Extended Lymphadenectomy During Pancreaticoduodenectomy for Cancer of the Pancreas: Summary of "How I Do It" Session

MODERATOR: L. William Traverso, M.D., Virginia Mason Medical Center, Seattle, Wash.
PARTICIPANTS: Yoshifumi Kawarada, M.D., and Suji Isaji, M.D., University of Mie, Mie, Japan; Sergio Pedrazzoli, M.D., University of Padua, Padua, Italy; Fabrizio Michelassi, M.D., University of Chicago, Chicago, Ill.; and Charles J. Yeo, M.D., Johns Hopkins University, Baltimore, Md.

Introduction

The participants were asked to describe in a "How I Do It" session their methods of extended lymphadenectomy during pancreaticoduodenectomy for cancer of the pancreas. Each participant's lymphadenectomy was somewhat different from the others. Common elements for the standard resection vs. the radical lymphadenectomy appear to overlap. The standard resection of these investigators may be more radical than the procedure of the reader. The reader must be familiar with all of the lymph node stations around the pancreatic head (see Table I for an overview). For purposes of discussion, the anatomic locations of these lymph node stations can be named by location or the lymph node station numbers used by the Japanese Pancreas Society can be employed. Once these lymph node stations are categorized, then the real controversy can be analyzed—that is, as the lymphadenectomy is extended, where is the precarious balance between survival benefit and quality of life? The main detriment to extended lymphadenectomy is the appearance of severe diarrhea resulting in malnutrition and higher death rates as shown in the Japanese data. These investigators eliminated diarrhea by limiting the lymph node dissection to the right side of the superior mesenteric artery, thereby decreasing the amount of nerve plexi that were removed. Is the diarrhea related to resection of the lymph nodes or the nerve plexus? The Italian study was not associated with severe diarrhea, even though these investigators performed a more extensive resection than either the Japanese D1 + α resection or the radical pancreaticoduodenectomy used at The Johns Hopkins Hospital. Also intriguing in the Italian study was the improvement in survival among the small number of patients who had positive lymph nodes after extended resection of the lymph nodes. If a randomized trial could compare the Italian lymphadenectomy with a standard resection in a larger number of patients, then the survival benefit after lymphadenectomy in lymph node–positive patients could be more adequately supported. The Johns Hopkins group found no difference in survival with their radical procedure, which emphasized the retroperitoneum and did not involve excision of the celiac axis, common hepatic artery, or left side of the superior mesenteric artery lymph nodes (in an attempt to avoid severe diarrhea). After reading the three reports that follow, the groundwork for a prospective trial comparing standard lymphadenectomy to the ideal extended lymphadenectomy should be better defined.

Presented at the Annual Meeting of the Pancreas Club, Inc., Orlando, Fla., May 16, 1999.

Table I. Japanese Pancreas Society nodal stations

Station No.	Nodal location	Group*
1	Right cardiac lymph nodes	3
2	Left cardiac lymph nodes	3
3	Nodes along lesser curve	3
4	Nodes along greater curve	3
5	Suprapyloric lymph nodes	3
6	Infrapyloric lymph nodes	1
7	Nodes along left gastric artery	3
8	Nodes along anterior and posterior common hepatic artery	1
9	Lymph nodes along celiac artery	2
10	Lymph nodes at the splenic hilum	3
11	Nodes along the splenic artery	2
12	**Nodes in the hepatoduodenal ligament**	**NA**
12h	Nodes in the hepatic hilum	3
12a1	Superior hepatic artery nodes	2
12a2	Inferior hepatic artery nodes	1
12p1	Superior portal vein nodes, dorsal to portal vein	2
12p2	Inferior portal vein nodes, dorsal to portal vein	1
12b1	Superior portal vein nodes anterior to bile duct	2
12b2	Inferior bile duct nodes anterior to bile duct	1
12c	Nodes around cystic duct	2
13a,b	Posterior surface of pancreas, superior and inferior	1
14	**Nodes along the mesenteric root**	**NA**
14a	Superior mesenteric artery nodes (SMA origin)	2
14b-d	SMA nodes—remainder distal to first jejunal branch	1
14V	Superior mesenteric vein (SMV) nodes	1
15	Middle colic artery nodes	2
16a1, b_2	Para-aortic—above celiac, below IMA	3
$16a_2$, b_1	Middle para-aortic—below celiac, above IMA	2
17a,b	Anterior surface of pancreas, superior and inferior	1
18	Inferior pancreas—left of SMV	2

SMA = superior mesenteric artery; SMV = superior mesenteric vein; IMA = internal mammary artery; NA = not applicable.
*When the tumor is in the head of the pancreas.
From Japan Pancreas Society. Classification of Pancreatic Carcinoma, First English ed. Tokyo: Kanehara & Co., Ltd., 1996, p 11.

Modified Standard (D1 + α) Pancreaticoduodenectomy for Pancreatic Cancer

Yoshifumi Kawarada, M.D., Shuji Isaji, M.D.

In Japan, the extended (D2 operation) has been performed for pancreatic carcinoma, but the overall survival has been unsatisfactory.[1] D2 lymph node dissection means dissection of all regional lymph nodes plus the lymph nodes around the celiac trunk, superior mesenteric vessels, and middle colic vessels. Another description of the D2 operation would be removal of all of the group 1 and 2 nodes according to the Japanese Pancreas Society classification (EDITOR'S NOTE: see Table I [column 3] in the Introduction). The extended operation is a radical resection and a very stressful operation for patients in that their postoperative quality of life is severely affected because of diarrhea. For this reason we developed a modified standard operation, which is not associated with severe diarrhea. The lymph node dissection is midway between the standard (group 1 or D1 lymph node dissection) and the extended D2 operation, and is referred to as the D1 + α dissection. We have been performing it since 1993.

D1 + α OPERATIVE PROCEDURE

D1 + α operation is performed when the retroperitoneal lymph nodes are grossly negative for malignancy. First the duodenum is mobilized on the left side as far as the aorta to allow complete examination of the head of the pancreas. Before lymph node dissection, the root of the mesentery and the lymph nodes around the superior mesenteric vein (SMV) are examined by inspection and palpation. A blunt maneuver is undertaken to determine resectability, that is, whether the portal vein is mobile and free of the surrounding pancreas.

Lymph nodes around the common hepatic artery and in the hepatoduodenal ligament are dissected (nodal stations 8a, 8p, and all of 12 except 12h). Cholecystectomy is performed. The gastroduodenal artery is ligated and divided near its origin from the hepatic artery. The stomach is then divided with a stapler, and the fourth portion of the duodenum is mobilized beneath the mesenteric vessels by incising the ligament of Treitz. After transecting the jejunum, the freed jejunum is passed completely beneath the superior mesenteric vessels. The pancreas is then divided, and a plastic stent tube is inserted into the remnant pancreatic duct. The soft tissue on the right lateral aspect of the portal vein is carefully divided, and the right border of the superior mesenteric artery (SMA) is exposed longitudinally by retracting the portal vein and SMV to the left to enable dissection of the lymph nodes on the right side of the SMA (all nodal stations 14 on the right side).

The anterior pancreaticoduodenal lymph nodes (station 17), posterior pancreaticoduodenal lymph nodes (station 13), nodes around the pylorus of the stomach (stations 5 and 6), hepatoduodenal ligament (station 12 except 12h), common hepatic artery (station 8), and the right half of the superior mesenteric artery (station 14) are dissected. We do not dissect the lymph nodes around the celiac trunk (station 9), the left half of the superior mesenteric vessels (station 14 left), the middle colic vessels (station 15), or in the para-aortic region (station 16) as is done in the D2 resection.

RESULTS

Postoperative quality of life and survival rates after D2 dissection and D1 + α dissection were compared. In 1981, all patients undergoing D2 resection (included complete resection of the nerve plexus around the SMA along with D2 lymph node dissection) developed severe diarrhea that was very difficult to control even with opium, and resulted in dehydration and malnutrition. As a result of this experience, complete removal of the nerve plexus around the SMA was avoided in the remaining 49 patients in whom D2 dissection was performed (only the right half of the nerve plexus around the SMA was resected along with

From the First Department of Mie University School of Medicine, Mie, Japan.

lymph node dissection). None of these 49 patients had severe diarrhea. None of the 20 patients in whom D1 + α dissection was performed have had diarrhea.

Comparison of hospital stays and hospital deaths in the D2 and D1 + α cases revealed a hospital stay of 115 ± 55 days in the D2 cases, as opposed to 42 ± 20 days in the D1 + α cases ($P < 0.01$), and a hospital death rate of 20.3% in D2 cases vs. only 4.8% in the D1 + α cases. Significant differences in the 3- and 5-year survival rates were not found between the D2 (18% and 14%, respectively) and the D1 + α (36% and 26%, respectively) groups.

SUMMARY

We believe that the effect of extended dissection, especially dissection of the nerve plexi around the celiac trunk, superior mesenteric vessels, and middle colic vessels, is impairment of intestinal motility that results in severe diarrhea postoperatively. Long term this results in a negative nutritional and possibly immunologic effect, which may have a long-term effect on outcome and patient survival. The best way to prevent severe diarrhea is to avoid complete resection of the nerve plexus around the SMA. To avoid diarrhea, we have been especially careful not to dissect the nerve plexus around the SMA in patients undergoing D1 + α resection. The most important consideration is to avoid stripping the SMA bare. Dissection of the superior mesenteric lymph nodes with preservation of the superior mesenteric nerve plexus is theoretically possible because 95% of the lymph nodes are located outside the nerve plexus.[2] From survival studies we concluded that D1 + α dissection for pancreatic cancer is an adequate lymph node dissection in terms of the postoperative quality of life and survival rate for patients with pancreatic cancer.

REFERENCES

1. Naganuma T, Isaji S, Kawarada Y. Staging and extended resection for pancreatic cancer. Pancreas 1998;16:335-362.
2. Kawabata A, Hamanaka Y, Suzuki T. Potentiality of dissection of the lymph nodes with preservation of the nerve plexus around the superior mesenteric artery. Hepatogastroenterology 1998;45:236-241.

Extent of Lymphadenectomy in the Surgical Treatment of Adenocarcinoma of the Head of the Pancreas

Sergio Pedrazzoli, M.D., Fabrizio Michelassi, M.D.

Pancreatic resection is the treatment of choice for pancreatic cancer. Unfortunately it can be performed only in 5% to 40% of pancreatic cancer patients undergoing exploratory laparotomy. The extent of lymphadenectomy to be associated with pancreatic resection is widely debated. Palliative, standard, traditional, extended, D1, D2, D3, regional, extended retroperitoneal, standard radical, and extended radical are only some of the terms used to indicate the extent of lymph node dissection. To try to overcome the problems created by this "Babel" of terms, a Consensus Conference took place in Castelfranco Veneto, Italy, in May 1998. The results have been published[1] and are summarized herein (the Japanese Pancreas Society rules for the study of pancreatic cancer were chosen to define the lymph node stations to be removed[2]).

Common Elements of the Kausch-Whipple Pancreatoduodenectomy*

- Removal of the gallbladder along with transection of the common hepatic duct
- Transection of the pancreas, at least 1 cm from the tumor, over the portal vein/superior mesenteric vein junction (HPV-SMV) for the "standard" procedure and to the left of the HPM-SMV for the "radical" and "extended radical" procedures (a tumor-free margin by frozen section should be obtained)
- The ligament of Treitz is divided and the first part of the jejunum is transected and divided.
- The pylorus-preserving procedure is to be avoided for cancers located in the dorsal part of the head of the pancreas.
- Mesentericoportal vein resection and/or accessory organ resection (stomach, spleen, colon, kidney, jejunum) may be necessary to obtain clear margins.

Lymph Nodes Removed With the Standard Kausch-Whipple Pancreatoduodenectomy

- Kocher maneuver is used to mobilize the head of the pancreas and to perform an en bloc resection of the following lymph node groups:
  - Lymph nodes of the right side of the hepatoduodenal ligament (stations 12b1, 12b2, and 12c)
  - Anterior (stations 17a and 17b) and posterior (stations 13a and 13b) pancreaticoduodenal nodes
  - Nodes to the right side of the superior mesenteric artery (stations 14a and 14b)
- Removal of the lymph nodes of the anterior region of the common hepatic artery (station 8a)

Radical Kausch-Whipple Pancreatoduodenectomy

- Gerota's fascia is removed en bloc with the head of the pancreas and, in addition to the lymph nodes removed with a "standard" pancreatoduodenectomy, the following lymph node groups are removed:
  - Circumferentially around the common and proper hepatic artery (stations 8a and 8p)
  - Lymph nodes of the celiac axis (station 9)
  - Lymph nodes of the hepatoduodenal ligament (all station 12 nodes)
  - Circumferential superior mesenteric artery (all station 14 nodes)
  - Resection of all lymph nodes of the anterolateral aspect of the aorta and of the inferior vena cava, in continuity with Gerota's fascia, between the celiac axis and the inferior mesenteric artery (stations 16a2 and 16 b1)

*EDITOR'S NOTE: Although A.O. Whipple first popularized pancreaticoduodenectomy beginning in 1935, the first successful procedure was accomplished by W. Kausch in 1909.

From the Dipartimento di Scienze Mediche e Chirurgiche, Semeiotica Chirurgica, University of Padua, Padua, Italy (S.P.), and the Department of Surgery, University of Chicago, Chicago, Ill. (F.M.).

Extended Radical Kausch-Whipple Pancreatoduodenectomy

- En bloc resection of the following lymph node groups, in addition to those removed with a radical pancreatoduodenectomy:
  - All station 16 nodes: Clearance of all connective and lymphatic tissue, starting from 3 cm to the right of the duodenum, and extending to the midportion of the left kidney, and from the inferior margin of the liver across the diaphragmatic hiatus, well above the origin of the celiac trunk to the origin of the common iliac arteries. Clearance of all lymphatic and neurovascular tissue anterior to the aorta including the tissue around the celiac axis.
  - Left adrenalectomy is optional.

All participants in the Consensus Conference agreed that the word "radical" should be avoided for pancreatoduodenectomies that do not include skeletonization of the common and proper hepatic artery and of the superior mesenteric artery in view of the frequency of tumor invasion of the nerve plexus around the pancreatic head (>60%) and metastatic lymph nodes around the superior mesenteric artery (23% to 38%).

Appropriate Extent of Lymphadenectomy Associated With Pancreatoduodenectomy in the Surgical Treatment of Adenocarcinoma of the Head of the Pancreas

Between 1991 and 1994, the authors participated in a multicenter prospective randomized study[3] conducted to determine whether the performance of an extended lymphadenectomy in association with a pancreatoduodenal resection improves the long-term survival of patients with a potentially curable adenocarcinoma of the head of the pancreas. The study comprised 81 patients randomized to a standard (n = 40) or extended (n = 41) lymphadenectomy. The standard and the extended lymphadenectomies performed during the study were quite similar to the "standard" and "radical" lymphadenectomy defined by the Consensus Conference[1] with the only difference represented by the inclusion of lymph node station 16a1 of the Japanese classification (para-aortic above the origin of the celiac axis) in the extended lymphadenectomy group.

Demographic (age, sex) and histopathologic (tumor size, stage, differentiation, oncologic clearance) characteristics were similar in the two patient groups. Performance of the extended lymphadenectomy added time to the procedure, although the difference did not reach statistical significance. Transfusion requirements, postoperative morbidity and mortality rates, and overall survival did not differ between the two groups. When subgroups of patients were analyzed using an *a posteriori* analysis that was not planned at the time of study design, there was a statistically significant ($P < 0.05$) although clinically modest longer survival rate in the node-positive patients after an extended rather than a standard lymphadenectomy. The survival curve of node-positive patients after an extended lymphadenectomy could be superimposed onto the curves of node-negative patients. Survival in node-negative patients did not differ according to the magnitude of the lymphadenectomy.

Although disabling watery diarrhea has been reported by Ishikawa[4] as a common postoperative complication after an extended lymphadenectomy, our patients did not experience this adverse occurrence. In comparing our technique to that reported by Ishikawa,[4] we did not resect the thoracic duct, nor did we perform a wide excision of the transverse mesocolon. Although skeletonization of the superior mesenteric artery is commonly claimed as the reason for severe postoperative diarrhea, it is possible that these differences in technique might explain the lack of disabling postoperative diarrhea in our patients.

REFERENCES

1. Pedrazzoli S, Beger H, Obertop H, Andrén-Sandberg Å, Fernandez Cruz L, Henne Bruns D, Lüttges J, Neoptolemos JP. A surgical and pathological based classification of resective treatment of pancreatic cancer: Summary of an international workshop on surgical procedures in pancreatic cancer. Dig Surg 1999;16:337-345.
2. Japan Pancreas Society. General Rules for the Study of Pancreatic Cancer, 4th ed. Tokyo: Kanehara & Co, Ltd, 1993, pp 25-35.
3. Pedrazzoli S, Di Carlo V, Dionigi R, Mosca F, Pederzoli P, Pasquali C, Klöppel G, Dhaene K, Michelassi F, and the Lymphadenectomy Study Group. Standard versus extended lymphadenectomy with pancreatoduodenectomy in the surgical treatment of adenocarcinoma of the head of the pancreas. Ann Surg 1998;228:508-517.
4. Ishikawa O. Surgical technique, curability and postoperative quality of life in an extended pancreatectomy for adenocarcinoma of the pancreas. Hepatogastroenterology 1996;46:320-325.

The Johns Hopkins Experience With Pancreaticoduodenectomy With or Without Extended Retroperitoneal Lymphadenectomy for Periampullary Adenocarcinoma

Charles J. Yeo, M.D.

Surgical resection by means of pancreaticoduodenectomy provides the only chance for cure in patients with periampullary adenocarcinoma. In most reported series of patients treated for periampullary adenocarcinoma, the results have been obtained using standard pancreaticoduodenal resection without radical (extended) lymph node dissection. Radical pancreatic resection was proposed by Fortner in 1973 as a means of improving the resectability rates and cure rates for patients with cancer of the pancreas. Currently, a variety of radical or extended resections are being performed. These procedures have been used most extensively in Japan and have gained some advocates in Europe and the United States. At The Johns Hopkins Hospital, there is currently an active and ongoing prospective, randomized study that has been designed to evaluate the impact of standard vs. radical pancreaticoduodenal resection on short-term outcomes (i.e., mortality and postoperative complications) and long-term outcomes (i.e., survival and quality-of-life issues).[1]

Between April 1996 and December 1997, a total of 276 patients underwent pancreaticoduodenectomy at The Johns Hopkins Hospital, with 190 of these patients undergoing resection for periampullary adenocarcinoma. Of these, 114 patients were enrolled in this single-institution study. Patients were randomized during surgery after standard pancreaticoduodenal resection to one of two surgical procedures: (1) *standard* pancreaticoduodenal resection (i.e., pylorus preservation or classic resection, whereas nodal groups resected included the anterior pancreaticoduodenal lymph nodes, posterior pancreaticoduodenal lymph nodes, nodes in the lower hepatoduodenal ligament, and nodes along the right lateral aspect of the superior mesenteric vessels) or (2) *radical* pancreaticoduodenal resection. For the purposes of this study, radical pancreaticoduodenal resection included a 30% to 40% distal gastrectomy, lymph nodes in the corresponding lesser and greater omentum, and a retroperitoneal lymph node dissection extending from the hilum of the right kidney to the left lateral border of the aorta in the horizontal axis, and from the portal vein to below the third portion of the duodenum (level of the internal mammary artery) in the vertical axis.*

Vagotomy, tube gastrostomy, and feeding jejunostomy were not used. Postoperatively, patients received histamine H_2-receptor antagonists as prophylaxis for stress and marginal ulceration, and erythromycin lactobionate as prophylaxis against delayed gastric emptying. Approximately 75% of the patients received postoperative chemoradiation therapy.

Of the 114 patients randomized, 56 underwent the standard resection and 58 underwent the radical resection. The two groups were statistically similar with respect to age and sex. In regard to intraoperative factors, the extent of pancreatic resection, type of pancreatic-enteric anastomosis, and amount of intraoperative blood loss were similar between the two groups. The operative time was approximately 35 minutes longer in the radical group. In the standard resection group 70% of patients were found to have positive lymph nodes, whereas in the radical group positive nodes were identified in 59% of patients. Eleven additional lymph nodes were harvested, on average, in the radical group, but in no patient did the additional harvested lymph nodes change the tumor staging.

*EDITOR'S NOTE: A celiac node was removed for histologic analysis; otherwise the nodes around the common hepatic artery, celiac artery, or to the left of the superior mesenteric artery were not removed.

From the Johns Hopkins University School of Medicine, Baltimore, Md.

The overall postoperative in-hospital and 30-day mortality rate was 4.4%, and the overall complication rate was 37%. There were no differences between the standard and radical groups. The most common postoperative complication was early delayed gastric emptying (10%) with other complications being pancreatic fistula (9%), wound infection (8%), and intra-abdominal abscess (7%). The median postoperative hospital length of stay was 10 days in the standard group and 12 days in the radical group, a difference that approached significance.

Survival was assessed for the 109 patients who survived the immediate postoperative period. The 1-year and 2-year survival rates for all patients were 77% and 47%, respectively, for the standard group and 83% and 56%, respectively, for the radical group ($P = 0.6$; not significant). Assessing only those patients with pancreatic adenocarcinoma (n = 34), the 1- and 2-year survival rates were 71% and 39%, respectively, for the standard group and 80% and 48% for the radical group ($P = 0.5$; not significant). For that group of patients with node-positive pancreatic adenocarcinoma (n = 36), the 1-year survival rate was 72% for both the standard and radical groups.

At the time of this report, this series represented the largest prospective, randomized comparison between standard and radical pancreaticoduodenectomy. Enrollment is not yet complete, and the randomization has not yet made the two groups statistically similar. Nonetheless, with a few exceptions, the current data support the premise that the addition of a distal gastrectomy and retroperitoneal lymph node dissection to a standard pancreaticoduodenal resection is relatively safe and does not affect overall morbidity and mortality. The early survival analysis has failed to confirm a survival benefit for patients with node-positive pancreatic cancer treated with the radical procedure. However, we continue to accrue patients into this trial, and the outcomes to be assessed at the final evaluation include not only morbidity and mortality but long-term survival and quality of life.

REFERENCE

1. Yeo CJ, Cameron JL, Sohn TA, Coleman J, Sauter PK, Hruban RH, Pitt HA, Lillemoe KD. Pancreaticoduodenectomy with or without extended retroperitoneal lymphadenectomy for periampullary adenocarcinoma: Comparison of morbidity and mortality and short-term outcome. Ann Surg 1999;229:613-624.

Is CT Angiography Sufficient for Prediction of Resectability of Periampullary Neoplasms?

Pierre F. Saldinger, M.D., Melissa Reilly, R.N., Kevin Reynolds, M.D., Vassilios Raptopoulos, M.D., Ram Chuttani, M.D., Michael L. Steer, M.D., Jeffrey B. Matthews, M.D.

The optimal preoperative evaluation of periampullary neoplasms remains controversial. The aim of this study was to analyze the accuracy of helical computed tomography (CT) and CT angiography with three-dimensional reconstruction in predicting resectability. Between March 1996 and May 1999, a total of 100 patients with periampullary neoplasms were prospectively staged by helical CT and CT angiography with three-dimensional reconstruction. Vascular involvement was graded from 0 to 4, with grade 0 representing no vascular involvement and grade 4 total encasement of either the superior mesenteric vein or artery. Patients with grade 4 lesions were considered unresectable. Sixty-eight patients underwent surgical exploration with intent to perform a pancreaticoduodenectomy. Forty-four lesions were grade 0, five were grade 1, eight were grade 2, and 11 were grade 3. Resectability for grades 0 to 3 was 96%, 100%, 50%, and 9%, respectively, for an overall resectability rate of 76%. Resectability in patients with vascular encroachment (grade 2) is usually determined by the extent of local disease rather than the presence of extrapancreatic disease. Resection is rarely possible in patients with evidence of vascular encasement (grade 3). Additional imaging modalities such as diagnostic laparoscopy are superfluous in patients with no evidence of local vascular involvement on CT angiography (grades 0 and 1) because of the high resectability rate and infrequency of unsuspected distant metastatic deposits. (J GASTROINTEST SURG 2000;4:233-239.)

KEY WORDS: Pancreas cancer, CT, surgery, pancreaticoduodenectomy

Pancreaticoduodenectomy is currently the only potentially curative treatment for patients with periampullary neoplasms. Advances in perioperative management and surgical technique have significantly decreased the morbidity and mortality for this operation.[1,2] Despite these advances, the overall prognosis remains bleak.[1] An important goal of the initial evaluation of these patients is to select patients in whom curative resection is potentially feasible and to avoid laparotomy in patients with disease that has progressed beyond the planned resection field or disease that is technically unresectable because of vascular encasement. A number of methods, including helical computed tomography (CT), magnetic resonance imaging (MRI), endoscopic ultrasonography, and laparoscopy with or without laparoscopic ultrasonography, have been evaluated in recent years to improve preoperative staging. Several groups have advocated the use of combinations of these modalities in order to achieve an accurate perioperative assessment of the resectability of periampullary neoplasms.[3-5] The most important end point of these studies is the avoidance of unnecessary laparotomy in patients who cannot benefit from resection. The present study has prospectively evaluated the potential of a single modality, helical CT with CT angiography, to predict vascular involvement of periampullary neoplasms and their resectability.

MATERIAL AND METHODS

Between March 1996 and May 1999, a total of 100 patients (46 men and 54 women; median age 69 years, range 36 to 80 years) with periampullary neoplasms

From the Division of General and Gastrointestinal Surgery (P.F.S., M.R., M.L.S., and J.B.M.), Department of Surgery, Department of Radiology (K.R. and V.R.), and Division of Gastroenterology (R.C.), Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Mass.
Reprint requests: Jeffrey B. Matthews, M.D., Division of General and Gastrointestinal Surgery, Beth Israel Deaconess Medical Center, 330 Brookline Ave., Boston, MA 02215. e-mail: jmatthew@caregroup.harvard.edu

Table I. Grading of vascular involvement in 68 patients undergoing surgical exploration with intent to perform pancreaticoduodenectomy according to helical CT with CT angiography*

Grade	Finding	No.
0	Normal, preserved fat plane around the portal vein, superior mesenteric vein, their confluence, the superior mesenteric artery and celiac axis	44
1	Loss of fat plane, smooth displacement of the vessel	5
2	Flattening or irregularity on one side of the vessel	8
3	Encased narrowed vessel with tumor >50% of the circumference	11
4	Occluded vessel	NA

NA=not applicable.
*Adapted from Raptopoulos V, Steer ML, Sheiman RG, et al. The use of helical CT and CT angiography to predict vascular involvement from pancreatic cancer: Correlation with findings at surgery. AJR Am J Roentgenol 1997;168:971-977.

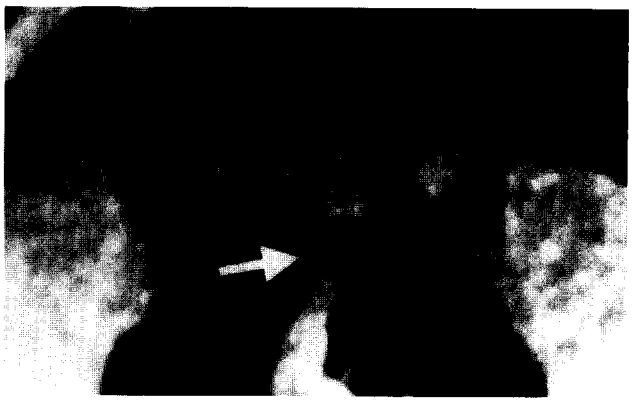

Fig. 1. Three-dimensional reconstruction of a CT angiogram showing a grade 2 lesion with flattening on one side of the vessel *(arrow)*.

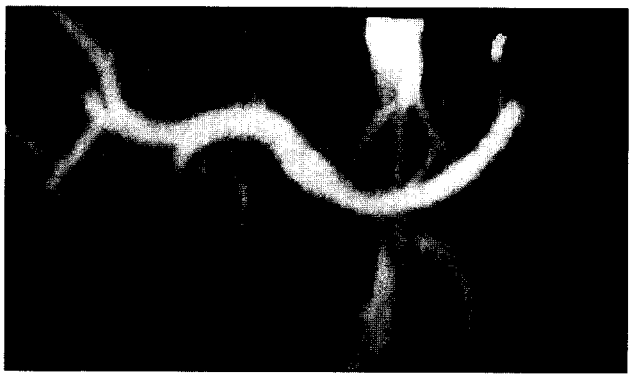

Fig. 2. Three-dimensional reconstruction of a CT angiogram showing a grade 3 lesion with narrowing of the vessel by the tumor of greater than 50% of the circumference *(arrow)*.

were prospectively staged for resectability by helical CT and CT angiography with three-dimensional reconstruction. Most of these patients had undergone a prior study (CT, ultrasonography, or endoscopic retrograde cholangiopancreatography) that suggested the presence of a periampullary neoplasm, but these studies had not identified either metastatic disease or locally unresectable disease. The studies were reviewed by a staff radiologist and vascular involvement was graded from 0 to 4[6] (Table I, Figs. 1 and 2). Patients with grade 0 to 3 lesions were considered potentially resectable, and they were scheduled to undergo a potentially curative pancreaticoduodenectomy. Grade 4 vascular involvement was considered an absolute radiologic criterion for unresectability; these patients did not undergo surgical exploration. Laparoscopy was performed at the surgeon's discretion but not routinely as part of the surgical exploration. Criteria for unresectability at operation included hepatic metastases, encasement of the superior mesenteric vein or artery, encasement of the portal vein, and hilar or celiac lymph node involvement confirmed by frozen section. A key step in the assessment of resectability was the ability of the surgeon to develop a plane between the anterior surface of the superior mesenteric vein/portal vein and the neck of the pancreas. Cases in which this was not possible were considered unresectable. Segmental portal vein resection for posterolateral involvement of the superior mesenteric vein/portal vein, as described by others,[7-9] was performed in one case. Patients with resectable lesions underwent a pancreaticoduodenectomy.

Operative and clinicopathologic data were recorded in a prospective pancreaticobiliary database maintained by the Division of General and Gastrointestinal Surgery at Beth Israel Deaconess Medical Center. Logistic regression was used to assess whether the CT grade was predictive of resectability. Patients with grade 4 lesions were not included in this analysis.

RESULTS

Sixty-eight patients were considered potentially resectable on the basis of the CT angiography and underwent surgical exploration with intent to perform a pancreaticoduodenectomy. There were 32 male and 36 female patients whose mean age was 66 years (range 44 to 80 years). Fifty-two patients had pancre-

Table II. Distribution of tumors among all patients undergoing surgical exploration (n = 68)

Tumor	Total	Grade 0	Grade 1	Grade 2	Grade 3
Pancreatic adenocarcinoma	52	32	3	6	11
Ampullary carcinoma	5	5			
Cystic neoplasm	5	3	1	1	
Endocrine neoplasm	2	1		1	
Duodenal adenoma	2	2			
Duodenal carcinoma	1		1		
Intraductal papillary tumor	1	1			

atic adenocarcinoma, five had ampullary carcinoma, five had a cystic neoplasm, two had an endocrine neoplasm, two had a duodenal adenoma, one had a duodenal carcinoma, and one had an intraductal papillary tumor (Table II).

Pancreaticoduodenectomy was performed in 52 patients resulting in an overall resectability rate of 76%. The resectability rates for grades 0 to 3 were 95%, 100%, 50%, and 9%, respectively (Fig. 3). This trend was highly significant ($P < 0.001$). The rate of positive margins in patients with pancreatic adenocarcinoma was 22%. All patients with unresectable tumors had pancreatic adenocarcinoma, whereas the 16 patients with other tumors were all resectable (Table III). The TNM classification of 36 resected pancreatic cancers is listed in Table IV. Both patients with grade 0 lesions who were unresectable had unsuspected liver metastases. In one of these patients, the hepatic lesions would have been readily apparent on diagnostic laparoscopy, but in the other there was only a small lesion at the dome of the liver that might have been difficult to visualize without the aid of palpation. The basis for unresectability in all four patients in the grade 2 category who did not undergo pancreaticoduodenectomy was the degree of local vascular involvement rather than the presence of peritoneal, nodal, or hepatic tumor deposits. One of the 10 unresectable patients in the grade 3 group had liver metastases, one had a malignant celiac node, and the remaining eight had extensive vascular involvement (Table V).

Four of the 16 patients who were ultimately found to have unresectable disease underwent laparoscopy as the first stage of their procedure. In three of them the laparoscopic findings were negative, but these patients were found to have extensive vascular involvement precluding resection. The fourth patient was found to have an enlarged celiac lymph node, but results of laparoscopic biopsy were inconclusive. Of the 12 unresectable patients who did not undergo laparoscopy, two had liver metastases that could likely have been detected by laparoscopy.

Table III. Resectability according to tumor type

Tumor type	No.	Resectability rate (%)
Pancreatic adenocarcinoma	52	69
Other	16	100

Table IV. TNM classification of 36 resected pancreatic cancers*

Stage	No. (%)
T1	4 (11)
T2	11 (30)
T3	20 (56)
T4	1 (3)
N0	10 (28)
N1	26 (72)

*The rate of positive margins for this group was 22%.

Table V. Reasons for unresectability according to grade of vascular involvement

Patient	Finding	Grade
V.B.	Liver metastases both lobes	0
R.D.	Liver metastasis, dome	0
V.S.	SMV encased	2
T.R.	SMV and SMA encased	2
J.B.	SMV encased	2
M.R.	SMV encased	2
J.E.	SMV encased	3
M.P.	SMV encased	3
R.S.	SMV encased	3
C.S.	SMV, portal vein, splenic vein encased	3
E.M.	Liver metastases left lobe	3
W.W.	SMV encased	3
J.F.	SMV encased	3
M.S.	SMV encased	3
C.T.	Celiac node encased hepatic artery	3
L.M.	Encased SMA, infiltrated aorta, para-aortic lymph nodes	3

SMV = superior mesenteric vein; SMA = superior mesenteric artery.

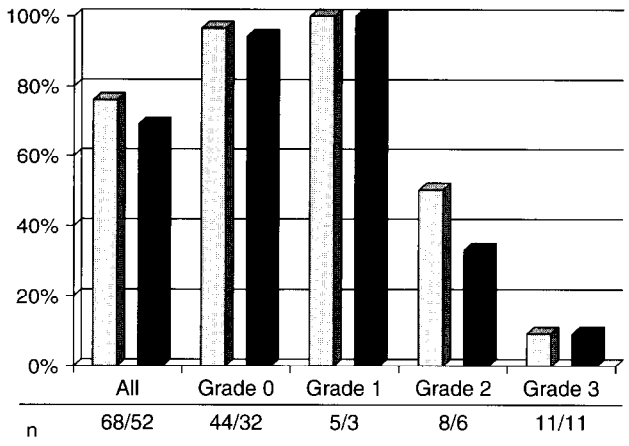

Fig. 3. Resectability according to CT grading system. Overall resectability was 76%. For grades 0 to 3 resectability rates were 95%, 100%, 50%, and 9%, respectively *(white bars)*. Resectability for pancreatic adenocarcinoma *(gray bars)* was 69%. For grades 0 to 3 resectability rates were 94%, 100%, 33%, and 9%, respectively.

DISCUSSION

Complete tumor removal by means of pancreaticoduodenectomy remains the only potentially curative treatment for periampullary neoplasms. However, the vast majority of patients who present with this disease are unresectable because of either distant spread or locally advanced disease. Many of these patients suffer from obstructive jaundice, which can be palliated by endoscopic stent placement, obviating the need for a surgical biliary-enteric bypass.[10,11] It is therefore essential to stage patients preoperatively as precisely as possible in order to avoid laparotomy in unresectable patients.

The advent of sophisticated imaging techniques has improved detection of both locally advanced disease and liver metastases. However, the specific utility of these modalities in the preoperative assessment of patients with periampullary neoplasms remains to be clearly defined. Published experiences with the use of CT report resectability rates ranging from 30% to 75%.[12-20] No doubt a major factor accounting for the wide variation in resectability is the quality of the CT itself. The current state of the art consists of helical CT plus CT angiography, with images acquired at 3 mm intervals, a technique that has not consistently been used in most published studies. In many cases CT scans of questionable quality may have been obtained prior to evaluation by the specialty center at which the operation is contemplated; repeat imaging with a specifically tailored CT imaging protocol may yield substantially more accurate assessment.

Several groups have tried to improve the resectability rate by combining CT with other modalities. Warshaw et al.[3] used MRI, angiography, and laparoscopy in addition to CT. They found a 78% resectability rate if all tests were negative and a 5% resectability if any of them were positive. The predominant reason for unresectability was vascular involvement. Conlon et al.[13] were able to increase the resectability rate from 53% after CT staging to 88% by adding laparoscopy. Laparoscopic ultrasound can also be added to conventional diagnostic laparoscopy to enhance its accuracy. Callery et al.[4] were able to markedly increase the predictive value of staging by using a combination of CT scan, laparoscopy, and laparoscopic ultrasound in patients with hepatobiliary and pancreatic malignancies. This trend has also been observed in other studies.[18,21] It is unclear, however, how information on vascular involvement obtained by laparoscopic ultrasound compares to that obtained by CT angiography, since some of the studies included unresectable patients with extensive vascular involvement.

Our study shows that helical CT with CT angiography yields accurate information sufficient for prediction of resectability in the vast majority of cases. Patients with grade 0 and 1 vascular involvement had a resectability of greater than 96%. The two patients who were found to be unresectable had liver metastases. Laparoscopy could potentially have been helpful in one patient, whereas the single lesion identified in the other was on the dome and not easily within reach for laparoscopic visualization or biopsy. Our resectability rate falls to 50% for grade 2 lesions and 9% for grade 3 lesions. Most of these patients were unresectable because of locally advanced disease and vascular encasement. Liver metastases were found in only one of these patients. One patient with encasement of the hepatic artery by a cancerous celiac node underwent laparoscopy. The node was visualized and biopsies were performed but frozen sections were inconclusive. A limited directed exploration was performed to obtain a definite tissue diagnosis. Of note is that all patients with unresectable disease had pancreatic adenocarcinoma, whereas all patients with other pathologic findings were resectable (see Table III).

Our results are comparable to those of Friess et al.[12] who analyzed 159 patients scheduled for pancreaticoduodenectomy for periampullary neoplasms. These investigators found that most unresectable patients had vascular infiltration by the tumor and concluded that routine laparoscopy could not be recommended since it would have achieved benefit in only 10% of their patients. Holzman et al.[19] reported comparable results in a smaller series.

Based on our results and the reports of others, we believe that helical CT scan with CT angiography is sufficient for the assessment of patients with periampullary neoplasms. Patients who are found to have grade 0 or 1 lesions do not require routine laparoscopy since the resectability rate exceeds 96%. Patients with signs of vascular encroachment or encasement (grades 2 and 3, respectively) could potentially benefit from additional investigations including diagnostic laparoscopy in order to decrease the number of unnecessary laparotomies. However, the most common factor that determines nonresectability in these subgroups is the locally advanced disease and not the presence of unsuspected distant spread.

We emphasize that all patients included in our study underwent imaging at our institution according to an identical CT protocol. This series thus has allowed the establishment of a standard against which other methods can be more directly compared. Studies reporting a high yield for laparoscopy have not uniformly applied state-of-the-art CT technology; these series may have included patients who would have been excluded from our study by the presence of more advanced vascular involvement (grade 4) or metastatic lesions below the limits of resolution of other studies, including earlier generation CT.[5,13,18]

Helical CT with CT angiography represents a powerful tool for the evaluation of periampullary neoplasms. The absence of vascular involvement (grades 0 and 1) demonstrable by this modality is highly predictive of resectability, rendering additional preoperative staging studies superfluous. Imaging technology is ever-improving, and it is likely that newly emerging techniques will eventually equal or eclipse CT. In this regard, endoscopic ultrasound has shown promising results that may complement or refine the information currently obtainable by CT angiography.[22]

REFERENCES

1. Conlon KC, Klimstra DS, Brennan MF. Long-term survival after curative resection for pancreatic ductal adenocarcinoma. Clinicopathologic analysis of 5-year survivors. Ann Surg 1996; 223:273-279.
2. Yeo CJ, Cameron JL, Sohn TA, et al. Six hundred fifty consecutive pancreaticoduodenectomies in the 1990s: Pathology, complications, and outcomes. Ann Surg 1997;226:248-257.
3. Warshaw AL, Gu ZY, Wittenberg J, Waltman AC. Preoperative staging and assessment of resectability of pancreatic cancer. Arch Surg 1990;125:230-233
4. Callery MP, Strasberg SM, Doherty GM, et al. Staging laparoscopy with laparoscopic ultrasonography: Optimizing resectability in hepatobiliary and pancreatic malignancy. J Am Coll Surg 1997;185:33-39.
5. Bemelman WA, de Wit LT, van Delden OM, et al. Diagnostic laparoscopy combined with laparoscopic ultrasonography in staging of cancer of the pancreatic head region [see comments]. Br J Surg 1995;82:820-824.
6. Raptopoulos V, Steer ML, Sheiman RG, et al. The use of helical CT and CT angiography to predict vascular involvement from pancreatic cancer: Correlation with findings at surgery. AJR Am J Roentgenol 1997;168:971-977.
7. Fuhrman GM, Leach SD, Staley CA, et al. Rationale for en bloc vein resection in the treatment of pancreatic adenocarcinoma adherent to the superior mesenteric-portal vein confluence. Pancreatic Tumor Study Group. Ann Surg 1996;223: 154-162.
8. Leach SD, Lee JE, Charnsangavej C, et al. Survival following pancreaticoduodenectomy with resection of the superior mesenteric-portal vein confluence for adenocarcinoma of the pancreatic head. Br J Surg 1998;85:611-617.
9. Harrison LE, Klimstra DS, Brennan MF. Isolated portal vein involvement in pancreatic adenocarcinoma. A contraindication for resection? Ann Surg 1996;224:342-347.
10. Lichtenstein DR, Carr-Locke DL. Endoscopic palliation for unresectable pancreatic carcinoma. Surg Clin North Am 1995;75:969-988.
11. Smith AC, Dowsett JF, Russell RC, et al. Randomised trial of endoscopic stenting versus surgical bypass in malignant low bileduct obstruction. Lancet 1994;344:1655-1660.
12. Friess H, Kleeff J, Silva JC, et al. The role of diagnostic laparoscopy in pancreatic and periampullary malignancies. J Am Coil Surg 1998;186:675-682.
13. Conlon KC, Dougherty E, Klimstra DS, et al. The value of minimal access surgery in the staging of patients with potentially resectable peripancreatic malignancy. Ann Surg 1996; 223:134-140.
14. Freeny PC, Traverso LW, Ryan JA. Diagnosis and staging of pancreatic adenocarcinoma with dynamic computed tomography. Am J Surg 1993;165:600-606.
15. McCarthy MJ, Evans J, Sagar G, Neoptolemos JP. Prediction of resectability of pancreatic malignancy by computed tomography. Br J Surg 1998;85:320-325.
16. Furukawa H, Kosuge T, Mukai K, et al. Helical computed tomography in the diagnosis of portal vein invasion by pancreatic head carcinoma: Usefulness for selecting surgical procedures and predicting the outcome. Arch Surg 1998;133:61-65.
17. Andren-Sandberg A, Lindberg CG, Lundstedt C, Ihse I. Computed tomography and laparoscopy in the assessment of the patient with pancreatic cancer. J Am Coll Surg 1998; 186:35-40.
18. John TG, Greig JD, Carter DC, Garden OJ. Carcinoma of the pancreatic head and periampullary region. Tumor staging with laparoscopy and laparoscopic ultrasonography. Ann Surg 1995;221:156-164.
19. Holzman MD, Reintgen KL, Tyler DS, Pappas TN. The role of laparoscopy in the management of suspected pancreatic and periampullary malignancies. J GASTROINTEST SURG 1997;1: 236-244.
20. Bluemke DA, Cameron JL, Hruban RH, et al. Potentially resectable pancreatic adenocarcinoma: SpiraI CT assessment with surgical and pathologic correlation. Radiology 1995;197: 381-385.
21. Minnard EA, Conlon KC, Hoos A, et al. Laparoscopic ultrasound enhances standard laparoscopy in the staging of pancreatic cancer. Ann Surg 1998;228:182-187.
22. Legmann P, Vignaux O, Dousset B, et al. Pancreatic tumors: Comparison of dual-phase helical CT and endoscopic sonography. AJR Am J Roentgenol 1998;170:1315-1322.

Discussion

Dr. M. Callery (Worcester, Mass.). You have shown that helical CT is a powerful tool for predicting resectability. I imagine that we would probably all pick this as our first choice if we were given one study for evaluating these patients. The goals of this and all staging modalities are really twofold. The first is to try to equalize the rates of operability and resectability, and then to eliminate unnecessary laparotomy in patients who cannot benefit if their lesions are unresectable.

I would like you to reconcile some of the differences between this study and several others with respect to the use of laparoscopy in staging. Dr. Conlon from the group at Memorial Sloan-Kettering Cancer Center has done a great deal of work identifying the value of laparoscopy. Also, Warshaw et al.[3] demonstrated a 20% rate of occult tumor spread in patients with pancreatic neoplasms. I would agree that for early-grade lesions (0 through 2½) laparoscopy may be superfluous. But if laparoscopy is used critically in your grade 2½ to 3 lesions, based on the images you presented, unnecessary laparotomy can be avoided in those patients, not only on the basis of miliary disease but also because there will be a higher yield with the use of the laparoscopic ultrasound probe in determining unresectability based on local vascular invasion. Thus the patient can be spared additional procedures such as endoscopic stenting by using laparoscopic palliative biliary and gastric bypass techniques.

Dr. P. Saldinger. I absolutely agree that the weakness of this method lies with patients who have lesions that are grade 2 and higher. If these patients can be identified beforehand, they can certainly benefit from either laparoscopy or laparoscopic ultrasound, or possibly endoscopic ultrasound, in an attempt to avoid surgery.

Dr. J. Bowen (New Orleans, La.). Can you clarify what you mean by local invasion preventing recurrence, specifically in regard to the superior mesenteric vein. There are cases where the superior mesenteric vein is involved to some degree and the tumor is still resectable. I would like to know if tumors with vein involvement were considered unresectable in your study or were you sometimes able to resect a portion of the vein with the tumor?

Dr. Saldinger. Intraoperatively, tumors were considered unresectable if we were unable to mobilize the superior mesenteric vein off the pancreatic neck. We have not encountered any cases where once the neck was transected, the vein was attached to the side of the uncinate process and one could consider resection of the vein. That did not occur in this series. If we deemed a patient to be unresectable because of local invasion, it was essentially because we were unable to mobilize the pancreatic neck.

Dr. M. Sarr (Rochester, Minn.). Can you tell us more about the grade 3 findings? In those patients, did you note the formation of any collateral vessels? Collateral vessels are a good sign of functional obstruction of the vein and equate with local unresectability.

Dr. Saldinger. There was no sign of collateral vessels in these patients. There were 11 of these patients and one underwent resection and 10 did not. One might ask whether it makes sense for these patients to undergo surgical exploration at all. In fact, the one patient who had a resection had a questionable grade 3 lesion according to one cut, so it was more of a grade 2 + than a grade 3 lesion. If we continue to analyze patients of this sort, at some point we might find that it is not justified to subject them to surgical exploration at all. If these patients are taken out of the analysis, of course, then the prediction of resectability with CT scan will go way up.

Dr. C. Fernandez-del Castillo (Boston, Mass.). I would like you to clarify your resectability rate for pancreatic cancer. Your overall resectability rate was 76%, but that included other periampullary tumors, which we know have a much higher likelihood of resectability. To underscore the value of laparoscopy in the group of patients with pancreatic cancer, not ampullary, bile duct, or duodenal carcinoma, our data show that 19% of patients with pancreatic head tumors will have unsuspected metastasis detected at the time of laparoscopy. Thus laparotomy will be avoided in 19% and if, in addition, peritoneal cytologic studies are also considered, there will be another 6% or 7% of patients who will not benefit from laparotomy. It is true that the application of laparoscopy can be stratified. If the tumor is smaller than 2 cm, the yield is very low. This is not just based on whether the tumor is touching the vein or not, because it is possible to have a 3 cm tumor that is not touching the vein, but the chance that these patients will have peritoneal or liver metastasis is probably about 20% or more.

Dr. Saldinger. The resectability rate for pancreatic cancer was 69%. All patients who were unresectable had pancreatic cancer. You are correct about laparoscopy and distant metastases. We had only three patients with liver metastasis and that caused some bias in our series. In two of them the lesions certainly could have been visualized by laparoscopy; the third patient had a lesion high up on the dome that probably could not have been seen or biopsied. For low-grade lesions, we probably still would not perform laparoscopy, but in the others its use is certainly justified.

Dr. Y. Fong (New York, N.Y.). In these patients, how effective was CT angiography in terms of depicting aberrant arterial anatomy? Could you see celiac stenosis or replaced right hepatic arteries coming behind the pancreas, to help you in planning surgery?

Dr. Saldinger. Absolutely. Virtually all of the anatomic variations can be seen with this method.

Dr. J. Hoffman (Philadelphia, Pa.). The vein is important, but I am not sure it is the only important criterion for determining local resectability. The status of the superior mesenteric artery and the celiac axis and hepatic artery would be just as important and you have not given us any details of those assessments. Second, I wonder if the procedure would identify patients with reversal of flow due to celiac axis compression.

Dr. Saldinger. With respect to the superior mesenteric artery, I did not mention that a grade 4 lesion indicates either superior mesenteric vein, portal vein, or superior

mesenteric artery involvement and we had, in fact, two patients with encasement of the superior mesenteric artery who were excluded prior to surgery. I do not know whether reversal of flow could be seen on the CT angiogram.

Dr. H. Reber (Los Angeles, Calif.). Your experience is very similar to what we have reported. We have more than 100 patients with pancreatic adenocarcinoma in the head of the gland in which helical CT prediction of resectability is approximately 85%. Dr. Warshaw and I have discussed the issue of the additional value of laparoscopy many times. His conclusions are different from ours, which probably reflects different institutional experiences with the reliability of CT. In our experience, with a high accuracy of prediction of resectability by CT, to perform laparoscopy after the CT is simply not economical.

You mentioned using CT angiography with three-dimensional reconstructions to obtain the reconstituted computerized image of the vessels. Our radiologists initially did this as well, but as they gained more experience, they found that simply interpreting the plain CT images without performing reconstructions is just as accurate and is less expensive because of the time and additional effort spent in trying to create the reconstructed images. Have your radiologists had a similar experience or do you still think it necessary to perform the reconstruction?

Dr. Saldinger. In many cases, just studying at the cuts is sufficient. If the lesion is grade 0 or 1, the fancy reconstruction is not necessary to figure out that the tumor is resectable. But we used reconstructions in all of the patients, and in a fair number of patients it really helps to determine more precisely where the problem will be.

Dr. A. Warshaw (Boston, Mass.). It may come as no surprise to you that my experience has been very similar to that of Dr. Fernandez. I guess it is a question of whether the glass is half full or half empty.

Dr. L. Traverso (Seattle, Wash.). Your data emphasize the radiologist's assessment of the superior mesenteric vein/portal vein system, not the arterial system. Is that true?

Dr. Saldinger. No, it includes the superior mesenteric artery as well.

Dr. Traverso. In a recent issue of *American Journal of Surgery* (1999;177:428-432), the UCLA, Mayo Clinic, and Virginia Mason Medical Center experience with predicting operative findings by CT scans for pancreatic cancer was published. We show that portal vein involvement is frequently overcalled, but when arterial involvement is noted, it is important and it affects long-term survival. If you could divide your data into venous and arterial assessment, you might find different resectability rates.

I have a caveat for those who rely on helical CT angiography. As you know, the helical CT captures the arterial phase on the way down and the portal venous phase on the way back. But the hypodense tumor may be totally missed. The tumor will not be seen in many cases unless it is captured right in the middle, somewhere between the arterial phase and the portal venous phase. Even if CT angiography is performed and the extra time and the extra cuts and the extra interpretation and reconstruction are used, the tumor will not be seen. That is a problem that should be worked out with the radiologist, to actually design the CT study so that the tumor can be seen, rather that just the loss of fat planes.

Dr. L. Way (San Francisco, Calif.). Despite the accuracy of the helical CT, occasionally there are studies that are difficult to interpret. I want to mention that magnetic resonance angiography usually resolves the ambiguity.

Therapy for Microcirculatory Disorders in Severe Acute Pancreatitis: Comparison of Delayed Therapy With ICAM-1 Antibodies and a Specific Endothelin A Receptor Antagonist

Thomas Foitzik, M.D., Guido Eibl, M.D., Heinz J. Buhr, M.D.

Many of the complications in severe acute pancreatitis result from the amplifying effects of microcirculatory disruption. The pathogenesis of these microcirculatory disorders is multifactorial and involves various vasoactive mediators. Thus questions arise as to which vasoactive mediators are most important and how long after the onset of disease vasoactive mediator blockade may be effective. The present study compares the effect of delayed therapy with two vasoactive mediator antagonists, previously tested with promising results in other studies in a well-established rodent model of severe acute pancreatitis. Twelve hours after induction of acute pancreatitis, rats were randomized to therapy with intracellular adhesion molecule-1 (ICAM-1) antibody (2 mg/kg IA-29), endothelin A receptor antagonist (ET-RA) (40 mg/kg LU 135252), or saline solution (volume equivalent). After 12 hours of fluid resuscitation, animals underwent repeat laparotomy for intravital microscopic determination of capillary blood flow, leukocyte rolling, and capillary permeability in the pancreas and colon. Other measurements included cardiorespiratory parameters, hematocrit, pleural effusions, ascites, urine production, and survival. Compared to saline treatment, both ICAM antibody and ET-RA significantly enhanced capillary blood flow in the pancreas and colon, reduced leukocyte rolling, and stabilized capillary permeability. These beneficial effects on microcirculation were associated with decreased fluid loss into the third space and improved renal function and survival. Although both antagonists likewise enhanced capillary blood flow and reduced leukocyte rolling, ET-RA was significantly more effective than ICAM antibody in counteracting capillary leakage, thereby further reducing fluid sequestration. The present study confirms the beneficial effects of endothelin and ICAM antagonists in severe acute pancreatitis, even with delayed therapy, suggesting that both compounds are candidates for further clinical testing. Selective endothelin A receptor blockade appears to be especially attractive for clinical use not only because it was superior to ICAM antibody in the present study but also because of its favorable pharmacologic properties and (preliminary) positive results in clinical phase 2 studies currently underway for other diseases. (J GASTROINTEST SURG 2000;4:240-247.)

KEY WORDS: Acute pancreatitis (experimental), microcirculation, vasoactive mediators, endothelin, adhesion molecules

Microcirculatory disorders are a hallmark of severe acute pancreatitis. They are not confined to the pancreas, where they may promote acinar cell necrosis, but have also been demonstrated in the colon, lung, and liver, where they are believed to contribute to organ dysfunction.[1-3] Assuming that many of the complications and deaths associated with acute pancreatitis result from the amplifying effects of organ-specific microcirculatory impairment, improving microcirculation appears to be a logical therapeutic approach. In addition to adequate fluid resuscitation, which is essential for compensating hypovolemia and hemoconcentration, novel strategies are aimed at inhibiting the vasoactive substances that cause vasoconstriction, higher vascular permeability, and pathologic interaction between blood cells and the endothelium. Vasoactive substances activated during acute pancreatitis include cytokines such as interleukins (IL-1, IL-6,

From the Department of Surgery, Benjamin Franklin Medical Center, Freie Universität Berlin, Berlin, Germany.
Presented at the Fortieth Annual Meeting of The Society for Surgery of the Alimentary Tract, Orlando, Fla., May 16-19, 1999.
Reprint requests: Dr. Thomas Foitzik, Chirurgische Klinik I, Universitätsklinikum Benjamin Franklin, Freie Universität Berlin, Hindenburgdamm 30, D-12 200 Berlin, Germany. e-mail: foitzik@ukbf.fu-berlin.de

IL-8), tumor necrosis factor, and platelet-activating factor, as well as secondary mediators such as bradykinin, prostaglandin, nitric oxide, endothelin, and adhesion molecules.[1-7] This study is part of a series evaluating which of these vasoactive mediators are most important for the microcirculatory disorders seen in acute pancreatitis and which factor, when blocked, is most effective in counteracting these microcirculatory changes and subsequent organ dysfunction. We also investigated which component of disturbed microcirculation (capillary blood flow, capillary permeability, or leukocyte-endothelial interaction) is mainly affected and how long after the onset of disease vasoactive mediator blockade can be effective. The study applies a well-established model of acute necrotizing pancreatitis in the rat that has proved suitable for evaluating therapy in previous studies.[8-10] The model involves extended organ monitoring and intravital microscopy using a novel computer-assisted image analysis system for the quantitative assessment of microcirculation.[11] The tested vasoactive mediator inhibitors are a specific endothelin A antagonist (ET-RA) and a monoclonal antibody against intercellular adhesion molecule-1 (ICAM-AB), which were previously shown to be most effective in this model of acute pancreatitis.[12,13]

MATERIAL AND METHODS

All experiments were conducted in accordance with the national guidelines for the use and care of laboratory animals and approved by the local ethics committee. After overnight fasting, 40 male Sprague-Dawley rats (300 to 350 kg), housed individually in metabolic cages, were anesthetized with intraperitoneal pentobarbital (20 mg/kg) and ketamine (40 mg/kg). Polyethylene catheters (inside diameter 0.5 mm) were inserted into both jugular veins and the left carotid artery, subcutaneously tunneled to the neck, and advanced through a steel tether, which allowed blood sampling and intravenous access in the unrestrained animals.

