Common Bile Duct Stones: Magnetic Resonance Cholangiopancreatography vs. Endoscopic Retrograde Cholangiopancreatography for Detection

Alan N. Barkun, M.D.

Magnetic resonance imaging (MRI) has come into increasing use for the assessment of patients with suspected intra-abdominal visceral disease. Magnetic resonance cholangiopancreatography (MRCP) is a newly developed application of MRI that provides both high-quality cross-sectional images of extraductal structures and composite projectional (coronal) images of the biliary tree and pancreatic duct.[1] Unlike direct cholangiography, the technique is noninvasive and the images can be obtained without the administration of oral or intravenous contrast agent. As the modality continues to evolve, it is not yet known whether it will replace more traditional methods of imaging the pancreaticobiliary system or remain an adjunctive diagnostic tool.

The Technology

Early results of MRCP were discouraging. However, more recent technical refinements have greatly improved the diagnostic accuracy and broadened the clinical applications of MRCP. The MRCP examination does not require special patient preparation or the routine administration of premedication or contrast medium injection. The technique is based on heavily T2-weighted pulse sequences that result in stationary fluids exhibiting high signal intensity, whereas solid organs demonstrate a low signal intensity.[1,2] In addition, flowing blood results in little or no measurable signal. This combination provides optimal contrast between the hyperintense signal of the bile and the hypointense signal of the background. Magnetic resonance cholangiograms are best reviewed at a diagnostic workstation rather than on hard-copy films. MRCP may also be carried out in conjunction with a more conventional magnetic resonance examination to provide details on surrounding structures (e.g., staging of tumors). A certain proportion of patients will be unable to undergo MRI, especially those with severe claustrophobia.

MRCP and Detection of Biliary Obstruction

MRCP in its current stage of development can diagnose the presence of bile duct obstruction in 91% to 100% of cases and can determine the level of obstruction in 85% to 100% of cases.[1,2] The bile ducts distal to the obstruction are routinely identified, including the distance from the site of obstruction to the ampulla. Furthermore, MRCP may often depict the intrahepatic biliary tree, particularly in cases of complete or high-grade obstruction when endoscopic retrograde cholangiopancreatography (ERCP) may not. Indeed, underfilling of the intrahepatic bile ducts often occurs with ERCP in these situations. In addition, the risk of sepsis, inherent to direct cholangiography in an obstructed system is obviated with MRCP.[1]

MRCP and Detection of Choledocholithiasis

Despite significant technical advances in recent years, the diagnostic accuracy of CT and ultrasound imaging in choledocholithiasis remains low, with modest sensitivities reported in most studies. Consequently ERCP remains a preoperative modality of choice for establishing the diagnosis of common bile duct (CBD) stones in selected settings. In addition, ERCP has the added advantage of allowing therapeutic intervention at the time of initial diagnosis. However, significant complications occur in 4% to 10% of patients after endoscopic sphincterotomy, with an overall mortality rate of 0.2% to 1.5% depending on the patient population studied.[3] Additionally, the sensitivity (90.4%) and specificity (95.8%) of ERCP in the diagnosis of CBD stones is not perfect, although

From the Division of Gastroenterology, McGill University, and the McGill University Health Center, Montreal, Quebec, Canada.

the use of prospectively validated clinical and laboratory predictors may allow for more accurate estimates of probabilities of choledocholithiasis.[4]

However, because all of these tests are highly specific, no further imaging tests are needed when choledocholithiasis is diagnosed by means of CT or ultrasound. Until recently, little data have been available regarding the accuracy of MRCP as a diagnostic test for CBD stones. In a recent series of 110 patients, MRCP diagnosed the presence of choledocholithiasis with a sensitivity of 90%, a specificity of 100%, and an overall diagnostic accuracy of 97%.[5] These figures represent a considerable improvement over the results obtained in previous studies with older magnetic resonance techniques, and suggest that MRCP is an excellent preoperative method in the diagnosis of CBD stones. Some groups have reported more modest results with particular difficulty in detecting smaller biliary ductal stones.[6]

Conclusion

MRCP is a very precise imaging technology for diagnosing CBD stones. However, its cost-effectiveness and optimal role remain undefined. Decision analyses and randomized trials are underway to assess which subgroups of patients, if any, may benefit from pre- or postoperative noninvasive imaging methods such as MRCP.[7] Any conclusions must take into consideration the ongoing evolution and the availability of all competing diagnostic and therapeutic technologies, such as MRCP, endoscopic ultrasound, intraoperative ultrasound, or cholangiography followed by laparoscopic CBD stone removal and selective preoperative or postoperative ERCP.[8-10]

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Intraoperative Detection: Intraoperative Cholangiography vs. Intraoperative Ultrasonography

Nathaniel J. Soper, M.D.

Intraoperative Cholangiography

Intraoperative screening of the common bile duct (CBD) for stones during laparoscopic cholecystectomy can be done using cholangiography (IOC) or ultrasonography (IUS). Application of one of these modalities for screening of the duct may be performed either routinely or selectively, a debate that has been reawakened by the emergence of laparoscopic cholecystectomy. Recently, a number of studies have compared IOC to IUS for screening of the CBD during laparoscopic cholecystectomy.

When performing IOC, radioiodinated contrast material may be injected either into the gallbladder directly or into the cystic duct, and radiographic images may be obtained by static or dynamic (fluoroscopic) techniques. Most authorities believe that fluoroscopic cholangiography performed with intubation of the cystic duct should be the primary method for IOC.[1] The overall success rate for obtaining adequate images by laparoscopic intraoperative cholangiography varies from 60% to 98% with false positive and false negative rates of less than 5%.[2] Potential negative aspects of IOC are the requirement to intubate the cystic duct, the possibility of ductal injury occurring as a direct result of the intubation, and the time and cost expended on the examination itself.

Intraoperative Ultrasound

During open cholecystectomy, several studies have shown IUS to be more accurate than IOC in assessing the CBD for stones (97% to 99% vs. 89% to 94%), respectively.[3,4] However, few surgeons have adopted ultrasound for this purpose. Laparoscopic IUS has been used in several centers to scan the biliary tree during laparoscopic cholecystectomy. With IUS, the transducer is generally a higher frequency with enhanced resolution compared to that used with transabdominal ultrasonography. IUS may be used to map the extrahepatic ductal system, identify anomalies of ductal and vascular structures, quantify ductal diameter, and visualize CBD stones and sludge.[5-7] In experienced hands, laparoscopic intracorporeal ultrasonography appears to be more sensitive than IOC for demonstrating choledocholithiasis, whereas cholangiography may be more accurate for demonstrating biliary anatomy.[6,8] Thus the two studies appear to be complementary for the delineation of biliary tract disease. Several studies have shown laparoscopic IUS to screen the bile duct more rapidly than IOC, and the cost is approximately $145 less than the cost of IOC per examination.[8] Despite these promising data, more clinical experience will be necessary to establish the appropriate role for laparoscopic IUS in screening the common bile duct during laparoscopic cholecystectomy.

Many surgeons are unfamiliar with ultrasound physics, technique, and image interpretation. Several introductory courses are currently being offered by the American College of Surgeons that cover basic ultrasound principles as well as techniques for performing IUS examinations. During the early learning phase of laparoscopic ultrasound, it may be helpful to enlist the assistance of experienced ultrasonographers as we have done.[7] The "learning curve" to achieve proficiency in the performance and interpretation of laparoscopic IUS of the bile duct has been reported as more than 10 cases,[9] but perhaps as many as 25 to 30 cases are required before the surgeon feels comfortable with the technique.

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From the Department of Surgery, Washington University School of Medicine, St. Louis, Mo.

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Tools of the Trade: The Common Bile Duct Stone Cart

Joseph B. Petelin, M.D.

Laparoscopic common bile duct (CBD) exploration requires extra effort, skill, tools, and organization if it is to be performed efficiently and successfully. The extra effort and skill used to perform laparoscopic CBD exploration techniques may be applied through either a transcystic or a transductal approach. The techniques include flushing the duct after sphincter relaxation, manipulation of balloon-tipped catheters to push the stone into the duodenum or pull it out of the duct proximally, fluoroscopic-guided basket manipulation and stone extraction, and choledochoscopy. All of the tools that might be needed for CBD exploration should be organized in a central location—a location that may be moved from place to place (i.e., room to room) as necessary. Just as "code carts" have facilitated emergency cardiopulmonary resuscitation, the CBD exploration cart promotes efficient CBD exploration (and reduces the stress on the operating room team dramatically).

To facilitate the creation of a cart, a list of the necessary equipment and a brief description of its use is supplied below. In my experience, the best way to set up such a cart is to have the interested surgeon work with the "can do" person from the operating room staff.

The equipment needed for laparoscopic CBD exploration may include any or all of the following:

1. Glucagon. Glucagon, 1 to 2 mg, is given intravenously by the anesthetist at the time a CBD stone is detected by intraoperative cholangiography (IOC). The drug works within minutes to relax the sphincter of Oddi.
2. Lidocaine 1%. At the time the glucagon is given, 10 cc of lidocaine is injected into the IOC catheter and may assist in sphincter of Oddi relaxation.
3. Balloon-tipped catheters (4 Fr preferred over 3 Fr and 5 Fr). Once the sphincter has been relaxed and flushing has not pushed the stones into the duodenum, then these catheters may assist in pushing them into the duodenum or retrieving them through the cystic duct or the choledochotomy.
4. Hydrophilic guidewire with flexible tip, 0.035 inch diameter. Once flushing has failed, this "slime wire" is advanced through the cystic duct into the duodenum. The IOC catheter can then be safely advanced over the wire into the distal common bile duct. A Segura-type basket can then be deployed through the catheter into the duct to troll back and engage the stone. The wire can also be used to advance the mechanical or pneumatic dilators through the cystic duct to allow placement of the choledochoscope into the CBD. The basket can then be introduced through the scope into the CBD and manipulated under visual control. (NOTE: After gaining some experience, the surgeon can actually perform many of the actions described above with the standard nonhydrophilic guidewire with equal success and much less expense; however, successful cannulation of the difficult cystic duct is almost assured with the hydrophilic wire.)
5. Segura-type baskets (four-wire, flat, straight in-line configuration). Although I prefer this basket configuration, others prefer a basket with a helical Dormia-type configuration. Both should be available on the cart to determine which will work best in a given situation.
6. Mechanical "over-the-wire" dilators. Sets of these dilators contain a series of graded sizes from 7 to 12 Fr.
7. High-pressure "over-the-wire" pneumatic dilators. Although these are very effective and generally safe, they are very costly.

From the Department of Surgery, University of Kansas School of Medicine, Shawnee Mission, Kan.

8. Intravenous tubing, three-way stopcocks, and saline solution. Saline irrigation or contrast infusion is used throughout the laparoscopic CBD exploration either through the choledochoscope or the cholangiocatheter.
9. Atraumatic grasping forceps (for choledochoscope manipulation). These are supplied by manufacturers of the various scopes.
10. Flexible choledochoscope. Several companies supply this complex but delicate instrumentation. Each must have its own light source and should have an outside diameter of ≤ 3 mm and a working channel of ≥ 1.1 mm to allow transcystic duct maneuvers.
11. Extra viewing equipment for the choledochoscope. These items are necessary when using a choledochoscope, that is, once flushing, transcystic fluoroscopic-guided baskets, or balloon tipped catheters have been considered or have failed.
    - Second video camera for choledochoscope
    - Extra monitor (or second viewing area on the primary laparoscopic monitor)
    - Video switcher (for simultaneous display of choledochoscopic and laparoscopic images on the same monitor)
12. Water Pik. Some surgeons use this device (Teledyne Water Pik, Newport Beach, California) for flushing assistance rather than using 20 cc syringes.
13. Electrohydraulic lithotriptor. During choledochoscopy the electrohydraulic lithotriptor can be used to fragment a CBD stone if the stone is impacted and cannot be grasped with the basket or if it is too large to enter the basket. This technology must be used under direct vision as the energy generated can perforate the duct.
14. Equipment for laparoscopic choledochotomy. This is used when transcystic techniques fail and the surgeon does not wish to use postoperative ERCP.
    - Absorbable suture (polyglycolic acid suture, 4-0 or 5-0 size)
    - T-tube (transductal) or C-tube (transcystic)
    - Stent (straight, 7 Fr or 10 Fr)
15. Sphincterotome (for antegrade sphincterotomy).

Since approximately 10% of patients undergoing elective cholecystectomy and routine cholangiography have a CBD stone, the above-listed equipment is only needed every month or two for an individual surgeon. However, if many cholecystectomy procedures are done at a health care facility, then the common bile duct stone cart may be needed weekly. The time and energy invested in organizing this cart will be well spent for the operating room, its personnel, the surgeon(s), and the facility.

Laparoscopic CBD exploration techniques are relatively straightforward to learn, and many instructional videotapes and CD-ROM self-taught courses are available.

Transrectal Ultrasound: Accurate Staging for Rectal Cancer

W. Douglas Wong, M.D.

Continued evolution of the instrumentation for endorectal ultrasound has made it more accurate and user friendly. This, combined with the exploration of less invasive treatments of colorectal disease, has led to the wider adoption of this imaging technology for the staging of rectal and anal cancer.

The accuracy of endoluminal ultrasound for staging of rectal cancer has been established from several studies comparing endoluminal ultrasound with pathologic staging from surgical specimens. Endoluminal ultrasound is particularly good for determining the depth of invasion of lesions. Reported accuracy of endoluminal ultrasound ranges from 81% to 94%, with overstaging in 0% to 12% and understaging in 1% to 9% of cases.

Overstaging depth of invasion results from inflammation at the deep edge of the tumor, preoperative radiation, hemorrhage in the rectal wall following biopsy, tangential scanning rather than scanning at 90 degrees to the rectal wall, or a tendency of the observer to fear understaging the depth of invasion. Overstaging occurred in 25% of patients in one study of T2 tumors as a result of desmoplastic inflammation and retraction of the muscularis propria.

Understaging depth of invasion occurs less frequently than overstaging particularly for minimally invasive tumors and when determining lymph node status. Although endoluminal ultrasound is accurate for preoperative staging of rectal cancer, it is less accurate for assessment of downstaging of the tumor after preoperative chemoradiation. After preoperative chemoradiation, the positive predictive value of endorectal ultrasound was 72% for wall penetration and 56% for lymph node status. Overstaging following chemoradiation is likely caused by inflammation secondary to radiation.

The efficacy of endoluminal ultrasound is only fair for determination of metastasis to perirectal lymph nodes. Ultrasound characteristics recently suggested to distinguish benign from malignant nodes may prove to be helpful but have not yet been validated. Unfortunately, the size of a lymph node is not highly predictive of the presence of metastasis. Reported accuracy of endoluminal ultrasound for detection of lymph node metastasis ranges from 58% to 80%, which remains superior to other imaging methods such as CT scanning. Transrectal biopsy of lymph nodes for preoperative staging is not usually performed because of difficulty in accessing the node without transgressing the primary tumor in the rectal wall. Thus the level of experience of the observer is very important to achieve accurate staging of rectal cancer by means of endoluminal ultrasound, particularly since this staging may influence the choice of therapy including less invasive options for traditional resection (e.g., local excision, cautery ablation, endocavitary radiation) or the decision to use preoperative radiation.

