-abdominal leaks, intestinal obstruction, and intestinal necrosis) were noted.

The estimated cost of tube feeding was calculated based on the costs of operating room time, supplies, and services at our institution. The jejunostomy procedure was estimated to require an additional 10 minutes of operating room time. The added expense of the feeding tube and suture material was included in the operating room costs. Nursing time for maintenance of feeding tubes, changing of tubing and dressings, and preparation of the formula was estimated at 1 hour/day. The cost of nursing care was estimated at $33/hr including benefits. The cost of the feeding bag, tubing, and pump rental was estimated at $10/day, whereas the cost of Osmolite NH and Impact is $2 and $19/L, respectively.

Subgroup analyses for each of the three primary malignancies, esophageal, gastric, and pancreas, were performed for each of the symptoms or complications mentioned previously. Statistical tests were conducted using the InStat software package (GraphPad Software, San Diego, Calif.). Comparisons were made using chi-square analysis, Fisher's exact test, and analysis of variance with a Newman-Keuls post-test analysis where appropriate. A P value of <0.05 was required for statistical significance.

Table I. Comparison of enteral formulas*

	Osmolite HN	Traumacal†	Impact
Protein (g)	44	62	59
Lipids (g)	37	49	28
Carbohydrates (g)	141	107	132
Osmolality (mOsom/kg H_2O)	300	338	375
Calories (kcal)	1060	1120	1000

*Values are per liter of formula.
†Based on using three-fourths strength Traumacal.

RESULTS

Information from 167 patients with esophageal, gastric, and pancreatic malignancies who had operative placement of a feeding jejunostomy was analyzed. A summary of patient characteristics from each of the three primary diseases is presented in Table II. The mean length of postoperative hospital stay was not significantly different among the three groups.

Table III presents a comparison of symptoms associated with the use of supplemented and standard formulas. Although a higher percentage of patients in the supplemented group had symptoms compared to patients in the standard group, these differences were

Table II. Patient characteristics

	Primary disease		
	Esophagus	Gastric	Pancreas
No.	67	44	56
Sex (M/F)	54/13	25/19	34/22
Age (yr)*	63.0 ± 1.5	66.8 ± 2.0	59.3 ± 2.0
Weight (kg)*	77.7 ± 2.4	67.6 ± 2.4	71.4 ± 1.8
Hospital stay (days)*	19.4 ± 1.5	17.2 ± 1.5	19.4 ± 1.1
Stage			
0	2	0	0
1	8	10	11
2	24	8	11
3	25	11	11
4	8	8	8

*Values are mean ± standard error.

Table III. Tolerance of jejunal tube feeding: Comparison of supplemented and standard formulas

Symptoms	Formula	% of symptomatic patients* (No.)	% of symptomatic days† (No.)
Nausea	Supplemented	24 (17/70)	5 (24/518)
	Standard	18 (13/73)	4 (22/521)
Diarrhea	Supplemented	66 (46/70)	29 (152/518)
	Standard	55 (40/73)	21 (108/521)
Cramping	Supplemented	50 (20/40)	9 (28/308)
	Standard	36 (16/45)	10 (31/325)
Distention	Supplemented	60 (24/40)	19 (59/308)
	Standard	44 (22/45)	17 (56/325)
Occluded	Supplemented	0	0
	Standard	4 (3/40)	2 (8/325)
Interrupted	Supplemented	39 (27/70)	9 (48/518)
	Standard	49 (36/73)	15 (76/521)

*Percentage of all patients who experienced any grade of symptoms through postoperative day 8.
†Percentage of all potential feeding days during which patients had symptoms.

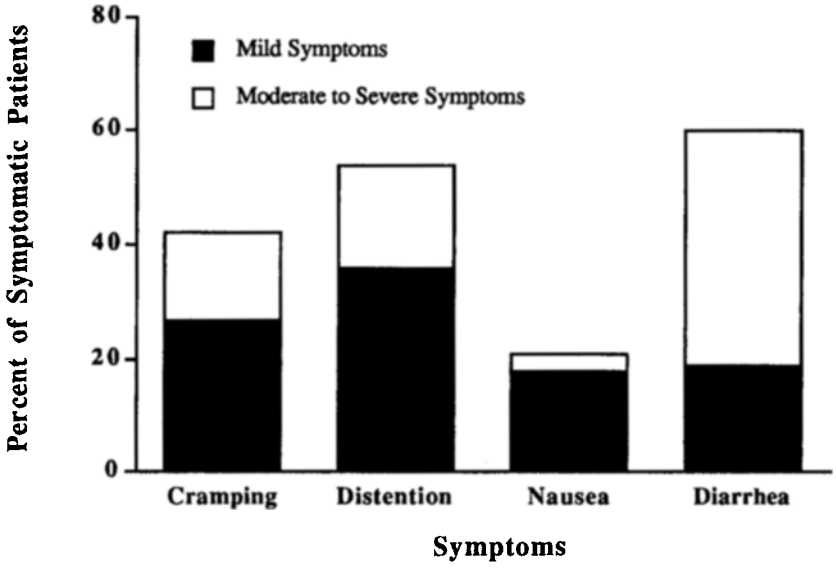

Fig. 1. Percentage of patients experiencing mild vs. moderate or severe symptoms.

not statistically significant. Furthermore, the percentage of symptomatic days did not differ among the groups.

In analyzing the percentages of patients experiencing any grade of symptoms during the first eight postoperative days, we found that 42% of patients overall had some abdominal cramping, 54% had abdominal distention, 21% had some nausea, and 60% had some episodes of diarrhea. Breaking this down into the percentages of patients with mild vs. moderate or severe symptoms, we found that the majority of patients had mild symptoms with the exception of diarrhea where more than half of the symptomatic patients had grade 2 or 3 diarrhea (Fig. 1). In analyzing the percentages of all days during which patients had symptoms, we observed a similar trend with diarrhea occurring on 24% of all feeding days and patients experiencing grade 2 or 3 symptoms on 13% of all feeding days (Fig. 2).

Symptoms attributed to tube feeding were also analyzed by postoperative day according to the type of surgery. Only minor differences were noted among the three groups of patients with regard to cramping, nausea, and distention. However, as shown in Fig. 3, patients undergoing pancreatectomy had significantly more diarrhea (all grades) on postoperative days 4, 5, and 6 compared to patients undergoing esophagectomy and gastrectomy ($P < 0.05$, chi-square analysis).

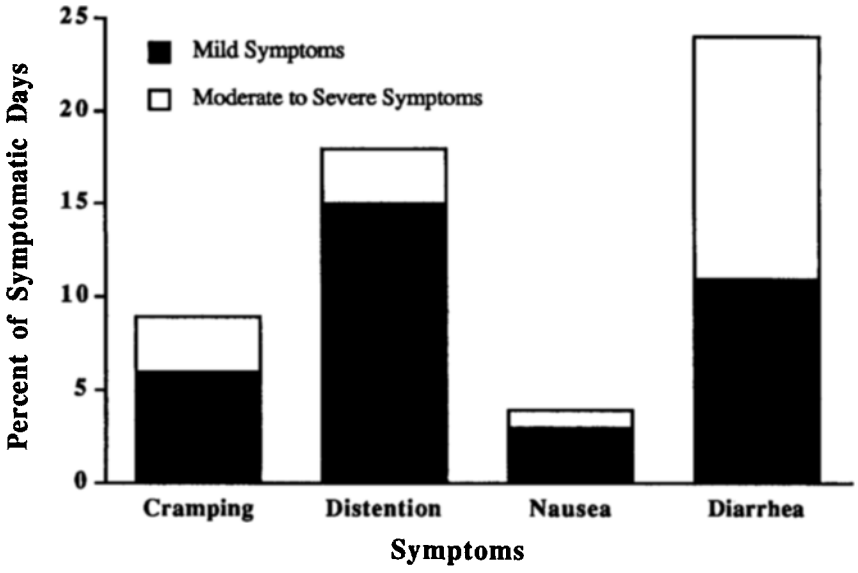

Fig. 2. Percentage of days patients had mild vs. moderate or severe symptoms.

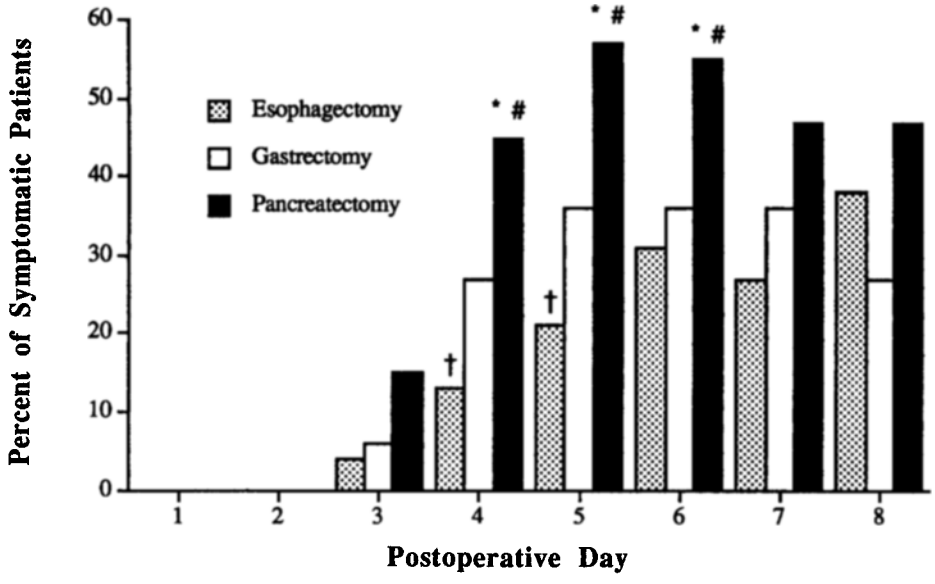

Fig. 3. Postoperative diarrhea by day and type of surgery. * = $P < 0.05$ (by large table chi-square analysis); # = $P < 0.05$ for pancreatectomy vs. esophagectomy plus gastrectomy; † = $P < 0.05$ for esophagectomy vs. pancreatectomy plus gastrectomy (chi-square analysis).

Those patients undergoing esophagectomy had significantly less diarrhea on postoperative days 4 and 5 compared to patients undergoing pancreatectomy and gastrectomy.

Throughout these studies the routine use of a feeding jejunostomy added no major morbidity or mortality inasmuch as there were no serious complications (intra-abdominal leaks, intestinal obstruction, or intestinal necrosis) or deaths associated with the feeding tube. Mechanical difficulties such as malfunctioning, dislodgment, or occlusion of tubes occurred in 6% of patients, representing 1% of all potential feeding days.