Severe (necrotizing) acute pancreatitis was induced by a standardized retrograde infusion of 0.5 ml of 10 mmol/L glycodeoxycholic acid (GDOC; Sigma, St. Louis, Mo.) into the biliopancreatic duct for 10 minutes, followed by intravenous infusion of 5 μg/kg/hr cerulein (Farmitalia, Freiburg, Germany) over 6 hours.

After 6 hours of acute pancreatitis induction, animals were observed for another 6 hours without any intervention or therapy. After 12 hours, cardiorespiratory parameters were monitored and blood was drawn to determine hematocrit and trypsinogen activation peptide (TAP) values in plasma.[14] Thereafter animals were randomized to therapy with the ICAM-1 antibody IA-29 (Boehringer, Ingelheim, Germany; Ridgefield Branch, Conn.; 2 mg/kg intravenous; n = 10), the specific endothelin A receptor antagonist LU 135252 (Knoll AG, Ludwigshafen, Germany; 50 mg/kg; slow intravenous injection; n = 10), or normal saline solution (volume equivalent; n = 12). After 24 hours (12 hours after the start of therapy), during which time the animals received continuous fluid resuscitation with Ringer's lactate (4 ml/kg/hr), cardiorespiratory and laboratory parameters were reevaluated and animals underwent repeat laparotomy to determine ascites and for intravital microscopy. Other measurements included pleural effusion determination at autopsy, urine collection from the metabolic cages, and histologic examination of pancreatic specimens harvested at autopsy.

Healthy sham-operated animals (intraductal and intravenous saline infusion) treated with saline, ICAM antibody, and ET-RA served as additional control subjects (data not shown). The dosage and type of antagonist administration were chosen according to the experience gained with these specific compounds in previous studies.[12,13]

Assessment of disease severity included the amount of trypsinogen activation and the extent of acinar cell necrosis as indicators of pancreatic injury, as well as systemic measurements representing the animals' fluid status and organ functions. TAP levels in plasma reflecting premature intrapancreatic protease activation were measured 12 hours after the start of acute pancreatitis induction (before therapy) using an enzyme-linked immunosorbent assay with affinity-purified rabbit anti-TAP antibodies.[14] Pancreatic acinar cell necrosis was evaluated by histologic examination and scored according to previously described criteria.[8] Fluid sequestration into the third space was estimated from repeated hematocrit measurements and the amount of free fluid collected from the abdomen before intravital microscopy and the thorax at autopsy. Cardiorespiratory function was assessed by monitoring arterial blood gases, mean arterial pressure, and heart rate at several time points during the experiment (0, 12, 18, and 24 hours before and after intravital microscopic examination), and renal function by collecting urine from the metabolic cages.

For intravital microscopy, animals were placed on a heated operating table. The abdomen was opened by a small midline incision. First, the duodenum and the head of the pancreas were mobilized and exteriorized, placed in an immersion chamber with Ringer's lactate maintained at 37° C, and positioned under a fluorescence microscope (Leitz, Wetzlar, Germany) with a heat protection and excitation filter (450 to 490 nm) connected to a video recorder. Following exposure of the pancreas, 0.5 ml/kg erythrocytes labeled with fluorescein isothiocyanate (FITC; Sigma, Deisen-

hofen, Germany) was injected intravenously. After a 5-minute stabilization period, three randomly chosen regions in the head of the pancreas (400 × 325 μm) were recorded for off-line analysis of pancreatic capillary blood flow. Thereafter the duodenal loop was repositioned, and the ascending colon was exposed for assessment of colonic capillary blood flow in the mucosa. Following the assessment of capillary blood flow, leukocyte-endothelial interaction was recorded in postcapillary venules of the adjacent mesentery after injection of 1 ml/kg 0.02% rhodamine (Sigma, Deisenhofen, Germany). Leukocyte rolling, defined as the number of cells passing a previously defined vessel segment within 1 minute, was assessed off line in 8 to 10 video segments per animal. Leukocyte sticking, defined as the number of cells attached to the endothelial lining with no movement over a longer observation period, was not assessed in this experiment to limit recording time. Capillary permeability was determined in the ascending colon following an injection of 0.2 ml 5% FITC-Dextran 150 (Sigma, Deisenhofen, Germany; molecular weight 150,000 daltons) by quantifying the increase in perivascular fluorescein intensity in the same field (5 fields per animal) over 30 minutes by means of CAP-Image (Zeintl, Heidelberg, Germany).[11] This novel computer-assisted video frame analysis system for dynamic capillaroscopy allows off-line analysis of a variety of microcirculatory parameters and calculates capillary permeability from the changes in perivascular density caused by extravasation of the fluorescent-labeled dextran (molecular weight 150,000 daltons) over a defined observation period. Details of the equipment, techniques, and methods of calculating the microcirculatory parameters have been described elsewhere.[15,16] Since continuous extravasation of FITC-dextran obliterates the microscopic image, which compromises further measurements, capillary permeability could only be determined at the end of the experiment in one organ bed per animal. The colon (rather than the pancreas, which we examined in previous studies) was chosen because its extensive surface and capillary density make it a major site of fluid extravasation in capillary leakage syndrome. The total time needed for exposure and recording of the microcirculatory beds was 90 to 120 minutes per animal. Heart rate, arterial pressure, and blood gases were measured before and after intravital microscopy.

Only data from animals with stable cardiorespiratory conditions were included in the analysis of the microcirculatory parameters to avoid bias possibly resulting from systemic cardiorespiratory derangement. Exclusion criteria were mean arterial pressure less than 80 mm Hg, pO_2 less than 80 mm Hg, pCO_2 more than 50 mm Hg, and pH <7.3 or >7.5.

Statistical Analysis

All results are expressed as mean ± standard error of the mean (SEM). Variables were tested for group differences by means of Student's *t* test, Mann-Whitney rank-sum test, and chi-square test where appropriate. A P value <0.05 was considered significant.

RESULTS

Eight of the 40 animals died within 12 hours, leaving 32 animals that were randomized to the three treatment groups. Seven of 12 saline-treated animals died before intravital microscopy after 24 hours, and one died at the end of the observation period. With ICAM antibody and ET-RA, two animals died and one in each group developed signs of severe cardiorespiratory distress during intravital microscopy and were subsequently excluded from further analysis (see exclusion criteria).

Compared to healthy control animals, rats with severe acute pancreatitis developed a significant decrease in urine production and an increase in hematocrit and plasma TAP levels (data not shown). The comparison of mean arterial pressure, respiratory parameters, urine production, hematocrit (data not shown), and TAP levels in plasma (saline, 4.6 ± 0.6 nmol/L; ICAM antibody, 4.4 ± 0.8 nmol/L; and ET-RA, 4.8 ± 0.9 nmol/L) before the administration of the test substances showed no significant group differences, indicating that the severity of pancreatitis was comparable in all animals at the time of randomization.

After 24 hours (12 hours after the start of therapy), animals treated with either ICAM antibody or ET-RA had a higher urine output, lower hematocrit, and less ascites and pleural effusion than animals given normal saline solution (Table I). Differences between animals treated with ICAM antibody and those treated with ET-RA were significant (P <0.05) for hematocrit values and urine production.

TAP values determined in plasma after 24 hours were lower than those measured after 12 hours and did not differ between treatment groups (data not shown). Scores for acinar cell necrosis likewise showed no differences (see Table I).

Compared to values in healthy control rats, pancreatic capillary blood flow was significantly reduced 24 hours after induction of acute pancreatitis (2.0 ± 0.04 vs. 1.3 ± 0.04 nl/min/cap; P <0.05). ICAM antibody significantly improved pancreatic capillary blood flow by 24% (1.6 ± 0.04 nl/min/cap; P <0.05) and ET-RA by 36% (1.9 ± 0.05 nl/min/cap; P <0.05 vs. ICAM antibody. The increase in capillary blood flow in the colon was 10% in the ICAM antibody group, and 15% in the ET-RA group (P <0.05 vs. saline-treated control rats; ET-RA vs. ICAM antibody P = NS).

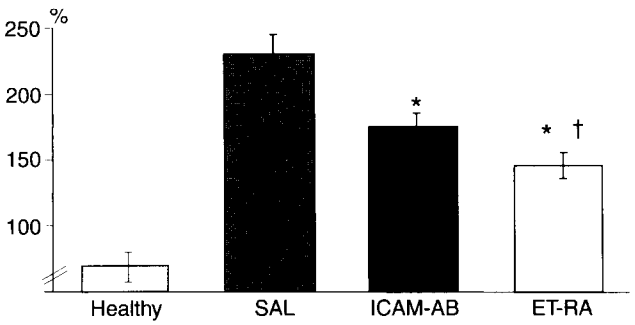

Fig. 1. Capillary permeability in the colonic mucosa in healthy control animals and animals with severe acute pancreatitis treated with saline (SAL), intracellular adhesion molecule-1 antibody *(ICAM-AB)*, and endothelin A receptor antagonist *(ET-RA)* (mean ± SEM). * = $P < 0.05$ vs. SAL; † = $P < 0.05$ ET-RA vs. ICAM-AB.

Table I. Target parameters after treatment with normal saline (SAL), IA 29 (ICAM-AB), or LU 135252 (ET-RA) (mean ± SEM)

	SAL (n = 5)	ICAM-AB (n = 8)	ET-RA (n = 8)
Mean arterial pressure (mm Hg)	118 ± 10	116 ± 7	114 ± 4
pO_2 (mm Hg)	90 ± 5	94 ± 8	96 ± 4
pH	7.42 ± 0.05	7.43 ± 0.04	7.40 ± 0.03
Urine (ml)*	1.6 ± 0.5	2.5 ± 0.5†	3.3 ± 0.5†‡
Hematocrit (%)	49 ± 1	45 ± 1†	42 ± 1†‡
Ascites (ml)	2.0 ± 0.3	1.4 ± 0.3	1.1 ± 0.2†
Pleural effusion (ml)	1.4 ± 0.1	0.7 ± 0.2†	0.7 ± 0.3†
Necrosis (points)	2.4 ± 0.3	2.1 ± 0.2	2.2 ± 0.2

*Collected between 12 and 24 hours.
†$P < 0.05$ compared to saline.
‡$P < 0.05$ ET-RA vs. ICAM-AB.

Leukocyte endothelial interaction was markedly enhanced in saline-treated animals with acute pancreatitis (16 ± 2 vs. 4 ± 1 rolling cells/min in healthy control rats). ICAM antibody and ET-RA significantly reduced the number of rolling leukocytes to 7 ± 2 and 8 ± 2, respectively.

Capillary permeability in the colon (Fig. 1) was significantly increased in saline-treated animals with acute pancreatitis (235 ± 14% vs. 68 ± 8% in healthy control rats). ICAM antibody reduced permeability by 25% (176 ± 8% vs. 235 ± 14; $P < 0.001$) and ET-RA by 37% (147 ± 12%; $P < 0.05$ vs. ICAM antibody).

DISCUSSION

Impairment of pancreatic blood supply, microcirculation, and tissue oxygenation contributes to the development of severe acute pancreatitis.[17,18] Microcirculatory disorders, however, are not confined to the pancreas. In animals, at least, they can also be demonstrated in the colon, lung, and liver, where they have been associated with organ dysfunction.[1-3] Therefore improving microcirculation appears to be a logical therapeutic approach. Because the pathogenesis of microcirculatory disorders in acute pancreatitis involves several components and causes, different therapeutic measures may be suitable, for example, hemodilution and the use of low-molecular-weight colloids, which directly counteract hypercoagulability and reduce abnormal leukocyte-endothelial interaction at the microcirculatory level.[18,19] Better understanding of the cytokine cascade activated during the systemic inflammatory response to pancreatic injury has given rise to novel strategies for directly interfering with the vasoactive substances that cause vasoconstriction, increased vascular permeability, and pathologic interaction between blood cells and the endothelium at the capillary level. Candidates that may be influenced include interleukins, tumor necrosis factor, and platelet-activating factor released mainly from tissue macrophages and monocytes, as well as secondary mediators such as endothelin and nitric oxide released directly from the endothelial cells in response to cytokine activation, endothelial injury, circulating substances (including proteases liberated from the injured pancreas), and changes in tissue perfusion.[2-5,12,13,20-23] Adhesion molecules that affect leukocyte-endothelial cell interaction at the capillary level have likewise been postulated to play a crucial role in causing microvascular dysfunction and inflammatory tissue injury in acute pancreatitis. They are expressed by a variety of cells (including the vascular endothelium), for example, in response to endotoxin

or cytokines, and are likewise activated during acute acute pancreatitis both in animals and humans.[24-26] The significance of these vasoactive factors for the development and course of severe acute pancreatitis has been underlined by studies demonstrating that vasoactive mediator blockade not only improves microcirculation inside and outside the pancreas, but also organ function, local pancreatic and systemic (e.g., lung) injury, and survival in acute pancreatitis.[2,12,13,20-23] Thus the question arises as to which vasoactive mediators are most important and how long after the onset of disease vasoactive mediator blockade is effective.

Monoclonal ICAM-1 antibodies and a specific endothelin-A receptor antagonist (ET-RA) were chosen for the present experiment because of positive experience reported with these specific compounds in recent studies. Werner et al.[13] found reduced leukocyte infiltration and tissue injury in the pancreas and lungs after treatment with ICAM-1 antibodies initiated 6 hours after disease onset in the same model of acute pancreatitis as that used in the present study. Studies by Frossard et al.[26] performed in wild-type and ICAM-1–deficient mice with secretagogue- and diet-induced acute pancreatitis likewise underlined the role of ICAM-1 for neutrophil-mediated local and systemic injury. In addition, they found evidence of an ICAM-1–induced increase in microvascular permeability in the lungs (which is in accordance with our present findings in the colon). However, their results also made it clear that neither pancreatitis nor pancreatitis-associated injury (including changes in permeability) are completely prevented by ICAM-1 deficiency, which supports the opinion that multiple mediators are involved. Our hypothesis that endothelin-1 is one of the decisive candidates has recently been confined by Plusczyk et al.,[27] who found that topical superinfusion of the exteriorized pancreas with endothelin-1 dramatically reduces functional capillary density and red blood cell velocity. This leads to focal acinar cell necrosis similar to that seen in acute pancreatitis. Beneficial effects of ET-RA in acute pancreatitis have previously been reported by Todd et al.,[23] who used a nonselective endothelin A and endothelin B receptor antagonist. Our most recent study,[28] however, suggests that endothelin A and endothelin B receptors mediate different even adverse effects and that combined endothelin A and endothelin B receptor blockade is less effective than selective endothelin A receptor blockade. This appears to explain the even better results achieved with the specific antagonist used in this study and previous ones in which we demonstrated improved survival in association with enhanced microcirculation (especially stabilized capillary permeability) inside and outside the pancreas, less intravascular fluid loss and extravascular fluid sequestration, as well as better renal and respiratory function in ET-RA–treated animals with acute pancreatitis than in untreated control animals.[12,29]

In addition to the question of which mediator is most important for the microcirculatory changes seen in severe acute pancreatitis, there is controversy as to which microcirculatory component (capillary blood flow, permeability, or postcapillary leukocyte-endothelial interaction) is crucial for the development of tissue injury and organ dysfunction. Our group found that ET-RA was especially effective probably because it almost normalized increased capillary permeability in severe experimental pancreatitis, which we believe is responsible for many of the systemic disease sequelae.[29] Other groups postulated that pathologic leukocyte-endothelial interaction is the crucial step in pancreatitis-associated tissue injury and organ dysfunction and suggested that ICAM antibodies are the superior treatment.[13] Consequently the present study compares the effect of ET-RA and ICAM-1 antibodies on capillary blood flow and permeability, leukocyte-endothelial interaction, fluid homeostasis, and organ function in the same model of severe acute pancreatitis, applying a protocol in which therapy was delayed until 12 hours after the onset of disease. The suitability of the model for evaluating therapy in severe acute pancreatitis has been demonstrated previously in several studies.[8-10] Comparing the time course of trypsinogen activation, enzyme release, cytokine activation, and the development of necrosis and systemic disease sequelae in small rodents and humans, the 12-hour therapy-free interval is supposed to be equivalent to more than 48 hours in the clinical setting. Organ function was monitored by standard measurements (mean arterial pressure, heart rate, arterial blood gases, urine output, creatinine, hematocrit, pleural effusion and ascites) also reflecting clinical practice. At the microcirculatory level we focused on the intestine because its large surface and capillary density make it a major site for fluid loss and because previous findings suggested that decreased capillary blood flow in the colonic mucosa contributes to gut failure with increased bacterial translocation.[1,30] Furthermore, the gastrointestinal tract seemed especially interesting for our experiment for two reasons: (1) the splanchnic circulation is one of the largest sources of soluble adhesion molecules such as isoform ICAM-1, and (2) the gastrointestinal tract has been shown to exhibit marked increases in endothelial ICAM-1 expression in response to endotoxin and cytokine stimulation.[25,31,32]

Despite comparable macrohemodynamic parameters (with 4 ml/kg/hr fluid resuscitation), animals given either ICAM antibody or ET-RA had significantly better microcirculatory parameters than saline-

treated rats. Stabilizing microcirculation was associated with increased intravascular volume (as assessed by hematocrit values), decreased fluid loss into the third space (as assessed by ascites and pleural effusion), and improved renal function and survival. In contrast (and despite the improvement seen at the capillary level), treatment with the antagonists had no significant impact on the extent of acinar cell injury. These findings agree with previously discussed observations[12,29] and suggest that pancreatic injury may not be influenced by delayed therapy because by then necrosis has already developed. The beneficial effects seen with ICAM antibody and ET-RA therapy can be explained by an improvement in systemic disease sequelae associated with the stabilization of systemic microcirculation. The hypothesis that systemic rather than local disease sequelae determine the outcome has recently been supported by clinical studies, which did not confirm a causal relationship between the extent of pancreatic necrosis and systemic complications.[33]

Although both ICAM antibody and ET-RA improved capillary blood flow and reduced leukocyte endothelial interaction, ET-RA was significantly more effective in counteracting capillary permeability. The latter was associated with improved intravascular volume reflected by lower hematocrit values and translated into improved urine output. This appears to strengthen our hypothesis that capillary leakage (rather than leukocyte endothelial interaction) is a crucial factor in the development of systemic disease sequelae in severe acute pancreatitis. The clinical finding that capillary leakage persists long after the initial events triggering pancreatic injury have subsided also explains why blockade of ET-RA (and ICAM antibody) is still effective with delayed therapy. The observation that ICAM antibody reduces capillary permeability supports the hypothesis that there is a correlation between leukocyte-endothelial interaction and increased capillary permeability, although the mechanism is not known at present.[25] It is likewise unknown how endothelin affects capillary permeability. An endothelin-associated increase in postcapillary resistance might elevate the hydrostatic pressure in the capillary bed, thereby enhancing fluid transfer from the intravascular to the extravascular space.[34] Alternatively, capillary leakage may be explained by increased hydraulic conductivity of the microvascular membrane secondary to interendothelial cell gap formation.[35] Majno et al.[36] postulated that mediators of capillary leakage contract vascular elements such as actin and myosin contained in endothelial cells and surrounding pericytes to open these gaps. Calcium required for contraction could be released by endothelin, since endothelin has been found to mobilize intracellular calcium and open calcium channels.[37] Although its precise mechanism of action remains unknown, this study and previous ones have clearly identified endothelin as a major and previously not well-recognized mediator of capillary permeability. Endothelin (rather than ICAM) may therefore be blocked to reverse capillary leakage, which accounts for many of the local and systemic sequelae of severe acute pancreatitis.

CONCLUSION

The present findings underline the role of ICAM and endothelin in the development of severe acute pancreatitis and suggest that ICAM antibody and ET-RA may become powerful new tools in the treatment of severe acute pancreatitis. This assumption is supported by the fact that both compounds were effective even after a therapy-free interval of 12 hours (equivalent to approximately 48 hours in human acute pancreatitis) and may be explained by the observation that they stabilize increased capillary permeability, which contributes to many of the sequelae of acute pancreatitis that develop and persist after the initial events triggering pancreatic injury have subsided. Endothelin receptor blockade may be especially attractive for clinical use not only because it was superior to ICAM antibody in the present study but also because of its favorable pharmacologic properties and (preliminary) positive results in several of the clinical phase 2 studies that are currently underway.

REFERENCES

1. Hotz HG, Foitzik T, Rohweder J, Schulzke JD, Fromm M, Runkel NSF, Buhr HJ. Intestinal microcirculation and gut permeability in acute pancreatitis: Early changes and therapeutic implications. J GASTROINTEST SURG 1998;2: 518-525.
2. Yamanaka K, Saluja AK, Brown GE, Yamaguchi Y, Hofbauer B, Steer ML. Protective effects of prostaglandin E1 on acute lung injury of caerulein-induced acute pancreatitis in rats. Am J Physiol 1997;272 (part 1):G23-G30.
3. Dobosz M, Hac S, Wajda Z. Does nitric oxide protect from microcirculatory disturbances in experimental acute pancreatitis in rats? Int J Microcirc 1996;16:221-226.
4. Kingsnorth A. Role of cytokines and their inhibitors in acute pancreatitis. Gut 1997;40:1-4.
5. Gross V, Leser H-G, Heinisch A, Schölmerich J. Inflammatory mediators and cytokines. New aspects of the pathophysiology and assessment of severity of acute pancreatitis? Hepatogastroenterology 1993;40:522-530.
6. Yotsumoto F, Manabe T, Ohshio G, Imanishi K, Ando T, Kyogoku T, Hirano T, Tobe T. Role of pancreatic blood flow and vasoactive substances in the development of canine acute pancreatitis. J Surg Res 1993;55:531-536.
7. Weidenbach H, Lerch MM, Gress TM, Pfaff D, Turi S, Adler G. Vasoactive mediators and the progression from edematous to necrotizing experimental acute pancreatitis. Gut 1995;37: 434-440.

8. Schmidt J, Rattner DW, Lewandrowski K, Compton CC, Mandavilli U, Knoefel WT, Warshaw AL. A better model of acute pancreatitis for evaluating therapy. Ann Surg 1992;215: 44-56.
9. Foitzik T, Stufler M, Hotz HG, Klinnert J, Wagner J, Warshaw AL, Schulzke JD, Fromm M, Buhr HJ. Glutamine stabilizes intestinal permeability and reduces pancreatic infection in acute experimental pancreatitis. J GASTROINTEST SURG 1997;1:40-47.
10. Foitzik T, Fernandez-del-Castillo C, Ferraro MJ, Mithofer K, Rattner DW, Warshaw AL. Pathogenesis and prevention of early pancreatic infection in experimental acute necrotizing pancreatitis. Ann Surg 1995;222:179-185.
11. Klyscz T, Jünger M, Jung F, Zeintl H. Cap Image—ein neuartiges computerunterstütztes Videobildanalysesystem für die dynamische Kapillarmikroskopie. Biomed Technik 1997;42: 168-175.
12. Foitzik T, Faulhaber J, Hotz HG, Kirchengast M, Buhr HJ. Endothelin receptor blockade improves fluid sequestration, pancreatic capillary blood flow and survival in severe experimental pancreatitis. Ann Surg 1998;228:670-675.
13. Werner J, Z'graggen K, Fernandez-del Castillo C, Lewandrowski K, Compton CC, Warshaw AL. Specific therapy for local and systemic complications of acute pancreatitis with monoclonal antibodies against ICAM-1. Ann Surg 1999;229: 834-842.
14. Hurley PR, Cook A, Jehanli A, Austen BM, Hermon-Taylor J. Development of radioimmunoassays for free tetra-l-aspartyl-L-lysine trypsinogen activation peptides (TAP). J Immunol Methods 1988;111:195-203.
15. Mithöfer K, Schmidt J, Gebhard MM, Buhr HJ, Herfarth C, Klar E. Measurement of blood flow in pancreatic exchange capillaries with FITC-labeled erythrocytes. Microvasc Res 1995;49:33-48.
16. Hotz HG, Schmidt J, Ryschich EW, Foitzik T, Buhr HJ, Warshaw AL, Herfarth H, Klar E. Isovolemic hemodilution with dextran prevents contrast medium induced impairment of pancreatic microcirculation in necrotizing pancreatitis of the rat. Am J Surg 1995;169:161-166.
17. Warshaw AL. Ischemia- and reperfusion-related injury in pancreatitis. Dig Dis Sci 1996;41:821-822.
18. Klar E, Messmer K, Warshaw AL, Herfarth C. Pancreatic ischemia in experimental acute pancreatitis: Mechanism, significance and therapy. Br J Surg 1990;77:1205-1210.
19. Niederau C, Crass RA, Silver G, Ferrell LD, Grendell JH. Therapeutic regimens in acute experimental hemorrhagic pancreatitis. Effects of hydration, oxygenation, peritoneal lavage, and a potent protease inhibitor. Gastroenterology 1988;95:1648-1657.
20. Formela LJ, Wood LM, Whittaker M, Kingsnorth AN. Amelioriation of experimental acute pancreatitis with a potent platelet-activating factor antagonist. Br J Surg 1994;81:1782-1785.
21. Norman J, Franz M, Messina J, Riker A, Fabri PJ, Rosemurgy AS, Gower WR Jr. Interleukin-1 receptor antagonist decreases severity of experimental acute pancreatitis. Surgery 1995;117:648-655.
22. Hughes CB, Grewal HP, Gaber LW, Kotb M, Mohey El-din AB, Mann L, Gaber AO. Anti-TNF-α therapy improves survival and ameliorates the pathophysiologic sequelae in acute pancreatitis in the rat. Am J Surg 1996;171:274-280.
23. Todd KE, Lewis MPN, Gloor B, Lane JS, Ashley SW, Reber HA. An ETa/ETb endothelin antagonist ameliorates systemic inflammation in a murine model of acute hemorrhagic pancreatitis. Surgery 1997;122:443-450.
24. Kaufmann P, Tilz GP, Smolle KH, Demel U, Krejs GJ. Increased plasma concentrations of circulating intercellular adhesion molecule-1 in patients with necrotizing pancreatitis. Immunobiology 1996;195:209-219.
25. Panes J, Granger DN. Leukocyte-endothelial cell interactions: Molecular mechanisms and implications in gastrointestinal disease. Gastroenterology 1998;114:1066-1090.
26. Frossard JL, Saluja A, Bhagat L, Lee HS, Bhatia M, Hofbauer B, Steer ML. The role of intercellular adhesion molecule 1 and neutrophils in acute pancreatitis and pancreatitis-associated lung injury. Gastroenterology 1999;116:694-701.
27. Plusczyk T, Bersal B, Westermann S, Menger M, Feifel G. ET-1 induces pancreatitis-like microvascular deterioration and acinar cell injury. J Surg Res 1999;85:301-310.
28. Foitzik T, Eibl G, Forgacs B, Faulhaber J, Hotz HG, Kirchengast M, Buhr HJ. Therapy of microcirculatory disorders in severe acute pancreatitis. Endothelin-A but not endothelin-B receptor blockade reduces capillary leakage [abstr]. Pancreas 1998;17:433.
29. Foitzik T, Kirchengast M, Buhr HJ. Therapy for microcirculatory disorders in severe acute pancreatitis: Effectiveness of platelet-activating factor receptor blockade vs. endothelin receptor blockade. J GASTROINTEST SURG 1999;3:244-251.
30. Eibl G, Forgacs B, Hotz H, Kirchengast M, Wagner J, Buhr HJ, Foitzik T. Therapy of microcirculatory disorders in acute pancreatitis: Improvement of colonic capillary blood flow reduces bacterial translocation [abstr]. Pancreas 1998; 17:431.
31. Panes J, Perry MA, Anderson DC, Manning A, Leone M, Cepinskas G, Rosenbloom CL, Miyasaka M, Kvietys PR, Granger DN. Regional differences in constitutive and induced ICAM-1 expression in vivo. Am J Physiol 1995;269: H1955-H1964.
32. Henninger DD, Panes J, Eppihimer M, Russell J, Geritsen M, Anderson DC, Granger DN. Cytokine-induced VCAM-1 and ICAM-1 expression in different organs of the mouse. J Immunol 1997;158:1825-1832.
33. Tenner S, Sica G, Hughes M, Noordhoek E, Feng S, Zinner M, Banks PA. Relationship of necrosis to organ failure in severe acute pancreatitis. Gastroenterology 1997;113:899-903.
34. Filep JG, Sirois MG, Rousseau A, Fournier A, Sirois P. Effects of endothelin-1 on vascular permeability in the rat: Interactions with platelet-activating factor. Br J Pharmacol 1991;104: 797-804.
35. Grega GJ, Adamsk SW, Dobbins DE. Physiological and pharmacological evidence for the regulation of permeability. Fed Proc 1986;45:96-100.
36. Majno GS, Shea SM, Leventhal M. Endothelial contraction induced by histamine-type mediators: An electron microscopic study. J Cell Biol 1969;42:647-672.
37. Masaki T, Kimura S, Yanagisawa M, et al. Molecular and cellular effects of endothelin regulation. Implications for vascular function. Circulation 1991;84:1457-1468.

Discussion

Dr. J. Werner (Heidelberg, Germany). I think it is important to stress the significance of microcirculatory disorders in pancreatitis—that is, once the microcirculatory disorder is treated, the pancreas is actually treated as well, thereby reducing mortality. I was very surprised that you were so successful in reducing mortality with endothelin antagonist, because your earlier studies did not show such a great reduction. I have two questions. First, in our previous studies we demonstrated not only a reduction in systemic complications with the use of monoclonal antibodies against ICAM-1, but also a reduction in local pancreatic injury and by this, in the long run, a reduction in pancreatic infection. So treatment with monoclonal antibodies against ICAM-1 showed both local effects and systemic reduction of injury and mortality, which has not been shown for endothelin antagonists. Thus inhibition of ICAM-1 seems to be more effective. Do you see a role for the combination of those two agents in this respect? My second question concerns capillary leakage, which you measured in the colon, and what it really means in terms of severity in acute pancreatitis?

Dr. T. Foitzik. In this experimental setting, we did not see any significant effect on pancreatic necrosis, and this is probably because we started the therapy very late. In your experiments and in our previous experiments, therapy was started 6 hours after the onset of disease, or the antagonists were even given prophylactically. In such a setting necrosis can be reduced. Regarding your question of whether we should combine ET-RA and ICAM antibodies, these experiments are currently underway in our laboratory. The problem with the ICAM antibody is that it is very expensive. To treat one rat costs us about $300 and I have no idea how expensive it would be to treat humans. To answer your second question, about capillary permeability, we believe that capillary leakage is very important. For example, if a patient is admitted for severe pancreatitis and has a hematocrit value of 55, he or she is likely to develop renal insufficiency, and therefore large amounts (sometimes 10 liters) of Ringer's lactate must be infused. We stabilize renal function by restoring the intravascular volume, but by the next day pulmonary edema can develop if we have not counteracted the capillary leakage. We have been confronted with this capillary leakage problem even during the second week after disease onset, so we believe treating it is very important.

Dr. M. Sarr (Rochester, Minn.). Have you evaluated an endothelin-2 receptor antagonist? Have you studied other models of pancreatitis that do not involve cerulein infusion?

Dr. Foitzik. We have tried other endothelin receptor antagonists; we have blocked endothelin-B receptors and observed different effects. It is important to selectively block endothelin-A receptors. Blocking endothelin-B receptors produces adverse effects, and blocking endothelin-A A and B receptors combined diminishes the beneficial effects of endothelin-A receptor blockade on capillary permeability. We are currently performing studies in a closed duodenal loop model of acute pancreatitis.

Dr. R. Prinz (Chicago, Ill.). Do you have any information on how effective your ICAM antibody or your ET-RA is in accomplishing your goal of blocking each of these specific pathways?

Dr. Foitzik. We have been performing experiments with endothelin receptor antagonists for the past 3 years, so we have gotten some information on how they act and what dosages we must use to avoid pressor effects and influence capillary permeability. As far as the ICAM antibodies are concerned, we do not have that information. These antibodies are pretested by the pharmaceutical company from which they are obtained, and we used exactly the same dose that was used by other groups who saw positive effects with this specific compound. How ICAM antibodies affect vascular permeability is not known.

Dr. M. Zenilman (Bronx, N.Y.). The other mediator of microvascular collapse or disorder during acute pancreatitis is platelet-activating factor. How does ICAM-1 receptor relate to platelet-activating factor receptor? Are they two separate mechanisms? Are you treating one entity?

Dr. Foitzik. There are not just two or three factors that increase capillary permeability; there are many, and nobody knows their mechanisms, how these mediators work, or how they interact. We are currently conducting several experiments in an attempt to investigate the effects of all factors we have knowledge of. For example, my colleague Dr. Eibl presented a paper yesterday in which he compared the effects of a platelet-activating factor receptor antagonist, our endothelin receptor antagonist, and ICAM antibodies and showed that the platelet-activating factor antagonist was not as effective in this model. There are other substances, however, such as bradykinin and substance P, which should also be investigated.

Temporal Correlation of Tumor Necrosis Factor-Alpha Release, Upregulation of Pulmonary ICAM-1 and VCAM-1, Neutrophil Sequestration, and Lung Injury in Diet-Induced Pancreatitis

Andrew H. Lundberg, M.D., D. Neil Granger, Ph.D., Janice Russell, B.S., Scott Callicutt, M.D., Lillian W. Gaber, M.D., Malak Kotb, Ph.D., Omaima Sabek, Ph.D., A. Osama Gaber, M.D.

Lung injury is a major cause of patient morbidity in acute pancreatitis. The purpose of this study was to examine the mechanism of pulmonary infiltration and lung injury in acute pancreatitis. Mice were fed a choline-deficient/ethionine-supplemented (CDE) diet for 144 hours to induce severe acute pancreatitis. Serum samples were collected for measurement of biochemical markers of disease and for the detection of tumor necrosis factor-alpha (TNF-α). Cell surface adhesion molecule expression was quantified by the sensitive radiolabeled dual monoclonal antibody technique. Neutrophil sequestration in lung tissue was measured by the myeloperoxidase assay. Lung injury was determined histologically and lung edema was assessed by wet/dry ratios. Pancreatic injury was demonstrated to occur in all CDE-fed mice, which developed significant hyperamylasemia and hypoglycemia by 48 hours ($P < 0.0001$). Serum TNF-α levels increased significantly by 48 hours over baseline values ($P < 0.02$). Expression of intracellular adhesion molecule (ICAM-1) in pulmonary endothelia was significantly increased above baseline by 30% at 48 hours ($P < 0.02$) and peaked at 120 hours by 100% ($P < 0.0001$). Vascular cellular adhesion molecule (VCAM-1) was constitutively expressed at baseline and was upregulated threefold by 48 hours ($P < 0.0001$). Neutrophil infiltration increased gradually 24 hours after ICAM-1 and VCAM-1 were upregulated with significant elevation of myeloperoxidase activity over baseline at 72 hours (7.2 ± 1.2 vs. 18.1 ± 2.2 activity units/gram tissue; $P < 0.05$). Neutrophil infiltration peaked at 144 hours (26.24 ± 10.49 activity units/gram tissue $P < 0.0001$), and its kinetics correlated with the onset and progression of morphologic injury as well as increased lung edema. These results show that acute pancreatitis is associated with a systemic release of inflammatory cytokines, followed by increased expression of pulmonary ICAM-1 and VCAM-1, neutrophil infiltration, and histologic lung injury. The adhesion molecule axis may be a potential target for practical intervention to ameliorate lung injury and morbidity in acute pancreatitis. (J GASTROINTEST SURG 2000;4:248-257.)

KEY WORDS: Pancreatitis, lung injury, ICAM-1, VCAM-1

Acute lung injury remains a major complication of severe acute pancreatitis (AP). Death in patients with severe forms of AP is commonly associated with respiratory failure, which mimics adult respiratory distress syndrome.[1,2] Lacking specific therapies that target the pathogenesis of acute lung injury, supportive management remains the only treatment option for morbid complications associated with the disease. Nonspecific and general supportive measures, however, still cannot prevent the morbid pulmonary complications, and mortality rates remain at 30% to 50% in severe cases.[3]

From the Departments of Surgery (A.H.L., S.C., M.K., O.S., and A.O.G.) and Pathology (L.W.G.), University of Tennessee-Memphis, Memphis, Tenn.; and the Department of Physiology (D.N.G. and J.R.), Louisiana State University Medical Center, Shreveport, La.
Supported by the Transplant Surgical Immunology Laboratory at the University of Tennessee at Memphis and by grant PO1 DK-43785 from the National Institutes of Health (Dr. Granger).
Presented at the SSAT/Ross Resident Research Symposium at Howey-in-the-Hills, Fla., (oral presentation), and the Fortieth Annual Meeting of The Society for Surgery of the Alimentary Tract, Orlando, Fla., May 16-19, 1999.
Reprint requests: A. Osama Gaber, M.D., Department of Surgery, Division of Transplantation, University of Tennessee, Memphis, 956 Court Ave., Suite A202, Memphis, TN 38163.

The central role of inflammatory cytokines in mediating the progression of distant organ injury during AP is well documented.[4-7] It is believed that these mediators, which are released in response to pancreatic injury, are responsible for the systemic manifestations including acute lung injury.[8] The mechanism of distant organ injury is thought to be related to upregulation of adhesion molecule expression and leukocyte infiltration in cytokine-stimulated tissues.[9,10] The first step for inflammatory cell migration requires the P- and E-selectin expression on endothelial cells, which mediates leukocyte cellular rolling. Once this step occurs, endothelial cell intracellular adhesion molecule (ICAM-1) and vascular cellular adhesion molecule (VCAM-1) expression mediates leukocyte attachment and migration into the tissue.[11] It is widely believed that stimulated leukocytes release harmful products such as reactive oxygen species, which subsequently cause organ injury through this cascade of events.[12,13] This hypothesis is supported by the fact that inhibitors of cytokines,[4,14,15] leukocyte function, and/or migration[16,17] tend to ameliorate organ injury associated with AP and other inflammatory disorders.

This study was designed to evaluate the kinetics of pulmonary ICAM-1 and VCAM-1 molecule expression in animals with severe AP and to determine the temporal relationship of upregulation of these molecules to neutrophil infiltration and progressive lung injury. Understanding the role of ICAM-1 and VCAM-1 molecules with regard to organ injury can afford novel and effective treatments of AP.

MATERIAL AND METHODS

Animals

Young female Swiss-Webster mice were purchased from Harlan-Teklad (Madison, Wis.) and used for all subsequent experiments. All animals were housed in wire-bottom cages and fed standard mouse chow and water ad libitum until the beginning of the experiment. The room was maintained at 21° C with 12-hour light/dark cycles, and strict biohazard precautions were observed. Animals were cared for in accordance with the "Guidelines for the Care and Use of Laboratory Animals" (NIH Publication 85-23, revised 1985).

Induction of Acute Pancreatitis

All mice (n = 140) were fasted for 24 hours before feeding with the choline-deficient/ethionine-supplemented (CDE) diet was begun. The CDE diet was obtained from Harlan-Teklad and DL-ethionine (USB Specialty Biochemicals, Cleveland, Ohio) was supplemented at a concentration of 0.5%, as described by Lombardi et al.[18] This was kept at 4° C in powder form. Control animals (n = 20) were fed mouse chow ad libitum.

Experimental Protocol

Experimental animals (n = 120) were fed the CDE diet and killed at 24-hour intervals until 144 hours was reached (n = 20 per each time period). Groups 1 and 2 were used to measure the expression of ICAM-1 and VCAM-1 molecules in lung tissue. Group 3 was used to evaluate biochemical parameters as well as serum cytokines, lung myeloperoxidase activity, histologic findings, and wet/dry ratios of lung tissue.

Antibodies and Radiolabeling Procedure

Monoclonal antibodies used for ICAM-1 and VCAM-1 measurements were YN-1 and MK1.9.1, respectively (Pharmingen, San Diego, Calif.). Using the iodogen method,[19] the monoclonal antibodies used in this study, both binding and nonbinding, were radiolabeled with ^{125}I and ^{131}I, respectively (DuPont NEN, Boston, Mass.). Iodogen was dissolved in chloroform at a concentration of 0.5 mg/ml. A total of 125 μg of iodogen was added to 250 μg of the monoclonal antibody and then incubated with 250 μCi for 20 minutes at 4° C to get a 1 μCi/μg protein mixture. The radiolabeled monoclonal antibodies were then filtered through a Sephadex PD-10 column (Pharmacia, Uppsala, Sweden) to separate the monoclonal antibody from the free ^{125}I and ^{131}I. After equilibration of the column, 1% bovine serum albumin was used to elute the column. The second of four fractions (2.5 ml) was collected and contained the radiolabeled antibody. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis showed normal heavy- and light-chain moieties of expected molecular weight. Previous studies have shown that the iodogen method for labeling mAbs does not interfere with their functional activity.[20]

Surgical Procedure

For quantitative evaluation of ICAM-1 and VCAM-1 adhesion molecules, animals were anesthetized and their jugular vein and carotid artery were cannulated using polyethylene (PE) PE10 and polyethylene PE50 tubing, respectively. For measurement of ICAM-1 expression, 10 μg of radiolabeled ICAM-1 and 40 μg of cold ICAM-1 were used with 0.5 to 5 μg of the nonbinding monoclonal antibody P-23 labeled with ^{131}I. VCAM-1 measurement required 10 μg of radiolabeled monoclonal antibody and 20 μg of nonradiolabeled antibody. This mixture was injected into

the animal through the venous catheter. A blood sample was obtained from the carotid artery 5 minutes after the initial injection of the monoclonal antibody mixture. The animals were then heparinized with 40 units of heparin and exsanguinated through the carotid artery catheter while a bicarbonate-buffer saline solution was infused through the venous catheter. At this point the organs of interest were harvested and weighed (wet and dry).

Adhesion Molecule Expression

The method used to determine the expression of ICAM-1 and VCAM-1 has been described in previous studies.[21] Briefly, the ^{125}I binding and ^{131}I nonbinding monoclonal antibody activities are measured in different tissues, and 50 μl samples of serum are counted in a 14800 Wizard 3 gamma counter (Wallace, Turku, Finland), with correction for background activity and spillover. The activity of the injected dose is measured prior to injection into the animal. The activity remaining in the tube used for mixing the monoclonal antibodies and the activity in the syringe used to inject the mixture into the animal are counted and subtracted from the total injected activity. On average, this is less than 1% of the total injected activity. The accumulated activity of each monoclonal antibody in an organ is expressed as the percentage of the injected dose (%ID) per gram of dry weight of tissue. Expression in terms of micrograms of monoclonal antibody per dry tissue (μg mAb/g) can then be calculated. The equation used to calculate ICAM-1 and VCAM-1 expression is as follows:

$$\text{Expression } (\mu g \text{ mAb/g}) = \frac{(^{125}I \text{ \%ID/g})}{(^{125}I \text{ \%ID injected})} \times \frac{(^{131}I \text{ \%ID/g})}{(\text{\%ID } ^{131}I \text{ injected})} \times \frac{\text{Total injected}}{\text{binding mAb } (\mu g)} \times 100$$

Tumor Necrosis Factor Assay

TNF-α in serum samples was measured by the enzyme-linked immunosorbent assay. A 96-well plate was prepared using the coating antibody (clone XT3; Endogen, Woburn, Mass.) and allowed to incubate overnight at room temperature. After this step was completed, the plate was blocked with assay buffer for 1 hour. Serum samples and standards were placed in the wells in duplicate with the biotin-labeled antibody (clone XT22; Endogen) and allowed to incubate for 2 hours at room temperature. Following this step, streptavidin enzyme was added to each well and the plate was allowed to incubate for 30 minutes. The substrate tetramethylbenzidine (TMB; Sigma, St. Louis, Mo.) was added to each well for 30 minutes, and the reaction was stopped with 0.18 mol/L H_2SO_4. Absorbency of the plate was read at 450 nm. Levels are reported as picograms per milliliter ± standard error.

Histologic Findings

At the time of sacrifice, the pancreatic tissue was removed and placed in formalin fixative until paraffin embedding was performed. Following this step, 4 μm paraffin sections were cut and stained with hematoxylin and eosin (H and E) at which time morphologic changes were assessed by a pathologist who was blinded to the animals' groupings.

For histologic evaluation of lung tissue, the trachea of each animal was cannulated with a 20-gauge angiocatheter, and 10% formalin solution was injected into the lung tissue under slow constant pressure to ensure homogeneous lung distention. Once fully inflated, the lung tissue was excised and placed into a formalin solution until paraffin embedding and H and E staining were performed. At this time, 4 μm sections were cut and stained with H and E and subsequently examined by a pathologist who was blinded to the animals' groupings. As previously described,[22] lung sections were examined for extent of alveolar collapse, hypercellularity of the alveolar membrane, and alveolar septal thickening. A total of five different sections from each specimen were examined to obtain an overall injury score. A numerical score ranging from 1 to 4 was assigned based on the severity of morphologic changes, which were viewed by light microscopy under an oil immersion lens at 100× magnification. Lung morphology was scored as follows: 1 = normal; 2 = mild; 3 = moderate; and 4 = severe injury.

Lung Edema

The wet/dry ratio of tissue (lung edema) was calculated by weighing portions of wet lung and then taking successive measurements of oven-dried lung tissue. When the weight of the lung tissue remained unchanged after further drying, values were used as organ dry weight.

Neutrophil Quantitation

Myeloperoxidase extraction was performed according to previously described methodology.[23] Frozen tissue was thawed and placed in 20 mmol/L (pH 7.4) potassium phosphate buffer. Homogenization with subsequent centrifugation at 20,000 relative centrifugal force was performed at 4° C for 15 minutes. The supernate was discarded and the pellet was resuspended in 50 mmol/L phosphate buffer (pH 6.0) containing 0.5% hexadecylmethylammonium bro-

mide (HETAB; Sigma). Sonication was performed on the samples for 40 seconds while they were on ice. The samples were recentrifuged and the supernate was assayed for myeloperoxidase activity using a mixture of water, HETAB 0.5%, TMB, sodium acetate buffer, and H_2O_2. The reaction was stopped with catalase and the samples were placed on ice. Myeloperoxidase activity was measured at 655 nm after 3 minutes of incubation. Activity units per gram of tissue (AU/g) were calculated and to ensure the quality of the assay reagents, a standard curve was constructed using human myeloperoxidase (Sigma).

Biochemical Measurements

Amylase was measured by a quantitative enzymatic assay (Sigma, No. 575-UV). Ten microliters are required per determination, and results are expressed as International units per liter (U/L). Serum glucose levels were measured by the Accu-check 111 kit (Boehringer-Mannheim Biochemica, Indianapolis, Ind.) and reported in milligrams per deciliter.

Statistical Methods

All values are reported as the mean ± standard error (SE). To determine statistical significance, analysis of variance (ANOVA) was performed on all parametric data and significance was set at $P < 0.05$ to determine the significance of data within each group; Student's t test was performed to determine significance between different time points within each group. The Mann-Whitney U test was used to determine the significance of nonparametric data (histologic grading).

RESULTS

Acute pancreatitis occurred in all CDE-fed animals. Biochemical measurements consistent with this model of AP included elevated serum amylase and decreased serum glucose levels (Table I). Peak amylase was observed at 72 hours during the feeding period. Glucose levels decreased throughout the study reaching 80 ± 25 mg/dl vs. control values (277 ± 88 mg/dl; $P < 0.0001$). Mild to moderate histologic changes occurred in the pancreas as early as 24 to 48 hours after the CDE diet was begun (data not shown). When compared to pancreatic tissue from normal animals (Fig. 1, *A*), CDE-fed mice had evidence of severe injury in the pancreas at 72 hours, with characteristic histologic changes including acinar cell vacuolization, pyknosis, cellular necrosis, and intralobular hemorrhage (Fig. 1, *B*).

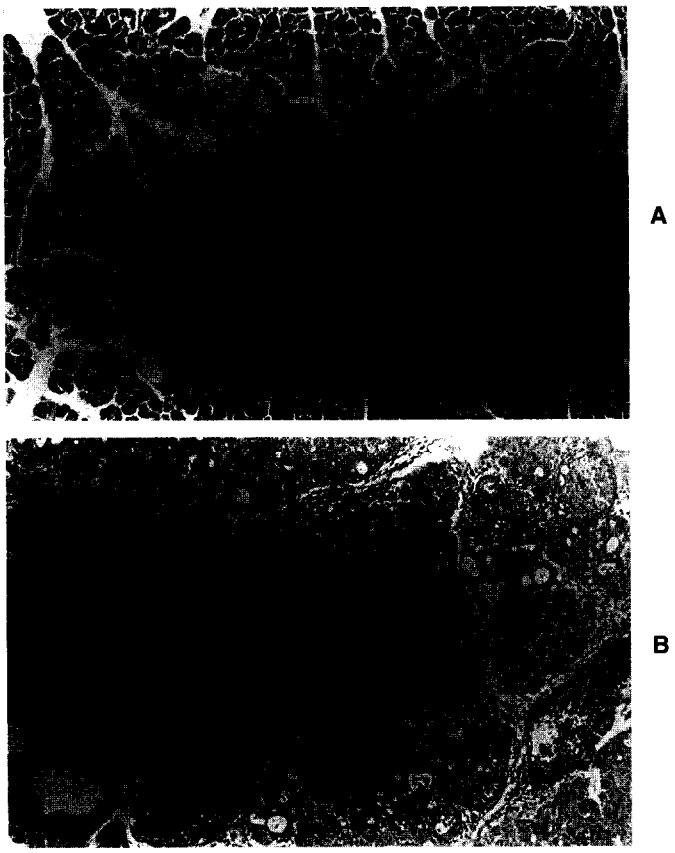

Fig. 1. Histologic sections of pancreatic tissue from normal **(A)** and CDE-fed **(B)** mice. The normal mouse was fed standard mouse chow. Pancreatic tissue from the CDE-fed mouse demonstrates widespread vacuolization and necrosis of the acinar cells. Edema of the interlobular septae accompanied by inflammatory infiltrates and extravasation of red blood cells is readily apparent. (Hematoxylin and eosin stain; original magnification ×20.)

Table I. Biochemical markers of acute pancreatitis

	Time (hours)						
	0	24	48	72	96	120	144
Amylase (U/L)	1659 ± 268	1033 ± 568	9230 ± 6013*	20791 ± 7505*	5182 ± 5019	848 ± 338	1339 ± 927
Glucose (mg/dl)	277 ± 88	275 ± 91	101 ± 34†	88 ± 23†	108 ± 33†	80 ± 25†	97 ± 34†

*$P < 0.0001$ by analysis of variance.
†$P < 0.0001$ by analysis of variance.
All values are expressed as means ± standard error.
Analysis of variance for the entire group compared to baselines (0 hours).

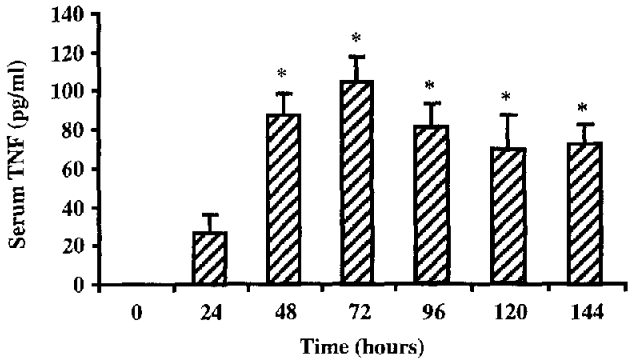

Fig. 2. Serum cytokine (TNF-α) levels in animals with acute pancreatitis (values are expressed as pg/ml ± SE). Statistical significance of the group was determined by ANOVA. * = values that are significant compared to baseline (0 hours) (P <0.0001).

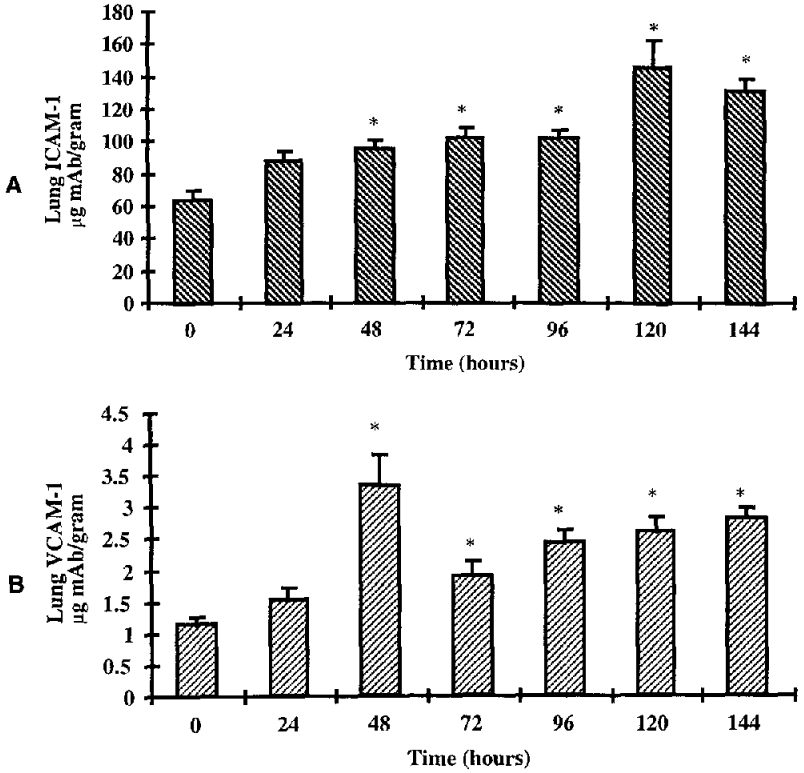

Fig. 3. ICAM-1 and VCAM-1 expression in lung tissue of mice with acute pancreatitis. **A,** Pulmonary ICAM-1 expression in mice with acute pancreatitis caused by the CDE diet (values are expressed as μg mAb/gram dry tissue weight ± SE). Statistical significance of the group was determined by ANOVA. * = P <0.0001 for the entire group; individual Student's t tests were performed between each group and compared to baseline values at 0 hours. Significance was less than 0.05 for experimental groups at 48 hours and beyond. **B,** Pulmonary VCAM-1 expression in mice with acute pancreatitis. * = P <0.0001 for this group, and starting at 48 hours all groups were demonstrated to have P values <0.05 as calculated by Student's t test.

Serum TNF-α levels increased by 24 hours and significance was reached by 48 hours (P <0.0001) (Fig. 2). Peak levels were elevated 100-fold over baseline values (0 vs. 105 ± 11 pg/ml; P <0.01) at 72 hours. Pulmonary ICAM-1 and VCAM-1 expression paralleled serum TNF-α levels (Fig. 3). ICAM-1 expression in lung tissue increased 1.5-fold over baseline by 48 hours (95.5 ± 5.2 vs. 64.1 ± 6.2 μg mAb/g tissue; P <0.02). Peak expression was observed at 120 hours after the CDE diet was begun (146.3 ± 16.3 μg mAb/g tissue; P <0.0001 compared to baseline). Concomitantly, VCAM-1 expression in lung tissue increased threefold over baseline values by 48 hours (P <0.0001) and continued to remain elevated throughout the remainder of the study. Lung neutrophil sequestration as measured by myeloperox-

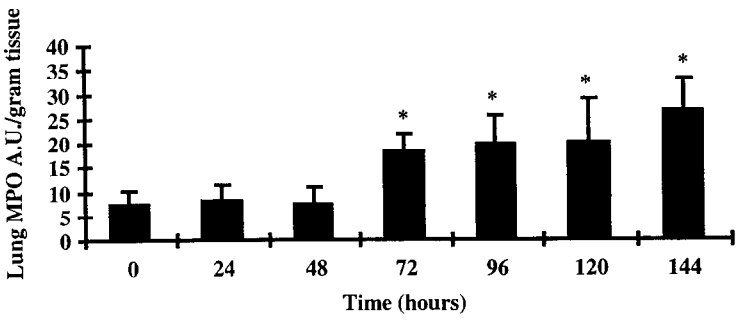

Fig. 4. Neutrophil sequestration in lung tissue of mice with acute pancreatitis. Lung neutrophil sequestion, measured by myeloperoxidase *(MPO)* activity, is elevated in mice with acute pancreatitis and is expressed as activity units *(A.U.)*/gram of tissue (mean values ± SE are shown). Statistical significance for the group was determined by ANOVA (* = $P < 0.0001$). Student's t test was performed to determine differences between each group and baseline values; significance was reached at 72 hours with a P value < 0.05.