Additional uses for endoluminal ultrasound include staging of anal cancer, with a reported accuracy of 86%, and the determination of malignant transformation of rectal villous adenomas. In one study of villous rectal tumors, the positive predictive value of endorectal ultrasound for detecting malignancy was 67%, whereas the negative predictive value was 89%. For this use, large exophytic lesions or proximity to the anal verge can limit accuracy. The high negative predictive value of endorectal ultrasound suggests that neoplasms not invading the submucosa on ultrasound can be treated by submucosal excision with confidence that the lesion has been adequately excised, even if in situ carcinoma is present. A final important use for endoluminal ultrasound is for follow-up of rectal cancer following surgery and for anal cancer after chemoradiation.

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Current Status of Laparoscopic Colectomy—Is It Experimental?

Heidi Nelson, M.D.

The introduction of laparoscopic cholecystectomy ushered in dramatic changes in the approach to abdominal surgery. The ability to remove abdominal organs through small incisions, thereby reducing pain and time for recovery, rapidly became popular among patients and effectively revolutionized the approach to cholecystectomy. Its continued popularity encourages applications of laparoscopic surgery in other fields. Developments in the field of laparoscopic colon and rectal procedures have paralleled those of laparoscopic cholecystectomy, the latter of which has proceeded at a more controlled pace and with more prospective assessments.

The introduction and acceptance of laparoscopic colectomy was more gradual for a number of reasons including the fact that it is technically challenging, has less dramatic patient benefits, and perhaps most significantly may represent a compromise as an oncologic procedure. Early clinical series with cancer patients are encouraging as they show that, with proper technique, adequate margins and lymph node harvests can be achieved. Studies based on animal models, however, have indicated that patients may incur a risk as well when laparoscopy is used in the treatment of colon cancer. Some studies have shown a promotion of tumor growth and dissemination with CO_2 pneumoperitoneum, whereas other researchers see no impact of laparoscopy on tumor growth and even propose an oncologic benefit from the documented preservation of the host immune response with the less invasive laparoscopic surgery. Today most researchers agree that the phenomenon of port-site implantation is mostly related to the well-described wound trophic effects or to poor surgical technique—problems that are not unique to laparoscopic cancer surgery.

What has been learned about laparoscopic colectomy so far is that it is feasible—that is, it can be performed with acceptable operative times and conversion rates. To learn the technique and achieve proficiency requires between 20 and 100 cases for most surgeons. As the next generation of laparoscopically facile surgeons emerges from residency and fellowship training, the "learning curve" has become considerably lower than that experienced by the previous generation. Most trainees can now be taught a segmental colectomy within 10 to, at most, 15 cases. Fortunately safety, as measured by morbidity and mortality, has not been a serious problem in previous series. These studies found at least equivalent rates of complications to the open procedure even during the "learning" phase. Those practicing laparoscopic colectomy continue to be encouraged about its future, as it is clear that patient-related benefits can be realized. Patients typically recover more quickly with reduced requirements for narcotics, reduction in length of ileus, and a shorter hospital stay, 2 to 4 days on average.

Finally, it must be admitted that when the benefits were not initially compelling and the oncologic results were reason for concern, a general slowdown was encouraged. The controlled introduction of laparoscopic colectomy has allowed better definition of patient-related benefits, and as well has allowed for a critical assessment of cancer risks. Thus, although it is established that laparoscopic colectomy is feasible, safe, and associated with patient-related advantages, the oncologic risks are not yet established. A formal risk/benefit analysis can only be forthcoming at the close of ongoing national and international prospective studies. Early results of at least three of these randomized prospective trials show equivalent results in recovery, margins, complication rates, and local/regional recurrences. There are, to date, no reports describing the impact of the laparoscopic approach on long-term survival. Because of this, at least for now, the answer to the question "Is it experimental?" must be "no" for benign conditions but "yes" for cancers.

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Transanal Endoscopic Microsurgery: Current Indications and Techniques

Lee Swanstrom, M.D.

Transanal endoscopic microsurgery (TEM) was introduced in 1984 as a direct-imaging alternative to traditional treatments of rectal cancer. Traditional treatments have ranged from the low-morbidity (but difficult) transanal excision to the permanent morbidity of an abdominal perineal resection. The ability to perform full-thickness excisions with magnification, good illumination, and the increased exposure afforded by insufflation and long instruments has dramatically increased the indications for and efficacy of transanal excisions of malignant and premalignant lesions.

The instrument set includes a 4 cm diameter operating rectoscope with either a 12 or 22 cm long barrel. Instrumentation replicates the tools of the advanced laparoscopic surgeon and includes graspers, cautery, scissors, needle holders, and a suction device. High-resolution imaging is obtained either with the originally described stereoscopic optics, which suffer from requiring the ergonomic disaster of an immobile, bent-over position, or by the use of an adaptor that permits a standard 25-degree laparoscope and camera system to be used. This permits a heads-up view of the projected image on video screens, which are accessible to the entire operating room team. The primary advantage of TEM is the ability to accurately determine margins and execute wide, full-thickness excisions under high magnification. Equally important is the ability to execute formal, non-narrowing, transverse closures of a full-thickness defect. Closure of the full-thickness excision site is even more technically demanding than standard laparoscopic suturing, but does allow accurate repair of even large defects including those after intraperitoneal full-thickness excision. TEM has permitted some investigators to perform primary reanastomosis following endoscopically performed sleeve resections. Current indications for TEM include the following:

- Adenomatous and villous polyps, which are unresectable endoscopically
- Stage I and II cancers (with adjuvant therapy)
- Simple prolapse
- Rectal ulcers and other benign lesions that require wide excision

Operative success is high with these excisions and conversions to open procedures are rare, in most series less than 1%. Cumulative experience in the literature now totals more than 1000 procedures and documents an average length of stay of less than 48 hours and an average perioperative complication rate of 5%. Postoperative pain is uncommon and the main complications reported are infection (2.5%), dehiscence of the incisions (0% to 5%), and bleeding (5% to 10%). Recurrence rates following benign tumor excision are between 1% and 8%, which is much lower than the rates reported for standard transanal excisions.

The role of TEM in the treatment of rectal cancer is not fully defined. Advocates cite the improved ability of imaging studies to accurately stage rectal cancers and the efficacy of modern chemoradiation protocols to justify this less invasive surgical approach. The reported actuarial 5-year cure rate for T1 cancers after TEM is 90%, which is comparable to more radical excisions. The local/regional recurrence rate for T3 lesions, however, approaches 65%, which underscores the importance of accurate staging of these patients to allow selection of those who will truly benefit. Current protocols rely on CT scans and endorectal ultrasound to determine the stage. Therefore TEM for cancer is subject to the accuracy limitations of these tests. Future developments such as positron emission tomographic scanning and radioimmunoguided imaging may allow selection of rectal cancer patients who would achieve optimal results from image-guided full-thickness TEM resection.

From Legacy Health System, Portland, Ore.

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Photoablation of Barrett's Esophagus

Kenneth K. Wang, M.D.

Photoablative therapy of Barrett's esophagus has been performed for almost a decade. The therapy can be categorized as either *thermal* or *photochemical* photoablative techniques. Thermal photoablative therapy is conducted with lasers, which heat tissue causing epithelial cell death. Lasers that have been reported to produce this type of injury include argon gas lasers, Nd:YAG lasers, and frequency-doubled YAG lasers termed KTP:YAG lasers. These lasers differ in the wavelength or color of light produced. The longer the wavelength of light used (deeper red color), the deeper the tissue penetration. This leads to more tissue injury, which may be needed for the treatment of mucosally invasive disease. Complications have been limited to bleeding and stricture formation, which may occur less frequently with the treatments that do not deeply penetrate tissue.

Most of these lasers have been used to treat Barrett's esophagus without dysplasia or with low-grade dysplasia. These patients usually require four to eight separate treatment sessions to completely treat the Barrett's segment. Recently, the KTP:YAG laser was used to treat patients with low-grade or high-grade dysplasia as well as two patients with superficial cancers. Overall, the results of this therapy have been good with reepithelialization of treated areas with normal squamous mucosa. Response rates to laser therapy have been virtually 100%, although complete responses appear less likely. However, this type of therapy can miss small amounts of Barrett's epithelium leading to 20% to 70% of these patients having Barrett's mucosa underlying squamous mucosa on subsequent biopsies. Because of this, the significant incidence of complications, and the possibility of missing an early cancer, this technique should still be considered either experimental or investigational. A summary of the data concerning these lasers is presented in Table I.

Photochemical ablation of Barrett's esophagus has centered on photodynamic therapy, which was first used clinically in 1960 at the Mayo Clinic. Photodynamic therapy involves three components: light, drug, and oxygen. The drug can be delivered orally (5-aminolevulinic acid) or intravenously as a hematoporphyrin derivative such as porfimer sodium. These drugs are porphyrin derivatives that circulate in the body and concentrate in epithelial tissue. The drug acts as a photosensitizer because it absorbs light energy and transfers it to oxygen. This high-energy oxygen, termed singlet oxygen, can in turn interact with tissue causing necrosis. The amount of the photosensitizer in the tissue and the intensity of light delivered to the tissue surface are the normal clinical determinants of photodynamic effect. After administration of light, tissue necrosis can be seen within 24 hours. This type of therapy produces a more uniform injury and less Barrett's mucosa is found underlying squamous mucosa. The majority of patients treated with photodynamic therapy have had high-grade dysplasia. The overall response of high-grade dysplasia to a *single* session of photodynamic therapy is close to 90%, whereas total elimination of Barrett's mucosa occurs in about a third of patients. However, the treatment parameters have not been clearly established and there are significant complications. The most important of these is esophageal stricture, which occurs in one fourth of the patients and requires multiple dilatations to reverse. Photosensitizers also make patients susceptible to cutaneous photosensitivity, which can result in significant sunburn if precautions are not taken. A summary of the studies conducted to date using photodynamic therapy for high-grade dysplasia is presented in Table II.

From the Division of Gastroenterology and Hepatology, Mayo Clinic, Rochester, Minn.

Table I. Thermal laser therapy for Barrett's esophagus

Reference	No. of patients	Type of laser	Response rate	Complete response
Berenson et al.[1]	10	Argon	95% (38/40 areas)	None
Luman et al.[2]	8 (4 controls)	Nd:YAG	0%	None
Barham et al.[3]	16	KTP:YAG	100%	5/16 (31%)
Salo et al.[4]	17 (6 controls)	Nd:YAG	100%	9/11 (81%)
Gossner et al.[5]	10	KTP:YAG	100%	8/10 (80%)

Table II. Photochemical therapy for Barrett's esophagus

Reference	No. of patients	Drug	HGD response	Barrett's response	Adjuvant therapy	Complications
Overholt et al.[6]	73	Porfimer sodium	88%	43%	83% YAG	34% strictures 3% severe photoxicity
Gossner et al.[5]	10	ALA	100%	0%	mTHPC (cancers)	47% nausea 65% elevated AST
Wang[7]	26	Hematoporphyrin derivative	88%	35%		81% chest pain 27% strictures
Barr et al.[8]	5	ALA	100%	0%		None

ALA = 5-aminolevulinic acid; HGD = high-grade dysplasia; mTHPC = meta-tetra (hydroxyphenyl) chlorin.

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Modern Imaging for the Assessment of Gastroesophageal Reflux Disease Begins With the Barium Esophagram

Jeffrey H. Peters, M.D.

Successful antireflux surgery is largely defined by just two outcomes—achieving the long-term relief of reflux symptoms and the absence of complications or complaints induced by the operation. In practice, achieving these two deceptively simple goals is difficult. Both are critically dependent on establishing that the symptoms for which the operation is performed are due to excessive esophageal exposure to gastric juice, as well as the proper performance of the appropriate antireflux procedure. Success can be expected in the vast majority of patients if these two criteria are met, that is, reflux is really present and an adequate operation has been employed.

The diagnostic approach to patients considered for antireflux surgery has four important goals: (1) to determine that gastroesophageal reflux is the underlying cause of the patient's symptoms; (2) to determine the presence or absence of esophageal shortening; (3) to evaluate the propulsive function of the esophagus and, occasionally, that of the stomach; and (4) to provide an assessment of the underlying severity of disease as an aid in patient and procedure selection. Radiographic assessment is an integral part of achieving these goals.

The best imaging study for gastroesophageal reflux is not magnetic resonance imaging, positron emission tomography, or computer-enhanced three-dimensional tomography. Barium sulfate esophagography remains the best imaging study. A carefully performed video esophagram can provide an enormous amount of information on the structure and function of the esophagus and stomach. The "modern" barium swallow emphasizes motion recording (video), uses a tightly controlled examination protocol, and requires an understanding of esophageal physiology. The quality of these imaging results is related to careful attention to the patient's body position and the technique used in the examination (Table I). Videotaping the study greatly aids the evaluation, providing the surgeon with a real-time assessment of swallowing function, bolus transport, and the size and reducibility of a hiatal hernia. Given routine review prior to antireflux surgery, its value becomes increasingly clear.

The significance of gastroesophageal reflux varies depending on whether reflux is spontaneous or induced by various maneuvers. Spontaneous reflux, that is, reflux of barium from the stomach into the esophagus with the patient in the upright position, is observed by the radiologist in only 40% of patients with classic symptoms of gastroesophageal reflux disease. In most patients who show spontaneous reflux on radiography, the diagnosis of increased esophageal acid exposure is confirmed by 24-hour esophageal pH monitoring. Therefore the radiographic demonstration of spontaneous regurgitation of barium into the esophagus in the upright position is a reliable indicator that reflux is present. Failure to see this does not indicate the absence of disease.

A video barium esophagram will provide structural information including the presence of obstructing lesions or anatomic abnormalities of the foregut. A hiatal hernia is present in more than 80% of patients with gastroesophageal reflux and is best demonstrated with the patient in the prone position. This position increases abdominal pressure and promotes distention of the hernia above the diaphragm. The presence of a hiatal hernia is an important component of the underlying pathophysiology of gastroesophageal reflux. Other relevant findings include a large (>5 cm) or irreducible hernia suggesting the presence of a shortened esophagus, a tight crural "collar" that inhibits barium transit into the stomach suggesting a possible cause of dysphagia, and the presence of a paraesophageal hernia.

Lower esophageal narrowing due to a ring, stricture, or obstructing lesion is optimally viewed with full distention of the esophagogastric region. A full-column technique with distention of the esophageal

From the Department of Surgery, University of Southern California School of Medicine, Los Angeles, Calif.

Table I. University of Southern California protocol for video esophagram studies

Patient position	Purpose	Technique
Prone right anterior oblique position	Esophageal body function	Five separate 10 cc swallows, 15 seconds between each, follow bolus on videotape
		Video swallow over thoracic inlet and another over distal third of esophagus without panning
	Esophageal diameter	Rapid swallow of several gulps to maximally distend esophagus
	Gastric function	Video record activity of stomach and duodenum for 30 seconds in prone position
Supine	Relationship of gastroesophageal junction to hiatus	Two to three individual swallows focused on distal esophagus and gastroesophageal junction
Erect	Cricopharyngeal function	Lateral and anteroposterior views of oropharynx and upper esophagus
	Mucosal injury	Spot of collapsed esophagus for mucosal detail
	Reducibility of hernia	Video images of one to two swallows focused on distal esophagus and gastroesophageal junction
		Gas distention of distal esophagus
Erect	Solid bolus transport	Record video images of passage of two contrast-coated "hamburger" boluses from oropharynx to stomach

wall can be used to discern extrinsic compression of the esophagus. Mucosal relief or double-contrast films should be obtained to enhance the detection of small esophageal neoplasms, mild esophagitis, or esophageal varices. The pharynx and upper esophageal sphincter are evaluated in the upright position, and an assessment of the relative timing and coordination of pharyngeal transit is possible.