Overall, 44% of patients had their feedings interrupted at some point during their postoperative course. Interruptions in tube feeding occurred on 12% of all potential feeding days.

Although there are some daily differences in vol-

ume and calories received among patients undergoing operations for esophageal, gastric, and pancreatic malignancies, there were no significant differences among the groups (Figs. 4 and 5). As a whole, the patients achieved an average caloric intake of 1365 kcal/day by postoperative day 4 (78% of the calculated target) and maintained their intake at this level for the remainder of the study.

The estimated costs associated with tube feeding are shown in Table IV. The estimated costs of routine jejunostomy feeding for 1 week with a standard enteral formula was $443 vs. $626 for the supplemented formula.

Table IV. Estimated costs for routine tube feeding

	Cost
Time in operating room (10 min)	$120
Nursing care (1 hr/day @ $33/hr × 7 days)	$231
Tubing/pump (@ $10/day × 7 days)	$ 70
Formula (× 7 days)	
Osmolite HN	$ 22
Impact	$205
Total cost (1 week of feeding)	
Osmolite HN	$443
Impact	$626

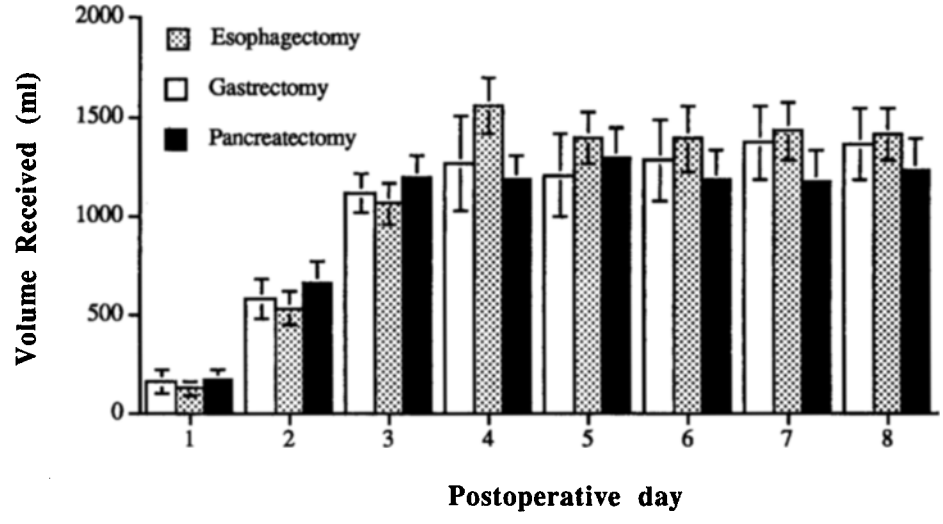

Fig. 4. Postoperative volume of formula received by day and type of surgery. Values are mean ± standard error of the mean.

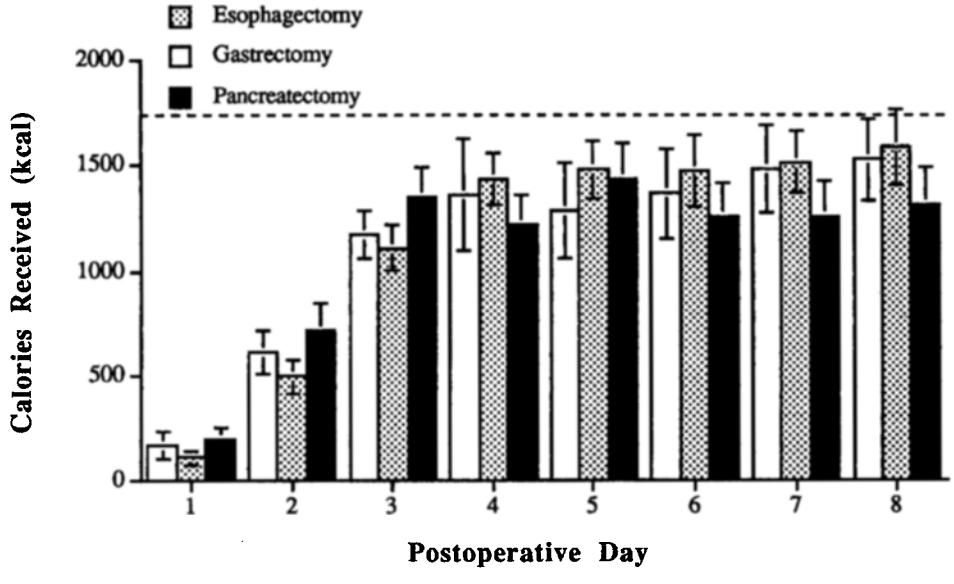

Fig. 5. Postoperative calories received by day and type of surgery. Values represent mean ± standard error of the mean. Broken line indicates mean calculated target caloric intake for all patients.

DISCUSSION

Early tube feeding beginning the morning of postoperative day 1 was successful in patients undergoing major resections for esophageal, gastric, and pancreatic malignancies. The safety of using jejunal feeding tubes is an important consideration whenever their use is contemplated. In our series the use of jejunal feeding tubes proved to be quite safe and there were no serious tube-related complications. Although some series have found unacceptably high rates of complications associated with the use of these tubes,[10] the majority of investigators have found low complication rates.[11] In a review of 10 previously published series examining jejunal tube complications, Eddy and Snell[12] found tube-related complication rates ranging from 0% (in 5 of the 10 published series) to 8% of patients with an average complication rate of 2%.

The use of early enteral nutrition following trauma, burns, or major intestinal surgery has gained favor in recent years. One reason for this surge in interest is the increasingly recognized central role played by the gut in terms of both maintaining nutritional status and regulating the immune system.[13] As expected, early postoperative jejunal feeding provides enhanced nutritional benefits following surgery or trauma.[14,15] Supplying nutrition alone (as occurs with total parenteral nutrition), however, does not restore a natural equilibrium because the route of feeding can influence the host's response to injury and intestinal function.[16,17] Other investigators have shown that the combination of extended bowel rest and treatment with total parenteral nutrition alters the natural gastrointestinal immunity and barrier function allowing for increased bacterial translocation.[18,19] Furthermore, some of these alterations can be prevented by the use of early enteral nutrition.[18,20]

The proposed nutritional and gastrointestinal immune benefits of early enteral nutrition may not be limited to trauma and burn patients. For example, cancer patients, especially those with upper gastrointestinal malignancies, are known to have high rates of malnutrition.[21] Providing these types of patients with early enteral nutrition reduces the incidence of infectious complications.[5,8,9]

Although our patients did not experience any serious complications as a result of jejunal tube feeding, a significant number had symptoms that could be attributed to the use of enteral feeding. As seen in Figs. 1 and 2, many patients had at least 1 day of abdominal cramping, distention, nausea, or diarrhea. When symptoms are analyzed by postoperative day, there are some differences between groups such as the occurrence of more diarrhea in patients undergoing pancreatectomy. However, these differences were relatively transient and did not result in a significant reduction in volume or calories delivered. Few other studies have specifically examined such symptoms related to jejunal feeding. In one study comparing patients with abdominal trauma who were fed an elemental formula through a jejunostomy tube with trauma patients who received total parenteral nutrition or no enteral nutrition, 83% of patients in the enterally fed group had some gastrointestinal symptoms (nausea, cramping, distension, and/or diarrhea) compared to 50% of patients in the control group.[22] The relatively high rate of gastrointestinal symptoms in the control group is not unique and may be related to the inclusion of patients with abdominal trauma and those receiving total parenteral nutrition, whereas the relatively higher rate in the treatment group may be related to the use of an elemental formula.[22,23] Up to 60% of patients had at least one of the four symptoms during jejunal feeding. The direct correlation between jejunal feeding and all of the symptoms experienced in this study is not clear because the percentage of symptomatic patients in our study is comparable to the percentage in the control group without jejunal feeding in the study by Jones et al.[22] Furthermore, in analyzing symptoms in greater detail according to the grade of severity, we found that the majority of patients had grade 1 symptoms of cramping, distention, and nausea. The exception was diarrhea, which had the highest percentage of symptomatic patients (any grade) as well as the greatest proportion with grade 2 or 3 symptoms.

Differences in abdominal cramping, distention, diarrhea, or nausea in our study are not attributed to the use of different enteral formulas because there were no significant differences in symptoms when the formulas were compared individually. This would suggest that any transient differences in symptoms detected during the study were more likely related to the primary disease or the extent of surgery rather than to the specific formula used.

A significant percentage of our patients (44%) had at least one interruption in their tube feeding regimen during the study period. Although the reasons for each interruption were not specifically recorded, tube feedings were routinely withheld because of the development of severe symptoms, malfunctioning jejunostomy tubes, or the need for reoperation or other studies. The increased percentage of patients with diarrhea identified among those undergoing pancreatectomy did not correlate with any significant increase in feeding interruptions in this group. It is worth noting that patients in this study were not routinely supplemented with pancreatic enzymes and could have been suffering from a relative deficiency of pancreatic secretory enzymes. Although mechanical problems with the jejunostomy tube contributed to an interruption of feeding in 6% of patients, this affected only 1% of all potential feeding days. Therefore mechani-

cal problems associated with feeding jejunostomy tubes accounted for relatively few days of missed nutrition.

Despite differences in symptoms and interruptions in tube feedings among some of the patients in these groups, these minor differences did not result in a significant impact on the volume of formula or calories delivered. Patients undergoing major resections for esophageal, gastric, and pancreatic malignancies received 78% of their calculated target calories by postoperative day 4 and maintained this level through postoperative day 8. Attaining specific caloric goals may not be absolutely necessary because the presence of any enteral nutrition (as opposed to the more traditional no enteral nutrition) may be sufficient to provide some benefit. Furthermore, patients who received the supplemented formula achieved a positive nitrogen balance by postoperative day 6.[8]

The use of enteral as opposed to parenteral nutrition has been favored for a number of physiologic reasons as previously discussed. There are also sound economic reasons favoring enteral nutrition in that it represents a more cost-effective method of feeding patients.[24] Bufo et al.[25] found that patients who tolerated early oral feeding following colon surgery had a shorter postoperative length of stay in the hospital (5.7 days) than those who did not tolerate early oral feeding (8 days), thus demonstrating another potential benefit of early enteral feeding. Although this study did not directly analyze the cost of enteral feeding (since all patients were fed enterally), we believe that the use of routine jejunostomy tube feeding in patients undergoing major upper abdominal operations for malignancy is economically sound. Based on our estimates, the cost of using routine jejunostomy feedings in these patients for 1 week (including operating room time) is approximately $443. If early enteral feeding lowers the rate of infectious complications and shortens the length of hospital stay, then the additional expense in a select group of patients at increased risk for prolonged hospitalization would be well worth the cost. In addition, the use of jejunostomy feeding in those patients who develop major postoperative complications (wound or anastomotic) obviates the use of parenteral nutrition and provides a simpler means of discharging them to their homes or to an extended care facility.