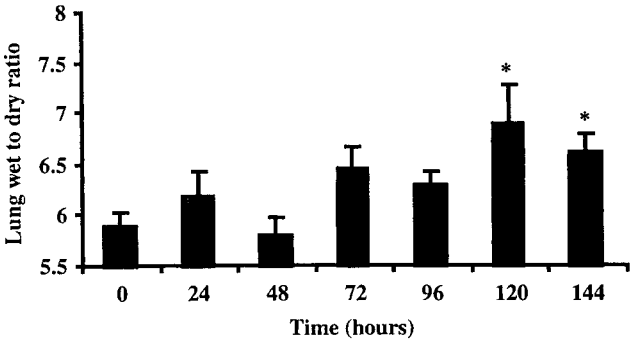

Fig. 5. Lung edema in animals with acute pancreatitis. Wet/dry ratios were recorded and expressed without units. * = Statistical significance for the group at 120 and 144 hours compared to baseline (0 hours) ($P < 0.005$) as determined by the Student's t test.

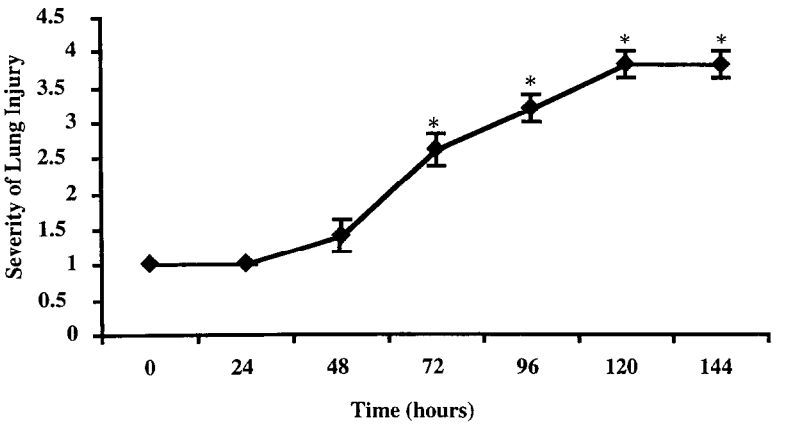

Fig. 6. Graded histologic lung injury in animals with acute pancreatitis. Hematoxylin and eosin–stained sections of lung tissue were graded based on the amount of alveolar collapse, hypercellularity of the alveolar membrane, and septal thickening. Lung sections were examined under an oil immersion lens at 100× magnification. Scores of 1 to 4 were assigned based on the relative extent of injury in comparison to normal lung tissue (score = 1). Mild to moderate changes were observed at 72 hours and progressed to severe changes by 144 hours. Significance between the groups in comparison to baseline normal control values was determined by the Mann-Whitney U test. Significance was set at $P < 0.05$ and all values marked with an asterisk (*) were < 0.005.

idase activity increased over baseline levels at 72 hours (7.2 ± 1.2 vs. 18.1 ± 2.2 AU/g; $P < 0.05$. Peak myeloperoxidase levels were observed at 144 hours (26.2 ± 3.3 AU/g; $P < 0.0001$) (Fig. 4).

Pulmonary edema as measured by the wet/dry ratio of lung tissue increased from 5.9 ± 0.1 to 6.8 ± 0.4 ($P < 0.003$) by 120 hours (Fig. 5) and remained elevated, indicating an increase in lung edema concomitant with progression of AP.

Lung injury was histologically graded from prepared lung sections. Severity of injury progressively worsened throughout the study (Fig. 6). Significant alveolar collapse and hypercellularity of mild to moderate severity was noted by 72 hours ($P < 0.005$) after

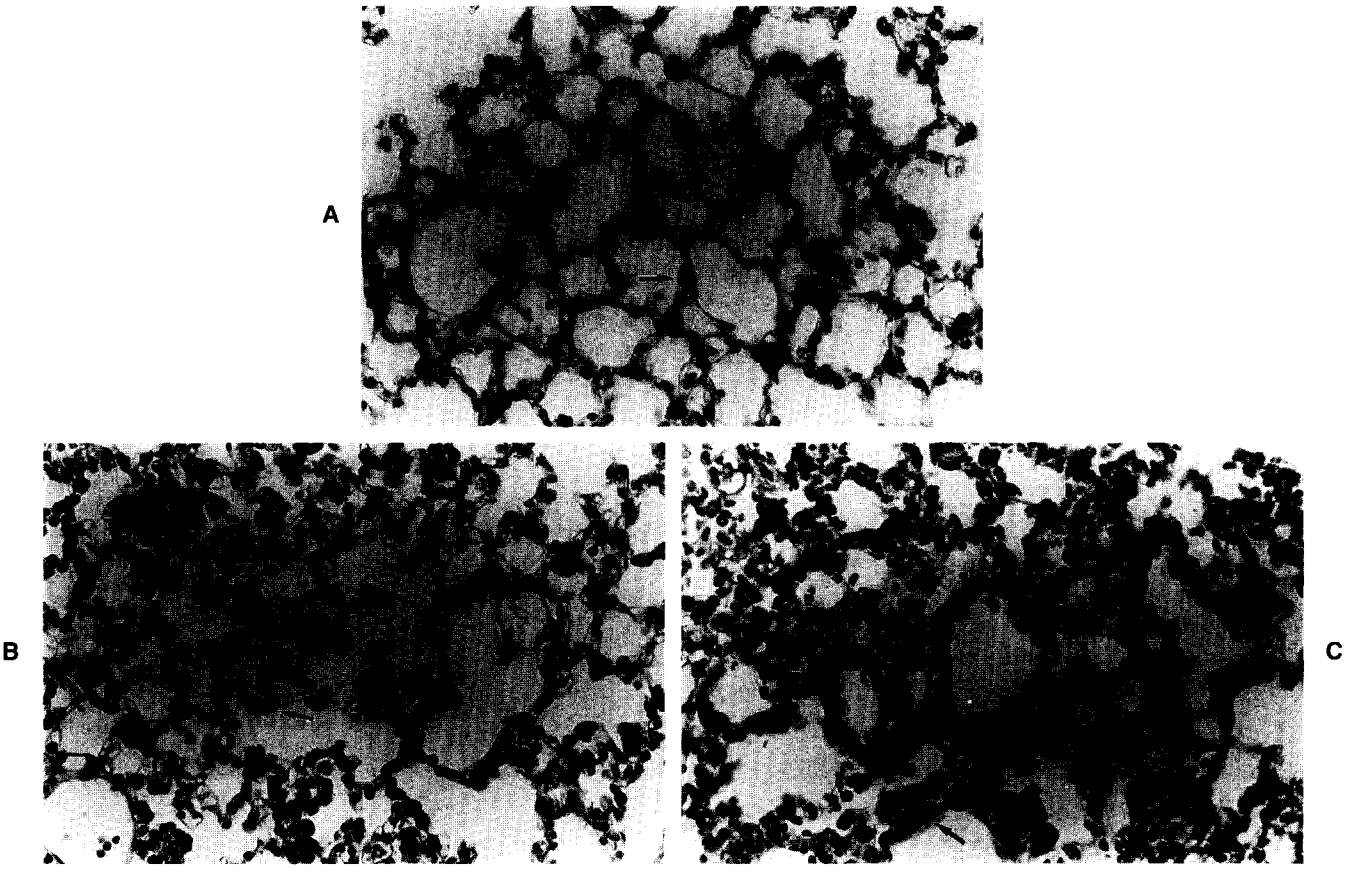

Fig. 7. Representative sections of lung tissue (4 μm) from animals with acute pancreatitis at 0, 72, and 144 hours after the CDE diet was started. Normal lung tissue **(A)** at 0 hours was observed to have thin alveolar membranes and very few cells in the septae *(arrow)*, whereas lung tissue at 72 hours **(B)** was demonstrated to have significant alveolar collapse and the alveolar membranes were hypercellular *(arrow)*. Lung tissue collected at 144 hours **(C)** was demonstrated to have thickened alveolar septae and hypercellular alveolar membranes *(arrow)* with alveolar collapse. (Hematoxylin and eosin stain; original magnification ×40.)

the CDE diet was begun. By 144 hours, the condition of the lungs had progressively worsened to severe injury ($P < 0.004$); changes included severe alveolar collapse, hypercellularity, and alveolar septal thickening. Fig. 7 demonstrates the changes that occur in the lung after the CDE diet is begun.

DISCUSSION

Severe forms of AP can result in life-threatening distant organ dysfunction including acute lung injury.[24] The pathophysiology of the resulting organ dysfunction remains to be elucidated. However, because of the similarities between lung injury associated with AP and lung injury seen in sepsis, it is believed that adhesion molecule upregulation is, in part, a mediator of the systemic manifestations in AP through the recruitment and activation of leukocytes.[25,26] In this study, we demonstrated a correlation between the expression of serum TNF-α, pulmonary endothelial cell adhesion molecules, neutrophil recruitment, and organ injury, which supports this theory. Although ICAM-1 cell *surface* expression has been demonstrated to be upregulated in AP by staining and immunofluorescence, this is the first study to show a quantitative measurement of cell surface expression of ICAM-1 and VCAM-1 molecules in animals with AP by using the sensitive radiolabeled dual monoclonal antibody technique. From these results we can determine the temporal relationship between the onset of inflammatory cell infiltration and organ injury with TNF-α expression and endothelial cell adhesion molecule expression. Inasmuch as our findings show that upregulation of ICAM-1 and VCAM-

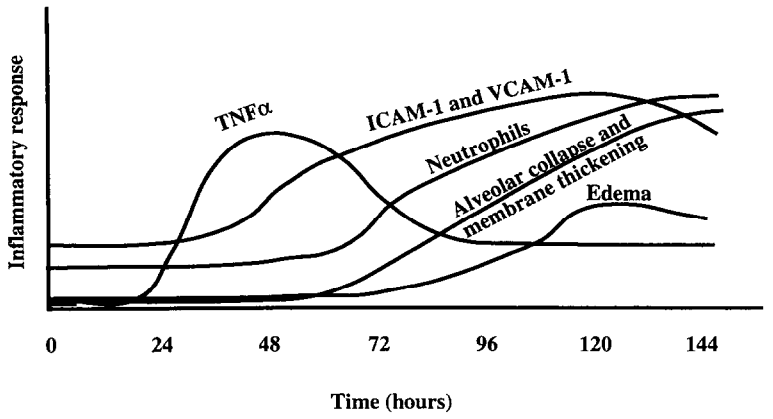

Fig. 8. Schematic diagram demonstrating the temporal kinetics between serum TNF-α, pulmonary ICAM-1 and VCAM-1, lung neutrophil sequestration, morphologic changes, and lung edema.

1 precedes neutrophil sequestration and lung injury, our data suggest that these adhesion molecules may be a good target for clinical intervention.

The CDE diet model of AP has been previously demonstrated to cause a severe hemorrhagic necrotizing pancreatitis and results in 100% mortality in young female mice by day 5.[18,27] It has been reported that altering the model with respect to age and weight of the animals and duration of feeding the animals decreases mortality.[28,29] In this study we modified the model described by Lombardi and Rao[28] by using older and larger mice, and this resulted in a mortality rate of less than 50%. In addition, we provided biochemical and histologic characterization of the changes associated with AP in this modified model. We observed that feeding the CDE diet to 18 g female mice causes manifestations of severe AP that are readily apparent, but the mortality rate is reduced to 15% by day 6. Therefore the modified CDE diet model has many advantages including its noninvasiveness, reproducibility, and slow progression, all of which allow comprehensive evaluation of the morphologic and inflammatory changes that occur in pancreatitis.[30-32]

The goal of this study was to characterize specific immunologic and inflammatory events preceding lung injury in AP. We reasoned that elucidating the temporal events leading to systemic manifestations could provide insight into appropriate and practical targets for clinical intervention. Inflammatory cytokines are produced in response to pancreatic injury[33] and appear to be important mediators of distant organ injury in AP.[8,34] In this model of AP, serum TNF-α levels began to rise by 24 hours and were significantly elevated by 48 hours. The rise in serum TNF-α levels appeared to precede other inflammatory manifestations of disease including the upregulation of ICAM-1 and VCAM-1 in lung tissue.[11]

The cell surface expression of ICAM-1 and VCAM-1 on pulmonary endothelia preceded the cascade of events that ultimately resulted in lung injury during AP. A significant upregulation of pulmonary ICAM-1 and VCAM-1 occurred at 48 hours. This was followed by neutrophil migration into the lung tissue at 72 hours. Only at the time of neutrophil infiltration (72 hours), did we begin to see significant morphologic changes in the lung tissue. These findings are different from our previously reported observations[22] in that neutrophil infiltration and organ injury occurred at an earlier time period. However, we can account for this difference because the animals that were used were a different species and the CDE diet model is modified in this study. The development of edema in the lung was demonstrated to be the final event, which becomes significant by 120 hours after the CDE diet is begun. Edema appears to be a direct result of fluid shifts out of the intravascular space and into the interstitial space, which implies pulmonary endothelial cell injury and capillary leakage. This finding suggests that neutrophil-mediated injury is required prior to endothelial cell damage and subsequent capillary leakage and edema.

Several recent studies have implicated the neutrophil as an important effector cell in lung injury associated with AP,[35] because elimination of the neutrophil prior to the onset of AP results in reduced lung injury.[17,36] We propose that once the inflammatory response is triggered and inflammatory mediators are upregulated, tissue infiltration of neutrophils and other leukocytes occurs though adhesion molecule–directed mechanisms. Thus our results, which demonstrate a temporal correlation of adhesion molecule expression, neutrophil migration, and organ injury, support this hypothesis. A schematic diagram of these events can be reviewed in Fig. 8.

Other investigators have provided evidence that supports our results, which indicate that blocking ICAM-1 expression reduces the local and systemic injury associated with AP.[17,37,38] However, none of the studies show complete amelioration of AP-associated injury, thereby suggesting that multiple pathways for leukocyte recruitment and organ injury exist in this disease.[16,39,40] Other possible mediators in leukocyte recruitment include the P- and E-selectin molecules, which we have demonstrated to be upregulated in multiple organs during AP.[22] However, it appears that the selectin molecules are not fully accountable for the organ injury observed in AP. This suggests the role of other adhesion molecules, which also regulate inflammatory cell migration. The participation of numerous components of the inflammatory cascade in causing systemic injury in AP may explain why many investigators cannot fully decrease AP-associated injury by eliminating one arm of the cascade.[4,16,41] It also appears that chemokines have a direct chemoattractant effect on leukocytes without utilizing adhesion molecules.[39,42,43] We believe that effective therapy may be achieved by targeting a combination of molecules that may synergize in producing the systemic injury in AP.

Unlike selectin and ICAM-1 upregulation, expression of VCAM-1 had not yet been studied in pancreatitis in vivo. Although several investigators have demonstrated the important actions of VCAM-1 expression through blocking studies in acute and chronic inflammatory syndromes by demonstrating decreased organ injury through inhibition of leukocyte migration,[44,45] this is the first study to demonstrate that VCAM-1 upregulation occurs in the lung during AP. Furthermore, we demonstrated that VCAM-1 expression is temporally correlated with distant organ injury in AP. Of particular interest to this study is a recent report by Masamune et al.[46] who showed the potentially important role of this adhesion molecule in AP during in vitro studies. They demonstrated that VCAM-1 and ICAM-1 are upregulated on cultured endothelial cells, which were stimulated with ascites from animals with AP. In our study, VCAM-1 expression followed the same pattern of upregulation and temporal sequence of expression as ICAM-1. This suggests a common role for both of these molecules during neutrophil infiltration and progressive organ injury in AP similar to other inflammatory diseases.[47,48]

In summary, our results strongly suggest, through temporal analysis, that these adhesion molecules, which are overexpressed during the acute inflammatory response in AP, are one of the links between the localized pancreatic injury and acute lung injury. Inasmuch as interfering with cytokine production in AP may not always be clinically feasible, we believe our data suggest that adhesion molecules may be a practical target of intervention with respect to distant disease progression during AP.

REFERENCES

1. Renner IG, Savage WT III, Pantoja JL, Renner VJ. Death due to acute pancreatitis. A retrospective analysis of 405 autopsy cases. Dig Dis Sci 1985;30:1005-1018.
2. Steinberg W, Tenner S. Acute pancreatitis [see comments]. N Engl J Med 1994;330:1198-1210.
3. Lankisch PG, Rahlf G, Koop H. Pulmonary complications in fatal acute hemorrhagic pancreatitis. Dig Dis Sci 1983;28:110-116.
4. Hughes CB, Grewal HP, Gaber LW, et al. Anti-TNFalpha therapy improves survival and ameliorates the pathophysiologic sequelae in acute pancreatitis in the rat. Am J Surg 1996;171:274-280.
5. Norman JG, Fink GW, Franz MG. Acute pancreatitis induces intrapancreatic tumor necrosis factor gene expression. Arch Surg 1995;130:966-970.
6. Norman JG, Fink GW, Denham W, et al. Tissue-specific cytokine production during experimental acute pancreatitis. A probable mechanism for distant organ dysfunction. Dig Dis Sci 1997;42:1783-1788.
7. Kingsnorth A. Role of cytokines and their inhibitors in acute pancreatitis. Gut 1997;40:1-4.
8. Norman J. The role of cytokines in the pathogenesis of acute pancreatitis. Am J Surg 1998;175:76-83.
9. Etzioni A. Adhesion molecules—their role in health and disease. Pediatr Res 1996;39:191-198.
10. Korthuis RJ, Anderson DC, Granger DN. Role of neutrophil-endothelial cell adhesion in inflammatory disorders. J Crit Care 1994;9:47-71.
11. Granger DN. Cell adhesion and migration. II. Leukocyte-endothelial cell adhesion in the digestive system. Am J Physiol 1997;273:G982-G986.
12. Doerschuk CM, Quinlan WM, Doyle NA, et al. The role of P-selectin and ICAM-1 in acute lung injury as determined using blocking antibodies and mutant mice. J Immunol 1996; 157:4609-4614.
13. Granger DN, Kubes P. The microcirculation and inflammation: Modulation of leukocyte-endothelial cell adhesion. J Leukoc Biol 1994;55:662-675.
14. Guice KS, Oldham KT, Remick DG, et al. Anti-tumor necrosis factor antibody augments edema formation in caerulein-induced acute pancreatitis. J Surg Res 1991;51:495-499.
15. Norman JG, Franz MG, Fink GS, et al. Decreased mortality of severe acute pancreatitis after proximal cytokine blockade. Ann Surg 1995;221:625-631; discussion 631-634.
16. Frossard JL, Saluja A, Bhagat L, et al. The role of intercellular adhesion molecule 1 and neutrophils in acute pancreatitis and pancreatitis-associated lung injury. Gastroenterology 1999;116:694-701.
17. Bhatia M, Saluja AK, Hofbauer B, et al. The effects of neutrophil depletion on a completely noninvasive model of acute pancreatitis-associated lung injury. Int J Pancreatol 1998; 24:77-83.
18. Lombardi B, Estes LW, Longnecker DS. Acute hemorrhagic pancreatitis (massive necrosis) with fat necrosis induced in mice by DL-ethionine fed with a choline-deficient diet. Am J Pathol 1975;79:465-480.

19. Fraker PJ, Speck JC Jr. Protein and cell membrane iodinations with a sparingly soluble chloroamide, 1,3,4,6-tetrachloro-3a,6a-diphrenylglycoluril. Biochem Biophys Res Commun 1978;80:849-857.
20. Eppihimer MJ, Wolitzky B, Anderson DC, et al. Heterogeneity of expression of E- and P-selectins in vivo. Circ Res 1996;79:560-569.
21. Eppihimer MJ, Granger DN. Ischemia/reperfusion-induced leukocyte-endothelial interactions in postcapillary venules. Shock 1997;8:16-25.
22. Lundberg AH, Granger DN, Russell J, et al. Quantitative measurement of P- and E-selectin adhesion molecules in acute pancreatitis: Correlation with distant organ injury. Ann Surg 2000;231:213-222.
23. Bradley PP, Priebat DA, Christensen RD, Rothstein G. Measurement of cutaneous inflammation: Estimation of neutrophil content with an enzyme marker. J Invest Dermatol 1982;78:206-209.
24. Beger HG, Rau B, Mayer J, Pralle U. Natural course of acute pancreatitis. World J Surg 1997;21:130-135.
25. Donnelly SC, Haslett C. Dransfield I, et al. Role of selectins in development of adult respiratory distress syndrome [see comments]. Lancet 1994;344:215-219.
26. Fassbender K, Kaptur S, Becker P, et al. Adhesion molecules in tissue injury: Kinetics of expression and shedding and association with cytokine release in humans. Clin Immunol Immunopathol 1998;89:54-60.
27. Lerch MM, Adler G. Experimental animal models of acute pancreatitis. Int J Pancreatol 1994;15:159-170.
28. Lombardi B, Rao NK. Acute hemorrhagic pancreatic necrosis in mice. Influence of the age and sex of the animals and of dietary ethionine, choline, methionine, and adenine sulfate. Am J Pathol 1975;81:87-99.
29. Niederau C, Luthen R, Niederau MC, et al. Acute experimental hemorrhagic-necrotizing pancreatitis induced by feeding a choline-deficient, ethionine-supplemented diet. Methodology and standards. Eur Surg Res 1992;24:40-54.
30. Steer ML. Workshop on experimental pancreatitis. Dig Dis Sci 1985;30:575-581.
31. Bilchik AJ, Leach SD, Zucker KA, Modlin IM. Experimental models of acute pancreatitis. J Surg Res 1990;48:639-647.
32. Banerjee AK, Galloway SW, Kingsnorth AN. Experimental models of acute pancreatitis. Br J Surg 1994;81:1096-1103.
33. Saluja AK, Steer ML. Pathophysiology of pancreatitis: Role of cytokines and other mediators of inflammation. Digestion 1999;60(Suppl)S1:27-33.
34. Grewal HP, Kotb M, el Din AM, et al. Induction of tumor necrosis factor in severe acute pancreatitis and its subsequent reduction after hepatic passage. Surgery 1994;115:213-221.
35. Barie PS, Tahamont MV, Blumenstock FA, Malik AB. The role of neutrophils in the pathogenesis of pulmonary vascular injury after acute pancreatitis. Curr Surg 1982;39:411-413.
36. Guice KS, Oldham KT, Caty MG, et al. Neutrophil-dependent, oxygen-radical mediated lung injury associated with acute pancreatitis. Ann Surg 1989;210:740-747.
37. Inoue S, Nakao A, Kishimoto W, et al. Anti-neutrophil antibody attenuates the severity of acute lung injury in rats with experimental acute pancreatitis. Arch Surg 1995;130:93-98.
38. Inoue S, Nakao A, Kishimoto W, et al. LFA-1 (CD11a/CD18) and ICAM-1 (CD54) antibodies attenuate superoxide anion release from polymorphonuclear leukocytes in rats with experimental acute pancreatitis. Pancreas 1996;12:183-188.
39. Gerard C, Frossard JL, Bhatia M, et al. Targeted disruption of the beta-chemokine receptor CCR1 protects against pancreatitis-associated lung injury. J Clin Invest 1997;100:2022-2027.
40. Wells TN, Lusti-Narasimhan M, Chung CW, et al. The molecular basis of selectivity between CC and CXC chemokines: The possiblity of chemokine antagonists as anti-inflammatory agents. Ann N Y Acad Sci 1996;796:245-256.
41. Manabe T, Steer ML. Protective effects of PGE2 on diet-induced acute pancreatitis in mice. Gastroenterology 1980;78: 777-781.
42. Ward PA. Role of complement, chemokines, and regulatory cytokines in acute lung injury. Ann N Y Acad Sci 1996;796: 104-112.
43. Luster AD. Chemokines—chemotactic cytokines that mediate inflammation. N Engl J Med 1998;338:436-445.
44. Essani NA, Bajt ML, Farhood A, et al. Transcriptional activation of vascular cell adhesion molecule-1 gene in vivo and its role in the pathophysiology of neutrophil-induced liver injury in murine endotoxin shock. J Immunol 1997;158:5941-5948.
45. Allen S, Khan S, Al-Mohanna F, Batten P, Yacoub M. Native low density lipoprotein-induced calcium transients trigger VCAM-1 and E-selectin expression in cultured human vascular endothelial cells. J Clin Invest 1998;101:1064-1075.
46. Masamune A, Shimosegawa T, Kimura K, et al. Specific induction of adhesion molecules in human vascular endothelial cells by rat experimental pancreatitis-associated ascitic fluids. Pancreas 1999;18:141-150.
47. Henninger DD, Panes J, Eppihimer M, et al. Cytokine-induced VCAM-1 and ICAM-1 expression in different organs of the mouse. J Immunol 1997;158:1825-1832.
48. Alon R, Kassner PD, Carr MW, et al. The integrin VLA-4 supports tethering and rolling in flow on VCAM-1. J Cell Biol 1995;128:1243-1253.

Do Preoperative Biliary Stents Increase Postpancreaticoduodenectomy Complications?

Taylor A. Sohn, M.D., Charles J. Yeo, M.D., John L. Cameron, M.D., Henry A. Pitt, M.D., Keith D. Lillemoe, M.D.

It has been suggested that the placement of endoscopic or percutaneous biliary stents prior to pancreaticoduodenectomy increases postoperative morbidity. A retrospective review of a prospectively collected database was performed. Patients undergoing preoperative biliary stenting were compared with patients who did not undergo stenting. In addition, outcomes after endoscopic and percutaneous stenting were compared. Patients who had undergone operative biliary bypass prior to pancreaticoduodenectomy were excluded from the analysis. Between January 1994 and December 1997, 567 patients underwent pancreaticoduodenectomy without prior operative biliary bypass. Preoperative biliary stenting was performed in 408 patients (72%), whereas the remaining 159 patients (28%) did not undergo biliary stenting. In the stented group, 64% had stents placed via a percutaneous approach and 36% had stents placed endoscopically. The stented patients were older (mean 63.1 years vs. 61.4 years; $P = 0.05$) and were more likely to be white (92% vs. 82%; $P = 0.005$). Those who had stents placed were more likely to have jaundice (67% vs. 38%; $P < 0.0001$) and fever (5% vs. 1%; $P = 0.03$) as presenting symptoms. There were no differences in multiple intraoperative parameters when the two groups were compared. Patients who had stents placed had a perioperative mortality rate of 1.7% compared to 2.5% in those who did not ($P = 0.3$). Although the overall complication rates were 35% in those who had stents placed and 30% in those who did not (P = NS), patients with stents experienced a significantly increased incidence of pancreatic fistula (10% vs. 4%; $P = 0.02$) and wound infection (10% vs. 4%; $P = 0.02$). The incidences of other postoperative complications were similar between the stented and unstented groups. Eight patients (3%) in the percutaneously stented group developed significant hemobilia after stent placement, whereas none of the patients undergoing endoscopic stent placement developed hemobilia ($P = 0.03$). There were no statistical differences in other complications between the percutaneously and endoscopically stented groups. Preoperative biliary stenting did not increase the overall complication rate or mortality rate in patients undergoing pancreaticoduodenectomy. Stenting does appear to increase the rate of pancreatic fistula formation, possibly as a result of pancreatic inflammation related to the stenting procedure. Stenting also increases the rate of wound infection, likely secondary to contaminated bile (bactibilia) after instrumentation of the biliary tree. Preoperative biliary stenting is safe but should be used selectively because of the above-mentioned risks. The method of stenting should be based on local expertise. (J GASTROINTEST SURG 2000;4:258-268.)

KEY WORDS: Pancreatic surgery, pancreaticoduodenectomy, biliary stenting

Preoperative biliary stents are often used in patients with benign and malignant biliary obstruction. Such stents cross the ampulla of Vater and can be placed via endoscopic or percutaneous transhepatic routes. The decision to place a percutaneous or endoscopic stent is often based on local expertise. Biliary stenting is accompanied by its own procedure-related morbidity and has been suggested to increase postoperative complications after pancreaticoduodenectomy as well as proximal biliary tract surgery.[1-5] For these reasons the use of preoperative biliary stents remains controversial, with conflicting data in many prospective and retrospective series.

In several retrospective studies, the degree of jaundice has been associated with an increased risk of postoperative complications.[6-10] These studies evalu-

From the Department of Surgery, The Johns Hopkins Medical Institutions, Baltimore, Md.
Presented at the Fortieth Annual Meeting of The Society for Surgery of the Alimentary Tract, May 16-19, 1999, Orlando, Fla.
Reprint requests: Charles J. Yeo, M.D., Professor of Surgery and Oncology, The Johns Hopkins Medical Institutions, Blalock 606, 600 N. Wolfe St., Baltimore, MD 21287-4606. e-mail: cyeo@jhmi.edu

ated multiple operative procedures for benign and malignant biliary tract disease including pancreaticoduodenectomy, biliary bypass, common bile duct exploration, and transduodenal exploration of the bile duct. These data implied that the relief of jaundice by placement of preoperative biliary stents would improve the morbidity rate in patients with jaundice. Two prospective randomized trials demonstrated no decrease in morbidity after preoperative percutaneous biliary stenting,[1,9] whereas one study documented increased morbidity despite decreases in preoperative serum bilirubin levels.[2] Several nonrandomized series report an increase in infectious and other complications following preoperative stenting.[3-5] Further, endoscopic stenting was shown to be of benefit in one prospective randomized study,[11] but was shown to have no benefit in another.[10]

Although several studies have evaluated percutaneous[1,2,5,9] or endoscopic[4,5,10,11] biliary stents prior to pancreaticoduodenectomy, these studies often included other procedures and did not involve any direct comparison of the two methods of stent placement. The current report is a retrospective review evaluating all patients undergoing pancreaticoduodenectomy within a 3-year time period. Outcomes in patients undergoing preoperative stent placement, via either percutaneous or endoscopic techniques, were compared to outcomes in unstented patients. The stented group was then broken down into endoscopic and percutaneous subgroups in an attempt to better define and compare the complications seen in each group.

PATIENTS AND METHODS

Between January 1994 and December 1997 inclusive, pancreaticoduodenectomy was performed in 597 consecutive patients. Data were collected prospectively on all patients undergoing elective pancreaticoduodenectomy for benign or malignant disease. Of the 597 patients, 30 had undergone previous operative biliary bypass (including hepaticojejunostomy, choledochojejunostomy, or cholecystojejunostomy) and were excluded from the current analysis. Of the 567 patients included, 408 patients (72%) underwent preoperative biliary stenting via the percutaneous transhepatic or endoscopic route, whereas 159 patients (28%) did not undergo preoperative biliary stenting.

All patients undergoing transhepatic biliary stenting underwent percutaneous transhepatic cholangiography first, followed by placement of a transhepatic biliary drain under fluoroscopic guidance via the Seldinger technique.[12] The majority of the percutaneous transhepatic stenting procedures were performed at The Johns Hopkins Hospital. Patients undergoing endoscopic stenting typically had this procedure performed in association with endoscopic retrograde cholangiopancreatography (ERCP), with major papilla sphincterotomy. Many of the patients with endoscopic stents were referred to our institution with the biliary stents already in place. A small minority of patients (9%) underwent endoscopic stenting followed by percutaneous stenting, usually secondary to occlusion of the endoscopic stent with recurrent jaundice. These patients were included in the analysis as having undergone percutaneous stenting, since the percutaneous transhepatic stent was providing the definitive biliary drainage.

All patients included in the analysis underwent pancreaticoduodenectomy. Our preference has been to perform pylorus-preserving partial pancreatic resection, with pancreatic-enteric continuity being restored via pancreaticojejunostomy or pancreaticogastrostomy. Superior mesenteric or portal venous resection was not routinely performed.[13-15] All patients except those who were allergic to penicillin received cefotetan preoperatively and for 24 hours postoperatively. Octreotide was not used prophylactically in these patients.

The overall postoperative complication rates were evaluated. Perioperative mortality was defined as death during the index hospitalization or within 30 days of surgery. Specifically, the incidences of delayed gastric emptying, pancreatic fistula, bile leak, wound infection, intra-abdominal abscess, cholangitis, pneumonia, pancreatitis, and hemobilia were evaluated. The need for reoperation in the immediate postoperative period was also determined. Delayed gastric emptying, pancreatic fistula, and bile leak have been defined in previous articles.[13-15] A diagnosis of infectious complications required fever as well as positive cultures from the respective source. Wound infection required purulent drainage from the wound, which necessitated opening of the wound, pneumonia required radiographic evidence of an infiltrate, and an intra-abdominal fluid collection on computed tomography (CT) was needed to diagnose intra-abdominal abscess. Patients were considered to have postoperative pancreatitis if they had elevated serum amylase levels, abdominal pain, and abnormal peripancreatic fluid/edema on CT after postoperative day 5. Significant hemobilia was defined as hemobilia requiring upsizing of biliary stents and/or angiographic embolization.

The demographic characteristics, presenting symptoms, operative results, and short-term outcomes of patients undergoing preoperative biliary stenting were compared to those of patients who did not undergo stenting. In addition, the results of transhepatic

versus endoscopic stenting were compared. Subgroup analyses were performed in patients with a postoperative diagnosis of periampullary adenocarcinoma and in patients who had jaundice on presentation. Differences in categorical outcomes between groups were evaluated by means of chi-square analysis. All data are reported as mean ± standard error of the mean. Differences in means between subgroups were compared using an unpaired *t* test or analysis of variance where appropriate. Significance was accepted at the 5% level. Multivariate analysis using a logistic regression model was used to determine the independent significance of various factors in outcomes including operative mortality, complications, pancreatic fistula formation, wound infection, and other infectious complications.

RESULTS

Between January 1994 and December 1997 inclusive, 597 patients underwent pancreaticoduodenectomy. Five hundred sixty-seven of those did not undergo previous operative biliary bypass. Preoperative biliary stenting was performed in 408 (72%) of the 567 patients, whereas the remaining 159 patients (28%) did not undergo stenting. In the stented group, 262 had percutaneous stents placed (64%) and 146 had endoscopic stents placed (36%). Forty-nine patients (9%) underwent endoscopic stenting followed by percutaneous stenting, usually secondary to occlusion of the endoscopic stent.

The demographics are summarized in Table I. Patients with preoperative stents were significantly older (63.8 years) compared to those without stents (61.4 years; $P = 0.05$). The sex distribution was similar in the two groups, with 54% of patients in the stented group being male compared to 49% in the unstented group. Ninety-two percent of patients in the stented group were white as compared to 83% in the unstented group ($P = 0.005$).

Patients undergoing preoperative stenting were significantly more likely to present with jaundice (67% vs. 38%; $P < 0.0001$), whereas the incidences of weight loss (40% vs. 37%), abdominal pain (38% vs. 37%), nausea/vomiting (18% vs. 22%), and fevers/chills (5% vs. 1%) were similar in the stented and unstented groups, respectively (Table II). On presentation to The Johns Hopkins Hospital, stented patients were more likely to have liver function test abnormalities including higher total bilirubin (5.4 mg/dl vs. 2.4 mg/dl; $P = 0.004$), direct bilirubin (2.7 mg/dl vs. 1.5 mg/dl; $P = 0.005$), aspartate aminotransferase (79 IU/L vs. 61 IU/L; $P = 0.05$), alanine aminotransferase (118 IU/L vs. 78 IU/L; $P = 0.01$), and alkaline phosphatase levels (307 IU/L vs. 250 IU/L; $P = 0.04$). There were no differences in the incidence of comor-

Table I. Demographics in stented and unstented patients

	Stented (n = 408)	Unstented (n = 159)	*P* value
Age			
Mean (± SE)	63.8 ± 0.6 yr	61.4 ± 1.2 yr	0.05
Median	66 yr	65 yr	
Sex			
Male	54%	49%	NS
Female	46%	51%	
Race			
White	92%	83%	0.005
Black	5%	13%	
Other	3%	4%	

Table II. Presenting signs and symptoms in stented and unstented patients

	Stented (n = 408)	Unstented (n = 159)	*P* value
Jaundice	67%	38%	<0.0001
Weight loss	40%	37%	NS
Abdominal pain	38%	37%	NS
Nausea/vomiting	18%	22%	NS
Fever/chills	5%	1%	NS

bid conditions between the two groups including hypertension, myocardial infarction, diabetes, chronic obstructive pulmonary disease, peripheral vascular disease, pancreatitis, alcohol use, and smoking.

The operative data are summarized in Table III. Ninety-six percent of patients in each group underwent partial pancreatectomy, whereas the number of pylorus-preserving procedures was similar in the two groups (78% stented vs. 71% unstented; P = NS). Seventy percent of stented patients underwent pancreaticojejunostomy to restore pancreatic-enteric continuity and 30% underwent pancreaticogastrostomy. This was similar to the 75% of patients undergoing pancreaticojejunostomy and the 25% undergoing pancreaticogastrostomy in the unstented group. The incidence of superior mesenteric or portal venous resection was similar in the stented and unstented groups (2% and 1%, respectively; P = not significant [NS]). The mean estimated blood loss was 970 ml in the stented group and 980 ml in the unstented group (P = NS), with similar mean red blood cell transfusion requirements (1.0 units stented vs. 1.1 units unstented; P = NS) and operative times (6.7 hours stented vs. 6.8 hours unstented; P = NS).

The postoperative diagnoses are summarized in Table IV. Patients undergoing preoperative stenting were significantly more likely to have periampullary adenocarcinoma (pancreatic, distal bile duct, ampullary, and duodenal cancers). Seventy-four percent (n = 301) of stented patients had periampullary cancer as the final diagnosis, with 190 pancreatic, 58 distal bile duct, 44 ampullary, and nine duodenal cancers.

Table III. Operative data in stented and unstented patients

	Stented (n = 408)	Unstented (n = 159)	*P* value
Extent of pancreatectomy			
Partial	96%	96%	NS
Total	4%	4%	
Type of resection			
Pylorus-preserving	78%	71%	NS
Classic	22%	29%	
Pancreatic reconstruction			
Pancreaticojejunostomy	70%	75%	NS
Pancreaticogastrostomy	30%	25%	
Vein resection	2%	1%	NS
Estimated blood loss (mean ± SE)	970 ± 90 ml	980 ± 120 ml	NS
Transfusion (mean ± SE)	1.0 ± 0.1 units PRBCs	1.1 ± 0.2 units PRBCs	NS
Operative time (mean ± SE)	6.7 ± 0.1 hr	6.8 ± 0.1 hr	NS

PRBC = packed red blood cells.

Table IV. Postoperative diagnoses in stented and unstented patients

	Stented (n = 408)		Unstented (n = 159)	
	No.	Percent	No.	Percent
Periampullary adenocarcinoma	301	74	90	57*
Pancreatic	190	47	60	38
Distal bile duct	58	14	8	5
Ampullary	44	11	11	7
Duodenal	9	2	11	7
Chronic pancreatitis	44	11	24	15
Cystic neoplasm	15	4	8	5
Islet cell tumor	13	3	12	8
Ampullary adenoma	13	3	3	2
Gastrointestinal stromal tumor	4	1	7	4
Metastatic tumors to pancreas	4	1	2	1
Other	14	3	13	8

*P = 0.0001; stented vs. unstented patients.

Table V. Mortality and morbidity in stented and unstented patients

	Stented (n = 408)	Unstented (n = 159)	*P* value
Mortality	1.7%	2.5%	NS
Complications			
Overall complication rate	35%	30%	NS
Pancreatic fistula	10%	4%	0.02
Wound infection	10%	4%	0.02
Delayed gastric emptying	12%	11%	NS
Abscess	4%	6%	NS
Cholangitis	3%	3%	NS
Bile leak	3%	4%	NS
Pneumonia	2%	1%	NS
Pancreatitis	2%	1%	NS
Reoperation	3%	5%	NS
Postoperative length of stay			
Mean	14.3 ± 0.4 days	14.1 ± 0.8 days	NS
Median	11 days	11 days	

Only 57% (n = 90) of unstented patients had periampullary cancer ($P = 0.0001$), with 60 pancreatic, eight bile duct, 11 ampullary, and 11 duodenal cancers.

Morbidity and mortality data are summarized in Table V. There were 11 deaths in the entire cohort, for an overall perioperative mortality rate of 1.9%. Seven deaths occurred in stented patients (1.7%) compared to four in unstented patients (2.5%; P = NS). Seven deaths were secondary to sepsis and associated multisystem organ failure, three deaths were from hemorrhage (one of which was directly related to hemobilia in a stented patient), and the final death was due to pulmonary artery rupture from a Swan-Ganz catheter.

The overall incidence of postoperative complications was 35% in stented patients and 30% in unstented patients (P = NS). Of note, stented patients had a significantly higher incidence of pancreatic fistula (10% vs. 4%; $P = 0.02$) and wound infection (10% vs. 4%; $P = 0.02$). Twelve percent of stented patients experienced delayed gastric emptying, 4% developed an intra-abdominal abscess, 3% developed cholangitis, 3% had a bile leak, 2% developed pneumonia, and 2% had pancreatitis. This is similar to the 11% delayed gastric emptying, 6% abscess, 3% cholangitis, 4% bile leak, 1% pneumonia, and 1% pancreatitis rates seen in unstented patients (P = NS). Three percent of stented and 5% of unstented patients required reoperation in the immediate postoperative period most often for bleeding, intra-abdominal sepsis, or wound/anastomotic dehiscence. The postoperative length of stay was similar in the two groups, with stented patients staying in the hospital an average of 14.3 days (median 11 days) and unstented patients staying an average of 14.1 days (median 11 days).

Using multivariate analysis, preoperative stenting remained an independent variable associated with postoperative wound infection ($P = 0.03$), and it approached significance for pancreatic fistula formation ($P = 0.06$). As in univariate analysis, preoperative stenting was not shown to increase the overall complication rate, operative mortality, and other specific complications. Preoperative jaundice, the method of stenting used, and the benign or malignant nature of the lesion did not influence these outcomes. The final multivariate model analyzing postpancreaticoduodenectomy complications is shown in Table VI.

Given the higher percentage of patients with periampullary adenocarcinoma in the stented group, a subgroup analysis was performed on the 391 patients with pancreatic (n = 250), distal bile duct (n = 66), ampullary (n = 55), and duodenal (n = 20) adenocarcinomas (Table VII). Three hundred one of these patients had stents placed (77%), whereas 90 did not (23%). Sixty-three percent of the stented patients and 67% of the unstented patients had pancreatic primary lesions (P = NS). The incidence of positive nodes and positive resection margins was similar in the two groups, with 69% and 23% of stented patients having positive nodes and positive margins, respectively, compared to 66% and 20% in unstented patients. The overall complication rates were similar for the stented and unstented patients. Stented patients had a significantly higher incidence of wound infection (10% vs. 2%; $P = 0.02$), whereas the incidence of pancreatic fistula formation in these patients approached significance (9% vs. 3%; $P = 0.07$). The in-

Table VI. Multivariate analysis: Factors influencing postpancreaticoduodenectomy complications

Outcome	Factors in multivariate analysis	*P* value
Operative mortality	Preoperative stenting	0.46
	Preoperative jaundice	0.87
	Method of stenting (endoscopic vs. percutaneous)	0.21
	Benign vs. malignant pathology	0.16
Overall complication rate	Preoperative stenting	0.38
	Preoperative jaundice	0.49
	Method of stenting (endoscopic vs. percutaneous)	0.37
	Benign vs. malignant pathology	0.73
Pancreatic fistula	Preoperative stenting	0.06
	Preoperative jaundice	0.70
	Method of stenting (endoscopic vs. percutaneous)	0.32
	Benign vs. malignant pathology	0.12
Wound infection	Preoperative stenting	0.03
	Preoperative jaundice	0.68
	Method of stenting (endoscopic vs. percutaneous)	0.07
	Benign vs. malignant pathology	0.39

Table VII. Mortality and morbidity in stented and unstented patients: Periampullary adenocarcinoma only (N = 391)

	Stented (n = 301)	Unstented (n = 90)	*P* value
Overall complication rate	34%	27%	NS
Pancreatic fistula	9%	3%	0.07
Wound infection	10%	2%	0.02
Delayed gastric emptying	13%	11%	NS
Abscess	4%	2%	NS
Cholangitis	3%	3%	NS
Bile leak	2%	3%	NS
Pneumonia	1%	1%	NS
Pancreatitis	0.3%	0%	NS
Reoperation	4%	3%	NS

cidences of delayed gastric emptying (13% vs. 11%), intra-abdominal abscess formation (4% vs. 2%), cholangitis (3% vs. 3%), bile leak (2% vs. 3%), pneumonia (1% vs 1%), and pancreatitis (0.3% vs. 0%) were similar between the two groups.

Because of the discrepancy in the number of patients with jaundice in the stented and unstented groups (67% vs. 38%, respectively; $P < 0.0001$), a subgroup analysis was performed in patients with jaundice only. Of the 330 patients who presented with jaundice, 272 patients underwent stent placement preoperatively (82%) and 58 patients did not (18%). Those undergoing stent placement were more likely to have fevers/chills preoperatively (3% vs. 0%; $P = 0.04$) but had similar frequencies of weight loss (42% vs. 36%; P = NS), abdominal pain (30% vs. 19%; P = NS) and nausea/vomiting (15% vs. 24%; P = NS). Again, patients with stents were more likely to have periampullary cancers (86% vs. 75%; $P = 0.002$). The data in this subgroup analysis are consistent with the previous analysis in that higher incidences of postoperative wound infection and pancreatic fistula were noted in the stented group. Of the patients with jaundice, 10% of those with stents developed wound infections and 10% developed pancreatic fistulas. This is compared to a 2% wound infection rate ($P = 0.04$) and a 2% pancreatic fistula rate in the unstented group ($P = 0.05$).

The demographic and operative data comparing percutaneous (n = 262) and endoscopic (n = 146) stents are summarized in Table VIII. There was a significantly lower incidence of periampullary adenocarcinoma in those undergoing percutaneous stent placement (71% vs. 80%; $P = 0.001$). The operative time,

Table VIII. Percutaneous vs. endoscopic biliary stenting: Demographic and operative data

	Percutaneous (n = 262)	Endoscopic (n = 146)	*P* value
Demographics			
Mean age	63.1 ± 0.8 yr	65.1 ± 0.9 yr	0.12
Male	56%	51%	NS
White	89%	96%	0.07
Operative data			
Extent			
Partial	97%	96%	NS
Total	3%	4%	
Type of pancreatectomy			
Pylorus-preserving	80%	75%	NS
Classic	20%	25%	
Pancreatic reconstruction			
Pancreaticojejunostomy	72%	68%	NS
Pancreaticogastrostomy	28%	32%	
Mean estimated blood loss (± SE)	1050 ± 130 ml	830 ± 70 ml	NS
Mean transfusions (± SE)	1.2 ± 0.2 units PRBCs	0.6 ± 0.1 units PRBCs	0.06
Mean operative time (± SE)	6.7 ± 0.1 hr	6.8 ± 0.1 hr	NS
Periampullary adenocarcinoma	71%	80%	0.001

PRBC = packed red blood cells.

Table IX. Percutaneous vs. endoscopic biliary stenting: Mortality and morbidity

	Percutaneous (n = 262)	Endoscopic (n = 146)	*P* value
Mortality	1.1%	2.8%	NS
Overall complication rate	35%	35%	NS
Hemobilia	3%	0%	0.03
Pancreatic fistula	10%	12%	NS
Wound infection	10%	10%	NS
Delayed gastric emptying	13%	9%	NS
Abscess	3%	5%	NS
Cholangitis	3%	3%	NS
Bile leak	3%	3%	NS
Pneumonia	2%	3%	NS
Pancreatitis	0.3%	0%	NS
Reoperation	4%	3%	NS
Postoperative length of stay			
Mean (± SE)	15.8 ± 0.8 days	14.0 ± 0.7 days	NS
Median	11 days	11 days	

estimated blood loss, and transfusion requirements were similar in the two groups. There were three deaths in the percutaneous group for a perioperative mortality rate of 1.1%, whereas there were four deaths in the endoscopic group for a perioperative mortality rate of 2.8% (*P* = NS). Both groups had an overall complication rate of 35% (Table IX). Of note, eight patients (3%) in the percutaneously stented group developed significant hemobilia after stent placement. These patients required upsizing of their biliary stents and/or angiography with embolization to control the bleeding. None of the patients in the endoscopically stented group had hemobilia. The incidences of all other postoperative complications in-

cluding wound infection (10% in each group) and pancreatic fistula formation (10% percutaneous vs. 12% endoscopic) were similar in the two groups.

DISCUSSION

In the 1970s pancreaticoduodenectomy was associated with high morbidity and mortality and poor long-term survival.[16,17] Significant improvements have been reported in the perioperative mortality, with several series reporting mortality rates of less than 5%.[13,18-24] Despite significantly improved mortality rates, these series still report high postoperative complication rates ranging from 25% to 50%.

Biliary stents are often placed during the workup of a patient expected to undergo pancreaticoduodenectomy. There are many reasons to consider placement of preoperative biliary stents. Preoperative cholangiography allows accurate definition of the level of biliary obstruction and aids in formulating the operative strategy. Biliary drainage increases patient comfort by decreasing bilirubin levels, thereby relieving the often problematic symptom of pruritus. Drainage of the biliary tree can provide relief of cholangitis. In addition, biliary drainage can serve as a temporizing measure if surgery is to be delayed for a significant length of time, allowing for normalization of liver function tests and hepatic function.

Despite these theoretical advantages, preoperative biliary stenting remains controversial. Although some authors report improvement in operative outcome following stent placement, several report no benefit from preoperative stent placement and others document increased morbidity associated with this practice.[1-5,9-11] If preoperative biliary stenting has no benefit and contributes to the observed high morbidity following pancreaticoduodenectomy, it should not routinely be performed.

There have been several prospective randomized trials comparing preoperative percutaneous biliary stenting to no preoperative stenting in both proximal and distal biliary tract surgery. Most patients in these studies had obstructive jaundice secondary to periampullary adenocarcinoma and underwent resection via pancreaticoduodenectomy or palliative biliary bypass. In 1982, Hatfield et al.[1] reported the results of a randomized trial in which 22 patients underwent preoperative biliary drainage and 25 patients did not undergo drainage. Procedures included pancreaticoduodenectomy, biliary bypass, exploratory laparotomy, and common bile duct exploration. Although serum bilirubin levels were lowered in the stented group, the postoperative morbidity and mortality rates were similar in the two groups. In 1985 Pitt et al.[9] compared 37 patients undergoing preoperative stent placement to 38 patients not undergoing stent placement. Both groups demonstrated similar morbidity, mortality, and lengths of stay but higher hospital costs were documented in stented patients. McPherson et al.,[2] in 1984, randomized 65 patients to receive or not receive preoperative percutaneous biliary stents. Again the operative procedures performed varied. Although the mortality rates were similar in the two groups, the morbidity rate was 32% in those with stents and only 19% in those without stents, which was statistically significant.

Two recent nonrandomized retrospective reviews from Memorial Sloan-Kettering Cancer Center again documented increased morbidity following biliary stenting.[4,5] The first, in 1998, evaluated biliary stenting prior to pancreaticoduodenectomy.[5] The majority of patients underwent endoscopic stenting, but patients with percutaneous stents were included as well. Patients with biliary stents had an increased risk of wound or intra-abdominal complications when these were evaluated together as an end point. The individual incidences of wound infection, pancreatic fistula, and intra-abdominal abscess were not significantly different between the two groups. In addition, there was a trend toward increased length of stay in the stented group. No comparison between endoscopically and percutaneously stented patients was performed. The second report evaluated preoperative biliary stenting for proximal cholangiocarcinomas.[4] Preoperative stenting increased the risk of contaminated bile (bactibilia) and postoperative infectious complications, as had been shown in previous studies.[25-29]

Two randomized studies evaluated preoperative endoscopic stenting in comparison to no stenting. Lai et al.[10] randomized 87 patients, only 33 of whom underwent pancreaticoduodenectomy. No differences in postoperative morbidity and mortality were observed between the two groups. Lygidakis et al.[11] randomized 38 patients to undergo or not undergo preoperative endoscopic stent placement. These investigators found that preoperative endoscopic drainage decreased postoperative complications. However, this study was flawed in that it included patients with cholangitis in the unstented group. In most settings such patients would undergo preoperative biliary drainage prior to surgery.

A more recent review from Memorial Sloan-Kettering Cancer Center evaluates 240 consecutive pancreaticoduodenectomy patients over a 3-year period with regard to preoperative biliary drainage.[30] Fifty-three percent of the patients underwent preoperative biliary drainage and the postoperative morbidity and

mortality rates were 48% and 5%, respectively. By univariate and multivariate analysis, preoperative biliary drainage was shown to be the only statistically significant variable associated with morbidity ($P = 0.025$), infectious complications ($P = 0.014$), and postoperative mortality ($P = 0.037$). The current retrospective study evaluates a much large number of patients undergoing pancreaticoduodenectomy, allowing comparison of preoperatively stented and unstented patients. In our analyses, the use of preoperative biliary stenting influenced only the rates of pancreatic fistula formation and wound infection. Unlike the data from Memorial Sloan-Kettering Cancer Center, our analyses do not associate preoperative stenting with overall morbidity or with postoperative mortality. It may be that the larger numbers of patients in our study (567 patients vs. 240 patients) or the lower rates of morbidity (34%) and mortality (1.9%) have an impact on the factors found to be associated with the stenting.

In the current study, the overall morbidity, mortality, and lengths of stay were similar in the stented and unstented groups. However, the incidence of wound infection was significantly higher in the patients with stents. This observation held true in the overall cohort, as well as in patients with jaundice and in patients with periampullary adenocarcinoma. As has been suggested by other investigators, this is likely explained by the high incidence of biliary contamination, or bactibilia, following instrumentation of the biliary tree. All stents traverse the ampulla of Vater, allowing contamination of hepatic bile. At the time of pancreaticoduodenectomy, the bile duct is transected, with the potential for contamination of the surgical wound and the peritoneal cavity with colonized bile.

An increase in pancreatic fistula formation was also noted in patients with stents. One can postulate that stent placement may lead to partial obstruction of the pancreatic duct. This may result in inflammation of the pancreas, with subsequent increased susceptibility to anastomotic leakage. This increased risk of pancreatic fistula was significant in the univariate analysis and in the subgroup analysis of patients with preoperative jaundice, and it approached significance in the multivariate model ($P = 0.06$).

Patients undergoing endoscopic versus percutaneous stenting had similar overall perioperative morbidity and mortality rates. The most striking difference in the two groups was the 3% incidence of hemobilia in the percutaneously stented group. Hemobilia is a direct result of the transhepatic access route and the fact that the stents remain in place in the postoperative period. Such hemobilia typically originates from intrahepatic branches of either the portal vein or the hepatic artery. Other than hemobilia, there were no differences in complications between the two groups. As many of our endoscopically stented patients were referred with stents already in place, a referral bias may exist. Patients who had major complications related to the endoscopic stenting procedure may never have been referred to our institution. In experienced hands, the rate of major procedure-related morbidity associated with endoscopic stenting is less than 5%, including duodenal perforation, hemorrhage, or severe post-ERCP pancreatitis.

In summary, preoperative biliary stenting did not increase the overall complication rate or mortality rate in patients undergoing pancreaticoduodenectomy. However, stenting does appear to increase the rates of wound infection and pancreatic fistula. In addition, the stenting procedure itself is not without risk, as demonstrated by the hemobilia seen in percutaneously stented patients.

The current data support the contention that routine preoperative biliary stenting is of no benefit and carries potential risk. Stenting should be considered in patients with obstructive jaundice who will have a delay of more than 2 weeks between initial presentation and definitive surgery, and in patients with major hepatic or hematologic abnormalities that preclude immediate safe resection. Preoperative stenting is indicated in patients with primary suppurative cholangitis, which is a rare finding in the absence of prior biliary instrumentation. Although percutaneous stenting has been argued to make the biliary anastomosis technically easier, the above-demonstrated risks have changed the practice patterns at our institution, with less frequent placement of preoperative percutaneous or endoscopic stents. Given the increased risks, preoperative biliary stenting should be used selectively. When performed, the method of biliary decompression should be based on local expertise.