Esophageal body function is assessed in both the recumbent and upright positions. During normal swallowing, a stripping wave (primary peristalsis) is generated that completely clears the bolus. Residual material can stimulate a secondary peristaltic wave, but usually a second pharyngeal swallow is required. The assessment of peristalsis on video esophagram often adds to, or compliments, the information obtained by esophageal motility studies. This is, in part, because the video barium study can be carried out with the patient in either the upright or supine position and with liquid or solid bolus material, which is not true of a stationary motility examination. This is particularly true with subtle motility abnormalities. Motility disorders with disorganized or simultaneous esophageal contraction have "tertiary waves" and provide a segmented appearance to the barium column this is often referred to as beading or a corkscrew. In a patient with dysphagia, a barium "burger" (barium-impregnated marshmallow, bread, or hamburger) is a useful adjunct that can discern a functional esophageal transport disturbance not evident on the liquid barium study. Reflux is not easily seen on the video esophagram and motility disorders that cause retrograde barium transport may be mistaken for reflux.

Assessment of the stomach and duodenum during the barium study is a necessity for proper preoperative evaluation of the patient with gastroesophageal reflux disease. Evidence of gastric or duodenal ulcer, neoplasm, or poor gastroduodenal transit has obvious importance in the proper preoperative evaluation.

Esophagoscopy for Surgeons

Tom R. DeMeester, M.D.

Endoscopy has revolutionized the management of digestive disease. Surgeons now realize the need to be part of this endoscopic revolution but find involvement difficult because endoscopy straddles the time-honored divide between orthodox "cutting" abdominal surgery and "thinking" gastroenterology. The reasons that drive a surgeon's interest in endoscopy are as follows:

1. Endoscopy helps the surgeon understand the patient's problem. Seeing, measuring, and believing is the best foundation on which to construct a rational basis for therapy.
2. Endoscopy allows the surgeon the possibility of discovering an unrecognized finding that could alter the therapeutic approach. After visual observation the endoscopist learns from the experience. Consequently a surgeon's perspective and a gastroenterologist's concepts of a disease state can differ markedly.
3. Endoscopy contributes to the surgeon's planning of the patient's operation. The ability to visualize rather than imagine pathology is a giant step toward solving a clinical problem.
4. Endoscopy helps the surgeon educate the patient about his or her problem. Informing patients about disease by reviewing with them video of their own endoscopy procedures is an excellent way to involve them in therapy.
5. Endoscopy helps the surgeon gain the patient's confidence. Entrusting oneself to a surgeon for endoscopy makes it easier to also have that surgeon perform the surgery.

Esophagoscopy is an indispensable aid to the foregut surgeon prior to, during, and after surgical therapy. Prior to surgery, endoscopy often forms the basis of the patient's examination. It allows an inspection of the geometry of the distal esophagus and cardia, and an assessment of mucosal damage and the discovery of anatomic variations. Esophagoscopy aids in the passage of motility catheters. Endoscopic ultrasound is made possible by delivering the ultrasound probe into the esophagus and then the skill to manipulate it. During surgery, endoscopy can help to identify the location of the gastroesophageal junction, to check the integrity of an anastomosis, to access organ injury, and to monitor the performance of a thoracoscopic myotomy. During the immediate postoperative period, the surgeon can use endoscopy to check on the viability of a pulled-up stomach or interposed colon and the healing of an anastomosis. During the follow-up period, endoscopy can be used to inject a paralyzed vocal cord, dilate an anastomotic stricture, assess a patient who is having difficulty swallowing, check on the integrity of an antireflux repair, and detect signs of recurrent or progressive mucosal disease.

Having dropped the "endoscopic" ball initially, surgeons should now reposition themselves to benefit from the myriad endoscopic innovations. Most patients and payers now want to consider endoscopic procedures that appear to be as effective as open procedures but are quicker, safer, and less expensive. Examples of these procedures are endoscopic variceal ligation, endoscopic insertion of stents, endoscopic gastrostomy, endoscopic mucosal ablation or resection,[1] endoscopic resection of gastric lesions, or endoscopic antireflux procedures.[2]

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From the Department of Surgery, University of Southern California School of Medicine, Los Angeles, Calif.

Hepatic Resection at a Community Hospital

Melvin E. Stone, Jr., M.D., Saif Ur Rehman, M.D., Gail Conaway, R.N., Armando Sardi, M.D.

Hepatic resection remains the "gold standard" in the primary management of primary and metastatic tumors to the liver. Advanced surgical techniques along with more modern and sophisticated equipment have led to an increasing number of hepatic resections being performed with a concomitant decrease in morbidity and mortality. We followed prospectively 18 consecutive hepatic resections performed over a period of approximately 2.5 years. The setting was a community teaching hospital with a low volume of referrals for hepatic resection. Sixteen (88%) had metastatic disease and two had primary liver disease. There were four trisegmentectomies, four lobectomies, four segmentectomies, and six large wedge resections. Average estimated blood loss was 608 ml. Seven patients required transfusions. Complications occurred in five patients (27%). In-hospital mortality was 0%. Our experience suggests that liver resections in a low-volume community hospital can be performed safely provided an experienced surgical team with familiarity of advanced surgical techniques and sophisticated equipment used in hepatic resection is readily available. (J GASTROINTEST SURG 2000;4:349-354.)

KEY WORDS: Hepatic resection, liver metastasis

For the management of primary and metastatic neoplasms of the liver, hepatic resection is the mainstay of therapy. Hepatic resection is a high-risk surgical procedure with varying morbidity and mortality related to extent of surgery, nature of disease, and comorbid conditions.[1] Although few studies have looked specifically at hepatic resection, other high-risk surgical procedures, such as cardiac bypass, have been shown to have more favorable outcomes when performed at institutions managing high volumes of these cases.[2-6] On the basis of number of liver resections per year, hospitals were arbitrarily classified as high-volume or low-volume providers (high volume, >15 per year; low volume, ≤15 per year). An alternative stratification was performed for high-, medium-, and low-volume provider groups (high volume, >15 per year; medium volume, between 7 and 15 per year; and low volume, <7 per year) for relative risk analysis. It has been suggested that major hepatic resection, being a high-risk surgical procedure, should only be performed at academic centers with a high volume of referrals.[7]

With advanced surgical techniques and more sophisticated equipment, overall long-term survival along with mortality and morbidity for liver malignancies has improved.[8] This has been followed by an increased number of liver resections in addition to a more aggressive surgical approach.[7-9] Whether these liver resections are performed at high-volume or low-volume centers is of economic relevance in today's health care climate. However, before regionalization can be addressed, safety, as evidenced by low mortality and morbidity, is of paramount importance. Described herein is the experience of hepatic resection at a community teaching hospital with low-volume referral.

MATERIAL AND METHODS

This study examined the outcomes for all patients who underwent hepatic resection at an urban community teaching hospital with an average of 6.72 liver resections annually. A total of 18 patients had liver resections performed by the same surgical oncologist and various senior residents over the period from April 1995 to December 1997 (Table I). Data were collected prospectively beginning with the primary hospital admission and continuing with outpatient follow-up visits until August 1998. Patients were selected on the basis of whether they had resectable

From the Department of Surgery, St. Agnes HealthCare, Baltimore, Md.
Presented at the Annual Meeting of the American College of Surgeons, Orlando, Fla., October 1998.
Reprint requests: Armando Sardi, M.D., St. Agnes HealthCare, Box 207, 900 Caton Ave., Baltimore, MD 21229.

Table I. Summary of method and results

	Total	Range	Median	Average
Patients	18			
Per year	9			
Male	10			
Female	8			
Age (yr)		29-81	70	59
Type of cancer				
Primary	2			
Metastatic	16 (88%)			
Comorbid diseases	14 (78%)			
Procedures				
Major	8 (44%)			
Lobectomies	4			
Trisegmentectomies	4			
Minor	10 (55%)			
Segmentectomies	4			
Single wedge resections	2			
Multiple wedge resections	4			
Estimated blood loss (ml)		300-2000		608
Transfusions	7 (38%)			
Complications	5 (27%)			
Hospital stay (days)		3-24	7	
Mortality	0			

liver disease. The criteria for resectability were determined by finding no evidence of extrahepatic malignancy. Exceptions included two patients with synchronous colonic primary lesions and one patient with recurrent renal cell cancer adherent to the abdominal wall, which were completely resected at the same time. Preoperatively all patients underwent a diagnostic screening to search for extrahepatic disease; this screening included CT of the chest and abdomen and CT portography. In addition, intraoperative ultrasound was performed in all patients.

The cohort consisted of 8 female and 10 male patients ranging in age from 29 to 81 years (mean 59.4 years). Sixteen (88%) of the 18 patients underwent hepatic resection for metastatic disease to the liver. Fifteen patients had colorectal sources for their metastatic disease; one patient had a recurrence of renal cell carcinoma with metastasis to the liver. Of note is that among the three patients with metastatic disease, two had postoperative pathologic findings that showed additional hemangiomas and one had a bile duct adenoma. Those patients with primary liver neoplasms included one patient with hepatocellular carcinoma and one patient with an adenoma.

The majority of patients, 14 (78%) of 18, presented with a wide variety of comorbid conditions that included diabetes, hypertension, sarcoidosis, cholelithiasis, chronic obstructive pulmonary disease, stroke, gout, gastroesophageal reflux disease, and a previously repaired abdominal aortic aneurysm. Several patients had a significant history of coronary artery disease including two patients whose status was post coronary bypass and one patient with mitral valve stenosis and a history of congestive heart failure. One patient suffered from radiation proctitis secondary to radiation therapy for rectal cancer. Four patients had a history of malignancies at sites other than the liver—two patients with melanoma and two patients with renal cell carcinoma. The one patient with hepatocellular cancer also had cirrhosis of the liver and chronic hepatitis. The extent of liver resections was categorized as either major or minor. We defined a minor resection as a simple wedge resection or segmentectomy. Four patients required more than one wedge resection with a maximum of three total wedge resections per patient. Major resections were defined as a lobectomy or trisegmentectomy. There were 10 minor (55%) and eight major (44%) liver resections. Of the eight major resections, there were four lobectomies and four trisegmentectomies. One patient with recurrent renal cell metastatic disease underwent cryoablation of a lesion in the right lobe, in addition to large wedge resection of the left lobe.

Outcomes and Results

We defined mortality as death occurring perioperatively or during the primary hospital stay. In our series mortality was 0%. However, five deaths occurred in our series. Three patients died of primary disease, two patients died of sepsis caused by a gastric rupture secondary to an obstruction by a previously placed Angelchik prosthesis, and one died of a new primary malignancy, respectively. Overall survival ranged from 4 to 42 months with a median survival of 26 months. Of those patients who survived, seven were alive with disease at last follow-up (Table II).

Five (27%) of 18 patients suffered from perioperative complications during the primary hospital stay. Two patients had atrial fibrillation, and one of these patients had postoperative ascites. One patient had an episode of postoperative pancreatitis requiring a second hospitalization of two days with no further sequelae. Another patient experienced urinary retention and was also discovered to have pseudomonas bacteremia, which resolved with intravenous antibiotic

Table II. Patient characteristics and follow-up

Patient	Age (yr)	Diagnosis	Type of operation	No. of lesions	Metachronous/ Synchronous	Survival	Follow-up (mo)
1	70	Colonic metastasis	Wedge resection	2	S	AWD (last visit 8/97)	26+
2	72	Renal cell carcinoma	Wedge resection	3	M	DWD	24+
3	58	Colonic metastasis	Lobectomy	2	M	AWD	42
4	70	Colonic metastasis	Segmentectomy	3	M	Died (breast cancer)	26
5	53	Colonic metastasis	Lobectomy	4	M	NED	39
6	54	Colonic metastasis	Trisegmentectomy	8	M	AWD (pulmonary metastasis)	36
7	74	Colonic metastasis	Segmentectomy	3	M	NED	36
8	79	Colonic metastasis	Lobectomy	1	M	AWD (last visit 4/98)	24
9	81	Colonic metastasis	Wedge resection	1	M	Died (perforation of stomach due to Angelchik prosthesis)	4
10	69	Colonic metastasis	Segmentectomy		S	AWD	33
11	64	Colonic metastasis	Lobectomy	5	M	DWD	8
12	29	Hepatic adenoma	Wedge resection	4	Adenoma	NED	29
13	80	Hepatoma	Wedge resection	1	Hepatoma	NED	26
14	68	Colonic metastasis	Trisegmentectomy	1	M	AWD (pancreatic mass)	25
15	70	Colonic metastasis	Segmentectomy	2	M	NED (recurrent pulmonary and chest wall metastasis)	22
16	49	Colonic metastasis	Lobectomy	1	M	NED	22
17	43	Colonic metastasis	Wedge resection	2	S	NED	20
18	44	Colonic metastasis	Trisegmentectomy	1	M	DWD	11

AWD = alive with disease; NED = no evidence of disease; DWD = died with disease.

therapy. The patient with the longest hospital stay of 24 days suffered the most complications, including pneumonia, delirium tremens, heparin-induced thrombocytopenia, and deep venous thrombosis. Hospital stays ranged from 4 to 24 days with an average of 8.1 days. Seven (38%) of the 18 patients required transfusion of a blood product either during surgery or afterward. Estimated blood loss ranged from 300 to 2000 ml with an average of 608 ml. Of the patients who underwent major resections, all except one had an estimated blood loss of 500 ml or greater. Six (50%) of 12 patients with an estimated blood loss of 500 ml or greater received transfusions of autologous blood.

DISCUSSION

Early series reported mortality rates following liver resection ranging from 10% to 20%[10]; this has now decreased to 2% to 5%.[11-13] Higher mortality rates had been attributed to a greater number of perioperative and postoperative complications,[11-16] such as excessive blood loss and sepsis.[17] More advanced surgical techniques, sophisticated equipment, and increasing surgical expertise have led to gradually decreasing complications and death. This has set the stage for an increasing number of liver resections being performed and more aggressive surgical approaches being attempted.[7-9,18]

With this emerging trend, the question arises as to whether liver resection, a high-risk surgical procedure, should be regionalized and performed only at high-volume referral centers.[7] Certainly it has been shown that for other high-risk surgical procedures performed at high-volume centers, there is lower mortality and morbidity[2-6]; however, little work has been directed specifically at hepatic resection and its relation to volume of referrals. In one of the few series reported in the literature, Choti et al.[7] found a 7.9% mortality rate for low-volume centers compared to a 1.5% rate for high-volume centers when performing hepatic resections. This mortality rate was based on stratified data that grouped high-, medium-, and low-volume referral centers (high volume, >15 per year; medium volume, between 7 and 15 per year; and low volume, <7 per year) for relative risk analysis.

