Editorial

Surgical Treatment of Inguinal Hernia, 1997

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Repair of a hernia is an exercise in applied anatomy. The essential principle is to close the defect without tension. Tension leads to pain following hernia repair and sets the stage for subsequent disruption and recurrence. Thus, during the conduct of each repair an intraoperative decision needs to be made as to whether the defect can be closed by suture without inducing tension or, alternatively, a prosthesis needs to be inserted to bridge the gap.

Hernia repair is one of the most frequent operations performed by general surgeons. More than half a million groin hernias are repaired in the United States annually. Nearly every surgeon views himself as an expert in hernia repair. But if expertise were so abundant, the rate of recurrence would certainly be less. Frustration with the continuing problem of recurrence has led to employment of alternative techniques of operative management, each touted to reduce the risk of recurrence.

Some surgeons have turned to routine rather than selective use of darning techniques and insertion of mesh devices. But, rote adherence to a single method of repair, a "one size fits all" approach, does not always result in an optimal repair since the anatomic situation in each individual patient varies sufficiently that a specific judgment is needed to choose the best technique of repair. The applicable techniques and principles of successful hernia repair are well known and can be taught. The fact that problems continue indicates that surgical educators have not done their job. Teaching of groin hernia repair is currently the greatest pedagogical failure in surgical education.

The major problem is that anatomic descriptions and concepts as usually taught to surgical residents continue to disseminate error. My hypothesis about the sociopolitical genesis of this situation has been described elsewhere.[1] For this editorial, I reviewed the current edition of a major American textbook of surgery and found three errors of conception and a dozen errors of description in the discussion of groin anatomy. Since hernia repair is an anatomic exercise, accurate knowledge is essential to success. We need to stop publishing anatomic disinformation. I believe every general surgeon would benefit from undertaking a number of dissections of unenbalmed material in order personally to discover authentic structure. I contend that the resultant increase in expertise would diminish the tendency to uncritical adoption of the latest technical variation reported in the literature. It would also break the cycle of disinformation in surgical education.

During the last decade, laparoscopic repair of groin hernia has become accepted largely because of the enthusiasm of surgical proponents of the method as well as of patients who anticipated advantages akin to those following laparoscopic cholecystectomy: less pain and a quicker return to normal activity. Whether these anticipated advantages actually accrue following laparoscopic hernia repair remains a lively topic of debate. There has been, however, one clearly positive result of surgeons taking up the laparoscopic approach to hernia repair. Appreciation of the anatomy of the groin, deriving from the experience of viewing the regional structures from the preperitoneal aspect, has improved. Unfortunately, the wonderfully enhanced understanding of structure has been accompanied by needless introduction of eponyms and novel terminology, further contributing to the babel of nomenclature that impairs communication between surgeons on the subject. Limiting, or at least referencing, terminology to the *Nomina Anatomica* would be of great help.

Enough clinical experience resulting from controlled comparative clinical trials of laparoscopic versus open groin hernia repair[2,3] has now accumulated so that some tentative summary conclusions can be made. There is a wide variance of outcome in terms of

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pain relief and return to work; in some studies these aspects clearly are better with the laparoscopic approach, while in others there does not seem to be much difference. What is consistent throughout all studies is the fact that the laparoscopic approach is more expensive than traditional open hernia repair. Compared with the standard open operation, laparoscopic hernia repair has been associated with an increased incidence of nerve injury, a problem that has continued even after identification of the genesis of most of these events.[4] Recurrence in many studies also has been relatively high. Early on, most of these were technical failures. As experience increased, recurrence has diminished but still occurs more frequently than is desirable. At this stage of its evolution, I do not perceive any advantage to laparoscopic repair sufficient to recommend the method for general routine use.

How should a hernia in an adult be repaired? Each available method of repair will succeed when appropriately utilized. Unlike advocates of one method or another, I find it impossible to provide blanket advice since, in my view, individual judgment needs to be applied by a knowledgeable surgeon to the specific circumstances presented by each patient. To the extent that I can generalize, I prefer an anterior open approach to repair of most small (internal ring dilated less than 3 cm) indirect hernias. In conducting the repair, I would not omit placing a stitch lateral to the cord when reconstructing the deep inguinal ring. I see no need to use any mesh device for this clinical situation. Further, routine extension of the repair to "reinforce" all of the posterior wall of the inguinal canal is meddlesome and leads both to undesirable tension and unwanted recurrence. Unless there is an actual hernia present, prophylactic "repair" accomplishes no real good.

For larger indirect and direct hernias, a prosthesis is likely to be needed so that a preperitoneal approach, either open or laparoscopic, would seem suitable. Femoral hernias should always be approached preperitoneally since this facilitates control of the contents of the hernia sac and permits direct suture repair. It makes no sense to approach a femoral hernia through the inguinal canal, necessitating an incision through the posterior wall in an area that is itself subject to herniation; approaching a femoral hernia through the thigh is the hard way to accomplish an otherwise straightforward task. Bilateral indirect hernias, if small, can be repaired in a single operative session using separate open approaches to each hernia. Larger bilateral hernias are preferentially managed by insertion of a Stoppa mesh chevron prosthesis.[5]

Most recurrent hernias have previously been repaired via an open anterior approach. Using the preperitoneal approach to a recurrent hernia has the advantages of dissection through virgin tissue and, since a prosthesis is needed in nearly all of these cases, ease of placing the prosthesis. I prefer an anterior approach in treating recurrence following laparoscopic repair. Whenever there are large bilateral recurrent hernias, the Stoppa technique works best.

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Laparoscopic Toupet Fundoplication for Gastroesophageal Reflux Disease With Poor Esophageal Body Motility

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Impaired esophageal body motility is a complication of chronic gastroesophageal reflux disease (GERD). In patients with this disease, a 360-degree fundoplication may result in severe postoperative dysphagia. Forty-six patients with GERD who had a weak lower esophageal sphincter pressure and a positive acid reflux score associated with impaired esophageal body peristalsis in the distal esophagus (amplitude <30 mm Hg and >10% simultaneous or interrupted waves) were selected to undergo laparoscopic Toupet fundoplication. They were compared with 16 similar patients with poor esophageal body function who underwent Nissen fundoplication. The patients who underwent Toupet fundoplication had less dysphagia than those who had the Nissen procedure (9% vs. 44%; $P = 0.0041$). Twenty-four-hour ambulatory pH monitoring and esophageal manometry were repeated in 31 Toupet patients 6 months after surgery. Percentage of time of esophageal exposure to pH <4.0, DeMeester reflux score, lower esophageal pressure, intra-abdominal length, vector volume, and distal esophageal amplitude all improved significantly after surgery. Ninety-one percent of patients were free of reflux symptoms. The laparoscopic Toupet fundoplication provides an effective antireflux barrier according to manometric, pH, and symptom criteria. It avoids potential postoperative dysphagia in patients with weak esophageal peristalsis and results in improved esophageal body function 6 months after surgery. (J GASTROINTEST SURG 1997;1:301-308.)

Laparoscopic minimal access surgery has been used in the treatment of gastroesophageal reflux disease (GERD) since 1991.[1] In the wake of the success of laparoscopic Nissen fundoplication, the laparoscopic approach has been used to perform other antireflux operations. These operations include Toupet posterior partial fundoplication, anterior partial fundoplication, and Hill gastropexy.[2-5] The principles of each procedure are the same with reduction of the hiatal hernia, narrowing of the esophageal hiatus, and creation of a fundoplication/gastropexy to increase the length of the intra-abdominal esophagus, which usually increases the resting pressure at the lower esophageal sphincter (LES). The essential difference between Nissen and Toupet fundoplication is the circumference of the abdominal esophagus that is covered with the wrap. Lundell et al.[6] has shown that 180- to 200-degree Toupet fundoplication results in less postoperative dysphagia 3 months after surgery compared to 360-degree Rossetti fundoplication; however, it is not clear whether Toupet fundoplication is as effective as an antireflux barrier since it produces a significantly lower LES pressure postoperatively compared to the Rossetti procedure.

Ineffective esophageal peristalsis is a complication of long-standing gastroesophageal acid reflux and can lead to worsening of GERD presumably as a result of ineffective clearance of acid from the esophagus.[7] It appears that patients with poor esophageal body function are likely to be at greater risk for postoperative dysphagia if a complete 360-degree fundoplication is performed. For this reason it seems sensible to carry out a partial fundoplication in patients with poor esophageal body function. Stein et al.[8] showed that patients with esophageal body peristaltic amplitudes below the fifth percentile of normal showed no im-

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provement in peristaltic function after Nissen fundoplication. It is, however, not clear whether a partial wrap will allow for improvement in the peristaltic function of the esophagus. There is also little information on how this procedure affects postoperative dysphagia compared to Nissen fundoplication in patients with poor esophageal body function.

PATIENTS AND METHODS

Patients

Laparoscopic antireflux surgery was first performed at Creighton University in August 1991 (Fig. 1). In the first 168 patients, Nissen fundoplication was used exclusively. Sixteen of these patients had poor esophageal body motility as defined below. Because of the high incidence of postoperative dysphagia in these patients, 28 of the subsequent 303 surgical GERD patients with impaired esophageal body function were offered a partial Toupet fundoplication. Patients with poor body motility who underwent Toupet fundoplication were also accumulated from the University of Innsbruck[9] where, in April 1994, surgeons trained at Creighton University began using laparoscopic antireflux surgery. It appears that European gastroenterologists refer patients for surgery at a more advanced stage of disease, as reflected by the higher percentage of patients with poor body motility in this group of patients (18 [44%] of 41 patients; see Fig. 1). Since identical techniques of evaluation, testing, and surgery were used at the two centers, all of the patients with poor body motility were evaluated together. The demographics of patients with poor body motility compared to those with normal esophageal body motility are presented in Table I. The presenting symptoms, with the exception of dysphagia, were similar in the two groups (Fig. 2). Patients had been receiving medical treatment for a median duration of 2 years (range 3 months to 20 years).

All patients underwent preoperative ambulatory 24-hour esophageal pH monitoring, esophageal manometry, endoscopy, and barium fluoroscopy. Patients with impaired esophageal body motility had more severe esophagitis, with significantly more patients with Barrett's esophagus (Fig. 3). Thirty-one patients, after the Toupet procedure, agreed to repeat esophageal manometry and 30 patients agreed to repeat 24-hour esophageal pH testing at least 6 months after surgery

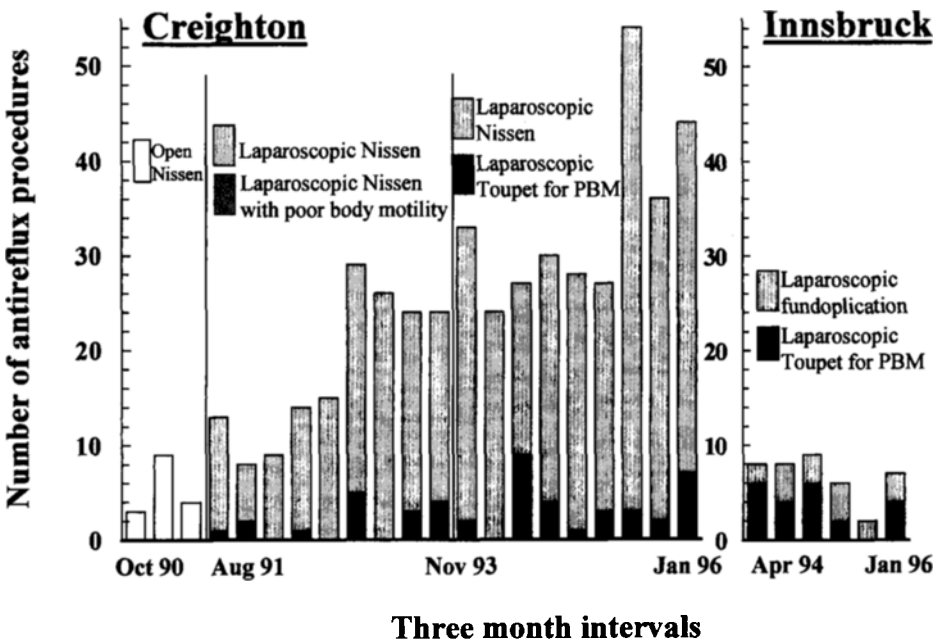

Fig. 1. Number of antireflux procedures performed at Creighton University and the University of Innsbruck from October 1991 to January 1996 divided into 3-month periods. Sixteen open Nissen fundoplications were performed between October 1990 and August 1991. One hundred sixty-eight laparoscopic Nissen fundoplications were performed between August 1991 and November 1993. Sixteen of these patients had poor esophageal body motility *(PBM)*. Between November 1993 and January 1996, a total of 28 patients with PBM underwent Toupet fundoplication and 275 patients with normal esophageal body motility underwent Nissen fundoplication. Between April 1994 and January 1996, at the University of Innsbruck, of 41 laparoscopic antireflux operations performed, 18 were laparoscopic Toupet fundoplications for PBM.

Table I. Demographics of the patient population

	Sex ratio (M:F)	Age (yr) (range)	Duration of symptoms (mo) (range)
Surgery for GERD with normal esophageal motility (n = 427)	236:191	50 (11-92)	109 (3-600)
Surgery for GERD with poor esophageal motility (n = 62)	42:20	53 (18-86)	137 (6-480)
P value	NS	NS	NS

NS = not significant.

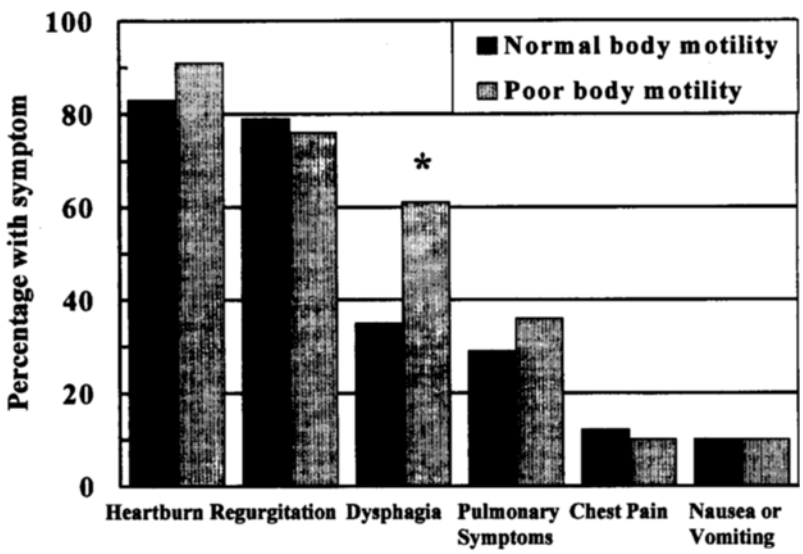

Fig. 2. Incidence of presenting symptoms expressed as a percentage of patients with normal esophageal body motility (n = 427) compared to patients with poor esophageal body motility (n = 62). **P* <0.001 vs. normal esophageal body motility.

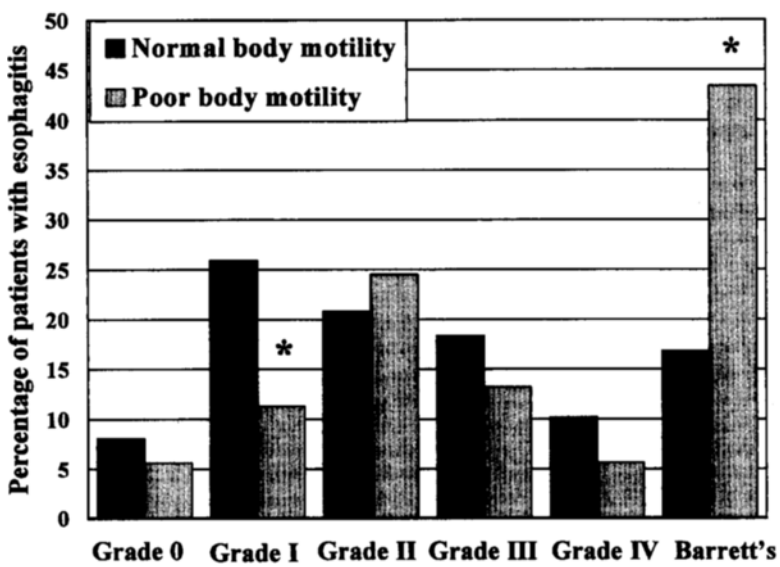

Fig. 3. Preoperative grade of esophagitis in patients with normal esophageal body motility compared to patients with poor esophageal body motility according to the Savary-Miller classification.[20] **P* <0.05 vs. normal esophageal body motility.

(range 6 to 12 months). All patients completed a symptom questionnaire 6 months after surgery. Pre- and postoperative dysphagia was graded as mild (occasional, with ingestion of coarse foods, and lasting only a few seconds), moderate (requiring clearance with liquids, no weight loss), and severe (semiliquid diet, history of weight loss). Patients requiring postoperative dilatation were considered to have severe dysphagia.

Manometry

Esophageal manometry was performed using a five-channel water-perfused catheter by the stationary pull-through technique as previously described.[10] The ports were spaced 5 cm apart. The LES resting pressure at the respiratory inversion point and the intra-abdominal sphincter length were measured. The LES was considered to be defective if the average resting pressure was less than 6.1 mm Hg and/or the intra-abdominal sphincter length was less than 1.2 cm. Esophageal peristaltic function was assessed manometrically at 3, 8, 13, and 18 cm above the upper border of the LES. Body motility was considered poor if more than 20% of the esophageal contractions following 10 wet swallows (5 ml water each) were defective. Defective clearance function was defined as a peristaltic amplitude of less than 30 mm Hg in the distal esophagus in response to a wet swallow and/or peristaltic propagation greater than 20 mm/sec between adjacent channels (simultaneous waves) and/or interrupted peristaltic waves (contraction amplitude <15 mm Hg). Three-dimensional pressure-length vector volume assessment was also performed. This consists of an assessment of the LES with four circumferentially arranged ports pulled at a constant rate through the LES.[11]

24-Hour Esophageal pH

Continuous ambulatory 24-hour esophageal pH was measured as previously described.[12] A DeMeester score of more than 14.8 was considered abnormal.[12] This was seen in 44 of the 46 Toupet patients and in 362 of the 427 Nissen patients tested before surgery. The mean score was 60.8 (range 3 to 240.5) for the Toupet patients and 41.6 (range 0.2 to 274.8) for the Nissen patients.

Operative Technique for Fundoplication

Toupet fundoplication was performed under general anesthesia with patients in the lithotomy position in the reverse Trendelenburg position. Placement of five 10 mm trocars, establishment of a pneumoperitoneum, division of the gastrohepatic ligament, dissection of the esophagus and hiatal crura, and establishment of a window behind the esophagus were performed as previously described for the Nissen procedure.[13] Hiatal hernias were reduced and the hiatal crura loosly approximated behind the esophagus using one to three 2-0 Prolene sutures.The short gastric vessels were usually divided within 15 cm of the angle of His along the greater curvature of the stomach. A Babcock grasper was passed from the right side through a window created behind the esophagus, and the gastric fundus was grasped near the short gastric vessels and advanced behind the esophagus, leaving the posterior vagus nerve outside of the fundic wrap. The right side of the wrap was first sutured to the adjacent right crus using three interrupted 2-0 silk sutures and then to the right side of the abdominal esophagus with another three silk sutures. The left limb of the wrap was then sutured to the left side of the abdominal esophagus using three 2-0 silk sutures. A segment of approximately 90 degrees of the anterior esophagus was not covered by the wrap. Suturing was performed using intracorporeal knot tying with two needle holders.

Nissen fundoplication was carried out over a 58 to 60 F Maloney bougie in the esophageal lumen using the technique previously described.[14]

Statistical Analysis

Comparison of esophageal manometry and pH monitoring data obtained before and after surgery was performed by means of the Wilcoxon rank-sum test. Data from patients who underwent Nissen fundoplication were compared with those in the Toupet fundoplication group by means of Fisher's exact test. A P value of <0.05 indicated statistical significance.

RESULTS

In the 46 patients undergoing Toupet fundoplication for poor esophageal body motility, the mean operative time was 2.6 hours (range 1.2 to 5 hours) (Creighton = 2.3 hours; Innsbruck = 2.8 hours). Average blood loss was 62 ml (5 to 500 ml) and the average postoperative hospital stay was 3 days (range 1 to 12 days) (Creighton = 1.8 days, Innsbruck = 4.3 days). All operations were completed by the laparoscopic route. Operative complications occurred in four patients (8.7%). These included a small gastric perforation, which was successfully repaired laparoscopically with uneventful recovery and discharge on postoperative day 5. An intra-abdominal hematoma with a decrease in the hemoglobin concentration to 8.4 g/L 3 days after surgery was seen in one patient

Table II. Dysphagia in patients with poor esophageal body motility: Toupet fundoplication vs. Nissen fundoplication

	Toupet fundoplication (n = 46)		Nissen fundoplication (n = 16)	
Dysphagia	**Preoperative**	**Postoperative**	**Preoperative**	**Postoperative**
None/mild	30 (65%)	42 (91%)	10 (62.5%)	9 (57%)
Moderate/severe	16 (35%)	4 (9%)	6 (37.5%)	7 (44%)*
P value	<0.005		NS	

NS = not significant.
*P = 0.0041, postoperative dysphagia in Toupet patients vs. Nissen patients.

Table III. Laparoscopic Toupet fundoplication: Preoperative vs. postoperative physiologic testing

	Preoperative	**Postoperative**	***P* value**
DeMeester score (normal <14.8)	61.4 ± 10.3 (3-240)	12.5 ± 5.2 (0.3-119.5)	0.0002
LES resting pressure (mm Hg)	4.7 ±0.8 (0-21)	10.9 ± 1.4 (0.1-35.3)	<0.0001
LES intra-abdominal length (cm)	1.1 ± 0.2 (0-4.4)	2.5 ± 0.2 (1-4.8)	<0.0001
Vector volume (mm Hg^2.mm)	1083 ± 230 (80-2190)	2965 ± 665 (210-7140)	0.009
Body amplitude 8 cm above LES (mm Hg)	27.9 ± 3.1 (0-69.6)	39.9 ± 3.1 (14.9-80.1)	0.001
Body amplitude 3 cm above LES (mm Hg)	26.4 ± 2.8 (0-72.8)	42.6 ± 3.0 (15.2-83.4)	<0.0001
% Simultaneous waves (n = 10)	25	2.6	<0.0001

Data are expressed as mean ± standard error of the mean; range is shown in parentheses.

who required a transfusion of two units of blood. The other two complications were pneumonia in one patient and deep venous thrombosis in another patient who had suffered a severe knee injury 1 year previously.

At follow-up assessment, patients with impaired esophageal body motility who underwent Nissen fundoplication had a higher rate of moderate-to-severe dysphagia (7 [44%] of 16) compared to patients with normal body motility (65 [15%] of 427; P = 0.007). After Nissen fundoplication, there was a greater need for esophageal dilatation in patients with poor body motility (4 [25%] of 16) compared to patients with normal body motility (53 [12%] of 427). Furthermore, in patients with poor esophageal body motility there was a higher rate of dysphagia among those who underwent Nissen fundoplication compared to those who underwent Toupet fundoplication (Table II). At 6-month follow-up of the Toupet patients, six complained of mild dysphagia and three complained of moderate dysphagia (see Table II). All of these patients had had dysphagia preoperatively. One additional patient who had mild dysphagia preoperatively complained of severe dysphagia postoperatively. This patient underwent balloon dilatation, which was unsuccessful, and at subsequent open laparotomy was found to have a hiatal crura that was too tightly approximated with formation of scar tissue in that area. After division of the hiatal crura anterior to the esophagus, this patient had an uneventful postoperative course with no heartburn or dysphagia.

Forty-two (91%) of 46 Toupet patients were pleased with the results of their operations. Postoperative endoscopy was carried out in 26 patients and showed no evidence of esophagitis. Postoperative esophageal manometry was performed in 31 patients and showed a significant increase in the mean resting pressure at the LES compared to preoperative values (Table III). This increase was not as high as the resting pressures recorded during postoperative evaluation of Nissen patients previously reported by Hinder et al.[13] (Fig. 4). Both the intra-abdominal length

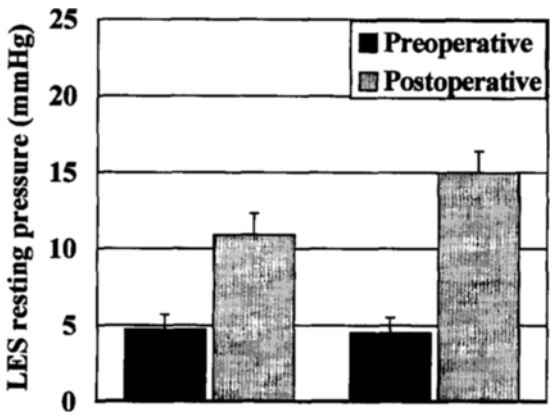

Fig. 4. Lower esophageal sphincter *(LES)* pressure before and after laparoscopic Toupet fundoplication showing a significant increase in the resting LES pressure at the respiratory inversion point after the procedure. The mean LES pressure after Toupet fundoplication is lower than the mean postoperative LES pressure after Nissen fundoplication.[13]

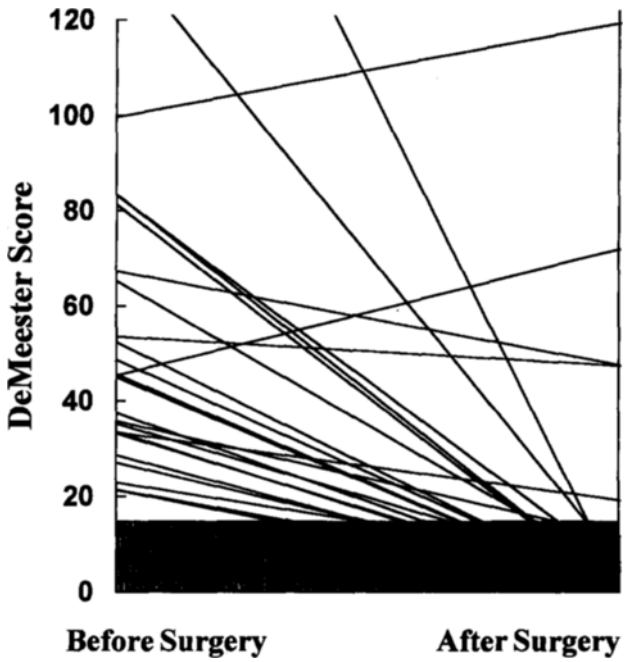

Fig. 5. The 24-hour esophageal pH composite score[12] before and after laparoscopic Toupet fundoplication. Normal score <14.8 (shaded). Twenty-five of 30 patients had a normal score after surgery. Three patients with positive scores were symptomatic.

of the LES and the total LES vector volume increased significantly postoperatively (see Table III). Esophageal body function as assessed by distal peristaltic amplitude and percentage of simultaneous or interrupted waves improved significantly compared to preoperative function (see Table III). Simultaneous waves in the distal esophagus were seen in 10 patients preoperatively. Six months after surgery, there was a significant reduction in simultaneous waves in these patients (see Table III).

Postoperative 24-hour esophageal pH testing in 30 patients revealed that the DeMeester score and the percentage of time of esophageal exposure to pH <4 were significantly improved compared to the preoperative values (Table III). There were five patients (17%) who had an abnormal DeMeester score after surgery (Fig. 5). Two of these patients were asymptomatic. Two complained of infrequent heartburn, but their LES pressure was normal and their symptoms were easily controlled with antacids. Another symptomatic patient with a positive pH score had no change in LES pressure; however, her esophageal body function had improved sufficiently 6 months after surgery to allow a Nissen fundoplication to be performed. This was easily accomplished laparoscopically and she is presently free of symptoms.

DISCUSSION

Long standing GERD may result in impaired esophageal body motility. An increase in the amount of submucosal collagen and a loss of muscle fiber have been demonstrated in these patients leading to a deterioration in esophageal contractility.[15] Poor esophageal body function may be seen in more than 50% of patients with severe esophagitis and in 25% of patients with mild esophagitis.[7] This is usually not thought to improve after healing of esophagitis by medical or surgical means.[8,16,17] The Nissen fundoplication is the most popular antireflux procedure performed laparoscopically and provides good results in approximately 90% of cases.[13] However, up to 8% of patients studied 1 to 10 years after Nissen fundoplication complain of severe dysphagia.[18] As shown in our study, this may be particularly true of patients with poor esophageal body motility. Furthermore, results of our study support those from a previous study, which found that Nissen fundoplication does not lead to an increase in esophageal peristaltic amplitude and creates a high outflow resistance in patients with impaired esophageal clearance function leading to dysphagia.[8] These previous investigators found that Nissen fundoplication improves body function only in patients with a contraction amplitude greater than 35 mm Hg in the distal esophagus and not when the peristaltic amplitude fell below the fifth percentile of normal. There was also no change in the amount of simultaneous or interrupted waves after Nissen fun-

doplication. We found that Toupet fundoplication results in a significant increase in LES pressure and intra-abdominal length and improvement in symptoms in 91% of patients with poor body motility. There was improvement in esophageal body function with an increase in esophageal body peristaltic amplitude and a decrease in the number of simultaneous contractions 6 months after surgery.

Our results make a good argument for tailoring the antireflux procedure depending on the peristaltic function of the esophagus. It is therefore important for all patients being considered for antireflux surgery to undergo a full manometric evaluation including assessment of LES pressure and peristaltic esophageal body function. If poor esophageal body function is found, then a partial fundoplication is indicated. This was found to decrease the dysphagia rate while still controlling reflux symptoms in the majority of cases. The improvement in esophageal body function found 6 months after surgery may also aid in preventing dysphagia.

The Nissen fundoplication results in a greater increase in LES pressure compared to the Toupet procedure and is our choice for the surgical treatment of GERD with normal esophageal body peristaltic function. Even though a good outcome was achieved in 91% of our patients undergoing laparoscopic Toupet fundoplication, 17% had a positive acid reflux score suggesting that it may not be as effective as Nissen fundoplication as an antireflux procedure. However, recent studies[6,19] of patients after open Toupet fundoplication have demonstrated it to be comparable to the Nissen procedure in preventing reflux, although it did not increase the mean resting pressure as much as the Nissen fundoplication. This suggests that mechanisms other than an increase in LES pressure are important in preventing reflux. These include reducing the hiatal hernia, increasing the length of the intra-abdominal esophagus, creating a posterior constriction on the esophagus by means of fundoplication, and decreasing the volume of functioning fundus, thereby increasing the rate of gastric emptying.

In one of our patients esophageal clearance function was improved sufficiently by the Toupet fundoplication that it was possible to perform a subsequent successful 360-degree Nissen fundoplication. The improvement in body function was probably due to the fact that reflux and esophagitis were decreased without there being an increase in outflow resistance, thereby allowing for restoration of peristaltic function to the esophagus.

Laparoscopic Toupet fundoplication provides an effective antireflux barrier as determined by manometric, pH, and symptom criteria. It decreases the potential for postoperative dysphagia in patients with weak esophageal peristaltic function and results in improved esophageal body function. Results of this study confirm the need for routine preoperative testing of all GERD patients to allow the selection of the most appropriate procedure for each patient to achieve the best possible results.

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Discussion

Dr. L. Way (San Francisco, Calif.). Your results parallel those recently presented by other investigators. I want to determine, however, whether we can agree on the definition of the terms being used. You have referred to the partial fundoplication performed on these patients as a Toupet procedure, yet in some patients the hiatus was closed posteriorly. The Toupet operation never includes a posterior closure of the hiatus, however, so if we wish to communicate precisely, some other name should be given to such operations (e.g., Guarner, Lind). To me, the Toupet procedure only has meaning if you insist that it not include closure of the hiatus, which is exactly the way Toupet meant his operation to be performed.

Dr. R. Hinder. It was interesting for us also to see the similarity in the results between two centers, indicating that partial fundoplication is effective for those patients with poor motility.

As far as the technique is concerned, we do not routinely close the esophageal hiatus. What we perform selectively is a true "Toupet" procedure without hiatal closure in patients without a very wide hiatus. Once we have completed our dissection, before we perform the fundoplication, if the hiatus is not very wide, we do not stitch it closed. But if there is a wide hiatus, then we do put stitches into the hiatus, which occurs approximately 50% of the time.

I agree that it is probably a good idea for us to start talking about partial fundoplication, and we should describe precisely what each of us is doing.

Dr. J. Meakins (Montreal, Canada). I simply do not understand how the esophageal body function improves as a result of partial fundoplication. Two previous reports have presented the same data, and I wonder if someone could explain this.

Dr. Hinder. I think there are probably two explanations for this. One is that there is esophageal lengthening. The body of the esophagus is, on average, elongated once the lower esophageal sphincter is replaced in the abdomen, and that allows the esophagus to work under better mechanical advantage. Second, and perhaps more important, when the reflux, the inflammation, the ulceration, and the esophagitis are all eliminated, there is better motor function.

Dr. K. Zucker (Albuquerque, N. Mex.). In that first group of 164 patients, before you discovered the Toupet procedure, you mention that you used Nissen fundoplication. What was the percentage of those patients who had a poor outcome?

Dr. Hinder. The poor outcome was mainly dysphagia. Approximately 40% of those patients had persistent dysphagia, some requiring dilatation, but none had permanent severe dysphagia. Some of those patients still complained of intermittent symptoms of dysphagia at 1 year, but these symptoms were not severe enough to disrupt their eating habits.

Dr. Zucker. Did any of them have to be reoperated?

Dr. Hinder. Only one, and in that patient the crus was too tight.

Dr. B. Miedema (Columbia, Mo.). If fundoplication improves the motility of the esophageal body, why is there still dysphagia after Nissen fundoplication?

Dr. Hinder. If you look at our data, you will see that the mean contraction amplitude increased significantly but did not enter the normal range. Our mean value was still in the 40 mm Hg range, whereas if you look at the normal population, the mean peristaltic amplitude in the lower esophagus is closer to 60 or 80 mm Hg. Although it has improved, it is not within the normal physiologic range, which may explain why some patients had dysphagia.

Comparison of Laparoscopic Total and Partial Fundoplication for Gastroesophageal Reflux

Marco G. Patti, M.D., Mario De Pinto, M.D., Mario de Bellis, M.D., Massimo Arcerito, M.D., Jenny Tong, M.D., Anne Wang, M.D., Sean J. Mulvihill, M.D., Lawrence W. Way, M.D.

Approximately 25% of patients with gastroesophageal reflux severe enough to be considered for surgical treatment have dysfunction of esophageal peristalsis in addition to dysfunction of the lower esophageal sphincter. A standard total (i.e., Nissen) fundoplication in these patients may be followed by dysphagia, so many experts recommend a partial fundoplication as an alternative. The goal of this study was to compare the clinical results and changes in esophageal function following laparoscopic total and partial fundoplication. Ninety-three patients with gastroesophageal reflux disease had laparoscopic antireflux operations. Total fundoplication was performed in 50 patients with normal esophageal peristalsis. Partial fundoplication was chosen for 43 patients with severe abnormalities of esophageal peristalsis. The same percentage of patients has resolution of heartburn (93%) and regurgitation (97%) after partial as compared to total fundoplication. Dysphagia developed in four patients (8%) after total fundoplication (one patient required dilatation) and in no patients after partial fundoplication. Both operations produced similar changes in lower esophageal sphincter function, but only partial fundoplication was associated with improvement in esophageal dysfunction. Esophageal acid exposure became normal in 92% of patients after total and in 91% of patients after partial fundoplication. Partial fundoplication improves lower esophageal sphincter pressure and esophageal body function and, in patients with abnormal esophageal peristalsis, it corrects reflux without producing dysphagia. Partial and total fundoplication are both indicated in patients with gastroesophageal reflux disease, and the choice of which procedure to use should be based on each patient's specific esophageal motor function abnormalities. (J GASTROINTEST SURG 1997;1:309-315.)

Approximately 25% of patients with gastroesophageal reflux have an especially severe form of the disease that is characterized by intractable symptoms and esophagitis, as well as frequent progression to stricture formation and Barrett's metaplasia.[1] Such patients are often found to have a panesophageal motor disorder that is characterized by abnormal esophageal peristalsis and incompetence of the lower esophageal sphincter (LES).[2-6] The advisability of antireflux surgery in those with peristaltic dysfunction has been a subject of debate because some clinicians question whether surgery might either fall short of correcting the reflux or result in dysphagia. Similar concerns have led most surgeon experts to conclude that in order to avoid postoperative dysphagia, the operative approach in these patients should be different from that in the average patient with reflux.

The goal of this study was to compare the clinical and physiologic changes following laparoscopic total and partial fundoplication in patients with normal and abnormal esophageal peristalsis.

PATIENTS AND METHODS

Between February 1993 and October 1995, ninety-three patients with gastroesophageal reflux disease (GERD) underwent laparoscopic fundoplication. Total (360 degrees) fundoplication was performed in 50 patients (28 men and 22 women; mean age 50 years) with normal esophageal peristalsis and partial (240 to 270 degrees) fundoplication was performed in 43 patients (23 men and 20 women; mean age 51 years) with abnormal esophageal peristalsis (amplitude of peristaltic pressure in the distal esophagus <40 mm

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Table I. Results of preoperative evaluation for laparoscopic total and partial fundoplication

	Total fundoplication (N = 50)	Partial fundoplication (N = 43)
Barium swallow	*No. of patients (%)*	*No. of patients (%)*
Normal	14 (28)	10 (23)
Hiatal hernia	30 (60)	31 (72)
Stricture	6 (12)	2 (5)
Endoscopy		
Grade 0	12 (24)	10 (23.5)
Grade 1	10 (20)	4 (9)
Grade 2	9 (18)	12 (28)
Grade 3	8 (16)	7 (16)
Grade 4*	11 (22)	10 (23.5)
Manometry		
LES		
Pressure (mm Hg)	10 ± 1.0	7.0 ± 0.5
Total length (cm)	2.2 ± 0.1	1.9 ± 0.1
Abdominal length (cm)	1.4 ± 0.1	1.1 ± 0.1
% Relaxation	82 ± 3	74 ± 7
Peristalsis		
Distal amplitude (mm Hg)	91 ± 6.0	36 ± 2.0
pH monitoring		
% time pH <4.0	14 ± 2.0	20 ± 2.0
DeMeester score	54 ± 7.0	77 ± 8.0

*Six patients in each group had Barrett's esophagus.

Hg, >20% waves segmented, >30% waves double peaked, and presence of waves triple peaked and/or dropped). Table I shows the results of preoperative evaluation. Each patient was questioned regarding the presence of symptoms suggestive of GERD (heartburn, regurgitation, dysphagia, cough, chest pain, and hoarseness). Each symptom was graded by means of a scoring system ranging from 0 to 4[7] (Fig. 1).

All patients underwent barium x-ray examination of the esophagus to look for hiatal hernias and esophageal strictures. The degree of esophagitis was graded according to the Savary-Miller classification.[8] Patients were studied after an overnight fast. LES pressure, length, and relaxation were measured using techniques previously described.[3,5,6] The percentage of LES relaxation was calculated from the following formula[9]:

$$\frac{\text{(LES resting pressure)} - \text{(LES residual pressure)}}{\text{(LES resting pressure)}} \times 100$$

Esophageal body function was assessed by giving 10 swallows of 5 ml of water at 30-second intervals. The data were analyzed by computer using a commercial software program (Gastrosoft, Synectics Medical, Irving, Tex.).