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REFERENCES

1. Mochizuki H, Trocki O, Dominioni L, Brackett K, Joffe S, Alexander J. Mechanisms of prevention of postburn hypermetabolism and catabolism by early enteral feeding. Ann Surg 1984;200:297-307.
2. Dominioni L, Trocki O, Mochizuki H. Prevention of severe postburn hypermetabolism and catabolism by immediate intragastric feeding. J Burn Care Rehabil 1984;5:106-112.
3. Moore F, Feliciano D, Andrassy R, McCardle A, Booth F, Morgenstein-Wagner T, Kellum J Jr, Welling R, Moore E. Early enteral feeding, compared with parenteral, reduces postoperative septic complications. Ann Surg 1992;216:172-183.
4. Gottschlich M, Jenkins M, Warden G, Baumer T, Havens P, Snook J, Alexander J. Differential effects of three enteral dietary regimens on selected outcome variables in burn patients. JPEN 1990;14:225-236.
5. Daly J, Lieberman M, Goldfine J, Shou J, Weintraub F, Rosato E, Lavin P. Enteral nutrition with supplemental arginine, RNA, and omega-3 fatty acids in patients after operation: Immunologic, metabolic, and clinical outcome. Surgery 1992; 112:56-67.
6. Reissman P, Teoh T, Cohen S, Weiss E, Nogueras J, Wexner S. Is early oral feeding safe after elective colorectal surgery? A prospective randomized trial. Ann Surg 1995;222:73-77.
7. Hayashi J, Wolfe B, Calvert C. Limited efficacy of early postoperative jejunal feeding. Am J Surg 1985;150:52-57.
8. Daly J, Reynolds J, Thom A, Kinsley L, Dietrick-Gallagher M, Shou J, Ruggieri B. Immune and metabolic effects of arginine in the surgical patient. Ann Surg 1988;208:512-523.
9. Daly J, Weintraub F, Shou J, Rosato E, Lucia M. Enteral nutrition during multimodality therapy in upper gastrointestinal cancer patients. Ann Surg 1995;221:327-338.
10. Smith R, Hartemink R, Hollinshead J, Gillet D. Fine bore jejunostomy feeding following major abdominal surgery: A controlled randomized clinical trial. Br J Surg 1985;72:458-461.
11. Meyers J, Page C, Stewart R, Schesinger W, Sirinek K, Aust J. Complications of needle catheter jejunostomy in 2,002 consecutive applications. Am J Surg 1995;170:547-551.
12. Eddy V, Snell J Jr. Analysis of complications and long term outcome of trauma patients with needle catheter jejunostomy. Am Surg 1996;62:40-44.
13. Wilmore D, Smith R, O'Dwyer S, Jacobs D, Ziegler T, Wang X. The gut: A central organ after surgical stress. Surgery 1988;104:917-923.
14. Moore E, Jones T. Benefits of immediate jejunostomy feeding after major abdominal trauma—A prospective, randomized study. J Trauma 1986;26:874-881.
15. Hoover HJ, Ryan J, Anderson E, Fischer J. Nutritional benefits of immediate postoperative jejunal feeding of an elemental diet. Am J Surg 1980;139:153-159.
16. Lowry S. The route of feeding influences injury response. J Trauma 1990;30:10-15.
17. Thompson J, Vaughan W, Forst C, Jacobs D, Weekly J, Rikkers L. The effect of route of nutrient delivery on gut structure and diamine oxidase levels. JPEN 1987;11:28-32.
18. Alverdy J, Chi H, Sheldon G. The effect of parenteral nutrition on gastrointestinal immunity: The importance of enteral stimulation. Ann Surg 1985;202:681-684.
19. Alverdy J, Aoys E, Moss G. Total parenteral nutrition promotes bacterial translocation from the gut. Surgery 1988; 104:185-190.
20. Gianotti L, Nelson J, Alexander J, Chalk C, Pyles T. Postinjury hypermetabolic response and magnitude of translocation: Prevention by early nutrition. Nutrition 1994;3:225-231.

21. Daly J, Redmond H, Gallagher H. Perioperative nutrition in cancer patients. JPEN 1992;16:100S-105S.
22. Jones T, Moore F, Moore E, McCroskey B. Gastrointestinal symptoms attributed to jejunostomy feeding after major abdominal trauma—A critical analysis. Crit Care Med 1989; 17:1146-1150.
23. Adams S, Dellinger E, Wertz M, Oreskovich M, Simonowitz D, Johansen K. Enteral versus parenteral nutritional support following laparotomy for trauma: A randomized prospective trial. J Trauma 1986;26:882-891.
24. Meguid M, Campos A, Hammond W. Nutritional support in surgical practice. Am J Surg 1990;159:345-358.
25. Bufo A, Feldman S, Daniels G, Lieberman R. Early postoperative feeding. Dis Colon Rectum 1994;37:1260-1265.

Discussion

Dr. H. Sax (Rochester, N.Y.). After pancreatic resection there may be a relative deficiency of intraluminal enzymes. Both of your enteral formulas were of the polymeric whole-protein type. Is there a role for a more defined diet, such as a peptide-based or elemental diet, in the subset of patients undergoing pancreatic resection? None of these formulas contained fiber. As you know, both soluble and insoluble fiber may be helpful in patients with diarrhea. Finally, were there any patients from whom, because of hypovolemia or a relative state of shock, you withheld feedings to reduce the chance of worsening ischemia?

Dr. M.D. McCarter. These are three excellent points you raised. You are correct in stating that there may be an advantage to elemental formulas, although the one other trial that actually reported symptoms associated with an elemental formula found that 87% of patients who were given an elemental formula complained of some similar abdominal symptoms. These investigators did not go into detail about that, but I think that even with elemental formulas patients can have those complaints. I should also point out that these patients did not receive any pancreatic supplements such as Pancrease, and that may have contributed to the more frequent symptoms. Finally, there is increasing evidence to suggest that adding fiber may help improve the diarrhea.

As far as stopping the tube feeding during shock or hypotensive states, yes that absolutely did occur. The interruptions in feeding could have occurred because of severe symptoms, for tests, or for hypotensive states.

Dr. B. Miedema (Columbia, Mo.). We have been looking at this same issue of prospectively determining how often we have to stop or slow the tube feeding, and our results have been the same as yours. About 50% of the patients cannot tolerate these tube feedings. But you have been much more aggressive in reinstituting feedings by starting them within 8 hours, whereas we usually wait several days. Have you had any problems with that? How many patients in whom you reinstituted the feedings after 8 hours had to have them stopped again? Are there any criteria that you might use to predict which patients will not tolerate tube feedings?

Dr. McCarter. As far as being very aggressive, that certainly is our approach, and we have been able to use it quite effectively. You are correct that a fair number of patients do not tolerate resumption of the tube feedings. I do not have a specific number for you, but anecdotally I would say that it is probably in the 50% range. Even after starting at a slower rate, occasionally we do have to stop again for another 8 hours and then resume the feedings again at a slower rate. Eventually all of these patients tolerate at least some enteral nutrition.

Dr. C.R. Fleming (Jacksonville, Fla.). Please tell us more about the tubes that you used and how they were inserted. Was there any correlation between postoperative complications and medications such as analgesics and antibiotics? What was the rate of sepsis following these operations and how might that have been related to the feeding that you used?

Dr. McCarter. The tubes are placed intraoperatively. A 12 F red rubber tube and Witzel jejunostomy is used for the most part. Some of the patients did receive a needle catheter jejunostomy. Overall, some sort of septic complication developed in approximately 5% to 10% of the patients. Again, in this trial none of those major complications occurred at the feeding tube or the jejunostomy, although other studies have found a low incidence of major complications related to the tube feeding.

Dr. Fleming. Were there associations between postoperative complications such as nausea and bloating and postoperative analgesia, and was diarrhea during the period of tube feeding associated with the use of antibiotics?

Dr. McCarter. We did not keep track of that. I think that is an excellent point. Although our database did not track all of those variables, I would say that for our purposes these tubes were used only for feeding, and routine medications were not given through the feeding tubes.

Dr. Sax. There have been criticisms of enteral feeding postoperatively because of intestinal ischemia. Did you encounter any of that? And if not, please stress the fact that it does not occur.

Dr. McCarter. That is a very good point. In this trial we did not see any instances of intestinal ischemia, although in our experience with other patients, on rare occasions we do see it. My take on the literature is that major complications occur, maybe 1% or 2% of the time, from the feeding tube. I would say anecdotally that we have seen one or two patients with intestinal necrosis that may have been attributed to tube feeding.

Endotoxin Temporarily Impairs Canine Jejunal Absorption of Water, Electrolytes, and Glucose

Joseph J. Cullen, M.D., Lynda L. Hemann, B.S., Kimberly S. Ephgrave, M.D., Marilyn M. Hinkhouse, B.S.

Enteral feeding during and after episodes of sepsis may be beneficial. The aim of our study was to determine the effects of a single sublethal dose of endotoxin on canine jejunal absorption. Following a 240 kcal liquid meal, absorption studies were performed in eight dogs with 75 cm jejunal Thiry-Vella fistulas. These fistulas were perfused with an isotonic solution containing polyethylene glycol to calculate absorption. Each dog was then given a single dose of *Escherichia coli* lipopolysaccharide, 200 μg/kg intravenously, and the studies were repeated for the next 3 days. Following endotoxin bolus infusion, net absorption of water, electrolytes, and glucose was decreased for 2 days and returned to baseline values on postendotoxin day 3. A single sublethal dose of endotoxin temporarily impairs canine jejunal absorption. Although enteral feeding may be advantageous, jejunal absorption may be temporarily impaired following an episode of endotoxemia. (J GASTROINTEST SURG 1997;1:286-291.)

Sepsis has been shown to adversely affect the barrier and metabolic functions of the small intestine.[1-4] Enteral nutrition may alter these effects, as experimental and clinical studies suggest that enteral feeding during and after episodes of sepsis may be beneficial. Experimentally, enteral feeding has been shown to alter the metabolic response to trauma, increase gut blood flow, and maintain gut barrier function.[5,6] Alexander et al.[7] showed that enteral feeding improved outcome compared with parenteral feeding in burned children. Similarly Moore et al.[8] demonstrated that enteral feeding improved outcome compared with parenteral feeding in adult trauma patients. Aditionally, improved survival has been demonstrated in septic animals if they are fed enterally rather than parenterally.[9] Thus it has been suggested that enteral rather than parenteral nutrition should be given to critically ill patients and that enteral nutrition should be started immediately after the insult to have any beneficial effects.[10] However, it is not known whether gut absorptive function of water and electolytes is impaired by sepsis or endotoxemia and, if so, to what degree and for how long.