Our center, with an average of only 6.72 hepatic resections a year, is considered a low-volume center. However, our in-hospital mortality rate was 0%. Furthermore, our rate of postoperative complications was a relatively low 28%. Nonfatal complications for partial hepatectomies have been reported to occur at a rate of 25% to 50%[1] so the 28% in our series falls within the lower end of the spectrum. In addition to a low rate of complications, only seven patients (38%) required blood transfusions. The reason for low mortality and morbidity despite low volume is likely multifactorial. Our center is a community teaching hospital where we have a surgical oncologist, anesthesiologist, and other support staff who are well versed in advanced surgical techniques with access to the modern equipment used in hepatic resection. Advanced surgical techniques and equipment, such as intraoperative ultrasound, cryosurgery, and ultrasonic dissection, which are available at our center, may not be readily available at most low-volume centers and may account for a reported higher mortality. Of equal importance is a surgical team that includes an anesthesiologist and nursing staff with broad experience in hepatic resection and its related postoperative management. Adverse outcomes can be avoided with early recognition of problems and familiarity with complex patients. A higher volume center is more likely to have an experienced surgical team along with availability of and familiarity with advanced surgical techniques and equipment for performing liver resection. One might safely assume that low-volume centers might achieve lower mortality and morbidity rates if they could provide surgical expertise with advanced surgical techniques and equipment despite having low referral numbers.

There are several limitations to this study. Although comorbidity, extent of resection, and nature of disease were included in our data set, they were not specifically looked at as individual variables to see how they might each affect mortality and morbidity. Second, most of the hepatic resections presented herein were performed for colorectal metastatic disease as opposed to primary hepatic malignancies. Choti et al.[7] found no significant differences in mortality between low-volume and high-volume centers for liver resections performed for colorectal metastatic disease. Third, our low rate of complications and zero mortality cannot automatically be assumed for other institutions of similar volume referral. A more appropriate approach to address this issue would be to examine regionally, nationally, or statewide all hospitals performing hepatic resections. Then one might establish a "minimum number of cases necessary" to safely perform hepatic resection.

CONCLUSION

The data presented here suggest that mortality and morbidity for liver resection are not affected solely by the volume of referrals to the institution and that other factors have significant influence. Moreover, safe hepatic resection with low mortality and morbidity may be accomplished at lower volume centers in the proper setting. Our experience lends support for

performing hepatic resection at low-volume referral centers provided an experienced surgical team who has expert familiarity with advanced surgical techniques and equipment is readily available.

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Commentary

Relating Surgical Volume to Outcomes in Hepatic Surgery

Michael A. Choti, M.D.

Organizations such as the Health Care Finance Administration have begun to emphasize the "centers of excellence" concept as a way to decrease health care costs and improve patient outcomes. Indeed, for many high-risk surgical procedures, patient outcomes have been shown to be better at hospitals where the procedures are performed more frequently compared to "low-volume centers."[1] Hepatic surgery is considered one of the more technically complicated and resource-intensive gastrointestinal procedures, and is often associated with high rates of morbidity and mortality.

In the study by Stone et al., reported in this issue, the authors present their series of 18 hepatic resections performed over a less than 3-year period at a community teaching hospital. This hospital was defined as a low-volume center (less than seven hepatic resections per year), and the authors reported no in-hospital deaths, a 27% complication rate, and an average hospital stay of 8.1 days. The authors conclude that these results reflect that, with surgical expertise and the use of advanced surgical techniques and equipment, favorable outcomes can be achieved in hospitals with low referral volume.

The authors are to be congratulated on the excellent results in this series of hepatic resections. However, caution must be exercised when generalizing outcomes based on such small numbers. For example, if the next two patients in this series died, the mortality rate would be 10%. This study reflects some of the many difficulties in determining unbiased estimates of the effect of volume on outcomes. A variety of factors,

From the Department of Surgery, Johns Hopkins Medical Institutions, Baltimore, Md.
Correspondence: Michael A. Choti, M.D., Department of Surgery, Johns Hopkins Hospital, 600 North Wolfe St., Halsted 614, Baltimore, MD 21287-5614. e-mail: mchoti@jhmi.edu

performing hepatic resection at low-volume referral centers provided an experienced surgical team who has expert familiarity with advanced surgical techniques and equipment is readily available.

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Commentary

Relating Surgical Volume to Outcomes in Hepatic Surgery

Michael A. Choti, M.D.

Organizations such as the Health Care Finance Administration have begun to emphasize the "centers of excellence" concept as a way to decrease health care costs and improve patient outcomes. Indeed, for many high-risk surgical procedures, patient outcomes have been shown to be better at hospitals where the procedures are performed more frequently compared to "low-volume centers."[1] Hepatic surgery is considered one of the more technically complicated and resource-intensive gastrointestinal procedures, and is often associated with high rates of morbidity and mortality.

In the study by Stone et al., reported in this issue, the authors present their series of 18 hepatic resections performed over a less than 3-year period at a community teaching hospital. This hospital was defined as a low-volume center (less than seven hepatic resections per year), and the authors reported no in-hospital deaths, a 27% complication rate, and an average hospital stay of 8.1 days. The authors conclude that these results reflect that, with surgical expertise and the use of advanced surgical techniques and equipment, favorable outcomes can be achieved in hospitals with low referral volume.

The authors are to be congratulated on the excellent results in this series of hepatic resections. However, caution must be exercised when generalizing outcomes based on such small numbers. For example, if the next two patients in this series died, the mortality rate would be 10%. This study reflects some of the many difficulties in determining unbiased estimates of the effect of volume on outcomes. A variety of factors,

From the Department of Surgery, Johns Hopkins Medical Institutions, Baltimore, Md.
Correspondence: Michael A. Choti, M.D., Department of Surgery, Johns Hopkins Hospital, 600 North Wolfe St., Halsted 614, Baltimore, MD 21287-5614. e-mail: mchoti@jhmi.edu

including sample size, case-mix adjustment, and timeliness of data, all need to be considered. Several studies have been reported in the literature examining the volume-outcomes relationship in patients undergoing hepatic resection.[2-4] In one study cited in this article, outcomes for hepatic resection in Maryland were analyzed in 606 patients from 52 hospitals.[3] The adjusted relative risk of in-hospital mortality in the low-volume group was 6.4 times that of the high-volume group. Another study compiled from California hospital discharge data examined liver resections performed for hepatocellular cancer.[4] These investigators found a risk-adjusted operative mortality rate of 9.4% in those centers performing more than 17 resections per 5 years compared to 22.7% in those performing one to two resections.

Although the presence of skillful surgeons with advanced equipment certainly contributes to improved outcome, the lower mortality seen at high-volume hospitals likely reflects many procedural and organizational aspects of surgical care including patient selection, anesthesia, and postoperative care. Ideally, patients who are potential candidates for hepatic resection should be referred to "centers of excellence" based on hospital performance rather than the proxy of hospital volume, particularly as some low-volume hospitals may have favorable outcomes. Unfortunately the ability to establish procedure-specific outcome measurements with adequate sample size and case-mix adjustment is difficult. Until such time when such databases are established, the preponderance of evidence for a variety of complex surgical procedures, including hepatic resection, suggests that centers performing high volumes have improved performance. The study by Stone et al. points to the need to better understand the attributes of high-volume providers that could lead to success in lower volume settings, as well as to further improve outcomes in the high-volume setting.

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Quality of Life and Long-Term Survival After Surgery for Chronic Pancreatitis

Taylor A. Sohn, M.D., Kurtis A. Campbell, M.D., Henry A. Pitt, M.D., Patricia K. Sauter, A.C.N.P., JoAnn Coleman, A.C.N.P., Keith D. Lillemoe, M.D., Charles J. Yeo, M.D., John L. Cameron, M.D.

The objective of this study was to evaluate the short-term and long-term outcome as well as quality of life in patients undergoing surgical management of chronic pancreatitis. Between January 1980 and December 1996, a total of 255 patients underwent surgery for chronic pancreatitis at The Johns Hopkins Hospital. The etiology of the disease, indications for surgery, patient characteristics and long-term survival were analyzed. A visual analog quality-of-life questionnaire containing 23 items graded on a scale of 0 to 10 (0 = worst and 10 = best) was sent to patients postoperatively. Visual analog responses relating to before and after the chronic pancreatitis surgery were compared using a paired *t* test. During the 17-year review period, 263 operations were performed for chronic pancreatitis in 255 patients. The most common presenting symptoms were abdominal pain (88%), weight loss (36%), nausea/vomiting (30%), jaundice (14%), and diarrhea (12%). The cause of the pancreatitis was presumed to be alcohol in 43%, idiopathic in 38%, pancreas divisum in 5%, ampullary abnormality in 4%, and gallstones in 3%. Pancreaticoduodenectomy was the most common procedure in 96 patients (37%), followed by distal pancreatectomy in 67 (25%), Puestow procedure in 52 (19%), sphincteroplasty in 37 (14%), and Duval procedure in five (2%). The overall mortality and morbidity rates were 1.9% and 35%, respectively. Two hundred twenty-seven (89%) of the 255 patients were alive at last follow-up. For the entire cohort of patients, the 5- and 10-year actuarial survivals were 88% and 82%, respectively. One hundred six (47%) of the 227 living patients responded to the visual analog quality-of-life questionnaire. Patients reported improvements in all aspects of the quality-of-life survey including enjoyment out of life, satisfaction with life, pain, number of hospitalizations, feelings of usefulness, and overall health ($P < 0.005$). In addition to improved quality of life after surgery, narcotic use was decreased (41% vs. 21%, $P < 0.01$) and alcohol use was decreased (59% vs. 33%, $P < 0.001$). However, patients often became insulin-dependent diabetics (12% vs. 41%, $P < 0.0001$) and required pancreatic enzyme supplementation (34% vs. 55%, $P < 0.01$) after surgical intervention. These data suggest that surgery for patients with chronic pancreatitis can be performed safely with minimal morbidity and excellent long-term survival. Moreover, this study evaluates quality of life in a standardized analog fashion, with highly significant improvement reported in all quality-of-life measures. We conclude that surgery remains an excellent option for patients with chronic pancreatitis. (J GASTROINTEST SURG 2000;4:355-365.)

KEY WORDS: Survival, quality of life, pancreatitis

Chronic pancreatitis is often characterized by persistent severe abdominal pain accompanied by progressive pancreatic endocrine and exocrine insufficiency often requiring multiple hospitalizations. The treatment of chronic pancreatitis remains a challenging problem. The disease is frequently the result of chronic alcohol abuse, and patients are often addicted to narcotics at the time of presentation to a surgeon. Although conservative management may be successful in some patients, the remission of pancreatic pain is uncommon and not consistently observed.[1] Those who do not experience pain relief with conservative management often suffer from progressive diabetes, pancreatic exocrine dysfunction, and

From the Department of Surgery, The Johns Hopkins Medical Institutions, Baltimore, Md.
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Reprint requests: Kurtis A. Campbell, M.D., Assistant Professor of Surgery, The Johns Hopkins Medical Institutions, Osler 625, 600 N. Wolfe St., Baltimore, MD 21287. e-mail: kcampbe@welchlink.welch.jhu.edu

unremitting pain, all of which adversely affect quality of life.

Over the past three decades, many advances have been made in the surgical management of this disease. A broad spectrum of surgical procedures has been applied, aimed primarily at the relief of pain and the management of complications associated with chronic pancreatitis. The procedures fall into three broad categories: (1) ablative procedures including pancreaticoduodenectomy and distal pancreatectomy; (2) drainage procedures including longitudinal pancreaticojejunostomy (Puestow procedure) and distal pancreaticojejunostomy (Duval procedure); and (3) sphincter procedures including major/minor papilla sphincteroplasty and pancreatic duct septoplasty. The choice of procedure is typically tailored to the pattern of disease based on preoperative imaging studies including endoscopic retrograde cholangiopancreatography (ERCP), computed tomography (CT), and magnetic resonance cholangiopancreatography (MRCP).

Because of the many surgical options, the results of various series are difficult to interpret. To assess the efficacy of various therapeutic maneuvers, Frey et al.[2] have suggested that multiple factors should be examined and reported. Outcomes should include the ease and safety of the procedures, the completeness and duration of the pain relief, the incidence and severity of the physiologic impairments, morbidity and mortality and their impact on quality of life. Unlike surgical outcomes and physiologic effects, the measurement of pain and quality of life are subjective. Many previous reports lack a standard method to assess these outcomes.[3-15] In addition to reporting the objective surgical outcomes, the current study assesses pain and quality of life in standardized analog fashion, in an attempt to better define the role of surgical therapy in this difficult disease.

PATIENTS AND METHODS

Between January 1980 and December 1996 inclusive, 255 patients underwent 263 operations for chronic pancreatitis at The Johns Hopkins Hospital. A retrospective chart review was undertaken to examine patient demographics, medical history, etiology of chronic pancreatitis, indications for surgery, type of procedure performed, and postoperative course.

Causes of chronic pancreatitis considered included alcohol abuse, gallstones, familial pancreatitis, pancreas divisum, hypercalcemia, hyperlipidemia, ampullary abnormalities (including sphincter of Oddi dysfunction and duodenal diverticulum), trauma (including ERCP-induced pancreatitis), and idiopathic and other miscellaneous causes. In patients with a history of alcohol abuse, a significant period of abstinence was strongly encouraged before surgery was undertaken. Patients were classified as having "idiopathic" pancreatitis when none of the other possible causes were clearly identified during careful chart review.

A variety of surgical procedures were performed including pancreaticoduodenectomy, distal pancreatectomy, the Puestow procedure, sphincteroplasty, and the Duval procedure. Most of the pancreaticoduodenectomies were pylorus-preserving partial pancreatectomies that left the body and tail of the pancreas in place. Distal pancreatectomy included splenectomy in the vast majority of cases. Sphincter procedures included major and minor papilla sphincteroplasties as well as pancreatic duct septotomy. Cholecystectomy was also often performed.

The choice of procedure was based on presenting symptoms, radiologic findings, and intraoperative findings. If a patient had no evidence of pancreatic ductal dilatation, an ablative procedure such as distal pancreatectomy or pancreaticoduodenectomy was preferred, with pancreaticoduodenectomy being performed for disease thought to be centered in the pancreatic head. In addition, ablative procedures were performed when malignancy was suspected. Drainage procedures were performed in patients with documented pancreatic ductal dilatation, pain, and no evidence of malignancy. Sphincter procedures were performed in patients with documented sphincter of Oddi dysfunction or pancreas divisum as the presumed cause of their pancreatitis.

The overall incidence of postoperative complications, mortality, and length of stay were evaluated. Perioperative mortality was defined as death during the initial hospitalization or within 30 days of surgery. Specifically, the incidences of reoperation in the immediate postoperative period, delayed gastric emptying, pancreatic fistula, intra-abdominal abscess formation, wound infection, urinary tract infection, pneumonia, and cholangitis were calculated. Delayed gastric emptying and pancreatic fistula were defined as previously described.[16-18] All infectious complications including intra-abdominal abscess formation, wound infection, urinary tract infection, pneumonia, and cholangitis were defined by fever with a documented positive culture from the suspected source. Intra-abdominal abscess required documentation by CT scan, whereas patients with pneumonia had radiographic evidence of an infiltrate. Wound infection required purulent drainage from the wound, necessitating opening of the wound.