Ambulatory 24-hour esophageal pH monitoring was performed using a pH probe with an electrode positioned 5 cm above the upper border of the manometrically determined LES. During the study patients consumed a normal diet and took no medications. Acid-suppressing medication was discontinued 3 (H_2-blocking agents) to 10 (proton pump inhibitors) days before the study. All patients had abnormal DeMeester scores.[10]

Operative Technique

Each operation entailed the following steps: (1) the short gastric vessels were divided from a point midway along the greater curvature of the stomach all the way to the angle of His; (2) if enlarged, the esophageal hiatus was narrowed with sutures behind the esophagus to a normal size; (3) to ensure that it was not too tight, the wrap was performed over a 56 to 60 F bougie; and (4) a 360-degree wrap was performed for total fundoplications and a 240- to 270-degree wrap for partial fundoplications (Fig. 2).

Statistical Analysis

Student's *t* test and Mann-Whitney U test were used for statistical evaluation of data. All results are expressed as mean ± standard error of the mean. Differences were considered significant at $P < 0.05$.

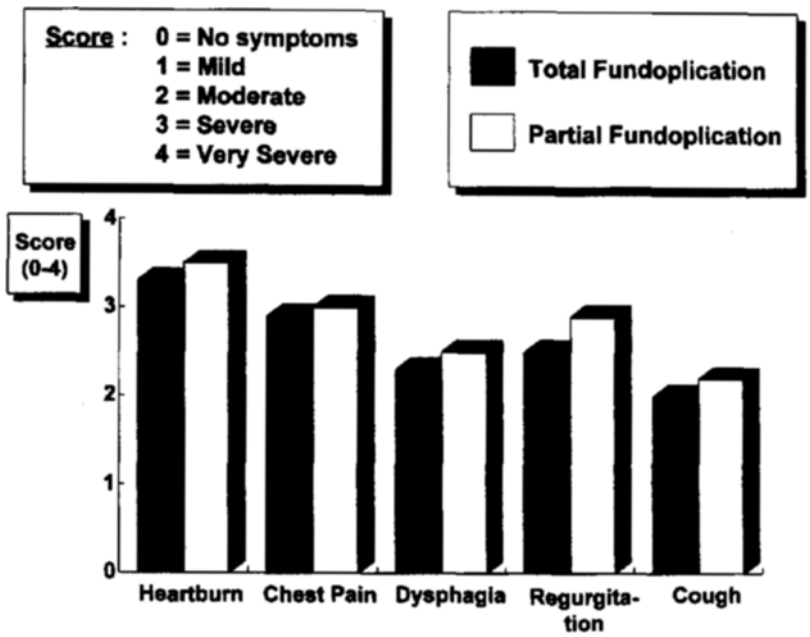

Fig. 1. Symptomatic assessment of patients with GERD before laparoscopic total and partial fundoplication.

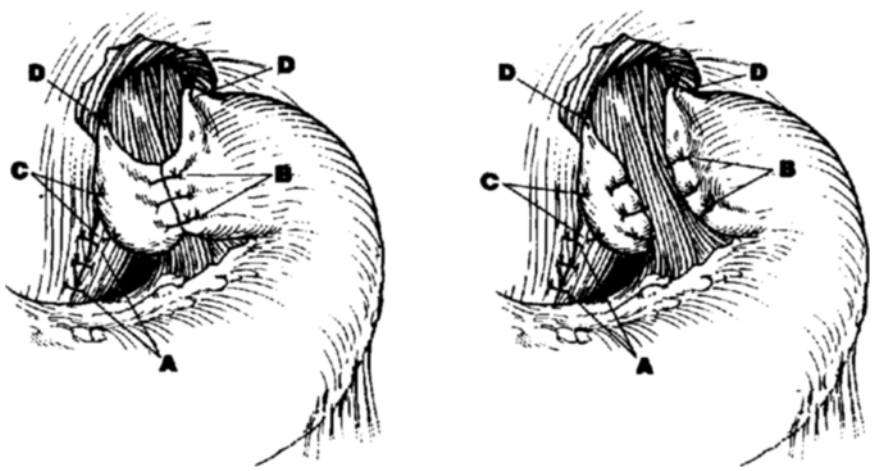

Fig. 2. Total (left) and partial (right) fundoplication. *A*, Stitches to approximate the crura. *B*, Stitches for creation of the wrap. *C*, Stitches from the right side of the wrap to the closed crus. *D*, Apical stitches (incorporating the crus, the esophagus, and the wrap).

RESULTS

The average operating time was similar for the two procedures: 169 ± 7 minutes for total fundoplication and 179 ± 8 minutes for partial fundoplication (P = NS).The average blood loss was less than 40 ml. Table II shows the hospital course of the two groups. The mean length of follow-up was 15 months.

Symptoms improved with both procedures (Table III). Dysphagia, which was present before surgery in 28 (56%) of the 50 patients who underwent total fundoplication, resolved in 25 patients (89%) and improved in three patients (11%). Four patients developed *de novo* dysphagia postoperatively, which resolved spontaneously in two patients and improved in one patient within 6 months after the operation. The remaining patients improved after one endoscopic dilatation.

Dysphagia was present preoperatively in 17 (40%) of the 43 patients (40%) who underwent partial fundoplication; postoperatively dysphagia resolved in 15

Table II. Hospital course

	Total fundoplication	Partial fundoplication
Time to regular diet (hr)	24 ± 2	24 ± 1
Time to discharge (hr)	42 ± 4*	39 ± 3†
Postoperative complications (No.)	4 (pulmonary aspiration, 2; severe vomiting, 1; myocardial infarction, 1)	4 (atelectasis, 1; urinary retention, 1; angina pectoris, 1; swollen labia, 1)

*Twenty-four patients (48%) were discharged within 23 hours.
†Twenty-two patients (51%) were discharged within 23 hours.

Table III. Postoperative symptom evaluation

	Total fundoplication (% of patients)	Partial fundoplication (% of patients)
Heartburn		
Resolved	93	93
Improved	7	7
Regurgitation		
Resolved	97	97
Improved	3	3
Cough		
Resolved	100	83
Improved	0	17
Chest pain		
Resolved	91	100
Improved	9	0
Hoarseness		
Resolved	100	100

patients (88%) and improved in the remaining two (12%). No patient in this group developed *de novo* dysphagia.

Esophageal manometry and ambulatory pH monitoring were performed postoperatively in 14 patients after total fundoplication and in 14 patients after partial fundoplication (Table IV). Four patients in each group had abnormal DeMeester scores postoperatively, although in all but one patient the score was markedly improved in comparison to the preoperative score. In the latter patient the wrap was found to have herniated above the diaphragm, and she was the only patient who was clinically worse after surgery.

DISCUSSION

These results show that (1) the clinical findings after laparoscopic total and partial fundoplications are good and similar, (2) the effects on LES function of these two operations are also good and similar, and (3) partial fundoplication corrected the reflux in patients with abnormal esophageal peristalsis without producing dysphagia.

Operative Technique and Postoperative Course

The two procedures differ only slightly with regard to their technical aspects (see Fig. 2). We believe it is important to close the esophageal hiatus, because the diaphragm is known to contribute to the gastroesophageal sphincter mechanism, behaving as an external sphincter.[11] Furthermore, closure of the hiatus helps to prevent postoperative paraesophageal herniation.[12] The short gastric vessels are divided in both procedures, and a short (2 cm) floppy 360- or 240-degree wrap is created around a 56 to 60 F bougie. We place additional stitches between the top of the wrap to the adjacent crus and the esophagus on each side (*C* in Fig. 2) and from the posterior surface of the wrap to the reapproximated crus to counteract rotational (*C* in Fig. 2) and cephalad traction forces (*D* in Fig. 2). The objective of these stitches is to stabilize the wrap, the main determinant of long-term outcome.[13]

The postoperative course after the two procedures was similar. Half of the patients were discharged within 23 hours and the other half within 2 days. These patients only required oral analgesics for pain, and they returned to their regular activities within 10 to 14 days.

Esophageal Function

Total and partial fundoplication produced a similar increase in the pressure and length of the LES. Neither procedure affected the ability of the sphincter to relax in response to swallowing. These results are in contrast to a previous report that laparoscopic fun-

Table IV. Preoperative and postoperative esophageal function tests

	Total fundoplication (n = 14)		Partial fundoplication (n = 14)	
	Preoperative	Postoperative	Preoperative	Postoperative
Manometry				
LES pressure (mm Hg)	8.5 ± 1.0	15 ± 1.0*	8.0 ± 1.0	12 ± 1.0*
LES length (cm)	1.9 ± 0.2	3.2 ± 0.2*	1.7 ± 0.2	3.1 ± 0.2*
LES relaxation (%)	82	79	74	79
Peristaltic amplitude (mm Hg)	101 ± 12	101 ± 14	32 ± 3	49 ± 4*
Ambulatory pH monitoring				
No. of reflux episodes	171 ± 30	58 ± 26*	241 ± 40	51 ± 14*
% time pH <4.0	19 ± 6	5 ± 3*	22 ± 3	3 ± 1*
Esophageal clearance (min)	1.7 ± 0.7	1 ± 0.6	1.2 ± 0.2	0.7 ± 0.2*
DeMeeste score (normal <15)	69 ± 21	21 ± 10*	89 ± 15	15 ± 5*

*P <0.05 vs. preoperative value.

doplication interfered with relaxation of the LES in 67% of patients and produced persistent dysphagia in 9% of patients.[14] The findings in that study may have been due to incomplete mobilization of the gastric fundus and use of the body rather than the fundus for the wrap (i.e., Rossetti modification).

Peristalsis was normal preoperatively among the patients subjected to total fundoplication and did not change postoperatively. After partial fundoplication, the amplitude of esophageal peristalsis and esophageal clearance improved by 53% and 42%, respectively (Table IV). Other authors have observed improvement in defective peristalsis after fundoplication, suggesting that the abnormal esophageal body motor function is at least partly a consequence of the hiatal hernia.[15,16]

Gastroesophageal Reflux

Several patients in each group still had minor amounts of reflux after surgery. In general, these were the patients who preoperatively had the most severe esophagitis, often with stricture formation and/or Barrett's changes (both indicating more advanced disease).

Symptomatic Outcome

Heartburn and rugurgitation resolved or improved in all patients, replicating the results obtained with open surgery[17] and confirming previous reports on laparoscopic antireflux operations.[18,19] Atypical symptoms such as chest pain, cough, and hoarseness also resolved. We have shown that reflux into the upper esophagus occurs more frequently in patients with a panesophageal moter disorder, and that these patients experience cough and hoarseness more often.[3,20] Cough resolved in 83% of patients and hoarseness cleared in all patients after partial fundoplication, suggesting that the creation of an effective antireflux valve and improvement in peristalsis decreased the upward extent of gastric reflux.

Dysphagia is common in GERD, even in the absence of a stricture. Because it probably results from abnormalities in the propagation and velocity of the peristaltic waves, it is referred to as nonobstructive dysphagia.[21] Dysphagia resolved or improved in all patients postoperatively. Although four patients had *de novo* dysphagia after total fundoplication, there were no instances of *de novo* dysphagia after partial fundoplication.

CONCLUSION

Therapy with proton pump inhibitors is unsatisfactory in approximately 25% of patients with esophagitis,[22] possibly because duodenal juice contributes to the injury.[23] The surgical creation of an effective antireflux mechanism may be a more logical treatment because it corrects reflux regardless of its chemical composition. Partial fundoplication provides the same symptomatic relief and control of reflux as total fundoplication, but the partial procedure avoids postoperative dysphagia.[4,5,24]

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Discussion

Dr. J. Hunter (Atlanta, Ga.). You suggested at the beginning of your presentation that 20% of your patients had severe esophageal dysmotility and yet approximately half of your patients underwent partial fundoplication. Did some of the patients with reflux and normal motility have a partial fundoplication and not a Nissen procedure? The second question relates to the very interesting finding that in some of the patients the esophageal motility improved postoperatively. As you know, this is a very mixed group of patients with some having primary motility disorders and some having secondary motility disorders, and probably the only way to sort them out is to follow them for a long period of time. We have found that the majority of patients had improved motility, but 10% of our patients had worsening motility after fundoplication, probably a manifestation of their primary motility disorder. Did you observe that phenomenon in your patients who had partial fundoplications?

Dr. M.G. Patti. The first slide refers to all patients who were referred to the Swallowing Center; among those patients we found a major dysmotility problem in 20%. Some of those patients were not referred for surgery and some were. Overall, among the patients who are referred for surgery, approximately 40% had a severe dysmotility disorder. We noticed that partial fundoplication worked very well. We are continuing to expand the indications for its use. We used to perform a total fundoplication in patients who had mild dysmotility; today we are more willing to perform partial fundoplication because it controls symptoms but does not seem to cause postoperative dysphagia. Some patients show improvement but we are unable to predict which patients will improve. It was suggested years ago

that there has been no improvement if the amplitude of peristalsis is less than 35 mm Hg. We have actually seen improvement even when the amplitude was 10 mm Hg; however, even when there is improvement, motility never returns to normal.

Dr. F. Quijano (Mexico City, Mexico). If the same physiologic results are achieved with partial and total fundoplication, why is it necessary to perform total fundoplication at all?

Dr. Patti. Short-term data similar to ours or Toupet fundoplication as performed by Dr. Hunter show that both procedures work equally well, but we are comparing these findings with long-term results such as the follow-up data presented by Dr. DeMeester in which 90% of patients had perfect control of symptoms at 10 years. Presently we use a selective approach, mainly for patients who have problems of abnormal peristalsis.

Dr. T. Pappas (Durham, N.C.). What about the 14 patients who were followed up with manometry. How were they selected since it was not the total group, and how long were they studied in follow-up? Finally, we are surprised that the LES pressure you observed after partial fundoplication was about the same as that encountered after total fundoplication.

Dr. Patti. We too were surprised to see that the increase in pressure was similar for both operations. We were able to bring back for esophageal function testing only one third of the patients, so these are patients who agreed to undergo postoperative testing even if they were asymptomatic. We routinely study patients 2 months after the operation.

Dr. A. Sicular (New York, N.Y.). I was also impressed with the results of your partial fundoplications and I wonder whether, with the improved peristalsis you observed, you associate this in any way with the restoration of normal length of the total esophagus and better fixation with two rows of stitches to the gastric position of your wraps?

Dr. Patti. The pressure that we recorded postoperatively was really a combination of many factors, as you suggested. Clearly, one is that the wrap is stable. Data from Finland have shown that this is the main determinant of long-term outcome. The other two factors are closure of the hiatus, because the pressure that is recorded is a combination of the action of the diaphragm plus the intrinsic pressure of the LES, and the reduction of the esophageal hiatal hernia. We have shown that reduction of the hiatal hernia is associated with improvement in the function of the sphincter and peristalsis, particularly in patients with large hiatal hernias.

Dr. H. Sugerman (Richmond, Va.). Do you believe that the long-term outcome of partial fundoplication will equal that of total fundoplication?

Dr. Patti. In the era of laparoscopic surgery, I do not think anybody has definitive data on outcomes as of yet. Dr. Hunter was one of the first to propose a selective laparoscopic approach selecting partial fundoplication only for those patients with weak peristalsis. Dr. Hinder and his associates are presenting their data for the first time on the selective use of Toupet fundoplication. There are, however, other data showing that open partial fundoplications, performed through either the abdomen or the chest, are actually quite effective in controlling reflux. We hope that we will achieve the same results when we perform this operation laparoscopically.

Expression and Prognostic Significance of HLA Class I, ICAM-1, and Tumor-Infiltrating Lymphocytes in Esophageal Cancer

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Most solid malignancies show some degree of lymphoid infiltration suggesting a specific immunologic host vs. tumor reaction. Tumor-infiltrating lymphocytes (i.e., CD3 + T-lymphocyte subsets), the human leukocyte antigen (HLA) class I molecules, and the intercellular adhesion molecule-1 (ICAM-1) are key factors involved in T-cell–mediated immune surveillance. The present study was designed to assess the expression pattern of intratumoral lymphocyte infiltrates and their relationship to HLA class I and ICAM-1 expression with regard to primary esophageal carcinoma and to evaluate their prognostic influence. Representative samples of primary tumors were obtained from 55 patients who had undergone radical en bloc esophagectomy. Frozen sections of these tumors were stained with monoclonal antibodies directed against CD3 for the assessment of tumor-infiltrating lymphocytes, HLA class I, and ICAM-1. The mean postoperative observation period was 19.5 months (range 5 to 45 months). Lymphocyte infiltration was absent in four tumors (8%), whereas 31 tumors (64%) showed moderate and 13 (27%) showed strong infiltration. HLA class I expression was deficient in 24 tumors (45%). Coexpression of HLA class I and ICAM-1 was significantly associated with lymphocyte infiltration of the tumor. Kaplan-Meier analyses revealed a significant beneficial influence on relapse-free survival for patients with lymphocyte infiltration of primary tumors compared to those with no lymphocyte infiltration of tumors (median 4 months vs. 18 months; $P < 0.002$) and for HLA class I^+ tumors compared to HLA class I^- tumors (median survival >18 months vs. 7 months; $P = 0.0081$). The present data support the hypothesis that T-cell–mediated immunity may influence the fate of patients with esophageal cancer. (J GASTROINTEST SURG 1997;1:316-323.)

Esophageal cancer is a very aggressive disease with a poor prognosis. Despite intentional curative surgery, 5-year survival rates still range from only 20% to 36%. Metastatic relapse remains the most frequent cause of cancer-related death.[1-5] It therefore must be assumed that minimal tumor cell dissemination occurs early in the course of the disease. The survival of these early metastatic "seed" tumor cells could be facilitated by circumvention of immune surveillance. At present, however, nothing is known about the potential role of the immune system as it relates to the onset or progression of esophageal cancer.

Major histocompatibility complex (MHC, HLA) molecules as well as the intercellular adhesion molecule-1 (ICAM-1) seem to play a key role in T-cell–mediated immune surveillance of tumor cells. Human leukocyte antigen (HLA) class I molecules are membrane glycoproteins associated with beta-2 microglobulins and are expressed in most nucleated cells of the body. They aid in recognition of foreign antigens, including tumor antigens, by cytotoxic T cells.

T cells are defined by either the expression of the T-cell receptor TCR_1 ($\chi\delta$-TCR) or the T-cell receptor TCR_2 ($\alpha\beta$-TCR). Most T lymphocytes present their T-cell antigen receptor ($\alpha\beta$-TCR or $\chi\delta$-TCR) together with the CD3 receptor subunit. CD3 consists of three different polypeptide chains (χ-chain, δ-chain, and ϵ-chain) with a molecular weight between 20 and 25 kilodaltons (KD). The $\alpha\beta$-TCR and CD3 are attached by a tight noncovalent bond. It is as-

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sumed that the intracellular epitopes of the CD3 peptide chains, together with an ξ-dimeric protein associated with the receptor complex, play an important role in signal transduction during T-cell activation.[6,7] Target cell binding by cytotoxic T cells via the TCR/CD3 receptor complex depends on the presentation of HLA class I molecules by the tumor cells. Downregulation of HLA class I surface markers leads to impairment of TCR/CD3–mediated tumor cell recognition. The loss of HLA class I molecules is a frequent occurrence in primary carcinomas and their metastase, which appears to be associated with a worse prognosis.

An important immunologic cofactor is the intercellular adhesion molecule-1 (ICAM-1, CD54), a ligand for the lymphocyte function–associated antigen-1 (LFA-1) that is present in T cells. Several cytokines can induce ICAM-1 expression in tumor cells, which alters the vulnerability of these cells to monocyte- and T-cell–mediated lysis.[8-10]

In the present study we investigated the expression pattern and prognostic influence of HLA class I and ICAM-1 and the correlation of these immunoregulatory molecules with tumor lymphocyte infiltration in esophageal cancer.

PATIENTS AND METHODS

Patients and Follow-Up

This study was approved by the ethics committee of the Chamber of Physicians of Hamburg. Informed consent was obtained from all patients before they were included in the study. Tumor specimens were collected from 55 patients with resectable thoracic esophageal carcinomas who had undergone radical en bloc esophagectomy with gastric tube reconstruction and cervical esophagogastric anastomosis between April 1992 and July 1995. The median age at the time of operation was 58 ± 9 years (standard deviation). Patients were reexamined every 3 months after esophagectomy for the first 2 years and at 6-month intervals thereafter. The evaluation included physical and chest x-ray examinations, endoscopy, endosonography, CT scan of the chest and abdomen, abdominal ultrasound, tumor markers and bone scan.

Tissue Preparation and Immunohistochemical Studies

Representative tumor samples were snap-frozen in liquid nitrogen immediately after resection and stored at −80° C. From each tumor 5 μm frozen sections were transferred onto glass slides prepared with 3-triethoxysilyl-propylamin (Merck & Co., Darmstadt, Germany) and stored at −20° C until use. For immunohistochemical analysis frozen sections were air dried, fixed in acetone, rehydrated with tris–phosphatase-buffered saline (TBS), and stained using the alkaline phosphatase-antialkaline phosphatase (APAAP) technique. Blocking of nonspecific binding was performed with normal human serum diluted 1:10 in TBS, which was applied for 30 minutes. Then the primary monoclonal antibody, in appropriate dilution with TBS, was applied for 45 minutes. This was followed by incubation with a linking antibody (Z-259, Dako Corp., Hamburg, Germany) for 30 minutes and subsequent application of the APAAP complex for an additional 30 minutes. After each incubation, specimens were washed in TBS. Antibody-linked alkaline phosphatase activity was detected with fast red TT dye (Sigma, Deisenhofen, Germany). Endogenous alkaline phosphatase activity was blocked by levamisole. Sections were counterstained with Gill's hematoxylin. All incubations were performed at room temperature in a humidified chamber.

Monoclonal Antibodies

The following mouse antihuman monoclonal antibodies were used: CD3, TS4B5 (Dako), which reacts with the 20 KD epsilon-chain of the T-cell–associated CD3 antigen, and HLA-A,B,C, W6/32 (Dako) directed against a monomorphic antigenic epitope of HLA class I heavy chains associated with beta-2 microglobulins. ICAM-1 was detected by 6.5B5 antibody (Dako) directed against the domain 1 of ICAM-1. To exclude nonspecific binding, negative control tests were performed with irrelevant mouse myeloma proteins of identical isotypes (MOPC 21, IgG_1 [Sigma] and UPC 10, IgG_2 [Sigma]).

Evaluation of Specimens

Slides were evaluated, in double-blind fashion, by two observers using light microscopy. Lymphocyte infiltration was considered deficient if less than 33% of the tumor showed $CD3^+$ T-cell infiltration. Moderate infiltration was assumed if 33% to 66% of the tumor showed lymphocyte infiltration, and infiltration was considered strong if more than 66% of the tumor displayed lymphocyte infiltration. HLA class I expression was considered to be deficient if less than 35% of the tumor cells were stained. ICAM-1 expression was considered to be induced if more than 25% of the tumor cells expressed the antigen. In 80% of the cases both observers obtained the same results; the remaining slides were reevaluated and a consensus decision was reached.

Statistical Analysis

Fisher's exact test was used to test the equality of two binomial proportions. Kaplan-Meier estimates of relapse-free survival were calculated. Univariate analyses of all prognostic factors were conducted using log-rank tests. The level of significance was set at $P < 0.05$.

RESULTS

Follow-up was completed for 53 patients, with a mean observation period of 19.5 months (range 5 to 45 months). Tumors were staged and graded according to the TNM classification of the International Union Against Cancer.[11] All patients in this study had stage pT_{1-4}, pN_{0-1}, M_0 tumors (Table I).

Table I. Patient and tumor characteristics

	No. of patients (%)*
Sex	
Male	43 (81)
Female	10 (19)
pT_1	9 (17)
pT_2	15 (28)
pT_3	26 (49)
pT_4	3 (6)
pN_0	23 (43)
pN_1	30 (57)
Tumor type	
Squamous cell carcinoma	36 (68)
Adenocarcinoma	17 (32)
Tumor grade	
1	2 (4)
2	38 (72)
3	13 (24)

*Median age of patients was 58 ± 9 years (standard deviation).

Expression of CD3 Antigen as a Marker for Tumor Lymphocyte Infiltration

Twenty-six percent of the tumors analyzed showed strong lymphocyte infiltration (>65% infiltration of the tumor) compared to the 65% that displayed moderate infiltration (33% to 66%), and the 8% with no evidence of tumor infiltration. No significant difference in lymphocyte infiltration was evident comparing squamous cell carcinomas and adenocarcinomas. In analyzing the correlation between lymphocyte infiltration and the clinicopathologic parameters, we observed no significant difference in relation to pT stage, pN stage, or grade of the primary tumors (Table II). However, a significant correlation between lymphocyte infiltration and expression of HLA class I could be seen. Seventy percent of tumors that displayed a strong infiltration had a normal expression of HLA class I and 61% of those with a moderate infiltration showed normal HLA class I expression; none of the four tumors with a lack of infiltration showed HLA class I expression ($P = 0.041$). Furthermore, induction of ICAM-1 in tumor cells was significantly correlated with lymphocyte infiltration. In 92% of tumors with intensive lymphocyte infiltration, ICAM-1 was expressed in tumor cells ($P = 0.006$). Regarding coexpression of HLA class I and ICAM-1, a significant correlation with lymphocyte infiltration was evident. Sixty-two percent of the tumors coexpressing both HLA class I and ICAM-1 showed a strong lymphocyte infiltration as compared to 31% expressing ICAM-1 exclusively and 7% expressing HLA class I exclusively. None of the tumors with no lymphocyte infiltration showed coexpression of both HLA class I and ICAM-1 ($P = 0.009$) (Table III).

Expression of HLA Class I Antigens

Deficient HLA class I expression was found in 45% of all tumors. There was a significant difference in HLA class I expression between adenocarcinomas and squamous cell carcinomas (Table II). Only 24% of the adenocarcinomas showed deficient expression of HLA class I antigens compared to 55% of the squamous cell carcinomas ($P = 0.028$). Assessment of the relationship between clinicopathologic factors and HLA class I expression revealed no significant correlation between HLA class I expression and pT stage. In analyzing tumors according to metastatic lymph node involvement that was verified by histopathologic examination, deficient HLA class I expression was significantly associated with metastatic nodal involvement. Sixty percent of node-positive patients showed HLA class I downregulation compared to only 26% of node-negative patients ($P = 0.013$) (see Table II). No correlation was seen between tumor grade and deficient HLA class I expression.

Expression of ICAM-1

Induction of ICAM-1 was evident in 60% of all esophageal carcinomas that showed expression of ICAM-1. There was no significant difference between the two histologic types. No correlation with metastatic lymph node involvement, tumor stage, or grade of the primary tumor was found (see Table II).

Table II. Expression of CD3 as a marker for tumor lymphocyte infiltration, HLA class I, and ICAM-1 in primary tumors: Correlation with tumor size (pT stage) and lymph node involvement (pN stage)

	No infiltration	Moderate (33%-66%) infiltration of the tumor	Strong (>66%) infiltration of the tumor	Deficient HLA class I expression*	Expression of ICAM-1†
Adenocarcinoma	1 (6%)	11 (65%)	5 (29%)	4 (24%)	9 (53%)
Squamous cell carcinoma	3 (9%)	22 (67%)	8 (24%)	20 (55)‡	23 (64%)
pT_1	0	6 (67%)	3 (33%)	3 (33%)	4 (44%)
pT_2	1 (7%)	8 (62%)	4 (31%)	4 (27%)	11 (73%)
pT_3	3 (12%)	16 (67%)	5 (21%)	16 (61%)	16 (61%)
pT_4	0	2 (67%)	1 (33%)	1 (33%)	1 (33%)
pN_0	0	15 (68%)	7 (32%)	6 (26%)	15 (65%)
pN_1	4 (15%)	17 (63%)	6 (22%)	18 (60%)§	17 (57%)

Statistical analyses were performed using Fisher's exact test.
*Less than 35% positive tumor cells.
†More than 25% positive tumor cells.
‡Significant difference between expression in adenocarcinoma and squamous cell carcinoma ($P = 0.028$).
§Significant difference between pN_0 and pN_1 ($P = 0.013$).

Table III. Correlation between tumor-infiltrating lymphocytes and expression of HLA class I and ICAM-1 in primary esophageal carcinomas

Tumor characteristics	No infiltration	Moderate (33%-66%) infiltration of the tumor	Strong (>66%) infiltration of the tumor	*P* value (χ^2 test)
HLA class I^+	0	19 (68%)	9 (32%)	
HLA class I^-	4 (20%)	12 (60%)	4 (20%)	0.041
ICAM-1^+	3 (11%)	13 (46%)	12 (43%)	
ICAM-1^-	1 (5%)	18 (90%)	1 (5%)	0.006
HLA class I^-/ICAM-1^-	1 (14%)	6 (86%)	0	
HLA class I^+/ICAM-1^-	0	12 (92%)	1 (8%)	
HLA class I^-/ICAM-1^+	3 (23%)	6 (46%)	4 (31%)	
HLA class I^+/ICAM-1^+	0	7 (47%)	8 (53%)	0.009

Prognostic Influence of Lymphocyte Infiltration, HLA Class I Antigens, and ICAM-1

Following a mean observation period of 19.5 months, 30 patients (56%) suffered a relapse. All patients with no lymphocyte infiltration of their tumors had a recurrence compared to 50% of those with moderate lymphocyte infiltration and 46% of those with strong infiltration ($P = 0.002$). The median relapse-free survival period for these three groups of patients was 4 months, 11 months, and 18 months, respectively. Among patients with tumors expressing HLA class I antigens homogeneously, only 41% had a recurrence following a median relapse-free survival of 18 months in contrast to 75% of patients with deficient HLA class I expression in their tumors who had a recurrence following a median relapse-free survival of 7 months ($P < 0.008$) (Fig. 1). Kaplan-Meier analyses further revealed that N stage and T stage had a significant influence on relapse-free survival, whereas ICAM-1 had no influence (Table IV).

In analyzing the prognostic impact of coexpression of HLA class I and ICAM-1, a significant interaction was found. Among patients with primary tumors coexpressing HLA class I and ICAM-1, 37% had a recurrence following a median relapse-free survival of

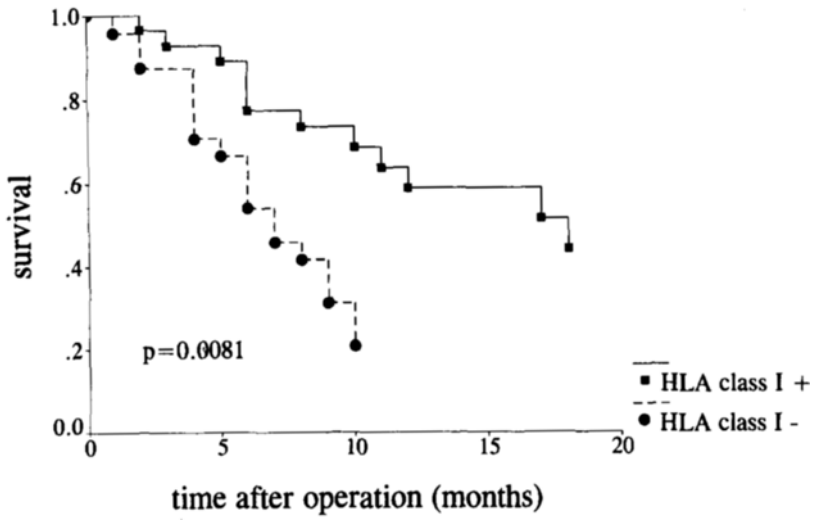

Fig. 1. Impact of HLA class I expression on relapse-free survival.

Table IV. Correlation between tumor relapse and tumor-infiltrating lymphocytes and expression of HLA class I antigens, ICAM-1, N stage, and T stage: Kaplan-Meier analyses

Tumor characteristics	No. of patients	Tumor relapse	Median relapse-free survival (mo)	*P* value (log-rank)
No infiltration	4	4	4	
Moderate infiltration	32	16	11	
Strong infiltration	13	6	18	0.0022
HLA class I^+	29	12	18	
HLA class I^-	24	18	7	0.0081
ICAM-1^+	32	18	10	
ICAM-1^-	21	12	10	0.751
HLA class I^-/ICAM-1^+	16	12	6	
HLA class I^+/ICAM-1^+	16	6	>18	0.017
N_0	23	4	>12	
N_1	30	26	6	<0.0001
T_{1-2}	24	10	17	
T_{3-4}	29	20	6	0.012

more than 18 months. In cases of HLA class I deficiency and ICAM-1 expression, 75% of the patients had a recurrence of their tumors after a median relapse-free survival of 6 months ($P = 0.017$), and if both HLA class I and ICAM-1 expression was deficient (n = 24), relapses occurred in 75% with a median relapse-free survival of 7 months (see Table IV).

When the relapse patterns were analyzed, a lymphocyte infiltration of tumors, HLA class I expression, and coexpression of HLA class I and ICAM-1 appeared to have a significant influence only on distant metastases but not on local recurrences. In addition, pN stage had a significant influence on the development of distant metastases, whereas pT stage did not (data not shown). Lymphocyte infiltration of the tumor, HLA class I expression, and coexpression of HLA class I and ICAM-1 also had a significant influence on overall survival (Table V). Furthermore, as expected, pN stage and pT stage were prognostic factors for overall survival (see Table V).

DISCUSSION

Despite radical surgical en bloc esophagectomy, patients with esophageal cancer frequently have an early recurrence of their disease. It must therefore be

Table V. Correlation between overall survival and tumor-infiltrating lymphocytes and expression of HLA class I antigens, ICAM-1, N stage, and T stage: Kaplan-Meier analyses

Tumor characteristics	No. of patients	Deaths	Median overall survival (mo)	*P* value (log-rank)
No infiltration	4	3	5	
Moderate infiltration	32	12	18	
Strong infiltration	13	6	19	0.0055
HLA class I^+	29	9	>15	
HLA class I^-	24	16	10	0.0072
ICAM-1^+	32	15	15	
ICAM-1^-	21	10	12	0.722
HLA class I^-/ICAM-1^+	16	11	10	
HLA class I^+/ICAM-1^+	16	4	>19	0.011
N_0	23	2	>17	
N_1	30	23	9	<0.0001
T_{1-2}	24	8	>18	
T_{3-4}	29	17	10	0.014

assumed that dissemination of tumor cells, which cannot be detected by current tumor staging modalities, is occurring early in the course of the disease. Our data suggest that some sort of "escape" mechanism that inhibits T-cell–mediated immune surveillance might be an important factor that is related to the prognosis of patients with esophageal cancer. In 51% of all tumors we observed a downregulation or complete loss of HLA class I antigen expression.

The observed correlation between metastatic lymph node involvement and primary tumor extension and deficient expression of HLA class I antigens indicates that tumor progression and tumor cell spread may be facilitated by a reduced ability of the immune system to recognize these cells, since tumor cell recognition by CD3+/CD8+ cytotoxic T lymphocytes is HLA restricted. Consequently, a deficiency in HLA class I antigens may account for an impaired antitumor response by tumor-infiltrating lymphocytes (TILs) with the result being a recurrence of the tumor. Several authors have demonstrated decreased cytotoxicity of TILs against autologous and allogenous tumor cells compared to peripheral blood lymphocytes.[12-16] The presence of T-suppressor cell subsets within the tumor, in addition to HLA downregulation, could be another explanation for this decreased cytotoxicity,[17] as well as the previously postulated tumor-derived immunosuppressive factors that may impair tumor-specific cytotoxity by TILs.[18-20]

Analysis of relapse rates showed a significant correlation between tumor recurrence and lymphocyte infiltration. This lymphocyte infiltration of the tumor accounted for a significantly reduced risk of developing a recurrence. Interestingly, there was an even more pronounced benefit for patients with strong infiltration compared to those with moderate infiltration. The significantly increased relapse rate that was observed in tumors lacking HLA class I expression could therefore be interpreted as resulting from impaired TCR/CD3-HLA tumor cell recognition with a consequent loss of antitumor response by the TILs.

Furthermore, coexpression of HLA class I and ICAM-1 was a prognostic factor in determining risk for the development of tumor recurrence. This significant interaction between HLA class I and ICAM-1 supports the hypothesis that a costimulatory signal is necessary for T-cell activation and subsequent T-cell–mediated antitumor response, as recently described in experimental and clinical reports.[9,21,22]

On the other hand, our findings indicate that induced ICAM-1 expression in tumor cells lacking HLA class I expression may enhance the metastatic capability of these cells. Patients in the subgroup that presented with HLA class I^-/ICAM-1^+ tumors had the worst prognosis. This finding may be explained by the fact that these tumor cells might be bound to leukocytes through the interaction between ICAM-1 and its ligand LFA-1, which is present in T cells. If the tumor cannot be recognized because HLA class I antigens are not present in sufficient quantities, the T cells fail to destroy the tumor cell. However, this heterotypic cell contact with migratory and invasive lymphocytes might enhance the ability of such tumor cells to reach secondary organs.[23] Interestingly, de novo expression of ICAM-1 in primary melanomas is also correlated with an increased risk of metastatic relapse.[23]

The demonstrated prevalence for the development

of distant metastases in patients who lack lymphocyte infiltration, as well as in patients with downregulation of HLA class I expression, leads to the assumption that minimal tumor cell dissemination is facilitated by circumvention of immune surveillance and probably occurs early in the course of the disease.

We are well aware that our data show only statistical correlations between the expression of immunoregulatory molecules in primary tumors, the effects of TILs, and prognosis. However, our data provide evidence that tumor-infiltrating T-cell populations exist in esophageal carcinomas and the statistical correlation with prognosis could be an indication of a T-cell–mediated antitumor response. It remains unclear to what extent intratumoral immunosuppressive factors are capable of attenuating the cytotoxic efficacy of TILs. Further investigations must be carried out to disclose the composition and status of activation of TILs.

CONCLUSION

We conclude that phenotyping of the primary tumor for immunologic effector molecules may identify patients at higher risk for early relapse with the potential consequences of adjuvant therapy. In consideration of the poor overall survival rate for patients with esophageal cancer, the search for new strategies in adjuvant therapy (e.g., immunotherapy), especially in patients with resectable primary tumors and a minimal residual tumor load, seems to be essential. Because coexpression of HLA class I and ICAM-1 seems to have a significant positive influence on the clinical outcome of the disease, the application of cytokines that induce expression of these molecules holds promise as a therapeutic strategy to increase the vulnerability of esophageal tumor cells. Furthermore, vaccinating immunocompetent patients with genetically modified tumor cells may induce systemic immunity, which might result in the destruction of unmodified disseminated tumor cells.[24] Our results indicate that patients with resectable esophageal cancer may be suitable candidates for such new therapeutic options.