There are a number of studies to suggest that gut absorption is affected during sepsis. Gut absorption of D-xylose and amino acids is depressed in rats after cecal ligation and puncture.[11,12] Additionally, Singh et al.[13] have shown decreased gut absorption of D-xylose in critically ill trauma patients. Thus gut absorptive capacity may be impaired during and after an episode of sepsis. The aim of our study was to determine the effects and time course of a single sublethal dose of endotoxin on canine jejunal absorption of water and electrolytes.

MATERIAL AND METHODS

Preparation of Animals

All procedures, care of animals, and conduct of experiments were carried out according to the protocol approved by the Veterans Administration and the University of Iowa Animal Care and Use Committees. Surgical procedures and experiments were performed in accordance with the "Guide for the Care and Use of Laboratory Animals" published by the National Institutes of Health (NIH Publication No. 80-23, revised 1985). Eight conditioned mongrel dogs weighing 12 to 18 kg were anesthetized with thiopental sodium (25 mg/kg) and halothane. Under aseptic operating conditions, the dogs underwent construc-

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tion of a jejunal Thiry-Vella fistula. Briefly, a 75 cm segment of jejunum located 30 cm from the ligament of Treitz was isolated and the anatomic continuity of the remaining bowel reestablished by end-to-end anastomosis. A metal perfusion cannula was inserted in the proximal end of the jejunal loop and the proximal end was oversewn. The distal end of the jejunum was connected to a modified Thomas cannula, which was then brought through the abdominal wall. The animals were allowed to recover for 2 weeks before baseline studies were begun. During the recovery period the cannulas were flushed periodically with 0.9% NaCl to prevent mucous plugging.

Conduct of Experiments

After an 18-hour fast, each dog was gavage fed a 240 kcal liquid meal consisting of 240 ml of Sustacal (Mead Johnson Nutritionals, Evansville, Ind.). The distal cannula was irrigated with 30 ml of 0.9% NaCl to clear it of debris. The Thiry-Vella fistula was perfused via the proximal cannula at 2.9 ml/min using a pump (Masterflex, Cole-Parmer Instrument Co., Niles, Ill.) with warmed (37° C) solution containing 120 mmol/L NaCl, 20 mmol/L $NaHCO_3$, 5 mmol/L KCl, 5.6 mmol/L glucose, and 5 g/liter polyethylene glycol (PEG). The infusion was continued for 1 hour before any measurements of transport were done. Thereafter 15-minute outputs from the distal stoma were collected for 1 hour. The volume of each 15-minute aliquot of effluent from the Thiry-Vella fistula was recorded and analyzed for Na^+, Cl^-, K^+, glucose, and PEG concentrations. Na^+, Cl^-, and K^+ concentrations were determined by ion-specific electrodes (EasyLyte, Medica, Bedford Mass.). Glucose concentrations were determined by the hexokinase method (Sigma Diagnostics, St. Louis, Mo.). The concentration of PEG in the sample was determined by a turbidimetric method[14] using a spectrophotometer (Coleman Junior II, Coleman Instruments, Maywood, Ill.) to calculate the recovery of PEG.

Following completion of two baselines studies on each dog, the endotoxin studies were begun. After an overnight fast, each dog was given a single bolus infusion of *Escherichia coli* lipopolysaccharide, serotype 055:B5 (Sigma Chemical Co., St. Louis, Mo.), 200 μg/kg, intravenously. Jejunal infusions and the liquid meal were then begun 2 hours after the endotoxin bolus was given to ensure that the dog would not vomit the meal. Vomiting characteristically occurs for less than 2 hours following this dose of lipopolysaccharide.[15] The absorption studies were then begun (postendotoxin day 1) and repeated each day for 2 additional days. All dogs were fasted with free access to water throughout the postendotoxin period. After completion of the endotoxin studies, all dogs were killed and postmortem examinations were performed. None of the dogs had any evidence of mechanical small bowel obstruction, perforations, or other intestinal abnormalities.

Analysis of Data

For each 15-minute interval, net water fluxes (in microliters per minute), electrolyte fluxes (in microequivalents per minute), and glucose fluxes (in micrograms per minute) were calculated from the change in PEG activity and the luminal perfusion rate (2.9 ml/min). Net water fluxes (F_{H_2O}) were calculated from the equation:

$$F_{H_2O} = 2.9 \left[1 - \frac{\text{PEG in the infusate}}{\text{PEG in the effluent}} \right]$$

Net fluxes of Na^+, Cl^-, K^+, and glucose were calculated from the equation:

$$F_{Ion} = 2.9 \left[[Ion]_{infusate} - [Ion]_{effluent} \left(\frac{\text{PEG in the infusate}}{\text{PEG in the effluent}} \right) \right]$$

Recovery (R) of PEG was calculated at 15-minute collection intervals by the equation:

$$R = \left(\frac{\text{PEG in the effluent} \times \text{effluent volume}}{\text{PEG in the infusate} \times \text{infusate volume}} \right) \times 100\%$$

Recovery was calculated to ensure that a steady state was reached, so that the flux equations would be valid and unaffected by changes in the volume recovered resulting from changes in motility or changes in intra-abdominal pressure. Recoveries not within 100% ± 20% were rejected and data from those study periods were discarded. Fewer than 10% of the study periods were discarded based on these recovery criteria. Each 1-hour study was considered to be a separate study for purpose of statistical analysis. All results are expressed as mean ± standard error of the mean (SEM). One-way analysis of variance with Tukey's test and Wilcoxon rank-sum test were performed using the Systat statistical software program (Systat Inc., Evanston, Ill.) to compare the baseline studies with the postendotoxin studies for each parameter.

RESULTS

General Aspects

All dogs recovered from the surgical procedure without incident; they remained healthy for the duration of the experiments and had normally functioning cannulas providing access to their jejunal Thiry-Vella fistulas. All experiments on each dog were performed within a 2-week period to minimize any vari-

Table I. Percentage recovery of marker (PEG) and concentration of marker (mg/ml) during baseline and postendotoxin studies

Study		Time period			
		0-15 min	16-30 min	31-45 min	46-60 min
Baseline	% Recovery	97.5 ± 2.8	103.4 ± 3.0	106.8 ± 4.7	96.3 ± 3.9
	Concentration	7.7 ± 0.2	7.7 ± 0.2	7.8 ± 0.1	7.5 ± 0.1
Postendotoxin					
Day 1	% Recovery	98.5 ± 4.2	100.4 ± 4.5	102.6 ± 5.1	98.9 ± 7.4
	Concentration	5.4 ± 0.3	5.4 ± 0.3	5.6 ± 0.4	5.7 ± 0.4
Day 2	% Recovery	105.4 ± 11.0	103.7 ± 3.9	100.3 ± 2.9	90.0 ± 3.3
	Concentration	6.3 ± 0.4	6.6 ± 0.3	6.7 ± 0.4	6.6 ± 0.4
Day 3	% Recovery	98.5 ± 3.7	93.7 ± 6.4	99.0 ± 2.9	99.4 ± 4.5
	Concentration	6.7 ± 0.4	6.8 ± 0.4	6.8 ± 0.4	7.1 ± 0.4

PEG = polyethylene glycol.

ance in the intestinal absorption data from any possible atrophy of the Thiry-Vella fistula.

Steady-state postprandial conditions during baseline and postendotoxin study days were confirmed by analyzing 15-minute intervals for recovery of the nonabsorbable PEG marker (Table I). A steady state of absorption during the baseline and postendotoxin 1-hour experiments was confirmed by showing that the concentration of marker changed little during the 1-hour experiments (Table I).

Absorption

Fig. 1 depicts postprandial water flux (F_{H_2O}) during the baseline and endotoxin experiments. Water absorption averaged 11.8 ± 0.7 μl/min/cm during the baseline studies and then decreased to 1.0 ± 2.4 μl/min/cm on postendotoxin day 1 and to 6.5 ± 1.0 μl/min/cm on postendotoxin day 2. Water flux returned to values found in healthy subjects on postendotoxin day 3.

Postprandial absorption of electrolytes demonstrated a pattern similar to that seen with water absorption (Fig. 2). Baseline sodium flux decreased from 1.6 ± 0.1 μEq/min/cm to 0.2 ± 0.3 μEq/min/cm and 1.1 ± 0.1 μEq/min/cm on postendotoxin days 1 and 2, respectively. Chloride absorption was also diminished for 2 days following endotoxin bolus (baseline = 1.2 ± 0.1 μEq/min/cm; postendotoxin day 1 = 0.03 ± 0.2 μEq/min/cm; and postendotoxin day 2 = 0.8 ± 0.1 μEq/min/cm). Finally, potassium flux was also diminished for 2 days following endotoxin bolus (baseline = 0.04 ± 0.01 μEq/min/cm; postendotoxin day 1 = −0.02 ± 0.01 μEq/min/cm; and postendotoxin day 2 = 0.02 ± 0.01 μEq/min/cm, data not shown). All electrolyte fluxes returned to values found in healthy animals on postendotoxin day 3.

Fig. 3 depicts postprandial glucose flux during baseline and endotoxin studies. Glucose absorption averaged 199 ± 5 nmol/min/cm during the baseline studies and then decreased to 127 ± 16 nmol/min/cm on postendotoxin day 1 and 174 ± 10 nmol/min/cm on postendotoxin day 2. Glucose absorption returned to baseline values on postendotoxin day 3.