To identify differences among groups of patients undergoing different operative procedures, the demographic and historic characteristics of the groups

were compared using chi-square test, analysis of variance, and *t* test when appropriate. The operative and postoperative courses were compared for the different groups in a similar fashion. All data are expressed as mean ± standard error of the mean. Survival information was obtained via surgeon, hospital, and United States Social Security Administration records, as well as direct patient contact. Fourteen (5%) of the 255 patients were lost to follow-up. Survival was analyzed by the method of Kaplan and Meier.[19]

A visual analog quality-of-life questionnaire was sent to the 227 patients alive at the time of survey. This instrument was patterned after those previously shown to be valid by Ferrell et al.[20] and modified in conjunction with the surgical staff and a small sample of chronic pancreatitis patients to ensure its content and construct validity. It uses a visual analog scale, which has been shown to be a reproducible method of quantitating subjective data. The questionnaire contained 23 items graded by the patient on a scale of 0 to 10, with 0 being the worst and 10 being the best. Patients were asked to respond to questions pertaining to their perception of multiple aspects of quality of life before and after surgery. The responses were compared using a paired *t* test, with each patient serving as his or her own control. Moreover, duplicate questionnaires were redistributed to a subset of our patients at a second point in time in order to confirm test-retest reliability of our instrument. In addition, the questionnaire addressed such issues as narcotic use, alcohol use, employment status, insulin dependence, and the need for pancreatic enzyme supplementation before and after surgery. A chi-square analysis was performed to identify differences in dichotomous variables before and after surgery.

RESULTS

Over the 17-year review period, 263 operations were performed for chronic pancreatitis in 255 patients. Eight patients underwent two different surgical procedures during separate hospital admission. An increasing volume was observed, with four procedures performed in 1980 and 30 in 1996.

The demographics, presenting signs and symptoms, as well as past medical history are summarized in Table I. The most common presenting symptom was abdominal pain seen in 88% of the patients. Of note, 58% of the patients were smokers, 53% were dependent on narcotics, and 45% had a history of alcohol abuse. The presumed etiology of the chronic pancreatitis is listed in Table II, with the largest number of patients having alcohol-related disease. The miscellaneous causes included cystic fibrosis, systemic lupus erythematosus, gastrinoma, and choledochal cyst.

Table I. Demographics, presenting signs and symptoms, and medical history (N = 255)

Demographics	
Age	
Mean ± standard error	47.9 ± 0.9 yr
Median	47.0 yr
Sex	
Male	53%
Female	47%
Race	
White	82%
Black	16%
Other	2%
Presenting signs and symptoms	
Abdominal pain	88%
Weight loss	36%
Nausea/vomiting	30%
Jaundice	14%
Diarrhea	12%
Steatorrhea	8%
Fevers/chills	6%
Gastrointestinal bleeding	4%
Medical history	
Smoking	58%
Narcotic dependence	53%
Alcohol abuse	45%
Peptic ulcer disease	21%
Hypertension	19%
Diabetes	18%
Myocardial infarction	7%
Chronic obstructive pulmonary disease	4%
Peripheral vascular disease	4%

Table II. Presumed etiology of chronic pancreatitis (N = 255)

Alcohol-related	43%
Idiopathic	39%
Pancreas divisum	5%
Ampullary abnormality	4%
Gallstones	3%
Trauma	2%
Familial	2%
Hyperlipidemia	0.8%
Hypercalcemia	0.4%
Other	2%

The indications for surgical intervention were chronic pain in 48%, acute recurrent episodes of pain and/or pancreatitis in 21%, possible malignancy with a history of chronic pancreatitis in 16%, possible malignancy without a history of chronic pancreatitis in 14%, and miscellaneous in 1%. Of the 255 patients,

Table III. Comparison of different operative procedures, demographics, and presenting symptoms

	Pancreaticoduodenectomy (N = 96)	Distal pancreatectomy (N = 67)	Puestow procedure (N = 52)	Sphincteroplasty (N = 37)
Demographics				
Age (mean ± standard error)	53.5 ± 1.6 yr	44.8 ± 1.5 yr*	46.1 ± 2.0 yr*	39.7 ± 2.2 yr*
% Male	56	46	67	32*
% White	83	88	77	94
Signs and symptoms				
Abdominal pain (%)	71	96*	100*	100*
Jaundice (%)	34	0*	0*	3*
Steatorrhea (%)	3	14*	8	11
Weight loss (%)	45	38	33	22*

*$P < 0.05$ when compared to pancreaticoduodenectomy.

36 (14%) had mechanical complications of chronic pancreatitis in addition to the above-mentioned indication for surgery. These included obstructive jaundice, cholangitis, duodenal obstruction, and pancreaticocutaneous fistula formation.

Preoperative workup was not standardized and was performed under the direction of the referring physician and the attending surgeon. Studies included ERCP in 78% of patients, CT scan in 68% of patients, mesenteric angiography in 27%, percutaneous transhepatic cholangiography/percutaneous biliary drainage in 21%, transabdominal ultrasound in 16%, and MRI in 4%.

Pancreaticoduodenectomy was the most common procedure, performed in 96 patients (37%), followed by distal pancreatectomy in 67 (25%), Puestow procedure in 52 (19%), sphincteroplasty in 37 (14%), and Duval procedure in five (2%). The remaining six patients (2%) underwent miscellaneous procedures including cholecystectomy alone, drainage of a pseudocyst, choledochojejunostomy, choledochoscopy, G-tube placement, pancreatic duct septotomy alone, and common bile duct exploration. Four of the 96 patients undergoing pancreaticoduodenectomy had a total pancreatectomy. Thirty-five percent of patients had undergone previous cholecystectomy, whereas 71% of the 178 patients with in situ gallbladders underwent cholecystectomy as part of their operative procedure. Eight patients underwent a second surgical procedure during a separate hospitalization. Two patients who had undergone pancreaticoduodenectomy were treated with completion distal pancreatectomy. The other six operations were distal pancreatectomy followed by completion pancreaticoduodenectomy, sphincteroplasty followed by pancreaticoduodenectomy, Puestow procedure followed by pancreaticoduodenectomy, Puestow procedure followed by sphincteroplasty, Puestow procedure followed by distal pancreatectomy, and pseudocyst drainage followed by distal pancreatectomy.

The patients undergoing pancreaticoduodenectomy, distal pancreatectomy, Puestow procedure, and sphincter procedures were compared. The results are summarized in Table III. Those undergoing pancreaticoduodenectomy were older, significantly less likely to present with pain, and more likely to present with jaundice as compared to those undergoing other procedures. Compared to those treated by pancreaticoduodenectomy, preoperative steatorrhea was noted significantly more frequently in patients undergoing distal pancreatectomy, whereas weight loss was significantly less common in those undergoing sphincteroplasty. The frequencies of other presenting symptoms were otherwise similar among the groups.

The intraoperative and postoperative results for the different procedures are summarized in Table IV. As would be expected, patients treated via pancreaticoduodenectomy had a significantly longer operative time than the three other procedures and a greater mean estimated blood loss than those treated via Puestow procedure or sphincteroplasty. Distal pancreatectomy was associated with the greatest estimated blood loss and largest transfusion requirement.

Five deaths occurred in the series for an overall perioperative mortality rate of 1.9%, with two deaths in the pancreaticoduodenectomy group (2.1%), two deaths in the distal pancreatectomy group (3.6%), and one death in the Puestow group (1.9%, P = not significant [NS]). The overall complication rate was 35%, with no significant differences being observed for the different procedures. Nine patients (3%) required reoperation in the immediate postoperative period with the indications for reoperation being hemorrhage in four patients, abscess or intra-abdominal sep-

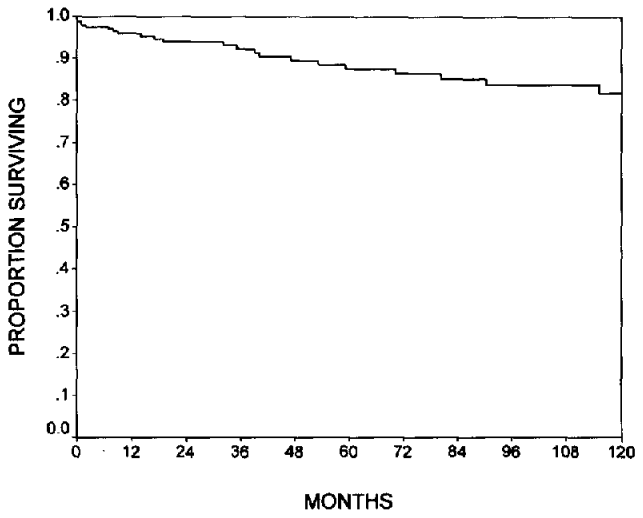

Fig. 1. Kaplan-Meier actuarial survival curves for patients undergoing surgery for chronic pancreatitis (n = 241; median survival not yet reached, 5- and 10-year survival rates of 88% and 82%, respectively).

Table IV. Comparison of different operative procedures: Operative and postoperative data

	Pancreaticoduodenectomy (N = 96)	Distal pancreatectomy (N = 67)	Puestow procedure (N = 52)	Sphincteroplasty (N = 37)
Operative data				
Estimated blood loss (mean ± SE)	995 ± 120 ml	1330 ± 210 ml	600 ± 180 ml*	190 ± 22 ml*
Transfusion of PRBCs (mean ± SE)	1.0 ± 0.2 units	1.9 ± 0.3 units*	0.5 ± 0.2 units	0.0 ± 0.0 units
Operative time (mean ± SE)	7.5 ± 0.2 hr	5.2 ± 0.2 hr*	5.2 ± 0.2 hr*	3.9 ± 0.2 hr*
Postoperative course				
Mortality	2.1%	3.6%	1.9%	0.0%
Overall complication rate	43%	34%	31%	40%
Reoperation	2%	9%	4%	0%
Delayed gastric emptying	18%	2%*	2%*	0%*
Pancreatic fistula	11%	9%	2%	20%
Wound infection	10%	2%	2%	20%
Intra-abdominal abscess	4%	4%	0%	0%
Pneumonia	7%	4%	4%	0%
Cholangitis	4%	0%	0%	0%
Urinary tract infection	5%	6%	0%	0%
Postoperative length of stay (mean ± SE)	15.4 ± 0.8 days	15.6 ± 1.3 days	12.4 ± 0.9 days*	10.3 ± 0.9 days*

PRBC = packed red blood cells.
*$P < 0.05$ when compared to pancreaticoduodenectomy.

sis in three patients, portal vein thrombosis in one patient, and small bowel obstruction in one patient. Three of the five postoperative deaths occurred in patients requiring reoperation. Delayed gastric emptying occurred most commonly in those undergoing pancreaticoduodenectomy (18%), whereas the incidences of other complications were similar among the groups (see Table IV). The postoperative length of stay was significantly longer for the patients undergoing pancreaticoduodenectomy and distal pancreatectomy.

Survival information was available for 241 patients (95%), with the remaining 14 patients being lost to follow-up. The mean follow-up was 55 months, with a median follow-up of 32.5 months. For the entire cohort of patients, the 5- and 10-year actuarial survival rates were 88% and 82%, respectively. The survival curve is depicted in Fig. 1. No differences in survival were observed for patients undergoing different procedures, with 5-year actuarial survival rates of 90% following pancreaticoduodenectomy, 86% following

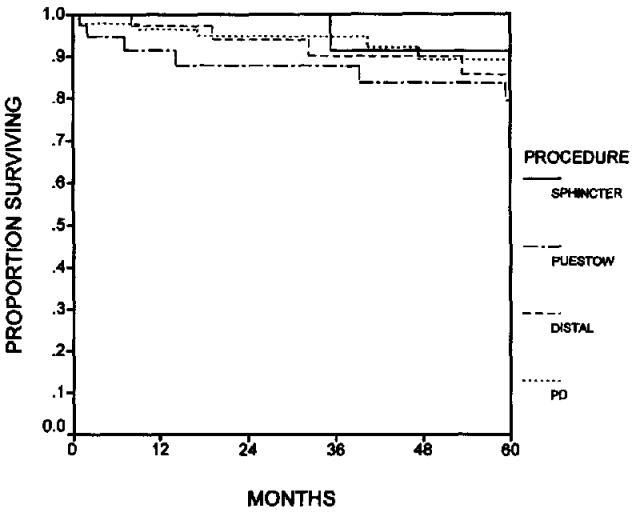

Fig. 2. Kaplan-Meier actuarial survival curves broken down by operative procedure. Five-year survival rates: 90% for pancreaticoduodenectomy (*PD*, n = 96), 86% for distal pancreatectomy (n = 67), 80% for Puestow procedure (n = 52), and 92% for sphincteroplasty (n = 37, *P* = NS).

Table V. Visual analog quality-of-life responses (N = 106)

	Prior to surgery	Now	Paired t test
Perception of quality of life			
Enjoyment out of life	4.2 ± 0.3	6.9 ± 0.3	<0.0001
Satisfaction with life	4.0 ± 0.3	7.0 ± 0.3	<0.0001
Quality of life	4.0 ± 0.3	7.1 ± 0.3	<0.0001
Ability to care for oneself			
Feelings of control	5.4 ± 0.3	7.5 ± 0.2	<0.0001
Feelings of usefulness	5.4 ± 0.3	7.5 ± 0.3	<0.0001
Ability to care for personal needs	8.6 ± 0.2	9.3 ± 0.2	<0.01
Participation in activities	6.2 ± 0.3	8.1 ± 0.3	<0.0001
Perception of health status			
Overall health	3.7 ± 0.3	6.6 ± 0.3	<0.0001
Absence of pain	3.2 ± 0.4	6.8 ± 0.3	<0.0001
Appetite	4.3 ± 0.4	7.4 ± 0.3	<0.0001
Bowel habits	5.4 ± 0.3	6.8 ± 0.3	<0.0001
Frequency of nausea	5.4 ± 0.4	7.7 ± 0.3	<0.0001
Frequency of vomiting	6.2 ± 0.4	8.6 ± 0.2	<0.0001
Objective measures			
Number of hospital admissions/year	4.4 ± 1.2	0.6 ± 0.1	<0.01
Weight (pounds)	152 ± 3.9	153 ± 3.3	0.767

Mean ± standard error.

distal pancreatectomy, 80% following Puestow procedure, and 92% following sphincteroplasty (Fig. 2). An alcoholic etiology of chronic pancreatitis did not adversely affect survival, with an 85% 5-year survival in alcoholic patients (n = 100) compared to 89% in nonalcoholic patients (n = 141, *P* = NS).

Thirty percent of patients underwent surgery for suspicion of malignancy (16% with a history of chronic pancreatitis and 14% without). As would be expected, these patients were significantly older than those without suspected malignancy (56.6 years vs. 43.5 years, *P* <0.0001), with similar sex and race distributions. This group was significantly less likely to present with abdominal pain (71% vs. 95%, *P* <0.0001) and significantly more likely to present with jaundice (37% vs. 3%, *P* <0.0001) and weight loss (57% vs. 27%, *P* <0.0001). There was no difference in survival between the two groups with 5-year survivals of 88% for both groups (suspected malignancy, n = 77; no suspected malignancy, n = 164; *P* = NS).