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Physiologic Determinants of Nocturnal Incontinence After Ileal Pouch–Anal Anastomosis

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The goals of the ileal pouch–anal anastomosis (IPAA) operation are the construction of a fecal reservoir and the preservation of anal function, without compromising continence. Some of the patients are incontinent at night. The aim of our study was to identify the mechanisms responsible for nocturnal incontinence. We analyzed patients undergoing IPAA for ulcerative colitis, who underwent anorectal tests between 1993 and 1995. All patients were subjected to pull-through manometry and pelvic floor function studies, and 33 patients underwent overnight ambulatory manometry. Among 44 patients (27 men and 17 women), 22 had complete continence, whereas 22 had nocturnal incontinence. Mean age was 40 ± 1 years. There were no differences with regard to sex, age, stool consistency, and ability to differentiate gas from stool between groups; only stool frequency was lower in the continent group (median [range] 6 [3 to 10] vs. 8 [5 to 25] stools/24 hours; $P = 0.011$). Resting and squeezing anal canal pressure did not differ ($P = 0.42$ and $P = 0.73$, respectively). Resting, squeezing, and defecating anorectal angle, percentage of pouch evacuation, and perineal descent, all measured scintigraphically, did not differ between groups (all $P > 0.05$). Ambulatory manometry showed that the mean anal canal pressure was higher in continent patients compared to incontinent patients, both during awake (88 ± 11 vs. 62 ± 8; $P = 0.032$) and sleep (81 ± 14 vs. 49 ± 9; $P = 0.029$) periods. The motility index was similar (awake, $P = 0.88$; sleep, $P = 0.95$), as was the number of episodes where the pouch pressure was greater than the anal canal pressure ($P = 0.28$). In otherwise continent patients after IPAA, the combination of high stool frequency and low basal anal canal pressure may be related to nocturnal incontinence. Moreover, standard anorectal physiology tests cannot identify these subtle differences. (J GASTROINTEST SURG 1997;1:324-330.)

The most common indication for surgery in patients with chronic ulcerative colitis is intractability of medical management, with dysplasia a distant second.[1] Several strategies to preserve fecal continence have been developed since total proctocolectomy became the standard operation for patients requiring proctocolectomy. Ileal pouch–anal anastomosis (IPAA) has gained increased acceptance as the procedure of choice for preserving continence in this setting.[2,3] IPAA is successful in approximately 94% of patients. However, occasional fecal incontinence is an ongoing complication, particularly at night. The cause of such nocturnal incontinence is unknown.

Most patients after IPAA are fully continent during the day, but some experience incontinence at night. Many previous studies have characterized incontinence after IPAA, but little data exist concerning patients who experience *only* fecal incontinence at night. Anal canal pressures are lower during sleep than when patients are awake,[4] but this is likely not the sole cause. Persistent nocturnal leaking after IPAA has been documented in 20% to 48% of patients.[5,6] The goal of this study was to characterize nocturnal incontinence in otherwise continent patients after IPAA.

METHODS

Forty-four patients with chronic ulcerative colitis who underwent IPAA between 1993 and 1995 were

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analyzed. The procedure performed was proctocolectomy, construction of a J-shaped ileal reservoir, and anastomosis to the anal canal using a hand-sewn or double-stapling technique. A diverting ileostomy was fashioned in all patients. Two or three months later, patients were readmitted for takedown of the ileostomy.

Follow-up ranged from 1 to 10 years. Patients were admitted at least 6 months following closure of the ileostomy so that the following studies could be performed: anal manometry (stepped pull-through manometry), nuclear medicine studies of pelvic floor function and pouch function, and ambulatory manometry.

In addition to these studies, each patient was asked to complete a questionnaire designed to determine continence status, the stool frequency during 24 hours, the ability to discriminate stool from gas, and stool consistency.

All patients were fully continent during the daytime. The dependent variable was the presence of nocturnal incontinence. This was defined as nocturnal leaking at least twice a week, even in minimal proportions. This finding was categorized as either occasional (<2 events/wk) or frequent (≥2 events/wk).

To facilitate the association of the independent variable with prognostic factors, patients were categorized by groups. Time and anastomosis types were not considered in the analysis.

Tests Performed

Patients were admitted to the Clinic Research Center and informed consent was obtained.

Nuclear Medicine Studies. Scintigraphic pouch–anal angle and defecation studies evaluated the angle formed between the pouch and the anal canal; this measurement assesses movement of the pelvic floor during attempted defecation and during squeezing.[7] In control subjects and in patients with normally functioning ileoanal anastomoses, the anal pouch angle becomes acute with squeezing and obtuse with straining.[7] Pouch emptying is also evaluated using radiolabeled artificial stool (Veegum).[8] This has the consistency of porridge. The patient is asked to evacuate the material during dynamic scanning; the percentage decrease in pouch volume from the beginning to the end of the evacuation is termed "evacuation efficiency."

Pull-Through Manometry. Using a perfused vector volume technique, anal canal resting and squeezing pressures were recorded.[9]

Ambulatory Manometry. Manometry was performed using a six-channel microtip pressure transducer catheter (Gaeltec Ltd., Isle of Skye, Scotland; distributed by Medical Measurement, Inc., Hackensack, N.J.). The catheter was positioned such that two channels were located in the anal canal and the other four in the pouch. The catheter was connected to a portable recorder, which stored 28 hours of recordings for each channel. The catheter was positioned using flexible sigmoidoscopy and taped in place. This technique has been extensively validated previously.[10,11] The patients were admitted to the Clinical Research Center the afternoon before the study. Catheters were placed the next morning. Patients then left the Clinic Research Center after breakfast. Recordings were made for 24 hours. The patients returned and their catheters were removed. Two standardized meals were given while patients were in the Center. The patients were instructed to pursue their usual daily activities. Also, bowel movements were not restricted.

Motor activity was expressed by a motility index (MI) calculated as MI = ln (sum of contraction amplitudes × number of contractions + 1).[11] Moreover, we measured the number of events per 10 hours in which the relationship (anal canal pressure > pouch pressure) was reversed (lasting 10 seconds or more).

Statistical Analysis

The analysis was designed to compare patients with and without nocturnal incontinence only. For parametric data Student's t test was used, either paired or unpaired according to the circumstances. For nonparametric data the Mann-Whitney rank-sum test was employed. When binomial variables were evaluated, chi-square and Fisher's exact tests were used if the expected value of any cell of the 2 × 2 table was less than five. In all cases a two-tailed P value was calculated; $P < 0.05$ was considered significant.

RESULTS

We identified 44 patients who were fully continent of stool during the day. Twenty-two of them were incontinent at night; 21 of the 22 had occasional leakage, whereas one had frequent leakage. Mean (± SD) age of the sample was 40 (± 9) years.

The mean age and the male:female ratio were the same in both groups (Table I). The only symptom that differed markedly between patients with and without nocturnal incontinence was the 24-hour stool frequency (Fig. 1). There were no differences in the other symptoms between groups (Table II).

Table I. Characteristics of the sample

	Continent (n = 22)	Incontinent (n = 22)	Chi-square test	*P* value
<45 years of age	12	8	1.46	0.226
>45 years of age	10	14		
Men	15	12	0.86	0.353
Women	7	10		

Table II. Anal function

	Continent (n = 22)	Incontinent (n = 22)	*P* value
Differentiate gas/stool			
Yes	13	12	0.761*
No	9	10	
Solid/semisolid stools	21	19	0.345†
Ambulatory manometry studies (median [range])			
Motility index			
Awake	16 (6-13)	14 (7-18)	0.88‡
Asleep	17 (3-20)	14 (7-20)	0.95‡
Reversals gradient			
Anal-pouch pressures	8 (0-50)	15 (0-70)	0.28‡

*Chi-square test.
†Fisher's exact test.
‡Mann-Whitney rank-sum test.

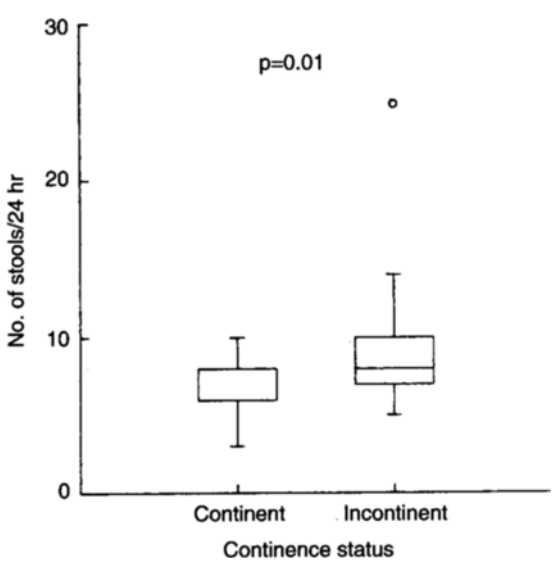

Fig. 1. Twenty-four–hour stool frequency.

Perineal descent, movement of the anal pouch angle, and pouch emptying showed no difference between patients who were continent and those who were incontinent at night (Figs. 2 and 3). Interestingly, resting and squeezing pressures of the anal canal, as measured by pull-through manometry, did not differ between continent and incontinent patients (Fig. 4).

Ambulatory manometry was performed in 33 patients, 16 of whom were incontinent. The mean (±SD) length of the recording sessions was 13.8 (±0.8) hours. In distinct contrast to the results of standard manometry, ambulatory manometry documented lower basal anal canal pressures in patients with nocturnal incontinence during both awake and sleep periods ($P = 0.034$ and $P = 0.029$, respectively) (Fig. 5). However, the MI did not differ between groups both during awake and sleep periods. Finally, the number of episodes in which pouch pressure was greater than anal canal pressure did not differ between groups (Table II).

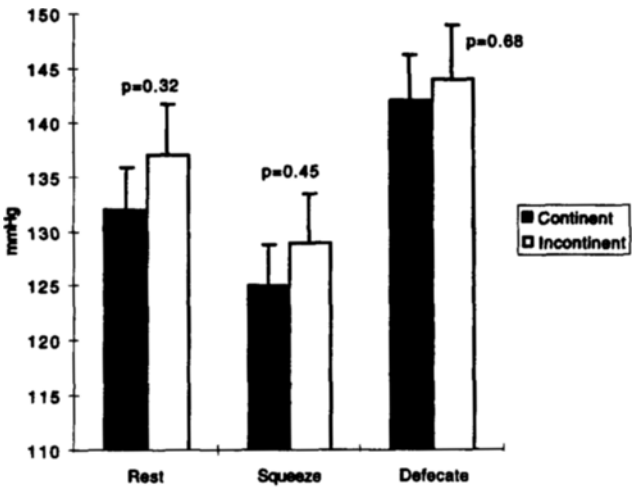

Fig. 2. Results of pelvic floor studies: Pouch–anal angle (mean ± SEM).

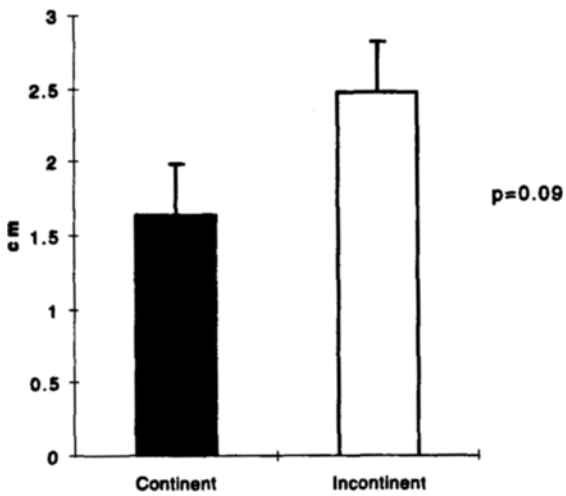

Fig. 3. Results of pelvic floor studies: Perineal descent.

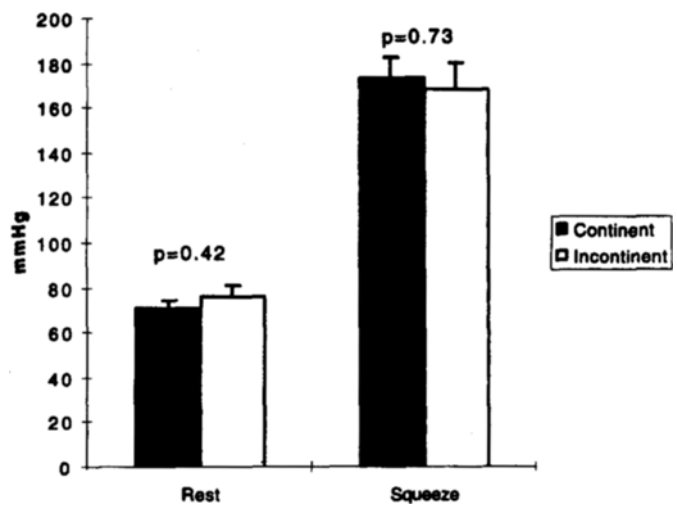

Fig. 4. Results of standard manometry: Anal canal pressure (mean ± SEM).

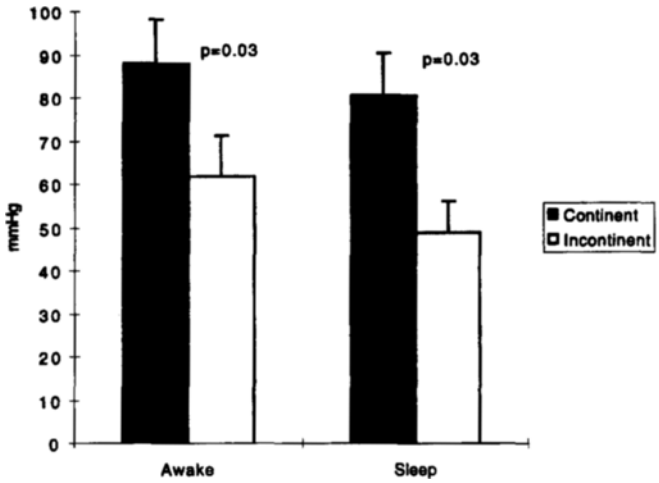

Fig. 5. *Results of ambulatory manometry: Basal anal canal pressure (mean ± SEM).*

DISCUSSION

That patients can be incontinent only at night after IPAA has been reported, but the etiology is obscure.[4,12] We have previously found that 50 (82%) of 61 patients were completely continent during the day; however, 12 (24%) of the 50 continent patients became incontinent at night.[13,14]

Orkin et al.[4] characterized the presence of nocturnal incontinence after IPAA in 1992. Using simultaneous anal canal pressure measurements and polysomnographic recordings of sleep stages, they demonstrated a decrease in anal canal pressure during rapid eye movement (REM) sleep. In a later study the authors demonstrated a decrease in anal canal pressures during REM sleep in healthy volunteers as well.[15] The possible contribution of age to incontinence was described by Pescatori and Mattana.[16] They found that older subjects (>45 years) had twice the rate of incontinence after IPAA compared to younger patients.[16] In the present study we compared patients over the age of 45 years with those 45 years of age or younger and found no difference (Table I). McHugh and Diamant[17] reached the same conclusion as Pescatori and Mattana but, in addition, found that women had higher rates of incontinence than men. In our series there was no trend toward increased nocturnal incontinence in women (Table I).

Ferrara et al.,[18] using techniques of ambulatory manometry identical to those used in this study, found that patients with fecal incontinence after IPAA had more reversals of the pouch–anal canal pressure gradient than those who were continent. Previously the same authors had shown that a gradient between the pouch and the anal canal, such that the anal canal pressure was greater than the pouch pressure, was preserved in patients who were continent.[10] In the rectum as in the pouch, strong contractions that increase rectal pressure trigger a rise in the anal canal pressure.[19-21] Failure of the anal canal to respond to such increases in pressure leads to fecal soilage because the pouch–anal canal pressure gradient is reversed.

Although the rectoanal inhibitory reflex has been postulated to be a primary mechanism controlling fecal continence,[22] all patients in our study demonstrated no rectoanal inhibitory reflex, yet all were continent during the day. The presence or absence of the rectoanal inhibitory reflex likely has little influence in maintaining gross fecal continence but may be at least partly responsible for fine control.

Of some interest is the increased 24-hour stool frequency among patients who are incontinent at night. Groom et al.[23] demonstrated that stool frequencies had prognostic implications in the outcome of IPAA; increased stool frequency was associated with a poor functional outcome. Our findings (see Fig. 1) support and extend these conclusions.

We showed that patients with nocturnal incontinence after IPAA have lower resting anal canal pressures compared to continent patients, not only during the day but also at night. During the day the external anal sphincter compensates for low resting pressures through conscious mechanisms (external anal sphincter and puborectal muscle contraction), which avoids fecal leakage.[4,24] At night no conscious mechanisms can be recruited, and the internal sphincter must face the challenge of bursts of high-pressure contractions in the pouch alone.[10,19] Thus, if such a partially damaged sphincter is exposed to high pouch pressures, leakage inevitably occurs.

Our findings confirm and extend the findings of others that standard pull-through manometry is unreliable in establishing the presence of fecal incontinence.[17,25] There are reports showing that up to 40% of incontinent patients have normal manometric findings.[17,25] However, we also confirmed what numerous authors have found—that ambulatory manometry, because it records dynamic pressures during the "normal activity of daily living" better qualifies episodes of fecal incontinence.

CONCLUSION

We found that after IPAA, patients with fecal incontinence occurring only at night have higher stool frequencies and lower resting pressures both during the day and at night compared to patients who are continent. However, reversals of the pouch–anal canal pressure gradient were no more frequent in patients with nocturnal incontinence than in patients who were continent. Ambulatory manometry and not standard manometry helps establish the presence of this unique manifestation of fecal incontinence.

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Discussion

Dr. J. Becker (Boston, Mass.). I am pleased that you have confirmed our findings regarding the relationship between leakage, stool frequency, and manometric findings. One of the things that is different is that we found a correlation between leakage and age. Could you comment on your larger series of patients undergoing IPAA, now well over 1000 patients, at the Mayo Clinic. In this larger cohort, have you found any correlation between age and leakage? Also, you did not comment on whether or not these patients had mucosectomy or a stapled low rectal anastomosis. You did not comment on the changes over time. Did you compare these postoperative manometric findings with preoperative values and late postoperative values? Was there any recovery over time?

Dr. J.M. Sarmiento. Everyone knows that patients with advanced age are less than ideal candidates for an ileal pouch procedure. This paper was concerned only with isolating terminal incontinence. Thus I cannot comment if we take into account the entire spectrum of incontinence.

Regarding mucosectomy, we did not include this in the comparison or in the analysis because in most of these patients a handsewn anastomosis is performed, and this includes a mucosectomy, as in 80% of these patients, so it is impossible for us to comment on that. Similarly, most of these patients (almost 80%) had an evaluation within the first postoperative year, so it is again impossible to say whether there is a difference between 1-year and 10-year postoperative results.

Dr. H. Sugerman (Richmond, Va.). In the past you presented results from a nonrandomized study, which showed no difference in leakage and incontinence rates between stapled and hand-sewn mucosectomy procedures, yet you have a very high rate of incontinence in the present data, much higher than that found by most of us who perform the stapled procedure. Have you excluded patients when they have episodes of pouchitis, which leads to incontinence? Did the incontinent patients have evidence of pouchitis at the time they were studied?

Dr. Sarmiento. We did not exclude patients based on the type of procedure; however, we did not include patients with pouchitis. These data are obtained while patients are going about their usual daily activities, so it is impossible to say.

We must remember that these are isolated events of nocturnal incontinence, and it is this fecal spotting, even in minimal proportion, that increases the rate or the frequency in incontinence that everyone is familiar with. Most people say that if we compared groups, we would find 25% to 40% of patients with incontinence and that is because of their definition. This is a very, very strict definition. As I said, even patients with minimal proportions of fecal spotting were classified as incontinent.

Dr. P.R. O'Connell (Dublin, Ireland). The incontinence is an episodic rather than a continuous problem at night, and with your brief sampling of the week-long nocturnal pattern, you may have missed brief transient increases in pouch pressure. Did you encounter episodes of incontinence during your monitoring period? Did you observe these high pressure waves that can occur and have been described in ileal pouches during daytime observation?

Dr. Sarmiento. We were measuring the big waves of the pouch, the long peak waves, and high pressure waves and we included them in the evaluation. With the inclusion of those waves we did not find an increased number of reversals of the gradients? This is a continuous measurement. How can I be more certain of the existence of reversals of the gradients. This is 14 hours of continuous tracing, so it is impossible to identify some reversal of the gradients that could be missed.

Dr. B. Orkin (Washington, D.C.). This type of study has been done previously by Dr. John Pemberton who examined the inversion of the pouch–anal gradient. I think the point that Dr. O'Connell was trying to get across is that these are individual events. Did you look at individual events during the night, when there is a change in gradient, and identify those as times when there was leakage?

Dr. Keith Kelly and I have also conducted studies examining sleep stages and identified those specific events during REM sleep. Did you look at individual events of incontinence at night and correlate them with any activity inversion?

Dr. Sarmiento. Yes we did. As you know, patients who are undergoing ambulatory manometry have a recorder, and they mark everything. They mark what they are doing during these episodes of incontinence, and there is no question that during these episodes there is a reversal of this gradient. In addition, this reversal of gradient does not occur in patients who are completely continent during the night.

Dr. Orkin. I think taking the mean over the course of the entire night is rather meaningless. You have to look at the individual events.

Direct Inhibitory Effect of Erythromycin on the Gallbladder Muscle

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Erythromycin, a macrolide antibiotic, stimulates motor activity in various parts of the gastrointestinal tract in humans and animals. This effect of erythromycin resembles that of motilin, a gastrointestinal hormone, in evoking contractions similar to phase 3 activity of the migrating motor complex. Motilin induces contractions in the canine gallbladder but fails to evoke any response, either in vivo or in vitro, in the human gallbladder. Surprisingly, erythromycin stimulates human gallbladder emptying in healthy volunteers and in persons with diabetic autonomic neuropathy. In the present study we examined the effect of erythromycin on chemically and electrically evoked contractions of isolated gallbladders from guinea pigs and humans by use of isometric force measurements. Carbachol, a muscarinic cholinergic agonist, evoked gallbladder contractions that were diminished by erythromycin in a concentration-dependent manner: at 200 μmol/L the contractions were 86% ± 20% of the control response, at 500 μmol/L they were 63% ± 21% of control, and at 1000 μmol/L they were 41% ± 20% of control ($P < 0.05$, N = 10, mean ± standard deviation). Electrically evoked gallbladder contractions were reduced to 68% ± 18% of the control response with the addition of 500 μmol/L of erythromycin and to 56% ± 19% of control after the addition of 1000 μmol/L ($P < 0.05$, N = 8). Guinea pig but not human gallbladders contracted after stimulation with the alpha-adrenergic agonist phenylephrine. Erythromycin reduced these contractions in a concentration-dependent manner but had no effect on gallbladder contractions induced by bradykinin. In human gallbladder strips, erythromycin at 500 μmol/L reduced the contractile response to electrical stimulation to 71% ± 16% of the control value (N = 10 [5 patients], $P < 0.01$) and the carbachol-evoked contractions to 53% ± 24% ($P < 0.01$, N = 32). The inhibitory effect of erythromycin persisted in the presence of the nerve blocker tetrodotoxin at 1 μmol/L. It is concluded that erythromycin has a direct inhibitory effect on guinea pig and human gallbladder contractions.
(J GASTROINTEST SURG 1997;1:331-336.)

The prokinetic activity of erythromycin was first described in 1984 and has since been studied by several groups.[1-3] This activity of erythromycin resembles that of the gastrointestinal hormone motilin, which is released periodically in the fasting state. In humans and dogs motilin produces phase 3 activity and is associated with the regulation of the migrating motor complex.[4] Erythromycin was shown to displace motilin from its receptors,[5] and thus it was suggested that erythromycin acts as a motilin agonist. Improvement of gastric emptying in patients with diabetic gastroparesis treated with erythromycin was the first clinical application of these properties.[6]

Motilin has been shown to stimulate canine gallbladder contraction in vivo[7] but has no effect on the human gallbladder.[8] However, erythromycin was reported to have a stimulatory effect on gallbladder contraction in normal volunteers and in persons with gallstones.[9] Erythromycin also reduces fasting gallbladder volume of patients with diabetic neuropathy.[10] The aim of the present study was to investigate the direct effect of erythromycin on the contractile activity of the isolated whole gallbladder of guinea pigs and on strips of human gallbladders.

MATERIAL AND METHODS

Male guinea pigs weighing 300 to 500 g were killed. Their gallbladders were then quickly removed and placed in Krebs solution of the following compo-

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sition (in millimoles per liter): NaCl, 120.7; KCl, 5.9; $MgSO_4$, 1.2; $NaHCO_3$, 14.4; NaH_2PO_4, 1.5; $CaCl_2$, 2.5; and glucose, 11.5. Whole gallbladders were placed in a bath filled with Krebs solution through which a mixture of 95% oxygen and 5% carbon dioxide was bubbled. The temperature was maintained at 37° C. One end of the gallbladder preparation was attached to a tissue holder and the other end was connected to an isometric force transducer (Statham UC2, Gould Instrument Systems, Inc., Valley View, Ohio). The resting force was 1 g.

Human gallbladder preparations were obtained from patients undergoing laparoscopic cholecystectomy because of symptomatic gallstones and from patients with normal gallbladders removed during surgery for other reasons such as a Whipple procedure or resection of echinococcal cysts. The gallbladder preparations were placed in Krebs solution immediately after resection. The gallbladder was cut into 3 × 20 mm strips. The serosa and the underlying connective tissue were removed. The strips of gallbladder tissue were fixed in the same manner as previously described for the guinea pig gallbladders. After reaching equilibrium, the preparations were electrically stimulated with two platinum ring electrodes using a Grass S88 stimulator (Grass Instrument Co., Quincy, Mass.). Stimulus parameters were 55 V amplitude, 0.5 msec duration at 1 to 10 Hz for 15 seconds. Chemical stimulation was achieved by adding drugs to the bath. The contractile responses were recorded on a Hewlett-Packard 7544-a recorder (Hewlett-Packard Co., Andover, Mass.). Erythromycin lactobionate was dissolved in distilled water immediately before use. The erythromycin vials nominally contained benzyl alcohol. Control experiments with benzyl alcohol at the same concentrations were also performed (see Results).

Each preparation, after reaching equilibrium, was electrically and chemically stimulated. The bathing solution was replaced several times until the preparation returned to basal activity and tone. Erythromycin was added to the bath and the preparation was restimulated. After each exposure to erythromycin, the electrical and chemical stimulations were repeated to verify return to control levels.

Statistics were calculated by means of analysis of variance multivariate tests with Bonferroni post-test correction. Each result was expressed as the mean ± standard deviation.

RESULTS

Effect of Erythromycin on the Guinea Pig Gallbladder Response to Carbachol

Carbachol at a concentration of 0.5 μmol/L caused contraction of the guinea pig gallbladder (Fig. 1*a*).

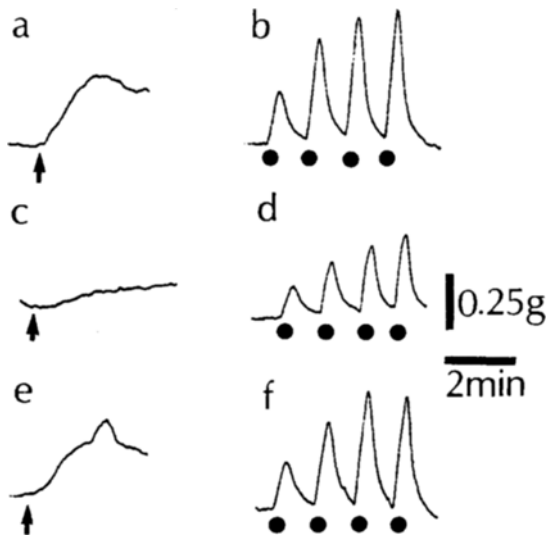

Fig. 1. Effect of erythromycin on the mechanical activity of the isolated guinea pig gallbladder. *a*, Response to carbachol (0.5 μmol/L) applied at the arrowhead; *c*, response of the same preparation to carbachol (0.5 μmol/L) in the presence of erythromycin (0.5 mmol/L); *e*, recovery of response to carbachol after washout; *b*, response to electrical stimuli (duration 15 seconds and frequency from left to right 1, 2, 5, and 10 Hz) marked by dots; *d*, response to the same stimuli in the presence of erythromycin (0.5 mmol/L); *f*, recovery of response after washout. Calibration bars for force *(0.25 g)* and time *(2 min)* are shown in *d*.

The addition of erythromycin to the preparation bath reduced this response in a reversible manner (Fig. 1*c* and *e*). This effect was concentration dependent: at 200 μmol/L the contraction was 86% ± 20% of the control response, at 500 μmol/L it was 63% ± 21%, and at 1000 μmol/L it was 41% ± 20% (N = 10, $P < 0.05$; Fig. 2). The addition of tetrodotoxin (TTX) at a concentration of 1 μmol/L, to the bath did not alter the response to carbachol or the inhibitory effect of erythromycin.

Effect of Erythromycin on the Guinea Pig Gallbladder Response to Electrical Stimulation

Electrical stimulation of guinea pig gallbladders at frequencies of 1, 2, 5, and 10 Hz evoked contractions (Fig. 1*b*). Erythromycin reduced these contractions in a reversible manner (Fig. 1*d* and *f*). The effect was concentration dependent (Fig. 3). Electrically evoked gallbladder contractions were reduced to 68% ± 18% of the control response with the addition of 500 μmol/L of erythromycin to the preparation bath and to 56% ± 19% after the addition of 1000 μmol/L

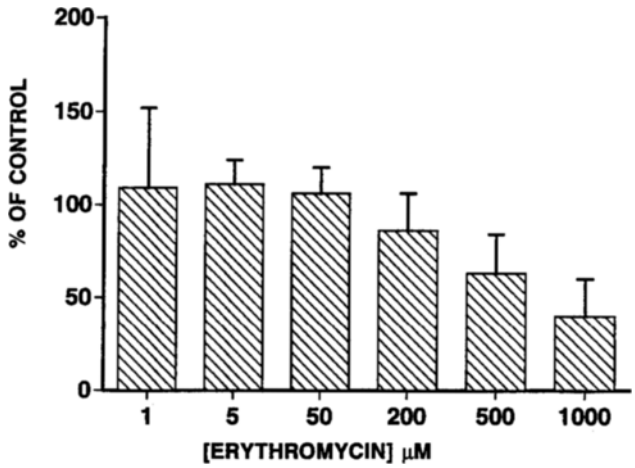

Fig. 2. Effect of various doses of erythromycin on the response of the guinea pig gallbladder to carbachol (0.5 mmol/L). Erythromycin was added to the bathing solution at concentrations ranging from 1 μmol/L to 1 mmol/L. Results are expressed as percentages of the control response and show that erythromycin at concentrations ranging from 0.5 to 1 mmol/L significantly reduced the response to carbachol ($P < 0.05$). Each bar represents the mean ± standard deviation of 10 different results. Asterisks indicate statistical significance as compared to control values ($P < 0.05$).

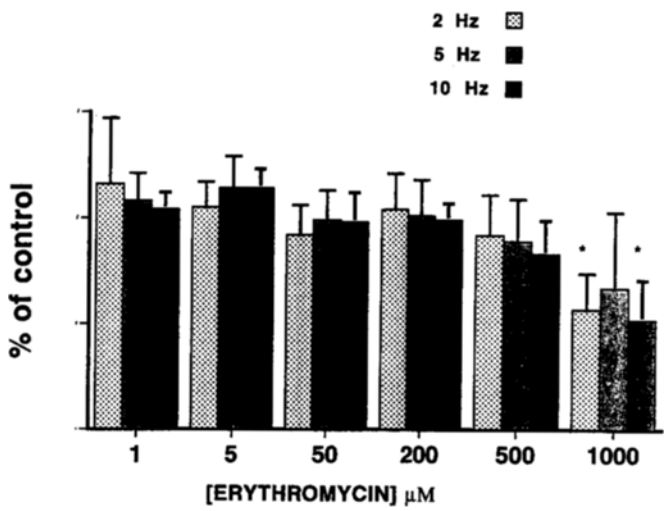

Fig. 3. Effect of erythromycin on the response of the guinea pig gallbladder to electrical stimuli at frequencies of 2, 5, and 10 Hz. Erythromycin was added to the bathing solution at concentrations ranging from 1 μmol/L to 1 mmol/L. Results are expressed as percentages of the control response and show that erythromycin at a concentration of 1 mmol/L significantly reduced the response to electrical stimulation ($P < 0.05$). Each bar represents the mean ± standard deviation of eight different results. Asterisks indicate statistical significance as compared to control values ($P < 0.05$).

($P < 0.05$, N = 8). The addition of atropine (1 μmol/L) or TTX (1 μmol/L) to the solution blocked the gallbladder responses to electrical stimulation, indicating that these responses were due to the release of acetylcholine.

Effect of Erythromycin on the Guinea Pig Gallbladder Response to Phenylephrine

The preceding results suggested that erythromycin blocked cholinergic responses in an atropine-like mechanism. To test this possibility the effect of phenylephrine, an alpha-adrenergic agonist, was examined. Phenylephrine at a concentration of 5 μmol/L, in the presence of atropine (1 μmol/L), caused contractions of the guinea pig gallbladder. These contractions were inhibited by erythromycin in a concentration-dependent manner (Fig. 4): at 200 μmol/L to 62% ± 27% and at 0.5 mmol/L to 21% ± 16% ($P < 0.05$, N = 5).

Effect of Erythromycin on the Gallbladder Response to Bradykinin

To further elucidate the mechanism of the erythromycin effect on the gallbladder, the response to bradykinin was studied. Bradykinin at a concentration

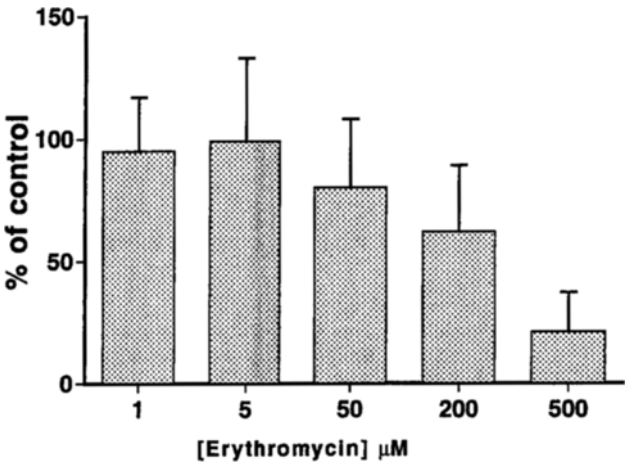

Fig. 4. Effect of erythromycin on the guinea pig gallbladder response to phenylephrine (5 μmol/L). Erythromycin was added to the bathing solution at concentrations ranging from 1 μmol/L to 0.5 mmol/L. Results are expressed as percentages of change in the control response and show that erythromycin at a concentration of 0.5 mmol/L significantly reduced the response to phenylephrine. Each bar represents the mean ± standard deviation of five different results. Asterisk indicates statistical significance as compared to control values ($P < 0.05$).

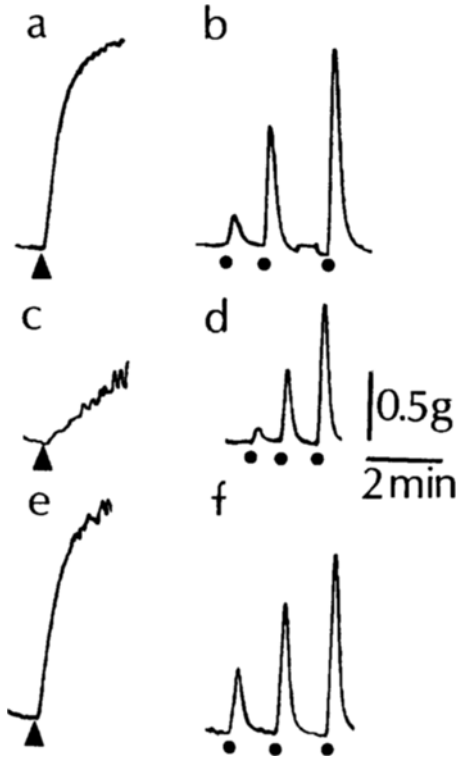

Fig. 5. Effect of erythromycin on the mechanical activity of the isolated human gallbladder. *a*, Response to carbachol (0.5 μmol/L) added at the arrowhead; *c*, response to carbachol (0.5 μmol/L) in the presence of erythromycin (0.5 mmol/L); *e*, recovery of response to carbachol after washout; *b*, response to electrical stimuli (duration 15 seconds and frequency from left to right 1, 2, 5, and 10 Hz marked by dots); *d*, response to the same stimuli in the presence of erythromycin (0.5 mmol/L); *f*, recovery of response after washout. Calibration bars for force *(0.5 g)* and time *(2 min)* are shown in *d*.

of 10 nmol/L, in the presence of atropine 1 μmol/L, evoked gallbladder contractions that were not altered following the addition of 100, 500, and 1000 μmol/L of erythromycin.

Effect of Benzyl Alcohol on the Gallbladder Response to Carbachol

To determine whether the effect studied was associated with the presence of benzyl alcohol in the erythromycin solution, we tested the response of the guinea pig and the human gallbladder to benzyl alcohol in the same concentration range as that used in the erythromycin solution. There was no change in the response to carbachol before and after the administration of benzyl alcohol.

Effect of Erythromycin on the Human Gallbladder Response to Carbachol and Electrical Stimulation

In human gallbladder strips erythromycin at 500 μmol/L reduced the contractile response to electric stimulation at 2, 5, and 10 Hz (Fig. 5). However, the effect was significant only at 10 Hz, where erythromycin reduced the responses to 71% ± 16% (N = 10 [5 patients], $P < 0.01$). Erythromycin at 500 μmol/L also reduced the carbachol-evoked contractions to 53% + 24% ($P < 0.01$, N = 32). The nerve blocker TTX at 1 μmol/L did not alter the inhibitory effect of erythromycin. The effect of erythromycin was reversible after washout. There was no significant difference between the responses of the gallbladders of patients with or without gallstones.

DISCUSSION

Our results demonstrate a direct, reversible inhibitory effect of erythromycin on isolated human and guinea pig gallbladders. Erythromycin significantly reduced the gallbladder contractile responses to electrical and chemical stimulation. The effect of erythromycin was concentration dependent, the threshold concentration being 50 to 100 μmol/L. There is relatively little information regarding the actions of erythromycin on isolated smooth muscle preparations. In a comprehensive study of the isolated guinea pig

small intestine, Minocha and Galligan[11] showed that in the longitudinal muscle erythromycin inhibited the responses to electrical stimulation but not to a muscarinic agonist or to substance P. However, in the circular muscle erythromycin reduced noncholinergic contractions and had little effect on cholinergic responses. An inhibitory effect of erythromycin was described in the isolated bronchial smooth muscle.[12] Percy and Christensen[13] showed that erythromycin, among other antibiotics, inhibited spontaneous contractions and reduced the tone of the distal colonic muscularis mucosae of the opossum. On the other hand, erythromycin and other macrolides were shown to induce contractions and to bind to motilin receptors in rabbit duodenal segments.[14] Hasler et al.[15] described contractions of rabbit colonic myocytes in response to erythromycin binding to motilin receptors. Amstrong et al.[16] described a stimulatory effect of erythromycin on ileal motility by activation of dihydropyridine-sensitive calcium channels. Thus there are different and even opposite direct effects of erythromycin on smooth muscles. The effect of erythromycin on gallbladder motility is also not fully understood. An in vivo study by Fiorucci et al.,[17] who used ultrasound measurements, demonstrated enhancement of gallbladder emptying following the administration of erythromycin. They also showed that motilin blood levels were elevated after the administration of erythromycin, suggesting a motilin-mediated effect. The inhibitory effect of erythromycin described in the present work is apparently not mediated by motilin receptors because this peptide has no effect on in vitro gallbladder strips from most species including humans[18] and guinea pigs.[19]

Another study by Fiorucci et al.[20] in diabetic patients with and without autonomic neuropathy failed to demonstrate the same effect in the patients with neuropathy. The enhancement of gallbladder emptying by erythromycin was also demonstrated by other groups who used in vivo ultrasonographic volume estimation.[9,10,21] Kaufman et al.[22] studied the effect of erythromycin on the prairie dog gallbladder. They concluded that erythromycin does not have any effect on the gallbladder as measured by means of a probe inserted directly into the gallbladder. Benzi et al.[23] studied pressure changes in the guinea pig gallbladder and showed pressure increases after administration of erythromycin. Our own results showed that in the guinea pig gallbladder erythromycin inhibited the contractions evoked by both carbachol (muscarinic cholinergic agonist) and phenylephrine (alpha-adrenergic agonist). In human gallbladder strips carbachol induced contractions that were significantly reduced after the administration of erythromycin. Phenylephrine failed to evoke contractions in human gallbladder strips. Bradykinin evoked contractions in both guinea pig and human gallbladders, which were not affected by erythromycin. Thus it appears that although erythromycin does not specifically block cholinergic muscarinic responses, it does have a certain degree of selectivity. We can hypothesize that it interferes with a postreceptor mechanism, possibly at the second messenger level.