DISCUSSION

Our present study demonstrates that a single sublethal bolus dose of endotoxin decreases subsequent jejunal absorption of water, electrolytes, and glucose in dogs. These effects are temporary, with patterns of jejunal absorption returning to those found in healthy animals on postendotoxin day 3. We have previously demonstrated a temporary dysfunction in both gastrointestinal motility and transit in dogs during endotoxemia. After the same dose of *E. coli* lipopolysaccharide (200 μg/kg intravenously), the interdigestive migrating myoelectric complex was abolished,[16] the frequency of action potentials was decreased, and gastric emptying of liquids and colonic transit were delayed for 2 days. These motility derangements also returned to normal on postendotoxin day 3.[15]

The effects of endotoxin on jejunal absorption are not surprising since previous studies have shown definite histologic damage of intestinal muscosa in the early stages of sepsis. Within 2 hours of systemic sepsis, small intestinal muscosal alterations, including submucosal edema, separation of the epithelium, and frank muscosal erosions, occur without concomitant hemodynamic changes.[17] Additionally, it has been shown that enterocyte glucose and glutamine utilization is depressed during sepsis, suggesting further enterocyte dysfunction.[18]

This study is in agreement with others exploring the effects of sepsis on gut absorptive function. Gardiner et al.[19] recently demonstrated that sepsis impairs intestinal absorption of all amino acids in rats. Additionally, in vivo studies demonstrated decreased ab-

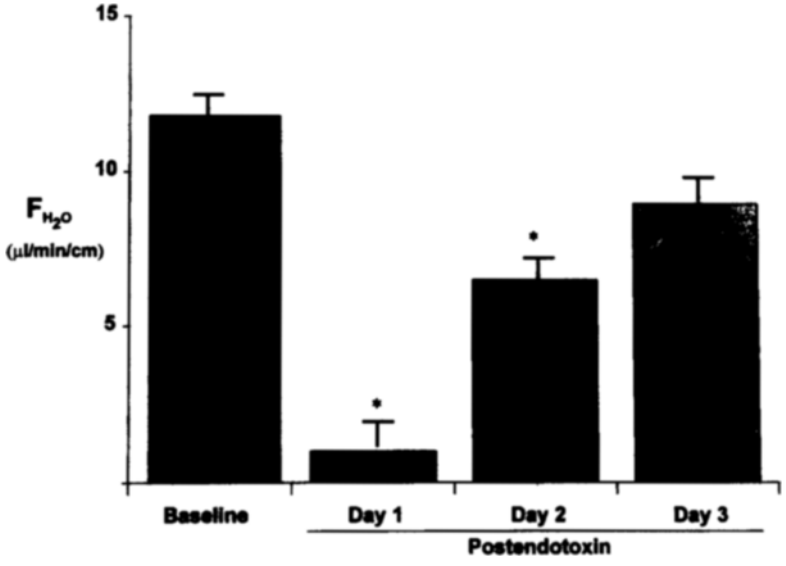

Fig. 1. Flux of water (F_{H_2O}) during baseline and endotoxin studies. Positive flux indicates absorption of H_2O from the lumen. Mean ± SEM; * = P <0.05 vs. baseline; n = 8.

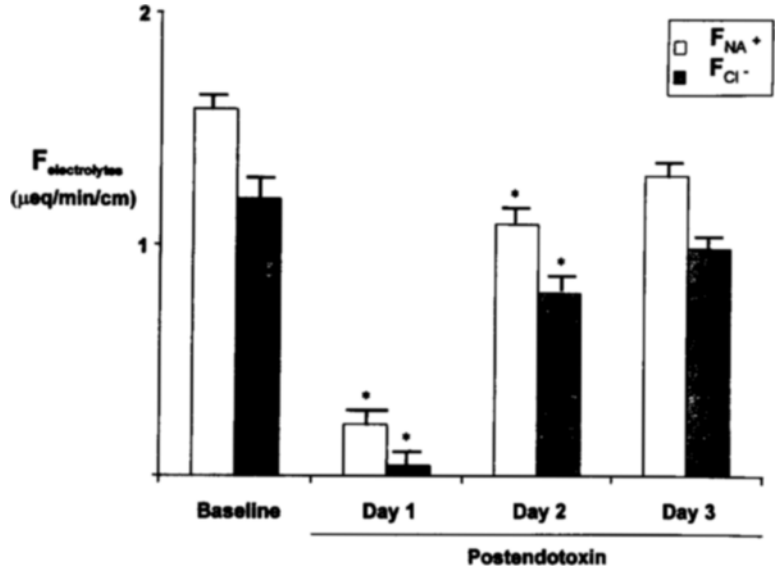

Fig. 2. Flux of electrolytes (F_{NA+}, and F_{Cl-}) during baseline and endotoxin studies. Positive flux indicates absorption of electrolytes from the lumen. Mean ± SEM; * = P <0.05 vs. baseline; n = 8.

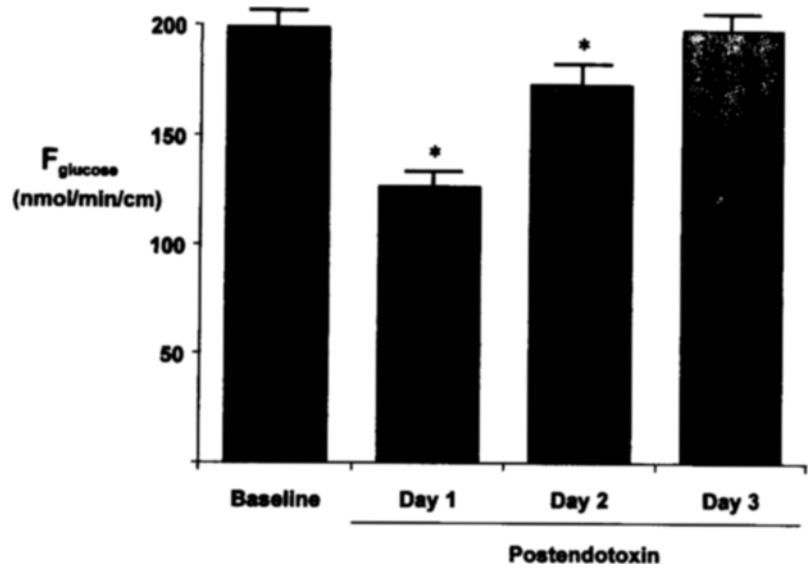

Fig. 3. Flux of glucose ($F_{glucose}$) during baseline endotoxin studies. Positive flux indicates absorption of electrolytes from the lumen. Mean ± SEM; * = P <0.05 vs. baseline; n = 8.

sorption of amino acids up to 72 hours after cecal ligation and puncture.[20] Part of the effect of decreased amino acid absorption in these studies may be explained by the decreased portal venous blood flow that occurred. In our present studies we did not measure portal venous or splanchnic blood flow. However, our previous data in this same canine experimental preparation of endotoxemia did not demonstrate a change in jejunal mucosal blood flow.[16]

Singh et al.[11] also demonstrated that at both 2 and 4 hours after the onset of sepsis in rats, gut absorption of D-xylose is decreased. In these investigations normal systemic and portal hemodynamics were definately maintained, thus suggesting an impairment in mucosal function rather than an alteration in splanchnic circulation. Additional studies confirmed these findings and also demonstrated that gut absorption was restored with diltiazem treatment.[21] Finally, Singh et al.[13] have extended their laboratory investigations to demonstrate depressed gut absorptive capacity in traumatized and septic patients, which was similar to their previous data in rats. In their patients D-xylose was decreased early after the onset of sepsis and did not return to normal until 1 to 3 weeks after the resolution of sepsis. The authors of this study speculated that a more severe episode of sepsis would further depress gut absorptive function and for longer time periods. Although our present canine study showed normalization of glucose absorption within 48 hours, it should be kept in mind that endotoxemia is generally more prolonged in clinical settings.

Our study demonstrated decreases in net sodium, chloride, and glucose absorption. Active transport of sodium into the intestinal absorptive cell can include a number of mechanisms including a single-entry process in the brush border membrane that involves direct coupling between sodium and chloride entry and sodium and chloride entry occurring via parallel exchange mechanisms, which may be indirectly coupled through the mediation of intracellular factors such as H^+. These mechanisms of active sodium transport may be impaired in our study of jejunal absorption during endotoxemia and in the study of Whang et al.,[22] which demonstrated an increase in chloride secretory flux in septic rats. In this study the ileum from septic rats was mounted in Ussing chambers. Treatment of septic rats with an endotoxin inhibitor reversed the sepsis-induced increases in short-circuit current. Thus endotoxin can impair electrolyte flux in the small intestine.

Our study also demonstrates decreases in glucose absorption during endotoxemia. Active sodium transport can also occur with processes in which sodium entry is coupled with the uptake into the cell of an organic nonelectrolyte such as glucose or amino acids. Salloum et al.[23] demonstrated that there is a decrease in sodium-dependent jejunal glutamine uptake and that the rate of jejunal transport of glucose was also decreased during endotoxemia. Their data suggested that there is a generalized downregulation of sodium-dependent, carrier-mediated substrate transport during sepsis, which may occur secondary to a decrease in transporter synthesis or an increase in the rate of carrier degradation. Thus endotoxin can also impair glucose flux in the small intestine.

A number of substances that are released during the septic state could explain the changes in jejunal absorption that we demonstrated. Vasoactive intestinal peptide is a potent secretagogue that has been shown to increase during sepsis.[24] Additionally, vasoactive intestinal peptide releases nitric oxide,[25] which could possibly result in these two secretagogues acting synergistically. Although nitric oxide has been shown to increase fluid absorption in physiologic states,[26] in pathophysiologic states nitric oxide may be produced at concentrations leading to net secretion.[27]

Several alternative explanations could account for the decrease in jejunal absorption demonstrated in the present study. Delivery of a meal stimulus into the stomach or small intestine has been shown to elicit a jejunal proabsorptive response,[28,29] which may have been blunted, since endotoxin has been shown to delay gastric emptying[15] and thus delivery of nutrients into the duodenum and jejunum. Additionally, rapid jejunal transit, as seen in a rat model of endotoxemia,[30] could contribute to decreases in jejunal transit. This may be less likely in this canine model since the percentage of recovery of the nonabsorbable marker polyethylene glycol varied little during the experiments.

CONCLUSION

A single sublethal dose of endotoxin temporarily impairs canine jejunal absorption. Following an endotoxin bolus infusion, net absorption of water, electrolytes, and glucose was decreased for 2 days and returned to baseline values on postendotoxin day 3. Thus, although enteral feeding may be advantageous, jejunal absorption may be temporarily impaired following an episode of endotoxemia.

REFERENCES

1. Souba WW, Herskowtiz K, Klimberg VS, Salloum RM, Plumley DA, Flynn TC, Copeland EM. The effects of sepsis and endotoxemia on gut glutamine metabolism. Ann Surg 1990;211:543-551.
2. O'Dwyer ST, Michie HR, Ziegler TR, Revhaug A, Smith RJ, Wilmore DW. A single dose of endotoxin increases permeability in healthy humans. Arch Surg 1988;123:1459-1464.