Of the 227 patients alive at the time of surgery, 106 (47%) responded to the quality-of-life questionnaire and the health and habits survey. Patients demonstrated a dramatic improvement in all questions regarding quality of life (Table V). The survey questions

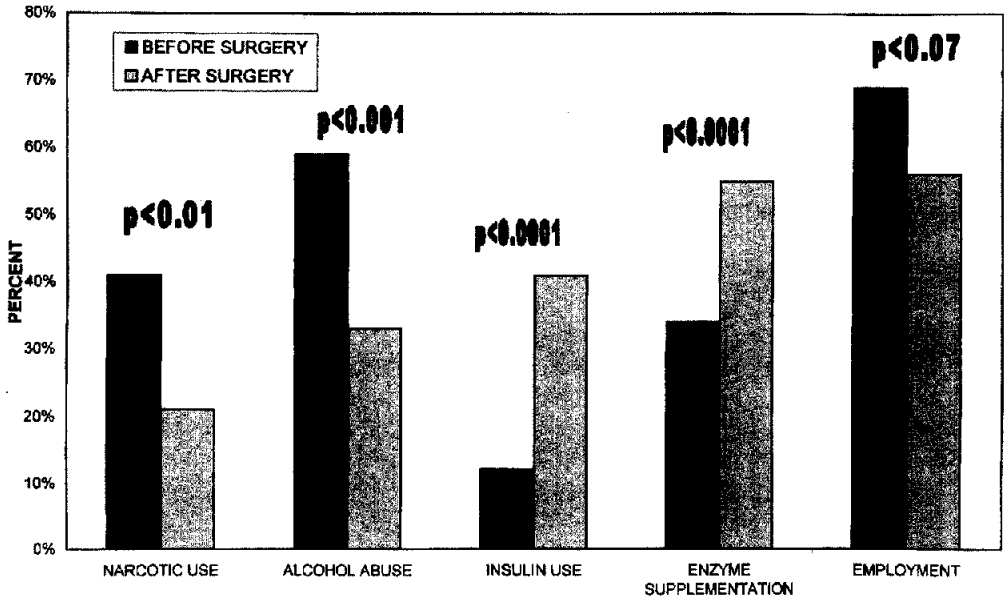

Fig. 3. Health and habits before and after surgery, including narcotic use, alcohol abuse, insulin use, enzyme supplementation, and employment status.

can be broken down into three broad categories: (1) perceptions of quality of life; (2) ability to care for oneself; and (3) perceptions regarding general health status. Patients showed dramatic improvement after surgery on all questions regarding subjective perception of quality of life. As for objective measures, the number of hospital admissions decreased significantly after surgery. Weight remained stable before and after surgery.

In addition, the results of the health and habits survey (Fig. 3) demonstrated a decrease in narcotic use (41% to 21%) and alcohol use (59% to 33%) following surgical intervention. However, surgery often exacerbated the pathophysiologic consequences of chronic pancreatitis. A significant portion of patients developed insulin-dependent diabetes (12% to 41%) and required pancreatic exocrine supplementation (34% to 55%) after surgery. In addition, the rate of employment declined (69% to 56%) after surgical intervention.

DISCUSSION

The medical management of chronic pancreatitis remains a difficult therapeutic problem. Chronic pancreatitis is often the result of alcohol abuse, and patients are often addicted to alcohol and narcotics at the time they are initially seen by a surgeon.[6] Most series report alcohol abuse as the cause of pancreatitis in 50% to 70% of patients, with the cause being idiopathic in 20% to 30%.[3-5,7,11,21,22] In the current series, the cause was thought to be alcohol related in 43% of patients, with the cause being idiopathic in an additional 39%. This slight difference from most other series may be due in part to the proportion of patients (30%) undergoing surgical exploration for a suspicion of malignancy. Sixteen percent of patients had symptoms of chronic pancreatitis with radiographic findings suspicious for malignancy. Fourteen percent of patients had no history of pancreatitis, presented with abdominal pain and a mass, and were thought to harbor a malignancy, but final pathologic findings were consistent with chronic pancreatitis. The latter group of patients was included in the analysis because the authors believe that presentation with an abdominal mass and abdominal pain, with or without jaundice, may represent an indolent form of chronic pancreatitis. The pathologic findings are indistinguishable from those seen in patients with a more classic history.

Multiple studies demonstrate that pancreatic surgery for benign disease can be performed safely.[21,23-26] In many previous reports the emphasis has been placed on the results of a particular approach or procedure,[8,9,11-15,27] whereas others report a variety of procedures, the choice of which is tailored to the patient's presentation and pathophysiologic findings.[3,5-7,10,26] This current report duplicates the latter, reviewing a series of procedures that were performed on the basis of the clinical presentation, radiographic studies, and intraoperative findings in individual patients. As would be expected, differences were observed in the presenting symptoms among those undergoing dif-

ferent procedures, reflecting the tailoring of the operative procedure to the underlying pathology. All procedures including pancreaticoduodenectomy, distal pancreatectomy, Puestow procedures, sphincter procedures, and Duval procedures were performed safely, with an overall mortality rate of 1.9% and procedure-specific mortality rates of less than 4% (see Table IV). The overall complication rate was 35%, with no statistical differences among the procedures. Although operative complications were not infrequent, most did not require reoperation and did not lead to death or significantly prolonged lengths of stay.

The reported late mortality after surgical intervention for chronic pancreatitis has been as high as 20% to 50%,[4,5,7,9,28,29] with the majority of late deaths being from end-stage liver disease, suicide, accidents, lung cancer, cardiac causes, and problems relating to brittle pancreatic endocrine insufficiency.[6,17,30-33] In the current series, long-term survival was acceptable, with 5- and 10-year actuarial survival rates of 88% and 82%, respectively. Of the 14 patients known to be deceased, one died of pancreatic adenocarcinoma, two died of myocardial infarction, one died of diabetic complications, and the remainder died of alcohol-related causes. This information suggests that the endocrine and exocrine complications of chronic pancreatitis, often accelerated by surgical intervention, were medically well managed and did not adversely affect long-term survival. In addition, the etiology of the pancreatitis and the operative procedure performed did not influence long-term survival. Despite a number of patients dying of alcohol-related causes, alcoholic patients shared a similar 5-year survival as those whose chronic pancreatitis was from other causes. This excellent 5-year survival in alcoholic patients differs from that seen in a recent study of surgical and medical patients with alcoholic pancreatitis in which 56 of 116 surgical patients and 54 of 91 medical patients died at a mean follow-up of 14.7 and 15.5 years, respectively.[22]

Medically intractable pain, which adversely affects quality of life, remains the most common indication for surgery in patients with chronic pancreatitis. Therefore any measurement of the success of surgical intervention should include assessments of pain control and patient perception of quality of life. Although numerous quality-of-life tools are available, several authors suggest that it is most appropriate to use tools that focus on patients' perceptions of their health status and nonmedical aspects (or quality) of life.[2,34,35] Many authors previously reported pain relief as "good" or "poor," or in categories such as "asymptomatic," "occasional pain," "disappearance," "alleviation," "improvement," or "no improvement."[3-8,11,13-15]

Evans et al.,[21] in a 1997 report, attempted to quantify quality of life in 44 patients. As does the present study, their report included various operative procedures individualized on the basis of preoperative data. The frequency and severity of abdominal pain were assessed at 6 weeks, 3 months, 6 months, 1 year, and then annually, graded on a scale of 0 to 4. Using an unpaired *t* test, a significant difference in pain score was consistently observed over the time intervals measured. In addition, there was an observed decrease in narcotic use, an increase in weight, and an increase in employment. Although objective measures of outcome were reproducibly measured, patient perceptions of subjective quality of life and overall health status were not assessed.

In a 1998 report from Izbicki et al.,[27] 61 patients with chronic pancreatitis were randomized to undergo longitudinal pancreaticojejunostomy combined with local pancreatic head resection (n = 31) or pylorus-preserving pancreaticoduodenectomy. Although the resolution of mechanical complications in adjacent organs was better following pancreaticoduodenectomy (100% vs. 93.5%), pancreaticoduodenectomy had a higher in-hospital complication rate (53.3% vs. 19.4%). Pain and quality of life were assessed on a scale of 0 to 100, with a 94% improvement in the pain score following drainage and local pancreatic head resection and a 95% improvement following pancreaticoduodenectomy. A reported 71% improvement in global quality of life was seen after longitudinal pancreaticojejunostomy and local pancreatic head resection vs. 43% after pancreaticoduodenum-preserving pancreatic head resection.

This review attempts to report pain control and quality of life in a standardized analog fashion. Because the symptoms associated with chronic pancreatitis are patient centered, no physiologic or clinical end point is satisfactory.[34] There is often no correlation between physiologic measures and patient-perceived outcome. The quality-of-life questionnaire was designed to be disease specific. We felt this was important, since generic quality-of-life scales such as the short form 36 are more appropriate when comparing outcomes across populations or a broad range of diseases and interventions. Disease-specific instruments better assess the responsiveness of a particular disease or condition to a specific intervention, in this case surgery.

Patients were asked, at a single point in time, to rate multiple aspects of quality of life before and after surgery. Ideally, patients would be asked quality-of-life questions before and after surgery to eliminate the inherent bias associated with retrospective review. In an attempt to minimize the bias, all quality-of-life data were paired for analysis using a

paired *t* test in which patients served as their own controls. Multiple questions were asked in each of three broad categories (perception of quality of life, ability to care for oneself, and perception of general health status) with highly significant results for each question, thus strengthening the data. Patients reported significant improvements in perception of quality of life, ability to care for themselves, and perception of health status. Patients had significantly decreased pain following surgical intervention for chronic pancreatitis. The fact that only 47% of patients responded to the quality-of-life questionnaire is a limitation of this study, which may have favorably biased the results.

Narcotic and alcohol addiction were noted to decrease in patients following surgery. It is, however, disappointing that several patients progressed to die of alcohol-related causes. On the other hand, the endocrine and exocrine insufficiency, which were likely accelerated by surgery, did not adversely affect long-term survival or patient perception of quality of life. Only one patient in the series died of complications directly related to brittle diabetes. In this series, employment status decreased. This decline may be due, in part, to a bias created by the age of the individuals who responded to the quality-of-life questionnaire. Many of the patients in this series who were operated on in the 1980s have since reached retirement age. Evans et al.[21] show an initial increase in employment, which declines toward preoperative baseline over time. In a series of patients with alcoholic chronic pancreatitis, a reduced postoperative employment status was noted.[22] Finally, in the study by Frey and Amikura[13] there was no change in the percentage of patients (59%) who were working preoperatively and postoperatively.

This retrospective review reports one of the largest series of patients undergoing operations for chronic pancreatitis. These data suggest that surgery for chronic pancreatitis can be performed safely, with acceptably low morbidity and mortality. Despite increases in endocrine and exocrine insufficiency, patients enjoyed acceptable long-term survival following operative intervention, with improved quality of life. The current study evaluates pain control and other subjective aspects of quality of life in a disease-specific and standardized fashion, with highly significant improvement reported in all quality-of-life measures. The choice of surgical therapy should be based on the individual's symptom complex and pattern of disease on radiographic studies. With careful patient and procedure selection, surgery is associated with improved quality of life and long-term survival and remains an excellent option for patients with chronic pancreatitis.

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Discussion

Dr. C. Frey (Sacramento, Calif.). I compliment the authors on the enormous effort they made in following this large group of patients with chronic pancreatitis, on a low surgical mortality and morbidity, and on an excellent 10-year actuarial survival of 80% and mean follow-up of 55 months. They are also to be complimented on their patient assessment, which used a visual analog scale to measure pain and quality of life in a health habit survey. Having said that, I am mystified as to how to weigh and interpret the significance of the very favorable results reported when only 106 or 47% of the 227 surviving patients operated on were assessed on the basis of pain, quality of life, and health habit survey. Of the 241 patients alive at the time of follow-up, 14 were lost to follow-up and 106 filled out a quality-of-life questionnaire and health habit survey. Do you have any information on the condition of the other 121 patients other than they were alive?

Could you tell us how many of your patients had concomitant pseudocysts at the time of operation and could you clarify the extent of your distal pancreatectomy? Were these 80% to 95% resections or were they limited to the body and tail of the pancreas? Last, I would like to compliment the authors on individualizing their operative procedures based on the structural abnormalities present and the liberal use of pancreaticoduodenectomy in patients for whom there is any suspicion of malignancy, without requiring histologic verification.

Dr. K. Campbell. As anyone who works with this group of patients knows, follow-up can be quite difficult. We have no data on our group of 121 patients other than they were alive. That knowledge was gained through tenacity as well as a number of different means, one of which was the United States Social Security Administration data, which are now available on the internet. Quality-of-life questionnaires were sent to all 227 patients, and patients were in fact sent questionnaires a second and sometimes even a third time to try and achieve a high rate of return. Despite our efforts, only 106 patients (47%) returned their questionnaires. I think the strength, however, comes from the fact that the data are internally controlled and the data are paired. So I think that the quality-of-life measures are relatively good.

With regard to the number of pseudocysts in these patients, there were actually very few. I am sure that some pseudocysts do cause chronic pain, although I am not as sure as the authors of a recent report (Ammann RW, et al. Gastroenterology 1999;116:1132-1140), which suggests that pseudocysts are related to the chronic pain in chronic pancreatitis. We think that patients with pseudocysts have had acute pancreatitis and are a different etiologic group with regard to pain. Last, the distal pancreatectomies were all significant resections, at least to the superior mesenteric vessels. Most of them were 85% to 95% pancreatectomies and none were small resections at the tail of the pancreas.

Dr. M. Buchler (Bern, Switzerland). This one of the largest series in the world on surgery for chronic pancreatitis, and the data speak for themselves. I have a question with regard to your policy of employing a Whipple resection in chronic pancreatitis. I am sure you are aware that there are randomized trials comparing the Whipple procedure with newer operations that preserve the duodenum (i.e., the Beger or the Frey procedure). In these trials we have seen that patients do better in terms of quality of life and many other aspects after such duodenum-preserving head resections. So why do you use the traditional Whipple resection in these patients and not shift to a more modern operative treatment of pancreatic head resection?

Dr. Campbell. Our experience with the Whipple procedure, be it for benign or malignant disease, speaks for itself. The low morbidity and mortality supports our use of the Whipple procedure as our resection of choice in patients who have an inflammatory mass in the head of the

pancreas. Certainly there are other ablative procedures that have been shown to have good quality-of-life measures, although not to my knowledge in a standardized fashion.

Dr. W. Nealon (Galveston, Texas). There are three general areas that I would consider worthy of focus. One is the quality of the population. Is this a typical population for chronic pancreatitis? As you mention in your article, there are features that are somewhat different. For example, there is a larger percentage of idiopathic pancreatitis (38%) and a lower rate of alcohol abuse as the etiology (43%) compared to most large populations. I was interested in the fact that you had a different distribution of males and females with 53% and 47%, respectively. Although you mentioned that ruling out cancer as one of your operative indications may result in a different distribution, I wonder if you could give it a little more thought. I am specifically interested in sphincter of Oddi dysfunction, which I would say is not typically represented in large volume; 68% of the patients in that group are women. If you extracted patients with sphincter of Oddi dysfunction, would you see anything different?

You told us that there are functional derangements after all of the operations. Have you looked at the specific operative procedures to see whether endocrine or exocrine dysfunction is more common after any particular operation?

I want to discuss the question of complication rate. I mention this because the reported complication rates in surgical references are often cited as a reason for employing nonsurgical interventions. You related an across-the-board complication rate of 35% to 40% and stated that the complication rates were essentially equal for all three operations. This worries me a little. It is certainly not unusual to have that rate of morbidity with the Whipple resection. But with the Puestow procedure, you appear, at least in your article, to report what appear to be considerably higher morbidity rates than usual in that subset of patients.