Our experiments demonstrate that erythromycin was effective in the concentration range of 50 to 1000 μmol/L. The plasma concentration of erythromycin after intravenous administration of 1 g in patients can reach 10 μg/ml (13 μmol/L). However, in bile the erythromycin concentration can reach a level as high as 250 μg/ml (300 μmol/L),[24] which is well within the range studied in vitro. Thus erythromycin may have a local inhibitory action on gallbladder smooth muscle. Erythromycin has multiple actions on smooth muscle contractions and gallbladder motility. The inhibitory effect described here contrasts with previous evidence on the excitatory effect of erythromycin on the gallbladder. The excitatory effect was observed in unstimulated isolated smooth muscle cells or, in vivo, in resting gallbladders where ultrasound volume measurements were used. None of these studies dealt with the responses to contractile stimulation. We found that the basal tone was not changed by erythromycin, which is consistent with the results of Kaufman et al.[22] Direct inhibitory effects of erythromycin on stimulated smooth muscle preparations have been described previously in small intestine and bronchial smooth muscle preparations.[11,12]

CONCLUSION

Erythromycin has multiple effects on smooth muscle, depending on the tissue and the experimental conditions. Apart from the stimulatory effect of erythromycin on the stomach and the duodenum, which is mediated via motilin receptors, there are other excitatory and inhibitory effects unrelated to motilin. These may include elevation of basal tone and pressure in the gallbladder and small intestine or inhibition of contractions evoked by chemical stimuli. The net response to erythromycin is probably the sum of all of these various effects.

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Needle Biopsy for Suspicious Lesions of the Head of the Pancreas: Pitfalls and Implications for Therapy

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Controversy continues to exist concerning the optimal diagnostic approach to a pancreatic head lesion suspected of being a neoplasm. The objective of this study was to evaluate the impact of needle biopsy in suspicious pancreatic head neoplasia and its effect on therapy and outcome. Seventy-three patients with symptoms or signs of periampullary neoplasia and a pancreatic head lesion identified on CT scan were reviewed retrospectively. Forty patients with potentially resectable lesions underwent intraoperative transduodenal core needle biopsy of the head of the pancreas. Thirty-three patients underwent CT-guided percutaneous fine-needle aspiration. The sensitivity and specificity of core needle biopsy were 76% and 100%, respectively. One death was directly related to the procedure and therapy was adversely affected in one patient with a false negative result. The sensitivity and specificity of percutaneous fine-needle aspiration were 85% and 92%, respectively, and were not significantly different from the core needle biopsy results ($P > 0.3$). Three false negative fine-needle aspiration biopsies occurred in patients with potentially resectable lesions and a low clinical suspicion for malignancy. In patients with a mass in the head of the pancreas on CT scan, fine-needle aspiration biopsy offers results similar to those of intraoperative transduodenal core needle biopsy. In patients estimated to have resectable disease, a pancreaticoduodenectomy should be performed without a biopsy. For patients with unresectable disease, cytologic examination of fine-needle aspirate should be performed. If this examination is positive, it offers the advantage of facilitating the construction of a rational plan for palliation. (J GASTROINTEST SURG 1997;1:337-341.)

Despite the recent development of a variety of radiologic, endoscopic, and laparoscopic tools, the pathologic diagnosis of a mass in the head of the pancreas remains elusive. Even at laparotomy, the nature of a palpable pancreatic mass may be difficult to determine. Attempts at pathologic diagnosis have included wedge biopsy performed operatively with frozen section examination,[1] core needle biopsy performed transduodenally with frozen sections obtained during surgery,[1,2] cytologic examination of fine-needle aspirate performed intraoperatively,[3-13] and preoperative cytologic examination of fine-needle aspirate performed under ultrasound or CT guidance.[2,4,10-12,14] Each of these procedures has been reported to have advantages and disadvantages. Among the intraoperative procedures, wedge biopsy may be associated with a higher rate of complications,[2] whereas transduodenal core needle biopsy offers the advantage of supplying a core piece of tissue rather than the few cells obtained on fine-needle aspiration and thus providing a possible higher diagnostic yield. A major advantage of the percutaneous technique over other pancreatic biopsy procedures is the possibility of avoiding surgery in selected cases. In this report we examine the value of intraoperative core needle biopsy and percutaneous CT-guided cytologic examination of fine-needle aspirate and determine how the biopsy results affected therapy and outcome.

MATERIAL AND METHODS

All pancreatic biopsies submitted to the pathology department at the Houston Veterans Administration Medical Center between October 1987 and 1994 were reviewed. Biopsies of the body or tail of the pancreas as well as wedge biopsies were excluded. Seventy-three patients were identified who had undergone one form of needle biopsy of the head of the pancreas, the choice of which was determined by the primary clinician. These patients were all men with a

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mean age of 62 years in whom a mass in the head of the pancreas was confirmed by CT scan.

Symptoms of periampullary neoplasia included jaundice in 23 patients (32%), weight loss in 21 (29%), and abdominal pain in 24 (33%) occurring alone or in combination. A history of alcohol abuse was confirmed in 35 patients (48%) with eight patients (11%) having had prior episodes of pancreatitis. Twenty-three patients underwent endoscopic retrograde cholangiopancreatography, which confirmed biliary obstruction in all of them and changes of chronic pancreatitis in eight. Nine patients underwent endoscopic biliary stent placement, whereas five patients underwent percutaneous transhepatic biliary drainage. These cases were not randomized but were divided into two groups retrospectively according to the type of biopsy performed.

Forty of these patients underwent an intraoperative transduodenal core needle biopsy of the head of the pancreas. All patients were judged to have a potentially resectable tumor based on a review of the CT scans obtained preoperatively. An average of three separate passes (range 2 to 7) were made through the clinically suspicious area, and a frozen section diagnosis was provided by the pathologist using a hematoxylin-eosin stain. The pathologic report on these frozen sections was then correlated with the final diagnosis obtained a few days later, which was based on the remainder of the tissue or the resected specimen.

Another 33 patients underwent CT-guided percutaneous fine-needle aspiration (FNA) of the head of the pancreas performed in the radiology department. The reason for performing this procedure was metastatic disease in six patients, unresectable disease in 11, poor medical condition in two, and a low suspicion for malignancy in 14 (Table I). Smears prepared from tissue aspirated with a gauge 20-22 needle at the time of the procedure were immediately fixed in 95% alcohol. Representative smears were stained with Papanicolaou stain, and the aspiration needle and syringe were rinsed with Sacchomanno fixative (an alcohol-based fixative) with a cell block prepared from the tissue fragments after centrifugation. Step sections of the cell block were prepared and stained with hematoxylin-eosin. Results were correlated with either operative or pathologic findings in the resected specimen, in cases where surgery was performed, or with biopsy specimens from other lesions thought to be metastatic.

For patients who did not undergo surgery or resection, a progressive decline in the clinical condition that resulted in death was considered a presumptive confirmation of malignancy. A course of continuing satisfactory health or symptoms compatible with chronic pancreatitis were considered confirmation of a cytologic examination that was negative for malignant neoplasm. Sensitivity and specificity for both techniques were determined and results compared by means of Fisher's exact test.

RESULTS

Among the patients who had core needle biopsies, 16 of them were considered to have a true positive result for malignancy (Table II). Treatment in this group consisted of palliative bypasses in seven patients, pancreaticoduodenectomy in four, and exploration alone in five (Table III). Overall the mean survival in this group was 6 months.

Nineteen patients followed for a mean of 19 months were considered to have true negative biopsies (see Table II). Six had undergone a biliary-enteric bypass, four had a pancreaticoduodenectomy for the associated pain of chronic pancreatitis, and the remaining nine underwent no additional operative procedures (see Table III).

Five patients had false negative results (see Table II). In two of these patients, the tumor was discovered in the pancreaticoduodenectomy specimen and no adverse outcome was noted. In two other patients, the initial frozen biopsy specimens were reported as

Table I. Indications and results of CT-guided percutaneous fine-needle aspiration of suspicious lesions of the head of the pancreas in 33 patients

	True positive	False positive	True negative	False negative
Metastatic disease	6	0	0	0
Unresectable tumor	11	0	0	0
Poor medical condition	0	0	2	0
No clinical signs of malignancy	1	1	9	3

chronic pancreatitis. In the first patient, the permanent section diagnosis revealed squamous cell carcinoma of the pancreas, whereas the second patient was confirmed to have adenocarcinoma during a second exploratory operation 5 months later for further biliary obstruction. Therapy was adversely affected in this patient who was found to have a potentially resectable tumor at the first operation. The last patient with a false negative result was confirmed to have adenocarcinoma of the pancreas through biopsy of a metastatic lesion in the liver. A subsequent review of the permanent section of the pancreatic core needle biopsy from this patient confirmed the presence of carcinoma (see Table I).

One patient with a true negative biopsy after five passes of the core needle died 14 days later of a retroperitoneal hematoma from an injured superior mesenteric vein. Attempts at controlling the bleeding and resuscitation failed. No other complications resulted from this procedure. The sensitivity and specificity of core needle biopsy were 76% and 100%, respectively.

Among the patients undergoing cytologic examination of fine-needle aspirate, 18 of 33 had positive cytology for carcinoma (see Table II). No surgical therapy was performed in any of these patients (see Table III). The mean survival in these 18 patients was 2.5 months.

One false positive result occurred in a patient who is currently doing well at 21 months' follow-up and whose cytologic examination results were interpreted as adenocarcinoma (see Table II).

Eleven patients had true negative cytologic findings for malignancy (see Table II). These patients were followed for a mean of 22 months and are currently doing well (see Table I).

Three patients had false negative cytologic findings. Metastatic nodules elsewhere in the body were found in two patients and following exploratory surgery in the third patient (see Tables I and II).

There were no complications encountered with the percutaneous FNA procedure. The sensitivity and specificity were, respectively, 85% and 92%, which was not statistically different from the core needle biopsy results ($P > 0.3$).

Table II. Results of intraoperative transduodenal core needle biopsy and preoperative percutaneous CT-guided fine-needle aspiration of suspicious lesions of the head of the pancreas

Diagnosis	Core needle biopsy (n = 40)	FNA (n = 33)
True negative	19	11
True positive	14	17
False positive	0	1
False negative	5	3
Equivocal	2*	1*,1†

*All patients were confirmed to have malignancy at repeat biopsy using the same technique and were ultimately counted as true positive.
†One patient had insufficient material at fine-needle aspiration, required surgical exploration, and was confirmed to have lymphoma by core needle biopsy. He was not entered in the final calculations for group 2.

DISCUSSION

Various approaches have been utilized in assessing and treating suspicious masses in the head of the pancreas based on different philosophies. Some surgeons advocate pancreaticoduodenectomy on clinical grounds without confirmation based on tissue samples. On the other hand, there are surgeons who rarely if ever elect to perform pancreaticoduodenec-

Table III. Impact of diagnostic needle biopsy on treatment of 73 patients with suspicious pancreatic head neoplasia

Therapy	Biopsy result: True negative	True positive	False positive	False negative
Biliary bypass	2 (1)	7	0	2
Biliary and gastric bypass	4	0	0	0 (2)
Pancreaticoduodenectomy	4 (1)	4	0	2
Exploration alone	9	5	0	1 (1)
Biliary stent or drainage	0	0 (9)	0	0
No intervention	0 (9)	0 (9)	0 (1)	0

Numbers in parentheses represent the results of percutaneous fine-needle aspiration; all other numbers represent results of core needle biopsy.

tomy without histologic verification of malignancy. Attitudes with regard to this question reflect an awareness of the shortcomings of biopsy. This study has evaluated two commonly used needle biopsy techniques and investigated how the results have influenced the management of patients with suspected malignant neoplasia in the head of the pancreas.

Based on the first diagnostic technique, which consisted of a core needle biopsy, 10 pancreaticoduodenectomies were performed (see Table III). Included among those patients who underwent a pancreaticoduodenectomy were two patients with the pain of chronic pancreatitis, a core needle biopsy that was negative for malignancy, and a final specimen confirming the presence of carcinoma that was missed at biopsy. This shows that intraoperative palpation of the tumor and the way the needle for core biopsy is directed to a specific area does not always offer an advantage. It can give a false sense of security that might adversely affect therapy, as occurred in one of our patients with a false negative result who underwent bypass for a potentially resectable tumor (see Table III). Major complications directly related to the core biopsy procedure, such as fatal bleeding in one of our patients and a reported morbidity of 10%,[3] can be avoided by using intraoperative FNA. However, intraoperative FNA still requires a major exploration and is subject to the same sampling errors that will result in the same pitfalls as core needle biopsy.

The second diagnostic technique based on percutaneous FNA under CT guidance and cytologic examination of the specimen offers the major advantage of avoiding surgical exploration while offering similar sensitivity and specificity as core needle biopsy without any of the major complications seen with it.[3] However, percutaneous FNA is still associated with the same sampling disadvantages as core needle biopsy. If one examines all 14 patients who had a suspicious mass on CT scan but no clinical signs of a malignancy, it becomes clear that four (29%) could be misdiagnosed as either false positive or false negative (see Table I). In addition, uncertainty could continue to prevail regarding nine patients (64%) who had a negative biopsy and in whom the doubt for a sampling error might continue to prevail (see Table I).

Based on these data we believe that patients with a suspicious lesion of the head of the pancreas that is potentially resectable, irrespective of the clinical presentation, should undergo resection without biopsy. Pancreaticoduodenectomy will remove any doubt concerning the presence or absence of a carcinoma and will relieve biliary and gastric obstruction if present.[14] It may also be performed with low morbidity and mortality.

In patients with metastatic or unresectable tumors, percutaneous FNA offers the advantage of facilitating a rational plan for palliation. Among our patients with unresectable cancer, five opted for no further therapy. Six other patients had endoscopic placement of a biliary stent and three had percutaneous transhepatic drainage of the biliary tract with good relief of jaundice in all of them (see Table III). Eight patients received a combination of chemotherapy and radiotherapy. Among these patients we did not observe any instances of needle tract or peritoneal seeding by the tumor, as has been reported by others.[15]

It is our belief that with the availability of percutaneous, endoscopic, and even laparoscopic palliation and with intraoperative biopsy results similar to those of percutaneous biopsy, routine surgical exploration cannot be justified. Resectability can be accurately assessed with the aid of high-resolution, thin-section CT scans and staging laparoscopy, with surgical laparotomy reserved for patients with potentially resectable disease.[16] Therefore, if the diagnosis can be obtained without laparotomy, the best approach for patients with unresectable pancreatic cancer may be no operation.

We conclude that in patients with a mass in the head of the pancreas demonstrated by CT scan, cytologic examination of the fine-needle aspirate offers results similar to those of intraoperative transduodenal core needle biopsy. In patients estimated to have resectable disease by preoperative staging, a pancreaticoduodenectomy should be performed without a preoperative or intraoperative biopsy. For patients with unresectable or metastatic disease, cytologic examination of percutaneous fine-needle aspirate should be performed. If positive, it offers the advantage of facilitating the formation of a rational plan for palliation in these patients. If negative, the treatment remains expectant, as these patients have been judged to be unresectable.

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Aggressive Surgical Management of Fibrolamellar Hepatocellular Carcinoma

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Fibrolamellar hepatocellular carcinoma (FLHC) is recognized as a distinct clinicopathologic variant of hepatocellular carcinoma. Ten consecutive patients with FLHC undergoing operative management at our institution were reviewed. At the initial presentation seven patients had stage II disease ($pT_2N_0M_0$), whereas three patients were in stage III ($pT_2N_0M_0$ or $pT_3N_0M_0$). Initial procedures included formal right or left hepatectomy in four patients, right or left trisegmentectomy in two patients, left lateral segmentectomy or nonanatomic resection in three patients, and in one patient considered for liver transplantation, only exploration with biopsy of positive nodes was performed. Four stage II patients required a second procedure for resection of recurrent disease from 8 months to 6 years after the initial resection and one patient required a third procedure after 13 years. Reoperations included hepatic re-resection, resection of extrahepatic disease, and liver transplantation. Overall 5- and 10-year Kaplan-Meier survival was 70%. There were no deaths among stage II patients (follow-up 96 to 180 months). All stage III patients (i.e., lymph node involvement, vascular invasion, or multiple tumors) died within 5 years. Patients with stage II disease had better survival than patients with stage III disease ($P = 0.011$, log-rank test). Aggressive treatment of FLHC including reoperation and liver transplantation is justified, especially in patients with stage II disease. (J GASTROINTEST SURG 1997;1:342-346.)

Fibrolamellar hepatocellular carcinoma (FLHC) is an unusual variant of hepatocellular carcinoma (HCC), initially described by Edmondson,[1] that has distinct clinicopathologic features.[2-5] Tumors are typically well-circumscribed masses, usually solitary, with bands of fibrosis that may coalesce to form a central scar. Microscopically lesions consist of polygonal cells with eosinophilic cytoplasm. The characteristic finding of FLHC is the separation of these neoplastic cells by fibrosis arranged in a lamellar pattern. FLHC occurs in a younger patient population than does the usual variant of HCC, has no association with hepatitis B, and generally arises in the noncirrhotic liver.[1,2,6] Initial reports on the management of FLHC described its apparent indolent course and the good results that could be achieved with surgical management.[2,6,7] A more recent report suggests that results of either resection or transplantation are no different for FLHC than for HCC when compared by stage.[8] We therefore reviewed our experience with FLHC to ascertain the prognosis and determine the role of surgical management in these unusual tumors.

PATIENTS AND METHODS

From 1982 to 1995, ten patients with biopsy-proved FLHC were seen at The Toronto Hospital, University of Toronto, with intention to treat surgically. This represents 9% of all cases of HCC seen during that time period with a similar intent to treat. The mean age of patients at presentation was 31 ± 11 years (range 20 to 56 years). There were equal numbers of men and women. No patients had associated hepatitis B or coexistent cirrhosis. Staging criteria used were those of the American Joint Committee on Cancer (AJCC).[9]

Results are reported as mean ± 1 standard deviation. Statistical analysis was performed using the SPSS 6.1 software package. Parametric statistical analysis was performed using Student's *t* tests, whereas nonparametric analysis was done by means of Fisher's exact test. Survival curves were generated using the Kaplan-Meier method with the difference between curves analyzed by means of the log-rank test. Significance was specified as $P = 0.05$.

Abdominal pain was the most common presenting

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Table I. Procedure, stage, and result of 10 patients with fibrolamellar hepatocellular carcinoma

Patient	First resection	TNM	Stage	Recurrence	Second procedure	Status
1	Right lobe	$T_2N_0M_0$	II	No	No	AFD, 13 yr
2	Left lobe + right wedge	$T_3N_1M_0$	III	8 mo	No	Dead, 1.5 yr
3	Right trisegmentectomy	$T_2N_0M_0$	II	41 mo	Nephrectomy + resection; extra-hepatic tumor	AFD, 13 yr
4	Right lobe	$T_3N_0M_0$	III	13 mo	No	Dead, 3 yrs
5	Segment 2,3	$T_2N_0M_0$	II	Positive margins	Left lobe + diaphragm	AFD, 12 yr
6	Segment 2,3	$T_2N_0M_0$	II	72 mo 130 mo	Liver transplant Segment 2, 3 + distal stomach	AFD, 13 yr
7	Right lobe	$T_2N_0M_0$	II	No	No	AFD, 10 yr
8	Nonanatomic wedge	$T_2N_0M_0$	II	15 mo	Right lobectomy	AFD, 9 yr
9	Left trisegmentectomy	$T_2N_0M_0$	II	No	No	AFD, 8.5 yr
10	Exploration only	$T_3N_1M_0$	III	—	No	Dead, 1.5 yr

AFD = alive and free of disease.

symptom and was found in six patients. An abdominal mass was palpable in four patients; two of the four patients were completely asymptomatic but were found to have an abdominal mass while being examined by a physician for unrelated complaints. One of these patients was in the early postpartum period. Nausea and bloating were observed in two patients and constitutional symptoms such as weight loss and fever were noted in two.

Results of liver function tests were normal in all patients. One patient had an elevated alpha-fetoprotein level (320 ng/ml). Ultrasound examination was performed as the initial diagnostic test in all patients and demonstrated a single large lesion is each case. CT scanning was performed in seven patients and angiography was done in five patients. Angiograms showed extensive neovascularization in all cases. In no case could a definitive diagnosis of FLHC be made on the basis of the radiologic features alone. Preoperative core needle biopsy was performed in four patients with a preoperative diagnosis of FLHC made in three of the four.

The initial procedures included right hepatic lobectomy in three patients, left lobectomy plus right wedge resection in one, right trisegmentectomy and left trisegmentectomy in one patient each, and left lateral segmentectomy in two. One patient had a nonanatomic local resection as the initial procedure. One patient with unresectable disease underwent surgical exploration as a potential liver transplant recipient; however, this patient was found to have extensive lymph node involvement and did not undergo transplantation (Table I).

Mean tumor size was 8 ± 4 cm. Four of the 10 tumors had a central scar. The tumor was encapsulated in two patients and multifocal in two patients. Vascular invasion was demonstrated in one patient.

RESULTS

Follow-up was 101 ± 55 months and was complete in all patients. There were no operative deaths. The patient who underwent left trisegmentectomy developed a bile leak that closed spontaneously at 2 weeks. There were no other complications. Actuarial 5- and 10-year survival was 70% (Fig. 1).

One patient (No. 5), who had had a segment 2,3 resection performed at another institution, had positive margins at the time of the first resection; this patient subsequently had a left lobectomy with regional lymphadenectomy plus diaphragmatic resection. Tumor recurrence was found in five patients at 8, 13, 15, 41, and 72 months postoperatively. Three recurrences were found in asymptomatic patients on routine follow-up ultrasound examination and these patients underwent resection. The other two patients presented with bony metastases and pulmonary metastases at 8 and 13 months, respectively, and were not candidates for resection. Of the three patients undergoing re-resection, one had a right hepatectomy 15 months after undergoing a nonanatomic wedge resection, one underwent radical nephrectomy and resection of a large retroperitoneal tumor recurrence 41 months after right trisegmentectomy, and one had a total hepatectomy and liver transplantation 72 months after a segment 2,3 resection. This last patient had an extrahepatic recurrence 6 years after liver transplantation that was subsequently resected with an en bloc resection of the tumor, distal stomach, and segment 2,3 of the transplanted liver. All of these patients are currently

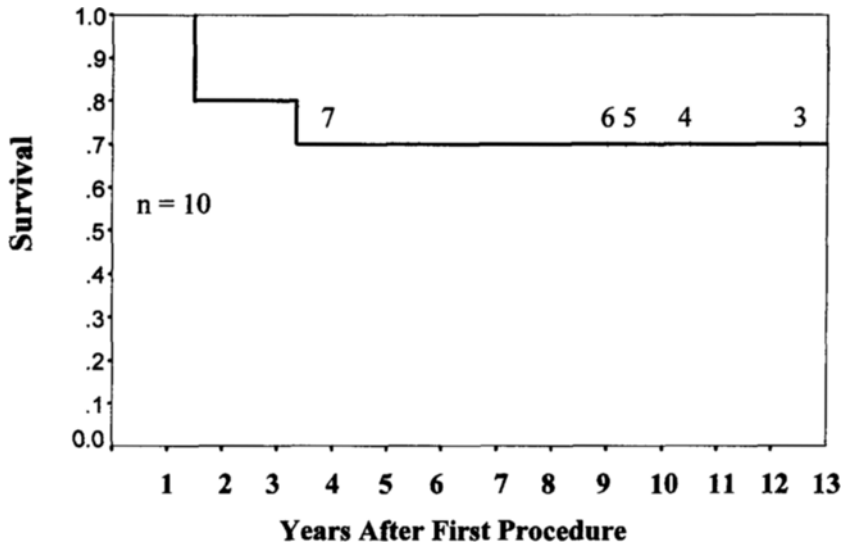

Fig. 1. Actuarial survival of all fibrolamellar hepatocellular carcinoma patients with intent to treat. Overall 5- and 10-year survival is 70%.

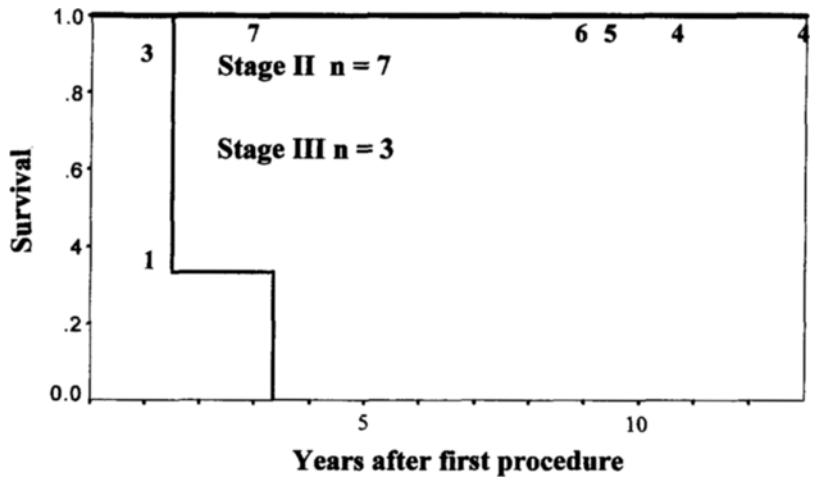

Fig. 2. Actuarial survival of patients demonstrated by American Joint Committee on Cancer stage. Extended survival is seen in all stage II patients, whereas no stage III patients survived for 5 years ($P = 0.01$).

alive and free of disease with follow-up ranging from 2 months to 9 years. The patients who were not candidates for re-resection, including the patient who underwent only exploration for possible transplantation, were given adriamycin-based chemotherapy, but died 24 ± 10 months after their initial procedure.

There were no differences in age, sex, liver function test results, or tumor size between patients who had prolonged survival and those who did not. Seven patients were considered to have AJCC pathologic stage II disease at the time of their initial procedure, whereas three patients were in stage III (see Table I). Actuarial 5- and 10-year survival was 100% for stage II patients, whereas there were no 5-year survivors among stage III patients ($P = 0.01$; Fig. 2).

DISCUSSION

Worldwide, HCC is the most common malignant hepatic tumor; however, the fibrolamellar variant is rare with a reported ratio of FLHC to HCC ranging from 4.8 to 23:100.[10-12] In patients under 35 years of age with no cirrhosis, however, FLHC accounts for up to 43% of all cases of HCC.[13] FLHC has histo-

pathologic and clinical features that distinguish it from standard HCC. Tumors consist of polygonal eosinophilic cells as well as fibrosis arranged in a lamellar pattern.[1] Other characteristics that distinguish FLHC from HCC are normal serum alpha-fetoprotein levels, increased unsaturated B_{12} binding capacity, and high serum neurotensin concentration.[4,6,14,15] An elevated alpha-fetoprotein level, however, does not rule out the possibility of FLHC. One of our patients who was found to have FLHC at resection did have an elevated alpha-fetoprotein level, and this has also been reported by others in a small number of patients with FLHC.[4,16]

Definitive preoperative diagnosis of FLHC is difficult and most often the disease is not diagnosed until surgery.[17] Preoperative imaging studies identified the location of the tumor in all instances in our series but were not specific for FLHC. In two cases multiple lesions were missed, presumably because the lesions were below the imaging resolution available at that time. Fine-needle biopsy has been reported as being helpful in diagnosis; however, a biopsy showing normal hepatocytes does not preclude a malignancy that might have been missed either because of a sampling error or because the characteristic finding of lamellar fibrosis may not be seen with the use of a fine needle.[18,19] Core biopsy was diagnostic in three of four cases in which it was used in our series; however, it is generally our policy not to biopsy lesions if the results of that biopsy will not preclude resection. Results of liver transplantation for hepatocellular tumors larger than 5 cm are poor[20]; therefore prior to consideration for transplantation, a definitive preoperative diagnosis of FLHC is required.

FLHC was initially reported to have a better prognosis than standard HCC.[6,21] Soreide et al.,[10] in reviewing the literature, reported a 58% overall resectability rate with a 56% five-year survival; even patients who did not undergo resection had a median survival of 13 months, which is better than the results for HCC. Nagorney et al.,[16] in a review of the Mayo clinic experience, found that long-term survival of patients with resected FLHC was no better than that of resectable noncirrhotic patients with HCC. More recently, the Hannover experience of Ringe et al.[8] has suggested that tumor stage must be taken into account in any discussion of the apparently more favorable outcome of patients with FLHC, but whether the FLHC variant had a better prognosis could not be confirmed. In our series survival was dependent on tumor stage at presentation; all patients with AJCC stage II tumors had prolonged survival and none of those with stage III tumors survived to 5 years. In spite of normal resection margins, three of seven patients with stage II tumors had a local recurrence up to 6 years after resection. An agressive approach to these recurrences, including re-resection, resection of extrahepatic tumor, and liver transplantation, resulted in long-term survival in all cases. We believe this is a point worth emphasizing since others have also reported long-term survival in all cases. We believe this is a point worth emphasing since others have also reported long-term survival after resection of locally recurrent disease.[10]

Liver transplantation for HCC has been undertaken with varying success rates; however, transplantation for FLHC has been reported to result in better survival than that for HCC.[20,22] Liver transplantation for FLHC was first reported by Starzl et al.[7] with three of six patients free of disease from 12 to 40 months after transplantation and one patient alive with disease at 31 months. Patients who did poorly with a liver transplant were those in whom extrahepatic tumor was present at transplantation. Ringe et al.[8] reported three of six long-term survivors with transplants with positive lymph nodes indicating poor prognosis. Our one patient in whom transplantation was considered as primary therapy had both extrahepatic tumor and positive lymph nodes and therefore was believed not to be a suitable candidate for transplantation. The other patient who underwent transplantation for recurrent disease confined to the liver developed a recurrence 7 years after her transplant but is currently disease free after en bloc resection of the tumor, distal stomach, and segment 2,3 of the transplanted liver. With the increasing shortage of donor organs, results achieved with transplantation for FHLC must approach those obtained for nonmalignant disease. This will require careful patient selection with exclusion of patients with extrahepatic disease or lymph node involvement. Although not included in this series because of the short length of follow-up (2 weeks), we have recently performed and additional resection in a 36-year-old man for FLHC that required a right trisegmentectomy, diaphragmatic resection, partial gastrectomy, and subtotal colectomy to remove all gross disease. He is currently receiving adjuvant chemotherapy since it appears that with resection alone patients with stage III and IV FLHC have a poor prognosis.

CONCLUSION

Aggressive surgical management of FLHC is warranted, especially in stage II lesions. Careful long-term follow-up is required since recurrence may be found years later in asymptomatic patients. Resection of recurrent intrahepatic and extrahepatic disease, or total hepatectomy with liver transplantation for unresectable intrahepatic disease, can provide extended

disease-free survival. Stage III FLHC has a much worse prognosis and it is likely that adjuvant systemic therapy will be required to improve outcomes.

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Hypotension During Septic Shock Does Not Correlate With Plasma Levels of Nitric Oxide Metabolites in the Conscious Rat

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Hypotension following administration of lipopolysaccharide may be due to excessive production of the potent vasodilator nitric oxide brought about by induction of nitric oxide synthase. The purpose of this study was to determine in conscious, fasted rats what role nitric oxide played in lipopolysaccharide-induced hypotension. When examined by Western immunoblot analysis, inducible nitric oxide synthase immunoreactivity was detected in the aorta at 3 hours and increased over time following administration of intraperitoneal lipopolysaccharide (20 mg/kg). When compared with saline-treated control rats, significant hypotension was observed at 2, 4, and 6 hours following lipopolysaccharide treatment. Blood pressure at 2 hours did not differ significantly from that at 6 hours. Using the Griess reaction to quantify plasma levels of nitrates and nitrites as an index of systemic nitric oxide production, an augmentation in the formation of these nitric oxide metabolites was demonstrated at 4 and 6 hours but not at 2 hours. Subcutaneous administration of the nitric oxide synthase inhibitor N^G-nitro-L-arginine methyl ester (5 mg/kg) prevented lipopolysaccharide-induced hypotension, an effect reversed by subcutaneous L-arginine but not D-arginine (350 mg/kg). However, nitric oxide synthase inhibition did not attenuate the ability of lipopolysaccharide to increase plasma nitrate/nitrite levels. These data indicate that lipopolysaccharide-induced production of nitric oxide metabolites does not correlate with lipopolysaccharide-induced hypotension. (J GASTROINTEST SURG 1997;1:347-356.)

Nitric oxide is produced through the enzymatic action of nitric oxide synthase. Production of nitric oxide in the vascular endothelium by the constitutive isoforms of nitric oxide synthase results in picomolar quantities of nitric oxide, which has a physiologic role in the regulation of systemic blood pressure.[1] During pathologic states, such as those encountered in sepsis, nitric oxide formation is markedly enhanced as a result of an upregulation of the inducible isoform of nitric oxide synthase (iNOS).[1,2] In comparison to the constitutive isoforms of nitric oxide synthase, generation of nitric oxide by iNOS results in nanomolar amounts of the potent vasodilator nitric oxide.[1,3] As a result it has been hypothesized that the profound hypotension that occurs during septic shock is a consequence of the tremendous burst in nitric oxide synthesis brought about by upregulation of iNOS.[4]

Once expressed, iNOS is capable of generating large quantities of nitric oxide for an extended period of time. However, the half-life of nitric oxide in vivo is exceedingly short, making direct measurement of this substance difficult.[1] Nevertheless, nitric oxide readily reacts with oxygen in plasma to form nitrates and nitrites, which are stable end products of nitric oxide metabolism.[5] Furthermore, the only known endogenous source of nitrates and nitrites in mammalian tissues is via the enzymatic conversion of L-arginine to nitric oxide.[1] Thus measurement of these stable nitric oxide metabolites represents an index of nitric oxide synthesis. Using such indices Ochoa et al.[6] demonstrated in septic patients that an inverse correlation existed between systemic vascular resistance and plasma nitric oxide metabolite levels. Moreover, Nava et al.[7] demonstrated that lipopolysaccharide

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(LPS) caused a sustained decrease in blood pressure that was accompanied by an increase in plasma nitrate and nitrite levels. In contrast, Van den Berg et al.[8] could not find any correlation between plasma levels of nitric oxide metabolites and hypotension following LPS administration to rats. Nonetheless, inhibition of nitric oxide synthase during septic shock has been reported by several laboratories to restore systemic blood pressure.[9-11] In addition, dexamethasone, a potent inhibitor of the induction of iNOS,[12] has also been shown to prevent LPS-induced hypotension.[13] However, in that same study, nitric oxide–mediated hyporeactivity to noradrenaline preceded the induction of iNOS.[13] Consequently the contribution of iNOS to the hypotension observed during endotoxemia is not entirely understood. This being the case, a series of experiments was undertaken in which a conscious rat model of sepsis was used[14,15] to determine whether or not hypotension during septic shock correlates with upregulation of iNOS and the resultant increased formation of plasma nitric oxide metabolites. The first set of experiments was performed to assess the effect of LPS on iNOS expression in the aorta. The second set of experiments was designed to ascertain what effect LPS has on mean arterial blood pressure and plasma nitrate/nitrite levels. In a third study the effect of nitric oxide synthase inhibition on LPS-induced hypotension and plasma nitrate/nitrite synthesis was examined.

METHODS

Animals and Experimental Model

Conscious Sprague-Dawley rats were used in all studies. Experimental protocols were approved by the Animal Welfare Committee of the University of Texas at Houston before any studies were begun. Animals were housed at a constant temperature, exposed to 12-hour light/dark cycles, and fed rat chow and water, ad libitum, during a 1-week acclimatization period. All experiments were performed in rats deprived of food for 18 to 24 hours but allowed free access to water up to the beginning of the studies. On the day of experimentation, all animals were randomly assigned to one of several groups. Sepsis was induced by intraperitoneal administration of LPS from *Escherichia coli*, serotype 0111:B4, given at a dose of 20 mg/kg body weight, a dose previously demonstrated in our laboratory to increase iNOS activity in rat ileum concurrent with a reduction in ileal contractility.[14] Control rats received a comparable volume of saline solution (0.9%). After completion of the experimental protocols, rats were anesthetized at the indicated time points described below with an intraperitoneal injection of 6 mg/kg xylazine and 70 mg/kg ketamine. Once satisfactory anesthesia was achieved, rats were killed by phlebotomy.

Effect of LPS on Aortic iNOS Immunoreactivity

To estimate and compare the content of the nitric oxide synthase enzyme induced in the aorta during sepsis, full-thickness segments of thoracic aorta were removed, immediately snap-frozen in liquid nitrogen, and stored at −80° C prior to protein extraction and Western immunoblot analysis for iNOS following 1, 3, or 5 hours of treatment with either saline solution (N = 4) or LPS (N = 5/group; 20 mg/kg). Protein in each sample was extracted by pulverizing the frozen tissue with a mortar and pestle in a liquid nitrogen slurry. This sample was then added to 1 ml of lysis buffer (10 mmol/L TRIS [pH 7.4], 100 mmol/L phenylmethyl sulfonyl fluoride [PMSF], and 1% sodium dodecyl sulfate [SDS]) and then subjected to two 15-second bursts of a polytron (Vertashear). These samples were then transferred to microfuge tubes and centrifuged for 10 minutes at 11,000 *g*. The supernate was removed and added to sample buffer (125 mmol/L TRIS [pH 6.8], 2% SDS, 5% glycerol, 1% beta mercaptoethanol [BME], and 0.003% bromophenol blue). Protein concentrations within each homogenate were determined using the BCA protein assay (Pierce Chemical Company, Rockford, Ill.) before adding the sample buffer to the supernate. The remaining proteins in these prepared homogenates were separated by SDS (7.5%)/polyacrylamide gel electrophoresis (PAGE) using 100 μg of protein per sample. Resultant proteins were electroblotted onto nitrocellulose paper and incubated for 1 hour at room temperature in blocking solution (5% nonfat dried milk and phosphate-buffered saline [PBS]). The resultant blot was then washed four times in 0.1% Tween-20/PBS followed by a 1-hour incubation with a specific polyclonal anti-iNOS antibody (1:2,000 dilution). Blots were then washed twice and incubated with horseradish peroxidase–conjugated, goat-anti-rabbit immunoglobulin as a secondary antibody (1:10,000 dilution) for 1 hour. After four final washes, the immune complexes were visualized using enhanced chemiluminescence (ECL) detection.