REFERENCES

1. Hatfield AR, Tobias R, Terblanche J, et al. Preoperative external biliary drainage in obstructive jaundice: A prospective controlled clinical trial. Lancet 1982;2:896-899.
2. McPherson GA, Benjamin IS, Hodgson HJ, et al. Pre-operative percutaneous transhepatic biliary drainage: The results of a controlled trial. Br J Surg 1984;71:371-375.
3. Temudom T, Sarr MG, Douglas MG, et al. An argument against routine percutaneous biopsy, ERCP, or biliary stent placement in patients with clinically resectable periampullary masses: A surgical perspective. Pancreas 1995;11:283-288.
4. Hochwald SN, Burke EC, Jarnagin WR, et al. Association of preoperative biliary stenting with increased postoperative infectious complications in proximal cholangiocarcinoma. Arch Surg 1999;134:261-266.
5. Heslin MJ, Brooks AD, Hochwald SN, et al. A preoperative biliary stent is associated with increased complications after pancreaticoduodenectomy. Arch Surg 1998;133:149-154.
6. Su CH, P'eng FK, Lui WY. Factors affecting morbidity and mortality in biliary tract surgery. World J Surg 1992;16:536-540.

7. Dixon JM, Armstrong CP, Duffy SW, et al. Factors affecting morbidity and mortality after surgery for obstructive jaundice: A review of 373 patients. Gut 1983;24:845-852.
8. Armstrong CP, Dixon JM, Taylor TV, et al. Surgical experience of deeply jaundiced patients with bile duct obstruction. Br J Surg 1984;71:234-238.
9. Pitt HA, Gomes AS, Lois JF, et al. Does preoperative percutaneous biliary drainage reduce operative risk or increase hospital cost? Ann Surg 1985;201:545-553.
10. Lai EC, Mok FP, Fan ST, et al. Preoperative endoscopic drainage for malignant obstructive jaundice. Br J Surg 1994; 81:1195-1198.
11. Lygidakis NJ, van der Heyde MN, Lubbers MJ, et al. Evaluation of preoperative biliary drainage in the surgical management of pancreatic head carcinoma. Acta Chir Scand 1987;153:665-668.
12. Rowlands PC. Stents in the biliary tree. Postgrad Med J 1995;71:79-80.
13. Yeo CJ, Cameron JL, Sohn TA, et al. Six hundred fifty consecutive pancreaticoduodenectomies in the 1990s. Pathology, complications, and outcomes. Ann Surg 1997;226:248-260.
14. Yeo CJ, Barry MK, Sauter PK, et al. Erythromycin accelerates gastric emptying following pancreaticoduodenectomy: A prospective, randomized placebo controlled trial. Ann Surg 1993;218:229-238.
15. Yeo CJ, Cameron JL, Maher MM, et al. A prospective randomized trial of pancreaticogastrostomy versus pancreaticojejunostomy after pancreaticoduodenectomy. Ann Surg 1995; 222:580-592.
16. Crile G Jr. The advantages of bypass operations over radical pancreaticoduodenectomy in the treatment of pancreatic carcinoma. Surg Gynecol Obstet 1970;130:1049-1053.
17. Shapiro TM. Adenocarcinoma of the pancreas: A statistical analysis of biliary bypass vs. Whipple resection in good risk patients. Ann Surg 1975;182:715-721.
18. Crist DW, Sitzmann JV, Cameron JL. Improved hospital morbidity, mortality, and survival after the Whipple procedure. Ann Surg 1987;206:358-365.
19. Trede M, Schwall G, Saeger H. Survival after pancreaticoduodenectomy. 118 consecutive resections without a mortality. Ann Surg 1987;221:447-458.
20. Grace PA, Pitt HA, Tompkins RK, et al. Decreased morbidity and mortality after pancreaticoduodenectomy. Am J Surg 1986;151:141-149.
21. Geer RJ, Brennan MF. Prognostic indicators for survival after resection of pancreatic adenocarcinoma. Am J Surg 1993: 165:68-73.
22. Cameron JL, Pitt HA, Yeo CJ, et al. One hundred and forty-five consecutive pancreaticoduodenectomies without a mortality. Ann Surg 1993;217:433-449.
23. Warshaw AL, Gu ZY, Wittenberg J, Waltman AC. Preoperative staging and assessment of resectability in pancreatic cancer. Arch Surg 1990;125:230-233.
24. Crist DW, Cameron JL. The current status of the Whipple operation for periampullary carcinoma. Adv Surg 1992;25: 21-49.
25. Robson MC, Bogart JN, Hegger JP. An endogenous source of wound infections based on quantitative bacteriology of the biliary tract. Surgery 1970;68:471-476.
26. Chetlin SH, Elliott DW. Biliary bacteremia. Arch Surg 1971;102:303-307.
27. McPherson GAD, Benjamin IS, Habib NA, et al. Percutaneous transhepatic drainage in obstructive jaundice: Advantages and problems. Br J Surg 1982;69:261-264.
28. Wells GR, Taylor EW, Lindsay G, et al. Relationship between bile colonization, high-risk factors and postoperative sepsis in patients undergoing biliary tract operations while receiving a prophylactic antibiotic. Br J Surg 1989;76:374-377.
29. Reiss R, Eiliashiv A, Deutsch AA. Septic complications and bile cultures in 800 consecutive cholecystectomies. World J Surg 1982;6:195-199.
30. Povoski SP, Karpeh MS Jr, Conlon KC, et al. Association of preoperative biliary drainage with postoperative outcome following pancreaticoduodenectomy. Ann Surg 1999;230:131-142.

Discussion

Dr. G. Tsiotos (Kansas City, Mo.). I think this is a very important paper since it comes from an institution where routine preoperative stenting has been advocated for years. I conclude from your presentation that preoperative stenting should not be used in the usual patient with periampullary cancer because it increases the rate of fistula and wound infection without offering any advantage whatsoever. This is especially important since most gastroenterologists and unfortunately several surgeons still use this method because, allegedly, it decreases perioperative mortality. This is not accurate, as you have stated, in patients other than those with hepatic hilar malignancies requiring hepatectomy. The practice of stenting really reflects pessimism about the ability of these patients to be resected safely; this attitude should be considered outdated. I have four questions. Obviously there was a very strong selection bias regarding which patients were stented and which were not, and you stated that patients with jaundice would more likely be stented. Can you elaborate on that? At what bilirubin level was stenting preferentially performed? The second question concerns the indications for stenting. Obviously cholangitis is a very grave indication in this group of patients. What are other conditions that might require stenting? You alluded to hematologic disorders. Can you elaborate on that? My third question concerns percutaneous stenting. Two thirds of your patients underwent percutaneous stenting. I would not expect that. Endoscopic stenting would be preferable, I suppose. My last question concerns the formation of pancreatic fistula. What do you think causes the increased incidence of pancreatic fistulas in patients with stents?

Dr. T. Sohn. With regard to the selection bias that is certainly true, as it is in any retrospective review. Approximately 40% of these patients were referred with stents already in place. We know their bilirubin levels when they arrived at our institution, but we do not know their bilirubin levels prior to referral, so we have not stratified the patients in terms of preoperative bilirubin levels. In terms of indications for stenting, one of the major indications at our institution is a delay between diagnosis and the time when

we can perform definitive surgery. Another indication is suppurative cholangitis, which, as you have mentioned, is rare in the primary setting. Hematologic abnormalities would include coagulopathies due to advanced liver disease from obstructive jaundice. One other indication that I did not talk about is the reoperative setting. In patients who have had a previous operative biliary bypass, it might aid dissection to have a stent in place. In terms of percutaneous and endoscopic stenting, the situation is a little unusual at our institution. We have very good interventional radiologists with whom we work very closely, and our preference had been to perform percutaneous stenting. We leave that stent in place postoperatively to decompress the anastomosis. But certainly endoscopic stenting is a reasonable option. The choice should be based on local expertise. With regard to pancreatic fistula formation, I believe that both endoscopic and percutaneous transhepatic stenting increase pancreatic inflammation. This small degree of pancreatitis related to the stenting procedure probably increases our rate of fistula formation after the operation.

Dr. G. Aranha (Maywood, Ill.). I want to ask you about the effect of stents on wound infection. How is that changing your intraoperative conduct in those who come to you with stents already in place? Do you close off the distal duct and place a clamp on the proximal duct? How can we change that high rate of infection?

Dr. Sohn. I think we now place stents in fewer people to begin with, but in patients who already have stents we are not doing anything different intraoperatively. All patients receive prophylactic antibiotics perioperatively.

Dr. S. Marcus (New York, N.Y.). We conducted a similar analysis at New York University and found that although our complication rate did not differ, the patients who were stented had a decreased length of stay. They seemed to have a faster recovery postoperatively. Do you have any data on length of stay?

Studies in animal models suggest that in those with obstructive jaundice, if liver function is to be recovered, the bile duct needs to be internally drained for at least 2 weeks and probably for as long as 6 weeks. Do you have any data on how long your patients underwent internal drainage between the time of stenting and the surgery?

Dr. Sohn. Our length of stay in all groups was similar. The median stay was 11 days overall. With regard to the length of stenting, it is variable. As I stated, many patients are referred with stents already in place. So there are patients who have had stents in place for well over 6 weeks and patients who have stents placed immediately before the operation.

Dr. G. Larson (Louisville, Ky.). Do you have any idea what the presence of a fistula does to the length of hospital stay? How significant is it in terms of cost and time spent in the hospital? Second, according to your data, is there any reason to place a stent in a patient who is not jaundiced? You had approximately 100 of these types of patients.

Dr. Sohn. Our experience is that the formation of a pancreatic fistula increases the length of stay by 4 to 5 days. Most of these patients have controlled leaks into Jackson-Pratt drains that are placed around the anastomosis. Reoperation is rarely necessary. In terms of stenting nonjaundiced patients, one indication would be a previous operative biliary bypass, where a difficult hilar dissection and the need for a redo biliary-enteric anastomosis are anticipated.

Dr. R. Prinz (Chicago, Ill.). It seems that infectious complications are increased after stenting. Is the length of preoperative stenting, the size of the stent, or the presence of preoperative cholangitis indicative of a greater likelihood of wound infection or other infectious complications?

Dr. Sohn. Again, we have many patients who are referred with stents in place. The patients have been stented for various lengths of time. We did not specifically look at any of the factors you mention to determine the rates of complications in those groups.

Ontogeny of Activin B and Follistatin in Developing Embryonic Mouse Pancreas: Implications for Lineage Selection

Thomas S. Maldonado, M.D., Alan S. Kadison, M.D., Christopher A. Crisera, M.D., Juan B. Grau, M.D., Susan L. Alkasab, B.A., Michael T. Longaker, M.D., George K. Gittes, M.D.

Activin, a member of the transforming growth factor-beta superfamily, has been shown to be a critical regulator in exocrine and endocrine pancreas formation. The purpose of our study was to describe the ontogeny of activin B and its inhibitor, follistatin, in developing pancreas and to elucidate potential mechanisms for exocrine and endocrine lineage selection. Mouse embryonic pancreata were dissected at various ages (day 10 [E10.5] to birth [E18.5]), sectioned, and immunostained for activin B (one of two existing isomers, A and B), follistatin, insulin, and glucagon. In addition, reverse transcriptase–polymerase chain reaction was employed to determine the messenger RNA expression of follistatin in isolated pancreatic epithelia and mesenchyme of various ages. Activin B was first detected at E12.5 in epithelial cells coexpressing glucagon. At E16.5 these coexpressors appeared as clusters in close proximity to early ducts. By E18.5 activin B was localized to forming islets where cells coexpressed glucagon and were arranged in the mantle formation characteristic of mature alpha cells. Follistatin was found to be ubiquitous in pancreatic mesenchyme at early ages by immunohistochemical analysis, disappearing sometime after E12.5. Follistatin reappeared in E18.5 islets and remains expressed in adult islets. Follistatin messenger RNA was first detected in epithelium at E11.5, preceding its protein expression in islets later in gestation. We propose that mesenchyme-derived follistatin inhibits epithelium-derived activin at early embryonic ages allowing for unopposed exocrine differentiation and relative suppression of endocrine differentiation. At later ages the decrease in the amount of mesenchyme relative to epithelium and the subsequent drop in follistatin levels liberates epithelial activin to allow differentiation of endocrine cells to form mature islets by the time of birth. (J GASTROINTEST SURG 2000;4:269-275.)

KEY WORDS: Activin, follistatin, development, pancreas, endocrine

The embryonic pancreas forms as an evagination of the foregut epithelium into its surrounding mesenchyme and subsequently gives rise to pancreatic islets, acini, and ducts. Several studies have established the importance of mesenchymal-epithelial interactions in morphogenesis as well as in differentiation during pancreas development.[1-5] Whereas whole E11.5 pancreas grown in a collagen matrix is capable of growth and differentiation, pancreatic epithelium isolated from its surrounding mesenchyme fails to develop in vitro.[1] Furthermore, a soluble "mesenchymal factor" has been implicated in pancreatic development. Transfilter experiments in which epithelia and mesenchyme separated by a porous membrane are capable of growth and differentiation confirm the existence of a soluble factor.[1,2] Despite persistent attempts to purify this mesenchymal factor by Rutter and others[3,6] in the 1970s, its identity has remained elusive, leading researchers today to focus on a candidate approach for describing the mesenchymal factor or factors.

Activins, members of the transforming growth factor-beta (TGF-β) superfamily, are known to regulate the growth and differentiation of a variety of cell

From the Laboratory of Developmental Biology and Repair, Department of Surgery, New York University Medical Center, New York, N.Y.
Supported by an American College of Surgeons Faculty Research Fellowship (Dr. Gittes) and a Tow Foundation Grant (Dr. Crisera).
Presented at the Fortieth Annual Meeting of The Society for Surgery of the Alimentary Tract, Orlando, Fla., May 16-19, 1999 (poster presentation).
Reprint requests: George K. Gittes, M.D., Laboratory of Surgical Organogenesis, Children's Mercy Hospital, 2401 Gillham Rd., Kansas City, MO 64108.

types. Originally isolated from ovarian fluid and identified based on its ability to regulate the release of follicle-stimulating hormone from the pituitary gland,[7,8] activin and its inhibitor, follistatin, have been shown to be important in the development of numerous organ systems including pituitary, thyroid, muscle, bone, and testis.[9-13] Activins (A and B) exist as dimers of two closely related inhibin β-subunits ($\beta_A\beta_A$ and $\beta_B\beta_B$, respectively)[7,14] and act through a transmembrane receptor with an intracellular serine/threonine kinase domain.[15]

Data are beginning to accumulate that implicate activin signaling in exocrine and endocrine pancreas differentiation as well. Exogenous activin added to gestational day 11 (E11.5) pancreatic anlage blocks ductal branching.[16] This inhibitory effect is entirely prevented by coincubation with follistatin, indicating that the effect of activin is specific and not toxic. The fate of endocrine cells was not specifically examined in these studies. However, activin signaling is clearly important for endocrine cell differentiation, as demonstrated in studies using transgenic mice expressing a dominant-negative form of the activin receptor (dn-ActR).[17] Mice with blocked activin signaling developed hypoplasia of pancreatic islets late in gestation. In addition, the dnActR mouse pups had a severely impaired insulin response to glucose, as well as a decreased 15-week survival rate. Interestingly, transgenic mice with overfunctioning (constitutive) activin receptor also develop hypoplastic islets, suggesting that an activin signal of the appropriate intensity (not too strong or too weak) is necessary for proper endocrine cell differentiation. In addition to their role in pancreas development, activin and follistatin may be important in maintaining endocrine homeostasis in adult pancreas. Indeed, exogenous activin A has been shown to stimulate insulin secretion in insulinoma cell lines[18] in a dose-dependent manner and to potentiate glucose-induced insulin release in rat pancreatic islets.[19,20]

The distribution of activin A and follistatin has been well described in mouse decidua, postimplantation embryos, as well as in various older embryonic tissues and in the adult.[21,22] In rat embryonic pancreas, activin A protein is detected by immunohistochemical analysis as early as E12 in the epithelium and is coexpressed with glucagon and insulin later in gestation.[23] In adult pancreas, follistatin and activin A are both expressed in beta cells.[24] Others have shown activin A to be present in secretory granules of alpha and delta cells by immunoelectron microscopy. The ontogeny of activin B and follistatin remains largely unknown in developing fetal pancreas.

Interestingly, activin B is thought to be an important notochord signal in the chick embryo that permits pancreas development to proceed by suppressing sonic hedgehog (Shh) expression, a known inhibitor of dorsal pancreas development.[25] Furthermore, Miralles et al.[26] noted that exogenous follistatin mimics the effect of mesenchyme on developing epithelia, driving exocrine and simultaneously suppressing endocrine differentiation. When added to in vitro developing E12.5 rat epithelium with its mesenchyme, follistatin results in a significant overgrowth of exocrine cells and a dramatic reduction in endocrine cells. Thus follistatin is likely to be an active component of the mesenchymal factor mediating mesenchymal-epithelial interactions, presumably through its inhibitory effect on epithelial activin.

The purpose of this study was to describe the ontogeny of activin B and follistatin in mouse embryonic pancreas to better understand possible autocrine and paracrine mechanisms involved in endocrine and exocrine lineage selection.

MATERIAL AND METHODS

Time-dated pregnant CD-1 mice were obtained from Charles River Laboratories (Wilmington, Mass.). The mice were killed on days 10.5, 12.5, 14.5, 16.5, and 18.5 of gestation (day 0.5 defined as the morning of discovery of the vaginal plug). All procedures were approved by the New York University Medical Center Animal Care and Use Committee in accordance with the Guide for the Care and Use of Laboratory Animals (Department of Health and Human Services, National Institutes of Health Publication No. 86-23, revised 1985). Embryos were harvested and preserved on ice in phosphate-buffered saline (PBS). Microdissection was performed on the individual embryos to isolate and remove the pancreas.[5,27]

Tissue Preparation

After the embryonic pancreata had been harvested, they were immediately preserved on ice until the entire litter had been dissected. A 4% paraformaldehyde (in PBS) solution was prepared and filtered with a 22 μm filter. The pancreata were fixed in the paraformaldehyde for 2 hours. They were then transferred to 30% sucrose (in PBS) for 4 to 12 hours. The pancreata were pooled according to their specific gestational age and finally frozen embedded for subsequent cryostat sectioning.

Immunohistochemistry

The frozen blocks of pooled embryonic pancreata were cut into sections 6 μm thick using a Leica cryostat (Leica, Wetzler, Germany). Except when indicated, all incubations were performed at 25° C. Antibodies against follistatin (rabbit antihuman) and in-

hibin beta-B (rabbit antimouse), specific for activin B, were generously provided by Dr. Bilezikjian.[9] Antibodies to insulin (sheep antihuman; The Binding Site, San Diego, Calif.) and glucagon (mouse monoclonal; Sigma, St. Louis, Mo.) had been quality tested and do not cross-react with other proteins. The antibodies were diluted in PBS, and their optimal concentrations for both immunoperoxidase and immunofluorescence were determined to be as follows: anti-inhibin beta-B (1:200), antifollistatin (1:200), anti-insulin (1:500), antiglucagon (1:500). Control tests for the primary antibodies were performed at each time point using normal serum rather than the individual antibodies.

For immunoperoxidase staining, the specimens were first rehydrated with PBS. The specimens were then rendered permeable in Tween-20 solution (0.5% in PBS) for 10 minutes and washed with PBS for 5 minutes. Heated sodium citrate (10 mmol/L, pH 3.0) was then used for antigen recovery. The specimens were incubated at 4° C with 4% blocking serum (goat serum; Vectastain, Vector Laboratories, Burlingame, Calif.) overnight prior to exposure to the primary antibody for 2 hours followed by secondary antibody (biotinylated goat antirabbit immunoglobulin; Vectastain) for 1 hour. The specimens were pretreated with H_2O_2 (0.3% in methanol) for 30 minutes to inactivate endogenous peroxidases, and then exposed to the ABC solution (Vectastain) for 1 hour. Prior to completing the immunohistochemical reaction with 3,3′-diaminobenzidine, the specimens were exposed to Triton-X100 for 30 minutes.

For double immunostaining, the specimens were also subjected to treatment with sodium citrate and Tween-20 (0.5% in PBS). Incubation with the blocking serum (4% normal donkey serum in PBS) took place overnight at 4° C. Both primary antibodies were applied together for 2 hours at 25° C after another exposure to Tween-20 (0.5% in PBS). Finally, the secondary antibodies (donkey antisheep tetrarhodamine isothiocyanate 1:200, and donkey antirabbit fluorescein isothiocyanate 1:200; Jackson Laboratories, Bar Harbor, Maine) were applied together after one more Tween-20 (0.5% in PBS) wash.

RNA Extraction and Reverse Transcriptase–Polymerase Chain Reaction

Pancreatic epithelia, ages E10.5 and E11.5, were isolated from their surrounding mesenchyme using the technique of Gittes et al.[5] Briefly, epithelia were microdissected from surrounding mesenchyme following brief incubation in 1% trypsin. Epithelia were immediately placed in a DNA lysis buffer and frozen in liquid nitrogen. Likewise, E10.5, E11.5, and E12.5 mesenchyme was obtained through microdissection and frozen. The E12.5 epithelium is unable to be reliably cleaned of its mesenchyme because of the many interdigitations of mesenchyme among the developing epithelial lobules (developing acini) at this gestational age. After the pancreatic epithelia and mesenchyme had been harvested and frozen, the messenger RNA from each was immediately extracted using the Qiagen Oligotex Direct mRNA Mini-Kit (Qiagen Inc., Santa Cruz, Calif.). A reverse transcriptase (RT) reaction was performed using random hexamers, and the resulting DNA was used in polymerase chain reactions (PCR).[27] The following mouse oligonucleotides were used as primers for follistatin: forward, 5′-TCATGGAGGACCGCAGCGCC-3′; reverse, 5′-ACAAGTGGAGCTGCCTGGAC-3′. These primers were designed to yield a 475 bp fragment from positive controls. For all the polymerase chain reactions, 10× PCR buffer (200 mmol/L Tris-HCl (pH 8.4), 500 mmol/L KCl) was used, and the concentration of $MgCl_2$ was 2.0 mmol/L. Forty cycles were performed. Amplification parameters included a 1-minute annealing step at 55° C and a 1:30-second extension step at 72° C. Negative controls were run for each primer and included PCR reactions with cyclic DNA prepared without RT. Primers to the common structural protein, beta tubulin, were used as positive controls for the RT-PCR reaction itself. The products of amplification were separated on a 2% agarose gel and compared to the known sequence.

RESULTS

Immunohistochemistry

Activin B. Expression of activin B was first detected in E12.5 pancreatic epithelium in a sparse and scattered distribution (Fig. 1, *A* and *B*). By E14.5 activin B was limited to distinct cell clusters in close proximity to early developing pancreatic ducts (Fig. 1, *C*). Later in gestation (E16.5 and E18.5), activin B expression was more organized and confined to newly forming islets of Langerhans (Fig. 1, *D*). Interestingly, in adult islets, activin B appeared in the mantle distribution typical of alpha cells (Fig. 1, *E*). Indeed, double immunostaining for activin B, and either insulin or glucagon, demonstrated activin B to be coexpressed with glucagon and not with insulin throughout gestation, as well as in the adult (Fig. 2, *A* through *E*). However, only a fraction of glucagon-positive cells coexpressed activin B, consistent with the notion that activin specifically directs pluripotential pancreatic cells toward endocrine commitment and is not important in more mature alpha cells. Likewise, activin B–positive cells coexpressing glucagon in adult islets may represent a subpopulation of glucagon-positive cells that may be a source of new cells for islet regeneration.

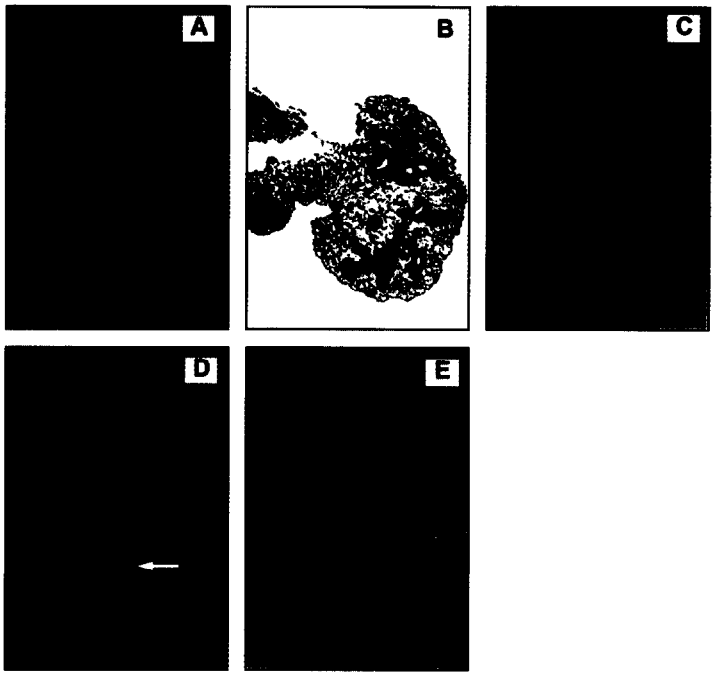

Fig. 1. Fluorescent and peroxidase immunohistochemistry of mouse embryonic and adult pancreas. Activin B is first expressed in a few scattered cells of E12.5 epithelium (fluorescein) **(A)**. Note abundant mesenchyme *(m)* compared with epithelium *(e)* at this age as seen on hematoxylin and eosin staining **(B)**. At later gestational ages (E14 and E16), activin B is found in the vicinity of forming ducts *(arrows)* (**C** and **D**). In the adult, activin B is expressed in peripheral islet cells (peroxidase) in a mantle distribution characteristic of alpha cells **(E)**.

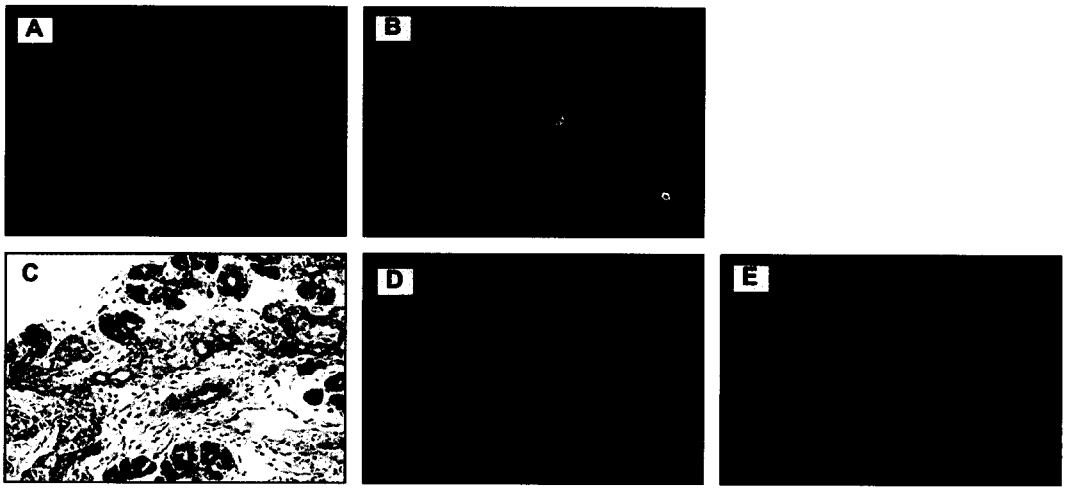

Fig. 2. Fluorescent immunohistochemistry of gestational age E16.5 and adult pancreas for activin B, insulin, and glucagon. Note that insulin-positive beta cells (rhodamine) do not coexpress activin B (fluorescein) in E16.5 pancreas or in the adult (**A** and **B**). As shown by hematoxylin and eosin staining **(C)**, at E16.5, the embryonic pancreas has already formed distinct ducts, acini, and clusters of endocrine cells. Some of these endocrine cells coexpress activin B **(D)** (rhodamine) and glucagon **(E)** (fluorescein). Note, not all glucagon-positive cells coexpress activin B.

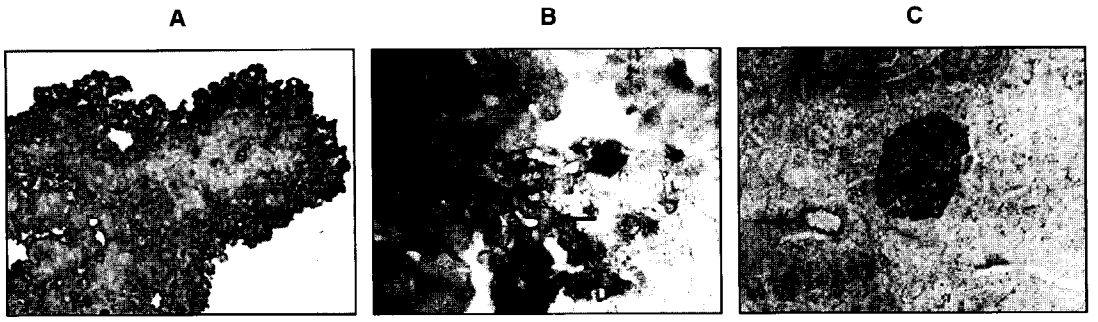

Fig. 3. Immunohistochemistry for follistatin in E12.5, E18.5, and adult pancreas. At E12.5, follistatin is expressed exclusively in the mesenchyme *(m)*. Note the epithelial-sparing *(e)* and distinct mesenchymal-epithelial interface. At 18.5, follistatin is expressed in forming islets emanating from ducts *(arrows)*. In adult pancreas, follistatin is found in the beta-cell distribution of islets.

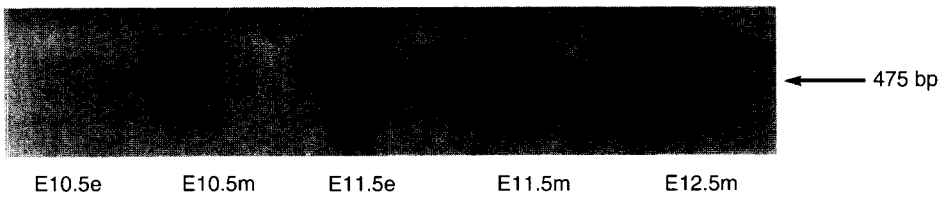

Fig. 4. RT-PCR for follistatin in pancreatic epithelia and its mesenchyme at various embryonic ages. Follistatin is present in pancreatic mesenchyme of all gestational ages examined. However, it is absent from E10.5 epithelia and is first detected in epithelia at E11.5. This epithelial messenger RNA expression of follistatin early in gestation is consistent with its protein expression in islets later in gestation and in the adult. NOTE: 475 bp represents the expected size of the PCR fragment generated using specific primers designed for follistatin from positive controls. The faint second band represents nonspecific binding of primers and is not significant.

Follistatin. Expression of follistatin was ubiquitous in pancreatic mesenchyme of all ages examined. Earlier gestational ages (i.e., E10.5 to E12.5), which contain abundant mesenchyme, showed strong mesenchymal expression of follistatin with distinct epithelial sparing (Fig. 3, *A*). As gestation proceeds (>E14.5) the relative proportion of mesenchyme compared to epithelium decreases dramatically, and follistatin becomes difficult to detect. Surprisingly, follistatin eventually reappears in the epithelium at E18.5 in clusters of cells emanating from ducts, and finally is expressed in the core of adult islets, characteristic of the beta-cell distribution (Fig. 3, *B* and *C*).

Reverse Transcriptase–Polymerase Chain Reaction

The apparent inconsistency between immunohistochemical localization of follistatin found in mesenchyme early in gestation and in epithelium late in gestation, as well as in the adult, prompted us to examine messenger RNA expression for follistatin in epithelia and mesenchyme of various ages. Messenger RNA for follistatin was detected in all pancreatic mesenchyme examined (E10.5, E11.5, and E12.5). Although it was absent in E10.5 pancreatic epithelium, follistatin messenger RNA was expressed in epithelium at E11.5 (Fig. 4). This epithelial expression is consistent with the positive immunohistochemical staining seen in the epithelium in late gestation (see above) and in the adult.

DISCUSSION

Numerous in vitro studies have shown follistatin to induce exocrine differentiation, whereas activin inhibits exocrine differentiation.[16,26] Similarly, transgenic mice with either a dominant-negative activin receptor or a constitutively active activin receptor both develop hypoplastic islets late in gestation, suggesting that a precise level of activin signaling is necessary for proper endocrine differentiation. Thus the dual role of activin as an inhibitor of exocrine lineage and a promoter of endocrine lineage appears to be regulated

by its known inhibitor, follistatin. In this study we have described the ontogeny of activin B and follistatin in developing pancreas, in order to better understand their function in vivo. Activin B was found to be present in pancreatic epithelium as early as E12.5 and persists in glucagon-expressing cells throughout gestation and in the adult. Follistatin was present in mesenchyme of early gestational ages (i.e., E12.5) and in beta cells of late gestation and the adult.

Glucagon has been thought to be an early marker of endocrine lineage, with its expression preceding the expression of insulin and the other endocrine hormones.[28] Nonetheless, glucagon's utility as an endocrine stem cell marker is limited because it is expressed in both immature and mature alpha cells. Interestingly, the coexpression of activin B with glucagon in only a fraction of glucagon-positive cells may represent a subset of endocrine stem cells and may serve as a more useful marker for these pluripotential cells. Moreover, activin B may be necessary for directing these cells toward a more mature endocrine cell fate.

A delicate balance appears to exist between activin and follistatin in mesenchymal-epithelial interactions of the developing pancreas. Early in embryonic life, mesenchymal follistatin may inhibit epithelial activin, resulting in unimpeded exocrine differentiation and relative suppression of endocrine lineage. However, as gestation proceeds and mesenchyme represents a smaller proportion of the total pancreas, follistatin may no longer be able to inhibit the proendocrine effect of activin, resulting in the greater proliferation of endocrine tissue that occurs at days 14 to 16. Not surprisingly, compensatory or redundant regulatory mechanisms for dictating lineage selection must exist. Indeed, transgenic mice deficient in follistatin, while suffering multiple defects as well as perinatal death, appear to have normal pancreata.[29,30] Furthermore, although transgenic pups deficient in the activin signaling have a decreased survival compared to littermate control pups, lack of activin signaling does not result in a lethal phenotype as might be expected if the endocrine lineage were solely dependent on activin.

Another potential role for activin B relates to its suppressive effect on sonic hedgehog (Shh), a secreted protein and potent patterning signal implicated in cell-cell interactions in various developing organ systems. Unopposed Shh has been shown to completely prevent the chick pancreas from developing early in gestation.[25,31] Moreover, activin B, as well as fibroblast growth factor-2, present in notochord at these early ages, has been shown to specifically block Shh expression, thereby allowing the pancreas to develop unimpeded. Interestingly, after fusion of the dorsal aortas at the 11-somite stage in the mouse, the developing pancreas is effectively sequestered from the protective effect of notochord-derived activin B against Shh. Yet Shh is never expressed in pancreatic endoderm at any age in vivo,[25,32] suggesting that some ongoing suppression of Shh is maintained even in the absence of notochord. One intriguing possibility is that inhibition of Shh results from the presence of endogenous activin B in midgestation (E11.5) epithelium, as shown in our study. Thus, in addition to its potential function in guiding lineage selection, activin B may be fundamentally important in maintaining a permissive environment for ongoing pancreas development.

In addition to their possible role in mesenchymal-epithelial interactions and lineage selection, activin and follistatin may help maintain endocrine homeostasis in the adult. Indeed, activin A has been shown to stimulate insulin secretion, even in the absence of glucose.[33] Interestingly, although follistatin is found predominantly in the mesenchyme at early gestational ages, it is eventually expressed in adult beta cells.[34] This apparent inconsistency may actually reflect the paracrine or autocrine mechanisms necessary for maintaining appropriate glucose levels in the adult.

CONCLUSION

Using an educated candidate approach to identifying factors involved in pancreas development, we have begun to assemble a growing list of factors, receptors, and inhibitors that may all play important roles in pancreas development. In this report we have shown activin B to be found exclusively in the epithelia, and its inhibitor, follistatin, in the mesenchyme of embryonic pancreas. As such, these two factors might be important in directing epithelial-mesenchymal interactions during pancreatic organogenesis. Specifically, activin B may drive pluripotential cells toward an endocrine cell fate, and follistatin may aid in promoting exocrine lineage selection through its inhibition of activin B. Employing these growth factors in vitro to identify a pluripotential stem cell and then driving those stem cells toward or away from a predetermined differentiated state remains the ultimate goal of the study of pancreas development and may someday provide a potential cure for diabetes mellitus.

Antisera to inhibin βB and follistatin was a generous gift from Drs. Louise Bilezikjian and Wylie Vale, Clayton Foundation Laboratories for Peptide Biology, The Salk Institute for Biological Studies.

REFERENCES

1. Golosow N, Grobstein C. Epitheliomesenchymal interaction in pancreatic morphogenesis. Dev Biol 1962;4:242-255.
2. Kallman F, Grobstein C. Fine structure of differentiating mouse pancreatic exocrine cells in transfilter culture. J Cell Biol 1964;20:399-413.
3. Rutter WJ, Pictet RL, Harding JD, et al. An analysis of pancreatic development: Role of mesenchymal factor and other extracellular factors. Symp Soc Dev Biol 1978;35:205-227.
4. Wessells N, Cohen J. Early pancreatic organogenesis: Morphogenesis, tissue interactions, and mass effects. Dev Biol 1966;15:237-270.
5. Gittes GK, Galante PE, Hanahan D, et al. Lineage-specific morphogenesis in the developing pancreas: Role of mesenchymal factors. Development 1996;122:439-447.
6. Ronzio RA, Rutter WJ. Effects of a partially purified factor from chick embryos on macromolecular synthesis of embryonic pancreatic epithelia. Dev Biol 1973;30:307-320.
7. Mason AJ, Hayflick JS, Ling N, et al. Complementary DNA sequences of ovarian follicular fluid inhibin show precursor structure and homology with transforming growth factor-beta. Nature 1985;318:659-663.
8. Robertson DM, Klein R, de Vos FL, et al. The isolation of polypeptides with FSH suppressing activity from bovine follicular fluid which are structurally different to inhibin. Biochem Biophys Res Commun 1987;149:744-749.
9. Bilezikjian LM, Corrigan AZ, Blount AL, Vale WW. Pituitary follistatin and inhibin subunit messenger ribonucleic acid levels are differentially regulated by local and hormonal factors. Endocrinology 1996;137:4277-4284.
10. Kotajima A, Miyamoto Y, Tsuruo M, et al. Effects of activin A on deoxyribonucleic acid synthesis, iodine metabolism, and cyclic adenosine monophosphate accumulation in porcine thyroid cells. Endocrinology 1995;136:1214-1218.
11. Link BA, Nishi R. Opposing effects of activin A and follistatin on developing skeletal muscle cells. Exp Cell Res 1997;233: 350-362.
12. Funaba M, Ogawa K, Murata T, et al. Follistatin and activin in bone: Expression and localization during endochondral bone development. Endocrinology 1996;137:4250-4259.
13. Meinhardt A, O'Bryan MK, McFarlane JR, et al. Localization of follistatin in the rat testis. J Reprod Fertil 1998;112: 233-241.
14. Kingsley DM. The TGF-beta superfamily: New members, new receptors, and new genetic tests of function in different organisms. Genes Dev 1994;8:133-146.
15. Mathews LS. Activin receptors and cellular signaling by the receptor serine kinase family. Endocr Rev 1994;15:310-325.
16. Ritvos O, Tuuri T, Eramaa M, et al. Activin disrupts epithelial branching morphogenesis in developing glandular organs of the mouse. Mech Dev 1995;50:229-245.
17. Yamaoka T, Idehara C, Yano M, et al. Hypoplasia of pancreatic islets in transgenic mice expressing activin receptor mutants. J Clin Invest 1998;102:294-301.
18. Shibata H, Kanzaki M, Takeuchi T, et al. Two distinct signaling pathways activated by activin A in glucose-responsive pancreatic beta-cell lines. J Mol Endocrinol 1996;16:249-258.
19. Totsuka Y, Tabuchi M, Kojima I, et al. A novel action of activin A: Stimulation of insulin secretion in rat pancreatic islets. Biochem Biophys Res Commun 1988;156:335-339.
20. Verspohl EJ, Ammon HP, Wahl MA. Activin A: Its effects on rat pancreatic islets and the mechanism of action involved. Life Sci 1993;53:1069-1078.
21. Feijen A, Goumans MJ, van den Eijnden-van Raaij AJ. Expression of activin subunits, activin receptors and follistatin in postimplantation mouse embryos suggests specific developmental functions for different activins. Development 1994; 120:3621-3637.
22. Albano RM, Arkell R, Beddington RS, Smith JC. Expression of inhibin subunits and follistatin during postimplantation mouse development: Decidual expression of activin and expression of follistatin in primitive streak, somites and hindbrain. Development 1994;120:803-813.
23. Furukawa M, Eto Y, Kojima I. Expression of immunoreactive activin A in fetal rat pancreas. Endocr J 1995;42:63-68.
24. Wada M, Shintani Y, Kosaka M, et al. Immunohistochemical localization of activin A and follistatin in human tissues. Endocr J 1996;43:375-385.
25. Hebrok M, Kim SK, Melton DA. Notochord repression of endodermal sonic hedgehog permits pancreas development. Genes Dev 1998;12:1705-1713.
26. Miralles F, Czernichow P, Scharfmann R. Follistatin regulates the relative proportions of endocrine versus exocrine tissue during pancreatic development. Development 1998;125:1017-1024.
27. Gittes GK, Rutter WJ. Onset of cell-specific gene expression in the developing mouse pancreas. Proc Natl Acad Sci USA 1992;89:1128-1132.
28. Alpert S, Hanahan D, Teitelman G. Hybrid insulin genes reveal a developmental lineage for pancreatic endocrine cells and imply a relationship with neurons. Cell 1988;53:295-308.
29. Edlund H. Transcribing pancreas. Diabetes 1998;47:1817-1823.
30. Matzuk MM, Lu N, Hannes V, et al. Multiple defects and perinatal death in mice deficient in follistatin. Nature 1995;374:360-363.
31. Kim SK, Hebrok M, Melton DA. Notochord to endoderm signaling is required for pancreas development. Development 1997;124:4243-4252.
32. Bitgood MJ, McMahon AP. Hedgehog and Bmp genes are coexpressed at many diverse sites of cell-cell interaction in the mouse embryo. Dev Biol 1995;172:126-138.
33. Furukawa M, Nobusawa R, Shibata H, et al. Initiation of insulin secretion in glucose-free medium by activin A. Mol Cell Endocrinol 1995;113:83-87.
34. Ogawa K, Abe K, Kurosawa N, et al. Expression of alpha, beta A and beta B subunits of inhibin or activin and follistatin in rat pancreatic islets. FEBS Lett 1993;319:217-220.

Gastroesophageal Reflux After Intact Vertical Banded Gastroplasty: Correction by Conversion to Roux-en-Y Gastric Bypass

Bruno M. Balsiger, M.D., Michel M. Murr, M.D., Jane Mai, R.N., Michael G. Sarr, M.D.

Symptomatic gastroesophageal reflux disease is common in our experience after vertical banded gastroplasty. Our aim was to determine the safety and efficacy of Roux-en-Y gastric bypass in the treatment of symptomatic gastroesophageal reflux disease complicating vertical banded gastroplasty. We evaluated prospectively collected data on 25 patients who underwent revisional bariatric surgery because of *severe* gastroesophageal reflux disease after vertical banded gastroplasty. Only 4 of 25 patients had gastroesophageal reflux disease symptoms prior to vertical banded gastroplasty. Endoscopic findings in 24 patients included esophagitis (58%), Barrett's esophagus (28%), pouchitis (29%), and gastritis (21%); 7 (28%) of 25 patients had evidence of stenosis at the pouch outlet. Mean follow-up (complete in all 25) after Roux-en-Y gastric bypass was 37 ± 7 months (range 3 to 102 months). There were no deaths. Postoperative complications occurred in six patients: pneumonia in two, wound infection in two, prolonged drainage of the defunctionalized stomach via gastrostomy in one, and fever in one. Median hospitalization was 7 days (range 5 to 43 days). At follow-up (37 ± 7 months), 24 (96%) of 25 are completely or almost completely symptom free. Body mass index was 33 ± 2 kg/m^2 before and 28 ± 2 kg/m^2 after Roux-en-Y gastric bypass ($P = 0.001$). Symptoms of gastroesophageal reflux disease are common after vertical banded gastroplasty. Conversion to Roux-en-Y gastric bypass is safe, relieves gastroesophageal reflux disease, and promotes further weight loss. Moreover, maladaptive eating (vomiting, and so forth) induced by vertical banded gastroplasty is relieved. (J GASTROINTEST SURG 2000;4:276-281.)

KEY WORDS: Bariatric surgery, obesity, gastroesophageal reflux, vertical banded gastroplasty, Roux-en-Y gastric bypass

Vertical banded gastroplasty (VBG) has been used widely in the treatment of morbid obesity for almost 20 years since the original report by Mason.[1,2] Indeed VBG is one of two proven effective operations condoned by a National Institutes of Health Consensus Conference in 1991.[3] One of the theoretical advantages of VBG is its alleged antireflux properties.[4] This presumed attribute of VBG has been suggested as important in choosing an operation for morbid obesity because of a higher prevalence of gastroesophageal reflux disease (GERD) in obese individuals. The vertical staple line in a VBG resembles a Collis gastroplasty; this partition is thought to create a functionally longer intra-abdominal "neo"-esophagus.

However, as a result of our initial experience with VBG as our primary bariatric operation from 1985 to 1989[5] and our further experience with reoperative bariatric surgery for procedure-related complications,[6,7] we have encountered a prominent subset of patients with refractory GERD after VBG. The current study was undertaken to evaluate the long-term results of converting patients with VBG and refractory GERD to a vertical disconnected Roux-en-Y gastric bypass (RYGB).[8] We hypothesized that conver-

From the Department of Surgery and the Gastroenterology Research Unit, Mayo Clinic, Rochester, Minn.
Supported by the Mayo Foundation, Astra Pharmaceuticals AG, Basel, Switzerland, and the Department of Visceral and Transplantation Surgery, University of Bern, Switzerland.
Presented in part at the Fortieth Annual Meeting of The Society for Surgery of the Alimentary Tract, Orlando, Fla., May 16-19, 1999, and published as an abstract in *Gastroenterology* 116:A1348, 1999.
Reprint requests: Dr. Michael G. Sarr, Professor of Surgery, Chair, Division of General and Gastroenterologic Surgery, 200 First Street SW, Rochester, MN 55905.

sion to RYBG both reduces GERD symptoms by diverting all acid and bile from the esophagus and results in further sustained weight loss.

METHODS

Prospectively collected data on all consecutive patients who underwent bariatric surgery at Mayo Clinic Rochester from 1985 to 1997 were analyzed. The medical records of 25 patients who underwent conversion from VBG to RYGB because of refractory GERD symptoms were reviewed. Patient characteristics, weight data, perioperative mortality and morbidity, and results of postoperative subjective assessment were analyzed. All patients were followed to date after RYGB; pertinent data points were collected prospectively by mailing patients a standard questionnaire at 3, 6, 12, 24, 36, and 48 months postoperatively. The questionnaire included detailed information on dietary and caloric intake, weight, level of activity, and a subjective assessment of quality of life. Patients were contacted to update follow-up at the time of completion of this study. Additional follow-up data were collected from on-site visits at our medical center. Follow-up was complete to date in all 25 patients.

VBG was converted to a vertical disconnected RYGB using minor modifications of our previously described technique.[8] Initially, after removal of the band or "ring" encircling the stoma of the VBG, the cardia was encircled and two rows of linear staples were applied across the cardia. The cardia was transected between the staple lines leaving as small a proximal pouch as possible ($\leq$15 ml). A retrocolic antegastric side-to-side cardiojejunostomy to a 150 cm Roux limb was constructed with a No. 21 EEA stapler (United States Surgical Corp., Norwalk, Conn.) (Fig. 1). If not previously performed, a cholecystectomy was carried out, and a tube gastrostomy placed in the defunctionalized distal stomach. Patients were started on clear liquids on the first or second postoperative day and advanced to pureed foods over 2 to 3 days. After discharge, they were maintained on pureed foods for 6 weeks after which their diet was advanced to regular food over the next 6 weeks.

The initial postoperative follow-up was at our facility and involved a visit with the surgeon, the medical physician in our Nutrition Clinic, and a dietitian. All patients had been strongly and repeatedly counseled and were started on a regimen of oral multivitamin supplements containing iron, monthly intramuscular injections of vitamin B_{12} (1000 μg), and oral calcium supplements. Postoperative data were pooled from the responses to the periodic questionnaires, return visits to Mayo Clinic, and phone interviews. Given the tertiary nature of our referral pattern, long-term follow-up directly at our facility was not always feasible for some patients who lived a great distance away. These patients underwent periodic assessment at their local health care facilities after we corresponded with their home physician counseling the latter in appropriate follow-up evaluation.

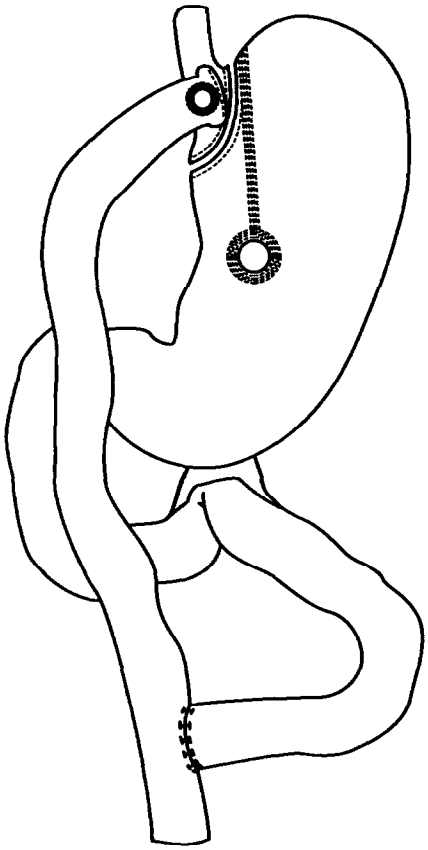

Fig. 1. Conversion of vertical banded gastroplasty (VBG) to vertical disconnected Roux-en-Y gastric bypass. Note removal of band at VBG stoma, stapling across cardia very proximal to VBG stoma, and disconnection of new proximal pouch of cardia from the remaining stomach.

Analysis of Data

All data are reported as mean $\pm$ standard error of the mean unless specified otherwise. When comparisons were appropriate, Student's t tests were employed; $P < 0.05$ was considered significant.

RESULTS

Clinical Presentation

Twenty-two women and three men (median age 47 years; range 28 to 70 years) underwent conversion of VBG to RYGB because of postoperative GERD. All 25 had severe, regular regurgitation and 22 suffered

Table I. Endoscopic findings in 24 patients after vertical banded gastroplasty*

	Stenosis (n = 6)	No stenosis (n = 18)
Esophagitis	3	11
Barrett's esophagus	3	4
Pouchitis	1	6
Gastritis	0	5
Esophagitis and ulcer	1	5
Pouch enlargement	0	2
Hiatus hernia	1	8

*Before Roux-en-Y gastric bypass.

from severe heartburn. Only four of these patients had reflux symptoms prior to the VBG, and in none was the reflux incapacitating.

The onset of post-VBG GERD tended to occur either early in the first year after VBG in 13 patients (median 1 month; range 1 to 12 months) or late after VBG in 10 patients at a median of 7 years (range 5 to 14 years). For the remaining two patients the onset of GERD could not be determined, but the period between VBG and RYGB was 3 and 8 years. The median interval between the onset of post-VBG GERD and conversion to RYGB was 24 months (range 4 to 180 months).

At the time of RYGB, 14 patients were maintained on antacids and/or proton pump inhibitors with unsuccessful relief of reflux symptoms. On upper gastrointestinal contrast studies, nine patients had a hiatal hernia, and two patients had proximal pouch enlargement. Some element of stenosis of the outlet, documented by contrast studies and/or gastroscopy, was present in only 7 (28%) of the 25 patients. Endoscopy in 24 patients revealed esophagitis, pouchitis, gastritis, and stomal ulcers in 58%, 29%, 21%, and 4%, respectively (Table I). In 42% (6 of 14) esophagitis was accompanied by ulceration, and in 29% (4 of 14) changes of Barrett's esophagus were present on histologic examination of biopsy specimens; seven patients were believed to have Barrett's changes on visual examination, but biopsy specimens were not obtained from three patients to confirm the presence of Barrett's esophagus. There were no differences in symptoms or endoscopic findings between patients with or without stenosis of the pouch outlet. In addition to GERD, 21 of the 25 patients had one or more of the following symptomatic comorbid conditions: hypertension in five, asthma in two, diabetes mellitus in three, and/or degenerative joint disease.[15] In addition, sleep apnea was present in one patient.

Hospital Morbidity and Mortality of Conversion to Roux-en-Y Gastric Bypass

There were no operative deaths. Postoperative complications occurred in six patients including pneumonia in two, wound infection in two, prolonged output of the defunctionalized stomach from the decompression tube gastrostomy in one, and fever of unknown origin in one. No reoperations for complications were required. Median length of postoperative hospitalization was 7 days (range 5 to 43 days); the patient who remained in the hospital for 43 days postoperatively was extremely debilitated and malnourished preoperatively and needed physical rehabilitation.

Follow-Up

Mean follow-up was 37 ± 7 months with a median of 18 months (range 3 to 12 months). Long-term operative-related complications were unusual. Incisional hernias requiring operative repair occurred in two patients. One patient underwent a single successful endoscopic dilatation for an alleged anatomic stricture. One patient was hospitalized with bleeding, which occurred at the site of the VBG band and was diagnosed at operation; this patient represents the one patient in whom the band was not removed at the time of RYGB. Three other patients had one episode of self-limited gastrointestinal bleeding, the source of which was unknown. One patient developed fatal strangulated intestinal obstruction secondary to adhesions 19 months postoperatively, whereas another developed locally advanced gastric cancer in the early months postoperatively that in retrospect probably was present at the time of RYGB because the stomach felt abnormally thick.

GERD Symptoms After Roux-en-Y Gastric Bypass (Table II)

All but one patient (96%) had complete or near-complete resolution of heartburn; one person still complained of frequent symptoms, whereas five others reported rare episodes of heartburn no more frequently than once a month and requiring no treatment. Three patients (12%) who took antacids before RYGB continued to use antacids postoperatively despite complete lack of GERD symptoms. Follow-up esophagogastroscopy performed in 5 (36%) of 14 patients because of endoscopically documented preoperative esophagitis showed complete resolution of esophagitis with normal esophageal mucosa. Surveillance endoscopy in all of the patients with Barrett's esophagus continues and has shown no progression to severe dysplasia. One patient reported vomiting as of-

Table II. Upper gastrointestinal symptoms after conversion from vertical bypass gastroplasty to Roux-en-Y gastric bypass (25 patients)

	Frequency			
Symptom	Once a day	Once a week	Once a month	None
Heartburn	0	1	5	19
Bile reflux	0	0	1	24
Nausea	0	2	0	23
Vomiting	1	4	2	18

Table III. Weight history before and after vertical banded gastroplasty and conversion to Roux-en-Y gastric bypass*

	Before bariatric surgery	After VBG†	After RYGB‡
Weight (kg)	129 ± 5	89 ± 5	76 ± 5
BMI (kg/m^2)	48 ± 2	33 ± 2	28 ± 1
EBW (kg)	69 ± 5	28 ± 4	15 ± 4
%EBW	113 ± 8	46 ± 7	24 ± 6
Loss %EBW	Not appropriate	59 ± 7	83 ± 6

BMI = body mass index; EBW = excess body weight.
*Mean ± standard error of the mean; n = 25 patients.
† Mean time after VBG = 7 ± 1 years.
‡ Mean time after RYGB = 34 ± 7 months.

ten as once a day, four patients about once a week, and two patients once a month.