3. Hurlbut DJ, Zhong R, Wang P, Chen H, Garcia B, Grant DR, Duff JH. Intestinal permeability with hemorrhagic shock, surgical trauma, and endotoxemia. Surg Forum 1989;40:93-95.
4. Fink MP, Antonsson JB, Wang H, Rothschild HR. Increased intestinal permeability in endotoxic pigs. Arch Surg 1991; 126:211-218.
5. Mochizuki H, Trocki O, Dominioni L, Brackett KA, Joffe SN, Alexander JW. Mechanism of prevention of postburn hypermetabolism and catabolism by early enteral feeding. Ann Surg 1984;200:297-310.
6. Inoue S, Lukes S, Alexander JW, Trocki O, Silberstein EB. Increased gut blood flow with early enteral feeding in burned guinea pigs. J Burn Care Rehabil 1989;10:300-308.
7. Alexander JW, MacMillan BG, Stinnett JD, Ogle CK, Boziam RE, Fischer JE, Oakes JB, Morriss MJ, Krummel R. Beneficial effects of agressive protein feeding in severely burned children. Ann Surg 1980;192:505-517.
8. Moore FA, Moore EE, Jones TN, McCroskey BL, Peterson VM. TEN versus TPN following major abdominal trauma—Reduced septic morbidity. J Trauma 1989;29:916-923.
9. Kudsk KA, Croce MA, Fabian TC, Minard G, Tolley EA, Poret A, Kuhl MR, Brown RO. Enteral and parenteral feeding: Effects on septic morbidity following blunt and penetrating abdominal trauma. Ann Surg 1992;215:503-513.
10. Cerra FB. Nutrient modulation of inflammatory and immune function. Am J Surg 1991;161:230-234.
11. Singh G, Chaudry KI, Chudler LC, O'Neill PJ, Chaudry IH. Measurement of D-xylose gut absorptive capacity in conscious rats. Am J Physiol 1991;261:R1313-R1320.
12. Sodoeyama M, Gardiner KR, Regan MC, Kirk SJ, Efron G, Barbul A. Sepsis impairs gut amino acid absorption. Am J Surg 1993;165:150-154.
13. Singh G, Harkema JM, Mayberry AJ, Chaudry IH. Severe depression of gut absorptive capacity in patients following trauma or sepsis. J Trauma 1994;36:803-809.
14. Hyden S. A turbidimetric method for the determination of higher polyethylene glycols in biological materials. K Lantbruks-Hogsk Ann 1956;22:139-141.
15. Cullen JJ, Caropreso DK, Ephgrave KS. Effect of endotoxin on canine gastrointestinal motility and transit. J Surg Res 1995;58:90-95.
16. Cullen JJ, Ephgrave KS, Caropreso DK. Gastrointestinal myoelectric activity during endotoxemia. Am J Surg 1996;171: 596-599.
17. Falk A, Myrvold HE, Lundgren O, Haglund U. Mucosal lesions in the feline small intestine in septic shock. Circ Shock 1982;9:27-35.
18. Ardawi MSM, Jammal YS, Ashy AA, Nasr H, Newsholme EA. Glucose and glutamine metabolism in the small intestine of septic rats. J Lab Clin Med 1990;115:660-668.
19. Gardiner KR, Ahrendt GM, Gardiner RE, Barbul A. Failure of intestinal amino acid absorptive mechanisms in sepsis. J Am Coll Surg 1995;181:431-436.
20. Sodeyama M, Gardiner KR, Regan MC, Kirk SJ, Efron G, Barbul A. Sepsis impairs gut amino acid absorption. Am J Surg 1993;165:150-154.
21. Singh G, Chaudry KI, Chudler LC, Chaudry IH. Sepsis produces early depression of gut absorptive capacity: Restoration with diltiazem treatment. Am J Physiol 1992;263:R19-R23.
22. Whang EE, Dunn JC, Mahanty HD, Zinner MJ, McFadden DW, Ashley SW. Endotoxin inhibitor prevents sepsis-induced alterations in intestinal ion transport [abst]. Gastroenterology 1995;108:A1251.
23. Salloum RM, Copeland EM, Souba WW. Brush border transport of glutamine and other substrates during sepsis and endotoxemia. Ann Surg 1991;213:401-410.
24. Fourtes M, Blank MA, Scalea TM, Pollock TW, Jaffe BM. Release of vasoactive intestinal peptide during hyperdynamic sepsis in dogs. Surgery 1988;104:894-898.
25. Murthy KS, Zhang K-M, Jin J-G, Grider JR, Makhlouf GM. VIP-mediated G protein-couples Ca2 + influx activates a constitutive NOS in dispersed gastric muscle cells. Am J Physiol 1993;265:G660-G671.
26. Barry MK, Aloisi JD, Pickering SP, Yeo CJ. Nitric oxide modulates water and electrolyte transport in the ileum. Ann Surg 1994;219:382-388.
27. Miller MJS, Sadowska-Krowicka H, Chotinaruemol S, Kakkis JL, Clark DA. Amelioration of chronic ileitis by nitric oxide synthase inhibition. J Pharmacol Exp Ther 1993;264:11-16.
28. Bastidas JA, Orandle MS, Zinner MJ, Yeo CJ. Small bowel origin of the signal for meal-induced jejunal absorption. Surgery 1990;108:376-383.
29. Anthone GJ, Orandle MS, Zinner MJ, Yeo CJ. Small bowel origin and calorie-dependence of the signal for meal-induced jejunal absorption [abst]. Gastroenterology 1991;100:A679.
30. Wirthlin DJ, Cullen JJ, Spates ST, Conklin JL, Murray J, Caropreso DK, Ephgrave KS. Gastrointestinal transit during endotoxemia: The role of nitric oxide. J Surg Res 1996;60:307-311.

Biliary Glycoprotein Is Overexpressed in Human Colon Cancer Cells With High Metastatic Potential

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Carcinoembryonic antigen (CEA) has been recently implicated in the process of human colon cancer liver metastasis by means of an adhesion mechanism. Based on the strong sequence and structural homology of biliary glycoprotein (BGP) to CEA, we hypothesized that BGP might be overexpressed at the RNA and protein level in tumor cells with high metastatic potential. We have found the BGP messenger RNA derived from highly metastatic colon cancer cells is constitutively overexpressed—nearly fourfold greater than poorly metastatic cells—and that BGP expression is induced by interferon-gamma. Similarly, we have demonstrated that BGP protein levels were constitutively elevated in highly metastatic human colon cancer cells when compared to poorly metastatic cells. Collectively these results suggest that the basal and interferon-stimulated expression of BGP transcripts may be regulated in a manner similar to CEA and that a potential role in the process of metastasis may be inferred. (J GASTROINTEST SURG 1997;1:292-298.)

Colorectal cancer is a common problem that afflicts nearly 140,000 new patients each year.[1] Unfortunately, a significant number of these patients will die of liver-metastatic disease. Few patients each year are actually eligible for curative liver resection; the majority require treatment with regional and/or systemic chemotherapy, which is generally noncurative. Currently, few if any specific molecular markers of liver-metastatic potential are available for routine clinical assessment. Anatomic and pathologic staging remains the only reliable means for determining which patients are at risk for the development of metastatic disease.

Carcinoembryonic antigen (CEA) has been reported to be a potential marker for metastatic disease.[2] Serum levels, when significantly elevated, may signal the presence of liver metastases in a large proportion of patients.[3] Furthermore, CEA has been demonstrated to play a role in the metastatic process by serving as a cell-surface adhesion molecule that may facilitate the adherence of colon cancer cells to the liver sinusoidal epithelium by means of the Kupffer cells lining these blood vessels.[4] CEA, however, is not always elevated in patients with liver metastases and may not be produced or released by some tumors.[5]

We hypothesized that other CEA family members, bearing similar DNA sequence and structural homology, might also play a role in the metastatic process (Fig. 1). In this regard we examined poorly liver-metastatic and highly liver-metastatic human colon cancer cells for their expression of CEA and two additional closely related family members, non–cross-reacting antigen (NCA) and biliary glycoprotein (BGP). We also examined their expression of these transcripts in the presence of interferon gamma, a cytokine known to strongly induce the expression of CEA.

MATERIAL AND METHODS

Cell Lines, Derivation, and Culture Conditions

Three human colon cancer metastatic variant cell lines were used in these experiments. The parental cell line, KM12C, which is poorly metastatic, was

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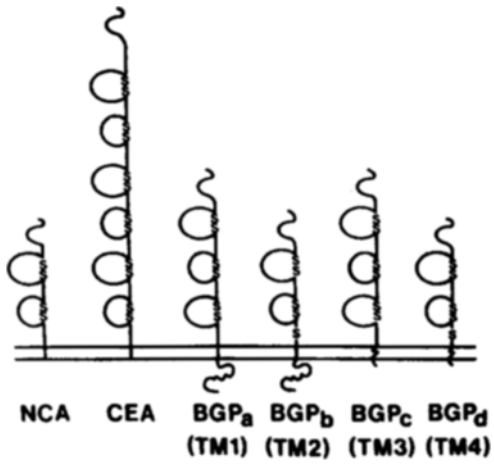

Fig. 1. CEA, NCA, and BGP all share very strong sequence and structural homology. All three are members of the immunoglobulin supergene family and share similar extracellular domains. BGP is unique in that it possesses a cytoplasmic tail. (Adapted from Thomas P, Toth CA, Saini KS, Jessup JM, Steele G. The structure, metabolism and function of the carcinoembryonic antigen gene family. Biochim Biophys Acta 1032:177-189, 1990; reprinted with permission of Elsevier Science-NL, Amsterdam, The Netherlands.)

originally derived from a Dukes' B2 colon cancer.[6] When implanted into the spleen of a nude mouse, few if any liver metastases form in the liver, whereas a spleen tumor is produced nearly 100% of the time. A subline, KM12SM, was derived from the parental line, KM12C, by injecting it into the cecum of the nude mouse with the subsequent production of a spontaneous liver metastasis. On reinjection into the nude mouse, KM12SM forms many metastatic liver foci in the majority of mice injected. A second subline, KM12L4A, was also derived from KM12C, but by a completely different mechanism. Tumor cells were originally injected into the spleen of a nude mouse, and when a metastatic liver focus was identified, it was harvested and serially reinjected using an enrichment protocol consisting of three more cycles—the end result being a highly liver-metastatic cell line. (Cell lines were a generous gift from I. Fidler.) Cell lines obtained at low passage numbers were tested immediately to prevent phenotypic drift using an intrasplenic injection assay.[7] All cell lines were grown as monolayers with RPMI (GIBCO, Grand Island, N.Y.) supplemented with 10% heat-inactivated fetal bovine serum (Hyclone Laboratories, Inc., Logan, Utah) and 0.1 mmol/L of L-glutamine (Sigma Chemical Co., St. Louis, Mo.).