Effect of LPS on Mean Arterial Blood Pressure and Plasma Nitrate/Nitrite Levels

To determine the effect of LPS on systemic blood pressure, carotid artery catheters were placed the day

before experimentation. Rats were anesthetized with an intraperitoneal injection of 6 mg/kg xylazine and 70 mg/kg ketamine. Using sterile surgical techniques, a neck incision was made and the carotid artery isolated. A catheter (PE 50) was placed in the carotid artery, tunneled to the back, and exteriorized at the base of the skull. A metal harness secured the line and allowed the rats free movement. The carotid line was continuously perfused with 0.9% saline solution at a rate of 0.5 ml/hr to prevent clotting. Rats were allowed to recover in individual cages with free access to water but no food for 18 to 24 hours before experiments were conducted.

On the day of study, rats were given either saline solution (N = 8) or LPS (N = 8; 20 mg/kg) intraperitoneally. Mean arterial blood pressure was recorded continuously following treatment with saline or LPS and reported at baseline and 2, 4, and 6 hours after the indicated treatment. These time points were chosen 1 hour after our iNOS protein determinations because the function of the enzyme will lag behind the appearance of the enzyme. In addition, blood samples of 0.5 ml volume were withdrawn from each rat into preheparinized tubes at baseline and 2, 4, and 6 hours after the rats received the saline or LPS. The volume withdrawn was replaced with 0.9% saline solution. Blood samples were centrifuged immediately and plasma was frozen at −70° C until quantification of plasma nitrate/nitrite levels as an index of systemic nitric oxide production.

Nitrate reductase, generated by anaerobic growth of *E. coli*, was used to reduce nitrate to nitrite. The amount of nitrite generated was determined by a colorimetric method based on the Griess reaction with sulfanillic acid and N-(1-naphthyl) ethylenediamine hydrochloric acid. Optical density at 540 nm was read before and 20 minutes after the addition of the chromagen as previously reported.[16] Standards were prepared with nitrates and taken through the full assay procedure.

Effect of Nitric Oxide Synthase Inhibition on LPS-Induced Changes in Blood Pressure and Plasma Nitrate/Nitrite Levels

To ascertain whether inhibition of nitric oxide synthase could restore mean arterial blood pressure during septic shock, a separate set of experiments was conducted using the nitric oxide synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME). For these studies the experimental protocol was identical to that previously described, with the exception of the subcutaneous administration of L-NAME, which was given simultaneously with either saline solution or LPS. L-NAME was given as a 5 mg/kg dose while control animals received a comparable volume of saline solution. Following the indicated pretreatments, mean arterial blood pressure, heart rate, and plasma nitrate/nitrite levels were determined at baseline and 2, 4, and 6 hours after pretreatment utilizing the methodology previously detailed. In addition to these four experimental groups (saline/saline [N = 4], saline/LPS [N = 6], L-NAME/saline [N = 6], and L-NAME/LPS [N = 6]), a fifth group (N = 4) received L-NAME (5 mg/kg subcutaneously) 3 hours after LPS administration. This latter group was included because nitric oxide synthase inhibition during LPS treatment can have differing effects depending on whether it is given concurrently or later in the course of endotoxemia,[17] that is, at a time point when iNOS is clearly upregulated (see Results). In still other experiments either L-arginine (350 mg/kg subcutaneously; N = 4) or D-arginine (350 mg/kg subcutaneously; N = 4) was given to assess whether excess substrate could reverse the actions of the competitive nitric oxide synthase inhibitor on mean arterial blood pressure during endotoxic shock.

Chemicals

Nitrocellulose filters were purchased from Schleicher & Schuell, Inc. (Keene, N.H.) and x-ray film (T-MAT) from Eastman Kodak Company (Rochester, N.Y.). The ECL system for Western immunoblot analysis was obtained from Amersham Corp. (Arlington Heights, Ill.). The BCA protein assay was from Pierce Chemical Company (Rockford, Ill.). All other reagents, including LPS (*E. coli* serotype 0111:B4) and L-NAME were of molecular biology grade and were purchased from Sigma Chemical Company (St. Louis, Mo.). LPS was dissolved in 0.9% saline (20 mg/ml) and L-NAME was dissolved in 0.9% saline to a concentration of 5 mg/ml. Both L- and D-arginine were likewise dissolved in 0.9% saline solution.

The polyclonal antibody against iNOS used in Western immunoblotting analysis was generated by a site-directed approach[18] to the oligopeptide "SLT" derived from a conserved region of iNOS from the rat beginning at LYS-117 and consisted of the sequence KSLTRGPRDK. The efficacy of this antibody probe has been previously published.[14]

Statistics

Statistical significance was determined using analysis of variance followed by appropriate post hoc testing. A P value of <0.05 was considered significant. Simple linear regression analysis was used to compare

plasma nitric oxide metabolite levels with mean arterial blood pressure.

RESULTS

LPS Enhances Aortic iNOS Immunoreactivity

Using a site-directed antibody to iNOS, the expression of iNOS in the rat thoracic aorta following 1, 3, or 5 hours of treatment with saline solution or LPS was examined. The results of these immunoblotting studies are illustrated in a representative Western blot analysis depicted in Fig. 1. As shown, nitric oxide aortic iNOS immunoreactivity was not detected in saline-treated animals. In contrast, administration of LPS increased aortic iNOS immunoreactivity in a time-dependent fashion. The detection of iNOS immunoreactivity was not readily apparent 1 hour after LPS administration. However, the expression of this enzyme clearly increased with the passage of time, as indicated by its presence at both 3 and 5 hours.

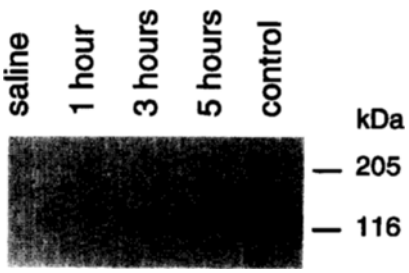

Fig. 1. Western immunoblot analysis of inducible nitric oxide synthase *(iNOS)* in full-thickness rat thoracic aorta after 1, 3, and 5 hours of pretreatment with either saline solution (control) or lipopolysaccharide (*LPS;* 20 mg/kg). The last lane represents a positive control from macrophage cell line RAW 264.7. Each lane was loaded with 100 μg protein. LPS induced expression of a protein having a molecular weight of 130 kilodaltons in thoracic aorta at 3 and 5 hours.

LPS Induces Hypotension and Increases Plasma Nitrate/Nitrite Levels

As show in Fig. 2, mean arterial blood pressure in the control group remained stable over the duration of the experiment. In contrast, administration of LPS caused a 22% decrease in blood pressure at 2 hours (from 102 ± 3 mm Hg to 80 ± 5 mm Hg). Blood pressure increased slightly at 4 and 6 hours but was not significantly different from blood pressure measurements at 2 hours, and was still significantly below baseline values and those obtained from saline-treated control rats.

LPS treatment also resulted in a significant increase in plasma nitrate/nitrite levels when compared to results in saline-treated control animals. As shown in Fig. 3, plasma nitric oxide metabolites remained relatively constant throughout the experimental protocol in animals receiving saline solution. In comparison, administration of LPS enhanced the formation of these metabolites, although the effect was not immediate. The augmentation in plasma nitrate/nitrite levels was present only at 4 and 6 hours after LPS treatment (148 ± 7 μmol/L and 267 ± 21 μmol/L,

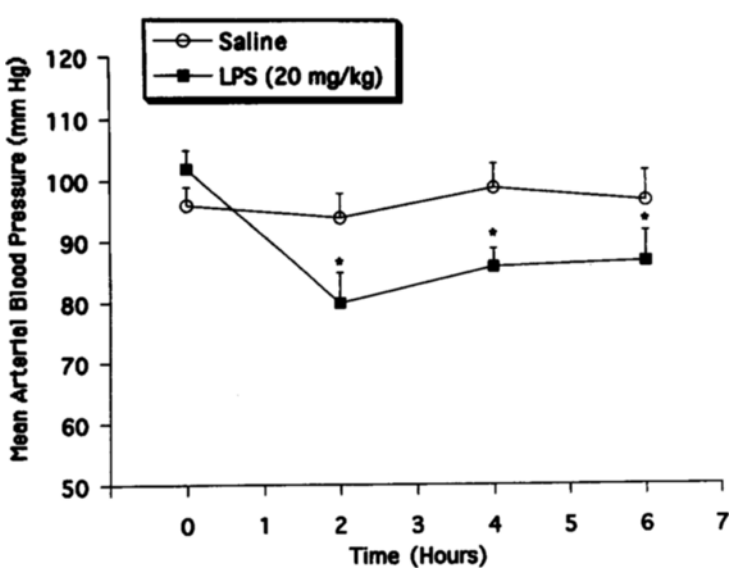

Fig. 2. Effect of intraperitoneal lipopolysaccharide (*LPS;* N = 8) and saline solution (N = 8) on mean arterial blood pressure at indicated time points. Values are expressed as mean ± SEM. *P = ≤0.05 vs. saline.

respectively; $P < 0.001$) but not at 2 hours, a time point that did not differ significantly from baseline determinations (11.9 ± 1 μmol/L). Thus these data indicate that LPS-induced hypotension occurs before any appreciable increase in plasma nitrate/nitrite levels. Furthermore, in additional animals receiving a lower dose of LPS (1 mg/kg; N = 3), mean arterial blood pressure was 106 ± 8 mm Hg 6 hours after administration of LPS, and plasma nitrate/nitrite levels were 121 ± 5 μmol/L compared to baseline measurements of 102 ± 2 mm Hg and 17 ± 4 μmol/L for blood pressure and plasma nitric oxide metabolite levels, respectively. These findings further suggest that elevation of plasma nitrates and nitrites is not necessarily associated with hypotension. Moreover, when linear regression analysis was used to compare plasma nitric oxide metabolite levels and mean arterial blood pressure in animals receiving LPS (20 mg/kg), no significant correlation was detected ($r = 0.404$; $P = 0.289$).

L-NAME Reverses LPS-Induced Hypotension But Not LPS-Stimulated Formation of Plasma Nitric Oxide Metabolites

As shown in Table I, administration of L-NAME not only prevented the ability of LPS to reduce mean arterial blood pressure but also significantly increased blood pressure from baseline values over the entire duration of the experiment. The restoration of blood pressure with L-NAME during LPS-induced hypotension was negated by the administration of L-

Table I. Mean arterial blood pressure determinations in the presence and absence of nitric oxide synthase inhibition following lipopolysaccharide (20 mg/kg) or saline pretreatment

	Mean arterial blood pressure (mm Hg)			
Group	Baseline	2 hr	4 hr	6 hr
Saline/saline	96 ± 3	94 ± 4	99 ± 4	97 ± 5
Saline/LPS	102 ± 3	80 ± 5*,†	86 ± 3*,†	87 ± 5*,†
L-NAME/saline	95 ± 4	108 ± 6*,†	106 ± 5*	102 ± 3
L-NAME/LPS	90 ± 3	101 ± 6*,‡	106 ± 6*,‡	108 ± 8*,‡

*$P \leq 0.05$ vs. baseline.
†$P \leq 0.05$ vs. saline/saline
‡$P \leq 0.05$ vs. saline/LPS.

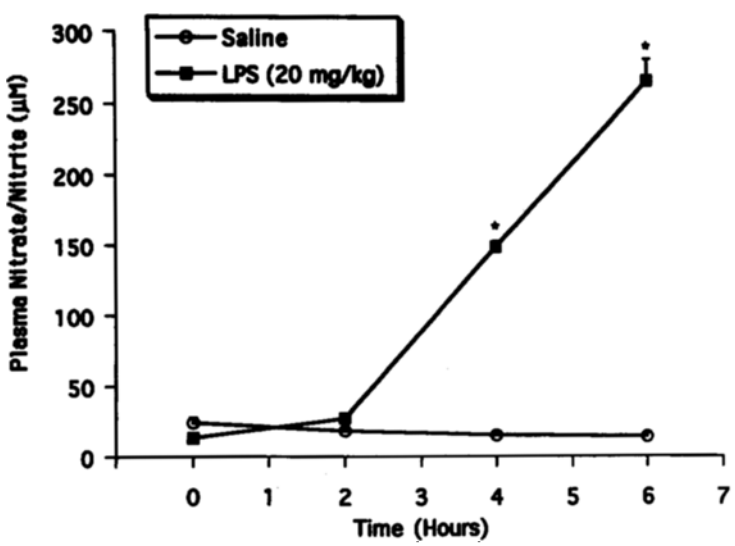

Fig. 3. Effect of intraperitoneal lipopolysaccharide (*LPS*; N = 8) and saline solution (N = 8) on plasma nitrate/nitrite levels at indicated time points. Values are expressed as mean ± SEM. *P = <0.05 vs. saline.

arginine but not D-arginine, as illustrated in Fig. 4.

Despite its ability to increase mean arterial blood pressure during endotoxic shock, L-NAME did not reverse or attenuate LPS generation of plasma nitric oxide metabolites (Fig. 5). As depicted, LPS significantly increased plasma nitrate/nitrite levels at 4 and 6 hours both in the presence and in the absence of the nitric oxide synthase inhibitor L-NAME. As shown in Fig. 6, when administration of L-NAME was delayed but it was given before the increase in nitric oxide metabolites, it also immediately restored mean arterial blood pressure to baseline levels and did not significantly diminish the subsequent generation of plasma nitric oxide metabolites.

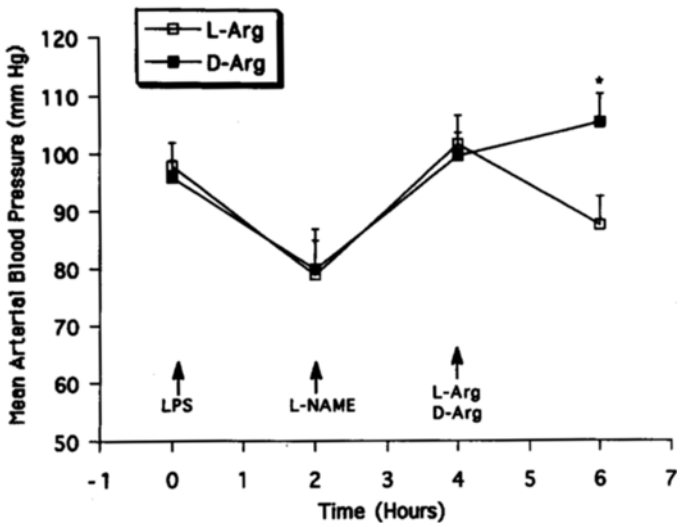

Fig. 4. Effect of subcutaneous L-arginine (L-*Arg*) or D-arginine (D-*Arg*) (350 mg/kg) on the ability of nitric oxide synthase inhibition (L-NAME; 5 mg/kg subcutaneously to reverse lipopolysaccharide (*LPS;* 20 mg/kg)–induced hypotension. Blood pressure reported as mean ± SEM. N = 4/group. **P* = ≤0.05 vs. D-arginine.

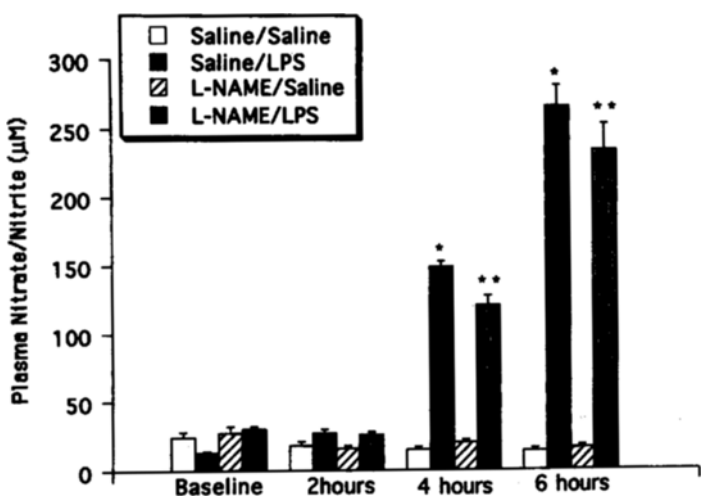

Fig. 5. Effect of lipopolysaccharide (LPS) (20 mg/kg) and saline solution on plasma nitrate/nitrite levels in the presence and absence of nitric oxide synthase inhibition (L-NAME; 5 mg/kg subcutaneously) at the indicated time points. Values are expressed as mean ± SEM. N ≥4/group. **P* = ≤0.05 vs. saline/saline; ***P* = ≤0.05 vs. L-NAME/saline.

DISCUSSION

This study demonstrated that a high dose of LPS results in upregulation of iNOS in the rat thoracic aorta. This upregulation occurred between 1 and 3 hours and increased with time. Administration of LPS also resulted in significant hypotension and enhanced production of plasma nitric oxide metabolites. However, the decrease in mean arterial blood pressure was evident before induction of iNOS in the aorta or elevation of plasma nitrates or nitrites was evident. This indicates that the hypotension observed during endotoxemia in conscious rats does not correlate with plasma nitric oxide metabolite levels.

Administration of the competitive nitric oxide synthase inhibitor L-NAME increased systemic blood pressure in saline-treated animals and completely reversed LPS-induced hypotension. Consequently these data confirm that nitric oxide plays a physiologic role in maintaining normal blood pressure homeostasis.[1] Furthermore, the ability of L-NAME to prevent LPS-induced hypotension implicates an overproduction of nitric oxide during endotoxic shock. Administration of L-arginine, but not the D-enantiomer, abolished the competitive inhibition of L-NAME by providing an excess of the required precursor necessary for nitric oxide synthesis. Thus the complete restoration of LPS-induced hypotension during concurrent administration of L-arginine and L-NAME further suggests that endotoxic shock is mediated by enhanced nitric oxide synthesis.

Several studies have reported that plasma levels of nitrates and nitrites, the stable metabolites formed during oxidation of nitric oxide, correlate with hypotension and a reduced systemic vascular resistance.[6,7,19] This excessive production of plasma nitric oxide metabolites is presumably due to enhanced expression of iNOS, which is known to be upregulated during pathologic states such as sepsis.[1,3] However, it is noteworthy that in two of these studies plasma nitrate and nitrite levels were determined 1 to 3 days after sepsis had been present in human patients.[6,19] In contrast, we measured plasma nitric oxide metabolites at 2, 4 and 6 hours after experimental creation of endotoxemia in rats. The other study, by Nava et al.,[7] demonstrated that plasma nitric oxide metabolites were elevated to approximately 50 and 100 μmol/L at 2 and 4 hours, respectively, after intravenous administration of *Salmonella typhosa* endotoxin (4 mg/kg) to anesthetized rats. In comparison, we chose a conscious rat model because anesthetized experimental conditions were shown to modify the spontaneously released nitric oxide contribution to blood pressure regulation in vivo.[20] Moreover, we used endotoxin from *E. coli*, the most frequent causative organism in septic shock,[21] and administered it intraperitoneally at a dose of 20 mg/kg.

In our experimental model of septic shock, we found that plasma nitric oxide metabolite levels increased between 2 and 4 hours after administration of endotoxin. Clearly the hypotensive response to LPS was present before an increase in plasma nitrates or nitrites was detected. Furthermore, administration of

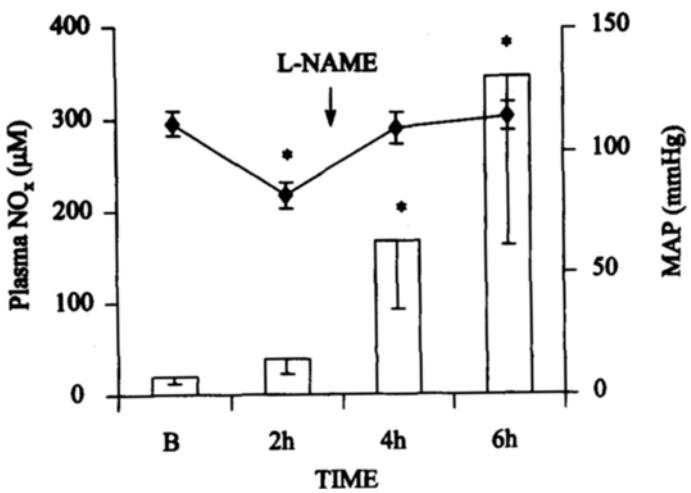

Fig. 6. Plasma nitrate/nitrite levels (NO_x; bar graph) and mean arterial blood pressure (*MAP*; line graph) during delayed (3 hours) administration of L-NAME (5 mg/kg subcutaneously) in lipopolysaccharide-induced sepsis (20 mg/kg intraperitoneally) at indicated time points. Values are mean ± SEM. N = 4/group. *P = <0.05 compared to baseline determinations.

L-NAME promptly restored mean arterial blood pressure but did not diminish the production of nitric oxide metabolites. It is possible that L-NAME never entered or reacted with the cells responsible for producing the large amounts of nitric oxide. However, if this were the case, our data would suggest that these cells do not participate in the hypotensive response to LPS. It might also be argued that this dose of L-NAME was not sufficient to inhibit the inducible isoform of nitric oxide synthase or was not effective in reducing plasma nitric oxide metabolite levels because of the lengthy duration of our experimental conditions. Apropos of this possibility, the delayed administration of L-NAME likewise restored mean arterial blood pressure during endotoxic shock and did not reverse or attenuate the stimulatory effect of endotoxin on formation of plasma nitric oxide metabolites. In addition, when the dose of L-NAME was increased eightfold and it was administered concurrently with LPS, all of the rats died before completion of the experimental protocol (unpublished observations). The effect of other nitric oxide synthase inhibitors in our experimental model was not studied. Last, another possible interpretation of our data is that plasma nitrate and nitrite levels are simply not a satisfactory proxy for the measurement of nitric oxide synthase activity. Nevertheless, our data clearly indicate that the hypotension that occurs following a high dose of LPS does not correlate with plasma nitric oxide metabolite levels. Thus our results confirm and extend the observations made by Vandenberg et al.[8] who demonstrated that hypotension and plasma nitric oxide levels did not correlate at 6 hours following administration of LPS to LPS-*resistant* rats.

The finding that L-NAME reverses LPS-induced hypotension indicates that the decrease in blood pressure observed during endotoxic shock is secondary to overproduction of nitric oxide. This enhanced release of nitric oxide, although not measured directly, must be through activation of the constitutive isoform of nitric oxide synthase, since iNOS immunoreactivity was not even apparent in the thoracic aorta at 1 hour after administration of LPS and only low levels were detected at 3 hours. These findings are also consistent with the observations of Szabó et al.[13] who demonstrated that calcium-independent (inducible) nitric oxide synthase *activity* was not appreciable until 3 hours after administration of intravenous *E. coli* LPS (10 mg/kg) to anesthetized rats. Moreover, in that same study, the nitric oxide–mediated hyporeactivity to noradrenaline preceded induction of nitric oxide synthase during endotoxic shock. Thus their results also suggested that the constitutive isoform of nitric oxide synthase is responsible for the early effect on systemic vascular resistance during endotoxemia.

The source of nitric oxide contributing to the hypotension following LPS treatment was not shown in this study. Nevertheless, the available evidence suggests that the constitutive isoform of nitric oxide synthase located in the vascular endothelium generates the nitric oxide responsible for hypotension during early septic shock. For example, endothelial cells exposed to LPS rapidly release a humoral factor that reduces the depolarization-induced calcium transients in cocultured vascular smooth muscle cells.[22] In addition, Salvemini et al.[23] demonstrated that bovine aortic endothelial cells also release, within minutes, a nitric oxide–like factor following exposure to LPS. Furthermore, local formation of small amounts of nitric oxide by the vascular endothelium could elicit its effects on vascular smooth muscle with resultant vasodilatation. Nitric oxide produced in such small amounts would have little, if any, impact on plasma nitric oxide metabolite levels.

The signal transduction mechanism responsible for LPS-induced activation of constitutive nitric oxide synthase also remains to be elucidated. It is possible that LPS elicits the release of other mediators, which then stimulate release of nitric oxide from vascular endothelium. Possible candidates include bradykinin, angiotensin II, histamine, 5-hydroxytryptamine, and others. All these substances are released in response to LPS and all are capable of stimulating nitric oxide release from endothelium.[23-27] Whether any or all of these mediators play a role in endotoxic shock warrants further investigation.

CONCLUSION

LPS induced iNOS expression in the rat thoracic aorta, an effect accompanied by an increase in plasma nitric oxide metabolites. Interestingly, LPS caused a reduction in mean arterial blood pressure before iNOS upregulation occurred or plasma nitric oxide metabolites became elevated. In addition, inhibition of nitric oxide synthase with L-NAME restored mean arterial blood pressure during endotoxic shock, an effect abolished by L-arginine but not by D-arginine. However, L-NAME did not diminish LPS-stimulated production of plasma nitrates and nitrites. Taken together these data indicate that although LPS-induced hypotension results from excessive production of nitric oxide, it does not correlate with plasma nitric oxide metabolites. We speculate that these seemingly paradoxical observations occur because LPS activates the constitutive isoform of nitric oxide synthase early

on. Later, the inducible isoform is upregulated and is responsible for the increased production of plasma nitric oxide metabolites.

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Discussion

Dr. N.E.P. Deutz (The Netherlands). Did you also measure iNOS in the gut or in the liver to determine whether there is a more rapid increase in iNOS activity? Did you resuscitate these rats to determine whether all of your effects would remain unchanged?

Dr. K. Klemm. The animals were given saline solution at a constant rate of approximately 0.5 ml/hr, so they were not really resuscitated. We measured blood flow to the intestine. What we found is that in this model of LPS-induced sepsis, there was a slight increase in blood flow to the duodenum and jejunum and a significant decrease in ileal blood flow. We also measured the iNOS protein content in the gut. There was normal iNOS in the ileum but no iNOS in the jejunum. When LPS is used for stimulation, there is an increase in all parts of the small intestine but mostly in the ileum. Upregulation of iNOS protein content in the gut occurred over the same time course as in the thoracic aorta.

Dr. E. Klar (Germany). If I understood your data correctly, you report that you first observe the hypotensive phase and then you see an upregulation of iNOS. Do you know enough about the kinetics of iNOS upregulation in hemorrhagic shock to be sure that this phenomenon is definitely unrelated to LPS infusion?

Dr. Klemm. What is known about iNOS upregulation is that after any stimulation it will be approximately 3 hours before iNOS is upregulated. The decrease in blood pressure at 2 hours cannot be related to iNOS upregulation. In hemorrhagic shock we do not see an upregulation of iNOS.

Dr. B. Schirmer (Charlottesville, Va.). Do you think that you are correct in measuring total nitrate metabolites? Obviously it took several hours for these to accumulate. They did not correlate very well with the blood pressure findings. Is it possible that attempting to measure something systemically that is occurring on a more local tissue level you have oversimplified the effect?

Dr. Klemm. Most likely that is what is happening. The hypotensive effect at 2 hours is clearly nitric oxide dependent, but the amount produced at this early time point has no effect on systemic nitrate/nitrite levels. On the other hand, the nitric oxide produced systemically by iNOS at 4 and 6 hours probably does not act at the endothelial level.

Dr. G. Kauffman, Jr. (Hershey, Pa.). Would you care to speculate, since you have ruled out iNOS as the mediator, what LPS-induced hypotension might be related to?

Dr. Klemm. Most likely the constitutively expressed endothelial isoform of nitric oxide synthase not otherwise specified is responsible for the hypotension observed, but I cannot prove that at the present time.

Transpapillary Stenting for Pancreaticocutaneous Fistulas

Richard A. Kozarek, M.D., Terrance J. Ball, M.D., David J. Patterson, M.D., Shirley L. Raltz, R.N., L. William Traverso, M.D., John A. Ryan, M.D., Richard C. Thirlby, M.D.

Because transpapillary stents have been successfully placed to treat the ductal disruptions associated with pseudocysts, pancreatic ascites and pleural effusions, and pancreaticoenteric fistulas, we reviewed our experience with endoscopically placed prostheses in patients who had persistent pancreaticocutaneous fistulas but an otherwise intact duct. Nine patients who underwent endoscopic transpapillary stent placement for ongoing pancreaticocutaneous fistulas at our institution were retrospectively reviewed. Fistulas were present for a mean (± SEM) of 35 ± 11 days and averaged 225 ± 55 ml of output daily. Etiology of the fistulas included percutaneous pseudocyst drainage in four patients, pancreatic necrosis in two, complications of pancreatic surgery in two, and perforation of the duct of Santorini at the time of minor sphincterotomy in one. All patients had an otherwise intact duct at the time of endoscopic retrograde cholangiopancreatography. Six patients had transpapillary stents placed that did not bridge the area of leakage and three had prostheses placed across the ductal disruption. Eight of nine fistulas were successfully closed by means of this technique including five within 48 hours. There was one instance of stent migration and one patient developed prosthesis occlusion and an infected pseudocyst, which was treated with stent exchange. Stents were retrieved 10 to 14 days after fistula closure and no patient has had a recurrence at a median follow-up of 3 years. Transpapillary stents appear to effect closure of pancreaticocutaneous fistulas that fail to respond to conventional therapy. (J GASTROINTEST SURG 1997;1:357-361.)

Transpapillary stents have been successfully placed in the treatment of pancreatic pseudocysts.[1-18] They have been successfully used to treat other consequences of pancreatic duct disruption including pancreaticoenteric fistulas, pancreatic ascites, and high amylase pleural effusions.[19-21] Theoretically such stents also have the ability to treat persistent pancreaticocutaneous fistulas that are the consequence of pancreatic necrosis, penetrating abdominal trauma, surgery, or percutaneous drainage of pseudocysts.

MATERIAL AND METHODS

All patients with persistent pancreaticocutaneous fistulas seen at Virginia Mason Medical Center between July 1986 and April 1996 were retrospectively reviewed. Specifically excluded from final analysis were patients who did not undergo diagnostic endoscopic retrograde cholangiopancreatography (ERCP) or in whom ERCP demonstrated complete duct occlusion with inability to bridge a disrupted duct (disconnected duct syndrome). Data collected included patient demographics, etiology of the ductal disruption, the endoscopic procedure performed and its complications, and the results of endoprosthesis placement including subsequent need for surgery.

Nine patients (three women and six men; mean age 51 years [range 29 to 78 years]) with persistent pancreaticocutaneous fistulas and an otherwise intact pancreatic duct were reviewed. Etiology of the fistulas included percutaneous pseudocyst drainage in four patients, pancreatic necrosis in two, postoperative complications in two, and duodenal/duct of Santorini perforation at the time of endoscopic minor sphincterotomy performed elsewhere in one (Fig. 1, *A* and *B*; Fig. 2, *A*). Fistulas were present for a mean (± SEM) of 35 ± 11 days and averaged 225 ± 55 ml of pure pancreatic juice output daily, and all patients had failed a trial of hyperalimentation plus somatostatin analogue (octreotide, Sandoz Pharmaceuticals, East Hanover, N.J.) prior to the attempt at endoscopic therapy.

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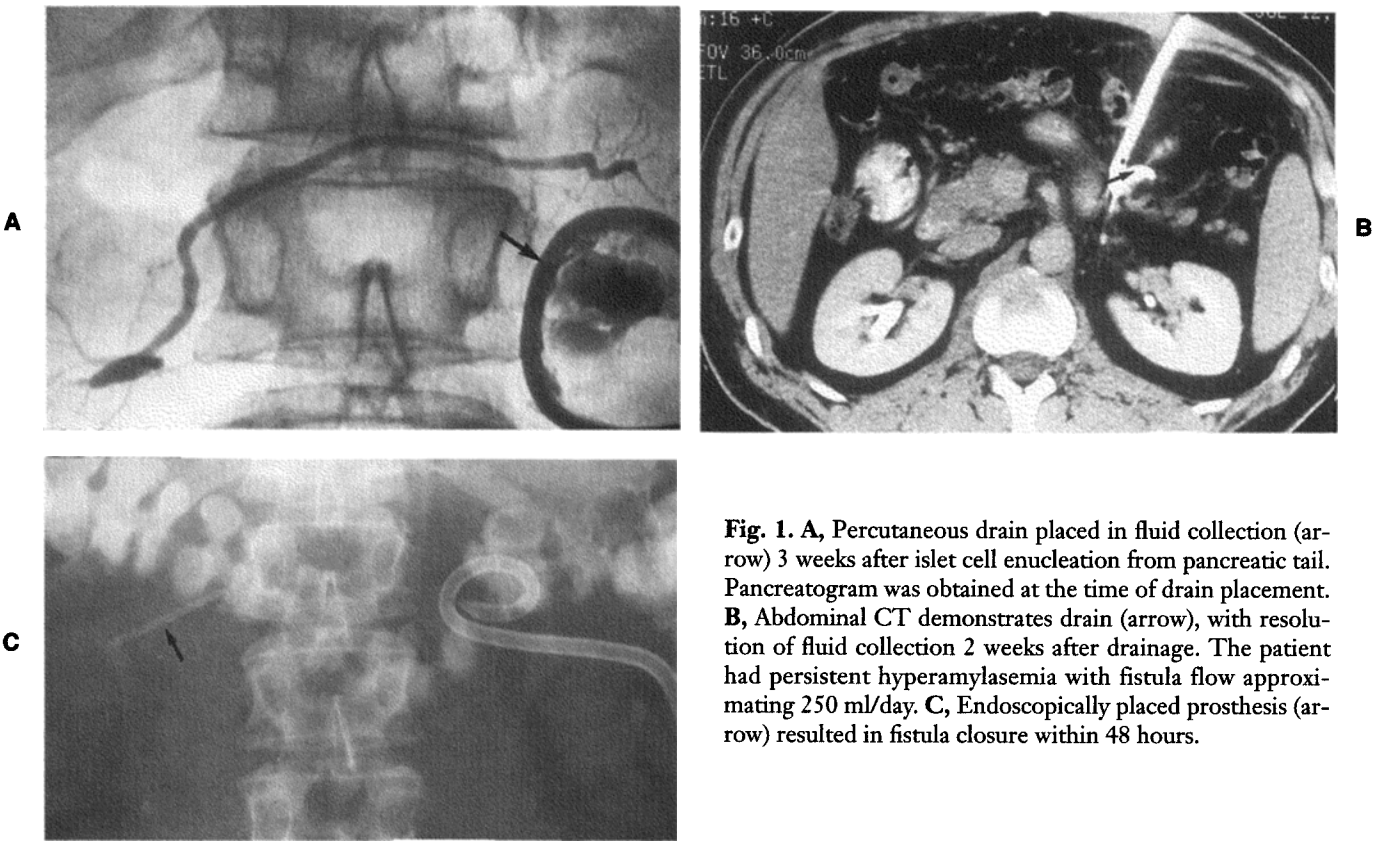

Fig. 1. A, Percutaneous drain placed in fluid collection (arrow) 3 weeks after islet cell enucleation from pancreatic tail. Pancreatogram was obtained at the time of drain placement. **B,** Abdominal CT demonstrates drain (arrow), with resolution of fluid collection 2 weeks after drainage. The patient had persistent hyperamylasemia with fistula flow approximating 250 ml/day. **C,** Endoscopically placed prosthesis (arrow) resulted in fistula closure within 48 hours.

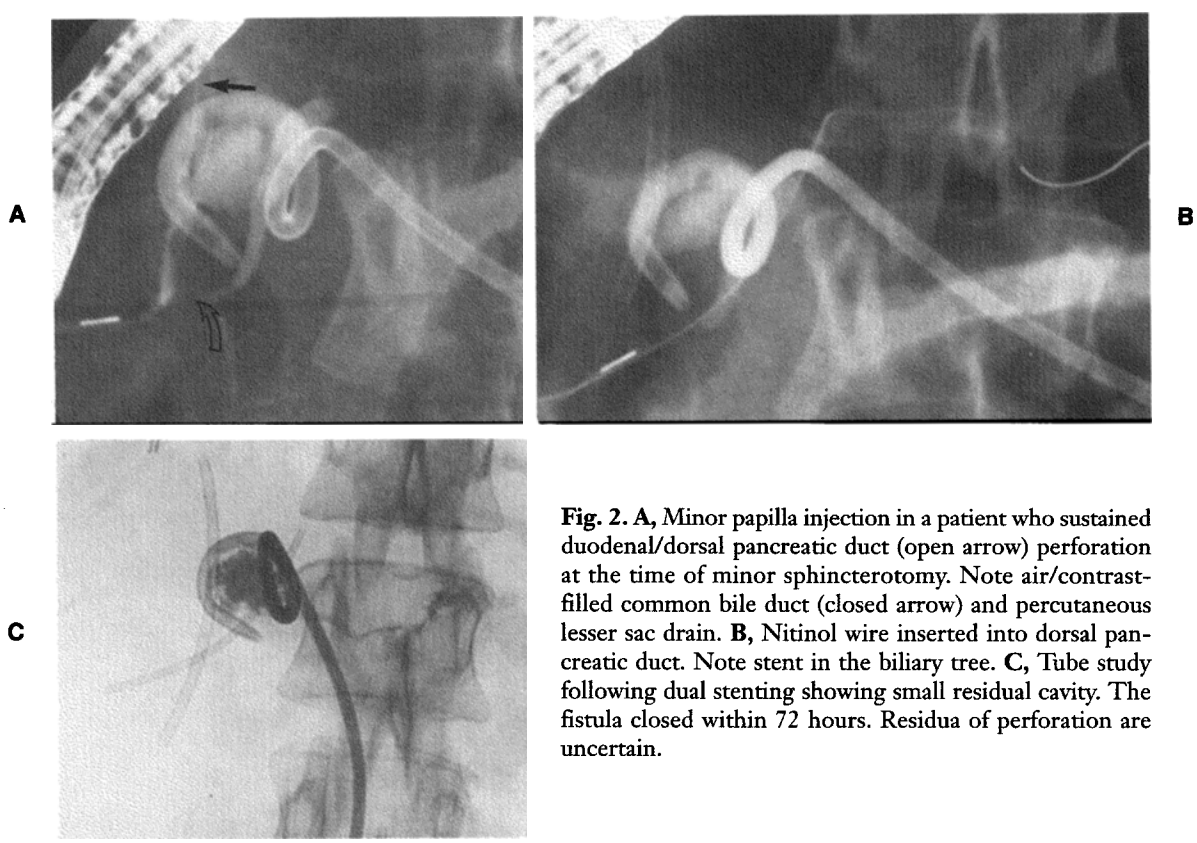

Fig. 2. A, Minor papilla injection in a patient who sustained duodenal/dorsal pancreatic duct (open arrow) perforation at the time of minor sphincterotomy. Note air/contrast-filled common bile duct (closed arrow) and percutaneous lesser sac drain. **B,** Nitinol wire inserted into dorsal pancreatic duct. Note stent in the biliary tree. **C,** Tube study following dual stenting showing small residual cavity. The fistula closed within 72 hours. Residua of perforation are uncertain.

Procedures were carried out after antibiotic precoverage with a second-generation cephalosporin at the time of pancreatography. All patients were noted to have residual duct disruption (pancreatic head in 3, body in 3, and tail in 3; side branch disruption in 3; and main duct disruption in 6). Stent sizes ranged from 5 to 7 Fr and were selected based on the size of the main pancreatic duct. Three patients had prostheses placed across the ductal disruption, whereas six had transpapillary stents placed in the pancreatic duct that did not bridge the area of leakage (Fig. 1, *C*; Fig. 2, *B* and *C*). Stents were retrieved 10 to 14 days after external fistula closure.