Weight History

Mean weight, excess body weight, and body mass index before VBG were 129 ± 6 kg, 113 ± 8%, and 48 ± 2 kg/m^2, respectively (Table III). At the time of conversion to RYGB, weight loss, percentage loss of excess body weight, and reduction of body mass index were 41 ± 6 kg, 59% ± 7%, and a decrease of 15 body mass index units (to 33 ± 2), respectively. At follow-up (37 ± 7 months), after conversion from VBG to RYGB, weight had decreased by another 13 ± 4 kg, total decrease in excess body weight was 83 ± 6%, and final body mass index was 28 ± 1. After RYGB, 21 of 25 patients either lost more weight or maintained their weight. All but two patients achieved at least a total of 50% loss of excess body weight compared to their pre–bariatric surgery weight; these latter two patients, however, lost 45% and 47% of their excess body weight, respectively.

Subjective Assessment of Quality of Life

At follow-up, 80% of the patients (22 of 25) were satisfied with the resultant weight loss after the operation and/or maintained that this procedure markedly improved the quality of their lives. Twenty patients indicated that they have recommended and will continue to recommend such operative treatment to similar individuals with clinically significant obesity.

DISCUSSION

Bariatric surgery is accepted as an acknowledged treatment modality for medically complicated obesity (morbid obesity).[3,9] Currently the two most commonly performed bariatric operations in North America are VBG and RYGB. We initially used VBG as our operation of choice from 1985 to 1989 but then abandoned VBG in favor of RYGB because of both an unsatisfactory weight loss (despite an intact anatomy) and unacceptable long-term complications and side effects of VBG, including frequent vomiting, a maladaptive eating syndrome, and a noticeable increase in symptomatic heartburn.[5] Our prospective study of VBG showed that only about 40% of our first 70 patients had lost at least half of their excess body weight. In addition, 38% complained of heartburn of varying severity after VBG despite an anatomically intact, nonobstructed anatomy; this incidence of GERD had increased significantly from the incidence of symptomatic heartburn of only 15% preoperatively

(before VBG).[5] These findings were initially surprising because others had suggested that VBG was in itself an antireflux operation.[4] Subsequently, over the past 10 years, we have treated an increasing number of patients with GERD after VBG that serves as the basis of this report.

Several investigators[10,11] have suggested that the incidence of GERD is similar to that of the general population, whereas others have maintained that GERD is more common in obese individuals.[12] Deitel et al.[4] conducted a study of the effects of VBG on symptoms of GERD and objective measures of lower esophageal sphincter function. This group suggested that by increasing the effective lower esophageal sphincter pressure after VBG and by creating a functional increase in the length of the intra-abdominal (neo) esophagus via the vertical staple line led to a markedly decreased prevalence of heartburn, aspiration, and gastroesophageal reflux.[13] Unfortunately our clinical experience and that of others[5,6,14,15] has been quite dissimilar. In addition, others could not reproduce the increase in functional lower esophageal sphincter pressure after VBG.[16,17] Ovrebo et al.[18] conducted a similar study before and after either VBG or gastric banding. Neither operation decreased symptomatic or objective gastroesophageal reflux. In this study the incidence of GERD did not increase after VBG, which differs from our experience with VBG that showed a progressive increase in symptomatic GERD from 15% preoperatively to 16%, 28%, and 38% at 1, 2, and 3 years postoperatively, respectively.[5] The differences between our study and that of Ovrebo et al.[18] may lie in selection bias; the patients of Ovrebo et al.[18] undergoing VBG had an incidence of GERD preoperatively of 32% (higher than our 15% incidence), yet their patients undergoing gastric banding had an incidence of only 14%, more in line with our experience.

Our experience with GERD after VBG showed two trends in onset of symptomatic GERD—early (<1 year) and late (median 7 years). The early onset of GERD in the absence of mechanical obstruction at the stoma strongly suggests that the anatomic and/or functional changes after VBG led to symptomatic reflux; however, we have not performed systematic pH studies to fully document our contention, and others did not find an increased pH after VBG.[17,19] The later development (median of 7 years) of GERD is consistent with our previous observation of an increasing yearly incidence of GERD after VBG. The reasons for GERD after VBG are not fully understood. Reflux symptoms may be related to stasis in the proximal lesser curvature pouch secondary to stomal stenosis; however, only 28% of our patients had any element of a stomal narrowing, and symptoms in those with and without this relative stenosis were similar (see Table I). Other potential factors involved in post-VBG GERD include the inclusion of acid-secreting parietal mucosa within the proximal pouch[20] (parietal cells occur along the proximal lesser curvature of the stomach) or operative damage to the lower esophageal sphincter. The extent of maneuvers of encircling the distal esophagus as suggested by Mason,[1] with mobilization of the gastroesophageal junction and the vertically oriented staple line, may vary among surgeons and of themselves disrupt the integrity/competence of the lower esophageal sphincter. We would doubt that the etiology of the development of post-VBG GERD lies in the maneuvers needed to encircle the distal esophagus because we did not mobilize the distal esophagus or encircle it in our prospective series of VBG operations[5] and did not measure proximal pouch volume as suggested by Mason.[1] Our previous work examining the motility of the proximal gastric pouch after VBG showed that this pouch did not exhibit a markedly increased resting pressure and accommodated to balloon distention.[21] These observations suggest that a markedly increased proximal pouch pressure was not the inciting factor in GERD.

Our study has several limitations. The lack of preoperative manometry and pH studies limits our ability to prove the presence of acid (or bile) reflux after VBG to account for the symptoms of GERD. Similarly these studies have not been performed postoperatively either, and thus our belief that the gastroesophageal reflux after VBG was the cause of post-VBG GERD remains somewhat conjectural and based on good symptomatic relief after conversion to RYGB, which prevents all acid-peptic or bile reflux. However, preoperative esophagoscopy showed severe esophagitis in more than half of the patients and, of those examined endoscopically postoperatively, the esophagitis had resolved. Most important, all but 1 of the 25 patients were relieved of severe GERD symptoms after RYGB. We believe that our technique of vertical disconnected RYGB with a 150 cm Roux limb effectively diverts all acid and bile from potential contact with the esophagus. We have previously documented the virtual absence of acid secretion from the very small pouch of the cardia created from the vertically constructed proximal pouch of the RYGB as performed by us.[22] Similarly the 150 cm Roux limb should prevent any bile reflux into the proximal pouch of the cardia and the esophagus. Indeed the anatomy of this form of RYGB should be one of the best possible "antireflux" operations to prevent acid/peptic and bile reflux into the esophagus. Consideration might even be given to using this operation in moderately obese individuals with severe GERD. In a

small series[23] of obese patients with GERD, RYGB with a small pouch as a primary procedure for GERD was successful in relieving symptoms. Buckwalter,[23] however, also incorporated a truncal vagotomy and pyloroplasty, a practice that we cannot support; nevertheless, he concluded that a pouch smaller than 25 ml (our pouch was 10 to 15 ml in volume) would render vagotomy/pyloroplasty unnecessary.

Some of the patients we converted to RYGB might have had symptoms of GERD unrelated to acid or bile reflux. After VBG, patients often develop a maladaptive eating syndrome or "pseudo-GERD." Intolerance to many solid foods is common, as is vomiting or ingestion of poorly chewed, rapidly eaten, or too great an ingested volume.[5] In our experience, many patients "adapt" to the food intolerances by changing their diets to include semisolid food and/or high-calorie liquids; unfortunately, with this type of diet, the amount (caloric intake) able to be ingested increases as does the patient's weight. For this reason and because of the increase in GERD symptoms, we no longer advocate VBG as our primary bariatric procedure. Indeed, in the past 16 months since we reviewed this experience, we have operated on an additional 10 patients with GERD after a gastroplasty, converting them to RYGB with similar good results.

The additional weight loss after conversion from VBG to RYGB was not investigated further. This additional weight loss is interesting because this group of patients already had been successful with a mean excess body weight loss of 59% 7 years after VBG. None of the patients required a reoperation because of unsatisfactory weight loss. The additional loss of a mean of 13 kg might best be explained by the additional effects of conversion to RYGB, that is, establishment of a dumping physiology that prevents, at least temporarily, ingestion of high-calorie liquids. This additional benefit supports our approach to using RYGB as the primary operation to treat morbid obesity in the majority of patients.

REFERENCES

1. Mason EE. Morbid obesity: Use of vertical banded gastroplasty. Surg Clin North Am 1987;67:521-537.
2. Mason EE. Vertical banded gastroplasty for obesity. Arch Surg 1982;117:701-706.
3. NIH Conference. Gastrointestinal surgery for severe obesity. Consensus Development Conference Panel. Ann Intern Med 1991;115:956-961.
4. Deitel M, Khanna RK, Hagen J, Ilves R. Vertical banded gastroplasty as an antireflux procedure. Am J Surg 1988;155: 512-516.
5. Nightengale ML, Sarr MG, Kelly KA, et al. A prospective evaluation of vertical banded gastroplasty as the primary operation for morbid obesity. Mayo Clin Proc 1991;66:773-782.
6. Kim CH, Sarr MG. Severe reflux esophagitis after vertical banded gastroplasty for treatment of morbid obesity (case report). Mayo Clin Proc 1992;67:33-35.
7. Behrns KE, Smith CD, Kelly KA, Sarr MG. Reoperative bariatric surgery. Lessons learned to improve patient selection and results. Ann Surg 1993;218:646-653.
8. Sarr MG. Vertical disconnected Roux-en-Y gastric bypass. Dig Surg 1996;13:45-49.
9. Brolin RE. Update: NIH consensus conference. Gastrointestinal surgery for severe obesity. Nutrition 1996;12:403-404.
10. Lundell L, Ruth M, Sandberg N, Bove-Nielsen M. Does massive obesity promote abnormal gastroesophageal reflux? Dig Dis Sci 1995;40:1632-1635.
11. Stene-Larsen G, Weberg R, Froyshov Larsen I, et al. Relationship of overweight to hiatus hernia and reflux oesophagitis. Scand J Gastroenterol 1988;23:427-432.
12. Hagen J, Deitel M, Khanna RK, Ilves R. Gastroesophageal reflux in the massively obese. Int Surg 1987;72:1-3.
13. Papavramidis ST, Theocharidis AJ, Zaraboukas TG, et al. Upper gastrointestinal endoscopic and histologic findings before and after vertical banded gastroplasty. Surg Endosc 1996;10:825-830.
14. Verset D HJ, Gay F, Elcheroth J, Bourgeois V, Van Gossum A. The place of upper gastrointestinal tract endoscopy before and after vertical banded gastroplasty for morbid obesity. Dig Dis Sci 1997;42:2333-2337.
15. Sugerman HJ, Kellum JM Jr, DeMaria EJ, Reines HD. Conversion of failed or complicated vertical banded gastroplasty to gastric bypass in morbidy obesity. Am J Surg 1996;171:263-269.
16. Backman L, Granstrom L, Lindahl J, Melcher A. Manometric studies of lower esophageal sphincter in extreme obesity. Acta Chir Scand 1983;149:193-197.
17. Naslund E, Granstrom L, Melcher A, et al. Gastrooesophageal reflux before and after vertical banded gastroplasty in the treatment of obesity. Eur J Surg 1996;162:303-306.
18. Ovrebo KK HJ, Viste A, Bassoe HH, Svanes K. Gastroesophageal reflux in morbidly obese patients treated with gastric banding or vertical banded gastroplasty. Ann Surg 1998;228: 51-58.
19. Lundell L, Ruth M, Olbe L. Vertical banded gastroplasty or gastric banding for morbid obesity: Effects on gastrooesophageal reflux. Eur J Surg 1997;163:525-531.
20. Jobe BA, Horvath KD, Swanstrom LL. Postoperative function following laparoscopic Collis gastroplasty for shortened esophagus. Arch Surg 1998;133:867-874.
21. Behrns KE, Soper NJ, Sarr MG, et al. Anatomic, motor, and clinical assessment of vertical banded gastroplasty. Gastroenterology 1989;97:91-97.
22. Smith CD, Herkes SB, Behrns KE, et al. Gastric acid secretion and vitamin B_{12} absorption after vertical Roux-en-Y gastric bypass for morbid obesity. Ann Surg 1993;218:91-96.
23. Buckwalter JA. Surgical treatment of morbid obesity with reflux esophagitis. Am Surg 1982;48:128-130.

Treatment of Esophageal Achalasia With Laparoscopic Heller Myotomy and Dor Partial Anterior Fundoplication: Prospective Evaluation of 100 Consecutive Patients

Giovanni Zaninotto, M.D., Mario Costantini, M.D., Daniela Molena, M.D., Francesca Buin, M.D., Alessandra Carta, M.D., Loredana Nicoletti, R.N., Ermanno Ancona, M.D., F.A.C.S.

In this article we report our experience in 100 consecutive achalasia patients who were treated with laparoscopic Heller myotomy and Dor antireflux fundoplication, with particular regard to the technical problems encountered, the learning curve, and the long-term follow-up. The operation was completed laparoscopically in 94 patients, with a median operative duration of 150 minutes, and a continuous steady reduction in the operating time from the first patients to the last. In six patients the operation was completed through "open" access. Postoperative complications were recorded in six cases. Follow-up was completed in all 100 patients, with a median follow-up of 24 months. Overall, actuarial life-table analysis showed a probability of 90% that patients would be symptom free over a 5-year period. Radiologic assessment showed a significant reduction in the esophageal diameter, and manometry showed a significant reduction in the lower esophageal sphincter resting pressure and residual pressure. Twenty-four–hour pH monitoring showed postoperative reflux in 6.9% of the patients. Persistent dysphagia or chest pain was reported by eight patients, which constituted treatment failures. Seven of these eight patients were eventually treated with multiple pneumatic dilatations, which were successful in six cases. It was concluded that laparoscopic Heller myotomy with Dor fundoplication is a feasible and effective treatment for achalasia, with an actuarial success rate of 90% at 5 years. (J GASTROINTEST SURG 2000;4:282-289.)

KEY WORDS: Esophageal achalasia, laparoscopic myotomy, laparoscopic surgery

Esophageal achalasia is a relatively uncommon disease with an annual incidence of approximately one per 100,000 and a remarkably uniform distribution throughout the Western world.[1] The underlying effects of this disease are loss of peristalsis in the esophageal body and absence of lower esophageal sphincter (LES) relaxation, leading to the inability to swallow; as a result, dysphagia is the predominant symptom of achalasia, which occurs in almost all patients with the disease.[2] Although the cause of achalasia is still not clear, the basic principle behind its treatment has been evident since the early 1900s—that is, disruption of the LES, by means of surgery (myotomy), forceful dilatation or, in more recent years, botulinum toxin (BOTOX) injections (although this method is probably less effective), which relieves dysphagia in most of the patients treated. The choice of treatment is left to the individual physician(s) or surgeon(s) after consultation with the patient and is also based on the known level of expertise, since the paucity of patients and the limited advantage of myotomy over dilatation[3] make it very difficult to recruit enough patients to conduct adequate randomized clinical trials (only one such trial has been reported in the medical literature).[4] In general, however, endoscopic dilatation is the treatment most often used for patients with achalasia because it is less expensive[5]; surgery is reserved for the treatment of patients in whom previous endoscopic therapy has failed. In the early 1990s laparoscopic surgery was also applied to esophageal myotomy for achalasia.[6,7] Alleviation of patient discomfort along with a reduction in overall costs with this technique

From the Department of Medical and Surgical Sciences (Clinica Chirurgica IV), University of Padua School of Medicine, Padua, Italy. Reprint requests: Giovanni Zaninotto, M.D., Department of Medical and Surgical Sciences, University of Padua School of Medicine, Via Giustiniani 2, 35128 Padua, Italy. e-mail: zaninot@ux1.unipd.it

suggests that surgery might become a more viable option.[7] As is the case with any "new" operation, particularly in the so-called laparoscopic era, a large number of technical reports have appeared in the medical literature, but only a few of the studies were based on groups of patients large enough to draw any significant clinical conclusions.[8-11] The aim of the present prospective study was to establish the feasibility of laparoscopic Heller myotomy, with particular regard to the technical difficulties encountered, the learning curve, and the long-term results achieved in 100 consecutive patients evaluated both clinically and objectively with esophageal function tests.

MATERIAL AND METHODS

From January 1993 to December 1998, a total of 112 patients were diagnosed as having achalasia (or had a prior diagnosis confirmed) at our institution. Ten of these patients were considered unfit for surgery (because of age or other medical problems) and underwent pneumatic dilatation; two other patients underwent surgical resection of a large sigmoid megaesophagus (>10 cm in diameter). The remaining 100 patients (56 men and 44 women; median age 40 years [range 14 to 73 years]) were treated laparoscopically and formed the study group. The median duration of symptoms was 24 months (range 6 to 240 months). Only 10 patients had undergone prior treatment elsewhere; seven had undergone esophageal balloon dilatation with a Rigiflex dilator (Microvasive/Boston Scientific Corp., Boston, Mass.) (median of 2 dilatations, range 1 to 6), one had received three BOTOX injections at the cardia, and one had undergone two dilatations and one BOTOX injection.

Diagnostic Studies

The diagnosis of primary achalasia was based on clinical history, barium swallow, and esophageal manometry. Clinical data from each patient were collected by means of a questionnaire, and each patient's symptoms (dysphagia, regurgitation of food, and heartburn in the postoperative control period) were assigned a score ranging from 0 to 8 according to severity and frequency (Table I). Chest pain was graded as absent, mild, or severe. A barium swallow was performed in each patient before and 1 month after treatment, and a standard anteroposterior image was obtained to measure the maximum esophageal diameter at the site of the barium-air level. Stationary manometry of the esophagus was performed before and after the operation using a low-compliance pneumohydraulic perfusion system. LES pressure was calculated by averaging the pressures recorded by four side holes positioned at the same level, 90 degrees apart, and withdrawing the catheter twice using a motorized pull-through technique at a constant speed of 1 cm/sec, from the stomach to the esophageal body, passing through the high-pressure zone[12] (LES pressure was therefore the average of eight pressure recordings). LES pressure was calculated as both the midexpiratory pressure at the respiratory inversion point and the average of all pressures recorded in the high-pressure zone (as analyzed by computer). Esophageal body motility and LES relaxation were

Table I. Symptom score according to severity and frequency*

Dysphagia		
0	None	
1	Mild	Occasionally with coarse foods (meat, sandwich, hard roll) lasting a few seconds
2	Moderate	Requires liquids to clear
3	Severe	History of meat impaction necessitating medical attention
Regurgitation		
0	None	
1	Mild	Occasionally after straining, after a large meal, or lying down after a meal
2	Moderate	Predictable with a position change, straining, or lying down
3	Severe	History of aspiration
Heartburn		
0	None	
1	Mild	Recognizable symptom, occasional episodes, no history of medical treatment
2	Moderate	Primary reason for medical visit or "medical problem"
3	Severe	Constant, marked disability in activities of daily living

*For each symptom, a score for frequency was added: 0 = never; 1 = occasionally; 2 = once a month; 3 = every week; 4 = twice a week; 5 = daily.

assessed, recording the changes in pressure elicited by 10 wet swallows, with the side holes of the catheter positioned inside the LES and 5, 10, 15, and 20 cm higher up, according to a technique described elsewhere.[13] LES residual pressure was defined as the minimal pressure (nadir) recorded in the LES during swallowing. Twenty-four–hour esophageal pH monitoring was performed only after the operation to evaluate any abnormal gastroesophageal reflux, positioning a glass electrode 5 cm above the previously detected upper border of the LES, according to the standard procedure used in our laboratory and reported elsewhere.[13] As suggested by Patti et al.,[11] all tracings from patients with abnormal reflux on computerized analysis were carefully reviewed to distinguish between true episodes of gastroesophageal reflux and false reflux due to stasis; recordings showing multiple decreases in pH to 3 or less were considered true reflux, whereas a slow decrease in esophageal pH forming a curve fluctuating between 4.5 and 3.5 was considered false reflux resulting from fermentation secondary to poor clearance. Upper gastrointestinal endoscopy was used to rule out any malignancy before the operation and to evaluate any signs of reflux esophagitis after the operation; when detected, esophagitis was graded according to the classification of Savary and Miller.[14]

Surgical Technique

All patients were operated on using the same previously described technique.[15] Briefly, only the anterior portion of the esophagus was dissected, the anterior vagus nerve was identified, and a myotomy 6 to 8 cm long, extending 1 to 1.5 cm on the gastric side of the cardia, was performed, preferably remaining on the left side of the nerve. An intraesophageal 30 mm balloon dilator (Rigiflex) was inserted using a guidewire positioned endoscopically after the patient had been intubated. During the esophageal myotomy, the balloon was gently inflated and deflated, thus facilitating identification of the circular fibers, which were stretched and then cut or torn apart. The inflated balloon was also useful in controlling minor bleeding from the submucosal vessels, thus reducing the need for cautery. An anterior partial (180-degree) Dor fundoplication completed the operation, with three stitches being placed on each side to suture the gastric wall to the edges of the myotomy.

Assessment of the Learning Curve

The overall institutional learning curve was assessed by measuring the operating time and plotting the conversions and complications observed on the operating time curve. For each of the four surgeons taking part in the study, the median operating time for the first and the last 10 operations was calculated.

Hospital Course

The duration of the postoperative hospital stay, the need for pain-relieving medication, and any adverse events occurring during this period were recorded. A swallow test using water-soluble contrast medium (Gastrografin) was performed on postoperative day 1 to rule out any perforation. The nasogastric tube was then removed and patients were allowed liquids by mouth. Patients were asked to remain on soft foods for 10 to 15 days and were then allowed a normal diet. They were discharged depending on the distance between their homes and the hospital (on day 2 if they lived within an hour's drive from the hospital or on day 4 if they lived further away).

Postoperative Follow-Up

Follow-up was scheduled as follows: patients were asked to return to the outpatient clinic for an interview after 1 month, at which time a barium swallow was also performed. They were asked to undergo repeat esophageal manometry and 24-hour pH monitoring 6 months after the operation, at which time they were interviewed for the second time and their symptoms were scored. Endoscopy was carried out after 1 year; thereafter clinical checkups were scheduled once a year. Patients who failed to report to the outpatient clinic were interviewed by phone.

Statistical Analysis

Data are expressed as median values (fifth to ninety-fifth percentiles). Wilcoxon and Mann-Whitney U tests were used, as appropriate, to compare data. The probability of a recurrence of symptoms after surgery was calculated using actuarial life-table analysis.

RESULTS

In 94 of the 100 patients who underwent laparoscopic treatment, the procedure was completed laparoscopically. Mortality due to the operation was nil. Reasons for conversion to an open procedure included adhesions from previous upper abdominal surgery in two patients and the finding of an unsuspected mass in the lower abdomen in one patient that could not be interpreted by laparoscopy and proved to be an ectopic kidney; in three patients conversion was necessary because of surgical complications. The most common complication we encountered was perfora-

Table II. Postoperative complications

Complication	No.
Trocar site bleeding	1
Pneumothorax	1
Vocal nerve palsy	1
External sciatic popliteal nerve palsy	1
Mucosal tear (unrecognized during myotomy)	1
Unexplained fever	1
TOTAL	6

tion of the esophageal mucosa (4%) while performing the myotomy. Two mucosal tears were repaired following conversion to an open operation and two were repaired laparoscopically using 4-0 reabsorbable sutures. In one patient the reason for conversion was a splenic intraoperative injury. All patients healed with no further consequences. The postoperative morbidity is shown in Table II. One additional mucosal leak was identified by water-soluble contrast swallow, which was routinely performed 24 hours after surgery. This patient was kept on parenteral nutrition and the nasogastric tube was left in place for 7 days, leading to healing of the leakage. None of the patients who had previously been treated with dilatation or BOTOX injections had mucosal tears during myotomy, and the duration of the operation was similar to that in the previously untreated patients, although all surgeons involved in the study had the subjective impression that the myotomy was more difficult to perform in previously treated patients.

The median operating time was 150 minutes (range 110 to 207 minutes). Fig. 1 shows the time required to complete the procedure. A steady reduction in the operating time was achieved after the first 20 operations. The median operating time for the first 20 procedures was 174 minutes (range 154 to 216 minutes), whereas the median operating time for the next 74 procedures was 145 minutes (range 108 to 194 minutes) ($P < 0.01$) (operations converted to laparotomy were excluded). Fig. 2 shows the median operating time for the first 10 and the last 10 patients for each participating surgeon. These surgeons did not start performing laparoscopic myotomy simultaneously; the second, third, and fourth surgeons were gradually introduced to this surgical approach by the senior surgeon (E.A.), who performed the first eight consecutive operations, so they benefited from his experience, clearly confirming that an institutional learning curve does exist.

The median hospital stay was 4 days (range 3 to 10 days). The median time from the operation to the patient's return to work was calculated in the 50 patients still employed and amounted to 12 days (range 6 to 21 days). After surgery, clinical follow-up was completed by all 100 patients; during follow-up three patients died (18, 44, and 56 months after surgery, respectively) of unrelated causes. A barium swallow 1 month after surgery was performed in 70 patients and 76 patients agreed to repeat manometry and 24-hour pH monitoring 6 months later.

Fig. 3 shows the decrease in the symptom scores after the operation. Concerning dysphagia, 70 patients were completely free of dysphagia, 22 had mild episodes of dysphagia less than once a week, and five patients had moderate (score = 2) to severe (score

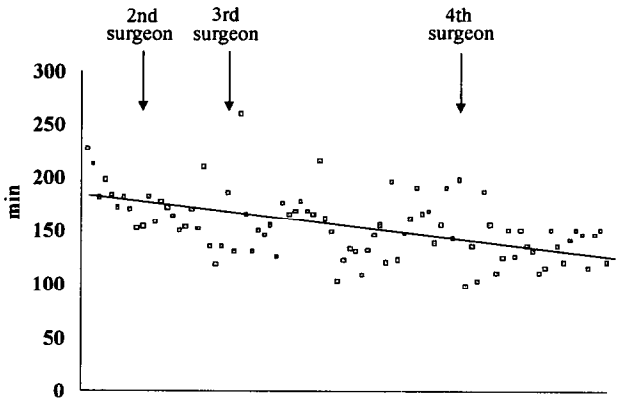

Fig. 1. Institutional learning curve for laparoscopic Heller-Dor operation. Time (minutes) required to complete each operation is plotted, and time at which the second, third, and fourth surgeon started performing the operation is also indicated. Note the steady reduction in the time required to complete the operation; each surgeon took advantage of previous experiences.

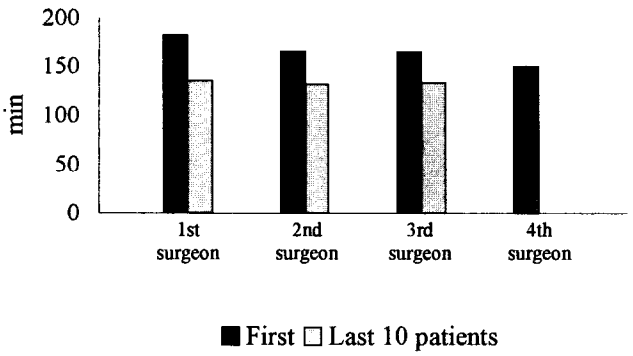

Fig. 2. Median time (minutes) required for each surgeon to perform the operation in the first 10 and the last 10 patients in his personal series. For the fourth surgeon, only the time required to complete the first 10 operations was available. These data confirm that each surgeon benefited from the experience of colleague(s) who started performing the operation earlier.

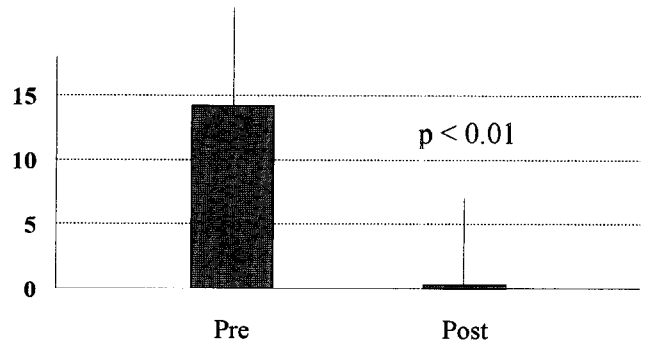

Fig. 3. Median and fifth to ninety-fifth percentiles of the preoperative and postoperative symptom scores in operated patients. Scores decreased after the operation from 14 (range 7 to 21) to 0 (range 0 to 7) ($P < 0.01$).

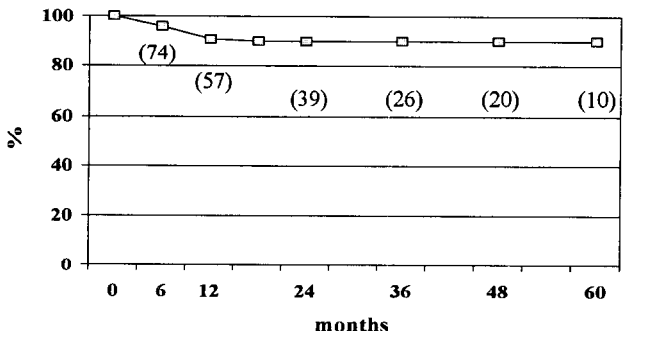

Fig. 4. Actuarial curve for control of dysphagia showing probability that patients would be symptom free at 5-year follow-up. Number of patients evaluated is indicated in parentheses. No patient was lost to follow-up, and three patients died of unrelated causes 18, 44, and 56 months, respectively, after operation.

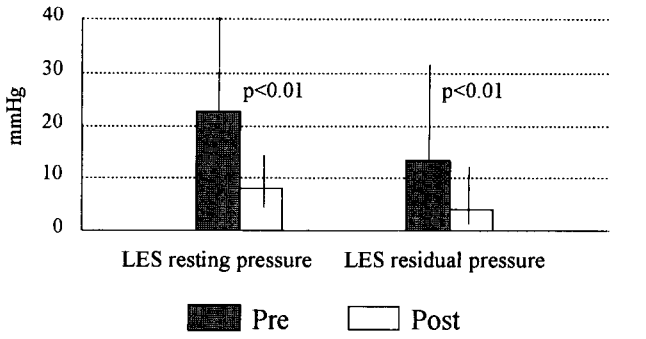

Fig. 5. Manometric characteristics of the LES at rest and during swallowing (residual pressure) before and after the operation in 72 patients. LES resting pressure decreased from 22 mm Hg (range 13 to 40) to 9 mm Hg (range 5 to 16) ($P < 0.01$) and LES residual pressure at swallowing decreased from 12.5 mm Hg (range 3 to 32) to 4 mm Hg (range 1 to 12) ($P < 0.01$).

= 3) dysphagia, occurring more than once a week or even daily. Three additional patients complained of mild postoperative dysphagia and severe chest pain on swallowing. These eight patients were considered laparoscopic myotomy failures. In these patients, symptoms recurred at a median of 4 months after the operation (range 2 to 13 months). Fig. 4 shows the probability of symptom recurrence or chest pain over a 5-year period. Esophageal diameter, as measured by barium swallow, decreased from 3.6 cm (range 2.5 to 6.0 cm) to 2 cm (range 1.2 to 3.4 cm) ($P < 0.01$). The pre- and postoperative LES pressure measurements are shown in Fig. 5. Twenty-four–hour pH monitoring revealed an esophageal acid exposure lasting more than 4.2% of the total time in 10 patients (13.2%). However, when the pH tracings were reviewed, five patients had only one lengthy episode with esophageal pH fluctuating between 3.5 and 4, and were considered to have "false" reflux, whereas five patients (6.6%) had genuine abnormal gastroesophageal reflux. Of the latter five patients, only three had symptoms (heartburn and acid regurgitation) requiring medical therapy with H_2 blockers or proton pump inhibitors. Only one of these patients had grade 1 esophagitis at endoscopy.

In none of the eight patients in whom laparoscopic myotomy failed, did pH monitoring show abnormal gastroesophageal reflux, and postoperative manometry showed abnormal LES pressure on swallowing in only three of eight patients (LES pressure nadir >6 mm Hg). The most important test to investigate the reason for failure was barium video fluoroscopy, which demonstrated difficulty in the passage of the barium above the presumed area of the myotomy in one patient and at the cardia in five.

Seven of the eight patients with recurrent symptoms after laparoscopic myotomy were treated with pneumatic dilatation using 3.5 and 4 cm Rigiflex balloons (median of 3 dilations, range 2 to 6). The treatment was successful in six cases. One patient is still symptomatic and repeat myotomy is under consideration. The last patient was treated elsewhere with BOTOX injections; at present, he is still symptomatic but has refused further treatment.

DISCUSSION

Despite recent progress in our understanding of the etiology of achalasia,[16] treatment of this disease is still (and will probably continue to be) based on methods aimed at mechanically relieving the obstruction at the LES level with a balloon or scalpel. The relative rarity of the disease and the similar efficacy of the two most commonly used methods for disrupting the LES

Table III. Results of laparoscopic treatment of esophageal achalasia (1996 to 1999)*

Study	No. of patients	Antireflux procedure	% with good results	% with reflux
Raiser et al.[9] (1996)	35	Dor/Toupet	62	0
Morino et al.[28] (1997)	21	Dor	81	5
Boulez et al.[29] (1997)	27	None	100	4
Graham et al.[30] (1997)	26	Dor	90	11.1
Hunter et al.[10] (1997)	40	Dor/Toupet	90	2.5
Vogt et al.[31] (1997)	20	Toupet	90	10
Patti et al.[11] (1998)	30	Dor	90	10
Rosati et al.[32] (1998)	61	Dor	98.2	7
Wang et al.[33] (1998)	27	None	89	11
Present series	100	Dor	92	6.9

*Only studies with more than 20 treated patients are reported.

(surgery and dilatation) are the reasons why controlled randomized trials are difficult to conduct and conclusions should be drawn only after careful evaluation of prospective case-series studies. The most important parameter to consider in this type of study is treatment efficacy, that is, the ability of the treatment to relieve symptoms, with little or no risk to the patient, few side effects, and little discomfort; perhaps the cost of treatment should also be considered. An additional factor to take into account is the availability of the technique and, with regard to surgery, its reproducibility and the fact that it can be taught. In terms of controlling symptoms, our study shows that, based on a 5-year follow-up, the probability of effecting a cure by means of laparoscopic myotomy is 90% and other authors report remarkably similar results (Table III).

Dysphagia in these patients was cured by lowering the LES resting pressure to less than 10 mm Hg and the LES nadir pressure at swallowing to less than 6 mm Hg. This was achieved with no deaths and a 6% morbidity rate, with only one undetected mucosal tear. This low morbidity rate compares well with the outcome of endoscopic dilatation, which carries a 1% to 8% perforation rate at the level of the cardia with an associated mortality rate of 0.2% to 0.4%. Most of these studies were performed retrospectively, however, and focused only on the primary complication (i.e., esophageal perforation). Recently a prospective study on the complications associated with forceful dilatation recorded an incidence of 14% for chest pain lasting several days after the procedure, 13% for traumatic diverticula, 3% for intramural hematoma, and 1.5% for both reflux and perforation.[17] Most of these complications have rarely, if ever, been observed after surgical myotomy. Those who favor forceful dilatation claim that this procedure is performed mainly as an outpatient procedure or with a 24-hour hospital stay. It should be taken into account, however, that many authors perform progressive esophageal dilatations in three or more sessions, and even those who perform dilatations in a single session report that a second dilatation is needed in 20% of patients. It may be estimated that, overall, half of the patients treated with dilatation require a second or even a third session. Probably the cost comparison between endoscopic dilatation and laparoscopic myotomy still favors the former (because of the high costs of the operating room and instrumentation), but the decrease in the hospital stay to a few days and the rapid return to work have markedly reduced this difference. Moreover, the hospital stay might be cut down to 1 day or even less[11] (as is the case in countries where the pressures on health services to shorten the hospital stay are much greater than in Italy and patients are more motivated to appreciate early discharge), thus further narrowing the gap between endoscopic dilatation and laparoscopic myotomy. Most studies report a 60% to 80% success rate for dilatation, but the previously mentioned prospective study on forceful dilatation indicated a less optimistic outcome, with only a 48% chance of remaining asymptomatic at 5 years, even under the most favorable conditions. These results of esophageal dilatation should be borne in mind when deciding on a treatment course for a patient with achalasia, especially when a "single-shot" treatment with little patient discomfort and a 90% probability of cure persisting at 5 years is available.

The pressure exerted by the LES in the distal esophagus is the main barrier to gastroesophageal reflux and weakening of the distal esophagus may lead to the abnormal presence of gastrointestinal contents in the gullet. In addition, patients with achalasia have poor peristalsis with inadequate clearance and gastroesophageal reflux disease (GERD) is potentially more

harmful than it is in normal subjects. The incidence of GERD and methods for its prevention have been (and still are) controversial issues. Ellis[18] reported effective control of dysphagia (90%) with a very low incidence of reflux (5%) using only a transthoracic myotomy without fundoplication. In their extensive review of the literature, Andreollo and Earlam[19] concluded that GERD occurred in 7% of patients after thoracotomic myotomy and in 13% after a laparotomic approach, if no antireflux procedure was added, concluding that the cause of reflux was a myotomy extending too far toward the stomach. Mattioli et al.[20] demonstrated, however, that this part of the operation (i.e., sectioning of the sling gastric fibers) and extension of the myotomy 1.5 to 2 cm toward the stomach is essential to reduce LES pressure. In most of the previous studies, moreover, assessment of reflux was based only on the presence of symptoms—and symptoms are not reliable markers of reflux.[21] When objective measurement of reflux was performed using 24-hour pH monitoring after a thoracotomy or a thoracoscopic approach, a much higher incidence was reported, ranging from 21% to 60%.[11,22,23] These and other reports, however, were based only on a handful of patients, and it is difficult to draw any definite conclusions from them. In our study we managed to perform 24-hour pH monitoring 6 months after the operation in more than three fourths of our patients, thus obtaining a sample large enough to determine the true incidence of postoperative GERD. Our results confirmed the low incidence of symptomatic reflux, but the objective incidence of abnormal esophageal acid exposure, with a clear GERD pattern, amounted to 6.9% in the patients studied. These data clearly show that GERD is a potential problem after myotomy (if extended through the sling gastric fibers) and that some additional measures beyond the myotomy should be implemented. The selection of a particular type of fundoplication was based on certain physiopathologic and technical considerations. First, fundoplication should not create a new obstacle to the passage of the bolus through the cardia—especially in those patients who have virtually no esophageal body motility. So partial fundoplication, rather than a full 360-degree fundoplication, seems more appropriate; the two most commonly used antireflux techniques after Heller myotomy are the partial anterior 180-degree Dor fundoplication[24] and the partial posterior 270-degree Toupet fundoplication.[25] Both procedures have been reported to prevent reflux after myotomy. From a technical standpoint, posterior fundoplication holds the myotomy open and probably offers better reflux control in the supine position,[10] but it requires full mobilization of the abdominal esophagus (which we do not routinely perform) along with mobilization of the gastric fundus. On the other hand, anterior fundoplication protects the mucosa and covers any undetected small perforation. Our preference for this technique stems from our previous experience with open surgery[26] and from the fact that because we do not perform any posterior dissection, anterior fundoplication is easier to perform. There is no evidence to support our choice, however, and only a trial comparing the two most frequently used techniques will resolve this issue.

The reasons for failure after myotomy are difficult to determine and three main mechanisms have been postulated: incomplete myotomy, scarring of the myotomy, and reflux esophagitis.[27] In our experience, manometry provided us with little information, since the pressure during swallowing is recorded only at one point of the myotomized sphincter, and small areas where muscle fibers are still present (incomplete myotomy) are difficult to demonstrate with this test. Patients, even if they undergo incomplete myotomy, report an improvement of their dysphagia, which returns (although in our experience it is less severe than preoperatively) a few months after the operation. The reasons for failure can be better investigated with barium swallow video fluoroscopy and by careful review of the videotape of the operation. Based on these two parameters (and on the absence of reflux on pH monitoring), we assumed that all but one of our failures were technical errors (incomplete myotomy), although we could not rule out the possibility of a fibrotic process. In one patient the myotomy probably should have been expanded further upward and in five patients downward. In the only patient who had a late recurrence, we presumed that this was due to a scarring process and in one last patient we were unable to assess the defect.

The most common criticism voiced against the widespread use of laparoscopic myotomy for the treatment of esophageal achalasia is that it is a "difficult" operation to perform and properly trained surgeons are not readily available. Our study shows that the learning curve for laparoscopic Heller myotomy is steep for about 20 patients and given the rarity of the disease, this figure is probably higher than the number of achalasia patients observed during the average career of most surgeons. However, our data also demonstrated that the institutional learning curve is relevant and that adequate proctorship can reduce the learning curve for individual surgeons. It is probably wise, therefore, to perform this operation only at designated referral centers, where experience can be gained and these centers can serve as "learning centers" for residents and other surgeons.

CONCLUSION

Our study demonstrates that laparoscopic Heller myotomy with partial Dor fundoplication is a feasible and effective treatment for achalasia with an actuarial success rate of 90% at 5 years. Postoperative gastroesophageal reflux remains a major concern and is detected, when properly investigated, in approximately 6% of patients. The operation is reproducible and can be taught, and the problem of the learning curve, which we estimated at 20 operations, can be overcome with the help of experienced preceptors.

REFERENCES

1. Sonnenberg A, Massey BT, McCarty DJ, Jacobsen SJ. Epidemiology of hospitalization for achalasia in the United States. Dig Dis Sci 1993;38:233-244.
2. Wong RKH, Maydonovitch CL. Achalasia. In Castell DO, ed. The Esophagus. Boston: Little, Brown, 1992, pp 233-260.
3. Vantrappen G, Janssens J. To dilate or to operate? That is the question. Gut 1983;24:1013-1019.
4. Csendes A, Velasco N, Braghetto I, Henriquez A. A prospective randomized study comparing forceful dilatation and esophagomyotomy in patients with achalasia of the esophagus. Gastroenterology 1981;80:789-795.
5. Parkman HP, Reynolds JC, Ouyang A, et al. Pneumatic dilatation or esophagomyotomy treatment for symptomatic achalasia: Clinical outcomes and cost analysis. Dig Dis Sci 1993;38:75-85.
6. Shimi S, Nathanson L, Cuschieri A. Laparoscopic cardiomyotomy for achalasia. J R Coll Surg Edinb 1991;36:152-154.
7. Ancona E, Peracchia A, Zaninotto G, et al. Heller laparoscopic cardiomyotomy with antireflux anterior fundoplication (Dor) in the treatment of esophageal achalasia. Surg Endosc 1993;7:459-461.
8. Anselmino M, Zaninotto G, Costantini M, et al. One-year laparoscopic Heller-Dor operation for esophageal achalasia. Surg Endosc 1997;11:3-7.
9. Raiser F, Perdikis G, Hinder RA, et al. Heller myotomy via minimal-access surgery. An evaluation of antireflux procedures. Arch Surg 1996;131:593-598.
10. Hunter JG, Trus TL, Branum GD, Waring P. Laparoscopic Heller myotomy and fundoplication for achalasia. Ann Surg 1997;225:655-665.
11. Patti M, Arcerito M, DePinto M, Feo CV, Tong J, Ganters W, Way LW. Comparison of thoracoscopic and laparoscopic Heller myotomy for achalasia. J GASTROINTEST SURG 1998;2:561-566.
12. Costantini M, Bremner RM, Hoeft SF, Crookes PF, DeMeester TR. The slow motorized pull-through. An improved technique to evaluate the lower esophageal sphincter [abstr.]. Gastroenterology 1992;103(A):1407.
13. Zaninotto G, DiMario F, Costantini M, et al. Oesophagitis and pH of the refluxate: Experimental and clinical study. Br J Surg 1992; 79:161-164.
14. Savary M, Miller G. The esophagus. In Handbook and Atlas of Endoscopy. Solothurn, Switzerland: Gassman, 1978.
15. Ancona E, Anselmino M, Zaninotto G, et al. Esophageal achalasia: Laparoscopic versus conventional open Heller-Dor operation. Am J Surg 1995;170:265-270.
16. Brookes SJ, Chen BN, Hodgson WM, Costa M. Characterization of excitatory and inhibitory motor neurons to the guinea pig lower esophageal sphincter. Gastroenterology 1996;111:108-117.
17. Eckardt VF, Kanzler G. Complications and their impact after pneumatic dilation for achalasia: Prospective long-term follow-up study. Gastrointest Endosc 1997;45:349-353.
18. Ellis FH Jr. Oesophagomyotomy for achalasia: A 22 years experience. Br J Surg 1993;80:882-885.
19. Andreollo NA, Earlam RJ. Heller's myotomy for achalasia: Is an added anti-reflux procedure necessary? Br J Surg 1987; 74:765-769.
20. Mattioli S, Pilotti V, Felice V, Di Simone MP, D'Ovidio F, Gozzetti G. Intraoperative study on the relationship between the lower esophageal sphincter pressure and the muscular components of the gastro-esophageal junction in achalasic patients. Ann Surg 1993;218:635-639.
21. Costantini M, Crookes PF, Bremner RM, et al. Value of physiological assessment of foregut symptoms in a surgical practice. Surgery 1993;114:780-787.
22. Streitz JM Jr, Ellis FH Jr, Williamson WA, Glick MF, Aas JA, Tiden RL. Objective assessment of gastroesophageal reflux after short esophagomyotomy for achalasia with the use of manometry and pH monitoring. J Thorac Cardiovasc Surg 1996;111:107-113.
23. Crookes PF, Corkill S, DeMeester TR. Gastroesophageal reflux in achalasia: When is reflux really a reflux. Dig Dis Sci 1997;42:1354-1361.
24. Dor J, Humbert P, Dor V, Figarella J. L'intéret de la technique de Nissen modifiée dans la prévention du reflux après cardiomyotomie extramuqueuse de Heller. Mem Acad Chir 1962;88:877-883.
25. Toupet A. Technique d'oesophago-gastroplastie avec phrenogastropexie appliquée dans la cure radicale des hernies hiatales et comme complement de l'operation de Heller dans les cardiospasmes. Acad Chir 1963;89:394-398.
26. Zaninotto G, Costantini M, Anselmino M, Boccù C, Ancona E. Onset of oesophageal peristalsis after surgery for idiopathic achalasia. Br J Surg 1995;82:1532-1534.
27. Fékété F, Breil PH, Tossen JC, et al. Reoperation after Heller's operation for achalasia and other motility disorders of the esophagus. A study of 81 re-operations. Int Surg 1982;67:103-110.
28. Morino M, Rebecchi F, Festa V, Garrone C. Preoperative pneumatic dilation represents a risk factor for laparoscopic Heller myotomy. Surg Endosc 1997;11:359-361.
29. Boulez J, Meeus P, Espalieu PH. Oesocardiomyotomie de Heller sans anti-reflux par voie laparoscopique. Analyse d'une serie de 27 cas. Ann Chir 1997;51:232-236.
30. Graham AJ, Finley RJ, Worsley DF, Dong SR, Clifton JC, Storseth C. Laparoscopic esophageal myotomy and anterior partial fundoplication for the treatment of achalasia. Ann Thorac Surg 1997;64:785-789.
31. Vogt D, Curet M, Pitcher D, Josloff R, Milne RL, Zucker K. Successful treatment of esophageal achalasia with laparoscopic Heller myotomy and Toupet fundoplication. Am J Surg 1997; 174:709-714.
32. Rosati R, Fumagalli U, Bona S, Bonavina L, Pagani M, Peracchia A. Evaluating results of laparoscopic surgery for esophageal achalasia. Surg Endosc 1998;12:270-273.
33. Wang PC, Sharp KW, Holzman MD, Clements RH, Holcomb GW, Richards WO. The outcome of laparoscopic Heller myotomy without antireflux procedure in patients with achalasia. Am Surg 1998;64:515-521.

Esophagogastric Adenocarcinoma in an E1A/E1B Transgenic Model Involves p53 Disruption

Mark D. Duncan, M.D., Tarik Tihan, M.D., Ph.D., David M. Donovan, Ph.D., Quy H. Phung, Ph.D., Dan L. Rowley, Ph.D., John W. Harmon, M.D., Patricia J. Gearhart, Ph.D., Kimberly L.K. Duncan, Ph.D.

We studied tumorigenesis and p53 immunostaining in a murine transgenic model introducing E1A/E1B under the control of the mouse mammary tumor virus–long terminal repeat (MMTV-LTR) promoter in which adenocarcinoma occurs at the squamocolumnar junction in the foregut, predominantly in males, and at no other site. Mutations of p53 are frequent in human esophageal adenocarcinoma and the E1B gene product interferes with p53-mediated apoptosis, inhibiting tumor suppression at the G_1/S checkpoint. Transgenic animals were generated utilizing a purified linear 6.7 kb fragment of plasmid DNA containing MMTV-LTR/E1A/E1B and were confirmed by dot blot hybridization of tail DNA to ^{32}P-labeled E1A/E1B probe and polymerase chain reaction (PCR) amplification of E1A. Transgenic and control animals were observed for morbidity and weight changes. Eleven of 45 animals were transgenic (24% efficiency) with an estimated 5 to 57 copies of the gene per genome. Profound weight loss (>20%) led to sacrifice or death of one of five females (at 12 weeks) and four of six males (at 16 to 17 weeks). Grossly visible tumors (2 to 10 mm) were noted in the forestomach at the visible margin between the proximal (squamous-lined) stomach and the distal glandular stomach. Histologic sections confirmed adenocarcinoma arising in each case at the squamocolumnar junction with glandular formation, pleomorphism, and frequent mitotic figures. Immunostaining was positive for p53 indicating accumulation of mutated or altered p53 protein. E1A/E1B transgenic animals developed macroscopic and microscopic adenocarcinoma at the squamocolumnar junction, which corresponds to adenocarcinoma at the human esophagogastric junction. Disruption of p53 was present in the transgenic model as in the human cancer. (J GASTROINTEST SURG 2000;4:290-297.)

KEY WORDS: Esophageal cancer, transgenic, p53

Esophagogastric adenocarcinoma has the most rapidly increasing incidence of any malignancy in the United States.[1,2] Although the sequence of genetic alterations in this tumor is not characterized, frequent mutations involve p53 and genes controlling DNA transcription.[3,4] To better understand esophagogastric tumorigenesis, we studied a murine transgenic model introducing E1A/E1B under the control of the mouse mammary tumor virus–long terminal repeat (MMTV-LTR) promoter in which adenocarcinoma occurs, predominantly in males, at the squamocolumnar junction in the foregut and at no other site.[5] This important finding was described as a model for gastric carcinoma.[6] As the human anatomic correlate of the squamocolumnar junction is the gastroesophageal junction, and the histology of the tumors demonstrated adenocarcinoma characteristics, we thought this transgenic system was worth pursuing as a model for esophagogastric adenocarcinoma.

From the Departments of Surgery (M.D.D., J.W.H., and K.L.K.D.) and Pathology (T.T.), The Johns Hopkins University School of Medicine, and the Laboratory of Molecular Genetics (Q.H.P. and P.J.G.) and the Transgenic and Knockout Facility (D.M.D. and D.L.R.), National Institute on Aging, National Institutes of Health, Baltimore, Md.

Supported by the Johns Hopkins University School of Medicine Discovery Fund and by the Richard S. Ross Clinician Scientist Career Development Award (M.D.D.), Baltimore, Md.

Presented at the Fortieth Annual Meeting of The Society for Surgery of the Alimentary Tract, Orlando, Fla., May 16-19, 1999, and published as an abstract in *Surgical Forum* 49:141-142, 1998.

Reprint requests: Mark D. Duncan, M.D., Section of Surgical Science, A-563, Johns Hopkins Bayview Medical Center, 4940 Eastern Avenue, Baltimore, MD 21224. e-mail: mduncan@jhmi.edu

Esophagogastric adenocarcinoma is increasing in incidence at a rate of 5% to 10% per year.[1,2] The prognosis for this disease is dismal and current therapies are inadequate. Most adenocarcinomas of the esophagus arise in regions of Barrett's metaplasia.[7-10] Esophagogastric adenocarcinoma evolves through disruption of mechanisms of cell growth control such as loss of the RB gene, loss of p53,[3,11-13] loss of MTS-1, cyclin D1 overexpression, or alterations in epidermal growth factor/epidermal growth factor receptor.[14,15] p53 is a tumor suppressor gene encoding for a protein that acts as a transcriptional activator of genes involved in cell cycle arrest and apoptosis. Changes in p53 expression through loss of heterozygosity at 17p or mutation occur in one third to two thirds of all cases of esophageal cancer.[3,13,16-18] and in patients with Barrett's esophagus.[3,14,17-19] p53 overexpression was found with increasing frequency in patients with Barrett's esophagus correlating with the severity of dysplasia.[13,18] Alterations in p53 likely affect progression of Barrett's esophagus to cancer.[13] Most p53 mutations lead to formation of an inactive protein product, which accumulates in the cell and can readily be detected by immunohistochemical analysis.[3,13,19]

There are few animal models in which to study either Barrett's esophagus or adenocarcinoma.[20] One promising model uses rats with different surgically induced reflux preparations receiving intravenous administration of the carcinogen methyl-N-amyl nitrosamine. These animals develop squamous cell and adenocarcinoma after 9 months.[21,22] The formation of esophageal adenocarcinoma in this model may be exacerbated by alkaline reflux.[21,23] A transgenic murine model expressing cyclin D1 leading to esophageal squamous dysplasia, but not adenocarcinoma, has recently been reported.[24] Earlier, Koike et al.[6] described a transgenic mouse wherein tumors with characteristics of adenocarcinoma formed at the squamocolumnar junction in the foregut. This mouse contains the human adenovirus 12 early region genes E1A/E1B under the control of the MMTV-LTR promoter. The E1A gene product is a transcriptional activator that binds to cyclins and to the Rb gene product, permitting transcription at the G_1/S checkpoint.[25] The E1B gene products interfere with p53-mediated apoptosis.[26] Together E1A and ElB are tumorigenic. Tumors in these animals were predominantly in males (10 of 12 vs. 2 of 13 in females), implicating a genetic predisposition by gender. No tumors or neoplasia were found in any tissue apart from the forestomach and intestinal metastases.[6] The original investigators were unable to breed the animals to create a stable line. The studies presented herein confirm, expand, and redefine the importance of Koike's observations.

METHODS

Generation of E1A/E1B Transgenic Animals

Dr. Gilbert Jay (American Red Cross, Rockville, Md.) kindly provided *Escherichia coli* bacteria harboring the plasmid vector *pMTVAd12* carrying the transgene of interest (Fig. 1). Plasmid DNA was isolated and digested with *Eco*R I and *Nde* I, which generated a 6.7 kb fragment containing the E1A/E1B genes together with the MMTV-LTR promoter region. DNA purification (Qiagen protocol, Qiagen Inc., Chatsworth, Calif.) followed by electroelution separation and chromatography and subsequent dialysis against injection buffer yielded a purified linear fragment for transgenic microinjection (Fig. 2). Pronuclear microinjections were performed on separate occasions into single-cell embryos (strain B6C3F1 × B6D2F1), which were implanted into pseudopregnant female mice. Successful E1A/E1B transgenic offspring were identified by Southern blot and dot blot hybridization of tail DNA to ^{32}P-labeled E1A/E1B probe and polymerase chain reaction amplification with a primer pair for the E1A gene with the sequences 5′-ACCCTCACCCCCGAAACT (position 422 to 442) and 5′-ATCAGACACCCCACAAACCATA (position 1988 to 2012). Gene copy numbers were estimated by densitometric quantification of hybridization in dot blots, and compared to a single copy per genome.

Monitoring for Tumor Occurrence

Animals were weighed twice a week, and tumor occurrence was assessed by weight loss and by careful observation for signs of anorexia or morbidity. Nontransgenic litter mates served as control subjects. Animals were killed by intraperitoneal barbiturate injection (pentothal, 240 mg/kg) if weight loss reached 20%. At necropsy, tissue was harvested from the gastrointestinal tract including the squamous lining of the esophagus and proximal stomach, the squamocolumnar junction, the columnar lining of the distal stomach, and the distal bowel. Tissue was divided and paired samples were either fixed in formalin for histologic examination or flash-frozen in liquid nitrogen and stored at −80° C for subsequent extraction of DNA and RNA. All procedures were carried out and animals were cared for in accordance with guidelines set forth in the "Guide for the Care and Use of Laboratory Animals," published in 1996 by the National Research Council, and also in accordance with specifications in the handbook entitled, "The Use of Experimental Animals at Johns Hopkins University," published in 1996 by the Division of Comparative Medicine at Johns Hopkins University. The protocol was approved by the Animal Care and Use Committee of the Johns Hopkins University School of Medicine.