Cell Treatment with Interferon Gamma

Prior to analysis of CEA, BGP, and NCA messenger RNA levels, tumor cells were treated with 50 or 100 U/ml of interferon gamma (Genentech Inc., South San Francisco, Calif.) for 4 days per the published studies of Takahashi et al.[8] Viability was assessed by trypan blue dye exclusion to be >90% following treatment with interferon gamma.

Northern Blot Analysis

Total cellular RNA was extracted from 1×10^7 treated and untreated tumor cells growing in culture using an acid-phenol thiocyanate–based kit (GIBCO, Gaithersburg, Md.). RNA was then fractionated on a 1% denaturing formaldehyde-agarose gel, transblotted to nylon membranes,[9] and UV cross-linked with 120,000 μjoules/cm^2 using a UV Stratalinker 1800 (Stratagene, La Jolla, Calif.). Filters were prehybridized with a solution (containing 50% formamide, 6x SSC, 5x Denhardt's solution, 0.2% sodium dodecyl sulfate [SDS], and sheared salmon sperm DNA [100 μl/ml]) at 42 degrees for at least 4 hours. Specific non–cross-reacting probes for CEA, NCA, and BGP (generous gift of J. Shively) were labeled by random priming with [α-^{32}P]dCTP to high specific activity (DECAprime random labeling system, Ambion, Inc., Austin, Tex.). The labeled probes were then added to the prehybridization solution and hybridized overnight at 42° C. The membranes were then washed with 1x SSC and 0.1% SDS for 15 to 30 minutes at room temperature followed by two washes with 0.1 to 0.5x SSC and 0.1% SDS for 15 minutes at 55° C. Membranes were then exposed to a phosphorimager for analysis. Membranes were consecutively hybridized with the three different probes to directly

compare the expression levels of each of the transcripts relative to the housekeeping transcript, GAPDH.

Western Blot Analysis

Cell monolayers were harvested and immediately resuspended in ice-cold lysis buffer (50 mmol/L HEPES, pH 7.5, 150 mmol/L NaCl, 0.1% Tween-20, 1 mmol/L EDTA, 2.5 mmol/L EGTA, 0.1 mmol/L PMSF, 10% glycerol, 10 μg/ml leupeptin, and 1 mmol/L dithiothreitol, vortexed vigorously, and stored on ice for 20 minutes. Following centrifugation to remove cellular debris, the crude cell extract (10 μg) was separated on 10% discontinuous SDS-polyacrylamide gels and the separated proteins were transferred to nitrocellulose membrane by electrophoretic blotting. Prestained molecular weight markers (Bio-Rad, Hercules, Calif.) were used to verify the efficiency of the transfer. Nonspecific binding was prevented by blocking the membrane in BLOTTO (5% dry milk in 1x phosphate-buffered saline/Tween-20 [PBS-T;1x PBS,0.1% Tween-20]) and incubated with the primary antibody (1:5000 dilution in PBS-T) for 1 hour at room temperature. The principal antibody used in this study was a murine monoclonal anti-BGP (generous gift of J. Shively). After washing in PBS-T, the membranes were incubated with sheep antimouse-horseradish peroxidase (1:10,000) for 1 hour, washed, and visualized by electrogenerated chemiluminescence, as recommended by the supplier (Amersham Corp., Arlington Heights, Ill.).

RESULTS

Prior to initiating experiments with the human colon cancer metastatic variants, we performed the nude mouse metastasis assay to ensure the absence of phenotypic drift. We previously documented the large differences in metastatic potential between the poorly metastatic KM12C cell line and the two highly metastatic cell line variants (KM12SM and KM-12L4A) derived from it.[10] These tested cell lines were subsequently used for the current analyses.

Unstimulated Cells

Northern blot analyses were performed using three cDNA molecular probes (CEA, NCA, and BGP) to test the three human colon cancer cell lines (KM12C, KM12SM, and KM12L4A) of variable metastatic potential grown under standard (basal) culture conditions without the influence of exogenous cytokines other than those present in 10% fetal bovine serum and under culture conditions employing 50 to 100 U/ml of interferon gamma (Fig. 2). Analysis of these lines under basal conditions demonstrated that CEA was the most highly expressed transcript of the three tested relative to (normalized to) the housekeeping transcript, GAPDH (Table I). This observation held up for all three cell lines. Further analysis, however, demonstrated that when each of the two highly liver-metastatic cell lines (KM12SM and KM12L4A) was compared with the poorly metastatic cell line KM12C from which they were originally derived using basal conditions, BGP appeared to be the transcript most upregulated of the three tested in the highly metastatic cell line variants. After normalization of all three transcripts to GAPDH, biliary glycoprotein appeared to be elevated in the KM12SM and KM12L4A cell lines 2.3- and 3.8-fold greater than in the poorly metastatic KM12C parental line. The magnitude of this difference was greater than that observed for both NCA and CEA when all three cell lines were compared.

To confirm that elevations in BGP transcripts were coincident with elevated protein levels, we performed Western blot tests using a monoclonal antibody that cross-reacts with CEA and BGP but not with NCA

Table I. Analysis of basal and interferon gamma–stimulated mRNA levels in poorly and highly liver metastatic human colon cancer cells

	Basal and IFN-g–stimulated mRNA levels*					
	NCA		CEA		BGP	
Cell line	Basal	IFN-g	Basal	IFN-g	Basal	IFN-g
KM12C (poorly metastatic)	1.0	1.1	1.0	1.5	1.0	6.9
KM12SM (highly metastatic)	0.5	0.8	1.0	3.1	2.3	13.4
KM12L4A (highly metastatic)	1.0	1.5	1.8	2.9	3.8	13.9

*Data expressed as fold increase over basal low metastatic cell line levels.
IFN-g = interferon gamma; NCA = non–cross-reacting antigen; CEA = carcinoembryonic antigen; BGP = biliary glycoprotein.

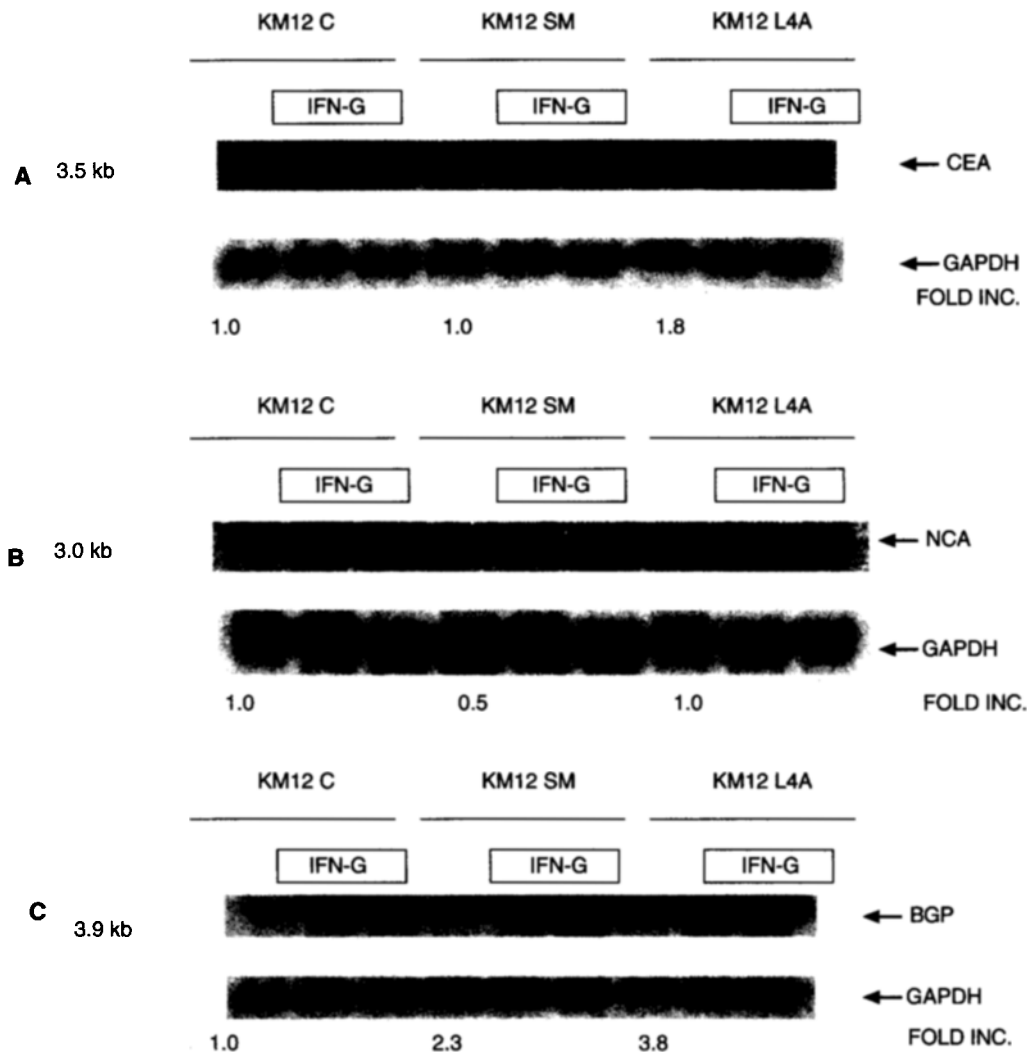

Fig. 2. Northern blot analysis of gene expression by three human colon cancer cell lines with low *(KM12C)* and high *KM12SM* and *KM12L4A)* liver-metastatic potential in the nude mouse model. Three lanes were devoted to each of the three cell lines. Lanes are as follows for each cell line: lane 1 = untreated; lane 2 = treated with 50 U/ml interferon gamma; lane 3 = treated with 100 U/ml interferon gamma. All three transcripts (CEA, NCA, and BGP) were analyzed on the same blot relative to GAPDH to directly compare expression levels for each of the three transcripts. **A,** The CEA probe hybridized to a primary 3.5 kb message and a slightly smaller secondary message. Their constitutive (basal) expression was upregulated in the highly liver-metastatic variants. The expression of both transcripts was upregulated by interferon gamma at both 50 and 100 U/ml dose levels. **B,** The NCA probe identified a single 3.0 kb transcript, the expression of which was essentially unaffected by the metastatic potential of the three cell lines and was erratically affected by treatment with interferon gamma. **C,** BGP was the most upregulated of the three transcripts tested when unstimulated, poorly metastatic *(KM12C)* cells were compared with highly metastatic *KM12SM* and *KM12L4A)* cell line variants. The BGP probe hybridized with a solitary 3.9 kb message. Its expression was also significantly affected by both doses of interferon gamma.