RESULTS

Eight of nine pancreaticocutaneous fistulas were successfully closed by means of this technique including five within 48 hours and three others within a week. Complications included one instance of stent migration into the duodenum and one patient who developed an infected pseudocyst that was related to stent occlusion. The latter was successfully treated with prosthesis exchange. Stents were retrieved 10 to 14 days after external fistula closure. One patient required elective pancreatic resection for residual duct leak, although a second patient (depicted in Fig. 2) continues to have mild pain and low-grade hyperamylasemia.

DISCUSSION

Endoscopic therapy for a disrupted pancreatic duct has primarily dealt with internal as opposed to external pancreatic fistulas.[22,23] Thus pseudocysts have been drained by needle-knife incision to create a fistula through the gastric or duodenal wall or by use of transpapillary drains or stents; pancreatic ascites and pleural effusions have been treated with a combination paracentesis/thoracentesis and endoprosthesis placement, and pancreaticoenteric fistulas have undergone spontaneous healing after pancreatic juice diversion through a transpapillary stent.

External pancreatic fistulas, in turn, are most often iatrogenic. Usually the consequence of percutaneous pseudocyst drainage or partial pancreatic resection, external fistulas occasionally occur in the setting of pancreatic necrosis, neoplasm, and penetrating abdominal trauma.[24] Most external fistulas resolve spontaneously or eventually heal in conjunction with a decrease in the output of pancreatic juice, which can be achieved with octreotide and/or hyperalimentation.[25-28] Alternatively, Garcia-Puges et al.[29] initiated high-dose pancreatic enzymes in five patients with external pancreatic fistulas. Each of these fistulas had a significant reduction in flow and trypsin output and closed after 1 to 12 days.

There is a subset of patients, however, who prove to have refractory external fistulas. Such patients may have downstream duct obstruction from either a stricture, stone, or neoplasm, or they may have a true "disconnected duct" syndrome in which necrosis or enzymatic digestion has left the pancreas in two unconnected pieces. The latter condition may eventually result in glandular involution, atrophy, and fibrosis with ultimate fistula closure, particularly with disruptions of the pancreatic tail.[30] Alternatively, patients with complete disruptions in the midbody or head of the pancreas may ultimately require partial pancreatectomy. Because these latter situations are amenable to endoscopic therapy only by virtue of draining concomitant fluid collections using a combination of transpapillary and transenteric techniques,[31] they were specifically excluded from our current series.

Our retrospective review clearly demonstrates the efficacy of small-diameter prostheses to facilitate drainage of pancreaticocutaneous fistulas. Whether these stents work by directly occluding major duct leaks or simply equalize the pancreatic duct and duodenal pressures to facilitate flow is uncertain. The latter appears to be more likely inasmuch as five of the six patients in whom only transpapillary stents were placed had resolution of their fistulas. This has also been seen with cystic duct leaks where transpapillary stenting proved as effective as prostheses placed into the common hepatic duct to effect leak closure.[32,33] Transpapillary stents would be unlikely to work, however, in the event of a ductal stenosis downstream from the leak. As such, three of our patients had prostheses placed that bridged the leak site proper. Two of these patients had inflammatory stenoses and the third had duodenal/duct of Santorini perforation following a minor papilla sphincterotomy at another institution. All three patients had rapid fistula closure without recurrence, although it is uncertain whether the latter patient will be left with a significant stenosis at the sphincterotomy site because she currently has chronic pain and low-grade hyperamylasemia.

Despite the ability to treat a subset of chronic pancreaticocutaneous fistulas endoscopically, some caution must be exercised. On the one hand, efficacy seems unlikely if the duct is completely disrupted and the pancreatic parenchyma is in two separate pieces as a consequence of enzymatic necrosis. On the other hand, procedures were not uncomplicated; there was one instance of spontaneous stent migration into the duodenum that required replacement plus an additional stent occlusion resulting in infection of a concomitant pseudocyst. The latter was successfully treated with antibiotics and stent exchange. Prosthe-

ses within the pancreatic duct, however, can themselves be problematic. These problems include not only occlusion and internal or external migration, as previously noted, but also localized duct inflammation from iatrogenic lesions.[34,35] The latter include not only side branch ectasias from occlusion but also inflammatory stenoses, some of which fail to resolve even after the prosthesis is retrieved. It was fear of inducing lesions such as those previously cited rather than an inability to manipulate the prosthesis through a tortuous ductal system that led us to place short transpapillary stents in lieu of longer ones that actually bridged the disrupted area. As previously noted, these short stents proved effective in all but a single instance, and their rapid retrieval 10 to 14 days after fistula closure was associated with a minimum of iatrogenic changes.

Although some of these fistulas may have "spontaneously" resolved with additional time, octreotide, and hyperalimentation, we believe that endoscopic therapy has a role in patients who fail to respond to conventional techniques. Its application may preclude the need for surgery or transform an urgent and potentially complicated surgical situation into one that can be treated electively. Further experience with this technique, therefore, appears reasonable to better define patient and ductal selection criteria and prospective closure and complication rates.

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Dopexamine Maintains Mesenteric Blood Flow During Systemic Hypoxemia in the Neonatal Piglet

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In adults, dopexamine is a specific dopaminergic and β_2-adrenergic agonist; its effects in neonates are unknown. Ultrasonic flow probes were placed around the ascending and descending aorta and cranial mesenteric artery of 0- to 2-day-old and 2-week-old piglets. Animals of each age group (9 to 14 per group) were subjected to (1) dopexamine infusion (5 μg/kg/min); (2) 30 minutes of hypoxia (inspired oxygen content 0.12) followed by 30 minutes of reoxygenation; and (3) dopexamine infusion during hypoxia and reoxygenation. In both age groups dopexamine alone increased ascending aorta blood flow (cardiac output minus coronary artery blood flow), mildly decreased mean arterial pressure, and increased cranial mesenteric artery blood flow. Compared to baseline values, 30 minutes of hypoxia produced significant ($P < 0.05$, analysis of variance) decreases in cranial mesenteric artery blood flow in 0- to 2-day-old (58 ± 13 ml/min vs. 30 ± 8 ml/min) and 2-week-old (125 ± 18 ml/min vs. 60 ± 11 ml/min) piglets. In all cases blood flow returned to baseline values after reoxygenation. In both animal groups treated with dopexamine before hypoxia, the decreases in cranial mesenteric artery blood flow were eliminated (47 ± 5 ml/min vs. 44 ± 6 ml/min in 0- to 2-day-old piglets; 140 ± 27 ml/min vs. 117 ± 18 ml/min in 2-week-old piglets). Dopexamine may prove to be of clinical benefit when neonates are threatened by hypoxemia-induced decreases in intestinal blood flow. (J GASTROINTEST SURG 1997;1:362-370.)

Neonatal necrotizing enterocolitis is a major cause of infant morbidity, particularly in infants who are born prematurely and/or of low birth weight. Clinical studies have identified several factors that are associated with the development of necrotizing enterocolitis including hypoxemia, hypothermia, and some of the vasoconstrictors used in neonatal intensive care units.[1] Each perturbation is thought to invoke a redistribution of blood flow away from less critical organs (e.g., skin, intestine) to those vital to survival (e.g., heart, brain). If the insult is prolonged, intestinal mucosal ischemia ensues and luminal bacteria invade the intestinal wall and proliferate, leading to abscess formation and full-thickness intestinal necrosis. Improvements in patient outcome can be expected once the mechanisms of injury are elucidated, and treatments are designed to interrupt this pathophysiologic process.

Lloyd[2] was among the first to postulate that hypoxia was responsible for changes in regional blood flow to the intestine of human neonates, similar to the mammalian diving reflex. Touloukian et al.[3] reported that asphyxia caused newborn piglet blood flow to decrease by 35% to 50% throughout the gastrointestinal tract. In newborn piglets Alward et al.[4] found that, although cardiac output remained stable, flow decreased to the gastrointestinal tract, which on histologic examination showed ischemic changes. Others have shown that moderate degrees of hypoxia induce vasodilation in newborn piglet mesentery, whereas severe hypoxia causes vasoconstriction.[5]

Dopamine is a commonly used vasoactive agent that, in adults at least, possesses the following dose-dependent activities: dopaminergic (<3 μg/kg/min), β_1-adrenergic (3 to 10 μg/kg/min), and α-adrenergic (>10 μg/kg/min). We have shown that, within this

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range, dopamine produces a dose-dependent increase in mesenteric blood flow in neonatal piglets.[6] However, others have data to suggest that, at high infusion concentrations, the appearance of peripheral vasoconstriction, tachycardia with increased myocardial consumption, and dysrhythmias may limit the effectiveness of dopamine.[7] Moreover, long-term use of dopamine has been shown to suppress pituitary function in infants.[8]

Dopexamine hydrochloride is a novel synthetic analogue of dopamine that has potent β_2-adrenoceptor and dopaminergic activity. Of note, this agent lacks appreciable β_1- or α-adrenergic activity.[9] Among the clinically relevant end points of these pharmacologic activities are afterload reduction and a mild positive inotropic effect.[7] As such, dopexamine has been used in European countries to manage cardiac failure[10,11]; its use in the United States has yet to be approved. Dopexamine has a rapid onset of action, and its hemodynamic effects are typically observed within 1 minute; in normal adult animal and human volunteers, its half-life is 6 to 7 minutes. Side effects have been minimal and include slight increases in heart rate.[12] Of interest, dopexamine has also been shown to produce vasodilation of the mesenteric vascular bed in adult animals, although its hemodynamic effects in newborn animals are not well delineated. For these reasons the present study was undertaken to determine the impact of dopexamine on regional blood flow monitored in newborn piglets subjected to a severe hypoxic challenge.

MATERIAL AND METHODS

All experimental procedures were completed in accordance with the guidelines and approval of the Tulane University School of Medicine Advisory Committee for Animal Resources.

Surgical Preparation and Instrumentation

Standard breed 0- to 2-day-old (1.2 ± 0.2 kg) and 2-week-old (3.1 ± 0.6 kg) piglets were brought to the laboratory on the day of experimentation. The animals were placed in a warmed environment and observed in the fasted state for 4 hours. Piglets with obvious signs of physical illness such as diarrhea and lethargy were excluded from the study. Each piglet was anesthetized with intraperitoneal pentobarbital sodium (35 mg/kg). Intraperitoneal pancuronium bromide (0.5 mg/kg) was administered in order to perform tracheostomy and intubation. The animals were placed on a small animal ventilator (Harvard Apparatus Inc., South Natick, Mass.) preset to deliver 8 to 12 ml/kg tidal volume, 20 to 30 breaths/min, and an inspired oxygen content of 1.0. Ventilator settings were adjusted to maintain serially obtained arterial blood gas values within desired limits. Temperature, monitored with a rectal probe, was maintained with the aid of external heating sources as needed.

After left groin cutdown, the femoral vein was cannulated to administer maintenance intravenous fluid (lactated Ringer's solution with 5% dextrose, 1 to 2 ml/kg/hr) and, in select groups, continuous infusion of dopexamine hydrochloride. The ipsilateral femoral artery was cannulated and connected to a pressure transducer (Statham model P23, Gould Instrument Systems, Inc., Valley View, Ohio) for the purpose of monitoring mean systemic arterial pressure (Grass model 7D polygraph, Instrument Co., Quincy, Mass.) and heart rate and to obtain blood samples. Through a left flank incision the cranial mesenteric artery (equivalent to the superior mesenteric artery in humans) was isolated and encircled by an ultrasonic transit time flow probe (Transonic Systems Inc., Ithica, N.Y.) for continuous measurement of blood flow using a two-channel small animal blood flowmeter (model T206, Transonic Systems Inc.). A left thoracotomy was then fashioned, and blood flow probes were positioned around the ascending and descending aorta to measure cardiac output (minus coronary artery blood flow) and descending aortic blood flow, respectively. All incisions were closed to minimize evaporative heat loss. On completion of the surgical preparation, all animals were allowed a minimum 15-minute period of time to ensure stabilization of vital signs and arterial blood gases.

Experimental Protocols

To determine the hemodynamic effects of varying doses of dopexamine in neonatal animals, instrumented 0- to 2-day old (n = 4) and 2-week-old (n = 4) piglets were given dopexamine hydrochloride (Speywood Pharmaceuticals, Berkshire, England) by continuous intravenous infusion (syringe pump model 351, Sage Instruments, Boston, Mass.) at clinically relevant adult doses of 2, 5, and 10 μg/kg/min. The dosage sequence was randomized and each animal was challenged with every dose several times. Once a peak response for each monitored hemodynamic variable was attained, the infusion was discontinued and sufficient time was allowed to permit all monitored hemodynamic variables to return to their respective baseline levels before a subsequent infusion dose was instituted. Based on data obtained from these trials, a dopexamine dose of 5 μg/kg/min was selected for use in subsequent experiments.

Piglets from both age groups were randomly assigned to one of the following experimental se-

quences: (1) 30 minutes of hypoxia (FIO_2 = 0.12)—induced by ventilating the animals with a nitrogen-oxygen gas admixture)—followed by a 30-minute period of reoxygenation at an FIO_2 concentration of 1.0 (0- to 2-day-old piglets [n = 10]; 2-week-old piglets [n = 14]) or (2) continuous infusion of dopexamine (5 μg/kg/min) instituted 10 minutes before a 30-minute period of hypoxia and continued through a 30-minute period of reoxygenation (0- to 2-day-old piglets [n = 9]; 2-week-old piglets [n = 10]).

Chemical Compound Preparation

Dopexamine hydrochloride (Speywood Pharmaceuticals) was dissolved in 0.9% normal saline solution, then diluted to a final concentration of 0.1 mg/ml. To ensure freshness the drug was frequently reconstituted from its stock and kept refrigerated when not in use.

Data Analysis

Dose-response data were subjected to Student's paired *t* test, with statistical significance set at $P < 0.05$. For all other variables monitored, within- and between-group mean values (± 1 standard error) were subjected to analysis of variance. When a significant F ratio was reached, Newman-Keuls test was applied to detect specific differences. Significance was established at $P < 0.05$.

RESULTS

The effects of varying doses of dopexamine in newborn piglets are shown in Table I. Dose-dependent increases in cardiac output were observed. In addition, all doses produced increases in heart rate, decreases in systemic mean arterial pressure, and increases in cranial mesenteric artery blood flow. Based on interpretation of these data, it was decided that the 5 μg/kg/min dose of dopexamine induced the most favorable effects on cardiac output and mesenteric blood flow while minimizing what appeared to be adverse consequences on systemic mean arterial pressure and heart rate. This dose was therefore used throughout the remainder of the experiments.

Displayed in Table II are the arterial blood gas values and temperature recordings obtained from the neonatal piglets exposed to the hypoxic challenge. An immediate and significant decrease in P_{O_2} was observed in all animal groups, an effect that was completely reversed by the end of the reoxygenation period. In contrast, pH, P_{CO_2}, and temperature changed little during the course of the experimental protocols.

In the 0- to 2-day-old animals exposed to hypoxia alone, there was a significant decrease in mean arterial pressure at 30 minutes of hypoxia, which returned to baseline levels 15 minutes after reoxygenation (Fig. 1, *A*). Animals of the same age treated with dopexamine prior to the hypoxic insult again showed an immediate decrease in mean arterial pressure. That this hypotensive effect persisted throughout reoxygenation is consistent with the aforementioned impact of dopexamine on systemic mean arterial pressure. In contrast, the 2-week-old piglets appeared more tolerant of the hypoxic episode, as they displayed no significant changes in mean arterial pressure during hypoxia or reoxygenation (Fig. 1, *B*). As occurred with the 0- to 2-day-old animals, there was an immediate, significant decrease in mean arterial pressure in animals given dopexamine, an effect that lasted throughout the hypoxia/reoxygenation period.

The position of each transit time flow probe placed on the ascending aorta of the neonatal piglets is such that it monitors cardiac output (minus coronary artery blood flow). In both age groups hypoxia induced significant decreases in cardiac output that were not completely reversed by reoxygenation (Fig. 2). On the other hand, dopexamine pretreatment resulted in maintenance of cardiac output at or above baseline levels throughout the duration of the experiments in both age groups. Hence, when compared at each observation point, the cardiac outputs of both age groups treated with dopexamine prior to hypoxia were significantly higher than those noted in the animals subjected to hypoxia alone, virtually throughout the experimental observation periods. Hypoxia induced mild, albeit insignificant, decreases in descending aortic blood flow that were not apparent in the animals pretreated with dopexamine (Fig. 3).

Cranial mesenteric artery blood flow markedly decreased during hypoxia in the 0- to 2-day-old piglets, only to recover during reoxygenation (Fig. 4, *A*). In this same age group, prehypoxia treatment with dopexamine resulted in maintenance of cranial mesenteric artery blood flow throughout the experimental time frame. When compared with cranial mesenteric artery blood flow values observed in animals exposed to hypoxia alone during the period of hypoxic stress, those recorded in animals pretreated with dopexamine were significantly higher at each observation point. Likewise, the 2-week-old piglets exposed to hypoxia alone demonstrated significant reductions in cranial mesenteric artery blood flow during the hypoxic period, in contrast to the well-preserved blood flow noted in animals pretreated with dopexamine (Fig. 4, *B*).

Table I. Dopexamine dose responses

Variable	Age group	Dopexamine dose		
		2 μg/kg/min	5 μg/kg/min	10 μg/kg/min
HR (beats/min)	0-2 days	10.02 ± 5.73	9.97 ± 3.96*	13.31 ± 2.53*
	2 weeks	5.92 ± 0.77*,†	11.13 ± 3.24*,†	25.08 ± 2.30*
MAP (mm Hg)	0-2 days	−19.35 ± 3.10*	−12.95 ± 2.38*	−22.00 ± 5.46
	2 weeks	−15.89 ± 2.97*	−16.49 ± 2.43*	−20.25 ± 2.56*
CO (ml/min)	0-2 days	−4.05 ± 2.33†	8.98 ± 2.63*,†	19.75 ± 4.53*
	2 weeks	9.60 ± 1.28*,†	10.73 ± 2.13*,†	25.09 ± 3.73*
CMA (ml/min)	0-2 days	14.39 ± 10.36	6.43 ± 3.48†	18.28 ± 2.38*
	2 weeks	11.58 ± 4.28*	13.19 ± 4.06*	6.48 ± 2.51

HR = heart rate: MAP = mean arterial pressure: CO= cardiac output; CMA = cranial mesenteric artery blood flow.
*P <0.05 vs. baseline
†P <0.05 vs. 10 μg/kg/min.

Table II. Arterial blood gases

Variable	Treatment	Age group	Time 0	15′ Hypoxia	30′ Hypoxia	15′ Reoxygenation	30′ Reoxygenation
pH	HYP	0-2 days	7.39 ± 0.06	7.36 ± 0.03	7.36 ± 0.02	7.36 ± 0.02	7.37 ± 0.02
		2 weeks	7.42 ± 0.02	7.35 ± 0.02	7.29 ± 0.03	7.30 ± 0.03	7.32 ± 0.03
	DPX/HYP	0-2 days	7.41 ± 0.04	7.39 ± 0.03	7.37 ± 0.03	7.35 ± 0.03	7.36 ± 0.0
		2 weeks	7.44 ± 0.02	7.37 ± 0.01*	7.33 ± 0.01*	7.31 ± 0.01*	7.33 ± 0.0
P_{CO_2} (mm Hg)	HYP	0-2 days	42 ± 4	39 ± 2	34 ± 2	34 ± 3	36 ± 1
		2 weeks	32 ± 1	35 ± 2	38 ± 2	35 ± 1	35 ± 1
	DPX/HYP	0-2 days	36 ± 4	34 ± 3	33 ± 3	30 ± 2	33 ± 1
		2 weeks	26 ± 1	30 ± 2	27 ± 3	28 ± 2	30 ± 2
P_{O_2} (mm Hg)	HYP	0-2 days	479 ± 15	34 ± 3*	35 ± 2*	309 ± 29*	445 ± 67
		2 weeks	429 ± 33	29 ± 2*	28 ± 1*	203 ± 39*	414 ± 42
	DPX/HYP	0-2 days	423 ± 21	34 ± 3*	32 ± 3*	294 ± 38*	365 ± 45
		2 weeks	429 ± 25	29 ± 2*	26 ± 2*	205 ± 25*	370 ± 31
Temperature (° C)	HYP	0-2 days	38.1 ± 0.1	38.2 ± 0.1	38.3 ± 0.1	38.4 ± 0.1	38.5 ± 0.2
		2 weeks	37.5 ± 0.2	37.6 ± 0.2	37.6 ± 0.2	37.8 ± 0.1	38.0 ± 0.1
	DPX/HYP	0-2 days	38.0 ± 0.1	38.0 ± 0.1	38.0 ± 0.1	38.2 ± 0.1	38.3 ± 0.2
		2 weeks	37.9 ± 0.1	37.8 ± 0.1	37.8 ± 0.1	38.0 ± 0.1	38.1 ± 0.1

HYP = hypoxia alone; DPX/HYP = dopexamine before hypoxia.
*P <0.05 vs. baseline.

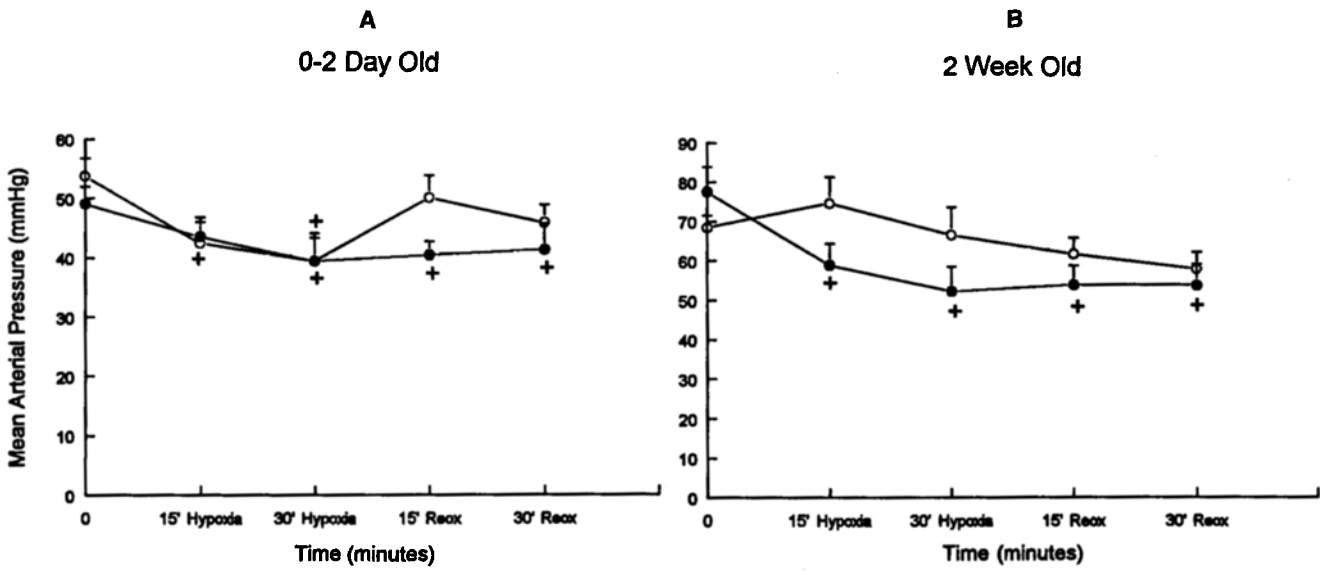

Fig. 1. Time course analysis of mean arterial pressure. Although hypoxia produced a significant decrease in mean arterial pressure in 0- to 2-day-old animals **(A)**, it was well tolerated in the older piglets **(B)**. In both age groups the systemic vasodilator effect of dopexamine was evident during the reoxygenation phase of the experiments. ○ = Hypoxia alone groups; ● = dopexamine pretreatment groups; *P = <0.05 vs. hypoxia alone; † = P <0.05 vs. baseline.

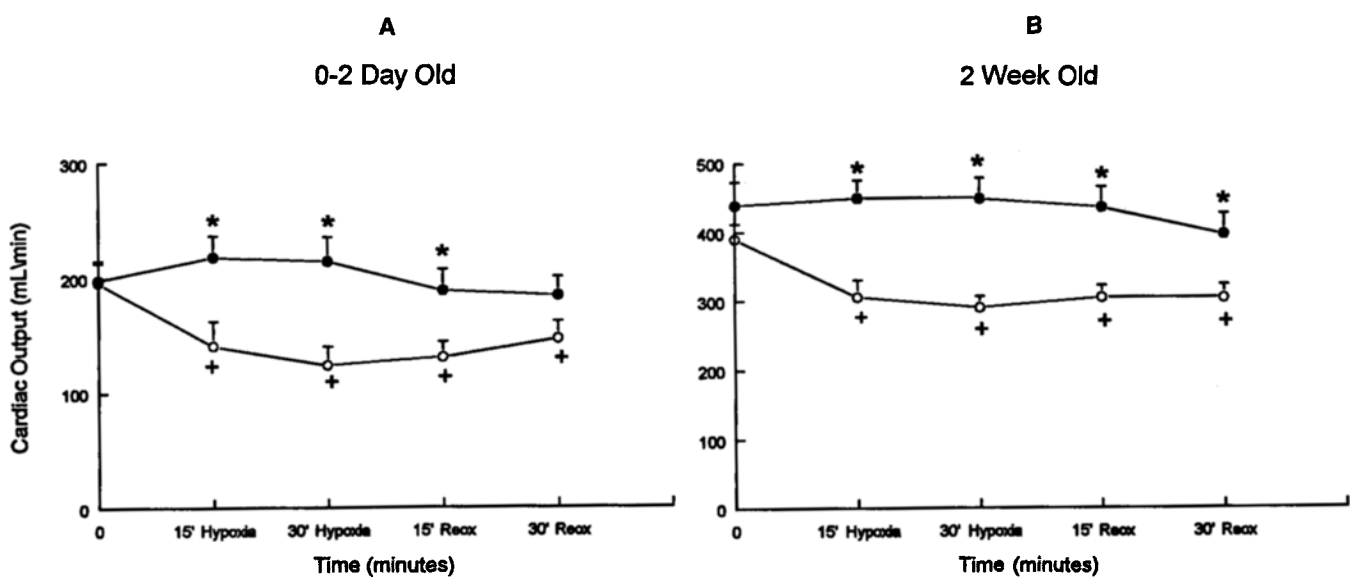

Fig. 2. Cardiac output (minus coronary artery blood flow) displayed over time. In both 0- to 2-day-old **(A)** and 2-week-old **(B)** piglets, dopexamine pretreatment blocked the otherwise significant reductions in cardiac output induced by hypoxia. ○ = Hypoxia alone groups; ● = dopexamine pretreatment groups; * = P <0.05 vs. hypoxia alone; † = P <0.05 vs. baseline.

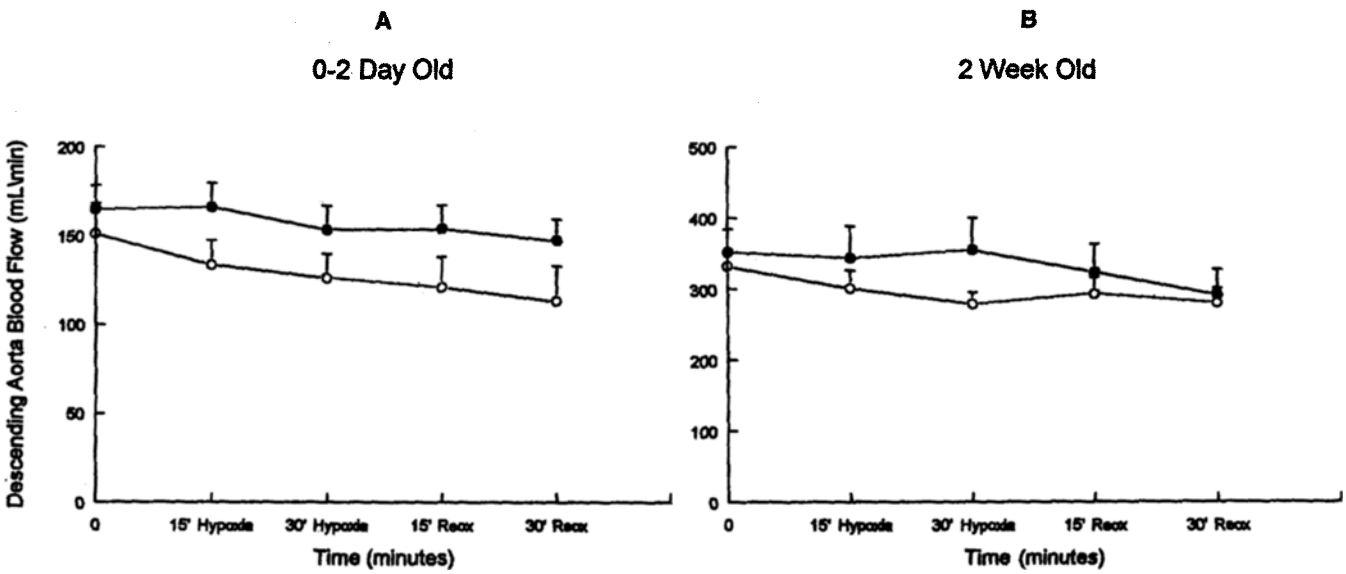

Fig. 3. Time course analysis of descending aortic blood flow. Although statistically insignificant, the slight reductions in descending aortic blood flow observed in both age groups were abrogated by dopexamine pretreatment. ○ = Hypoxia alone groups; ● = dopexamine pretreatment groups.

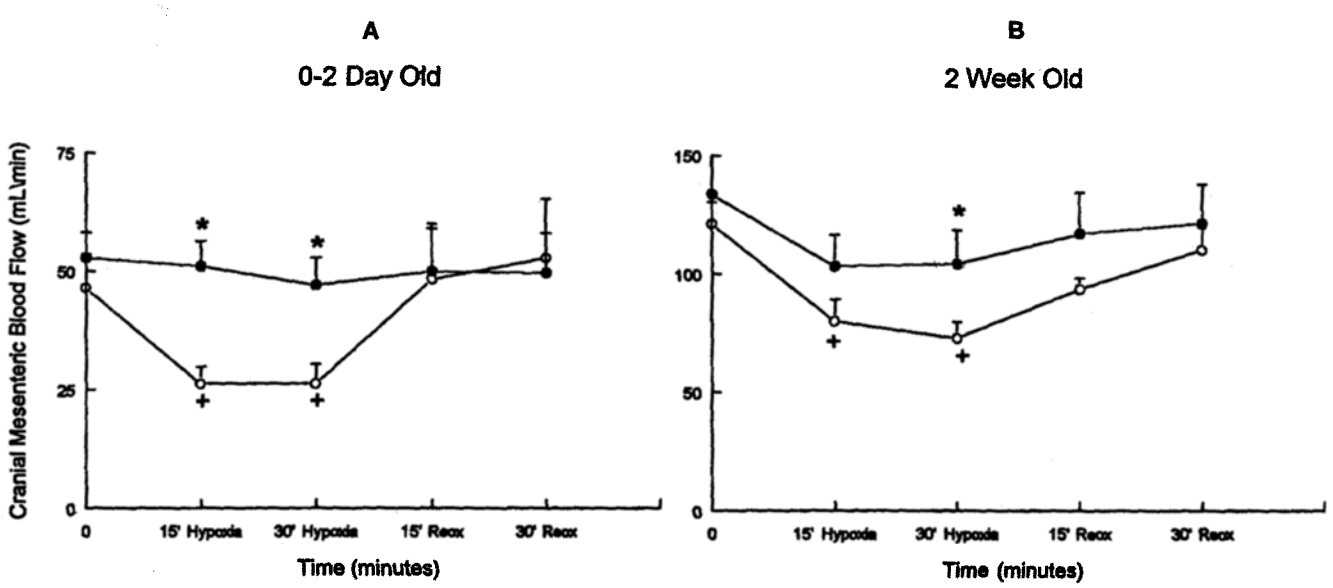

Fig. 4. Cranial mesenteric artery blood flow displayed over time. In both the 0- to 2-day-old **(A)** and 2-week-old **(B)** piglet groups, hypoxia induced marked reductions in cranial mesenteric artery blood flow that were reversed on reoxygenation. Dopexamine-treated groups maintained baseline cranial mesenteric artery blood flow throughout the hypoxia/reoxygenation period. ○ = Hypoxia alone groups; ● = dopexamine pretreatment groups; * = $P < 0.05$ vs. hypoxia alone; † = $P < 0.05$ vs. baseline.

DISCUSSION

Data reported herein suggest that the hemodynamic consequences of dopexamine when infused in newborn piglets are virtually identical to those documented in adult animal and human studies. These effects are mediated via several mechanisms related to the pharmacologic receptor profile of this agent. Cardiac output is thought to be increased through a variety of avenues. In an exhaustive series of in vitro and in vivo animal experiments, Brown et al.[9] demonstrated that, when contrasted with the potency of dopamine, dopexamine was 60 times more active at vascular β_2-adrenoceptor sites. Using a canine preparation, Smith et al.[13] showed that this peripheral vasodilator effect of dopexamine was enhanced directly by stimulation of vascular dopaminergic-1 (DA-1) receptors and indirectly through stimulation of prejunctional dopaminergic-2 (DA-2) receptors. The end result of these actions is a reduction in systemic mean arterial pressure, or afterload reduction. Brown et al. also demonstrated that dopexamine displays a direct inotropic effect through β_1-adrenoceptor agonist properties, whereas Smith et al. showed direct agonist activity at cardiac β_2-adrenoceptors. Finally, Bass et al.[14] used a canine model to show that an appreciable contribution to the inotropic effect of dopexamine comes from a baroreceptor-mediated reflex release of norepinephrine from sympathetic nerve terminals in combination with inhibition of its neuronal uptake (uptake-1 pathway). The end result of afterload reduction and heightened myocardial contractility is an increase in cardiac output, which is the primary reason for the clinical use of dopexamine. In experiments performed on conscious, instrumented dogs, it was revealed that the dopexamine-induced tachycardia—the primary side effect of this drug—is mediated through the aforementioned direct cardiac β_2-adrenoceptor agonist activity and baroceptor response.

Of specific interest, dopexamine was also shown to display mesenteric vasodilator activity in dogs.[12] Amenta et al.[15] used ligand binding and autoradiographic techniques in rats to demonstrate that the increased mesenteric blood flow and decreased mesenteric vascular resistance following dopexamine infusion was due to binding onto DA-1 receptors and β_2-adrenoceptors. In this regard, although moderate doses of dopamine also produce mesenteric vasodilation in adults, higher doses induce an α_1- and α_2-adrenoceptor–mediated peripheral vasoconstriction.[7] That dopexamine has no demonstrable α-adrenergic activity avoids this potentially deleterious side effect of dopamine.

Data from this study demonstrate that the hemodynamic profile of dopexamine documented in adult animal and human experiments can be replicated in newborns. These findings are consistent in some respects with those reported by Taylor et al.[16] who found that 1 to 100 μg/kg/min dopamine infusions in awake, chronically instrumented newborn sheep produced dose-related decreases in mean arterial pressure and increases in heart rate. However, these effects were appreciated only at the highest (e.g., superpharmacologic) dose; moreover, the cardiac index was not appreciably altered at any dose in the newborn lambs. It is suspected that differences in species and experimental design account for the apparent discrepancies between their data and our own observations. As such, it is believed that a more detailed analysis of the effects of dopexamine in newborn animal models be undertaken before recommending that this agent be used in humans.

Each newborn animal group responded in similar fashion to the severe hypoxic insult, with moderate decreases in cardiac output and more profound decreases in mesenteric blood flow. That dopexamine maintained baseline cardiac output throughout the periods of hypoxia and reoxygenation represents a novel observation that exposes another avenue for scientific investigation in that, to date, dopexamine has been used almost exclusively to treat adults with low-cardiac-output syndromes stemming from primary congestive heart failure.[7] Data from these experiments indicate that afterload reduction played an appreciable role in this effect, as the dopexamine-treated groups maintained their cardiac output concomitant with decreases in mean arterial pressure that persisted throughout the hypoxia and reoxygenation phases. In contrast, the mean arterial pressures recorded at the end of the reoxygenation phase in the hypoxia alone animal groups were equivalent to baseline values, yet the cardiac output in these animals remained significantly lower at those same respective observation points.

The most striking finding in this study was the ability of dopexamine to maintain baseline newborn piglet mesenteric blood flow in the face of a severe hypoxic insult. Although it is suspected that this mesenteric vasodilator response is mediated through direct β-adrenoceptor and DA-1 receptor activation, the extent to which the dopexamine-induced increase in cardiac output plays a contributing role is unknown. To help address this issue, experiments are currently being conducted in which hypoxic piglets are given direct intramesenteric infusions of dopex-

amine. Regardless of its mechanism(s) of action, this heretofore unreported observation is thought to possess significant potential for additional experimental endeavors. Foremost, although we were unable to identify a dose-response effect of dopexamine relative to mesenteric blood flow in control newborn piglets, it is considered prudent to reexamine this issue in hypoxic animals. Whether dopexamine can reverse already established hypoxia-induced mesenteric vasoconstriction needs to be addressed. Moreover, it remains to be determined whether this dopexamine-related preservation of neonatal mesenteric blood flow during hypoxia translates into an improvement in tissue (e.g., small intestinal) oxygenation. In this regard Cain and Curtis[17] have shown that oxygen extraction was not improved in dopexamine-treated endotoxic dogs subsequently exposed to hypoxic insult. Although there are several obvious differences between the experimental designs outlined in that report and our own, their observations do raise an important issue that must be addressed.

Finally, as it is well recognized that prematurity and low birth weight are primary epidemiologic factors associated with the development of neonatal intestinal ischemic diseases, the same experimental paradigm is being applied to piglets delivered at 90% of their gestational age, using our previously described techniques.[18] At present, however, it is concluded that the data reported herein are of sufficient interest to warrant further investigation.

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Discussion

Dr. B. Schirmer (Charlottesville, Va.). Have you done any studies to examine the effect of any dopamine antagonists in the system? Does that negate the effect of the dopexamine?

Dr. T.V. Thomas. That will be one of our future areas of investigation. We plan to perform tests using direct intramesenteric arterial injections of the drug as well as antagonists.

Dr. Schirmer. How far away from being able to use this in humans are you?

Dr. Thomas. It has been used in Great Britain for several years in adults with acute congestive heart failure. I am not sure how far away we are from clinical approval in this country.

Dr. L. Cicalese (Pittsburgh, Pa.). I realize that you maintain blood flow in the mesenteric vessels and you examine the animals only after 30 minutes of reperfusion, which may be too early to detect any histologic change, but did you look at free radical production or infiltration of neutrophils?

Dr. Thomas. We plan to examine infiltration of neutrophils with our next phase of the study. Of the background studies that I mentioned, several looked at the histology of intestinal segments after exposure to hypoxia of varying degrees. Histologic changes of vascular congestion were seen after as little as 15 minutes of hypoxia.