Dr. Campbell. With regard to your question regarding the population, our institution is in an urban area, but the high incidence of idiopathic causes and periampullary abnormalities and a lower incidence of alcohol abuse may have to do with the fact of tertiary referral. I cannot answer you regarding sphincter of Oddi dysfunction and the female and male distribution. Our complication rate was equally low across the group. I think we have performed fewer ductal drainage procedures recently, and perhaps the higher complication rates with the Puestow procedures relates to them being performed longer ago.

Dr. R. Prinz (Chicago, Ill.). I would like to echo Dr. Nealon's comments about etiology in your series. How does it differ from most series on chronic pancreatitis? Have you looked at the visual analog results in the alcoholic patients and in the patients with idiopathic pancreatitis, and did you find a difference? Second, you have a larger number of patients in whom the indication for surgery was a possible malignancy. Certainly finding out that they did not have a malignancy would improve the quality of life for most people. I wonder if you would comment on that?

Dr. Campbell. There was no difference in quality-of-life measures in the group with alcohol-related disease compared to the idiopathic group. With regard to malignancy, those patients underwent resection for pain and what appeared to be a lesion in the head of the pancreas or a periampullary mass. If the histologic analysis was negative for malignancy and showed only chronic pancreatitis, those patients were included in the group. But they are a different subgroup that we hope to study more in the future.

Dr. M. Zenilman (Bronx, N.Y.). In these patients the first operation does not always work. Were there reoperations, for example, when a Puestow procedure was unsuccessful and the patient underwent resectional therapy? The second question has to do with diabetes and the quality of life. Have you noticed a difference in the incidence of diabetes when comparing resectional surgery with a Whipple procedure versus a conservative operation such as a Puestow procedure, or if an attempt is made to conserve the pancreas using a Frey procedure?

Dr. Campbell. In regard to reoperation, patients who had undergone ductal drainage procedures initially but continued to have pain went on to have an ablative procedure. Of those eight patients, approximately half of them went on to have a second ablative procedure of the remaining portion of the gland after the first resection had failed. The incidence of endocrine abnormalities was certainly higher in those patients who underwent ablative procedures as opposed to ductal drainage procedures. However, the choice of procedure was based on what was thought to be the etiology of the chronic pancreatitis.

Two-Stage Trauma Pancreaticoduodenectomy: Delay Facilitates Anastomotic Reconstruction

Leonidas G. Koniaris, M.D., Aloke K. Mandal, M.D., Ph.D., Thomas Genuit, M.D., John L. Cameron, M.D.

A case of a gunshot wound to the head of the pancreas and superior mesenteric vein requiring pancreaticoduodenectomy is discussed. Managing such an injury is challenging, first because of the ongoing hemorrhage and second because of the technical difficulty in working with a normal pancreas and bile duct. In the case presented herein, enteric reconstruction was performed 72 hours after the initial surgery. A delay in reconstruction resulted in tissue changes that facilitated enteric reconstruction. A two-stage pancreaticoduodenectomy may be considered if the surgeon is faced with an unstable patient. (J GASTROINTEST SURG 2000;4:366-369.)

KEY WORDS: Pancreaticoduodenectomy, pancreas, bile duct, trauma

Major pancreatic injuries are associated with a mortality rate of 10% to 30%.[1,2] Injuries to the head of the gland may result in an even higher mortality rate because they sometimes require both pancreaticoduodenectomy and repair of an associated vascular injury to the portal region.[1-4] In the case presented here, a two-stage approach was used following a large-caliber gunshot wound to the duodenum, head of the pancreas, and the retropancreatic superior mesenteric vein (SMV). Initially, the patient was managed by primary resection and reconstruction of the SMV and pylorus-preserving pancreaticoduodenectomy; visceral reconstruction was then delayed until 72 hours after the first surgery. This approach resulted in considerable dilatation of the common bile duct and the formation of an edematous but firm pancreas with a large, dilated pancreatic duct. These changes allowed for technically easier bilary-enteric and pancreatic-enteric anastomoses. We suggest that a two-stage pancreaticoduodenectomy should be considered when a patient's condition or available resources preclude immediate reconstruction.

CASE REPORT

The patient was a 29-year-old man who had two large-caliber gunshot wounds to the right flank and buttock. He arrived at the hospital in shock with a rigid abdomen. The patient was resuscitated and taken for emergency celiotomy. A massive right-sided retroperitoneal hematoma with ongoing hemorrhage from a disrupted pancreatic head, a laceration to the right lobe of the liver, and a proximal jejunal–small bowel enterotomy were identified at exploration (Fig. 1, *A*). An extended Kocher maneuver was performed while manually compressing the mesenteric root and hepatoduodenal ligament to control the nonvisualized source of hemorrhage. Because of the massive rate of hemorrhage from the region of the pancreatic head following the Kocher incision, a diagnosis of either portal or retropancreatic SMV injury was made.

To quickly isolate the blood vessels in this region, the SMV was identified and dissected free from the pancreatic neck, and the bile duct was divided.[5,6] The early division of the bile duct allowed for rapid exposure of the retropancreatic portal vein, thereby enabling the quick separation of the portal confluence from the pancreatic neck. Next the first portion of the duodenum and the pancreatic neck were divided, exposing the through-and-through bullet injury to the retropancreatic SMV. With additional dissection, vascular control was obtained above and below the injury. A 3 cm segment of vein traumatized by the large-caliber bullet was resected. Mobilization of the SMV and division of its first jejunal and middle colic venous branches were performed to provide sufficient length for primary end-to-end anastomosis without the need for an interposition graft.

Following isolation and repair of the SMV injury, attention was directed to the injured pancreatic head. A pylorus-

From the Department of Surgery, The Johns Hopkins University School of Medicine, Baltimore, Md.
Reprint requests: Leonidas G. Koniaris, M.D., The Johns Hopkins University School of Medicine, 607 Preclinical Teaching Building, 725 N. Wolfe St., Baltimore, MD 21205.

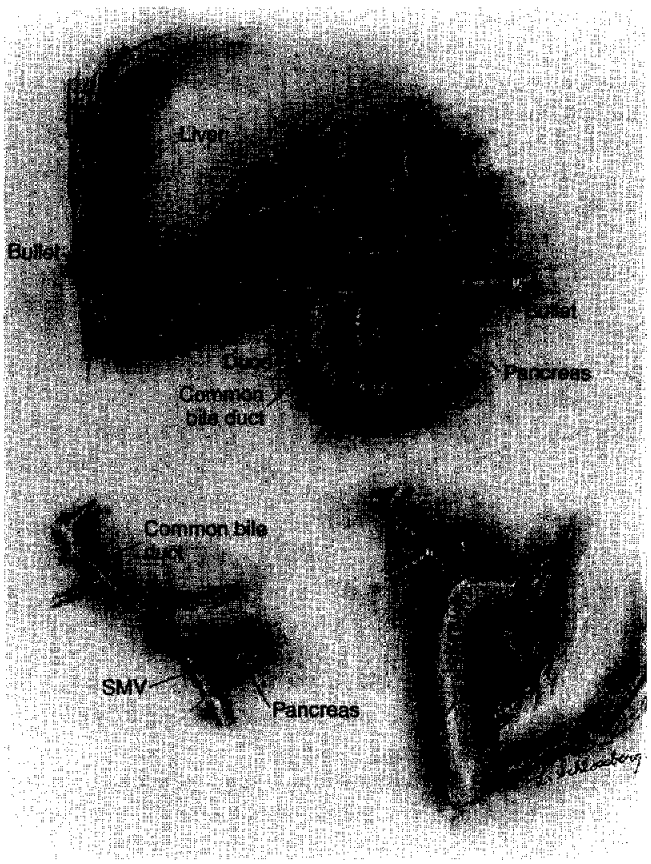

Fig. 1. Two-stage pancreaticoduodenectomy following traumatic injury. **A,** The destructive course of the bullet. Following penetration of the right flank, the bullet traversed the substance of the right hepatic lobe, the pancreas adjacent to the second portion of the duodenum, the retropancreatic superior mesenteric vein *(SMV)*, and the proximal jejunum. **B,** Anatomy after the completion of the first surgical procedure. The stapled duodenal bulb, transected common bile duct with T tube, stapled pancreatic neck, and reconstructed superior mesenteric vein *(SMV)* are represented. Two large silicone rubber drains and the stapled jejunum are not shown. **C,** Anatomy after the completion of the second surgical procedure. Depicted are the completed pancreaticojejunostomy, choledochojejunostomy, and duodenojejunostomy. Two large silicone rubber drains are not shown.

preserving pancreaticoduodenal resection was completed as previously described.[5,6] At this point, blood loss was estimated at 14 liters with the patient having received 20 units of packed red blood cells, 10 units of fresh-frozen plasma, and 8 units of platelets. The patient was requiring large amounts of crystalloid infusion, and exhibited a core temperature of 35° C and a moderate metabolic acidosis with a nadir pH of 7.17. The small bowel was massively dilated because of SMV clamping during reconstruction. Given the patient's instability and dilated bowel, it was elected to delay reconstruction. This first surgery was then completed by using a TA-35 mm stapler to close the normal pancreatic neck. A cholecystectomy was performed, an 8 F T tube was placed into the common bile duct, and a large clip was placed on the cut distal-end of the common bile duct. Two large silicone rubber drains were placed (Fig. 1, *B*). Fascia was closed with a running nonabsorbable suture; the skin was not reapproximated.

Following surgery, the patient was taken to the surgical intensive care unit. Within 3 hours of arrival, the patient became profoundly hypotensive and developed a worsening metabolic acidosis. Inotropic support was initiated and over the next 24 hours the patient received 16 liters of fluid (net 8 liters positive), including one additional unit of packed red blood cells and two units of fresh-frozen plasma. The patient's worsening clinical condition elicited suspicion of SMV thrombosis. However, 24 hours after the patient's arrival in the surgical intensive care unit, a duplex study demonstrated a patent SMV without evidence of thrombus or narrowing.

The patient improved over the ensuing 24 hours, resolving his need for inotropic support, and by postoperative day 3 he had a 9-liter negative fluid balance from his initial arrival to the intensive care unit. At that point, 72 hours after the initial surgery, the patient was returned to the operating room for enteric reconstruction. On reexploration the patient's small bowel was markedly less dilated. The previously placed pancreatic staple line was resected. The pancreas, which originally had been a normal soft gland without a discernible pancreatic duct, was found to be edematous but firm with a 0.7 cm dilated pancreatic duct. Similarly the bile duct that had originally allowed only the placement of an 8 F T tube had dilated, allowing placement of a 12 F T tube. A two-layer duct-to-mucosa pancreaticojejunostomy, a one-layer choledochojejunostomy, and a two-layer duodenojejunostomy were performed (Fig. 1, *C*). Two large silicone rubber drains were again placed.

The patient was extubated the following day. A cholangiogram 4 days after the second surgery demonstrated a patent biliary enteric anastomosis with no leaks. The patient's diet was advanced. He was discharged home 15 days after his initial surgery with only an internal T tube in place. The T tube was removed at the 6-week follow-up visit. Follow-up examination at 12 months demonstrated no evidence of biliary abnormality, diabetes, or pancreatic exocrine insufficiency. The patient reported returning to his normal lifestyle, which included active participation in sports.

DISCUSSION

Pancreaticoduodenectomy is indicated in unusual instances of severe trauma to the pancreatic head and duodenum with injury to the ampulla of Vater, retropancreatic portal vein, or main pancreatic duct.[1,3] In most large series of pancreatic trauma, pancreaticoduodenectomy is performed in less than 5% of cases.[1-4,7] Trauma was the indication for pancreaticoduodenectomy in only four of more than 700 (0.6%) of these procedures performed at the Johns Hopkins

Hospital over the past decade. Pancreaticoduodenectomy is usually reserved for neoplasms of the periampullary region.[8] At our institution, mortality rates for elective pancreaticoduodenectomy are currently below 2%, with a pancreatic fistula rate of 10%, and a major morbidity rate of 41%.[8,9] In most series of major pancreatic injury, including cases managed with distal pancreatectomy, pyloric exclusion with gastrojejunostomy, duodenal diverticulization, or pancreaticoduodenectomy, mortality rates of 10% to 30% with a pancreatic fistula rate of greater than 20% and a major morbidity rate of 60% to 80% have been reported.[1,2,4,7,10] This markedly increased morbidity and mortality in trauma series can be attributed to the presence of associated vascular or other solid organ injuries, and the additional difficulty of working with a normal soft pancreas.[1,2,11]

Fifty to 70% of reported deaths following pancreatic injury are due to hemorrhage from associated vascular injuries; such injuries occur in up to 40% of cases and usually result in mortality within the first 24 to 48 hours.[1-3,7,11,12] Following venous injury, lateral venorrhaphy is the preferred method of venous repair. In the case presented here, lateral venorrhaphy was not possible; therefore venous excision, mobilization of the distal SMV, and primary end-to-end reconstruction were performed. Other options included placement of an interposition graft, splenic vein transposition, portal-systemic shunting, or venous ligation.[2,12] Of note, early division of the common bile duct and an extended Kocher incision[5] allowed for rapid exposure of the portal vein and SMV. It is our impression that this approach may be performed more quickly and safely than either identification of the SMV by dissection into the lesser sac or by an extensive Cattell maneuver,[1,2,12] and therefore should be the preferred approach to a portal confluence or retropancreatic SMV injury. It must be stressed that the surgeon should be fairly certain that a major vascular injury or other indication for pancreaticoduodenectomy exists before dividing the common bile duct. Conversely, excessive dissection and devascularization around the bile duct prior to division is also not advisable as this will markedly increase the risk of late biliary stricture.

Postpancreatic injury sepsis and multisystem organ failure become the major causes of morbidity and mortality after the initial 48 hours, accounting overall for 25% to 50% of traumatic pancreatic deaths.[1,2,12] A pancreatic leak following trauma is the cause of 20% to 50% of these septic deaths.[1,2,4,7,11] The higher observed frequency of pancreatic fistula following trauma at least partly can be attributed to the increased difficulty encountered in working with a normal soft pancreas and nondilated pancreatic duct.[11] In the case described, we found that a 72-hour delay in the construction of a pancreatic-enteric anastomosis following stapled transection of the pancreatic neck allowed for a previously normal pancreas to undergo dilatation of the pancreatic duct and increased firmness of the gland with no elevations of serum amylase. This firmer pancreas with a larger pancreatic duct permitted a technically easier pancreatic-enteric anastomosis with the creation of a two-layered pancreatic duct to jejunal mucosal anastomosis.

Delayed reconstruction following traumatic pancreaticoduodenectomy has been previously reported.[13-15] In the reported cases, the pancreatic remnant was left ligated.[13,14] Although ligation of the pancreatic duct was a historical option, fistula and mortality rates in excess of 50% were reported, with most survivors developing a lifelong requirement for oral pancreatic supplementation.[10,12,17] For these reasons we advocate the more physiologic pancreatic-enteric reconstruction over ligation for a trauma victim who has many decades of life ahead and who should not be encumbered with the need to take oral pancreatic supplementation.

Pancreaticogastrostomy is a second option for reconstruction and low fistula rates using this technique following traumatic pancreaticoduodenectomy have been reported.[4] It is notable that in a prospective, randomized trial among elective pancreaticoduodenectomies, no difference in fistula rates between pancreatic-gastric or pancreatic-jejunal anastomoses were observed.[10] In this case a pancreatic-gastric anastomosis (because of the markedly swollen and edematous state of the small bowel) would have been our preference if reconstruction had been attempted at the initial surgery. Reconstruction was delayed because of the patient's overall clinical state and further because it was felt that technically superior biliary-enteric and duodenal-jejunal anastamoses could be performed if the small bowel swelling was allowed to resolve.