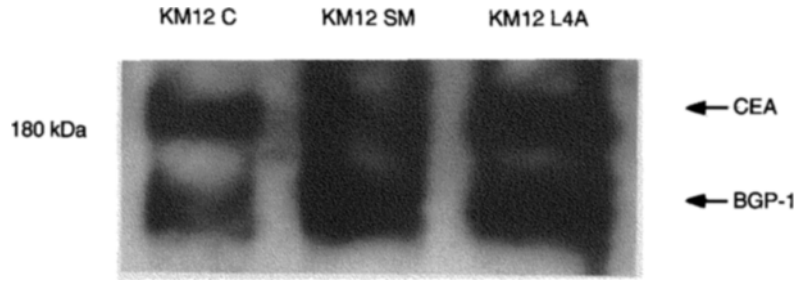

Fig. 3. Western blot analysis of BGP protein levels in three human colon cancer cell lines. Highly metastatic variants *(KM12SM* and *KM12L4A)* appeared to produce more constitutive BGP and CEA than the poorly metastatic parental cell line *(KM12C)* from which they were derived.

(Fig. 3). These experiments demonstrated that BGP protein levels were elevated in highly metastatic cells 2.5- to 4.7-fold over poorly metastatic cells.

Interferon Gamma–Stimulated Cells

Northern blot analysis of cells stimulated with interferon gamma showed increases in gene expression for each of the three transcripts tested, with BGP appearing to be the most responsive to this cytokine. BGP levels linked to pretreatment with interferon gamma were elevated 6.9- to 13.9-fold over basal unstimulated levels. Although CEA and NCA transcripts were also elevated with interferon-gamma treatment, the magnitude of change in levels was not as dramatic. No significant differences between poorly and highly metastatic cell lines in magnitudes of transcript overexpression were noted.

DISCUSSION

It has been suggested that cell adhesion molecules play an important role in the processes of tumor progression and metastasis simply because they govern the ability of tumor cells to adhere to surrounding cells, extracellular matrix, and end-organ endothelial cells such as those in the hepatic sinusoids.[11] Changes in relative cell-surface expression levels of various cell adhesion molecules such as integrins,[12] cadherins,[13] selectins,[9,14] and members of the immunoglobulin supergene family[15] have been demonstrated to participate in the development of metastatic disease. In particular, CEA has been well studied in this regard. Numerous reports have supported a role for CEA as an intercellular or homotypic adhesion molecule[16,17] that promotes the association of tumor cells as clumps and the association of tumor cells with end-organ endothelial cells. Similarly, exogenously administered CEA[18] has been shown to increase the metastatic potential of human tumor cells in the nude mouse. In addition, CEA has been demonstrated to play an accessory role in collagen binding[19] and may possess ecto-ATPase activity.[20]

BGP is a member of the immunoglobulin supergene family that was first isolated from human bile specimens.[21] It is closely related to CEA both in molecular sequence and in structure[22] (see Fig. 1). In fact, the coding sequences of CEA and BGP are nearly identical (>80% homology) and in order to identify specific BGP transcripts, unique 3' untranslated sequence must be used as a probe. The extracellular protein domains, in particular, are strikingly similar. BGP is unique in that it possesses a cytoplasmic portion that may have signal transduction potential. The function of BGP, however, remains poorly understood. It has been hypothesized that BGP might be an adhesion molecule in that it has already been demonstrated to mediate calcium-dependent, temperature-dependent intercellular adhesion,[23] although its structure also resembles that of the neural cell adhesion molecule. Because of its cytoplasmic domain that bears threonine residues, in conjunction with its extracellular and transmembrane domains, it has also been hypothesized to play a role in signal transduction.[22]

With the knowledge that BGP was so closely related to CEA, which has been demonstrated to potentiate the human colon cancer metastatic process, we hypothesized that BGP, like CEA, might also participate in a similar fashion. Because no BGP-specific antibodies are available for study, we investigated BGP at the message level for over- or underexpression by cells that are known to be poorly liver metastatic as opposed to highly liver metastatic. Si-

multaneously we examined the expression of another immunoglobulin supergene family member, NCA. By Northern blot analysis of one poorly metastatic (KM12C) and two highly metastatic (KM12SM and KM12L4A) human colon cancer cell lines, all derived from the same original patient, we were able to compare the relative levels of gene expression for each of three transcripts. We found that BGP was, constitutively (unstimulated), the most overexpressed of the three messages, with highly metastatic cells expressing BGP 2.5- to 3.8-fold greater than poorly metastatic cells. We believe that this level of overexpression, taken together with the knowledge that BGP is similar in structure to CEA, suggests a similar functional role for both proteins.

Because it has been reported that CEA and BGP are both regulated by interferon gamma,[8] we decided to examine the effects of this cytokine on CEA, NCA, and BGP in our model system. Interferon gamma is known to be an important mediator of inflammatory responses, particularly those related to bacterial infections. It is also known to upregulate MHC class II antigens, which is a process critical to antigen presentation and immune response. It has been hypothesized that upregulation of proteins such as CEA and BGP by interferon gamma might provide an immunoprotective role by agglutinating pathogens. It has also been suggested that this sort of response may be responsible for metastatic behavior because inflammation is often a component of the metastatic process. For these reasons we hypothesized that there might be differences in interferon-stimulated expression of CEA, BGP, and NCA in tumor cells that metastasize as opposed to those that do not. Interestingly, we found that BGP was the most upregulated of the three transcripts relative to GAPDH when all three were examined at the same time (72 hours) following interferon treatment. These results are different from those previously published using different cell lines, where CEA and NCA levels were found to be even more elevated than BGP; however, these results may be explained by the fact that our experiments were performed at a time that precedes the peak of CEA expression simulated by interferon.[8] In any event we believe that the observation that BGP and CEA are both responsive to interferon-gamma treatment suggests that they may possess promoter elements responsive to interferon. This sort of shared homology again suggests a potential shared functional role for CEA and BGP.

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REFERENCES

1. Parker SL, Tong T, Bolden S, Wingo PA. Cancer statistics, 1996. CA Cancer J Clin 1996;46:5-28.
2. Garrett PE, Kurtz SR. Clinical utility of oncofetal proteins and hormones as tumor markers. Med Clin North Am 1986;70:1295-1306.
3. Steele G Jr, Zamcheck N. The use of carcinoembryonic antigen in the clinical management of patients with colorectal cancer. Cancer Detect Prev 1985;8:421-427.
4. Toth CA, Thomas P, Broitman SA, Zamcheck N. Receptor-mediated endocytosis of carcinoembryonic antigen by rat liver Kupffer cells. Cancer Res 1985;45:392-397.
5. Holyoke ED, Block GE, Elwood J, Sizemore GW, Heath H, et al. Biologic markers in cancer diagnosis and treatment. Curr Probl Cancer 1981;6:1-68.
6. Morikawa K, Walker SM, Jessup JM, Fidler IJ. In vivo selection of highly metastatic cells from surgical specimens of different human colon carcinomas implanted into nude mice. Cancer Res 1988;48:1943-1948.
7. Giavazzi R, Jessup JM, Campbell DE, Walker SM, Fidler IJ. Experimental nude mouse model in human colorectal cancer liver metastases. J Natl Cancer Inst 1986;77:1303-1308.
8. Takahashi H, Okai Y, Paxton RJ, Hefta LJF, Shively JE. Differential regulation of carcinoembryonic antigen and biliary glycoprotein by γ-interferon. Cancer Res 1993;53:1612-1619.
9. McEver RP. Leucocyte interactions mediated by selectins. Thromb Haemost 1991;66:80-87.
10. Yeatman TJ, Cher ML, Mao W, Wloch M, Tedesco T. Identification of genetic alterations associated with the process of experimental colon cancer liver metastasis. Clin Exp Metastasis 1996;14:246-252.
11. Yeatman TJ, Nicholson GL. Molecular basis of tumor progression: Mechanisms of organ specific tumor metastasis. Semin Surg Oncol 1993;9:256-263.
12. Hynes RO. Integrins: Versatility, modulation, and signaling in cell adhesion. Cell 1992;69:11-25.
13. Takeichi M. Cadherin cell adhesion receptors as a morphogenetic regulator. Science 1991;251:5000, 1451-1455.
14. Yeatman TJ, Bland KI, Copeland EM III, Kimura AK. Tumor cell surface expression of galactose correlates with the degree of colorectal liver metastasis. J Surg Res 1989;46:567-571.
15. Obrink B. C-CAM (cell-CAM 105)—A member of the growing immunoglobulin super family of cell adhesion proteins. Bioassays 1991;13:227-234.
16. Benchimol S, Fuks A, Jothy S, Beauchemin N, Shirota K, Stanners CP. Carcinoembryonic antigen, a human tumor marker, functions as an intercellular adhesion molecule. Cell 1989;57:327-334.
17. Oikawa F, Inuzuka C, Kuroki M, Matsuoka Y, Kosaki G, Nakazato H. Cell adhesion activity of non-specific cross reacting antigen (NCA) and carcinoembryonic antigen (CEA) expressed on CHO cell surface: Homophilic and heterophilic adhesion. Biochem Biophys Res Commun 1989;164:39-45.
18. Hostetter R, Campbell DE, Kerckhoff S, Clearly K, Ullrich S, Thomas P, Jessup JM. Carcinoembryonic antigen enhances metastatic potential of human colorectal carcinoma. Arch Surg 1990;124:300-304.
19. Pignatelli M, Durbin H, Bodmer WF. Carcinoembryonic antigen functions as an accessory adhesion molecule mediat-

ing colon and epithelial cell-collagen interactions. Proc Natl Acad Sci USA 1990;87:1541-1545.
20. Lynn SH, Guidotti G. Cloning and expression of a cDNA coding for a rat liver plasma membrane ecto-ATPase. J Biol Chem 1989;264:14408-14414.
21. Svenberg T. Carcinoembryonic antigen-like substances of human bile. Isolation and partial characterization. Int J Cancer 1976;17:588-596.
22. Hindona Y, Neumaier M, Hefta SA, Drzeniek Z, Wagner C, Shively L, Hefta LJF, Shively JE, Paxton RJ. Molecular cloning of cDNA coding biliary glycoprotein. I. Primary structure of a glycoprotein immunologically cross reactive with carcinoembryonic antigen. Proc Natl Acad Sci USA. 1988;85:6959-6963.
23. Rojas M, Fuks A, Stanners CP. Biliary glycoprotein (BGP), a member of the immunoglobin supergene family, functions in vitro as a calcium-dependent intercellular adhesion molecule. Cell Growth Differ 1990;1:527-533.