Tumor Necrosis Factor-Alpha Regulates Inducible Nitric Oxide Synthase Gene Expression in the Portal Hypertensive Gastric Mucosa of the Rat

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Increased expression of both nitric oxide synthase (NOS) and tumor necrosis factor-alpha (TNF-α) have been implicated in the hyperdynamic circulation of portal hypertension. Since overexpression of these proteins would affect gastric mucosal defenses, which are impaired in portal hypertension, we examined the expression and interrelationships of TNF-α and NOS in the gastric mucosa of portal hypertensive rats. Following staged portal vein ligation, gastric strips from portal hypertensive rats were incubated in organ culture medium with or without TNF-α antibody. The expression of TNF-α and NOS mRNAs was assessed by reverse transcription–polymerase chain reaction (RT-PCR) at baseline and after 1, 2, and 6 hours of incubation. RT-PCR demonstrated a threefold increase in inducible NOS mRNA and a 50% increase in TNF-α mRNA expression at baseline in portal hypertensive animals as compared to sham-operated animals. In tissue incubated with TNF-α neutralizing antibody, inducible NOS mRNA expression was significantly decreased by 40%, 70%, and 80% after 1, 2, and 6 hours, respectively. Since increased TNF-α and NOS production could potentially impair gastric mucosal defenses, our findings suggest a major role for these proteins in the development of portal hypertensive gastropathy. (J GASTROINTEST SURG 1997;1:371-376.)

Upper gastrointestinal hemorrhage from abnormal gastric mucosa (gastropathy) has been recognized as a major cause of morbidity and mortality associated with portal hypertension.[1,2] Several studies have illustrated the increased susceptibility of the portal hypertensive (PHT) stomach to injury and have delineated the ultrastructural and microvascular changes (vasculopathy) in the PHT gastric mucosa.[3-5] These changes include marked endothelial cell hypertrophy and reduced capillary luminal area. The resultant hypoxygenation of the luminal surface of PHT gastric mucosa renders it highly susceptible to injury.[3,6,7] The mechanism responsible for endothelial abnormalities in the PHT gastric mucosa remains unknown.

Tumor necrosis factor-alpha (TNF-α) is an important mediator of inflammation, which is produced by mononuclear cells in response to tissue injury, bacterial endotoxin, and neoplasia.[8,9] Recent studies have implicated TNF-α as a major mechanism responsible for the development of the hyperdynamic circulation associated with portal hypertension.[10] Treatment with TNF-α neutralizing antibody was shown to reverse the hyperdynamic state in PHT rats.[10] In addition, factors that promote TNF-α synthesis increase the susceptibility of gastric mucosa to damage, whereas TNF-α inhibitors have the opposite effect.[11,12]

Although the precise mechanism of action of TNF-α in the gastric mucosa remains unknown, numerous in vitro studies have demonstrated that this cytokine exerts its effects through upregulation of the enzyme nitric oxide synthase (NOS) resulting in release of the potent vasodilator nitric oxide (NO).[13] Generation of NO is an important factor in modulating gastric mucosal blood flow and a key element in mucosal defenses.[14] Although the effects of NO at low levels are cytoprotective,[15] excessive NO production has cytotoxic potential and has been shown to increase gastric mucosal injury.[16,17] Numerous investigators have demonstrated increased NOS activity in PHT models and have suggested that increased re-

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lease of NO may be responsible for the hyperdynamic state.[18,19] Recently El-Newihi et al.[20] reported that gastric mucosal NOS activity and NO production are significantly increased in cirrhotic patients with PHT gastropathy compared to normal control subjects. In addition, we have demonstrated that NOS mRNA and protein are overexpressed in the PHT gastric mucosa and that moderate inhibition of the increased NOS activity by N^{ω}-nitro-L-arginine methyl ester normalizes ethanol-induced gastric mucosal injury in portal hypertension.[21,22] Therefore understanding the interactions of TNF-α and NOS in PHT gastric mucosa may lead to a better understanding of PHT gastropathy and vasculopathy, forming the basis of the present study.

To elucidate the interrelationship between TNF-α and NOS in the PHT gastric mucosa, we examined baseline expression of TNF-α and NOS mRNAs in PHT gastric mucosa and studied the effect of neutralizing TNF-α in organ culture on the regulation of NOS mRNA production.

MATERIAL AND METHODS

This study was approved by the subcommittee for animal studies of the Long Beach (Calif.) Department of Veterans Affairs Medical Center. Seventy male Sprague-Dawley rats (weight range 250 to 275 g) were used in this study. Rats were maintained individually in wire bottom cages with free access to standard rat chow (Rodent Diet No. 8504, Harlan Teklad, Madison, Wis.) and water. The room where the animals were housed was maintained on a 12-hour light-dark cycle. The room temperature was maintained at 18° to 22° C and the humidity at 60% to 70%.

Portal hypertension was produced in 35 rats that were anesthetized with Nembutal (50 mg/kg intraperitoneally) (Abbot Laboratories, North Chicago, Ill.) and subjected to staged portal vein occlusion accompanied by splenic vein ligation as previously described.[6] Thirty-five sham-operated (control) rats underwent laparotomy with exploration of the splenic and portal veins without ligation of these structures. After 2 weeks, the animals were fasted for 24 hours and anesthetized with Nembutal (50 mg/kg intraperitoneally) followed by laparotomy. Portal venous pressure was measured by cannulation with a PE-50 catheter through a peripheral mesenteric vein. The zero reference point was the inferior vena cava. After measurement of pressure, the stomach was removed and opened along the greater curvature. The forestomach and antrum were discarded, and the remainder of the specimen was cut into small strips (0.5 × 0.5 cm), which were used in all incubation experiments. Samples were incubated in organ culture medium composed of Dulbecco's modified Eagle's medium (90%) (Mediatech, Inc., Herndon, Va.), fetal bovine serum (10%) (Gemini Bioproducts, Calabasas, Calif.), and antibiotic/antimycotic solution (penicillin G, 10,000 U/ml; amphotericin B, 25 mg/ml; and streptomycin, 1000 U/ml) (Mediatech, Inc.). Falcon 3043 organ culture wells were purchased from Becton Dickinson (Lincoln Park, N.J.). One group of wells contained 4 ml of culture medium solution previously described. To a second group, TNF-α neutralizing antibody (Genzyme, Cambridge, Mass.) was added in excess (5000 U/ml). Gastric mucosal strips (6 samples/well) were incubated at 37° C in 5% carbon dioxide. Samples were removed from the incubator at 1-, 2-, and 6-hour time intervals.

RNA Isolation and Reverse Transcription–Polymerase Chain Reaction for NOS and TNF-α

Gastric specimens were imediately frozen in liquid nitrogen and stored at −80° C. Samples were homogenized with a Polytron homogenizer (Kinematica AG, Littau, Switzerland) in 4 mol/L guanidinium isothiocyanate, and total RNA was prepared after guanidinium isothiocyanate-phenol-chloroform procedure as previously described.[23] The total RNA concentration of each sample was determined from absorbance at 260 nm, and the quality of each RNA preparation was determined by agarose-formaldehyde gel electrophoresis and ethidium bromide staining.

Reverse transcription and polymerase chain reactions (RT-PCR) were carried out using a GeneAmp RNA polymerase chain reaction (PCR) kit and a DNA thermal cycler (Perkin-Elmer Corp., Norwalk, Conn.) according to standard techniques.[24,25] Briefly, 0.3 μg of total RNA was used as the template to synthesize complementary DNA with 2.5 units of Moloney murine leukemia virus reverse transcriptase, in 10 μl of buffer containing 10 mmol/L Tris-HCl, pH 8.3; 50 mmol/L KCl; 5 mmol/L $MgCl_2$; 1 mmol/L each deoxyribonucleoside triphosphate; 2.5 mmol/L of random hexamer; and 1.4 unit/ml of ribonuclease inhibitor. Reverse transcription was performed at room temperature for 20 minutes, then at 42° C for 15 minutes, at 99° C for 5 minutes, and 5° C for 5 minutes. The resulting complementary DNA was used as a template for subsequent polymerase chain reaction. The PCR was performed in 50 μl of buffer containing 10 mmol/L Tris-HCl, pH 8.3; 2 mmol/L $MgCl_2$; 50 mmol/L KCl; 0.2 mmol/L each deoxyribonucleoside triphosphate; 0.25 μg of each primer; and 1.2 units of *Taq* DNA polymerase. The amplification was performed for 34 cycles of 1 minute

at 94° C for denaturing, 1 minute at 63° C for annealing, and 2 minutes at 72° C for extension. The primers for constitutive NOS (cNOS) were defined by the following complementary DNA base sequences[26]: sense, 5′-TACGGAGCAGCAAATCCAC-3′; antisense, 5′-CAGGCTGCAGTCCTTTGATC-3′. The primers for inducible NOS (iNOS) were defined by the following complementary DNA base sequences[27]: sense, 5′-CACAAGGCCACATCGGATTTC-3′; antisense, 5′-TGCATACCACTTCAACCCGAG-3′. The primers for TNF-α were defined by the following complementary DNA sequences[28]: sense, 5′-TACTGAACTTCGGGGTGATTGGTCC-3′; antisense, 5′-CAGCCTTGTCCCTTGAAGAGAACC-3′. PCR of β-actin served as a positive control and an internal standard. The primers for β-actin were defined by the following complementary DNA base sequences[29]: sense, 5′-TTGTAACCAACTGGGACGATATGG-3′; antisense, 5′-GATCTTGATCTTCATGGTGCTAGG3′. Nine-microliter aliquots of the PCR amplified mixture were subjected to electrophoresis on 1.5% agarose gel and DNA was visualized by ethidium bromide staining. Location of the predicted PCR products (base pairs) was confirmed by using a 100-base pair ladder (GIBCO BRL, Gaithersburg, Md.) as a standard size marker. The gel was then photographed under ultraviolet transillumination.

For quantitation we determined the intensity of PCR products on the negative film of the gel photographs according to the method of Griffins et al.[27] Expression of the products was quantified using a video image analysis system (Image-1/FL, Universal Imaging Corp., Westchester, Pa.). An index of messenger RNA expression [Index = Area × Intensity] was determined for each sample. The index of NOS and TNF-α signals was standardized against that of the β-actin signal from the same RNA and expressed as the NOS or TNF-α/β-actin ratio.

Statistical Analysis

Results were expressed as mean ± standard deviation. Student's *t* test was used to determine the statistical significance between PHT and sham-operated rats. One-way analysis of variance was used to determine the sequential RT-PCR data.

RESULTS

The gross appearance of the stomach in sham-operated animals was normal in contrast to the PHT rats in which numerous collateral vessels could be visualized around the markedly dilated serosa, especially at the gastroesophageal junction.

Portal Pressure

Portal venous pressure in PHT animals was significantly higher as compared to sham-operated animals (22.9 ± 2.1 vs. 16.0 ± 1.6 cm H_2O, respectively; P <0.01).

Reverse Transcription–Polymerase Chain Reaction for iNOS, cNOS, and TNF-α

Baseline cNOS mRNA expression was increased by 75% (P <0.01) in the PHT group as compared to the sham-operated group. In PHT animals, RT-PCR revealed increases in both TNF-α and iNOS mRNA expression as compared to the sham-operated group, the relative increases being 50% and 300%, respectively (P <0.01; Figs. 1 and 2). In PHT gastric mucosal strips incubated with TNF-α neutralizing antibody, iNOS mRNA expression was significantly re-

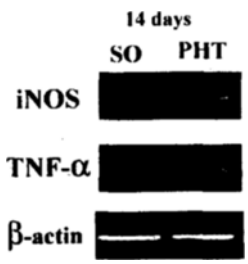

Fig. 1. mRNA expression for TNF-α and iNOS with RT-PCR at 14 days after portal vein ligation and sham operation. SO = sham operated rat; PHT = portal hypertensive rat.

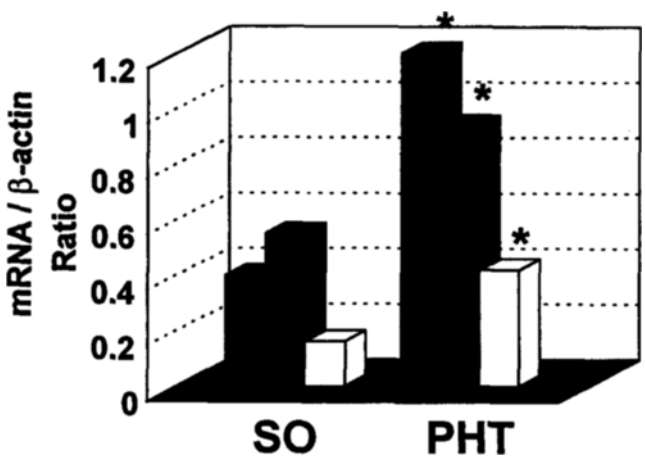

Fig. 2. Relative amounts of iNOS (■) cNOS (▨), and TNF-α (□) mRNAs quantified by the Image-1 system and expressed as the mRNA/β-actin ratio. Values are in mean video image units. SO = sham-operated rats; PHT = potal hypertensive rats; *P = <0.01 compared to the SO group.

Fig. 3. mRNA expression for iNOS with RT-PCR following incubation of PHT gastric strips in culture medium alone (−) or with TNF-α antibody (+) at 0, 1, 2, and 6 hours.

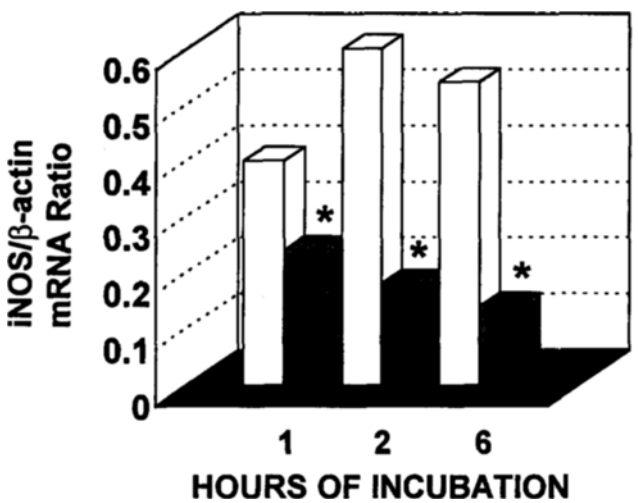

Fig. 4. Relative amounts of iNOS mRNA in PHT gastric strips incubated in culture medium alone (□) or in the presence of TNF-α antibody (▨), quantified by the Image-1 system and expressed in mean video image units. *P = <0.05 compared to culture medium alone.

duced at all time intervals as compared to gastric mucosal strips incubated in culture medium alone, the incremental decreases being 40%, 70%, and 80% after 1, 2, and 6 hours of incubation, respectively (P <0.05; Figs. 3 and 4). No significant change in cNOS or TNF-α mRNA expression was observed at any time interval following incubation with TNF-α antibody.

DISCUSSION

The present study demonstrates that both TNF-α and NOS mRNAs are overexpressed in the PHT gastric mucosa when examined in isolated gastric strips incubated in culture medium. It is important to note that by neutralizing TNF-α with its appropriate antibody added to the culture medium, iNOS mRNA expression is downregulated. This reduction of iNOS mRNA expression was the most marked after 6 hours' incubation as compared to 1 and 2 hours, and the effect seems to correlate directly with the length of time that the tissue was exposed to TNF-α antibody. Since the experiments were performed in isolated gastric strips, this finding implies that the interrelationship of TNF-α with iNOS in the gastric mucosa is direct and independent of vascular or central neurogenic influences.

In previous studies, quantitative ultrastructural analysis of the PHT gastric mucosa microvascular endothelial cells demonstrated marked abnormalities—collectively termed PHT gastric mucosal vasculopathy.[4,5] The endothelia have expanded cytoplasm, increased numbers of pinocytotic vesicles, and the capillary basement membranes are markedly thickened.[4] Because of the capillary luminal narrowing, we found that the PHT gastric mucosal surface—but not serosa—has significantly reduced oxygenation compared to sham-operated control rats,[7] similar to what is now found in PHT humans.[30,31] This chronic hypooxygenation may be the basis for impaired mucosal defense with increased susceptibility of PHT gastric mucosa to damage—a characteristic feature of gastric mucosal gastropathy.

TNF-α is a cytotoxic polypeptide produced by mononuclear cells activated by damaged tissue, tumor cells, and bacterial endotoxin. It is a mediator of the host response to sepsis and neoplasia. It increases vascular permeability, causing structural and metabolic changes in vascular endothelial cells.[8,9] It enhances macrophage attachment to endothelial cells, promoting the inflammatory response to tissue injury and infection. This protein also induces hypotension as a result of stimulating NO production.[32] Relevant to the role of TNF-α in PHT gastropathy and vasculopathy, there are several major lines of evidence that must be considered:

1. Recent studies have implicated this cytokine as a major contributor to the hyperdynamic circulation associated with portal hypertension in prehepatic PHT rats.[10]
2. Patients with portal hypertension and cirrhosis produce higher amounts of TNF-α.[33-35]
3. Factors that enhance TNF-α synthesis and/or release increase gastric mucosal susceptibility to injury, whereas those that inhibit its release (e.g., pentoxifylline) reduce gastric mucosal injury.[11,12]
4. TNF-α produces ultrastructural microvascular abnormalities in pulmonary microvessels that are similar to the vasculopathy of the PHT stomach that we have demonstrated.[4,36]

5. Also TNF-α increases vascular permeability and produces tissue edema, findings that are consistently found in PHT gastric mucosa.[4,8]
6. Our demonstration of the increased susceptibility of the PHT gastric mucosal microvasculature to damage is relevant in light of findings that TNF-a induces structural and metabolic injury to endothelial cells.[3,8,9]

The preceding evidence strongly supports the hypothesis that the mechanism leading to PHT gastric mucosal vasculopathy and gastropathy is activation of the gene for TNF-α with overproduction of its protein. However, the effects of TNF-α may be mediated by the NOS system. We have demonstrated that NOS mRNA and protein are overexpressed in the PHT stomach, and moderate inhibition of the increased NOS activity normalizes increased susceptibility of PHT gastric mucosa to damage.[21,22] In addition, a recent study demonstrated that thalidomide, which is a selective inhibitor of TNF production, decreases TNF-α plasma levels and NO production, ameliorating the systemic hyperdynamic circulation of portal hypertension.[37] Therefore our demonstration of the overexpression and close interaction of TNF-α and iNOS would suggest that these proteins in PHT gastric mucosa may be major factors in the development of the mucosal microvasculopathy and gastropathy in portal hypertension.

CONCLUSION

Our study demonstrated that portal hypertension activates the TNF-α and iNOS mRNAs in the PHT gastric mucosa and that overexpression of iNOS mRNA is significantly inhibited by anti–TNF-α neutralizing antibody. Since increased TNF-α and NOS production could potentially impair gastric mucosal defense, our findings suggest that these proteins play a major role in the development of PHT gastric mucosa.

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Selective Management of Hepatic Venous Outflow Obstruction

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To describe the outcome of selective management of hepatic venous outflow obstruction (HVOO), based on its presentation and liver function, we reviewed the records of 49 consecutive patients managed at our institution between 1984 and 1993. Twenty-six patients were managed surgically, 12 nonsurgically, and 11 were not treated. Portosystemic shunts (PSS) were performed in 18 patients (patency 83%). Two patients (11%) died postoperatively, 11 (61%) did well (mean follow-up 6.4 years), three (17%) required subsequent orthotopic liver transplantation, and two (11%) died of late liver failure. PSS remained patent if the preoperative pressure gradient between the portal vein and the infrahepatic inferior vena cava was greater than 10 mm Hg and across the intrahepatic inferior vena cava 18 mm Hg or less. All six orthotopic liver transplantations (three as primary treatment and three after failed PSS) were successful (mean follow-up 4.8 years). Five patients underwent other procedures. Nine (75%) of the 12 nonsurgically treated patients did well (mean follow-up 3.8 years). The most important predictor of successful outcome after PSS or medical management was the degree of liver function. All 11 untreated patients died either of end-stage liver failure (n = 7; 63%) or of severe comorbid disease (n = 4; 37%). In patients with preserved liver function, medical management of HVOO can be successful early in the course of the disease; a late presentation necessitates PSS. Orthotopic liver transplantation should be employed in patients with liver failure and may decrease the high mortality rate of HVOO. (J GASTROINTEST SURG 1997;1:377-385.)

Hepatic venous outflow obstruction (HVOO), historically referred to as the Budd-Chiari syndrome, is a complex clinicopathologic disease of variable etiology, characterized by thrombosis of the hepatic veins[1] and associated with progressive hepatocellular dysfunction. It is rare in the general population; patients with HVOO comprise less than 5% of those operated on for portal hypertension[2] and approximately 1% of those undergoing liver transplantation.[3] Regardless of the mechanism of HVOO, increased postsinusoidal resistance to hepatic blood flow causes centrilobular venous congestion, intrahepatic hypertension, and hepatocellular injury. Clinical evidence suggests that severity of hepatic dysfunction is probably related to the rapidity of onset and extent of HVOO.[4] Abrupt, complete HVOO leads to extensive hepatocellular necrosis and fulminant hepatic failure. In contrast, insidious, incomplete HVOO may be associated with portal decompression through hepatovenous collateral vessels and reduced hepatocellular injury. This pathophysiologic concept of HVOO suggests a broad clinical spectrum of disease.

We have been impressed by the variability in clinical presentation and the unpredictability of progression of HVOO. Consequently the management of patients with HVOO has been difficult. Numerous treatment options ranging from diuretic alone to orthotopic liver transplantation have proved effective, but few reports have evaluated an unselected patient cohort to assess the disease spectrum and outcome.[5-7] Although HVOO is being diagnosed with increasing frequency,[8] its low incidence makes a prospective study unrealistic. Thus our aims were to review the spectrum of clinical presentation and treatment of HVOO, to characterize factors associated with disease severity and chronicity, to determine the long-term clinical outcome at a referral center, and to propose an algorithm for clinical management.

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PATIENTS AND METHODS

The records of all patients with the diagnosis of HVOO, who were managed at the Mayo Clinic between March 1984 and December 1993, were reviewed. The diagnosis of HVOO was based on the absence of blood flow in the major hepatic veins as demonstrated by imaging. Patients' age, sex, symptoms and signs, liver tests, imaging studies, etiology of the disease, treatment, hospital stay, outcome, and quality of life were recorded. The time interval between the onset of symptoms and the diagnosis was estimated, and liver function was assessed by the Child-Pugh classification at the time of diagnosis and at the last follow-up examination. Preoperative liver biopsies obtained from all patients undergoing postsytemic shunt (PSS) or orthotopic liver transplantation (OLT) were correlated with the outcome of PSS. HVOO was documented by duplex ultrasonography, contrast-enhanced CT, or both. Preoperative hepatic angiography was performed selectively, complemented by manometry. Pressures in the suprahepatic and infrahepatic inferior vena cava (IVC) and hepatic vein (HV) were measured directly, and wedge HV pressure was used to estimate portal vein (PV) pressure. Patency of PSS was determined by duplex ultrasonography or angiography.

Treatment was classified as supportive, medical, or surgical based on the major focus of management. Treatment outcome was assessed by clinical performance status and hepatic function. Follow-up was complete to death or August 1995 and was based on the last office visit or standardized telephone interview. Statistical comparisons were made using Fischer's exact test or Pearson's chi-square test. Survival was estimated by the Kaplan-Meier method.

RESULTS

Between 1984 and 1993, 49 patients were diagnosed with HVOO at our institution. There were 18 men and 31 women (male:female ratio 1:1.7). The mean age was 43 years (range 17 to 73 years).

Clinical Presentation

Most patients presented with ascites (86%), followed by hepatomegaly (69%), lower extremity edema (55%), right upper quadrant abdominal pain and fatigue (51% each), jaundice (39%), and splenomegaly (24%). Encephalopathy (18%) and hemorrhage (8%) were less common. The exact time of onset of HVOO could only be estimated because clinical presentation was nonspecific. Eighteen patients had an acute onset (less than 6 weeks)[7] with the clinical findings of fulminant hepatic failure. Typically a prodrome of ascites and dull abdominal pain of increasing severity prompted evaluation.

We indentified a definite or probable etiology in 44 (90%) of our patients (Table I). A hypercoagulable state, including polycythemia rubra vera and paroxysmal nocturnal hemoglobinuria, was considered causative in 53% of patients. Use of oral contraceptive

Table I. Etiology of HVOO

		No. of patients	%
Hypercoagulable state		26	53
Polycythemia rubra vera	(n = 7)		
Paroxysmal nocturnal hemoglobinuria	(n = 5)		
Protein C deficiency	(n = 4)		
Lupus anticoagulant	(n = 4)		
Myeloproliferative disorder	(n = 4)		
Idiopathic	(n = 2)		
Cirrhosis		4	8
Webs		3	6
Cancer		3	6
Systemic lupus erythematosus		2	4
Polycystic liver disease		2	4
Pregnancy		2	4
Oral contraceptives*		1	2
Congestive heart failure		1	2
Unknown		5	10
		49	100

*Cofactor in three additional patients in a hypercoagulable state.

steroids was a cofactor in three patients with a concurrent hypercoagulable state but in only one patient without recognized coagulopathy. HVOO was caused by severe comorbid conditions in four patients: end-stage intrahepatic malignancy in three and severe cardiomyopathy (left ventricular ejection fraction 10%) in one. The cause was unknown in only five patients (10%).

Duplex ultrasound examination of the hepatic veins was diagnostic in 39 (87%) of 45 patients and contrast-enhanced CT in 24 (90%) of 27 patients. Angiography confirmed HVOO by demonstrating obstruction of major hepatic veins, webs, or a "spider web" injection pattern in 33 patients. Manometry confirmed HVOO with an elevated wedge-free HV pressure gradient in 21 patients. In the remaining 12, technical factors precluded wedge measurements. All patients who were treated for HVOO underwent angiography.

Liver biopsy was performed in 34 patients before treatment. Dilatation or congestion was present in nine patients (26%), fibrosis or necrosis in 21 (62%), and cancer or normal tissue in two each (6%). Liver function at the time of diagnosis was classified as Child-Pugh class A in 23 patients (47%), class B in 16 (32%), and class C in 10 (20%).

Management

The 49 patients were categorized into four groups for assessment of therapy: surgical (n = 26), medical (n = 12), supportive (n = 7), and severe comorbidity (n = 4) (Table II).

Surgical Management. Twenty-six patients underwent an operation for treatment of HVOO. PSS was performed in 18 patients. Fifteen patients had side-to-side portocaval shunts (PCS), one patient had a portocaval-cavoatrial shunt (PC-CAS) with spiral saphenous vein graft because of juxtahepatic IVC occlusion, and one patient underwent dorsocranial liver resection with hepatoatrial anastomosis (Senning procedure) and pulmonary valve replacement because of thrombosis extending into the suprahepatic IVC. One patient had a mesocaval shunt with ringed polytetrafluoroethylene graft because of PV thrombosis. Two patients (11%) died postoperatively: one of sepsis and one of hepatorenal failure from IVC thrombosis and shunt occlusion. The mean duration of symptoms was 100 ± 17 days. Fifteen patients were treated by PSS only; in three patients (17%) initially treated by PSS, treatment failed and these patients were converted to OLT. PSS failure was caused by fulminant hepatic failure in one Child-Pugh class C patient (immediately postoperatively) and by shunt thrombosis in two patients (1 week and 1 year postoperatively).

Table II. Management of patients with HVOO

		No. of patients
Surgical treatment		26
PSS as only treatment	(n = 15)	
OLT as primary treatment	(n = 3)	
OLT after PSS	(n = 3)	
Right hepatectomy	(n = 1)	
LeVeen/Denver shunt	(n = 4)	
Medical treatment		12
Anticoagulation, steroids, etc.	(n = 10)	
TIPS	(n = 1)	
Angioplasty, thrombolysis	(n = 1)	
Supportive care		7
Severe comorbidity		4
		49

Early patency of PSS was 83% and was predicted by preoperative PV-HV and infrahepatic-suprahepatic IVC pressure gradients. Two patients with early thrombosis of PCS who eventually underwent OLT had an infrahepatic-suprahepatic IVC pressure gradient of 19 mm Hg or greater. All remaining 13 patients had a portocaval pressure gradient (wedge HV pressure minus the infrahepatic IVC pressure) of 10 mm Hg or greater, and a transhepatic IVC pressure gradient (infrahepatic minus suprahepatic IVC pressure) of 18 mm Hg or less during preoperative manometry, and their shunts remained patent. Thrombosis of a PC-CAS occurred in an additional patient 1 year postoperatively because of extensive thromboses secondary to hypercoagulability and despite aggressive anticoagulant therapy.

Thirteen patients with PSS as the only and definitive therapy were discharged after initial hospitalization. Mean duration of hospitalization was 11 days (range 6 to 33 days). Mean postoperative follow-up has been 6.4 years (range 1.5 to 11.8 years). Two patients (11%) died of late liver failure (after 1.9 and 9.3 years, respectively), despite confirmation of shunt patency by duplex ultrasonography at last follow-up. Liver function in the remaining patients was well preserved and remained the same as that determined preoperatively (9 patients, 82%) or improved (2 patients, 18%). The two patients with late liver failure were Child-Pugh class A and B initially. Overall 5-year actuarial survival was 79%. The 5-year estimate of treatment success of PSS (including patients with failed PSS who were converted to OLT) was 66%. All survivors were asymptomatic at last follow-up and had an excellent performance status (Eastern Conference

Oncology Group [ECOG] = 0). Shunt patency has been confirmed by Doppler ultrasonography, angiography, or both in these patients.

Histopathologic findings from liver biopsies were not predictive among patients undergoing PSS. In two patients centrilobular congestion or dilatation was noted on preoperative liver biopsy and 13 had necrosis or fibrosis. The four patients who died after PSS (early and late death) belonged to the latter group, but fibrosis and necrosis did not prove to have a statistically significant correlation with early ($P = 0.55$), late ($P = 0.5$), or overall ($P = 0.36$) shunt patency and survival.

Four patients had peritoneovenous shunts (two LeVeen and two Denver shunts) for palliation of ascites. Other surgical options were precluded because of extensive portovenous and suprahepatic and infrahepatic caval thrombosis, or both, in two patients and refusal of OLT despite liver failure in one. These three patients died of liver failure at 1, 8, and 21 months, respectively. Interestingly, although PSS was precluded in the fourth patient because of extensive portovenous thrombosis, therapy for her myeloproliferative disorder with hydroxyurea and anticoagulation has been associated with a survival of 9.8 years. Hepatic veins have partially recanalized and liver function has returned to normal. Finally, one additional patient underwent left hepatectomy and cyst fenestration for polycystic liver disease causing HVOO and remains well 1.8 years postoperatively.

Six patients underwent OLT: three as initial treatment and three after failure of PSS as previously mentioned. Long-standing HVOO and poor hepatocellular function (Child-Pugh class B or C) was the indication for OLT in the first three patients. Fulminant hepatic failure and shunt occlusion (as previously described) necessitated the remainder. Liver biopsy demonstrated fibrosis or necrosis in all of these patients. However, clinical status and not histopathologic findings prompted OLT. Mean duration of symptoms before treatment was 78 ± 32 days. There were no perioperative deaths. Mean duration of hospitalization was 32 days (range 12 to 74 days). Mean postoperative follow-up has been 4 years (range 1.2 to 6.8 years). There was one late death from an unrelated cause (intravenous drug abuse) at 2.1 years. Overall 5-year actuarial survival was 75%. All patients had excellent liver function at follow-up (Child-Pugh class A) and an excellent clinical performance status (ECOG = 0). Although the patient who underwent OLT after thrombosis of the PC-CAS had a recurrence of ascites and lower extremity edema after OLT, as a result of thromboses of the superior mesenteric vein and infrarenal IVC, hepatic allograft function is normal.

Medical Management. Twelve patients had medical (nonoperative) treatment. Mean duration of symptoms in these patients at diagnosis was 41 ± 11 days. Most of these patients had recent-onset HVOO and preserved liver function. Eleven patients were Child-Pugh class A. One patient (Child-Pugh class C) who had HV and IVC thrombosis underwent a transjugular intrahepatic portosystemic shunt (TIPS) and placement of an expandable metallic stent in the IVC. Despite initial improvement with upgraded liver function to class B and hospital discharge, he died of sepsis 3 months later (early mortality rate 8%). One patient with IVC web was successfully treated with angioplasty and thrombolysis and remains asymptomatic with a patent IVC after 2.7 years. Ten patients have been managed with anticoagulation (n = 6), steroids (n = 4), diuretics (n = 4), or combinations (n = 5). All were discharged after a mean hospitalization of 10 days (range 0 to 40 days) with excellent liver function and resolution of symptoms.

Mean follow-up after discharge of these patients has been 3.8 years (range 0.3 to 10.3 years). Nine patients (75%) are alive, asymptomatic, and had excellent liver function at last follow-up. Their clinical performance status is normal. Two patients (17%) who had clinical progression of HVOO were not referred for surgical management and died of liver failure after 1.8 and 2.3 years respectively. Overall, medical management in this select group of patients led to a 92% initial success rate, and 75% are alive and well after a mean of 3.8 years. The 5-year actuarial survival was 71%.

Supportive Care. Seven patients presented with early HVOO and severe hepatocellular dysfunction (Child-Pugh class C). Mean duration of symptoms at presentation was 44 ± 15 days. All patients had encephalopathy and four had upper gastrointestinal hemorrhage. One patient had undergone OLT twice previously for primary biliary cirrhosis. One patient died of uncontrollable esophageal variceal bleeding on admission. Attempted thrombolysis using tissue plasminogen activator in one patient and anticoagulation in two patients was unsuccessful. Severe liver insufficiency with refractory coagulopathy prohibited PSS. Three patients were activated for OLT but died before a donor became available. The hospital course of these patients evolved rapidly with hepatorenal syndrome, multiple system organ failure, and death within 15 days of admission (mean 9 days).

Severe Comorbidity. In four patients HVOO was a

complication of other severe underlying disease (end-stage cancer in three and severe congestive heart failure in one). Treatment targeted specifically for HVOO was not implemented. All four died within 18 months (median 7 months) after diagnosis.

CORRELATION OF CLINICAL PRESENTATION AND MANAGEMENT

We stratified outcome on the basis of the timing of clinical presentation (early vs. late) and liver function (preserved vs. decompensated) to attempt to identify distinct subgroups for a management algorithm.

Early HVOO, Preserved Liver Function. Eleven of 12 patients treated medically belong to this subgroup. All were Child-Pugh class A. Medical management was associated with 100% early and 82% (9 of 11 patients) late success. Overall 5-year actuarial survival was 78%. Although this approach was effective for these patients, careful long-term management is essential. HVOO will progress in some patients and should prompt evaluation for surgical therapy.

Early HVOO, Decompensated Liver Function. The seven patients who received supportive care only and the patient who underwent TIPS and IVC stent placement comprise this group. Liver function deteriorated rapidly and all were Child-Pugh class C at diagnosis. All of these patients died. Although radiologic intervention was temporarily successful in one patient, only OLT offers definitive treatment for fulminant hepatic failure associated with HVOO. Medical and radiologic intervention in these patients should be used only to prepare them for OLT.

Late HVOO, Preserved Liver Function. Seventeen patients who were Child-Pugh class A (n = 11) or class B (n = 6) and who underwent PSS comprise this group. Excluding one patient who died of sepsis and two patients with PCS occlusion from unfavorable pressure gradients, the remainder fared well. Five-year actuarial survival was 79%. The duration of symptoms was longer in this group than in the group with early HVOO and preserved liver function (100 $\pm$ 17 days vs. 41 $\pm$ 11 days; $P = 0.01$). However, despite shunt patency, there were two late deaths from liver failure. PSS clearly provides effective therapy in most of these patients, but preservation of liver function is not ensured indefinitely and careful long-term observation is warranted.

Late HVOO, Decompensated Liver Function. Four patients comprise this subgroup and underwent OLT: three as their initial treatment and the fourth with fulminant hepatic failure after PCS. All were Child-Pugh class C. OLT has been associated with excellent liver function and quality of life after a mean follow-up of 4 years (range 1.2 to 6.8 years). OLT for patients with end-stage liver disease provides the only definitive therapy.

DISCUSSION

We have analyzed 49 consecutive patients with HVOO seen at the Mayo Clinic over a 10-year period. By careful examination for abnormal hematologic conditions and other risk factors, we were able to identify a likely cause in 90% of our patients. Medical treatment was successful only in patients with early disease and preserved liver function. Patients with chronic disease and preserved liver function were successfully treated by PSS. OLT was the only treatment for patients with decompensated liver function, whether presentation was early or late. Venography of the perihepatic vasculature with manometry was essential for selection of the appropriate PSS and correlated with shunt patency and patient outcome. Histopathologic findings on liver biopsy, although important, were not associated with outcome. In this unselected cohort of patients with HVOO, the mortality rate was high (45%), but the mortality rate for those who were treated was 29%. Our conclusions are schematically shown in Fig. 1 as a proposed algorithm for diagnosis, staging, and treatment of patients with HVOO.

The etiology of HVOO varies worldwide. Underlying coagulopathies must be carefully defined for ongoing treatment.Despite etiology, early or late presentation is characterized by symptom duration of less than or greater than 6 weeks. Clinical suspicion of HVOO can usually be confirmed by duplex ultrasonography and dynamic CT. Additionally, perihepatic venography and manometry should be used to further characterize the severity of HVOO and identify the best treatment option.