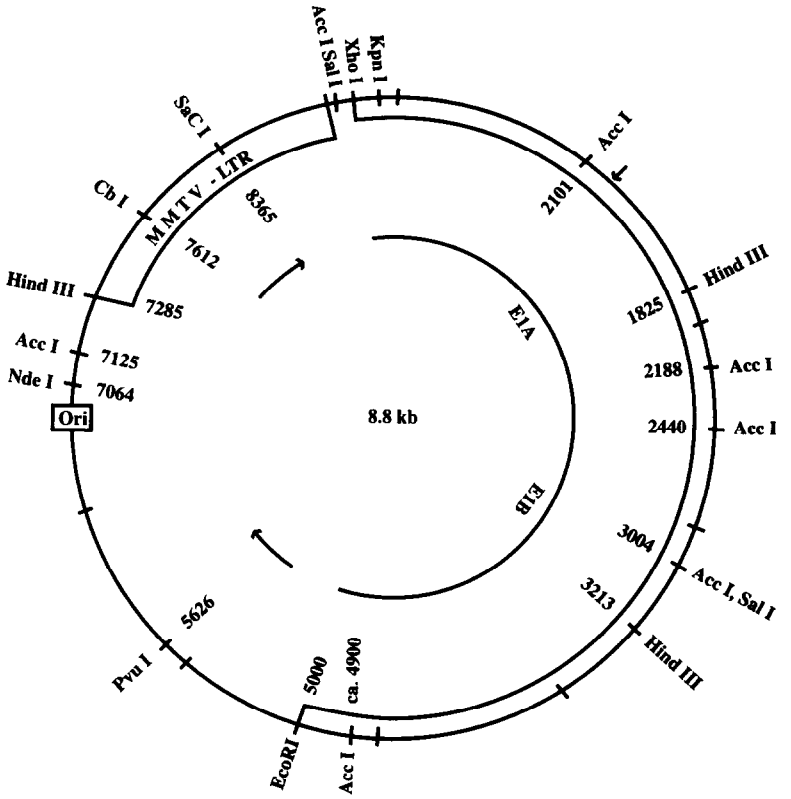

Fig. 1. pMTVAd12 plasmid containing MMTV-LTR, E1A, and E1B genes with an antibiotic resistance gene. (Restriction map provided by Dr. Gilbert Jay.)

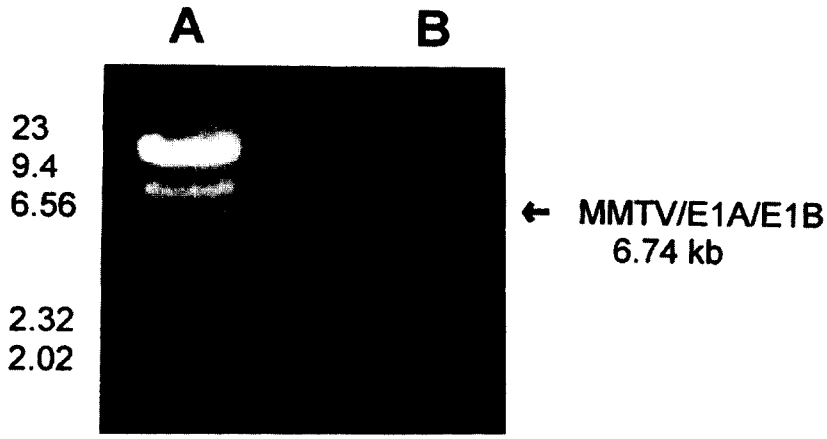

Fig. 2. Purified linear 6.7 kb MMTV-LTR/E1A/E1B fragment. Lane A = molecular weight markers, lane B= DNA for microinjection.

Immunohistochemistry

Formalin-fixed slides were subject to antigen retrieval methods using target unmasking fluid (PharMingen International, San Diego, Calif.), and an avidin-biotin peroxidase chromogen. Evaluation of immunohistochemical stains was performed by one pathologist. Analysis of p53 immunohistochemistry was performed using the D07 monoclonal antibody (DAKO Corp., Carpinteria, Calif.), which has been validated in neoplasia including esophageal and gastric adenocarcinomas. Mutations in the p53 gene can lead to an increased stability of the mutated gene product and accumulation of this protein within the nuclei. Immunohistochemical analysis of proliferating cell nuclear antigen (PCNA) was performed with the PC10 monoclonal antibody (DAKO Corp.). The DAKO animal research kit was used to mask endogenous murine immunoglobulin G and eliminate nonspecific background staining.

RESULTS

Eleven of 45 animals were transgenic (24% efficiency) with an estimated 5 to 57 copies of the gene per genome. PCR amplification of tail DNA with E1A primer sequences allowed definitive identification of transgenic animals (Fig. 3). Weight curves for transgenic males and nontransgenic litter mates demonstrated a steep decline in the weight of transgenic animals, which corresponded to the observed listlessness and ruffled fur (Fig. 4). Profound weight loss (>20%) led to sacrifice or death of one of five females (at 12 weeks) and four of six males (at 13 to 17 weeks) from our initial series. There was no significant

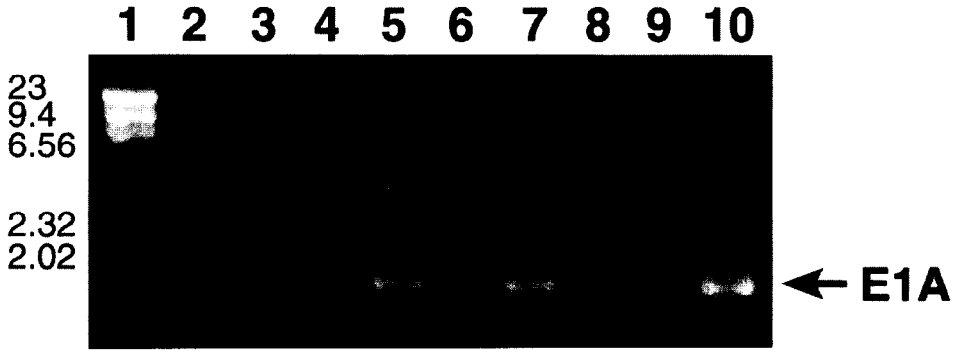

Fig. 3. PCR amplification of tail DNA with E1A primer pairs. A discrete band identifies transgenic animals (lanes 5 and 7) with a positive control of linearized E1A/E1B plasmid (lane 10); negative control wild-type C57 (lane 9) and *Hind* III markers (lane 1).

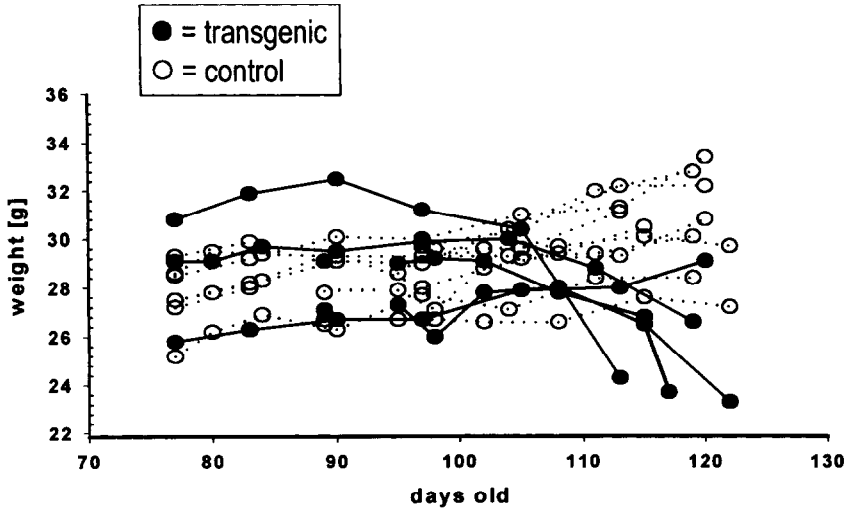

Fig. 4. Weight curves demonstrating weight loss in transgenic animals between 100 and 125 days.

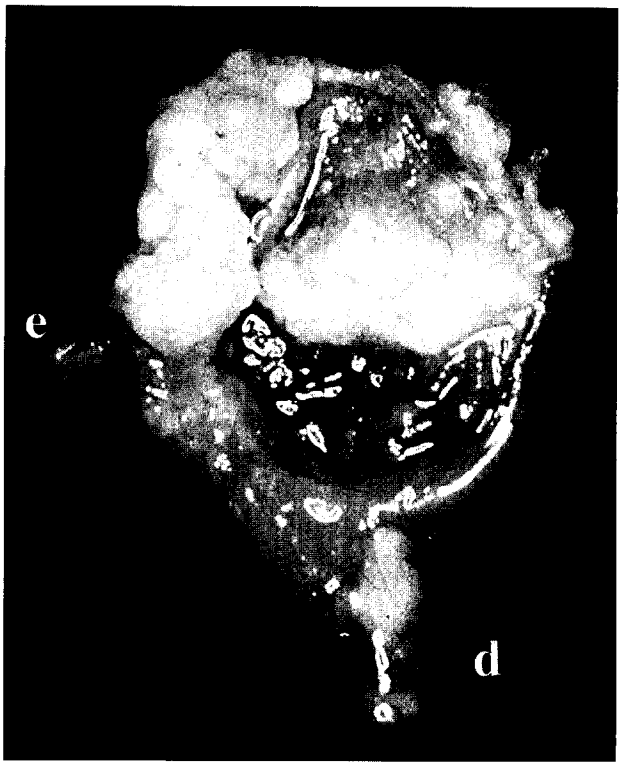

Fig. 5. Gross tumor in forestomach of transgenic mouse arising at the margin between the shiny proximal squamous-lined stomach and the thicker rugae of the distal columnar-lined stomach. e = esophagus; d = duodenum.

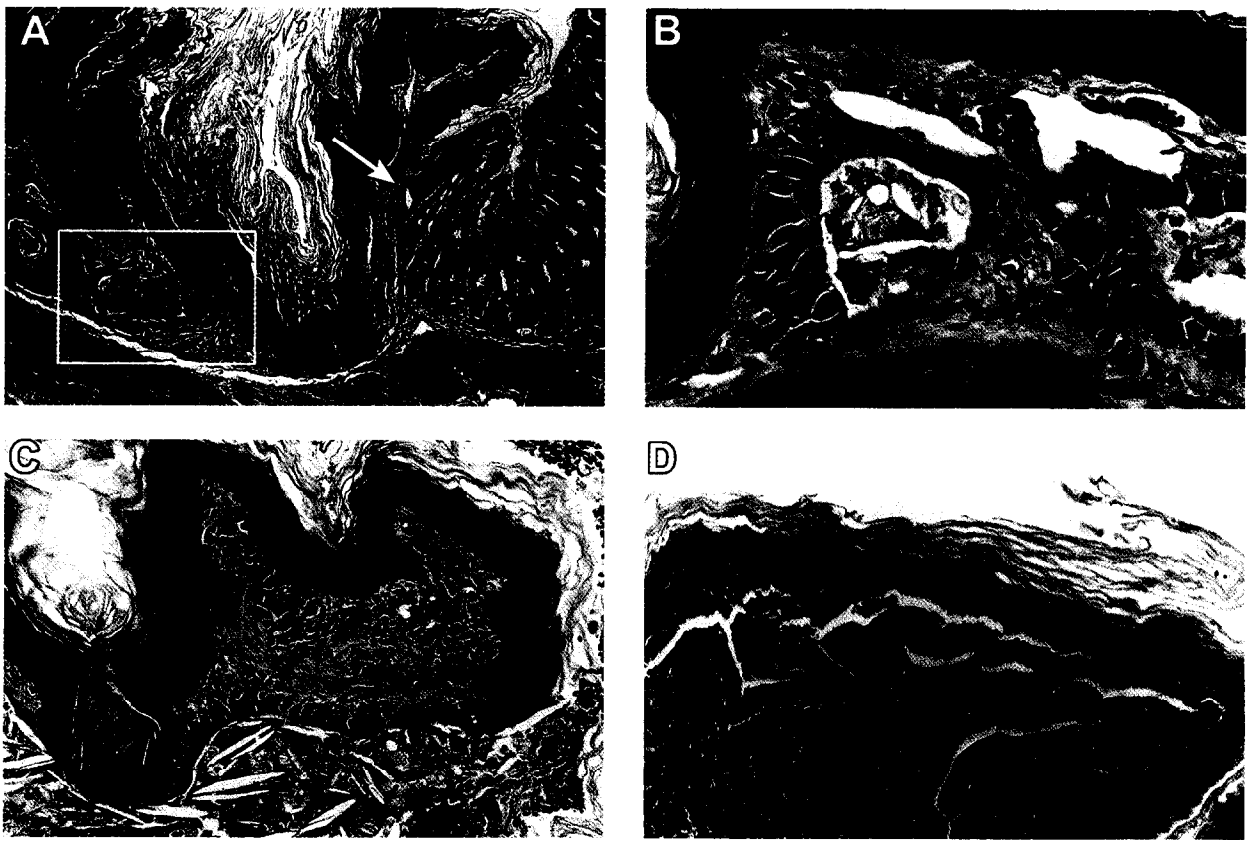

Fig. 6. A, Squamocolumnar junction in a transgenic mouse. A sharp transition *(arrow)* is seen between the squamous mucosa (left) and the glandular mucosa (right). A focus of adenocarcinoma is present beneath the squamous mucosa on the lower left *(inset)* (original magnification ×100). **B,** High-power view of malignant glands with marked pleomorphism, hyperchromasia, mitoses, and intraluminal necrotic material (original magnification ×400). **C,** Adenocarcinoma in another transgenic animal arising beneath the squamous mucosa adjacent to the squamocolumnar junction. Tumor cells show marked hyperchromasia and invasion into the submucosal layer (original magnification ×200). **D,** Adenocarcinoma in another transgenic animal with malignant glandular formation including some poorly differentiated areas occurring beneath normal squamous mucosa (original magnification ×200).

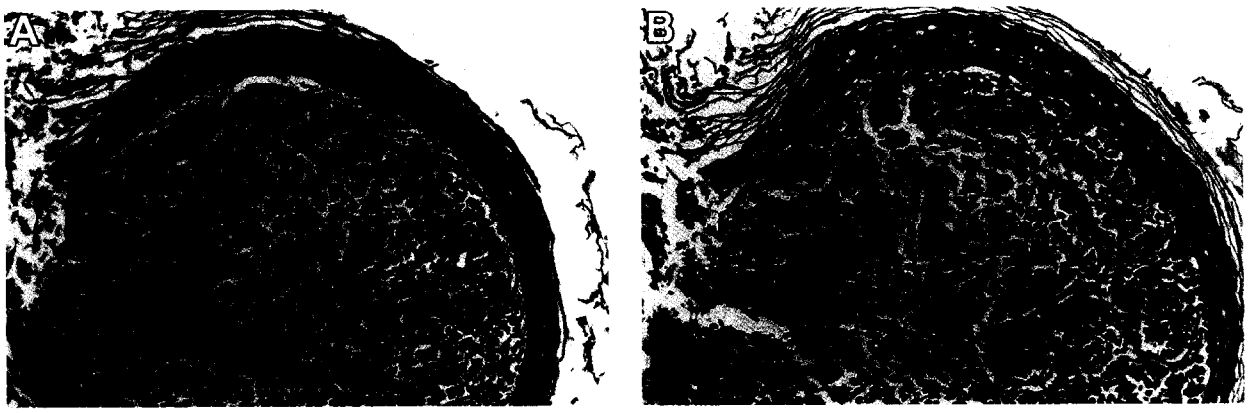

Fig. 7. A, Adenocarcinoma arising beneath normal squamous mucosa adjacent to the squamocolumnar junction (hematoxylin and eosin stain; original magnification ×200). **B,** Immunohistochemistry for p53 with monoclonal antibody D07 of the same section showing intense nuclear and some cytoplasmic staining (dark brown). Normal squamous mucosa does not stain for p53; basal cells of squamous mucosa (dark blue nuclei; some light brown cytoplasm) are normal (hematoxylin and eosin stain; original magnification ×200).

Table I. Pathologic findings and p53 immunostaining of transgenic animals

	Weight loss	Age (wk)	Pathology	p53
Male (n = 6)	4	16	Adenocarcinoma	+
		16	Adenocarcinoma	+
		17	Adenocarcinoma	+
		17	(unavailable)	
Female (n = 5)	1	12	Adenocarcinoma, stromal and poorly differentiated tumors	+

weight loss in nontransgenic animals. Grossly visible tumors (2 to 10 mm) were noted in the forestomach of the female and two of three examined males at the margin between the shiny proximal squamous-lined stomach and the thicker rugae of the distal stomach (Fig. 5). At necropsy, no other tissue showed evidence of tumors.

Histologic sections confirmed adenocarcinoma arising at the squamocolumnar junction in all transgenic animals. A sharp transition was seen between the squamous mucosa and the glandular mucosa with foci of adenocarcinoma present beneath the squamous mucosa (Fig. 6). Tumor cells showed marked pleomorphism, hyperchromasia, mitoses, and invasion into the submucosal layer. All males with tumors demonstrated microscopic and macroscopic adenocarcinoma, in each case arising in the area of the squamocolumnar junction. One female mouse developed a multiphenotype undifferentiated carcinoma with glandular and sarcomatoid features at the squamocolumnar junction in addition to adenocarcinoma.

Tissue sections were studied by immunohistochemical analysis for p53 protein and PCNA. Antibody for p53 protein stained positive in all four of the tumors from transgenic animals studied (Table I) and in one of two tumors from transgenic animals generated subsequent to the current study. Both cytoplasmic and nuclear staining were present with p53 (Fig. 7), although staining was not uniform and some cells within the tumors did not stain. There was no staining for p53 outside of the tumor cells. All tumors had strong diffuse positivity for PCNA as expected with proliferating tumor cells.

DISCUSSION

E1A/E1B transgenic animals developed macroscopic and microscopic adenocarcinoma at the squamocolumnar junction, which corresponds to adenocarcinoma at the human esophagogastric junction. Disruption of p53 was present in all tumors in this transgenic model with accumulation of altered p53

protein demonstrated by immunostaining. A male predominance in adenocarcinoma formation in these transgenic mice was suggested.

This tumor model represents an orthotopic model of adenocarcinoma arising at the site of the most rapidly increasing cancer in the United States, and provides insights into why tumors may form at the squamocolumnar junction. We have not demonstrated Barrett's changes in the mucosa; however, we have waited for a 20% weight loss before sacrificing animals. Because the squamocolumnar junction in rodents lies within the stomach, the junction and squamous mucosa are already exposed to gastric juice including acid and bile. In the future we expect to examine tissue at the squamocolumnar junction prior to the appearance of tumor. We will be able to characterize progressive stages of carcinogenesis using this approach.

The MMTV-LTR promoter is frequently used in transgenic models, as is, separately, the E1A/E1B sequence. It is only the unique combination that yields the specific phenotype of foregut adenocarcinomas. The squamocolumnar junction is not generally associated with hormonal regulation or with the MMTV promoter, yet tumors arose only at this site. The E1A/E1B transgene in this model was found by Koike et al.[6] to be uniformly expressed in the testes and in tumors at the squamocolumnar junction, with only sporadic expression in other tissues; messenger RNA for E1A and E1B was present in high levels in the stomach and testes, with low levels in the brain, and in some transgenic animals in the lung, liver, marrow, and other sites. Male transgenic mice are more susceptible to adenocarcinoma development, despite expression of the transgene in females. The MMTV promoter is hormone sensitive. This suggests that the squamocolumnar junction in the forestomach, like the testes, may be subject to hormonal stimulation. We believe hormonal stimulation of MMTV promoter-driven transcription may be a necessary stimulus for adenocarcinoma formation in this model, but was not sufficient, as no tumors formed in the testes. We are currently studying androgen and estrogen receptor status in the transgenic adenocarcinomas, as well as in the normal human esophagus and clinical esophageal adenocarcinoma. The squamocolumnar junction may also be uniquely sensitive to the tumorigenic potential of the E1A and E1B gene products.

The known oncogenic properties of the human adenovirus 12 E1A and E1B genes may elucidate pathways of carcinogenesis in the human disease. The E1B gene products interfere with p53-mediated apoptosis, inhibiting tumor suppression at the G_1/S checkpoint. E1B encodes two proteins of 55 kD and 19 kD. The E1B 55K product binds to the amino terminus of p53 blocking p53 transcription stimulating activity and thus suppressing apoptosis.[27] The 19K product inhibits p53-mediated apoptosis through a separate, undefined mechanism not involving direct binding.

The presence of accumulated p53 protein by immunostaining in this transgenic model may be explained in part by E1B binding. We have demonstrated in adenocarcinoma at the squamocolumnar junction in the E1A/E1B transgenic model that p53 staining is both cytoplasmic and nuclear. This may be accounted for by E1B effects, or may also include potential genetic alteration. If the squamocolumnar junction is uniquely prone to adenocarcinoma formation, it is likely in part mediated by the known mutations determined from clinical specimens; mutations in p53 are common in esophagogastric adenocarcinoma. Although E1B is known to interfere with p53 function, this may be incomplete. Furthermore, in the transgenic model, E1A and E1B are expressed in tissues other than the squamocolumnar junction without tumor formation, implying that the effects of E1A and E1B, even when transcribed, are not sufficient for tumorigenesis.

Each transgenic animal is the result of a separate microinjection, and experiments were initiated several months apart indicating the reproducibility of the model. We have demonstrated success with repeat microinjections in generating successive sets of MMTV/E1A/E1B transgenic mice, which develop adenocarcinoma at the squamocolumnar junction. The strength of this transgenic model lies in the remarkably specific localization of tumor formation, specifically adenocarcinoma predominantly in males, at the squamocolumnar junction. Genetic similarities with the human disease strengthen the role for this model in the study of the etiology of clinical esophageal adenocarcinoma.

CONCLUSION

E1A/E1B transgenic animals developed macroscopic and microscopic adenocarcinoma at the squamocolumnar junction, which corresponds to adenocarcinoma at the human esophagogastric junction. Disruption of p53 was present in the transgenic model as in the human cancer. This reproducible transgenic model of human esophageal adenocarcinoma at the esophagogastric junction allows identification and characterization of precursor genetic and histologic changes at this "hot spot" for carcinogenesis, and further implicates hormonal status as contributing to tumorigenesis at this site.

REFERENCES

1. Daly JM, Karnell LH, Menck HR. National Cancer Data Base report on esophageal carcinoma. Cancer 1996;78:1820-1828.
2. Blot WJ, Devesa SS, Fraumeni JFJ. Continuing climb in rates of esophageal adenocarcinoma: An update [letter]. JAMA 1993;270:1320.
3. Moskaluk CA, Heitmiller R, Zahurak M, Schwab D, Sidransky D, Hamilton SR. p53 and p21(WAF1/CIP1/SDI1) gene products in Barrett esophagus and adenocarcinoma of the esophagus and esophagogastric junction. Hum Pathol 1996; 27:1211-1220.
4. Wong DJ, Barrett MT, Stoger R, Emond MJ, Reid BJ. p16INK4a promoter is hypermethylated at a high frequency in esophageal adenocarcinomas. Cancer Res 1997;57:2619-2622.
5. Duncan MD, Donovan DM, Phung QH, Tihan T, Harmon JW, Duncan KLK. Tumorigenesis at the squamocolumnar junction in an E1A/E1B transgenic model of esophagogastric adenocarcinoma. Surg Forum 1998;49:141-142.
6. Koike K, Hinrichs SH, Isselbacher KJ, Jay G. Transgenic mouse model for human gastric carcinoma. Proc Natl Acad Sci USA 1989;86:5615-5619.
7. Rosenberg JC, Budev H, Edwards RC, Singal S, Steiger Z, Sundareson AS. Analysis of adenocarcinoma in Barrett's esophagus utilizing a staging system. Cancer 1985;55:1353-1360.
8. Hamilton SR, Smith RR, Cameron JL. Prevalence and characteristics of Barrett esophagus in patients with adenocarcinoma of the esophagus or esophagogastric junction. Hum Pathol 1988;19:942-948.
9. Barrett NR. Chronic peptic ulceration of the oesophagus and "oesophagitis." Br J Surg 1950;38:175-182.
10. Barrett NR. The lower esophagus lined by columnar epithelium. Surgery 1957;41:881-894.
11. Vogelstein B, Kinzler KW. p53 function and dysfunction. Cell 1992;70:523-526.
12. Blount PL, Galipeau PC, Sanchez CA, Neshat K, Levine DS, Yin J, Suzuki H, Abraham JM, Meltzer SJ, Reid BJ. 17p allelic losses in diploid cells of patients with Barrett's esophagus who develop aneuploidy. Cancer Res 1994;54:2292-2295.
13. Younes M, Lebovitz RM, Lechago LV, Lechago J. p53 protein accumulation in Barrett's metaplasia, dysplasia, and carcinoma: A follow-up study. Gastroenterology 1993;105:1637-1642.
14. al-Kasspooles M, Moore JH, Orringer MB, Beer DG. Amplification and over-expression of the EGFR and erbB-2 genes in human esophageal adenocarcinomas. Int J Cancer 1993;54: 213-219.
15. Filipe MI, Jankowski J. Growth factors and oncogenes in Barrett's oesophagus and gastric metaplasia. Endoscopy 1993;25: 637-641.
16. Casson AG, Kerkvliet N, O'Malley F. Prognostic value of p53 protein in esophageal adenocarcinoma. J Surg Oncol 1995;60: 5-11.
17. Casson AG, Mukhopadhyay T, Cleary KR, Ro JY, Levin B, Roth JA. Oncogene activation in esophageal cancer. J Thorac Cardiovasc Surg·1991;102:707-709.
18. Ramel S, Reid BJ, Sanchez CA, Blount PL, Levine DS, Neshat K, Haggitt RC, Dean PJ, Thor K, Rabinovitch PS. Evaluation of p53 protein expression in Barrett's esophagus by two-parameter flow cytometry [see comments]. Gastroenterology 1992;102:1220-1228.
19. Casson AG, Manolopoulos B, Troster M, Kerkvliet N, O'Malley F, Inculet R, Finley R, Roth JA. Clinical implications of p53 gene mutation in the progression of Barrett's epithelium to invasive esophageal cancer. Am J Surg 1994;167:52-57.
20. Rosenberg JC, Lichter AS, Leichman LP. Cancer of the esophagus. In De Vita VT, Hellman S, Rosenberg SA, eds. Cancer, Principles and Practice of Oncology. Philadelphia: Lippincott, 1989, pp 753-757.
21. Fein M, Peters JH, Chandrasoma P, Ireland AP, Öberg S, Ritter MP, Bremner CG, Hagen JA, DeMeester TR. Duodenoesophageal reflux induces esophageal adenocarcinoma without exogenous carcinogen. J GASTROINTEST SURG 1998;2: 260-268.
22. Pera M, Trastek VF, Carpenter HA, Fernandez PL, Cardesa A, Mohr U, Pairolero PC. Influence of pancreatic and biliary reflux on the development of esophageal carcinoma. Ann Thorac Surg 1993;55:1386-1392.
23. Yamashita Y, Homma K, Kako N, Clark GW, Smyrk TC, Hinder RA, Adrian TE, DeMeester TR, Mirvish SS. Effect of duodenal components of the refluxate on development of esophageal neoplasia in rats. J GASTROINTEST SURG 1998;2: 350-355.
24. Jenkins TD, Mueller A, Odze R, Shahsafaei A, Zukerberg LR, Kent R, Stoner GD, Rustgi AK. Cyclin D1 overexpression combined with N-nitrosomethylbenzylamine increases dysplasia and cellular proliferation in murine esophageal squamous epithelium. Oncogene 1999;18:59-66.
25. Nevins JR. Adenovirus E1A:Transcription regulation and alteration of cell growth control. Curr Topics Microbiol Immunol 1995;199:25-32.
26. White E. Regulation of p53-dependent apoptosis by E1A and E1B. Curr Topics Microbiol Immunol 1995,199:34-58.
27. Moran E. Interaction of adenoviral proteins with pRB and p53. FASEB J 1993;7:880-885.

Standard Roux-en-Y Gastrojejunostomy vs. "Uncut" Roux-en-Y Gastrojejunostomy: A Matched Cohort Study

Rodrigo A. Mon, M.D., Joseph J. Cullen, M.D.

Roux-en-Y gastrojejunostomy is a common method of reconstruction after subtotal gastrectomy. Maintaining myoneural continuity has been proposed to decrease the incidence of Roux stasis syndrome, with an "uncut" Roux-en-Y reconstruction. The aim of our study was to compare the clinical results in patients who have undergone uncut Roux-en-Y gastrojejunostomy with those in patients who have undergone a standard Roux-en-Y gastrojejunostomy. Eleven patients underwent gastrectomy and uncut Roux-en-Y gastrojejunostomy and were compared with a cohort of 14 patients who underwent gastrectomy and standard Roux-en-Y gastrojejunostomy. Patients were contacted and charts were reviewed for Visick grade, early and late morbidity and mortality, and incidence of staple line dehiscence. Early postoperative morbidity was 18% in patients undergoing uncut Roux gastrojejunostomy and 28% in patients undergoing standard Roux reconstruction. There were no early postoperative deaths in either group. In the patients undergoing the uncut Roux procedure, no cases of staple line dehiscence were detected clinically (mean follow-up 9 months, range 1 to 48 months). Visick grade improved following the uncut Roux procedure, but changed little after standard Roux reconstruction. Uncut Roux-en-Y gastrojejunostomy can be performed safely with improvement in symptoms. The uncut Roux procedure may provide an alternative for reconstructive gastric surgery. (J GASTROINTEST SURG 2000; 4:298-303.)

KEY WORDS: Roux-en-Y gastrojejunostomy, postgastrectomy syndrome

Roux-en-Y gastrojejunostomy is a common method of reconstruction after subtotal gastrectomy. The procedure has been used for alkaline reflux gastritis, dumping syndrome, and chronic gastric atony.[1-3] However, recent reports demonstrate that revisional gastric surgery is successful in less that 50% of patients, with many developing symptoms of stasis in the upper gastrointestinal tract.[4] This Roux stasis syndrome occurs in 10% to 67% of patients and consists of chronic abdominal pain, nausea, vomiting, and postprandial bloating.[5,6]

The etiology of the Roux stasis syndrome may be multifactorial. Clinical investigations suggest that stasis and marked motility abnormalities are present in the Roux limb.[7,8] The Roux limb may act as a functional obstruction resulting in reflux from the Roux limb to the gastric remnant producing slowed gastric emptying. These findings may result from transecting the jejunum and separating the Roux limb from the small intestinal pacemaker in the proximal duodenum. Transection of the jejunum results in the development of ectopic pacemakers distal to the transection, which results in orad propagating pacesetter potentials.[9] Orad propagating contractions of the jejunal limb influenced by the orad propagating pacesetter potentials lead to delayed transit through the limb and slowed gastric emptying.[10]

Maintaining myoneural continuity has been proposed to decrease the incidence of Roux stasis syndrome, with an "uncut" Roux-en-Y, a modified Billroth II gastrojejunostomy in which staples occlude the afferent jejunal lumen, whereas biliary and pancreatic secretions are diverted distally through a jejunojejunostomy. The staple lines function to divert pancreatobiliary secretions from the gastric remnant without transecting the jejunum, which is done during the standard Roux operation. This modification of the Roux-en-Y reconstruction was first reported in

From the Department of Surgery, University of Iowa College of Medicine, Iowa City, Iowa.
Presented at the Fortieth Annual Meeting of The Society for Surgery of the Alimentary Tract, Orlando, Fla., May 16-19, 1999.
Reprint requests: Joseph J. Cullen, M.D., 4622 JCP, University of Iowa Hospitals and Clinics, Iowa City, IA 52242.

1988[11] and has been investigated by a number of groups. Experimental work in dogs demonstrates that ectopic pacemakers do not appear in the uncut Roux limb, and gastric emptying and Roux limb transit are normal.[12] Additionally, the staple lines placed across the jejunum may not disrupt myoneural continuity to the uncut Roux limb. Early clinical results however, suggest an unacceptable rate of staple line dehiscence. Tu et al.[13] reported a staple line dehiscence rate of 36% with 1-year follow-up. All patients with staple line dehiscence had symptomatic alkaline reflux gastritis or esophagitis.

The aim of our study was to review our clinical experience with the uncut Roux-en-Y gastrojejunostomy and compare the clinical results in patients who have undergone the uncut Roux-en-Y gastrojejunostomy to a cohort of patients who have undergone a standard Roux-en-Y gastrojejunostomy. Additionally, we wanted to determine if patients had recurrent alkaline reflux gastritis or esophagitis suggestive of staple line dehiscence.

MATERIAL AND METHODS

Patient Demographics and Surgical History

Medical records for all patients who underwent a gastrectomy and Roux-en-Y gastrojejunostomy were abstracted for details including symptoms, nutritional status, diagnostic studies, operation performed, operative morbidity, and outcome. None of the patients had previously undergone gastric surgery for treatment of morbid obesity. This study was approved by the University of Iowa Institutional Review Board for human subjects on September 17, 1998.

Between 1993 and 1998, 11 patients (6 women and 5 men) underwent gastrectomy and uncut Roux-en-Y gastrojejunostomy. The mean patient age was 59 years with a range of 29 to 66 years. Indications for the operation included gastroparesis in four, gastric adenocarcinoma in three, bile reflux gastritis in three, and peptic ulceration with gastric outlet obstruction in one. These 11 patients had undergone a total of eight gastric operations before the gastrectomy and uncut Roux-en-Y gastrojejunostomy.

Between 1987 and 1998, 14 patients (11 women and 3 men) underwent gastrectomy and standard Roux-en-Y gastrojejunostomy. The mean patient age was 51 years with a range of 34 to 80 years. Indications for the operation included gastroparesis in four, bile reflux gastritis in three, peptic ulceration with gastric outlet obstruction in three, gastrointestinal bleeding in two, dumping syndrome in one, and gastric adenocarcinoma in one. These 14 patients had undergone a total of 20 gastric operations before the gastrectomy and Roux-en-Y gastrojejunostomy.

Nutritional Status

More than one half of the patients had been hospitalized previously for malnutrition, although none was dependent on enteral or parenteral nutrition prior to gastrectomy and either standard or uncut Roux-en-Y gastrojejunostomy.

Operation

The uncut Roux reconstruction was performed similarly as described by Van Stiegmann and Goff.[11] The length of the efferent jejunal limb from the gastrojejunostomy to the jejunostomy was 45 cm, a distance that ensured an efferent jejunal limb long enough to prevent reflux of pancreatobiliary secretions (Fig. 1). For construction of the uncut Roux limb, we applied four staple lines across the afferent jejunal limb just distal to the jejunojejunostomy by using two applications of a noncutting TA55 stapler (United States Surgical Corp., Norwalk, Conn.). The distance from the staple lines to the gastrojejunos-

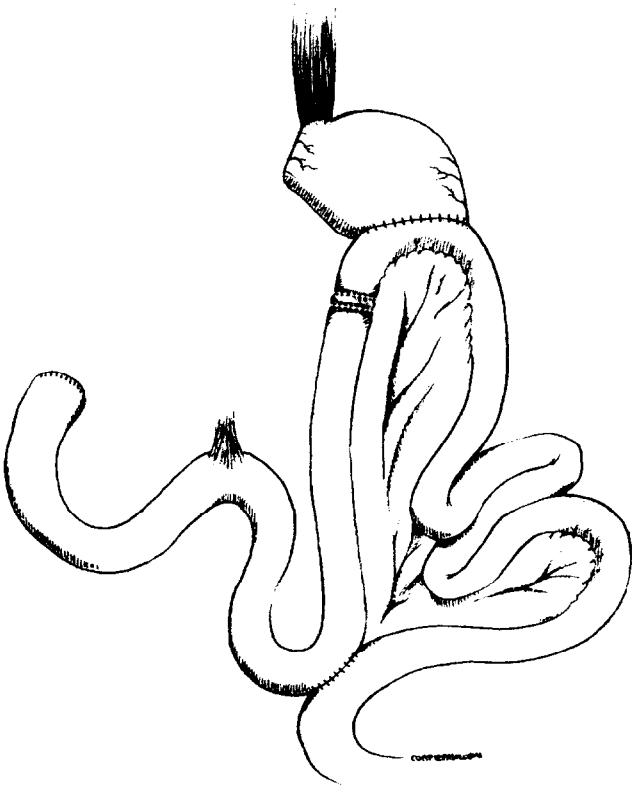

Fig. 1. Uncut Roux-en-Y gastrojejunostomy. The length of the efferent jejunal limb from the gastrojejunostomy to the jejunostomy is 45 cm. Four staple lines are placed across the afferent jejunal limb just distal to the jejunojejunostomy by using two applications of a noncutting stapler. The distance from the staple lines to the gastrojejunostomy is approximately 10 cm.

tomy was approximately 10 cm. The senior author (J.J.C.) was involved in all of the uncut Roux operations. Two patients had a concomitant jejunostomy tube placed at the time of operation. Gastrectomy and standard Roux-en-Y operations were performed using the technique described by Kirk[14] to restore gastrointestinal tract continuity.

Follow-Up and Outcome

Follow-up was completed using the medical records and interview. To measure outcome, patients were classified into four groups using a Visick grading system.[15] Briefly, grade 1 corresponded to no symptoms. Grade 2 included patients with intermittent symptoms, with or without continued use of medications, but not affecting life-style. Grade 3 corresponded to continuous, nondisabling symptomatology refractory to medical therapy, with or without supplemental use of parenteral or enteral nutrition. Grade 4 corresponded to severe disabling symptoms, with or without dependence on enteral or parenteral nutrition as the primary source of nutrition.

Additionally, a subjective severity of symptoms score based on seven gastrointestinal and five systemic symptoms, as described by Eckhauser et al.,[16] was also calculated before and after operation. The gastrointestinal symptoms included heartburn, abdominal pain, abdominal fullness, nausea, vomiting, diarrhea, and excessive flatulence. The systemic symptoms included fainting or dizziness, weakness or tremulousness, sleepiness, sweating, and palpitations. The severity of each symptom was graded on a scale of 1 to 3. An overall severity of symptoms score, as well as a separate severity of symptoms score for gastrointestinal and systemic symptoms, was calculated both before and after operation.

Data Analysis

Student's *t* test and the Wilcoxon rank-sum test were used where appropriate to determine differences between the uncut and standard Roux groups. Statistical significance of the differences between groups was tested using the computer program SYSTAT (Evanston, Ill.). All data are expressed as means ± standard error of the mean (SEM).

RESULTS

Postoperative Complications

There were no operative deaths or in-hospital deaths in patients undergoing either type of Roux reconstruction. In patients undergoing the uncut Roux gastrojejunostomy, three had early postoperative complications (partial small bowel obstruction, gastroparesis, and atrial fibrillation). The mean length of stay was 12 days (range 5 to 22 days). All patients were able to eat solid food at the time of discharge, and two of them required enteral nutritional supplementation via a feeding jejunostomy. One patient who had the uncut Roux reconstruction developed a late complication that was the result of a gastric outlet obstruction from a stricture at the gastrojejunostomy from continued nonsteroidal anti-inflammatory drug abuse. This condition necessitated placement of a feeding jejunostomy 3 years later.

In the standard Roux group, seven patients had early postoperative complications including anastomotic leak in one, subphrenic abscess in one, bleeding in one, and wound infection in one. The mean length of stay was 13 days (range 7 to 40 days; $P > 0.05$ vs. uncut Roux). At the time of discharge, 11 patients were able to eat solid food. Two of them required additional nutrition via a feeding jejunostomy. One patient was able to tolerate liquids only and was requiring supplementation via a jejunostomy. Two patients were unable to ingest liquids or solids and were being fed through an orogastric tube or by total parenteral nutrition. Late postoperative complications in the standard Roux group included stricture of the Roux limb in three patients who continued to use large quantities of nonsteroidal anti-inflammatory medications.

Follow-Up

Follow-up after hospitalization was possible in all of the patients. Of the patients who underwent an uncut Roux reconstruction, eight are still alive. The three patients who died had a preoperative diagnosis of gastric adenocarcinoma. Of the patients who had a standard Roux reconstruction, 13 are still alive. The patient who died in this group also had a preoperative diagnosis of gastric adenocarcinoma. Mean follow-up was 9 months (range 1 to 48 months) for the uncut Roux patients and 16 months (range 1 to 120 months) for the standard Roux patients ($P > 0.05$ vs. uncut Roux).

Outcome at Follow-Up

Visick Grade. In the uncut Roux group, the Visick grade decreased postoperatively at the time of follow-up (Fig. 2). Preoperatively the Visick grade was 3.0 ± 0.3 and decreased to 1.7 ± 0.2 postoperatively (mean ± SEM; $P > 0.01$ vs. preoperative Visick grade). Five of the patients (45%) reported excellent results and were without gastrointestinal complaints and had

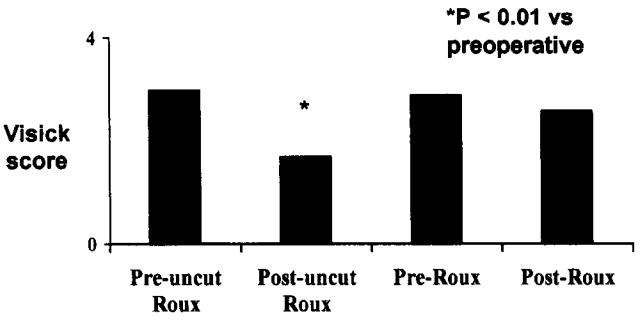

Fig. 2. Visick grade before and after Roux reconstruction (mean ± SEM). * = $P < 0.05$ vs. preoperative grade.

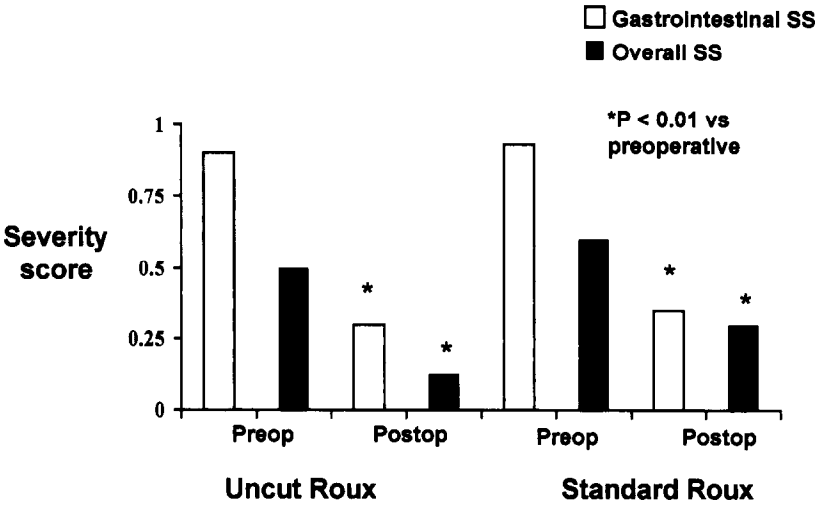

Fig. 3. Symptomatic response to Roux reconstruction using an overall and a gastrointestinal severity score (*SS*). The improvement seen in the overall severity score resulted from the improvement in gastrointestinal symptoms with negligible systemic symptoms present.

gained weight. Four patients in this group (36%) reported improvement on their symptoms, with occasional nausea and vomiting, or abdominal pain. At the time of follow-up, none of them had gained weight, and three had actually lost weight. Two patients (18%) reported unsatisfactory results with persistent nausea and vomiting.

In the standard Roux group the Visick grade changed little (see Fig. 2). Preoperatively the Visick grade was 2.9 ± 0.2 and it changed little postoperatively, to 2.6 ± 0.2 (mean ± SEM; $P > 0.05$ vs. preoperative Visick grade). None of these patients reported complete resolution of their symptoms. Seven patients (50%) demonstrated improvement in their Visick grade, but with persistent nausea, vomiting, heartburn, or abdominal pain. Only two of them had gained weight at the time of follow-up. Five patients (35%) had no change in their Visick grade, with persistent gastrointestinal symptoms after the surgery. Two patients (14%) reported worsening of their symptoms after the Roux reconstruction. Both patients had lost weight during the follow-up period.

Symptom Severity Score. An overall severity of symptoms score, as well as separate severity of symptoms scores for gastrointestinal and systemic symptoms, were calculated both before and after operation. In the uncut Roux group, the overall symptom severity score (Fig. 3) decreased from 0.53 ± 0.1 preoperatively to 0.13 ± 0.1 postoperatively (mean ± SEM; $P < 0.001$ vs. preoperatively). This reflected a decrease in the gastrointestinal symptom severity score (see Fig. 3), which decreased from 0.9 ± 0.2 preoperatively to 0.3 ± 0.1 (mean ± SEM; $P < 0.001$ vs. preoperatively). None of the patients in the uncut group had symptoms as described in the systemic symptom severity category. Specifically patients in the uncut Roux group did not have symptoms of alkaline reflux gastritis or esophagitis suggestive of staple line dehiscence.

In the standard Roux group, the overall symptom severity score (see Fig. 3) decreased from 0.6 ± 0.1

preoperatively to 0.28 ± 0.08 (mean ± SEM; $P < 0.001$ vs. preoperatively). This also reflected an improvement in their gastrointestinal symptom severity score (see Fig. 3), which was 0.93 ± 0.1 preoperatively, and 0.35 ± 0.1 postoperatively (mean ± SEM; $P < 0.001$ vs. preoperatively). In this group only two patients had symptoms that could be evaluated in the systemic symptom severity category.

CONCLUSION

Our study demonstrates that the uncut Roux gastrojejunostomy can be performed safely in patients who would traditionally undergo a Roux reconstruction for a variety of disorders. Patients undergoing uncut Roux reconstruction have an improvement in their Visick grade and in their overall and gastrointestinal symptom severity scores. In comparison, patients undergoing the standard Roux procedure have little change in their Visick grade but decrease their overall and gastrointestinal symptom severity scores. Although follow-up is short, the uncut Roux procedure may have some advantages over the standard Roux reconstruction.

Our present study demonstrated no mortality and low morbidity with the uncut Roux gastrojejunostomy, which corresponds to the report of Tu et al.[13] They reported 14 patients who underwent an uncut Roux reconstruction, with a complication rate of 28%, which was not related to the type of gastric reconstruction. Fifty-seven percent of the patients in their series reported excellent to satisfactory results from the operation. In our series 45% of patients reported excellent results without any postoperative complaints, whereas 36% of patients reported improvement in their symptoms, with occasional nausea and vomiting or abdominal pain.

Standard Roux-en-Y reconstruction in our series had little effect on improving the Visick grade. Fifty percent of patients demonstrated improvement in their Visick grade, but with persistent gastrointestinal complaints. Thirty-five percent of patients had no change in their Visick grade, with persistent gastrointestinal symptoms postoperatively. Roux-en-Y reconstruction has been used for a variety of disorders, with some series reporting relief of symptoms in 60% to 80% of patients.[3,5,16-18] However, recent reports suggest that completion gastrectomy and Roux-en-Y reconstruction is successful in less than half of patients,[14] which was also the case in our present study. Forstner-Barthell et al.[4] reported 62 patients who underwent near-total gastrectomy and Roux reconstruction. Nausea, vomiting, and postprandial pain were reduced in their series, similar to what we have demonstrated with improvement in the overall and gastrointestinal severity scores following the standard Roux-en-Y reconstruction. However, 57% of their patients remained in the Visick grade 3 or 4 categories, whereas only 43% had all or most of their symptoms relieved. Thus the standard Roux reconstruction may have more disappointing results with long-term follow-up than previously thought.

Unlike the previous report regarding the uncut Roux gastrojejunostomy, none of our patients developed symptomatic alkaline reflux gastritis or esophagitis consistent with staple line dehiscence. Perhaps a different stapling technique, as used in our study, to occlude the lumen and divert pancreatobiliary secretions, has decreased the risk of dehiscence. As Tu et al.[13] have pointed out, if nuclear medicine or contrast studies had been done in their study, the uncut Roux group may have had a higher rate of staple line dehiscence. Additionally, our present study reports a smaller number of patients with a shorter follow-up period.

The theoretical advantage of the uncut Roux reconstruction is that myoneural continuity may be maintained and ectopic pacemakers do not develop that will impede transit as demonstrated experimentally.[12] Other groups have suggested that myoneural continuity of the efferent limb of a gastrojejunostomy with the duodenal pacemaker is less important than luminal continuity of the afferent and efferent limbs in determining postgastrectomy gastric emptying.[19] Additionally, the experimental evidence for pacesetter potential propagation across staple lines has been demonstrated in canine preparations by some groups,[12] but disputed by others.[20] Evidence for pacesetter potential propagations across staple lines has not been demonstrated in humans.

In conclusion, the uncut Roux-en-Y gastrojejunostomy can be performed safely with improvement in symptoms and no clinical evidence of staple line dehiscence. Following the uncut Roux procedure, patients have an improvement in their Visick grade, whereas patients undergoing the standard Roux reconstruction have little change in their Visick grade. Our study should be approached with caution since follow-up is short and the occurrence of staple line dehiscence may be higher with more precise investigations using contrast or radionuclide studies. The uncut Roux gastrojejunostomy may provide an alternative for reconstructive gastric surgery.

We thank Sally Blackmon for secretarial assistance, Michael T. Napierkowski for medical graphics assistance, and the surgical residents of the University of Iowa for care of the patients presented in this study.

REFERENCES

1. Kelly KA, Becker JM, van Heerden JA. Reconstructive gastric surgery. Br J Surg 1981;68:687-691.
2. Vogel SB, Hocking MP, Woodward ER. Clinical and radionuclide evaluation of Roux-Y diversion for postgastrectomy dumping. Am J Surg 1988;155:57-61.
3. Karlstrom L, Kelly KA. Roux-Y gastrectomy for chronic gastric atony. Am J Surg 1989;157:44-49.
4. Forstner-Barthell AW, Murr MM, Nitecki S, Camilleri M, Prather CM, Kelly KA, Sarr MG. Near-total completion gastrectomy for severe postvagotomy gastric stasis: Analysis of early and long-term results in 62 patients. J GASTROINTESTINAL SURG 1999;3:15-23.
5. Herrington JL, Scott HW, Sawyers JC. Experience with vagotomy-antrectomy and Roux-en-Y gastrojejunostomy in surgical treatment of duodenal, gastric, and stomal ulcers. Ann Surg 1984;199:590-597.
6. Britton JP, Johnston D, Ward DC, Axon ATR, Barker MCJ. Gastric emptying and clinical outcome after Roux-en-Y diversion. Br J Surg 1987;74:900-904.
7. Mathias JR, Fernandez A, Sninsky CA, Clench MH, Davis RH. Nausea, vomiting, and abdominal pain after Roux-en-Y anastomosis: Motility of the jejunal limb. Gastroenterology 1985;88:101-107.
8. Miedema BW, Kelly KA, Camilleri M, Hanson RB, Zinsmeister AR, O'Connor MK, Brown MI. Human gastric and jejunal transit and motility after Roux gastrojejunostomy. Gastroenterology 1992;103:1133-1143.
9. Cullen JJ, Eagon JC, Hould F-S, Hanson RB, Kelly KA. Ectopic jejunal pacemakers after jejunal transection and their relationship to transit. Am J Physiol 1995;268:G959-G967.
10. Woodward A, Sillin LF, Bortoff A. Migrating myoelectric complex and jejunal slow-wave propagation after Roux gastrectomy in dogs. Dig Dis Sci 1993;1073-1078.
11. Van Stiegmann G, Goff JS. An alternative to Roux-en-Y for treatment of bile reflux gastritis. Surg Gynecol Obstet 1988;166:69-70.
12. Miedema BW, Kelly KA. The Roux stasis syndrome. Treatment by pacing and prevention by use of an "uncut" Roux limb. Arch Surg 1992;127:295-300.
13. Tu BN, Sarr MG, Kelly KA. Early clinical results with the uncut Roux reconstruction after gastrectomy: Limitations of the stapling technique. Am J Surg 1995;170:262-264.
14. Kirk RM. Roux-en-Y. World J Surg 1985;9:938-944.
15. Visick AH. Study of failures after gastrectomy: Hunterian lecture. Ann R Coll Surg 1948;3:266-294.
16. Eckhauser FE, Knol JA, Raper SA, Guice KS. Completion gastrectomy for postsurgical gastroparesis syndrome. Ann Surg 1988;208:345-353.
17. McCallum RW, Polepalle SC, Schirmer B. Completion gastrectomy for refractory gastroparesis following surgery for peptic ulcer disease. Dig Dis Sci 1991;36:1556-1561.
18. Gustavsson S, Ilstrup DM, Morrison P, Kelly KA. Roux-Y stasis syndrome after gastrectomy. Am J Surg 1988;155:490-494.
19. Cheng G, Vogel SB, Hocking MP. Efferent limb myoneural and luminal continuity and postgastrectomy gastric emptying. J Surg Res 1995;58:746-753.
20. Hocking MP, Harrison D, Vogel SB. Myoelectric effects and histology after stapled occlusion of the small bowel.

Surgical Resection Improves Survival in the Treatment of Early Gastric Lymphomas

Sarah Blair, M.D., Shimul Shah, M.D., Wael Tamim, M.D., Robert Quinlan, M.D., Richard Swanson, M.D.

Gastric lymphomas are a relatively rare form of malignancy and controversy about their optimum treatment still exists. To date, there have been no studies directly comparing results of medical therapy alone versus a combination of surgery plus medical therapy. We reviewed our experience in the three teaching hospitals of the University of Massachusetts Medical School to determine the role of surgery in the management of early gastric lymphoma. Statistics were evaluated by means of chi-square, log-rank, and Kaplan-Meier curve analysis where appropriate. Using tumor registry data, 39 patients were treated for early disease at our medical school from 1980 to 1998. Patients treated with surgery plus chemotherapy and radiation had a 90% 5-year survival compared to patients who received chemotherapy and radiation alone (55% 5-year survival; $P < 0.01$). When we compared all patients on an intention-to-treat basis (patients preoperatively thought to have early-stage disease), there was still a significant survival benefit with the addition of surgery to their management. Because this is an uncommon disease, there are no large prospective studies examining treatment. Based on our retrospective experience, surgical resection should be considered an important adjunct in the treatment of gastric lymphomas in early-stage disease. (J GASTROINTEST SURG 2000;4:304-309.)

KEY WORDS: Lymphoma, stomach, surgery, chemotherapy

Although the stomach is the most common site of extranodal non-Hodgkin's lymphoma, these tumors remain relatively rare. They represent only 1% to 7% of all gastric malignancies.[1,2] Because there are few series with large numbers of patients, controversy still exists regarding the optimal treatment of this disease. In the past, gastric lymphoma was considered a surgical disease.[3-6] However, with the advent of safe and effective chemotherapeutic regimens, chemotherapy (without surgery) has become the preferred method of treatment of this disease at some centers.[7-9] The purpose of our study was to retrospectively analyze treatment options used at our institution and how they affected survival in order to delineate the role of surgery in the treatment of early gastric lymphoma.

METHODS

We retrospectively reviewed the records of patients identified in the tumor registry from 1980 through 1998. Patient records from three teaching hospitals in the University of Massachusetts Coordinated Residency program were reviewed. Disease was staged according to the Ann Arbor gastric lymphoma scheme with Musshoff's modification (Table I).[10] Comparisons were made between patients who underwent chemotherapy and radiation and patients who underwent a combination of surgery plus chemotherapy

Table I. Staging classification for gastric lymphomas according to Musshoff's criteria

Stage Ie	Lymphoma limited to the stomach
Stage IIe1	Involvement of stomach and contiguous lymph nodes
Stage IIe2	Involvement of stomach and noncontiguous subdiaphragmatic lymph nodes
Stage III	Involvement of stomach and lymph nodes on both sides of the diaphragm
Stage IV	Hematogenous spread (diffuse or disseminated involvement of stomach and extralymphatic organs)

From the University of Massachusetts Medical School, Worcester, Mass.
Presented at the Fortieth Annual Meeting of The Society for Surgery of the Alimentary Tract, Orlando, Fla., May 16-19, 1999.
Reprint requests: Richard Swanson, M.D., Department of Surgery, University of Massachusetts, 55 Lake Ave., Worcester MA 01655.

and radiation. Patients who had surgery were staged by laparotomy, and patients who were treated medically were staged by endoscopy and abdominal computed tomography (CT). Statistics were evaluated by means of Student's *t* test, chi-square test, log-rank analysis, and Kaplan-Meier curves where appropriate.