Preoperative bilobar liver biopsy (percutaneous or transjugular) has a central role in the diagnosis and the pathologic staging of HVOO. Centrilobular congestion and dilatation are consistent with a recent obstruction, whereas necrosis and fibrosis are compatible with chronic advanced disease. Although the findings on liver biopsy have been emphasized as the "dominant factor" for selection among the surgical options,[9] our findings did not corroborate this postulate. In contrast, our findings suggested that preservation of hepatocellular function did not correlate with histopathologic findings herein. Our observation is supported by those of others who have demonstrated that hepatocellular function rather than histo-

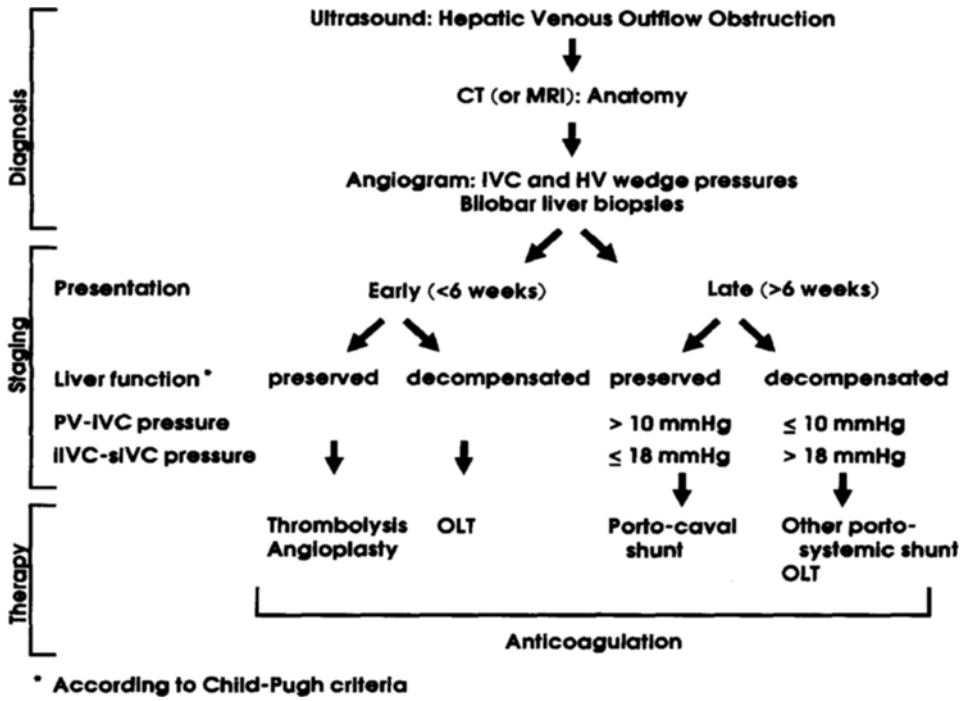

Fig. 1. Suggested algorithm for diagnosis, staging, and treatment of hepatic venous outflow obstruction. CT = computed tomography; HV = hepatic vein; IVC = inferior vena cava; MRI = magnetic resonance imaging; OLT = orthotopic liver transplantation; PV = portal vein.

logic findings should be the critical factor in deciding between PSS and OLT.[10,11]

In our opinion two primary factors determine the choice of therapy: (1) the relative timing between onset and diagnosis of HVOO and (2) the level of liver function and the rate of its deterioration. Patients who present with acute HVOO and fulminant hepatic failure usually deteriorate rapidly to hepatorenal failure. Medical therapy is not successful as illustrated by our seven acutely ill patients with Child-Pugh class C liver function who died within 2 weeks of diagnosis. PSS in this setting not only carries a prohibitive mortality rate but may aggravate the existing hepatic injury and prevent compensation as well. OLT is currently the only option for end-stage liver failure. Indeed we have successfully performed transplantation in one such patient, and other investigators have reported encouraging results with 1-year survival rates of 63% to 68%.[12,13]

Early HVOO with well-preserved and stable liver function at diagnosis is not uncommon and occurred in 25% of our patients. Recent thrombosis without impaired hepatic function may allow medical management directed toward thrombolysis and anticoagulation. Steroids, hydroxyurea, diuretics, or other agents can be useful adjuncts depending on the etiology of HVOO. This approach was used in 11 patients with Child-Pugh class A liver function and was initially successful in all of them. Nine patients (82%) were alive with excellent liver function after a mean of 3.8 years with a 5-year actuarial survival of 78%. Two late deaths from liver failure (1.8 and 2.3 years later) could possibly have been averted by early referral for PSS or OLT at the time of recurrence of symptoms. The often dismal results reported after medical therapy[4,14,15] are likely due to selection of patients with end-stage liver disease. Our data have shown that medical therapy was effective in these patients with early HVOO and well-preserved, stable liver function. Moreover, other nonoperative methods should be considered in patients with a stenotic or occluded segment of the IVC. The combination of TIPS and IVC metallic stent was initially successful in one patient, and angioplasty and thrombolysis comprised the definitive treatment in one of two such patients in our series. Percutaneous balloon angioplasty[16-18] and expandable metallic stents[19,20] have been successful in improving long-term results. TIPS for HVOO has been described[21,22] but the long-term patency of TIPS in patients with hypercoagulability is unknown.

If patients present after several months of symptoms, suggesting a chronic course, the role of medical management is minimal. The main goal of therapy is the restoration of hepatic function. The degree of he-

patic impairment at diagnosis dictates the choice between liver decompression or transplantation. If hepatocellular function is well preserved (Child-Pugh class A or B), PSS can halt deterioration[7,9,10] or even improve function,[23,24] regardless of biopsy findings.[7,10,11] Portal decompression must be nonselective. The choice of shunt is dictated by the anatomy and the preoperative manometric findings of the perihepatic vasculature. PV and IVC patency and appropriate pressure gradients between the IVC and the PV are critical for decision making.

Although perihepatic manometry has been advised prior to PSS for HVOO, limited data have defined the parameters for shunt patency. Our results support a PV-infrahepatic IVC gradient of at least 10 mm Hg.[9,25] Although preoperative gradients exceeding 20 mm Hg have been recommended,[7] flow through the PSS should be adequate with a gradient of 10 mm Hg if postoperative resistance across the PSS is nil. The degree of IVC stenosis acceptable for long-term patency of PCS is less well defined. We found that an infrahepatic-suprahepatic IVC gradient of 18 mm Hg or less was associated with PSS patency. Although other studies failed to show that gradients of 10[23] or 15 mm Hg[11] along the IVC correlated with patency, PCS is not recommended when the IVC gradient exceeds 20 mm Hg.[26]

With appropriate patient selection, PCS has been associated with 80% to 100% early and late patency,[9,10,24,25,27] stabilized or improved liver function,[9,24] 5-year actuarial or actual survival ranging from 57% to 85%,[11,28] and return to normal lifestyle in 75% to 100% of patients.[10,24,25] Stabilization or even regression of the pathologic changes has also been observed.[9,10,24] The perioperative mortality rate however, remains between 5% and 19%[11,24,27] (11% in our series) and is mainly attributed to multiple organ failure secondary to end-stage liver disease or to early shunt thrombosis.[25] We believe that the mortality rate may be lowered by better patient selection (OLT rather than PSS for patients with advanced liver disease) and shunt selection (tailoring the choice of PSS according to pressure gradients). One postoperative death in our series could have been prevented by applying our current criteria. More sophisticated selection may decrease early PSS failure. Conversion to OLT occurred in 17% of our patients, similar to others.[11] Indeed two of our three patients who underwent PSS and were converted to OLT would have undergone OLT or another kind of PSS based on our current assessment of pressure gradients.

If PCS is technically or anatomically precluded by PV thrombosis, an interposition mesocaval shunt is an alternative option. Our one mesocaval shunt (using artificial graft) remains patent with anticoagulation therapy, and liver function is excellent 11.8 years postoperatively. Although Bismuth and Sherlock[23] have reported 90% primary and 100% secondary patency and 95% 5-year actuarial survival after mesocaval shunt, others have reported high thrombosis rates ranging from 23% to 75%,[11,14,26] especially with synthetic conduits. Autogenous vein grafts may lead to improved patency.[11] Routine postoperative anticoagulation has been strongly recommended after PSS,[10,11,13,23,24] especially when prosthetic grafts are used, and has been our standard practice.

The independent venous drainage of the caudate lobe into the IVC leads to its enlargement, which may often have therapeutic implications. First, caudate hypertrophy may compress the IVC and cause infrahepatic venous hypertension, the degree of which in relation to PV pressure may determine the choice of surgical treatment. If the PV pressure exceeds the infrahepatic IVC pressure by more than 10 mm Hg, PSS will decompress the liver. If PV pressure and infrahepatic IVC pressure are similar, decompression is precluded.[11] Second, the presence of the enlarged caudate between the PV and the IVC may technically interfere with construction of the PCS.[27] We and others believe that if both venous structures are extensively and circumferentially mobilized, such difficulty usually will not occur and a side-to-side, tension- and angulation-free PCS can be performed.[24] Using this technique we accomplished a PCS in all 15 patients.

HVOO associated with high-grade stenosis or occlusion of the IVC poses a particularly difficult problem. Hepatic decompression cannot be achieved by PCS alone. Several options have been proposed to bypass the IVC obstruction. We performed a PC-CAS and one dorsocranial liver resection with direct hepatoatrial anastomosis (Senning procedure) to address this specific problem. Although PC-CAS is not commonly performed, excellent early results in a small series of five patients[29] have not been widely reproduced[30] Mesoatrial shunts, although appealing, proved to be associated with very high rates (63% to 67%) of early and late thrombosis[9,24] and low survival (37% to 49%).[24,26] Because mesoatrial shunts or PC-CAS require the use of long prosthetic conduits, which generally tend to thrombose over time,[11] OLT may emerge as the best therapy for this subgroup of patients. The Senning procedure[31] offers a direct solution to the problem of HVOO with segmental occlusion of the juxtahepatic IVC and uniquely decompresses the liver by excising the obstruction. Although very limited experience in 17 cases[32] has been reported, worldwide early results are promising.

OLT has been reserved for patients with severely compromised hepatocellular function. Fibrosis or necrosis in liver biopsy alone, when function is well

preserved, is not an indication for OLT.[10,11] In our experience OLT was associated with no perioperative deaths, 100% 2-year actual survival, and 75% 5-year actuarial survival. Our findings support those of others who reported actuarial survival rates of 69% to 88% for 1 year and 45% to 88% for 3 years.[3,10,33] A normal lifestyle has been resumed by 86%[10] to 100% (herein) of the surviving patients. Although OLT for HVOO carries a perioperative mortality rate of nearly 14%,[10,11,33] it provides the only treatment option in patients with end-stage hepatic failure and/or failed PSS. Because thrombosis of the HVs of the implanted liver can occur in 6% to 19% of patients,[3,11,33] long-term anticoagulation therapy is imperative. Anticoagulation may not be needed if OLT is performed for HVOO secondary to deficiency of antithrombin-3, protein-S, or other proteins that regulate coagulation and are produced in the liver,[3] but this remains unproved.

CONCLUSION

Medical therapy, shunt surgery, and liver transplantation may all be successful in appropriately selected patients chosen on the basis of time of presentation, degree of liver function, and pressure gradients within the perihepatic vasculature. In our opinion these treatment modalities do not compete but instead complement each other. The role of the physician and surgeon is to accurately identify the stage of HVOO in individual patients and to implement the most appropriate therapy.

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Discussion

Dr. M. Henderson (Cleveland, Ohio). I would take issue with your final slide and conclusion. I think one needs to be cautious, even in that early group of patients, that there may be more hepatic dysfunction than is revealed on laboratory tests. My question relates to that early assessment of the liver and the role you see for biopsy for ongoing evidence of hepatocyte necrosis. We found this to be probably the most important factor in dictating early decompression when there was significant ongoing central hepatocellular necrosis. Do you have any information on the role of the biopsy?

Dr. G.G. Tsiotos. We are very well aware of Dr. Henderson's data. In our experience we failed to prove any correlation between histopathologic findings and long-term outcome, either in the group as a whole or in the subgroup with PSS. There was no significant difference in long-term outcome between patients who had either congestion/dilatation or necrosis/fibrosis in liver biopsy, which was bilobar preoperatively in all patients who underwent either PSS, OLT, or aggressive medical therapy.

Dr. W. Meyers (Worcester, Mass.). Were all biopsies performed safely?

Dr. Tsiotos. Yes, there were no complications.

Mechanisms of Insulin-Induced Relaxation of the Canine Proximal Stomach After Proximal Gastric Vagotomy

Hideki Morimoto, M.D., Keith A. Kelly, M.D.

The aim of this study was to determine whether insulin-induced relaxation of the proximal stomach after proximal gastric vagotomy is mediated by vagal release of antral gastrin. In six conscious, fasted dogs following proximal gastric vagotomy, the effects of intravenous insulin (1 U/kg) and intravenous gastrin (1 μg/kg) on proximal gastric motility, as measured by a gastric barostat, on plasma glucose, and on plasma gastrin, as measured by radioimmunoassay, were assessed 1 hour before and for 2 hours after injection. The effects of a cholecystokinin (CCK)-A receptor antagonist and a CCK-B receptor antagonist on insulin-induced or gastrin-induced relaxation of the proximal stomach and on plasma glucose and gastrin were also determined. Intravenous insulin decreased plasma glucose (before [mean ± SD], 97 ± 5 mg/dl vs. after, 45 ± 3 mg/dl; $P < 0.05$), increased plasma gastrin (before, 240 ± 59 pg/ml vs. peak after, 387 ± 85 pg/ml; $P < 0.05$), and relaxed the proximal stomach (100% ± 0% barostat volume vs. 202% ± 15% volume; $P < 0.05$). Exogenously administered gastrin also relaxed the proximal stomach without decreasing plasma glucose. CCK-B blockade diminished, but did not abolish, the gastric relaxation caused by insulin or gastrin, whereas CCK-A blockade had little effect. It was concluded that insulin-induced relaxation of the proximal stomach after proximal gastric vagotomy is mediated, in part, by vagal release of antral gastrin. (J GASTROINTEST SURG 1997;1:386-394.)

Truncal vagotomy is still used by many surgeons to treat gastric and duodenal ulcers, even though it may disturb gastric motility and emptying and result in postsurgical disability. Truncal vagotomy impairs receptive relaxation of the stomach[1] and thus may speed gastric emptying of liquids.[2] Truncal vagotomy sometimes induces ectopic pacemakers in the distal stomach, which can drive gastric contractions in an aborad or reverse direction.[2] Truncal vagotomy also weakens gastric antral peristalsis. The net result is a slowing of gastric emptying of solids. Gastric emptying of solids is so slowed by truncal vagotomy that pyloroplasty is usually performed to combat it. However, pyloroplasty itself has adverse effects on gastric motility and is followed by a 10% to 30% incidence of dumping symptoms and alkaline reflux gastritis.[3]

Proximal gastric vagotomy (PGV) preserves gastric motility and emptying better than truncal vagotomy, results in fewer symptoms, and avoids the need for pyloroplasty. This is likely because PGV maintains antral and pyloric innervation and gastric emptying of solids,[4] whereas truncal vagotomy does not. In addition, PGV may preserve gastric receptive relaxation and gastric emptying of liquids better than truncal vagotomy. Insulin-induced relaxation of the proximal stomach is maintained after PGV.[4] These data suggest that relaxation of the proximal stomach may be mediated not only by vagal efferents to the gastric fundus and corpus, as previously thought,[1] but also by another vagal mechanism preserved by PGV. In this study we investigated the hypothesis that this additional mechanism is the vagal release of antral gastrin, a known relaxant of the proximal stomach.[5-7]

METHODS

The experimental protocol was approved by the Institutional Research Committee and the Institu-

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tional Animal Care and Use Committee of Mayo Clinic Scottsdale, and the following surgical procedures and experiments were performed in accordance with the guidelines of the National Institutes of Health and the Public Health Service, as outlined in the manual, "Guide for the Care and Use of Laboratory Animals."

Preparation of Animals

Six adult mongrel dogs, each weighing between 17 and 24 kg, underwent PGV and gastrostomy. After a 16-hour fast, dogs were anesthetized with methohexital sodium (12.5 mg/kg intravenously), and anesthesia was maintained with mechanical ventilation and a mixture of oxygen and halothane (0.5% to 1.5%) via an endotracheal tube. Through a midline celiotomy under sterile conditions, PGV was performed by dividing the neurovascular bundles to the lesser curvature of the stomach from a point 7 cm orad to the pylorus up to and including the esophagogastric junction.[8] The distal 2 cm of esophagus was mobilized, and all accessory vagal branches from the anterior, posterior, and intermediate vagal trunks to the gastric fundus and corpus were divided, carefully preserving the trunks themselves, the hepatic and celiac branches, and the nerves of Latarjet to the antrum and pylorus. A Thomas cannula with a diameter of 9 mm was then inserted through the anterior gastric wall close to the greater curvature and 10 cm proximal to the pylorus and secured with a pursestring suture. The external end of the cannula was brought to the surface through the anterior abdominal wall and secured with a suture. The Thomas cannula was kept sealed when not in use.

Butorphanol tartrate (0.25 mg/kg) was administered intramuscularly for analgesia just after the operation, on the evening of the operative day, and as needed thereafter. The dogs were allowed liquids by mouth but no solid food for 3 days postoperatively. Parenteral fluids were also given as needed during this period. The dogs were fed a soft diet on postoperative days 4 through 6 and a normal diet thereafter. They were allowed a 2 week recovery period after the operation, prior to beginning the postoperative tests. All dogs were weighed twice weekly. They steadily gained weight during the experiments.

Gastric Acid Output

The adequacy of the proximal gastric vagal denervation was assessed using an insulin stimulation test (1 IU/kg intravenously), during which gastric output of acid was measured. Gastric juice was collected in 15-minute aliquots from the freely draining gastric cannula or an orogastric tube used during the preoperative control tests. To assess the completeness of the collection, a 0.025% phenolsulfonphthalein (PSP) solution, a nonabsorbable marker, was infused at 1 ml/min during the tests through a soft Silastic catheter (2.5 mm in diameter) positioned in the gastric fundus alongside the gastric barostat, as previously described.[9] The concentration of PSP in gastric samples was determined by spectrophotometric analysis, and the amount of PSP recovered was calculated from the volumes and concentrations recovered over time. Portions of the gastric aliquots were titrated against 1.0 mol/L sodium hydroxide to measure the concentration of protons in the sample. The total acid output was calculated from the volume recovered per 15 minutes and the proton concentration, and the value was corrected for the proportion of gastric juice recovered, as determined by the PSP recovery. No or minimal (<2 mEq/15 min) acid output during the first hour after insulin injection was considered to confirm the completeness of PGV.[4] Three dogs were tested before PGV (control) and all six dogs were tested after vagotomy.

Proximal Gastric Motility

Proximal gastric motility was measured using an electronic barostat.[4,10-12] A compliant plastic bag was attached to the distal end of a 16 F plastic tube, the proximal end of which was connected to a rigid bellows containing air. The bag, catheter, and bellows formed a closed system (maximal volume 800 ml). An electronically driven motor continually adjusted the bellows to maintain the air pressure within the system at a value specified by the operator. When placed in the proximal stomach, the air pressure within the system expanded the bag to fill the proximal stomach to the extent that gastric muscular tone allowed. In the standing, awake dog a pressure of 3 mm Hg was sufficient to expand the barostat bag without disturbing gastrointestinal interdigestive motility.[11,12]

Plasma Insulin, Gastrin, and Glucose

Plasma insulin and gastrin levels were measured by radioimmunoassay[13,14] at the Mayo Clinic Research Center, Rochester Minnesota, and plasma glucose levels by a glucose analyzer (One Touch II, Lifescan, Johnson & Johnson, Milpitas, Calif.) at Mayo Clinic Scottsdale.

Conduct of Tests

Each conscious dog underwent repeated insulin and gastrin tests on different days. A minimal 2-day

interval was taken between experiments on a particular dog. After a 16-hour fast, the dogs were placed in a harness-sling. The dogs stood continuously on their forelimbs, while the sling supported the hind limbs without exerting pressure on the abdomen. The gastric cannula was opened and cleaned of any debris that had accumulated in the dead space within the cannula. A pneumograph was placed around the animal's chest. A collapsed barostat bag was introduced through the gastric cannula and positioned in the gastric fundus and proximal corpus. The pressure in the barostat was electronically set and maintained at 3 mm Hg. Continuous measurements of the pneumographic fluctuations and the barostat volume and pressure were made on an electronic pressure recorder. The signals were simultaneously converted to digital signals at 8 Hz and stored on magnetic media to be analyzed later.[4]

The dogs were studied with the preceding apparatus in place for at least 1 hour until the barostat volume became stable. Either insulin from bovine pancreas (Sigma Diagnostics, St. Louis, Mo.) or human synthetic sulfated gastrin-17 (Sigma) reconstituted with saline solution was given intravenously at 1 IU/kg and 1 μg/kg, respectively. These doses of insulin and gastrin were chosen because they have resulted in relaxation of the proximal stomach in previous experiments in intact dogs.[4,5] Cholecystokinin (CCK) receptor antagonists, L-364,718 (Merck & Co., West Point, Pa.)[14,15] and L-365,260 (Merck),[16,17] were reconstituted with 8 volumes percent of glycerol (J.T. Baker, Inc., Phillipsburg, N.J.), 56% polyethylene glycol (E.M. Science, Gibbstown, N.J.), and 36% distilled water, and one or the other was injected at 1 mg/kg intravenously 30 minutes before insulin or gastrin in experiments where the effects of CCK blockade were assessed. These doses were chosen because they have been shown by others to provide effective blockade of CCK receptors.[14-17]

At the beginning of each test, a blood sample (2 ml) was obtained from the cephalic vein for measurement of plasma glucose, insulin, and gastrin levels. Just before the insulin or gastrin injection, a second blood sample was obtained. Every 15 minutes for the following 2 hours, the gastric effluents were collected and sampled for determination of the concentration of PSP and protons, and blood samples were obtained. The recording from the gastric barostat continued over the 2-hour period.

Data Analysis and Statistics

The total amounts of acid produced during the hour preceding the insulin or gastrin injection and during each of 2 hours after insulin or gastrin administration were determined. The barostat volumes were averaged over each successive 15-minute period before and after the injection of insulin or gastrin. The plasma glucose, insulin, and gastrin levels were plotted against time.

The data were averaged across all replications (three times in each dog, except for the CCK receptor blockade experiments, which were performed one time in each dog), and the average measurements for each dog were used to calculate grand means for the six dogs. The results were summarized as mean ± standard deviation based on n = 6 dogs unless otherwise stated. When the baseline measurements obtained from each dog prior to the administration of the experimental conditions varied within a dog, the baseline measurements were used as covariates in the analysis.

The study was designed as a three-factor block design with repeated measures on all three factors: drug injected (insulin vs. gastrin), gastrin antagonist administered (no vs. yes), and time (before vs. after injection). Depending on the behavior of the responses, the measurements for a specific parameter obtained after the injection were analyzed as one individual parameter measurement over time or summarized and analyzed as one individual parameter over a 2-hour period following injection. Each parameter (e.g., gastric acid production or proximal gastric motility) was analyzed using a separate three-factor analysis of variance for repeated measurements to assess the main effect and interaction differences.[18] Specific comparisons of interest were made using Student's t test for paired differences. All P values calculated were two sided, and P values <0.05 were considered statistically significant. The Bonferroni correction for multiple comparisons was applied as appropriate.

RESULTS

Plasma Glucose

Insulin caused a prompt decrease in plasma glucose, whereas gastrin did not. The decrease in plasma glucose brought about by insulin usually had returned to the control level by 2 hours after injection (Fig. 1, Table I).

Gastric Acid Output

Insulin caused little or no increase in gastric acid secretion in dogs with PGV. Before vagotomy, basal acid output was 0.4 ± 0.3 mEq/15 min (n = 3 dogs). Output increased 13-fold to 5.2 ± 0.7 mEq/15 min by 45 minutes after insulin. In contrast, basal acid output only increased from 0.2 ± 0.4 mEq/15 min be-

Table I. Effects of insulin, gastrin, and CCK receptor antagonists after canine proximal gastric vagotomy

	Gastric barostat volume (%)	Gastric acid (mEq/15 min)	Plasma glucose (mg/dl)	Plasma insulin (IU/ml)	Plasma gastrin (pg/ml)
Baseline	100 ± 0	0.2 ± 0.4	97 ± 5	6 ± 2	240 ± 59
Insulin alone	202 ± 15*	0.9 ± 0.4	45 ± 3*	421 ± 87*	387 ± 75*
Insulin + L-364,718	195 ± 7*	0.7 ± 0.3	46 ± 4*	386 ± 75*	368 ± 55*
Insulin + L-365,260	143 ± 21†	0.4 ± 0.2	46 ± 6*	377 ± 59*	381 ± 58*
Gastrin alone	192 ± 7*	1.1 ± 0.4	94 ± 2	9 ± 2	322 ± 45*
Gastrin + L-364,718	218 ± 13*	0.7 ± 0.3	96 ± 3	8 ± 2	326 ± 45*
Gastrin + L-365,260	141 ± 6†	0.5 ± 0.3	96 ± 7	9 ± 3	317 ± 33*

All data are mean ± standard deviation 45 minutes after insulin or gastrin injection except for plasma gastrin, the peak values of which are 30 to 90 minutes after injection; n = 6 dogs.
L-364,718, a CCK-A receptor blocker, and L-365,260, a CCK-B receptor blocker, were given 30 minutes before insulin or gastrin injection. Reconstitution solution was given 30 minutes before insulin or gastrin injection in insulin alone or gastrin alone group.
*P <0.05 compared to baseline.
†P <0.05 compared to insulin alone or gastrin alone.

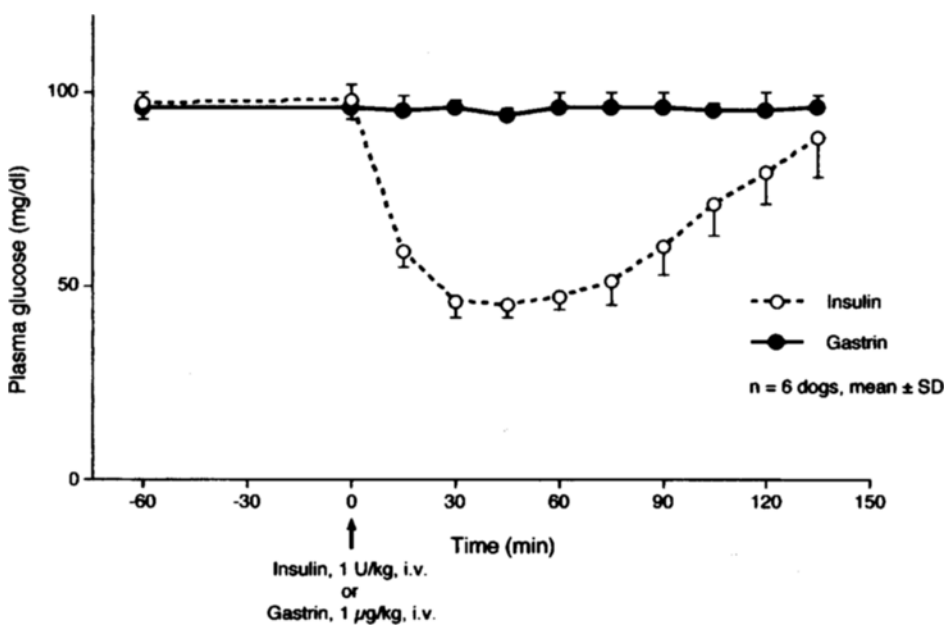

Fig. 1. Effect of insulin or gastrin on plasma glucose concentrations after canine PGV. Insulin or gastrin was injected intravenously at time zero.

fore insulin to 1.3 ± 0.4 mEq/15 min after insulin when PGV had been done (n = 6 dogs). PSP recovery ranged between 35% and 65% for each 15-minute period in any experiment. These data suggest that PGV had effectively vagally denervated the gastric fundus and corpus of the dogs.

Insulin- and Gastrin-Induced Relaxation of the Proximal Stomach

Insulin injection caused a prompt relaxation of the proximal stomach (Fig. 2). Proximal gastric tone was largely restored by 2 hours after insulin injection. Exogenously administered gastrin, like insulin, also relaxed the proximal stomach (Fig. 2). Proximal gastric tone was also restored by 2 hours after gastrin injection.

Plasma Insulin and Gastrin Concentrations After Insulin or Gastrin Injection

Plasma concentrations of insulin increased immediately after insulin injection and then declined exponentially over the ensuing 2 hours, returning to the control range between 60 and 90 minutes after injection (data not shown). Basal plasma gastrin (before injection of insulin or gastrin) was 227 ± 26 pg/ml before PGV and 240 ± 39 pg/ml after PGV ($P > 0.05$). Plasma gastrin levels increased and peaked between 30 and 60 minutes after insulin injection (Fig. 3). The increase in plasma gastrin did not clearly differ from baseline ($0.1 > P > 0.05$) at any specific point in time, but the peak gastrin levels after insulin were greater ($P < 0.05$) than the baseline levels before insulin injection.

After gastrin injection, plasma gastrin increased immediately. Gastrin was then eliminated exponentially from plasma, returning to the control level by 2 hours after injection (Fig. 3). Plasma concentrations of gastrin after gastrin injections were similar to those after insulin injections at periods of time between 30 minutes and 2 hours after the injections. Plasma insulin values were unchanged by the gastrin injections (data not shown).

These results suggest that insulin caused gastrin release in dogs with PGV and that the increased plasma gastrin concentration was accompanied by relaxation of the proximal stomach.

Effects of CCK-A and CCK-B Antagonist on Insulin- and Gastrin-Induced Relaxation of the Proximal Stomach

The reconstitution solution for CCK receptor antagonists without L-364,718 or L-365,260 did not af-

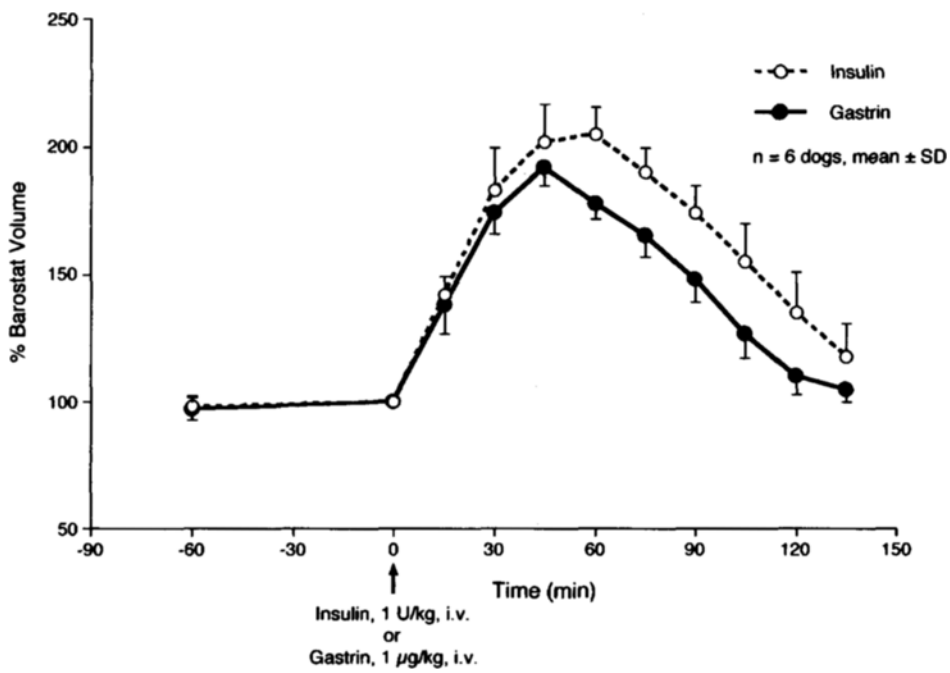

Fig. 2. Insulin- and gastrin-induced relaxation of the proximal stomach after canine PGV. Insulin or gastrin was injected at time zero. Proximal gastric barostat volume at time zero was standardized to 100% in each experiment.

fect plasma concentrations of glucose, insulin, or gastrin, nor did it alter proximal gastric tone. This solution alone did not interfere with the effects of insulin or gastrin injection on proximal gastric motility described previously (data not shown).

L-365,260, a CCK-B receptor antagonist, partially inhibited the relaxation of the proximal stomach brought about by insulin or gastrin ($P < 0.05$; Fig. 4, Table I), whereas L-364,718, a CCK-A receptor antagonist, had no consistent effect ($P > 0.05$). Neither

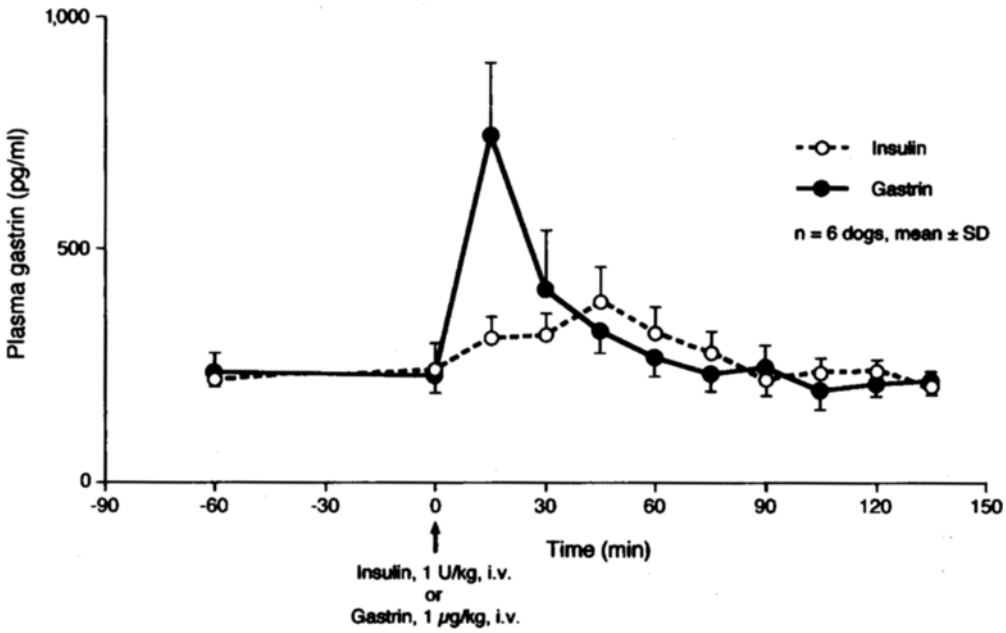

Fig. 3. Plasma gastrin after intravenous injection of insulin or gastrin at time zero in dogs with PGV.

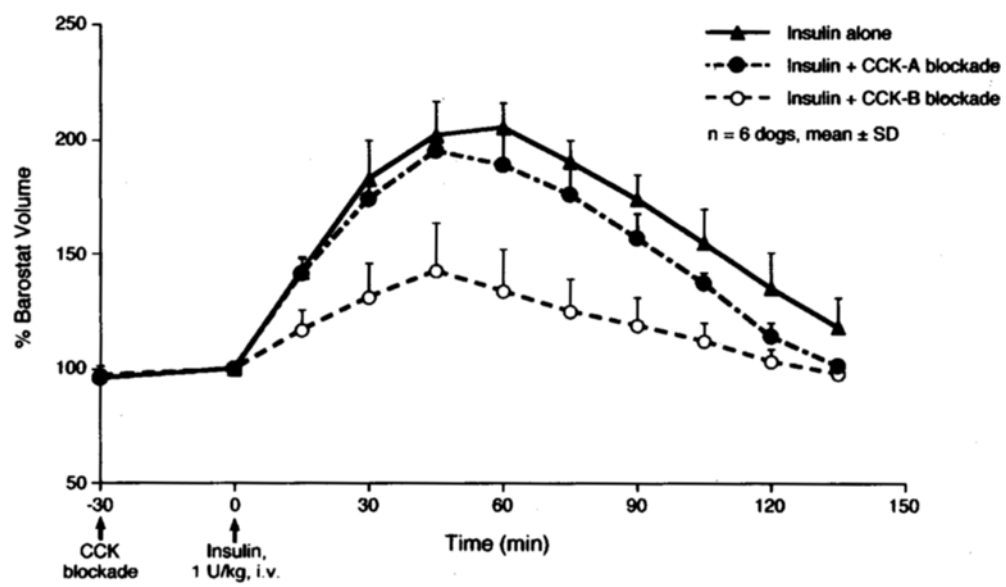

Fig. 4. Effect of CCK-A blockade (1 μg/kg) or CCK-B blockade (1 μg/kg) on insulin-induced relaxation of the proximal stomach after canine PGV. Insulin was given intravenously at time zero, after one or the other CCK blocker (1 mg/kg) had been given intravenously at time −30 minutes. CCK = cholecystokinin.

antagonist altered the other observed effects of insulin or gastrin, except for proximal gastric tone.

DISCUSSION

Insulin-induced hypoglycemia is thought to stimulate the vagal efferent neurons of the dorsal motor nucleus of the vagus.[19] Activation of these neurons has direct and indirect effects on distal gastric motility.[20] Vagal stimuli cause contraction of the smooth muscle of the distal stomach through cholinergic pathways.[20] Vagal stimuli also induce gastrin release from antral G cells[21] through cholinergic pathways.[20,22] The gastrin released further stimulates the contraction of smooth muscle of the distal stomach and changes the motility of the distal stomach from a fasting pattern to a "fed-like" pattern.[23] PGV preserves vagal innervation to the distal stomach and thus insulin-induced activation of distal gastric motility in dogs with PGV.

Proximal gastric motility is also controlled by vagal innervation. Truncal vagotomy abolishes receptive relaxation of the proximal stomach.[1] Vagal relaxation of the proximal stomach is thought to be controlled by noncholinergic signaling pathways.[24-26] The relaxation may be brought about, in part, by vagally induced release of vasoactive intestinal peptide, somatostatin, or nitric oxide.[27] Besides the direct neural control of relaxation by vagal efferents, other mechanisms may be involved in the relaxation of the proximal stomach. PGV preserves the relaxation,[4] suggesting that insulin-induced relaxation of the proximal stomach is conducted, in part, through mechanisms that are distinct from those mediated by vagal nerves to the proximal stomach. Gastrin is a known relaxant of the proximal stomach[5-7] and a contractant of the distal stomach. The present study provides evidence that insulin-induced relaxation of the proximal stomach is caused, in part, by vagally induced release of antral gastrin.

The gastrin receptor is identical to the CCK receptor type B (CCK-B receptor).[28,29] The receptor is present on gastric smooth muscle.[30,31] When gastrin molecules bind to the receptor on the smooth muscle cells of the proximal stomach, relaxation occurs and gastric emptying is slowed, although some believe that the concentrations of gastrin required to relax the proximal stomach and slow gastric emptying are not physiologic.[32]

The inhibitory effect of L-365,260 on the relaxation of the proximal stomach by gastrin was partial in our tests. Only one dosage and one administration schedule of L-365,260 were used in the present study. The method of administration may not have been the best regimen. Further, the affinity of L-365,260 for CCK receptors is reported to be less in the dog than in other species such as humans.[17] Thus the blockade of gastrin receptors by L-365,260 in our tests may have been incomplete. Repeating the experiments with several larger doses of L-365,260 would provide data on this point.

The pattern of the increase in plasma gastrin and the relaxation of the proximal stomach followed a similar time course after insulin was given intravenously, but the peak in plasma gastrin occurred before the peak of gastric relaxation when gastrin was given intravenously. The cause of this lag effect with gastrin is not known, but it may relate, in part, to the activation of slower onset intramural neuronal pathways by gastrin in addition to the effect of gastrin on proximal gastric smooth muscles.

Intravenous insulin has been reported to induce premature interdigestive migrating myoelectric complexes,[33] a prominent component of which are increases in proximal gastric tone and increases in the amplitude and frequency of "volume" waves in the proximal stomach.[10] These stimulatory changes in proximal gastric motility were not noted in our tests, suggesting that they may be mediated by vagal motor efferents to the proximal stomach, which were divided by the PGV. It would be of interest to follow proximal gastric tone throughout the interdigestive cycles in unstimulated animals after PGV. Presumably, increases in proximal gastric tone and cyclic waves would still be present. We have shown in previous tests that autotransplanted, extrinsically denervated pouches of proximal stomach continue to cycle with the main gastrointestinal tract,[34] likely because of the cyclical release of stimulatory hormones during the interdigestive complexes.

The return of proximal gastric tone after insulin-induced relaxation in our previous tests was slower in dogs with PGV than in dogs with intact vagal innervation.[4] Acetylcholine or other stimulatory neurotransmitters released by vagal efferents to the proximal stomach in healthy control dogs may antagonize the effect of gastrin after insulin injection and allow the dogs to regain proximal gastric tone more quickly. These possible controlling mechanisms are apparently lost or impaired after PGV.

Proximal gastric vagotomy decreases acid secretion from the proximal stomach, which in turn results in

less acid inhibition of antral gastrin release. Postoperative hyperplasia of gastrin-secreting cells (G cells) and hypergastrinemia usually result. The increases in serum gastrin that we found in our tests after proximal vagotomy, however, were small ($P > 0.05$). Perhaps the short, 2-week interval between the operation and testing did not allow sufficient time for G-cell hyperplasia to occur.

The maintenance of the ability of the proximal stomach to relax and contract after PGV and the loss of these functions after truncal vagotomy provide more experimental evidence favoring PGV over truncal vagotomy as the operative treatment of choice for chronic duodenal ulcers.

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