RESULTS

More than 600 patients with gastric cancer were listed in the tumor registry, and 71 of them were identified with primary gastric lymphoma. Thirty-nine patients had early-stage disease (Fig. 1). This was defined as disease confined to the stomach or stomach plus perigastric lymph nodes (stage Ie or IIe1). There were 19 women and 20 men in this series. Twenty-five patients underwent some combination of surgery, chemotherapy, and radiation therapy (surg group). Fourteen patients were treated with chemotherapy and radiation alone (chemo/rt group). Patients most commonly presented with pain (79%). Thirty-six percent of patients presented with a weight loss of more than 10 pounds, and 24% had significant upper gastrointestinal hemorrhage as their presenting symptom. Forty-six percent of patients had an upper gastrointestinal series as part of their workup, 90% had an endoscopic evaluation, and 46% also underwent CT prior to treatment. Eighty percent of patients had a bone marrow biopsy and aspirates from all of them were normal. Seventy-six percent of all early-stage tumors demonstrated large cell diffuse histology. Three patients had mucosa-associated lymphoid tissue (MALT) histology.

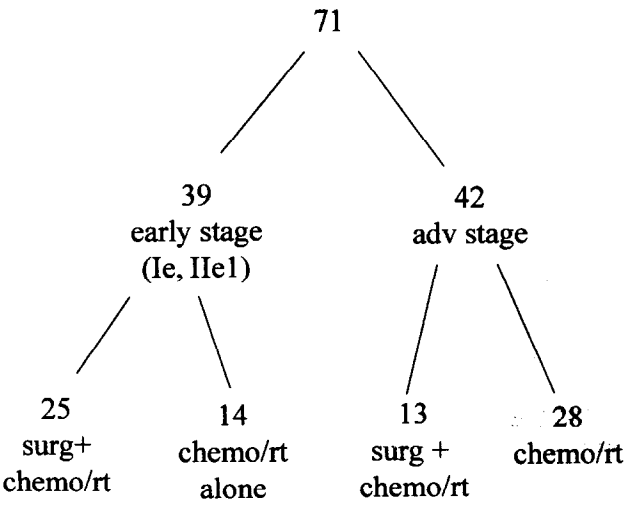

Fig. 1. Patients with primary gastric lymphoma divided according to disease stage (early or advanced) and type of treatment received.

The surg group included 14 men and 11 women whose mean age was 62.0 ± 11 years. The chemo/rt group included eight men and six women whose mean age was 62.6 ± 14 years. There were no significant differences between the two groups with regard to the age of the patients, the number of preexisting comorbid conditions, or the number of patients with suppressed immune systems (Table II). In the surg group, five patients underwent surgery alone and 12 patients had surgery plus chemotherapy. Three patients had surgery, chemotherapy, and radiation for high-grade tumors. Five patients had surgery plus radiation. In the chemo/rt group, five patients underwent chemotherapy alone, five patients had a combination of chemotherapy and radiation, and four patients underwent radiation alone.

Thirty-nine patients with all stages of disease underwent surgical intervention, with complete pathologic staging in all of them. Nine patients underwent emergency surgery for uncontrolled bleeding or perforation and were found to have advanced-stage disease. One patient underwent exploration for perforation and the final pathology report still showed early-stage disease. An additional five patients had preoperative CT scans suggestive of early-stage disease, but on surgical exploration they were found to have advanced disease. Four patients were overstaged by preoperative CT and on final pathologic examination were found to have localized disease. Only 43% of patients who underwent surgery had an endoscopic biopsy–confirmed diagnosis of gastric lymphoma prior to surgery. Thirty-one patients underwent partial gastrectomy and eight patients a total gastrectomy.

For all 71 patients with gastric lymphoma in this series, there was a direct correlation between stage at presentation and survival (Fig. 2). For all stages, patients who had surgery as part of their therapy had a significantly prolonged survival compared to patients who did not ($P < 0.02$) (Fig. 3). When we evaluated the patients with early-stage disease, those in the surg group (n = 25) had a 90% 5-year survival rate compared to only 55% in the chemo/rt group (n = 14; $P < 0.01$) (Fig. 4). Furthermore, when we examined disease-free survival in the surg group and the chemo/rt group, the addition of surgery still conferred a significant improvement in survival compared to chemotherapy and radiation alone ($P < 0.02$). Finally, in an attempt to compensate for bias in staging between patients who were surgically staged and patients who were staged by imaging only, we compared all patients on an intention-to-treat basis. We compared the 14 patients in the chemo/rt group, which included patients who were treated medically, to patients who were preoperatively staged with early disease. This group included 25 patients with early-stage

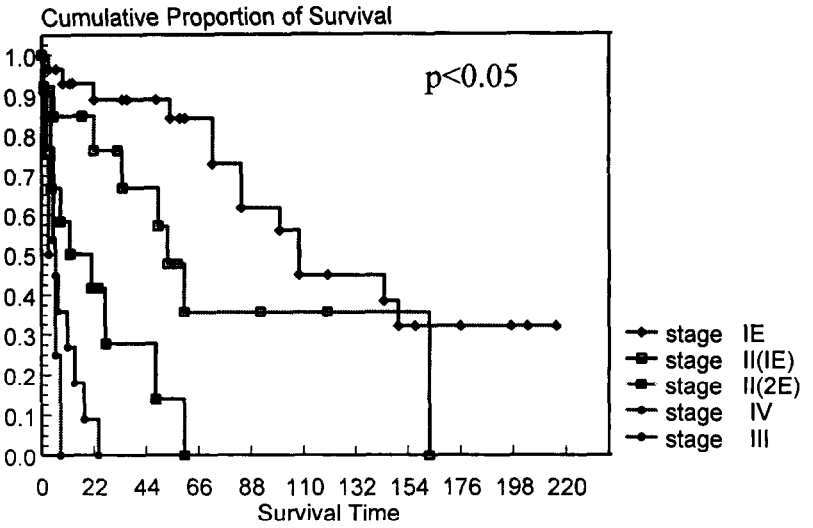

Fig. 2. Survival by stage for all 71 patients identified with gastric lymphoma.

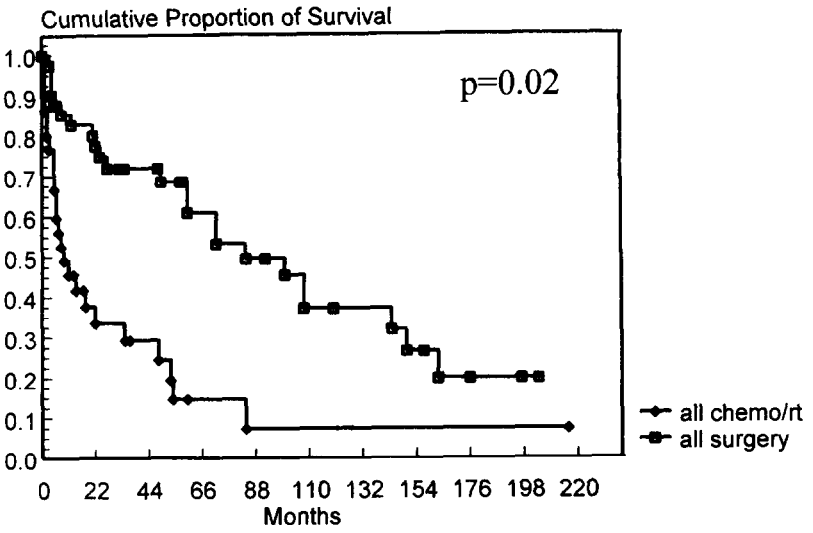

Fig. 3. Survival in patients with any disease stage who underwent surgery combined with chemotherapy and radiation *(all surg)* compared to patients treated with chemotherapy and radiation alone *(all chemo/rt)*.

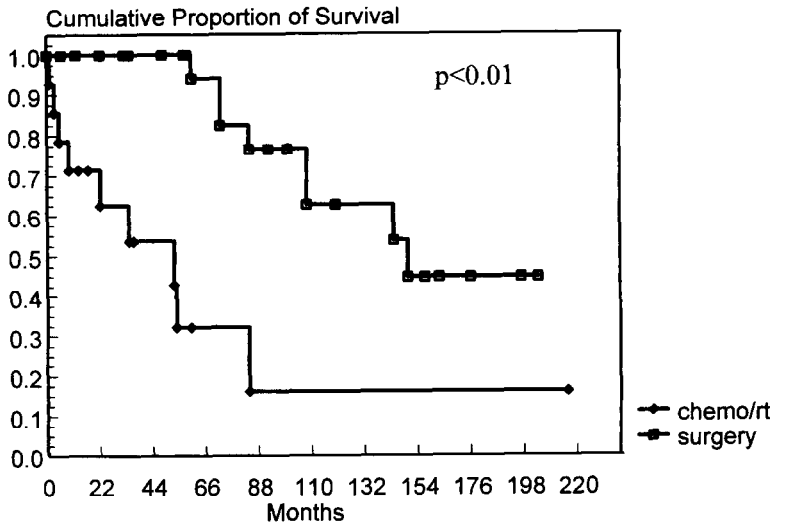

Fig. 4. Survival in patients with early-stage disease only (stage Ie or IIe1). Patients who were treated with chemotherapy and radiation alone *(chemo/rt)* are compared to patients treated with a combination of surgery plus chemotherapy and radiation *(surgery)*.

Table II. Preexisting comorbid factors in patients with early-stage gastric lymphoma

Patient	Age (yr)	Group	Comorbid conditions
1	72	Surg	Prostate cancer
2	83	Surg	Diabetes, hypertension, CAD
3	41	Surg	Seizures
4	76	Surg	MI, AF, hypertension
5	89	Surg	Seizures
6	72	Surg	Chronic lymphocytic leukemia
7	32	Surg	None
8	70	Surg	CAD, hypertension
9	64	Surg	CHF, breast cancer
10	69	Surg	Hypertension
11	69	Surg	Nfl, diabetes, hypertension
12	49	Surg	Laryngeal cancer
13	64	Surg	None
14	70	Surg	Pulmonary hamartoma
15	40	Surg	None
16	56	Surg	None
17	70	Surg	None
18	61	Surg	AAA repair
19	69	Surg	MI, CABG, diabetes
20	75	Surg	None
21	82	Surg	DVT, hypertension
22	70	Surg	Hemophilia
23	70	Surg	None
24	83	Surg	CAD, CABG, AAA repair
25	70	Surg	CAD, hypertension
26	82	Chemo/rt	None
27	76	Chemo/rt	Diabetes, hypertension, CAD
28	41	Chemo/rt	None
29	81	Chemo/rt	MI
30	91	Chemo/rt	None
31	37	Chemo/rt	HIV
32	78	Chemo/rt	AAA repair
33	88	Chemo/rt	None
34	71	Chemo/rt	Hypothyroidism
35	52	Chemo/rt	None
36	80	Chemo/rt	Multiple myeloma
37	61	Chemo/rt	CAD, CHF, AF, diabetes
38	70	Chemo/rt	None
39	35	Chemo/rt	HIV

CAD = coronary artery disease; MI = myocardial infarction; AF = atrial fibrillation; CHF = congestive heart failure; AAA = abdominal aortic aneurysm; CABG = coronary artery bypass graft; DVT = deep vein thrombosis; HIV = human immunodeficiency virus.

disease by pathologic staging plus five additional patients who were shown by preoperative imaging to have early-stage disease, but who were shown by pathologic staging to have advanced disease (combsurg group). We found that there was still a significant survival benefit with the addition of surgery to their management compared to the chemo/rt group ($P < 0.05$; Fig. 5).

Complications and Recurrence

There were no significant differences in morbidity and mortality between the surg and chemo/rt groups. Among patients with early-stage disease, there were no deaths. In the surg group the only postoperative complications were as follows: three patients had significant delayed gastric emptying prolonging their hospitalization more than 14 days or requiring readmission. One patient required readmission and medical management of a marginal ulcer in the remaining portion of the stomach. In the surg group there were no anastomotic leaks or wound infections documented in the immediate postoperative course. In the chemo/rt group, one patient developed deep vein thrombosis requiring hospitalization for anticoagulation therapy and one patient went into renal failure during chemotherapy.

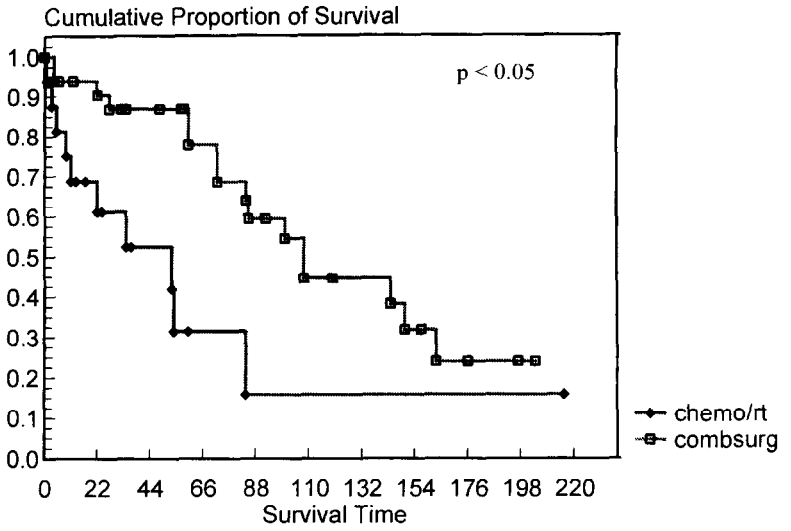

Fig. 5. Survival curve for patients on an intention-to-treat basis (i.e., patients thought preoperatively to have early-stage disease *[combsurg]*) compared to patients with early-stage disease treated with chemotherapy/radiation alone *(chemo/rt)*.

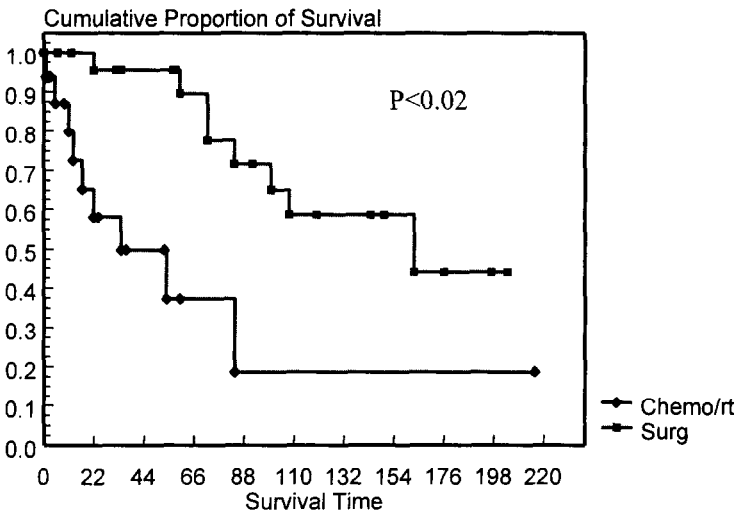

Fig. 6. Kaplan-Meier cumulative survival plot comparing disease-specific survival rates in patients with early-stage disease only (stage Ie or IIe1). Patients treated with chemotherapy and radiation alone *(chemo/rt)* are compared with patients treated with a combination of surgery plus chemotherapy and radiation *(surg)*.

None of the patients in the surg group had positive margins. Six patients had multisystem organ failure. There were no local recurrences.

DISCUSSION

Treatment of gastric lymphoma, like many tumors, has changed over the past 30 years. Because of the rarity of this tumor, earlier studies in the literature have grouped all types of gastrointestinal lymphomas together or have used different staging systems. In the past, surgery was considered the only method for staging and treatment.[3-6,11] Earlier chemotherapeutic regimens had reported high rates of bleeding or perforation. However, in the 1980s Maor et al.,[8] from M.D. Anderson Cancer Center, treated 34 patients with early-stage disease with chemotherapy and radiation. These patients had a 73% 5-year survival rate. They had a 17% morbidity rate and a 6% mortality rate related to their treatment. Bartlett et al.,[12] from Memorial Sloan-Kettering Cancer Center, treated 34 patients with either surgery or surgery plus some

combination of chemotherapy and radiation. They found no difference in survival between surgery alone versus surgery plus chemotherapy and radiation. The 10-year actuarial disease-free survival was 91% for stage I disease and 82% for stage II disease. This series reported a morbidity rate of 26% and no deaths. The largest series of patients with early-stage disease (n = 145) was from Germany. These investigators found no difference in survival between surgery alone and surgery plus chemotherapy and radiation.

Our series is the first to compare patients treated with a combination of surgery plus chemotherapy and radiation versus those treated with chemotherapy and radiation without surgery within one institutional system. Within the University of Massachusetts system, there are three different teaching hospitals; differences in treatment reflect differences in philosophies among various groups of physicians over time. According to our retrospective review, there were no differences in preexisting medical conditions between the two groups. The staging in the chemo/rt arm of this study may not be as accurate as it is in the surg group. However, in this study we did find an equal number of patients who were overstaged as were understaged by CT. The addition of endoscopic ultrasound may increase the accuracy of nonsurgical staging in the future. However, this modality was not widely available at our institution during the time of this review. We tried to compensate for this potential introduction of bias by analyzing patients on an intention-to-treat basis. We compared all of the patients who were found by preoperative imaging studies to have early-stage disease and then underwent pathologic staging to all of the patients who were staged by imaging only and treated medically. We found that patients who had surgery as part of their treatment had a significant survival benefit. We hypothesize that the differences in survival rates reflect the distinct treatment options employed by individual physicians.

For patients treated with surgery, we found similar 5-year survival rates for early-stage disease as has been previously reported in the surgical literature.[6,12,13] In our series, morbidity was low and there were no deaths in the group that underwent surgery, which reflects improvements in operative technique and postoperative care. Our complication rate in patients who had surgery did not differ from the complication rate associated with current chemotherapy and radiation protocols, and these patients showed significant improvement in both overall and disease-specific survival (Fig. 6).

CONCLUSION

Gastric lymphoma remains a relatively rare form of cancer and subsequently there have been no randomized studies proving the optimum treatment for this disease. Based on our retrospective analysis, the addition of surgery should be considered in the treatment of early-stage gastric lymphoma.

REFERENCES

1. Contreary Y, Nance FC, Becker WF. Primary lymphoma of the gastrointestinal tract. Ann Surg 1980;191:539-598.
2. Hockey MS, Powell J, Crocker J, Fielding JWL. Primary gastric lymphoma. Br J Surg 1987;74:484-487.
3. Brooks JJ, Enterline HT. Primary gastric lymphomas: A clinicopathologic study of 58 cases with long-term follow-up and literature review. Cancer 1983;51:701-711.
4. Fleming ID, Mitchell S, Dilawari RA. The role of surgery in the management of gastric lymphomas. Cancer 1978;43:1135-1141.
5. Hande KR, Fisher RI, DeVita VT, Chabner BA, Young RC. Diffuse histiocytic lymphoma involving the gastrointestinal tract. Cancer 1978;41:1984-1989.
6. Shiu MH, Nisce ZN, Pinna A, et al. Recent results of multimodal therapy of gastric lymphoma. Cancer 1986;58:1389-1399.
7. Burgers JM, Taal BG, van Heerde P, et al. Treatment results of primary stage I and II non-Hodgkin's lymphoma of the stomach. Radiother Oncol 1988;11:319-326.
8. Maor MH, Maddux B, Osborne BM, et al. Stages IE and IIE non-Hodgkin's lymphomas of the stomach. Comparison of treatment modalities. Cancer 1984;54:2330-2337.
9. Maor MH, Velasquez WS, Fuller LM, Silvermintz KB. Stomach conservation in stages IE and IIE gastric non-Hodgkin's lymphoma. J Clin Oncol 1990;8:266-271.
10. Musshoff K. Klinishe stadieneinteilung der nicht-Hodgidn-Lymphome. Strahlentherapie 1977;153:218-221.
11. Schwarz RJ, Conners JM, Schmidt N. Diagnosis and management of stage IE and stage IIE gastric lymphomas. Am J Surg 1993;165:561-565.
12. Bartlett D, Karpeh M, Filippa D, Brennan, M. Long-term follow-up after curative surgery for early gastric lymphoma. Ann Surg 1996;223:53-62.
13. Cogliatti SB, Schmid U, Schumacher UE, et al. Primary B-cell gastric lymphoma: A clinicopathological study of 145 patients. Gastroenterology 1991:101:1159-1170.
14. Gobbi PG, Dionigi P, Barbieri F, et al. The role of surgery in the multimodal treatment of primary gastric non-Hodgkin's lymphomas. Cancer 1990;65:2528-2536.

Role of a Hyaluronate-Based Membrane in the Prevention of Peritonitis-Induced Adhesions

Ali M. Ghellai, M.D., Arthur F. Stucchi, Ph.D., Daniel J. Lynch, B.S., Kevin C. Skinner, V.M.D., M. Jude Colt, B.A., James M. Becker, M.D.

Adhesions remain a significant postoperative complication of abdominal surgery; however, recent evidence suggests that physical barriers may reduce their incidence. Although these adhesion prevention barriers are efficacious when used under aseptic conditions, little is known about their use in the presence of peritonitis, which is associated with an increased incidence of abdominal adhesions. A sodium hyaluronate and carboxymethylcellulose bioresorbable membrane (HA membrane) has been shown recently to reduce postoperative adhesions in several animal models and in two clinical trials. To investigate the efficacy of HA membrane in the presence of peritonitis, generalized peritonitis was induced in rats by either cecal ligation and puncture (CLP) or cecal ligation (CL) alone. The ceca were resected after 12 hours, and animals were randomly assigned to receive or not receive HA membrane applied to the cecum. At day 7, abdominal adhesions and abscesses were scored. In the presence of peritonitis, HA membrane did not significantly reduce the number or tenacity of adhesions. A trend toward increased abscess formation was associated with HA membrane in the CL group. Although HA membrane has been shown to reduce the incidence and severity of abdominal adhesions under aseptic conditions, this study demonstrates that it is not efficacious in preventing abdominal adhesions in the presence of peritonitis. The association between HA membrane and abscess formation in the presence of experimental peritonitis requires further investigation. (J GASTROINTEST SURG 2000;4:310-315.)

KEY WORDS: Abdominal adhesions, peritonitis, carboxymethylcellulose, hyaluronic acid, Seprafilm

The incidence of peritoneal adhesions has been reported to be as high as 93% following general abdominal surgery.[1-3] Abdominal adhesions and their pathologic sequelae can result in significant postoperative clinical complications. Intraperitoneal adhesions are not only the leading cause of small bowel obstruction,[4] they are also a major source of infertility and abdominal pelvic pain.[5] Perhaps of greater relevance to the surgeon, abdominal adhesions can significantly hamper subsequent surgical access to the abdomen making reoperation both difficult and dangerous. Adhesiolysis and the treatment of adhesion-related conditions substantially increase health care costs, which have been estimated to be more than 1.3 billion dollars per year.[6] Although postoperative adhesions are the most common, intraperitoneal inflammation can account for up to 20% of abdominal adhesions in patients with no history of prior surgery.[7-9]

The significant morbidity associated with postoperative intraperitoneal adhesions has prompted extensive research into potential adhesion prevention modalities.[10,11] Of these, biodegradable physical barriers have been successfully used in preventing or reducing adhesion formation by mechanically limiting tissue apposition during the critical period of mesothelial repair and healing. By minimizing the development of a fibrin matrix between serosal tissue surfaces, such membranes can effectively prevent adhesion formation. One such physical barrier, Seprafilm bioresorbable membrane (Genzyme Corp., Cambridge, Mass.), a sodium hyaluronate and carboxymethylcellulose bioresorbable membrane (HA membrane), has

From the Department of Surgery (A.M.G., A.F.S., and J.M.B.), Boston University School of Medicine, Boston, Mass., and Genzyme Corporation (D.J.L., K.C.S., and M.J.C.), Cambridge, Mass.
Supported by the Genzyme Corporation, Cambridge, Mass.
Presented at the Fortieth Annual Meeting of The Society for Surgery of the Alimentary Tract, Orlando, Fla., May 16-19, 1999.
Reprint requests: James M. Becker, M.D., F.A.C.S., Chairman, Department of Surgery, Boston University School of Medicine, One Medical Center Place, Boston, MA 02118.

been shown to significantly reduce postoperative adhesions in preclinical studies[12] and in two clinical trials.[5,13] Although this adhesion prevention barrier is very efficacious when used under noncompromised aseptic conditions,[5,12,13] little is known about its use in the compromised surgical field, such as in the presence of peritonitis.

Peritonitis, or inflammation of the peritoneum or part of it, can also result in significant abdominal adhesions as well as abscess formation, both of which are associated with high morbidity. Most surgically related peritonitis is secondary to either intraperitoneal bacterial contamination from the gastrointestinal tract or intraperitoneally associated ischemic tissue, especially bowel. Both of these forms of peritonitis have been implicated in the formation of clinically relevant intra-abdominal adhesions. Hence the purpose of this study was to determine if a currently used and proven effective bioresorbable physical adhesion prevention film composed of hyaluronic acid and carboxymethylcellulose remains efficacious in preventing abdominal adhesions when either bacterial or ischemic peritonitis is present.

MATERIAL AND METHODS

Sixty-two female Sprague-Dawley rats (250 to 350 g; Charles River Laboratory, Wilmington, Mass.) were housed in groups of five and allowed to acclimatize for 5 days prior to surgery. Throughout the experimental period, the animals were allowed free access to water and were housed at a constant room temperature of 64 to 79° F (18 to 26° C) with 12-hour light/dark cycles. All experimental animals consumed a diet containing 70% lean ground beef, which has previously been shown to induce a polymicrobial cecal population.[14] All procedures were performed in accordance with recommendations outlined in the "Guidelines for the Care and Use of Laboratory Animals" (Department of Health and Human Services, National Institutes of Health, Publication No. 86-23, 1985). This study was approved by the Institutional Animal Care and Use Committee of the Genzyme Corporation (Cambridge, Mass.).

Experimental Design

Generalized peritonitis was induced in rats by one of two methods as follows: (1) cecal ligation and puncture (CLP), a commonly used model[15-17] that results in polymicrobial peritoneal sepsis (n = 38); or (2) cecal ligation only (CL alone), which produces an ischemia-induced peritonitis (n = 24). In each of the two groups, half of the animals were randomly assigned to receive (HA membrane) or not receive HA membrane (control) applied to the cecum as described below. All animals were killed on postoperative day 7 via carbon dioxide asphyxiation. Abdominal adhesions and abscesses were evaluated and scored as described below.

Operative Procedure

All surgical procedures in rats were performed by the same surgeon (A.M.G.) assisted by the same surgical technician. Animals were anesthetized with a single intramuscular injection of ketamine (85 mg/kg) and xylazine (6 mg/kg). The ceca were externalized through a 3 to 5 cm midline abdominal incision. In all rats, the ceca tips were devascularized and ligated with 4-0 Dexon (Davis & Geck, Wayne, N.J.). The cecum was either ligated or ligated and punctured in the same location in all animals as determined by a standard measurement (1.5 cm) from the tip of the cecum. In the CLP group, the ceca were punctured once using an 18-gauge needle on the antimesenteric side. The ceca were placed back into the abdomen and the abdominal wall was closed in one layer with 3-0 Dexon (Davis & Geck).

Rats were resuscitated with 10 ml of lactated Ringer's given subcutaneously. Twelve hours after surgery, all rats received 2 mg of gentamicin (Elkins-Sinn Inc., Cherry Hill, N.J.) and 15 mg clindamycin (Upjohn Company, Kalamazoo, Mich.) given in separate intramuscular injections and then every 8 hours for 3 days. One hour after the first postoperative dose of antibiotics, rats were weighed, anesthetized as above, and the ceca were externalized through the midline abdominal incision. Peritoneal fluid was collected for aerobic and anaerobic bacterial cultures. The portion of the cecum distal to the ligation was resected and subsequently closed in single layer with a running suture (5-0 Dexon, Davis & Geck) in a uniform fashion in all animals. After closure of the cecum, the peritoneal cavity was irrigated with 10 ml of normal saline solution and a 5 cm^2 piece of HA membrane (Seprafilm bioresorbable membrane, lot No. N5051B) was applied evenly over the suture line such that the film was distributed equally around the suture line and the cecum. The abdominal wall was closed as above. All animals were allowed to recover in an incubator and then were returned to group housing.

Evaluation of Abdominal Adhesions and Abscesses

In preliminary experiments characterizing CLP in rats as a model to study peritonitis-induced adhesions, we noted that both the number and tenacity of abdominal adhesions rose significantly by day 1 and re-

mained significantly elevated through day 28. Although adhesion tenacity remained constant, there was a reduction in the number of adhesions by day 28. Based on these studies, we decided that day 7 following CLP would be the optimal time point at which to study adhesiogenic events associated with peritonitis. Therefore animals were killed on day 7 following the second operation. Abdominal adhesions were evaluated and scored by a blinded observer in four categories as follows: (1) the total number of adhesions, defined as the number of individually identifiable and dividable adhesions between any two surfaces; (2) the number of cecal adhesions, defined as the number of individually identifiable and dividable adhesions between the cecum and any other surface; (3) the tenacity of the adhesions, scored based on the firmness of the adhesion's attachment as shown in Table I and described by Peck et al.[18]; and (4) the average length of adhesions to the cecum expressed in millimeters. While evaluating and scoring adhesions, the observer also noted the presence, location, and size of any abdominal abscesses, which were defined as purulent collections in the abdomen.

Evaluation of Cecal Integrity and Healing

After evaluation of adhesions, the portion of the intestine from which the cecum was resected, 3 cm proximal to and 7 cm distal to the anastomosis, was resected for evaluation of bursting pressure. In addition, a 10 cm proximal segment of intact bowel from each animal was resected. The intraluminal content of each segment was emptied and irrigated with normal saline solution. The bowel segments were then tested for hydrostatic bursting pressure as follows. The distal end of each bowel segment was cannulated with a 14 F Foley urinary catheter secured with 0-silk ligature, and the proximal end was occluded with an atraumatic bowel clamp. Normal saline solution was then infused into the bowel lumen through the Foley catheter at a rate of 5 ml/min with a Harvard infusion pump (Harvard Apparatus, Inc., South Natick, Mass.). One limb of the Foley catheter was connected to a Hewlett-Packard pressure transducer (Hewlett-Packard Medical Products Group, Andover, Mass.) and strip-chart recorder for continuous monitoring of intraluminal pressure. Hydrostatic bursting pressure was defined as the pressure at which the bowel segment leaked.

Table I. Scale for scoring adhesion tenacity

Adhesion tenacity	Description
0	No adhesions
1	Filmy adhesions with easily identifiable plane
2	Mild adhesions with freely dissectable plane
3	Moderate adhesions with difficult dissectable plane
4	Dense adhesions with nondissectable plane

Statistics

All data are reported as the mean ± standard error of the mean. Differences between groups were tested by analysis of variance and *t* test where appropriate. The difference in the rate of abdominal abscesses between the groups was tested by chi-square analysis. A *P* value of less than 0.05 was considered significant.

RESULTS

Following CLP, all rats showed physical signs of intra-abdominal sepsis, including apathetic behavior, lethargy, reduced food and water consumption, piloerection, ocular exudate, and diarrhea. Of the total of 38 rats from the bacterial peritonitis group that underwent CLP, two rats (10.5%) in the HA membrane group died, whereas there were no deaths in the control group. These deaths occurred early in the experiment and were not related to the HA membrane. The peritoneal cavity at autopsy typically contained 1 to 2 ml of cloudy fluid, which grew numerous enteric organisms such as *Escherichia coli*, *Proteus mirabilis*, *Enterococci*, *Streptococci*, *Clostridia*, and bacteroid species. There were no deaths among the 24 rats that had CL alone. As expected, peritoneal fluids collected from this group showed little or no bacterial growth since there was no puncture of the cecum.

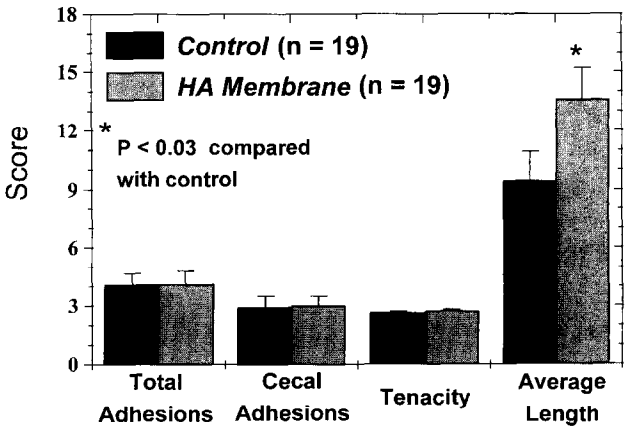

Fig. 1. Adhesion scores in HA membrane–treated animals compared with control rats following polymicrobial peritonitis resulting from cecal ligation and puncture.

A pressure monitoring system was used to test the integrity of the cecal closure and showed no leaks at pressures up to 75 to 100 mm Hg.

Adhesions

Our earlier work showed that peritonitis significantly increased the incidence of intra-abdominal adhesion formation.[19] Compared with control rats, the HA membrane was not effective in reducing total and cecal adhesions, or in reducing the tenacity or mean length of adhesions in both the CLP group (Fig. 1) and the CL-only group (Fig. 2). In fact, in the presence of bacterial peritonitis (CLP model), the mean length of adhesions to the cecum was significantly greater in the HA membrane group compared with control rats (see Fig. 1).

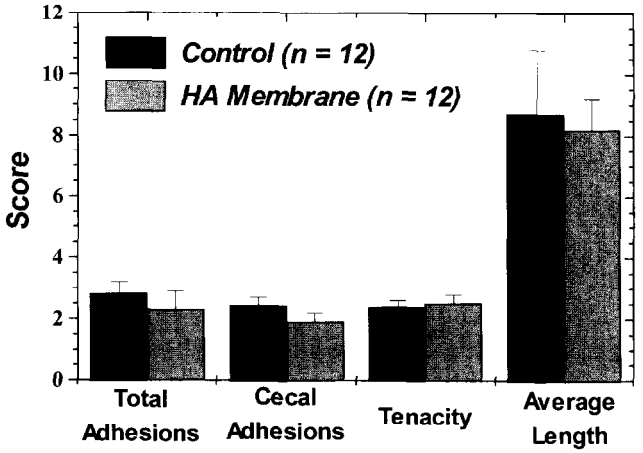

Fig. 2. Adhesion scores in HA membrane–treated animals compared with control rats following ischemic peritonitis resulting from cecal ligation alone.

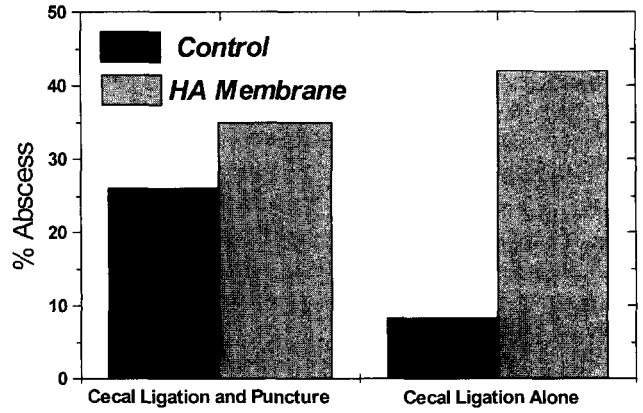

Fig. 3. Abscess rate in HA membrane–treated rats compared with control rats in both the CLP (polymicrobial peritonitis) and CL-only (ischemic peritonitis) models.

Abdominal Abscesses

In the CLP group, six animals (35%) treated with HA membrane developed abdominal abscesses as compared with five (26%) in the untreated (control) group ($P = 0.56$). Conversely, in the CL-only group, five animals (42%) treated with HA membrane developed abdominal abscesses, whereas only one (8%) control animal had an abdominal abscess ($P = 0.13$) (Fig. 3). Pooled analysis strongly suggested a greater risk of abdominal abscess formation ($P < 0.05$) when HA membrane was applied in the presence of peritonitis.

DISCUSSION

In a recent retrospective study involving nearly 19,000 patients who had undergone either an open colorectal or general abdominal surgical procedure, Beck et al.[4] concluded that the incidence of small bowel obstruction and adhesiolysis for obstruction as well as the requirement for repeated abdominal surgery after major abdominal resection is much higher than previously published. Retrospective studies such as this clearly identify the importance of reducing the occurrence of postoperative intra-abdominal adhesions not only in reducing future health care costs associated with adhesion-related complications, but also in reducing subsequent operative complexity and morbidity.

Adhesions may be defined as abnormal attachments between tissues and organs[20] and have been classified as congenital or acquired.[21,22] The development of acquired adhesions is a generalized phenomenon in response to trauma to the peritoneum. Acquired adhesions arise from either an intra-abdominal infection or inflammation such as diverticulitis or as a result of surgical trauma. Although most postsurgical adhesions are clinically silent, as mentioned, they are the leading cause of intestinal obstruction in the Western world and are responsible for more than 70% of all small bowel obstructions. Up to 93% of patients who had a previous laparotomy were found to have intra-abdominal adhesions.[1-3] Between 36% and 60% of all patients who present with adhesive intestinal obstruction require surgical treatment.[23,24]

More than 100 years ago, Thomas Bryant reported the occurrence of an episode of fatal intestinal obstruction due to intra-abdominal adhesions that appeared after removal of an ovarian cyst.[2,25,26] Since that time, various attempts have been made to prevent adhesion formation following abdominal surgery. These include the use of corticosteroids,[27] nonsteroidal anti-inflammatory drugs, dextran, anticoagulants, tissue plasminogen activator, and physical barriers.[10,11] Several barriers have demonstrated clinical efficacy in adhesion prevention and have become commercially available. More recently, promising re-

sults have been obtained with the use of a sodium hyaluronate and carboxymethylcellulose-based bioresorbable membrane (HA membrane), which has been shown to reduce the incidence and severity of adhesions in both preclinical[12] and clinical studies.[5,13] However, the efficacy of HA membrane has only been tested under aseptic conditions.

Most surgical peritonitis is secondary to bacterial contamination from the gastrointestinal tract. To stimulate intra-abdominal sepsis in animals, we used the well-established CLP model in rats.[15-17] The surgical technique consists of devascularization and puncture of a segment of the cecum, which is allowed to slough, thus mimicking a perforated or strangulated bowel with severe transmural infection. In this model the sepsis is due to peritoneal contamination with mixed flora in the presence of devitalized tissue and thus bears a resemblance to clinical situations such as perforated appendicitis and diverticulitis. The severity of the peritonitis depends on several factors including the rate of development and the bacterial content of the intestine at the time of rupture.

Although HA membrane has been shown to reduce the incidence and severity of abdominal adhesions under aseptic conditions,[13] this study demonstrates that the HA membrane is not efficacious in preventing the incidence or the severity of intra-abdominal adhesions in an animal model of either bacterial or ischemia-induced peritonitis. In fact, when the efficacy of HA membrane was assessed in the presence of polymicrobial peritonitis (CLP; see Fig. 1), it was associated with a significant increase in cecal adhesion length. HA membrane prevents the formation of intra-abdominal adhesions by functioning as a physical barrier that temporarily separates serosal surfaces during the critical early postoperative healing phase.[12] The mechanisms of peritoneal host defenses within the infected or inflamed peritoneal cavity involve clearance, phagocytosis, and sequestration with macrophages and polymorphonuclear leukocytes playing a significant role in this process.[28-30] The increased influx of these inflammatory cells may cause rapid degradation and clearance of the HA membrane, thus rendering it ineffective as an antiadhesion barrier.

Many forms of peritonitis are also associated with increased abscess as well as adhesion formation. An abscess represents an effective defense when the tissue injury and the number of bacteria are in excess of the host's capability to terminate a peritoneal infection. In the CLP model we observed a 35% increase in the abscess rate in the HA membrane–treated group, whereas in the CL-alone group, the abscess rate increased fivefold in the HA membrane–treated animals when compared with control rats. In contrast to our results, a similar study in rats utilizing the CLP model to induce polymicrobial peritonitis showed that there were no significant differences in adhesions between rats treated with HA membrane and control animals at either 7 or 21 days; additionally, abscess formation was similar in both groups at day 7 (6 of 12 in control rats vs. 4 of 12 in HA membrane–treated rats).[31] To further assess the efficacy of Seprafilm bioresorbable membrane (HA membrane) usage during serious intra-abdominal sepsis, Tzianabos et al.[32] recently demonstrated in rats with peritonitis that there were no differences in either mortality or abscess formation in animals treated with Seprafilm compared with control animals. These data suggest that the use of Seprafilm during serious intra-abdominal infection alters neither the outcome of the disease process nor the effects of antibiotic therapy. In a recently reported clinical trial by Becker et al.[13] in which HA membrane was used in the absence of intra-abdominal sepsis, abscesses only developed in 7 (7.7%) of 91 patients in the HA membrane–treated group.

CONCLUSION

Although HA membrane has been shown to reduce the incidence and severity of abdominal adhesions under aseptic conditions, this study suggests that it is not efficacious in preventing abdominal adhesions in the presence of intra-abdominal peritonitis. Pooled analyses of our data suggest a greater risk of abdominal abscess formation ($P < 0.05$) when HA membrane was applied in the presence of peritonitis, suggesting that the stimulus for adhesion formation can overcome the antiadhesion properties of HA membrane when peritonitis is present. Further studies are needed to determine the precise relationship between the use of HA membrane in the presence of peritonitis and abscess formation.

REFERENCES

1. Menzies D, Ellis H. Intestinal obstruction from adhesions—How big is the problem? Ann R Coll Surg Engl 1990;72:60-63.
2. Menzies D. Peritoneal adhesions. Incidence, cause and prevention. Ann Surg 1992;24 (part 1):29-45.
3. Menzies D. Postoperative adhesions: Their treatment and relevance in clinical practice. Ann R Coll Surg Engl 1993;75: 147-153.
4. Beck DE, Opelka FG, Bailey HR, Rauh SM, Pashos CL. Incidence of small bowel obstruction and adhesiolysis after open colorectal and general surgery. Dis Colon Rectum 1999;42: 241-248.
5. Diamond MP. Reduction of adhesions after uterine myomectomy by Seprafilm membrane (HAL-F): A blinded, prospective, randomized, multicenter clinical study. Seprafilm Adhesion Study Group. Fertil Steril 1996;66:904-910.
6. Ray FN, Denton WG, Thamer M, Henderson SC, Perry S. Abdominal adhesiolysis: Inpatient care and expenditure in the United States in 1994. J Am Coll Surg 1998;186:1-9.

7. Nemir P. Intestinal obstruction: Ten year statistical survey at the hospital of the University of Pennsylvania. Ann Surg 1952;135:367-375.
8. Perry JF, Smith GA, Yonehiro EG. Intestinal obstruction caused by adhesions. Ann Surg 1955;142:810-816.
9. Weibel M, Majno G. Peritoneal adhesions and their relation to abdominal surgery. Am J Surg 1973;126:345-353.
10. DiZerega GS. Contemporary adhesion prevention. Fertil Steril 1994;61:219-235.
11. Diamond MP, DeCherney AH. Pathogenesis of adhesions formation/reformation: Application to reproductive pelvic surgery. Microsurgery 1987;8:103-108.
12. Burns JW, Colt MJ, Burgess LS, Skinner KC. Preclinical evaluation of Seprafilm bioresorbable membrane. Eur J Surg Suppl 1997; 577:40-48.
13. Becker JM, Dayton MT, Fazio VW, Beck DE, Stryker SJ, Wexner SD, Wolff BG, Roberts PL, Smith LE, Sweeney SA, Moore M. Sodium hyaluronate-based bioresorbable membrane (HAL-F) in the prevention of postoperative abdominal adhesions: A prospective, randomized, double-blinded multicenter study. J Am Coll Surg 1996;183:297-307.
14. Onderdonk AB, Weinstein WM, Sullivan NM, Bartlett JG, Gorbach SL. Experimental intra-abdominal abscesses in rats: Quantitative bacteriology of infected animals. Infection Immun 1974;10:1256-1259.
15. Ryan NT, Blackburn GL, Clowes HA Jr. Differential tissue sensitivity to elevated endogenous insulin levels during experimental peritonitis in rats. Metabolism 1974;23:1081-1089.
16. Wichterman KA, Baue AE, Chaudry IH. Sepsis and septic shock—a review of laboratory models and a proposal. J Surg Res 1980;29:189-201.
17. Edmiston CE, Goheen MP, Kronhall S, Condon RE. Fecal peritonitis: Microbial adherence to serosal mesothelium and resistance to peritoneal lavage. World J Surg 1990;14: 176-183.
18. Peck LS, Quigg JM, Fossum GT, Goldberg EP. Evaluation of CMC and HA solutions for adhesiolysis. J Invest Surg 1995;8:337-348.
19. Ghellai AM, Chegini N, Dou O, Kaseta JM, Burns JW, Skinner KC, Stucchi AF, Becker JM. The role of transforming growth factor beta-1 in peritonitis-induced adhesions. Gastroenterology 1998;114:A1390.
20. Snoj M, Ar'Rajab A, Ahren B, Bengmark S. Effect of phosphatidylcholine on postoperative adhesions after small bowel anastomosis in the rat. Br J Surg 1992;79:427-429.
21. Ellis H. The causes and prevention of intestinal adhesions. Br J Surg 1982;69:241-243.
22. Ellis H. Prevention and treatment of adhesions. Infect Surg 1983;2:802-817.
23. Hofstetter SR. Acute adhesion obstruction of the small intestine. Surg Gynecol Obstet 1981;152:141-144.
24. Wolfson PJ, Bauer JJ, Gelernt IM, Kreel I, Aufses AH. Use of long tubes in the management of patients with small intestinal obstruction due to adhesions. Arch Surg 1985;120:1001-1006.
25. Bryant T. Clinical lectures on intestinal obstruction. Med Times Gaz 1872;1:363.
26. Monk BJ, Berman ML, Montz FJ. Adhesions after extensive gynecological surgery: Clinical significance, etiology and prevention. Am J Obstet Gynecol 1994;170:1396-1403.
27. Liao S, Svehiro GT, McNamara JJ. Prevention of postoperative intestinal adhesions in primates. Surg Gynecol Obstet 1973;137:816-818.
28. Dunn DL, Barke RA, Knight NB, Humphrey EW, Simmons RL. Role of resident macrophages, peripheral neutrophils, and translymphatic absorption in bacterial clearance from the peritoneal cavity. Infection Immun 1985;49:257-264.
29. Dunn CJ, Miller SK. The effects of cyclosporin A on leucocyte infiltration and procoagulant activity in the mouse delayed hypersensitivity response in vivo. Int J Immunopharmacol 1986;8:635-643.
30. Dunn DL, Barke RA, Ewald DC, Simmons RL. Macrophages and translymphatic absorption represent the first line of host defense of the peritoneal cavity. Arch Surg 1987;122:105-110.
31. Reijnen MPJ, Meis J, Postma V, van Goor H. Prevention of intra-abdominal abscesses and adhesions using a hyaluronic acid solution in a rat peritonitis model. Arch Surg 1999; 134:997-1001.
32. Tsianabos AO, Cisneros RL, Gershkovich J, Johnson J, Miller RJ, Burns JW, Onderdonk AB. Effect of surgical adhesion devices on the propagation of experimental intra-abdominal infection. Arch Surg 1999;134:1254-1259.

Role of Transforming Growth Factor Beta-1 in Peritonitis-Induced Adhesions

Ali M. Ghellai, M.D., Arthur F. Stucchi, Ph.D., Nasser Chegini, Ph.D., Chunfeng Ma, M.D., Christopher D. Andry, Ph.D., Jean M. Kaseta, James W. Burns, Ph.D., Kevin C. Skinner, V.M.D., James M. Becker, M.D.

Peritonitis is a major cause of intra-abdominal adhesion formation. The overexpression of transforming growth factor beta-1 (TGF-β1), a potent mitogen, chemoattractant, and stimulant for collagen synthesis by fibroblasts, has been linked to tissue fibrosis at various sites throughout the body including peritoneal adhesion formation. Hence we hypothesized that the mechanism(s) involved in peritonitis-induced adhesion formation may be mediated through the upregulation of TGF-β1 expression. Peritonitis was induced in rats by cecal ligation and puncture, while a control group underwent sham operation. Adhesions were scored and harvested from both groups at 0, 6 and 12 hours and at 1, 2, 4, 7, and 28 days. Tissue expression of TGF-β1 mRNA was determined by quantitative reverse transcription–polymerase chain reaction and TGF-β1 protein was localized by immunohistochemical analysis. Serum and peritoneal fluid TGF-β1 concentrations were quantified by enzyme-linked immunosorbent assay. Compared with sham operation, peritonitis was associated with a significantly greater incidence of abdominal adhesions and a significant increase in the levels of TGF-β1 mRNA expression at days 2, 4, and 7. Immunostaining intensity of TGF-β1 in adhesions from the peritonitis group also steadily rose through day 7. In peritoneal fluid, the ratio of active:total TGF-β1 was significantly increased in the peritonitis group on days 1, 2, and 4 compared with the sham group. These results suggest that peritonitis is associated with the upregulation of TGF-β1, a mechanism that may exacerbate adhesion formation. (J GASTROINTEST SURG 2000;4:316-323.)

KEY WORDS: TGF-β1, peritonitis, abdominal adhesions

Peritonitis is a major cause of intra-abdominal adhesion formation. Peritoneal adhesions are not only the leading cause of small bowel obstruction, but they also lead to difficult reoperation and are a major source of chronic abdominal pain and infertility in women. Although tissue trauma induced during surgical procedures is the single most common cause of peritoneal adhesion formation, previous studies have estimated that 18% to 20% of adhesions are caused by pelvic inflammation.[1] A recent study has estimated that the cost of medical treatment associated with adhesion-related conditions exceeds 1.3 billion dollars per year,[2] which has a substantial impact on health care costs. Despite recent advances in adhesion prevention,[3] the pathogenesis of adhesion formation is still imcompletely understood.

Although the cellular and biochemical responses to peritoneal injury are originally intended to repair the mesothelial surfaces, they can both initiate and exacerbate adhesion formation. Peritoneal ischemia and intra-abdominal sepsis, both of which exacerbate intra-abdominal adhesion formation, have also been associated with compromised peritoneal fibrinolytic activity.[4] The fibrin exudate formed following tissue injury not only provides the structural framework for normal tissue repair,[5] its prolonged existence due to reduced fibrinolytic activity may facilitate the development of adhesion formation.[6] The migration of

From the Departments of Surgery (A.M.G., A.F.S., and J.M.B.) and Pathology (C.D.A.), Boston University School of Medicine, Boston, Mass.; The Institute for Wound Research, University of Florida, Gainesville, Fla. (N.C. and C.M.); and the Genzyme Corporation (J.M.K., J.W.B., and K.C.S), Cambridge, Mass.

Supported in part by the Genzyme Corporation, Cambridge, Mass.

Presented in part at the Thirty-Ninth Annual Meeting of The Society for Surgery of the Alimentary Tract, New Orleans, La., May 17-20, 1998, and published as an abstract in *Gastroenterology* 114:A1390, 1998.

Reprint requests: James M. Becker, M.D., F.A.C.S., Chairman, Department of Surgery, Boston University School of Medicine, One Medical Center Place, Boston, MA 02118.

proinflammatory cells into the peritoneal cavity in concert with numerous growth factors secreted by these cells results in the chemotactic attraction of fibroblasts into the region, which following their proliferation, differentiation, and stimulation of extracellular matrix expression, further promotes the establishment of permanent adhesions.

Transforming growth factor beta-1 (TGF-β1), a polypeptide cytokine with potent chemoattractant and mitogenic activities for macrophages and fibroblasts, is capable of stimulating the expression of various extracellular matrix components by fibroblasts. The overexpression of TGF-β1 has been implicated in the pathogenesis of several fibrotic disorders at various sites throughout the body such as pulmonary fibrosis, glomerulonephritis, cirrhosis of the liver, and skin scarring,[7,8] as well as peritoneal adhesion formation.[9-13] Further evidence implicating a pathogenic role for the overexpression of TGF-β1 is derived from experiments in which the cutaneous[8] or intraperitoneal[14] application of neutralizing antibodies directed against TGF-β1 decreased scar formation at the site of dermal injury as well as intra-abdominal adhesions.

Because TGF-β1 appears to play a pivotal role in various fibrotic disorders including peritoneal adhesion formation, we hypothesized that in the presence of peritonitis, the expression of TGF-β1 is upregulated and contributes to the increased incidence of adhesion formation. This study investigates the role of TGF-β1 in the formation of peritonitis-induced adhesion formation.

MATERIAL AND METHODS

One hundred twenty female Sprague-Dawley rats (250 to 350 g; Charles River Laboratory, Wilmington, Mass.) were housed in groups of five and allowed to acclimatize for approximately 5 days prior to surgery. Throughout the experimental period, animals were housed at a constant room temperature with 12-hour light/dark cycles and allowed free access to water and standard rat chow (Prolab 3000, PMI Feed, St. Louis, Mo.). The study was approved by the Institutional Animal Care and Use Committee at the Genzyme Corporation and the University of Florida Animal Care Committee. All procedures were performed in accordance with recommendations outlined in the "Guidelines for the Care and Use of Laboratory Animals" (National Institutes of Health, NRC, 1996).

Experimental Design

Animals were randomly assigned to either the peritonitis (n = 80) or the sham-operated (n = 40) group. Peritonitis was induced by cecal ligation and puncture as described below.[15] At 0, 6, and 12 hours, and at 1, 2, 4, 7, and 28 days following the cecal ligation and puncture, 10 animals from the peritonitis group and five animals from the sham-operated group were anesthetized with a single intramuscular injection of ketamine (85 mg/kg) and xylazine (6 mg/kg). Phosphate-buffered saline (1 ml) was injected intraperitoneally followed by a gentle 1-minute abdominal massage after which peritoneal fluids were collected. Blood was also collected and centrifuged, and both the serum and peritoneal fluids were stored at −80° C for the quantification of TGF-β1. After in situ scoring of abdominal adhesions (see below), adhesions and intact peritoneum were harvested and prepared for immunohistochemical studies.

Surgical Procedure

All surgical procedures in rats were performed by the same surgeon (A.M.G.) assisted by the same surgical technician. On day 0, rats were weighed and anesthetized with a single intramuscular injection of ketamine (85 mg/kg) and xylazine (6 mg/kg). The ceca were externalized through a 3 to 5 cm midline abdominal incision. In all rats, the ceca tips were devascularized and ligated with 4-0 Dexon (Davis & Geck, Wayne, N.J.). The cecum was punctured once using an 18-gauge needle on the antimesenteric side, in the same location in all animals as determined by a standard measurement (1.5 cm) from the tip of the cecum. The ceca were placed back into the abdomen and the abdominal wall was closed in one layer with 3-0 Dexon (Davis & Geck). In the sham-operated group, the cecum was externalized, manipulated only, and returned to the abdomen.

Evaluation of Postoperative Adhesions

Total number and tenacity of abdominal adhesions were scored in situ by a blinded observer. The total number of adhesions was the number of all individually identifiable and dividable adhesions, whereas the tenacity was based on the firmness of the attachment of adhesions as described by Peck et al.[16] (Table I).

Quantitative Reverse Transcription–Polymerase Chain Reaction

All procedures used for the isolation of total cellular RNA, quantitative reverse transcription–polymerase chain reaction (RT-PCR), and construction of external cDNA standard template for quantitative RT-PCR have been previously described.[17,18] RT-PCR primers were synthesized by the University of Florida Core

Table I. Scale for scoring adhesion tenacity

Adhesion tenacity	Description
0	No adhesions
1	Filmy adhesions with easily identifiable plane
2	Mild adhesions with freely dissectable plane
3	Moderate adhesions with difficult dissectable plane
4	Dense adhesions with nondissectable plane

DNA Synthesis Facility according to the protocol described in detail by Dou et al.[17] To determine the level of TGF-β1 mRNA expression, total cellular RNA was isolated from adhesions and intact peritoneum from both the peritonitis and the sham-operated groups, and subjected to quantitative RT-PCR as previously described.[17] Briefly, cDNA was synthesized in a series of standard reactions, each containing 2 μg of total RNA prepared from each sample and several dilutions of competitive external cRNA standard (1×10^8 to 1×10^3 copies/μg of RNA).[17] The reactions were incubated at 25° C for 10 minutes, 37° C for 60 minutes, and 92° C for 5 minutes followed by the separation of the PCR products on 2% agarose gels containing ethidium bromide. The gels were photographed, scanned, and quantified using NIH-Image (version 1.54).[18] The ratio of the band intensities within each lane was plotted against the copy number of added standard template/reaction, and the quantity of the target mRNAs was determined where the ratio of template/target band intensities was equal to one and analyzed by equations of best-fit lines. The final quantity of the number of mRNA molecules (copies/cell) was calculated as previously described.[17,18]

Immunolocalization of TGF-β1

Adhesion and peritoneal tissues were fixed in Bouin's solution and embedded in paraffin. Tissue sections (5 μm thick) were prepared and immunostained for TGF-β1 using TGF-β1 polyclonal antibody (Santa Cruz Biochemical, Santa Cruz, Calif.) as described by Chegini et al.[10] Control specimens included tissue sections incubated with preabsorbed antibody. The cellular distribution and differences in immunostaining were qualitatively assessed by two blinded investigators (A.M.G. and C.D.A.).

Enzyme-Linked Immunosorbent Assay for TGF-β1

TGF-β1 content of peritoneal fluids and serum was determined using enzyme-linked immunosorbent assay kits specific for TGF-β1 (Promega Corp., Madison, Wis.). Peritoneal fluids and serum were assayed before and after acid treatment, a procedure that results in the activation of latent TGF-β1, allowing for the quantification of active and total TGF-β1 as previously described.[12,13]

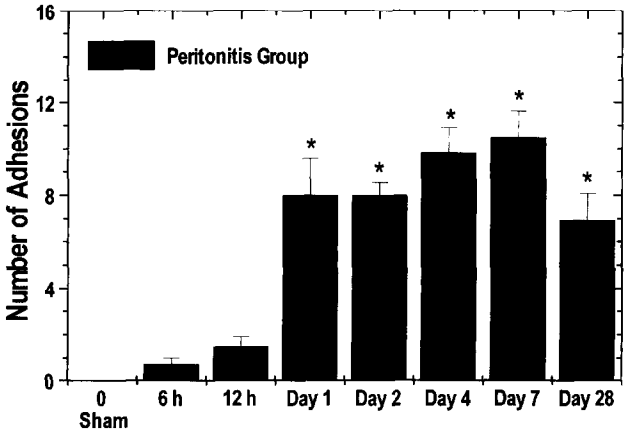

Fig. 1. Peritonitis-induced adhesion formation. All data are expressed as mean ± SEM (* = $P < 0.05$ compared with time 0 and sham-operated animals).

Statistics

All data are expressed as mean ± standard error of the mean (SEM). Group differences were detected by analysis of variance followed by a post hoc multiple mean separation. A P value of less than 0.05 was considered significant.

RESULTS

A total of 80 rats underwent cecal ligation and puncture, whereas 40 rats underwent sham operation. Following cecal ligation and puncture, all rats presented with physical signs of intra-abdominal sepsis including apathetic behavior, lethargy, reduced food and water consumption, piloerection, ocular exudate, and diarrhea. In animals that survived cecal ligation and puncture (78 of 80), the acute symptoms resolved within 3 to 5 days and at the time of sacrifice, the peritoneal cavity typically contained 1 to 2 ml of cloudy fluid containing numerous enteric organisms such as *Escherichia coli*, *Proteus mirabilis*, and *Enterococci*. There were no deaths in the sham-operated group.

Adhesions

In the peritonitis group, diffuse abdominal adhesions were observed as early as 6 hours and were significantly increased ($P < 0.05$) by day 1 compared either with day 0 or the sham-operated animals (Fig. 1). The number of identifiable adhesions remained signif-

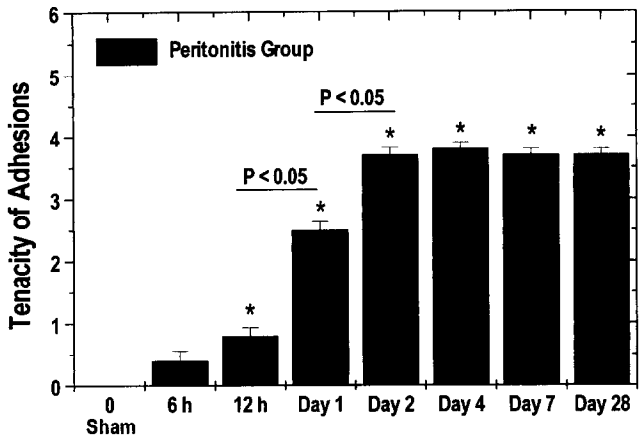

Fig. 2. Peritonitis-induced adhesion tenacity. All data are expressed as mean ± SEM (* = $P < 0.05$ compared with time 0 and sham-operated animals).

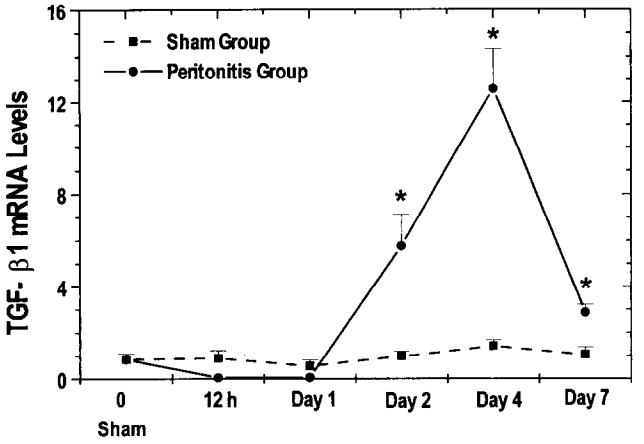

Fig. 3. TGF-β1 mRNA levels (copies per cell) in peritonitis-induced adhesions. All data are expressed as mean ± SEM (* = $P < 0.05$ compared with time 0, 12 hours, day 1, and sham-operated animals).

Table II. Active and total TGF-β1 levels in peritoneal fluid from sham-operated and peritonitis-induced animals

Time	Sham group		Peritonitis group	
	Active	Total	Active	Total
0	0.48 ± 0.08	1.23 ± 0.35	0.48 ± 0.08	1.23 ± 0.35
12 hr	0.73 ± 0.04	0.94 ± 0.14	0.85 ± 0.06	1.46 ± 0.05
Day 1	0.63 ± 0.03	1.17 ± 0.17	0.93 ± 0.07	1.17 ± 0.38
Day 2	0.82 ± 0.06	2.45 ± 0.25†	0.60 ± 0.07	0.65 ± 0.03
Day 4	0.85 ± 0.09	1.96 ± 0.44	1.25 ± 0.54	1.13 ± 0.18
Day 7	1.61 ± 0.3*	2.23 ± 0.48†	0.56 ± 0.10	1.37 ± 0.18
P value (analysis of variance)	0.0005	0.0004	0.08	0.06

Values are mean ± SEM. n = 5 at each time point in the sham-operated group, whereas n = 10 at each time point in the peritonitis group.
*$P < 0.05$ compared with all other time points within the group.
†$P < 0.05$ compared with 12 hours and day 1 within the group.

icantly elevated through day 28 (see Fig. 1). Although the cecum was the major site of adhesion involvement, intra-abdominal adhesions to other abdominal organs were noted. No adhesions were observed at time 0 or in the sham-operated group. In addition to the increased incidence of adhesions, peritonitis also significantly increased ($P < 0.05$) the tenacity (Fig. 2) of abdominal adhesions by 12 hours compared with either time 0 or the sham-operated animals. Adhesion tenacity also remained significantly elevated through day 28.

Adhesion and Peritoneal TGF-β1 mRNA Levels

The expression of TGF-β1 RNA in adhesions and intact peritoneum harvested from the peritonitis and sham groups is shown in Fig. 3. The results indicate that following the induction of peritonitis on day 0, the TGF-β1 mRNA levels were suppressed to undetectable levels through the day 1 (see Fig. 3). Subsequently, TGF-β1 mRNA levels significantly increased ($P < 0.05$) approximately 6-, 15-, and 3-fold compared with time 0 on days 2, 4, and 7, respectively. Compared with the sham-operated group, TGF-β1 mRNA levels in the peritonitis group increased approximately 6-, 9-, and 3-fold on days 2, 4, and 7, respectively. TGF-β1 levels in the intact peritoneum from the sham-operated group remained unchanged through day 7 (see Fig. 3).

Peritoneal Fluid and Serum TGF-β1 Levels

In the sham-operated group, active TGF-β1 levels in peritoneal fluids were unaffected until day 7, where levels were significantly higher ($P < 0.05$) compared with all other time points (Table II). Total TGF-β1 levels in the sham-operated group rose significantly ($P < 0.05$) on day 2 compared to day 1, and remained elevated until day 7. In contrast, in the peritonitis group, there were no significant changes in peritoneal fluid active or total TGF-β1 levels (see Table II).

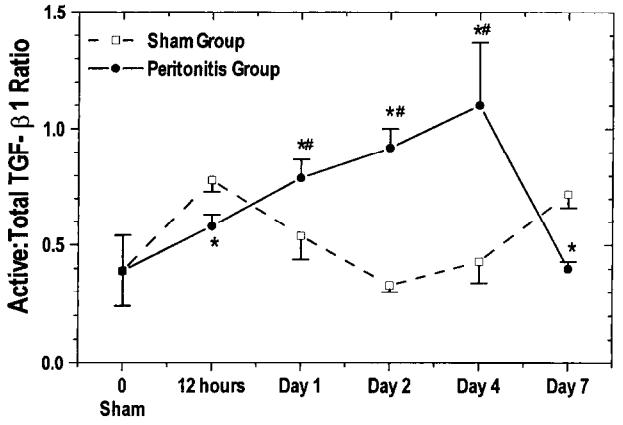

Fig. 4. Peritoneal fluid active:total TGF-β1 ratios. All data are expressed as mean ± SEM. n = 5 at each time point in the sham-operated group, whereas n = 10 at each time point in the peritonitis group (* = $P < 0.05$ compared with the sham group at each time point; # = $P < 0.05$ compared with 12 hours and day 7 within the peritonitis group).

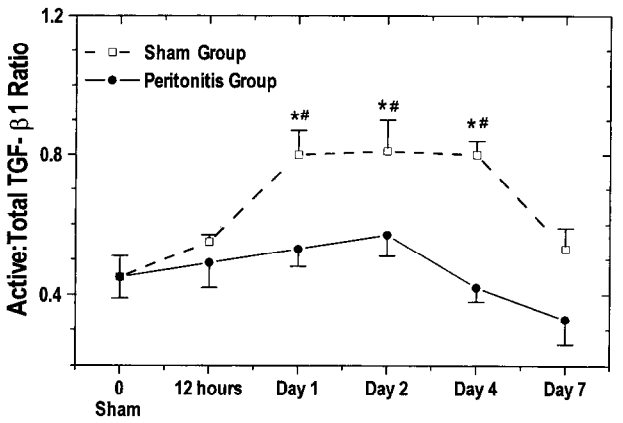

Fig. 5. Serum active:total TGF-β1 ratios. All data are expressed as mean ± SEM. n = 5 at each time point in the sham-operated group, whereas n = 10 at each time point in the peritonitis group (* = $P < 0.05$ compared with the peritonitis group at each time point; # = $P < 0.05$ compared with 12 hours and day 7 within the sham-operated group).

Most mammalian cells release TGF-β1 as a high-molecular-weight inactive or latent complex.[19] Although numerous mechanisms such as sepsis or low pH have been shown to activate the latent complex,[20] the amount of latent complex released as well as the proportion subsequently activated can vary greatly. Because only the bioactive TGF-β1 is able to bind to TGF-β1 receptor to exert its biologic effect, the active:total ratio of TGF-β1 is perhaps a better assessment of TGF-β1.[21] Interestingly, the results indicate that the active:total ratio of TGF-β1 steadily and significantly ($P < 0.05$) rose from time 0 and remained significantly elevated compared with sham-operated animals through day 4, at which time it dropped precipitously (Fig. 4). These data indicate that although total latent TGF-β1 levels are not significantly changing (Table II), the portion of the total that is activated is increasing during peritonitis compared with the sham-operated group (see Fig. 4).

In the sham-operated group, there were no significant differences in serum levels of active TGF-β1 (Table III). However, total serum TGF-β1 levels rose significantly on day 7 compared with time 0, 12 hours, and day 1. In the peritonitis group, both active and total serum TGF-β1 levels were significantly elevated ($P < 0.05$) on day 7 compared with all other time points within the group (see Table III). As shown in Fig. 5, the active:total ratio of TGF-β1 in serum rose significantly by day 1 in the sham-operated group and remained significantly elevated compared with both the sham group and time 0 through day 4, at which time it dropped sharply.

Immunohistochemical Analysis

Adhesions obtained from the peritonitis group and intact peritoneum from the sham-operated group all contained immunoreactive TGF-β1. Baseline staining was low in both the sham-operated and peritoni-

Table III. Active and total TGF-β1 levels in serum from sham-operated and peritonitis-induced animals

Time	Sham group		Peritonitis group	
	Active	Total	Active	Total
0	1.7 ± 0.40	3.53 ± 0.54	1.7 ± 0.40	3.53 ± 0.54
12 hr	1.57 ± 0.04	2.88 ± 0.08	2.41 ± 0.09	5.14 ± 0.69
Day 1	1.86 ± 0.19	2.44 ± 0.29	2.81 ± 0.27	5.23 ± 0.18
Day 2	3.82 ± 0.60	4.64 ± 0.47	2.96 ± 0.55	5.50 ± 1.17
Day 4	3.76 ± 0.71	5.0 ± 1.17	2.23 ± 0.30	5.76 ± 0.68
Day 7	3.60 ± 0.69	6.27 ± 0.63*	5.76 ± 1.42†	22.15 ± 7.49†
P value (analysis of variance)	0.004	0.0016	0.005	0.0031

Values are mean ± SEM. n = 5 at each time point in the sham-operated group, whereas n = 10 at each time point in the peritonitis group.
*$P < 0.05$ compared with time 0, 12 hours, and 1 day within the group.
†$P < 0.05$ compared with all other time points within the group.

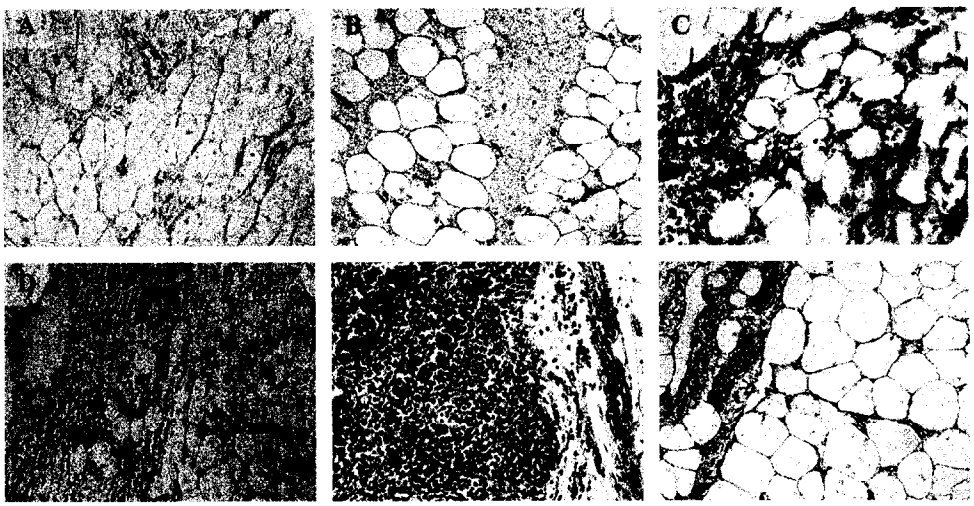

Fig. 6. Immunohistochemical localization of TGF-β1 in adhesion tissues and omentum. The immunoreactive TGF-β1 protein is present primarily in inflammatory cells, especially macrophages. Representative tissue and omentum sections are from days 0, 1, 2, 4, and 7 in peritonitis-induced animals and from day 7 in a sham-operated control rat. A representative tissue section from an experimental animal on day 0 prior to the induction of peritonitis showing minimal TGF-β1 staining in the omentum **(A)**. Sham-operated animals on day 0 were similar in appearance and showed little immunoreactive TGF-β1 (not shown). TGF-β1 staining in abdominal adhesions and omentum from the peritonitis group was similarly low on day 1 **(B)**. TGF-β1 staining became more steadily abundant, in concert with rising TGF-β1 mRNA levels by days 2 **(C)** and 4 **(D)**. Even though TGF-β1 mRNA levels dropped after day 4, TGF-β1 staining intensity increased substantially on day 7 **(E)**, suggesting that the express protein persists after the message has been reduced. There was minimal TGF-β1 staining in peritoneal tissues and omentum from sham-operated animals at all time points. A representative section of omentum from a sham-operated animal is shown in **F.** (Original magnification ×150.)

tis groups on day 0 (Fig. 6, *A*). In the peritonitis group (Fig. 6, *B* to *E*), the intensity of TGF-β1 immunostaining was substantially increased on day 4 (Fig. 6, *D*) and day 7 (Fig. 6, *E*) compared with day 1 (Fig. 6, *B*) and day 2 (Fig. 6, *C*), with inflammatory cells, especially macrophages, as the major site of TGF-β1 immunoreactivity. The intensity of TGF-β1 immunoreactivity appears to correspond with the upregulation of TGF-β1 mRNA expression (see Fig. 3). There was no significant immunostaining of TGF-β1 in tissues obtained from the sham-operated group at any time point, and a representative tissue section of omentum from a sham-operated animal on day 7 is shown in Fig. 6, *F*. In control rats, sections incubated with deletion of the primary antibody, or with preabsorbed antibody, resulted in substantial reduction in immunostaining (not shown).

DISCUSSION

Peritonitis or intra-abdominal sepsis is associated with numerous potentially serious and even life-threatening complications. Among these are the development of significant peritoneal adhesions, fibrosis, and abscess formation. In this study, in which a common rat model was used to induce generalized peritonitis, we demonstrated a significant increase in both the number (see Fig. 1) and tenacity (see Fig. 2) of intra-abdominal adhesions. Most surgically associated peritonitis is secondary to bacterial contamination from the gastrointestinal tract, and the resulting polymicrobial contamination leads to peritoneal insudation of numerous inflammatory cells. The subsequent release of chemotactic, mitogenic, and proinflammatory mediators that accompany the ensuing inflammatory response adversely affects the fibrinolytic system, which impairs normal wound healing and facilitates abdominal adhesion formation.[22]

Among these substances, TGF-β1 has been shown to induce fibrotic disorders at various sites throughout the body because of its overexpression. Our results indicate that peritonitis is also associated with the upregulation of TGF-β1 mRNA and protein, which may contribute to the increased incidence of peritoneal adhesion formation. Although our results indicate that there was a decline in TGF-β1 mRNA expression during the early stages of peritonitis (see Fig. 3), TGF-β1 mRNA expression subsequently increased ($P < 0.05$) on days 2, 4, and 7 compared with both baseline values and those in the sham-operated group. The reason for the initial decline in TGF-β1 mRNA expression up to day 1 is unknown.

TGF-β1 is a potent chemotactic agent for fibroblasts and inflammatory cells such as macrophages. TGF-β1 is one of five structurally related TGF-β isoforms and constitutes more than 85% of the total TGF-β in wound fluid.[23] As mentioned previously, TGF-β1 is synthesized and secreted as a high-molecular-weight biologically inactive or latent complex that readily associates with extracellular matrix or cell membranes. The latent TGF-β1 provides a reservoir for the peptide that can be activated by acid, heat, or enzymatically by sialidases and serine proteases, which are secreted by inflammatory cells that infiltrate ischemic, septic, or injured tissues.[8,21] Although numerous mechanisms including sepsis have been shown to facilitate activation of the latent complex, the amount of latent TGF-β1 released as well as the proportion subsequently activated can vary greatly. Only bioactive TGF-β1 is able to bind to the TGF-β1 receptor to exert biologic effects such as promoting angiogenesis, cell proliferation, and differentiation, as well as to stimulating the synthesis and deposition of extracellular matrix.[8,19] Dysregulation of the balance between the active and latent forms of TGF-β1 could result in excessive activity as has recently been described for human scar and hypertrophic scar fibroblasts.[24] Hence the active:total ratio of TGF-β1 is perhaps a better assessment of the bioactivity of TGF-β1. Although the levels of total or active TGF-β1 protein in the peritoneal fluid were not significantly altered during peritonitis (see Table II), the active:total ratio increased significantly up to day 4 following the induction of peritonitis (see Fig. 4), suggesting that the portion of the total latent TGF-β1 that is activated is increasing during peritonitis compared with the sham-operated group. The increased influx of inflammatory cells during this period may explain both the increased amounts of TGF-β1 mRNA observed during peritonitis as well as the increased expression of TGF-β1 protein through day 7 as demonstrated immunohistochemically. The increased influx of inflammatory cells may also represent the source of enzymatic activation of latent TGF-β1. Latent TGF-β1 has a considerably longer plasma half-life in vivo compared with active TGF-β1, which may explain the increase in serum levels of total TGF-β1 as well as the reduction in the active:total ratio in the peritonitis group compared with the sham-operated group. We have previously demonstrated that the peritoneal fluid levels of total and active TGF-β1 significantly increased during the first week after injury in surgically induced peritoneal injury in mice[12] as well as in patients with adhesions.[13]

Peritonitis is associated with persistent TGF-β1 expression in humans, which may cause persistent TGF-β1 mRNA expression,[25] especially since TGF-β1 can upregulate its own expression.[11] In fact, the message for TGF-β1 may persist long after the initiating peritoneal stimulus has subsided resulting in a sustained expression of TGF-β1. Furthermore, it has been shown that the formation of intra-abdominal adhesions is inhibited by antibodies to TGF-β1 providing further evidence implicating TGF-β1 solely in the initiation of intra-abdominal adhesion formation.[14] More recent evidence has also demonstrated increased levels of talc-induced adhesions in mice heterozygous for the TGF-β1 null allele (+/−) compared with wild type (+/+), suggesting that TGF-β1 plays a key role in regulating the extent of adhesion formation even under aseptic conditions.[26] In the present study, we have demonstrated that peritonitis is associated with a 15-fold increase in the expression of TGF-β1 mRNA in adhesions by day 4 compared to time 0 and sham-operated control values, respectively. The excessive production of TGF-β1, especially to this magnitude, can substantially increase the amount of intracellular and matrix-associated plasminogen activator inhibitor-1, even in vitro, which can impair normal peritoneal healing and promote matrix deposition and intra-abdominal adhesion formation.[27]

Although transcriptional regulation of TGF-β1 plays a key role in the synthesis of TGF-β1 protein, perhaps of more biologic relevance is the regulation of TGF-β1 protein activity. As mentioned earlier, this process involves principally the activation of latent forms of TGF-β1, which are secreted from cells, released from platelets, and sequestered by extracellular matrix.[28] TGF-β1 can associate with a variety of matrix proteins including biglycan, decorin, type IV collagen, fibronectin, and thrombospondin and form both covalent and noncovalent complexes with α_2-macroglobulin.[29] Mechanisms of activation include proteolytic activation, low pH, and binding to thrombospondin.[20,30] Thus the proteolytic environment characteristic of the early stages of wound healing or sepsis may potentiate the release and activation of TGF-β1 from its stores in pericellular matrix prior to transcriptional activation of the TGF-β1 gene.[31]

Immunohistochemically, TGF-β1 has also been localized in humans in postoperative fibrous adhesions induced under aseptic conditions.[10] Our immunohistochemical studies have demonstrated that most cell types, particularly the inflammatory cells infiltrated into the peritonitis-induced adhesions, contained immunoreactive TGF-β1 (see Fig. 6). The intensity of TGF-β1 immunostaining substantially increased on days 4 and 7 compared with days 1 and 2, and appears to correspond with the upregulation of TGF-β1 mRNA expression.

CONCLUSION

Peritonitis is a serious and potentially life-threatening event. The pathogenic events that accompany the acute inflammatory response are well characterized and appear to be mediated primarily by TGF-β1. Regulating the release of TGF-β1 from invading inflammatory cells, preventing the subsequent activation of TGF-β1, or neutralizing active TGF-β1 may represent viable alternatives that may minimize the pathogenic sequelae following peritonitis including the formation of intra-abdominal adhesions.

REFERENCES

1. Weibel M, Majno G. Peritoneal adhesions and their relation to abdominal surgery. Am J Surg 1973;126:345-353.
2. Ray FN, Denton WG, Thamer M, Henderson SC, Perry S. Abdominal adhesiolysis: Inpatient care and expenditure in the United States in 1994. J Am Coll Surg 1998;186:1-9.
3. Becker JM, Dayton MT, Fazio VW, Beck DE, Stryker SJ, Wexner SD, Wolff BG, Roberts PL, Smith LE, Sweeney SA, Moore M. Sodium hyaluronate-based bioresorbable membrane (HAL-F) in the prevention of postoperative abdominal adhesions: A prospective, randomized, double-blinded multicenter study. J Am Coll Surg 1996;183:297-307.
4. Vipond MN, Whawell SA, Thompson JN, Dudley HAF. Effect of experimental peritonitis and ischaemia on peritoneal fibrinolytic activity. Eur J Surg 1994;160:471-477.
5. Ellis H, Harrison W, Hugh TB. The healing of peritoneum under normal and pathological conditions. Br J Surg 1965; 52:471-476.
6. Thompson JN, Patterson-Brown S, Harbourne T, Whawell SA, Kalodiki E, Dudley HAF. Reduced human peritoneal plasminogen activating activity: As a possible mechanism of adhesion formation. Br J Surg 1989;76:328-384.
7. Border WA, Noble NA. Transforming growth factor beta in tissue fibrosis. N Engl J Med 1994;331:1286-1292.
8. Roberts AR. Transforming growth factor-b: Activity and efficacy in animal models of wound healing. Wound Repair Regeneration 1995;3:408-418.
9. Williams RS, Rossi AM, Chegini N, Schultz G. Effect of transforming growth factor β on postoperative adhesion formation and intact peritoneum. J Surg Res 1992;52:65-70.
10. Chegini N, Gold LI, Williams RS. Localization of transforming growth factor beta isoforms TGF-β1, TGF-β2, and TGF-β3 in surgically induced pelvic adhesions in the rat. Obstet Gynecol 1994;83:455-461.
11. Chegini N. The role of growth factors in peritoneal healing: Transforming growth factor beta (TGF-beta). Eur J Surg 1997;577:17-23.
12. Rong H, Tang XM, Zhao Y, Juneja SC, Fay MF, Williams RS, Chegini N. Postsurgical intraperitoneal exposure to surgical glove powders modulates inflammatory and immune-related cytokines production. Wound Repair Regeneration 1997;5:89-96.
13. Chegini N, Rong H, Bennett B, Stone IK. Peritoneal fluid cytokine and eicosanoid levels and their relation to the incidence of peritoneal adhesion. J Soc Gynecol Invest. 1999;6: 153-157.
14. Lucas PA, Warejcka DJ, Young HE, Lee BY. Formation of abdominal adhesions is inhibited by antibodies to transforming growth factor-β1. J Surg Res 1996;65:135-138.
15. Wichterman KA, Baue AE, Chaudry IH. Sepsis and septic shock—a review of laboratory models and a proposal. J Surg Res 1980;29:189-201.
16. Peck LS, Quigg JM, Fossum GT, Goldberg EP. Evaluation of CMC and HA solutions for adhesiolysis. J Invest Surg 1995; 8:337-348.
17. Dou Q, Zhao Y, Tarnuzzer RW, Rong H, Williams RS, Schultz GS, Chegini N. Suppression of transforming growth factor-β (TGF-β) and TGF-β receptor messenger ribonucleic acid and protein expression in leiomyomata in women receiving gonadotropin-releasing hormone agonist therapy. J Clin Endocrinol Metab 1996;81:3222-3230.
18. Dou Q, Williams RS, Chegini N. Expression of integrin messenger ribonucleic acid in human endometrium: A quantitative reverse transcription polymerase chain reaction study. Fertil Steril 1999;71:347-353.
19. Lawrence DA. Transforming growth factor-beta: A general review. Eur Cytokine Network 1996;7:363-374.
20. Schultz-Cherry S, Murphy-Ullrich JE. Thrombospondin causes activation of latent transforming growth factor-beta secreted by endothelial cells by a novel mechanism. J Cell Biol 1993;122:923-932.
21. Clark DA, Coker R. Transforming growth factor-beta (TGF-β). Int J Biochem Cell Biol 1998;30:293-298.
22. Ivarsson ML, Bergstrom M, Eriksson E, Risberg B, Holmdahl L. Tissue markers as predictors of postoperative adhesions. Br J Surg 1998;85:1549-1554.
23. Longaker MT, Whitby DJ, Ferguson MW, Lorenz HP, Harrison MR, Adzick NS. Adult skin wounds in the fetal environment heal with scar formation. Ann Surg 1994;219:65-72.
24. Messadi DV, Bertolami CN. CD44 and hyaluronan expression in human cutaneous scar fibroblasts. Am J Pathol 1993; 142:1041-1049.
25. Lin CY, Chen WP, Yang LY, Chen A, Huang TP. Persistent transforming growth factor-beta 1 expression may predict peritoneal fibrosis in CAPD patients with frequent peritonitis occurrence. Am J Nephrol 1998;18:513-519.
26. Krause TJ, Katz D, Wheeler CJ, Ebner S, McKinnon RD. Increased levels of surgical adhesions in TGF-beta 1 heterozygous mice. J Invest Surg 1999;12:31-38.
27. Rougier JP, Guia S, Hagege J, Nguyen G, Ronco PM. PAI-1 secretion and matrix deposition in human peritoneal mesothelial cell cultures: Transcriptional regulation by TGF-beta 1. Kidney Int 1998;54:87-98.
28. Miyazono K, Ichijo H, Heldin CH. Transforming growth factor-beta: Latent forms, binding proteins and receptors. Growth Factors 1993;8:11-22.
29. Noble NA, Harper JR, Border WA. In vivo interactions of TGF-beta and extracellular matrix. Prog Growth Factor Res 1992;4:369-382.
30. Harpel JG, Metz CN, Kojima S, Rifkin DB. Control of transforming growth factor-beta activity: Latency vs. activation. Prog Growth Factor Res 1992;4:321-335.
31. Grainger DJ, Wakefield L, Bethell HW, Farndale RW, Metcalfe JC. Release and activation of platelet latent TGF-beta in blood clots during dissolution with plasmin. Nat Med 1995; 1:932-937.

Hospital Procedure Volume and Teaching Status Do Not Influence Treatment and Outcome Measures of Rectal Cancer Surgery in a Large General Population

Marko Simunovic, M.D., Teresa To, Ph.D., Nancy Baxter, M.D., Andrew Balshem, B.A., Eric Ross, Ph.D., Zane Cohen, M.D., Robin McLeod, M.D., Paul Engstrom, M.D., Elin Sigurdson, M.D.

A clear benefit of increased hospital procedure volume or teaching hospital status on outcomes of rectal cancer surgery has yet to be shown. Few have examined treatment differences that may lead to varying outcomes. This study assessed the impact of hospital procedure volume and teaching status on both treatment and outcome measures of rectal cancer surgery in a large general population. Data were obtained for 1072 incident cases of rectal adenocarcinoma diagnosed in 1990 from Ontario, Canada, and treated with a major resection. Hospitals were classified by teaching status and procedure volume. Pathology reports were examined for 418 procedures. Abdominoperineal resections accounted for 31.0% of all procedures. There were no clinically significant differences in treatment measures, operative mortality, and long-term survival among the hospital groups according to both univariate and multivariate analyses. In conclusion, the absence of a hospital volume or teaching status effect on treatment and outcome measures suggests that for rectal cancer surgery in Ontario, centralization of procedures into high-volume or teaching centers is unlikely to improve surgical quality. (J GASTROINTEST SURG 2000;4:324-330.)

KEY WORDS: Rectal neoplasm, outcomes, database

Hospitals with higher vs. lower procedure volumes or hospitals with teaching vs. nonteaching status may have improved surgical outcomes. When better outcomes for oncologic procedures appear related to greater volume or teaching hospital status, clinicians and researchers have suggested particular operations be restricted to high-volume or teaching centers.[1-8] Such a policy response may result in the transfer of patients to high-volume centers with poor outcomes where their poor results are hidden by the superior results of other high-volume centers. In addition, in a large population, a lack of correlation between outcomes and gross hospital descriptors may lead to quality complacency, despite inferior outcomes in specific hospitals. More important, descriptors such as hospital teaching status or procedure volume shed little light on the actual treatment differences leading to improved outcomes.

A clear benefit has yet to be shown for increased volume or teaching status in terms of outcomes of rectal cancer surgery.[7-13] This study examined these relationships in Ontario, Canada—something that had not been done previously in a large general population. We created treatment measures to look for actual treatment differences among hospital groups in Ontario, and considered the potential role of treatment and outcome measures in quality initiatives.

METHODS

Data Source and Inclusion Criteria

The Ontario Cancer Registry collects data on all cancer cases in the province of Ontario, Canada (population 10.2 million). Relevant variables were abstracted from the Registry including patient age, sex, comorbidity, discharge status (alive or dead), long-

From the Fox Chase Cancer Center Outcomes Group, Philadelphia, Pa. (M.S., A.B., E.R., P.E., and E.S.), the Institute for Clinical Evaluative Sciences, Toronto, (M.S. and T.T.), and the University of Toronto, Mount Sinai Hospital, Toronto, Ontario, Canada (N.B., Z.C., and R.M.L.).

Presented at the Thirty-Ninth Annual Meeting of The Society for Surgery of the Alimentary Tract, New Orleans, La., May 17-20, 1998.

Reprint requests: Marko Simunovic, M.D., Department of Surgical Oncology, Fox Chase Cancer Center, 7701 Burholme Ave., Philadelphia, PA 19111. e-mail: m_simunovic@fccc.edu

term survival (alive, dead, or lost to follow-up), and hospital affiliation (teaching or nonteaching institution). We defined a major resection for rectal cancer as abdominoperineal resection (APR) or low anterior resection (LAR), if linked to a diagnosis of colon or rectal cancer (Canadian Classification of Diagnostic, Therapeutic and Surgical Procedures [CCP] codes 604 or 605, respectively,[14] linked to International Classification of Diseases, Clinical Modification Ninth Revision (ICD-9) diagnosis codes 153 or 154, respectively[15]). The inclusion of colon cancer if linked to a rectal procedure ensured the capture of high rectal lesions.

All incident cases of colorectal cancer diagnosed in calendar year 1990 were selected for analysis if they satisfied the preceding link, had no other cancer diagnosis, had histologic findings consistent with adenocarcinoma, underwent resection within 60 days of diagnosis, and if the patient was 20 years of age or more. Year 1990 was chosen because survival data were available only into year 1996. The 60-day window was designed to examine the usual standard of care in the province—there were only 54 major resections past our 60-day window. The administrative database lacked details on the presence of metastatic disease, use of adjuvant therapy, incidence of local tumor recurrence, and cancer-related mortality.

Pathology Reports

We reviewed pathology reports for 418 of our 1072 cases—resource limitations precluded the examination of all reports. An attempt was made to examine every APR report since we were interested in sphincter preservation as a treatment measure. Close examination of 344 of a potential 352 APR reports indicated that 20 cases were procedure LAR for a false APR rate of 5.8%. Appropriate coding adjustments were made resulting in the review of 324 APRs and 94 LARs; the original 74 LAR reports were selected using a random sample process. Tumor variables abstracted included size, grade, depth of wall invasion, margin status, lymph node status, number of lymph nodes reported, and distance between the tumor edge and distal resection margin.

Treatment and Outcome Measures

Treatment measures examined included number of sphincter-removing procedures (APR rate), number of lymph nodes assessed, anal verge–tumor distance (for APRs), and distal margin–tumor distance (for LARs). These measures considered two important aspects of rectal cancer surgery—sphincter preservation and an adequate oncologic resection. Provision of an APR, and thus loss of the anal sphincter, is ostensibly a function of tumor distance from the anal verge; tumors located 5 cm or more from the verge usually can be removed and the proximal bowel reanastomosed to the distal rectum or anal canal.[16] Also, work done prior to 1990 by various investigators supports the need for thorough dissection of the mesorectum—the lymph node–bearing portion of the rectum—and a minimal 2 cm distal resection margin.[16-20] Outcomes chosed included operative mortality and long-term overall survival. Operative mortality was any in-hospital death during the index admission and was not limited to 30 days. (The database used is highly accurate and complete when coding operative mortality and long-term survival.[21])

Analysis

The database did not include surgeon-specific data, and analyses were limited to the hospital level. Prior to assessing them for treatment or outcome differences, individual hospitals were placed into low-volume (11 or less), medium-volume (12 to 17)–, or high-volume (18 or more) groups based on the number of major resections performed during the study period, and into teaching (i.e., involved with the training of physicians) or nonteaching institutions. Volume cutoffs created three evenly sized groups; this combined the need for statistical stability and clinical relevance. Where appropriate, chi-square, Kruskal-Wallis, or Wilcoxon two-sample testing measured for differences among the groups in patient and tumor variables and in treatment and outcome measures.

Logistic regression models analyzed operative mortality, whereas proportional hazards models assessed long-term survival. All models included the explanatory variables of hospital volume, hospital teaching status, and patient age, sex, and comorbidity score. The Deyo et al.[22] validated modification of a comorbidity index for the ICD-9-CM database was used to define comorbidity. Radiotherapy services in Ontario are centrally controlled and delivered at one of only nine regional cancer centers. Therefore registration at a regional cancer center within 120 days of diagnosis was considered a proxy of advanced disease and included in the survival models. Twenty-three cases registered at a regional cancer center prior to surgery were excluded—possible very advanced tumors treated with neoadjuvant therapy. A separate survival model for the 418 cases with reviewed pathology also incorporated nodal status and depth of wall invasion.

A number of sensitivity analyses for our regression models tested the robustness of our results. First, volume cutoffs were varied by one to three procedures in all directions.[23] In addition, we created very low–

and very high–volume groups. For example, designating low- and high-volume cutoffs of five or less and 28 or more produced low- and high-volume groups encompassing 12.8% and 11.6% of all cases, respectively. We also wished to ensure that results for 1990 were not statistical aberrations vs. other years. Therefore data for years 1988 and 1991 were combined and used to construct regression models as above. Pathology reports were not available for these years.

RESULTS

There were 1072 incident cases of rectal cancer diagnosed in 1990 and treated with a major resection. APRs accounted for 332 or 31.0% of all resections. Fourteen high-volume and 86 low-volume hospitals provided 32.0% and 36.8% of procedures, respectively (Table I), whereas 26.3% of resections were done in 20 teaching hospitals (Table II). Age, comorbidity, sex, and tumor variables were evenly distributed among the volume and teaching groups (see Table I and II). The operative mortality rate for the province was 4.3%. For the 418 procedures with reviewed pathology, the average number of nodes reported was 6.3, the average distal margin–tumor distance was 3.2 cm (procedure LAR), and the average anal verge–tumor distance was 5.2 cm (procedure APR). In 75.1% of cases, tumors were node positive, invading through the bowel wall, or both. Tumor variables were evenly distributed by age and sex (data not shown). For example, for ages 20 to 59, 60 to 69, and 70 or older, the percentages of node-positive patients were 42.3%, 37.6%, and 45.2%, respectively ($P = 0.4$), whereas the percentages of full wall penetration were 67.6%, 63.1%, and 72.7% ($P = 0.2$), respectively. Postoperatively, 32.6% of all patients were registered at a regional cancer center for consideration of adjuvant therapy.

There were no significant differences in operative mortality rates and treatment measures among the volume groups (Table III). As shown in Table IV, operative mortality and average number of nodes reported were higher in teaching vs. nonteaching hospitals—6.4% vs. 3.5% ($P = 0.04$) and 7.1 vs. 5.9 ($P = 0.01$), respectively.

Hospital volume and teaching status did not significantly influence operative mortality when models controlled for patient age, sex, comorbidity, and procedure type (Table V). Long-term survival was worse for cases treated in low-volume vs. high-volume hospitals (risk ratio [RR] = 1.2, confidence interval [CI] = 1.0 to 1.5, $P = 0.04$) (Table VI). Hospital teaching status did not influence long-term survival (RR = 1.0, CI = 0.8 to 1.2, $P = 0.7$). With the inclusion of tumor variables, the greater risk of death in low-volume vs. high-volume institutions became nonsignificant (RR = 1.4, CI = 1.0 to 1.9, $P = 0.08$) (Table VII). As

Table I. Characteristics by volume group

	Low (≤11)	Medium (12-17)	High (≥18)	*P* value
For 1072 major resections				
No. of cases	394 (36.8%)	335 (31.3%)	343 (32.0%)	NA
No. of hospitals	86	24	14	NA
Age				
20-59 yr	23.4%	25.1%	31.5%	0.13
60-69 yr	34.5%	35.2%	30.6%	
≥70 yr	42.1%	39.7%	37.9%	
Comorbidity score				
0	82.7%	86.0%	85.4%	0.65
1	13.5%	11.1%	10.5%	
≥2	3.8%	3.0%	4.1%	
Female sex	35.5%	37.0%	38.2%	0.76
For 418 resections with pathology available				
No. of cases	163	124	131	NA
Node positive	62 (38.0%)	49 (39.5%)	64 (48.9%)	0.14
Full wall invasion	109 (66.9%)	85 (68.6%)	90 (68.7%)	0.93
Moderately differentiated	88 (53.4%)	77 (62.1%)	66 (50.4%)	0.16
Ulcerating morphology	105 (64.4%)	80 (65.0%)	92 (70.2%)	0.54
Vascular/lymphatic invasion	28 (17.2%)	13 (10.5%)	15 (11.5%)	0.19

NA = not applicable.

Table II. Characteristics by teaching group

	Teaching	Nonteaching	*P* value
For 1072 major resections			
No. of cases	282 (26.3%)	790 (73.7%)	NA
No. of hospitals	20	104	NA
Age			
20-59 yr	28.4%	25.8%	0.37
60-69 yr	35.1%	32.9%	
≥70 yr	36.5%	41.3%	
Comorbidity score			
0	81.2%	85.8%	0.11
1	13.5%	11.2%	
≥2	5.3%	3.1%	
Female sex	37.2%	36.7%	0.86
For 418 resections with pathology available			
No. of cases	105	313	NA
Node positive	40 (38.1%)	135 (43.1%)	0.37
Full wall invasion	69 (65.7%)	215 (68.7%)	0.57
Moderate differentation	64 (61.0%)	167 (53.4%)	0.18
Ulcerating morphology	70 (66.7%)	207 (66.4%)	0.95
Vascular/lymphatic invasion	13 (12.4%)	43 (13.7%)	0.72

NA = not applicable.

Table III. Operative mortality and treatment measures by volume group

	Low (≤11)	Medium (12-17)	High (≥18)	*P* value
Operative mortality	16 (4.1%)	12 (3.6%)	18 (5.3%)	0.54
Procedure APR	138 (35.0%)	93 (28.0%)	101 (30.4%)	0.08
Average ATD (cm)	5.1	5.2	5.1	0.68
Average DMTD (cm)	3.3	3.2	3.2	0.97
Average NON	6.2	6.1	6.5	0.56

APR = abdominoperineal resection; ATD = anal verge–tumor distance for 324 APRs; DMTD = distal margin–tumor distance for 94 low anterior resections; NON = number of nodes.

Table IV. Operative mortality and treatment measures by teaching group

	Teaching	Nonteaching	*P* value
Operative mortality	18 (6.4%)	28 (3.5%)	0.04
Procedure APR	85 (30.1%)	247 (31.3%)	0.73
Average ATD (cm)	5.0	5.2	0.47
Average DMTD (cm)	3.5	3.2	0.59
Average NON	7.1	5.9	0.01

APR = abdominoperineal resection; ATD = anal verge-tumor distance for 324 APRs; DMTD = distal margin–tumor distance for 94 low anterior resections; NON = number of nodes.

Table V. Logistic regression for operative mortality for 1072 major resections

Explanatory variable*	Likelihood of death: Odds ratio	95% Confidence interval	*P* value
Volume (procedures/yr)			
Medium (12-17)	0.9	0.4-2.0	0.90
Low (≤11)	0.9	0.4-1.9	0.71
Teaching	1.7	0.8-3.3	0.16
Male sex	1.7	0.9-3.7	0.13
Age			
60-69 yr	1.7	0.5-8.0	0.42
≥70 yr	5.7	1.9-24.2	<0.01
Comorbidity score			
1	3.4	1.7-6.6	<0.01
≥2	6.5	1.4-31.4	0.02
Procedure APR	0.3	0.1-0.7	0.02

APR = abdominoperineal resection.
*Reference comparisons are high volume (≥18 procedures/yr), nonteaching status, female sex, age 20 to 59 years, comorbidity score 0, and procedure low anterior resection.

Table VI. Proportional hazards for long-term risk of death for 1072 major resections

Explanatory variable*	Likelihood of failure: Risk ratio	95% Confidence interval	*P* value
Volume (procedures/yr)			
Medium (12-17)	1.1	0.9-1.4	0.38
Low (≤11)	1.2	1.0-1.5	0.04
Teaching	1.0	0.8-1.2	0.71
Male sex	1.0	0.9-1.2	0.66
Age			
60-69 yr	1.2	1.0-1.5	0.08
≥70 yr	1.9	1.5-2.4	<0.01
Comorbidity score			
1	1.4	1.2-1.8	<0.01
≥2	2.6	1.4-5.1	<0.01
Referral to RCC	1.1	0.9-1.3	0.35
Procedure APR	1.0	0.8-1.1	0.66

RCC = regional cancer center; APR = abdominoperineal resection; LAR = low anterior resection.
*Reference comparisons are high volume (≥18 procedures/yr), nonteaching status, female sex, age 20 to 59 years, comorbidity score 0, nonreferral, and procedure LAR.

Table VII. Proportional hazards for long-term risk of death for 418 resections with pathology available

Explanatory variable*	Likelihood of failure: Risk ratio	95% Confidence interval	*P* value
Volume (procedures/yr)			
Medium (12-17)	1.1	0.7-1.5	0.76
Low (<11)	1.4	1.0-1.9	0.08
Teaching	1.3	0.9-1.8	0.20
Male sex	1.1	0.8-1.4	0.63
Age			
60-69 yr	1.0	0.7-1.5	0.86
≥70 yr	1.5	1.1-2.1	0.02
Comorbidity score			
1	1.4	1.0-2.0	0.06
≥2	3.1	0.7-13.5	0.14
Full wall invasion	1.9	1.4-2.7	<0.01
Positive nodes	2.2	1.7-2.9	<0.01
Referral to RCC	0.7	0.5-1.0	0.03
Procedure APR	0.9	0.7-1.3	0.55

Abbreviations as in Table VI.
*Reference comparisons are high volume (≥18 procedures/yr), nonteaching status, female sex, age 20 to 59 years, comorbidity score 0, partial wall invasion, negative nodes, nonreferral, and procedure LAR.

expected, positive nodal status (RR = 2.2, CI = 1.7 to 2.9, $P < 0.01$) and full wall invasion (RR = 1.9, CI = 1.4 to 2.7, $P < 0.01$) were associated with worse survival.

Sensitivity analyses for regression models showed our results to be robust. Varying volume cutoffs by one to three procedures in all directions or even creating extreme volume groups mattered little. For example, in the low-volume vs. high-volume group, when cutoffs were designated as five or less and 28 or more, the likelihood of operative mortality (operative risk (OR) = 0.6, CI = 0.1 to 2.6, $P = 0.5$) and long-term survival (RR = 1.5, CI = 1.0 to 2.1, $P = 0.04$) varied minimally. When data for 1988 and 1991 were combined, long-term survival risk ratios for the low- and medium-volume groups in comparison to the high-volume group were 1.0 (CI = 0.8 to 1.1, $P = 0.8$) and 1.0 (CI = 0.8 to 1.1, $P = 0.5$), respectively (data not shown).

DISCUSSION

In 1990, for the province of Ontario, hospital volume and teaching status did not influence our selected treatment measures. Among the groups, resection margin–tumor distances and average number of nodes reported were almost identical. In the teaching hospitals, an average 1.2 more nodes were removed ($P = 0.01$), but this likely is of little clinical importance. In addition, hospital volume and teaching status did not have a major impact on our outcome measures of operative mortality and long-term survival. Patients treated in low-volume hospitals did have a slightly greater risk of long-term death (RR = 1.2, CI = 1.0 to 1.5, $P = 0.04$). However, when tumor variables were included in the 1990 model, this increased risk became nonsignificant and risk ratios for combined 1988 and 1991 data were also nonsignificant. Overall, for rectal cancer surgery in Ontario, our measures suggest a uniform standard of treatment and outcomes among hospitals when hospitals are defined by procedure volume or teaching status.

Our results concur with European outcomes studies analyzing rectal cancer surgery.[9-12] In these studies, marked variation in outcomes were noted at the surgeon or hospital level, but improved outcomes were not seen with increased procedure volume or consistently with teaching hospital status. In contrast, two recent North American studies found better survival and lower rates of local recurrence with increased volumes of surgery.[7,8] However, the data were from geographically limited jurisdictions, and in both instances a single volume cutoff was used precluding the observation of a trend effect for volume.

As with most outcomes research, the above-cited studies made minimal attempts to discern actual treatment differences among defined groups. We examined three measures of treatment—number of nodes reported, resection margin–tumor distance and APR rate. These measures were selected as reflections of two important, and potentially competing, aspects of rectal cancer surgery—sphincter preservation and an adequate oncologic resection.

Our provincial rate of sphincter resection was 31.0%. This is well above rates nearer 10% seen in expert series.[16,18,19] The average anal verge–tumor distance was 5.2 cm for APR specimens, indicating that a fair number of these procedures were done for tumors 5.0 cm or more from the anal verge; the surgical literature typically concludes that tumors at or above 5 cm can be resected with sphincter preservation.[16] Some investigators conclude that thorough dissection of the mesorectum is critical to decrease local recurrence and improve survival.[16-20] Our measure of 6.3 nodes reported on average is lower than the usual number of 15 to 20 nodes obtained during total mesorectal excision (Enker W, Personal communication, 1999). The lower number of nodes may indicate an inadequate resection or an inadequate assessment by the pathologist.[24]

The ultimate purpose of outcomes research is to improve the quality of care delivered in a health care system. Correlation between outcomes and procedure volume has important policy implications. For example, in pancreatic cancer surgery, the finding of improved outcomes with increased hospital volume has led clinicians and researchers to call for the centralization of procedures into high-volume centers—a marked shift in practice over traditional referral patterns.[1-4] The results of this study, taken together with work from Europe, suggests that centralization of rectal surgery into high-volume or teaching hospitals may not improve treatment or outcome measures.[9-12]

The lack of correlation between our hospital characteristics and measures should not lead to quality complacency. In Ontario, as in Europe, there are likely individual institutions or surgeons with either markedly superior or inferior outcomes. It would be advantageous to emulate practices of the former and support quality initiatives for the latter. Comparisons of our measures among individual surgeons, hospitals, regions, or isolated expert centers could afford insights into standards of care, incentive for further inquiry, or actual changes in surgical practice. Currently there are no ideal standards for these and other measures and research is needed in this area.

Our study lacked data on the presence of metastatic disease, use of adjuvant therapy, incidence of local

tumor recurrence, and cancer-related mortality because of the limitations of the database used. Among our hospital groups, the distribution of available patient and the tumor variables were remarkably similar. Thus it is likely that the distribution of distant metastatic disease would also be similar. Since our models included registration at a regional cancer center, where all radiotherapy in the province is administered, additional data on actual treatment, although ideal, is unlikely to change our results. Therefore we are satisfied that our regression models provided an accurate evaluation of relationships between the specified hospital descriptors and our treatment and outcome measures. However, chart-based research is needed in Ontario to assess the outcomes of local recurrence and cancer-specific survival.

CONCLUSION

For patients undergoing major resection for rectal cancer in the province of Ontario, treatment and outcome measures were not influenced by hospital procedure volume or hospital teaching status. Centralization of procedures into high-volume or teaching centers is unlikely to improve outcomes or overall quality; hospital or surgeon specific initiatives may be necessary. Our treatment and outcome measures may be practical and useful tools for the assessment of quality in rectal cancer surgery.

We thank Darlene Dale and Gordon Febringer for assistance and advice with data abstraction from the Ontario Cancer Registry, and for obtaining pathology reports from across the province. We thank Dr. Warren Enker for reviewing the manuscript.

REFERENCES

1. Lieberman MD, Kilburn H, Lindsey M, Brennan MF. Relation of perioperative deaths to hospital volume among patients undergoing pancreatic resection for malignancy. Ann Surg 1995;222:638-645.
2. Glasgow RE, Mulvihill SJ. Hospital volume influences outcome in patients undergoing pancreatic resection for cancer. West J Med 1996;165:294-300.
3. Gordon TA, Burleyson GP, Tielsch JM, Cameron JL. The effects of regionalization on cost and outcome for one general high-risk surgical procedure. Ann Surg 1995;221: 43-49.
4. Simunovic M, To T, Theriault M, Langer B. Hospital volume-outcome relationships in pancreatic resection for neoplasm in a publicly-funded health care system. Can Med Assoc J 1999;160:643-648.
5. Mathews HR, Powell DJ, McConkey CC. Effect of surgical experience on the results of resection for oesophageal carcinoma. Br J Surg 1986;73:621-623.
6. Choti MA, Bowman HM, Pitt HA, et al. Should hepatic resections be performed at high-volume referral centers? J GASTROINTEST SURG 1998;2:11-20.
7. Porter GA, Soskoine CL, Yakimets WW, Newman SC. Surgeon-related factors and outcome in rectal cancer. Ann Surg 1998;227:157-167.
8. Simons AJ, Ker R, Groshen S, et al. Variations in treatment of rectal cancer: The influence of hospital type and caseload. Dis Colon Rectum 1997;40:641-646.
9. Holm T, Johansson H, Cedermark B, et al. Influence of hospital- and surgeon-related factors on outcome after treatment of rectal cancer with or without preoperative radiotherapy. Br J Surg 1997;84:657-663.
10. Phillips RK, Hittinger R, Blesovsky L, et al. Local recurrence following 'curative' surgery for large bowel cancer: I. The overall picture. Br J Surg 1984;71:12-16.
11. McArdle CS, Hole D. Impact of variability among surgeons on postoperative morbidity and mortality and ultimate survival. B Med J 1991;302:1501-1505.
12. Hermanek P, Wiebelt H, Staimmer D, Riedl S. The German Study Group Colo-Rectal Carcinoma (SGCRC). Prognostic factors of rectum carcinoma—Experience of the German Multicentre Study SGCRC. Tumori 1995;81:60-64.
13. Kelly JV, Hellinger F. Physician and hospital factors associated with mortality of surgical patients. Med Care 1986;24:785-800.
14. Statistics Canada. Canadian Classification of Diagnostic, Therapeutic and Surgical Procedures. Ottawa: Ministry of Industry, Science, and Technology, 1993.
15. U.S. Department of Health and Human Services. ICD-9-CM: International Classification of Diseases, Clinical Modification, 9th Revision 4th ed. Los Angeles: Practice Management Information Corporation (PMIC), 1995.
16. Milsom JW, Ludwig KA. Surgical management for rectal cancer. In Wanebo HJ, ed. Surgery for Gastrointestinal Cancer: A Multidisciplinary Approach. Philadelphia: Lippincott-Raven, 1997, pp 639-665.
17. Quirke P, Durdey P, Dixon MF, Williams NS. Local recurrence of rectal adenocarcinoma due to inadequate surgical resection. Histopathological study of lateral tumour spread and surgical excision. Lancet 1986;1:996-999.
18. MacFarlane JK, Ryall RDH, Heald RJ. Mesorectal excision for rectal cancer. Lancet 1993;341:457-460.
19. Kockerling F, Reymond MA, Altendorf-Hofmann A, et al. Influence of surgery on metachronous distant metastases and survival in rectal cancer. J Clin Oncol 1998;16:324-329.
20. Enker WE, Thaler HT, Cranor ML, et al. Total mesorectal excision in the operative treatment of carcinoma of the rectum. J Am Coll Surg 1995;181:335-346.
21. Clarke EA, Marrett LD, Kreiger N. Appendix 3(c) Cancer registration in Ontario: A computer approach, the Ontario Cancer Registry; OCTRF, Toronto, Ontario. In Jensen OM, Parkin DM, MacLennan R, Muir CS, Skeet RG, eds. Cancer Registration: Principles and Methods. Lyon: International Agency for Research on Cancer, 1991, pp 245-257.
22. Deyo RA, Cherkin DC, Ciol MA. Adapting a clinical comorbidity index for use with ICD-9-CM administrative databases. J Clin Epidemiol 1992;45:613-619.
23. Basinski A. Use of hospital resources. In Naylor CD, Anderson GM, Goel V, eds. Patterns of Health Care in Ontario. The ICES Practice Atlas, vol I. Ottawa: Canadian Medical Association, 1994, pp 165-306.
24. Quirke P, Scott N. The pathologist's role in the assessment of local recurrence in rectal carcinoma. Surg Oncol Clin North Am 1992;1:1-17.