Our management also differs from the previously reported two-stage procedures in that a T tube rather than a cholecystostomy tube was used to decompress the normal biliary system after the initial surgery. A cholecystostomy tube would have been our preference if the patient had been in extremis. A T tube was used because it was felt that better control of the biliary tree could be obtained, and the amount of time needed to perform an open cholecystectomy and place a T tube in this patient, whose common duct had been already identified and transected, was minimal.[18] The presence of the small T tube allowed for some biliary dilatation presumably from partial bil-

iary obstruction due to the small size of the T tube, although no elevation in serum bilirubin was observed. During the first procedure the normal-caliber bile duct allowed only the snug placement of an 8 F T tube, but at return laparotomy as 12 F T tube fit loosely. It is our experience that the risk of complications from biliary leakage following complex reconstructions are minimized by the placement of the T tube.[18] Moreover, a T tube allows postoperative access to the biliary tree as well as the option to externally drain the biliary system if needed, a maneuver that would allow small biliary leaks to heal conservatively.[18] The use of either a T tube or transhepatic biliary catheter in the elective treatment of a variety of biliary abnormalities is routine at our institution, with excellent results.[6,8,9,18] Biliary stricture following choledochojejunal biliary reconstruction occurs at a rate of 5% to 10%.[18,19] It is widely believed that nondilated biliary trees are at greater risk for stricture.[18] It is our assumption that a dilated duct allowed for the creation of a biliary anastomosis that would be less likely to leak acutely or stricture over the long term.

As this case illustrates, reconstruction following pancreaticoduodenectomy may be successfully performed as a two-stage procedure with certain modifications from its popularized two-stage description by Whipple et al.[20] in 1935. The approach described led to changes in both the pancreas and bile duct that made reconstruction technically easier. This, in turn, may lead to the formation of nonleaking biliary and pancreatic anastomoses, thereby reducing the risk of late septic morbidity and mortality following pancreaticoduodenectomy. Finally, it is suggested that the described two-stage pancreaticoduodenectomy be considered if associated injuries, intraoperative misadventure, or limitations in resources preclude immediate reconstruction.

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Specific Pancreatic Enzymes Activate Macrophages to Produce Tumor Necrosis Factor-Alpha: Role of Nuclear Factor Kappa B and Inhibitory Kappa B Proteins

Colleen Jaffray, M.D., Cynthia Mendez, M.D., Woody Denham, M.D., Gay Carter, B.S., James Norman, M.D.

The triggering events by which mononuclear cells throughout the body are induced to produce large amounts of cytokines during acute pancreatitis are unclear. However, recent work in our laboratory demonstrated that three specific pancreatic enzymes (elastase, carboxypeptidase A, and lipase) induced dramatic tumor necrosis factor-alpha (TNF-α) protein production from macrophages, whereas all others could not. This series of experiments was designed to examine the second messenger system by which this occurs. The rat macrophage cell line NR8383 was incubated for 3 hours with elastase, carboxypeptidase A, lipase, trypsin, or lipopolysaccharide (positive control). Activation of nuclear factor kappa B (NF-κB) was demonstrated by electrophoretic mobility shift assay, presence of inhibitory kappa B alpha and beta (IκB-α and IκB-β) by Western blot analysis, and TNF-α protein production by enzyme-linked immunosorbent assay. Elastase, carboxypeptidase A, and lipase induced degradation of IκB-β (but not IκB-α), activation of NF-κB, and production of TNF-α protein, whereas inhibition of IκB with pyrrolidine dithiocarbamate attenuated this response. Trypsin was unable to elicit any of these responses. Macrophages can be induced by specific activated pancreatic enzymes—elastase, carboxypeptidase A, and lipase—to produce TNF-α. This process is dependent on IκB-β degradation and NF-κB activation, suggesting that these enzymes trigger this second messenger system through specific membrane-bound receptors. (J GASTROINTEST SURG 2000;4:370-378.)

KEY WORDS: Pancreatitis, enzyme, macrophage, NF-κB, IκB

The mortality associated with severe acute pancreatitis most commonly results from distant organ compromise and failure involving the lungs, kidneys, and liver. This end-organ dysfunction is due to the development of a systemic inflammatory response, which is believed to be mediated through diffuse activation of mononuclear cells throughout the body.[1] Systemic macrophages, monocytes, and polymorphonuclear cells produce a variety of inflammatory mediators including tumor necrosis factor-alpha (TNF-α), interleukin (IL)–1β, IL-6, nitric oxide, and platelet-activating factor during acute pancreatitis in response to a signal believed to be released from the pancreas.[2,3] Intrapancreatic and serum TNF-α and IL-1β concentrations have been shown to rise early in the course of acute pancreatitis, whereas a later predictable peak in cytokine production is noted in the lungs, kidneys, liver, and spleen.[1,4]

Identifying the signal from the pancreas that activates systemic mononuclear cells to produce these inflammatory mediators during acute pancreatitis has been the focus of a large body of research. Pulmonary TNF-α and IL-1β messenger RNA production increases in otherwise healthy rats after the systemic administration of sterile, cytokine-free ascites obtained from rats with bile salt pancreatitis, confirming that

From the Department of Surgery, University of South Florida, Tampa, Fla.
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Reprint requests: James Norman, M.D., Department of Surgery, University of South Florida, P.O. Box 1289, Tampa General Hospital, Tampa, FL 33601.

the stimulating factor is neither endotoxin nor cytokines.[3,5] Further work in our laboratory has come full circle to consider pancreatic enzymes as the systemic macrophage-activating signal. The inflamed pancreas releases pancreatic enzymes during acute pancreatitis, and increased levels have been measured in the thoracic duct, portal vein, ascites, and systemic circulation.[6-8] Plasma concentrations of pancreatic enzymes have been shown to correlate with lung injury score and lymph levels of cytokines, whereas portal vein levels can exceed peripheral concentrations by approximately 10%.[7,8] The importance of the lymphatic system as a major pathway of transport has been questioned because thoracic duct cannulation does not prevent an increase in peripheral serum concentrations of pancreatic enzymes. However, evidence suggesting that substances released from the diseased pancreas may have some effect on the liver prior to alveolar macrophage activation and lung injury has recently been provided by studies demonstrating decreased pulmonary neutrophil infiltration and alveolar macrophage production of TNF-α and nitric oxide in animals undergoing portosystemic shunting prior to the induction of hemorrhagic pancreatitis.[9] Our laboratory has recently shown that select pancreatic enzymes activate human monocytes and a rat alveolar macrophage cell line to produce cytokines in a very specific time- and dose-dependent manner.[10] Interestingly, many pancreatic proteases including amylase, trypsin, carboxypeptidase B, chymotrypsin A, chymotrypsin B, and cathepsin B are not believed to have the ability to induce in vitro cytokine production, whereas exposure of mononuclear cells to elastase, carboxypeptidase A, and lipase resulted in a significant increase in TNF-α production. In fact, elastase is at least as powerful an inducer TNF-α as lipopolysaccharide (LPS).

The transcription factor nuclear factor kappa B (NF-κB) is a regulatory substance that controls a wide range of genes. The primary level of control for NF-κB is through its interactions with a cytoplasmic inhibitor protein, inhibitory kappa B (IκB), which exists in multiple isoforms including IκB-α and IκB-β. These inhibitory proteins are associated with NF-κB in the cytoplasm and on stimulation, IκB becomes phosphorylated and subsequently degraded. Only then is NF-κB translocated into the nucleus (activated) where it binds to target DNA elements and assists in the initiation of gene transcription encoding proteins involved in immune responses, inflammation, and control of cell growth. NF-κB is believed to play a central role in multiple immunologic and disease processes including arthritis, atherosclerosis, oncogenesis, and sepsis. It can be activated by the exposure of cells to a variety of stimuli including LPS, inflammatory cytokines such as TNF-α or IL-1β, and viral infection.[11] During septic shock, LPS or other microbial products stimulate the expression of various inflammatory mediators by systemic macrophages through a process involving activation of NF-κB.[12] Although NF-κB activation and IκB degradation have been demonstrated in the pancreatic parenchyma in an animal model of hormone-induced acute pancreatitis,[13] the role of NF-κB and IκB in the development of a systemic inflammatory response during severe acute pancreatitis has not been studied. Therefore we aimed to determine the role of NF-κB and its inhibitory proteins (IκBs) in pancreatic enzyme–induced macrophage activation and inflammatory mediator production.

MATERIAL AND METHODS

Cell Culture

To examine the cell signaling mechanisms by which pancreatic enzymes stimulate macrophage production of cytokines in vitro, the rat alveolar macrophage cell line NR8383 (American Type Culture Collection, Rockville, Md.) was used. This macrophage cell line is particularly useful because these cells have a functional mannose receptor, the expression of which is believed to be tightly linked to the functional state of macrophages.[14] Recent work has demonstrated the importance of the mannose receptor in macrophage host defense properties and inflammation. This receptor has been characterized in human alveolar macrophages and monocytes[15] as well as in rat alveolar macrophages and bone marrow–derived macrophages. To date, the NR8383 rat alveolar macrophage–derived cell line is the only macrophage cell line to express mannose receptor activity, protein, and messenger RNA, and that expression is regulated by previously described positive and negative macrophage modulators. The commonly used murine macrophage cell line RAW 264.7 lacks levels of a functional and regulated mannose receptor, and therefore we chose to use the NR8383 cell line in this set of experiments. The NR8383 cell line was maintained at 37° C, 5% carbon dioxide in Ham's F-12K medium (Sigma, St. Louis, Mo.) supplemented with 15% heat-inactivated fetal calf serum (Atlanta Biologicals, Atlanta, Ga.) and 1X PSN antibiotic mixture (Sigma) and grown to semiconfluency.

Exposure to Pancreatic Enzymes

The NR8383 macrophage cell line was incubated with fresh medium alone (negative control) or medium with sterile, filtered pancreatic enzymes for 3 hours. This time point was selected on the basis of prelimi-

nary work in our laboratory demonstrating activation of NF-κB following incubation with pancreatic enzymes for 3 hours. These enzymes included elastase (1.0 U/ml), carboxypeptidase A (10 U/ml), lipase (10,000 U/ml), and trypsin (1200 U/ml), which were obtained from Sigma. Enzyme concentrations were selected on the basis of dose-response experiments performed previously in our laboratory.[10] Endotoxin or LPS (2 μg/ml) has been shown to induce IκB degradation, NF-κB activation, and TNF-α production in macrophages[16] and therefore served as a positive control.

Pyrrolidine Dithiocarbamate Treatment

In a subset of experiments, NR8383 cells were incubated with pyrrolidine dithiocarbamate (PDTC), an antioxidant that inhibits IκB degradation and therefore NF-κB activation,[17] for 3 hours before the addition of pancreatic enzymes or LPS to cell culture. Cells were subsequently incubated for 3 hours as above with media with and without enzymes or LPS prior to IκB-α, IκB-β, and TNF-α protein determination.

Extraction of Cytosolic and Nuclear Proteins

Cytosolic and nuclear extracts were obtained using a modification of the method described by Dignam et al.[18] Briefly, the macrophage cell line NR8383 was stimulated with media with and without enzymes or LPS for 3 hours as described above. Cells were washed with ice-cold phosphate-buffered saline solution and collected with a cell scraper in ice-cold buffer A (10 mmol/L HEPES, pH 7.9, 1.5 mmol/L $MgCl_2$, and 10 mmol/L KCl) with protease inhibitors (1 μmol/L aprotonin, 1 μmol/L leupeptin, 0.5 mol/L phenylmethyl sulfonyl fluoride, and 0.5 mmol/L dithiothreitol). After centrifugation, the cell pellet was resuspended in buffer A with 0.1% Nonidet P-40 (Sigma) and incubated on ice for 15 minutes. Nuclei were pelleted by centrifugation at 14,000 rpm for 10 minutes. The supernate, containing mostly cytosolic proteins, was diluted in two volumes of buffer D (20 mmol/L HEPES, pH 7.9, 0.2 mmol/L EDTA, 50 mmol/L KCl, and 20% glycerol) with protease inhibitors. The nuclear pellet was resuspended in buffer C (20 mmol/L HEPES, pH 7.9, 1.5 mmol/L $MgCl_2$, 0.42 mol/L NaCl, 0.2 mmol/L EDTA, and 25% glycerol) with protease inhibitors, incubated on ice for 15 minutes, and centrifuged. The supernate, containing a crude nuclear fraction, was diluted with two volumes of buffer D. Cytosolic and nuclear protein concentrations were determined by the Bradford method.

IκB-α and IκB-β Protein Determination by Western Blot Analysis

Cytosolic protein fractions (30 μg) were separated on a 12% polyacrylamide gel and transferred to Hybond nitrocellulose membranes (Amersham, Arlington Heights, Ill.). Membranes were blocked overnight in tris-buffered saline (40 mmol/L Tris, pH 7.6, and 300 mmol/L NaCl) containing 5% nonfat dry milk and then incubated for 3 hours at room temperature with rabbit polyclonal anti-IκB-α or anti-IκB-β antibody (Santa Cruz Biotechnology, Santa Cruz, Calif.). After washing, membranes were incubated for 1 hour with horseradish peroxidase–conjugated goat antirabbit immunoglobulin (BioRad, Hercules, Calif.) and washed again. Immunoreactive proteins were detected with an electrogenerated chemiluminescent detection system (Amersham). IκB-α and IκB-β bands were analyzed using GDS image analysis software (UVP, Upland, Calif.).

Determination of NF-κB Activation By Electrophoretic Mobility Shift Assay

NF-κB–specific consensus oligonucleotide (5′AGTTGAGGGTTTCCCAGGC 3′, Promega Corp., Madison, Wis.) was 5′ end-labeled with $\gamma^{32}P$ adenosine triphosphate (ICN, Costa Mesa, Calif.) using polynucleotide kinase (Gibco, Gaithersburg, Md.). Samples of 10 μg of nuclear protein extract were incubated in binding buffer (10 mmol/L Tris, pH .75, 100 mmol/L NaCl, 1 mmol/L EDTA, 4% glycerol, and 80 μg/ml sonicated sperm DNA) with or without excess unlabeled NF-κB–specific oligonucleotide for 15 minutes on ice. End-labeled NF-κB oligonucleotide (1.5×10^5 cpm) was added, and samples were incubated for an additional 45 minutes at room temperature. Free oligonucleotide and oligonucleotide-bound proteins were separated by electrophoresis on a native 6% polyacrylamide gel. Gels were dried under vacuum on Whatman paper and exposed to Kodak BioMax MS film (Sigma) for 3 to 6 hours at −80° C. Absence of binding in the presence of excess unlabeled NF-κB–specific oligonucleotide (competitor oligonucleotide) confirmed NF-κB–binding specificity.

TNF-α Protein Production by Enzyme-Linked Immunosorbent Assay

After incubation of NR8383 cells with sterile filtered pancreatic enzymes or LPS for 3 hours, the supernate was collected and assayed using a commercially available murine TNF-α enzyme-linked immunosorbent assay (ELISA) kit (Quantikine M, R&D Systems, Minneapolis, Minn.) according to the man-

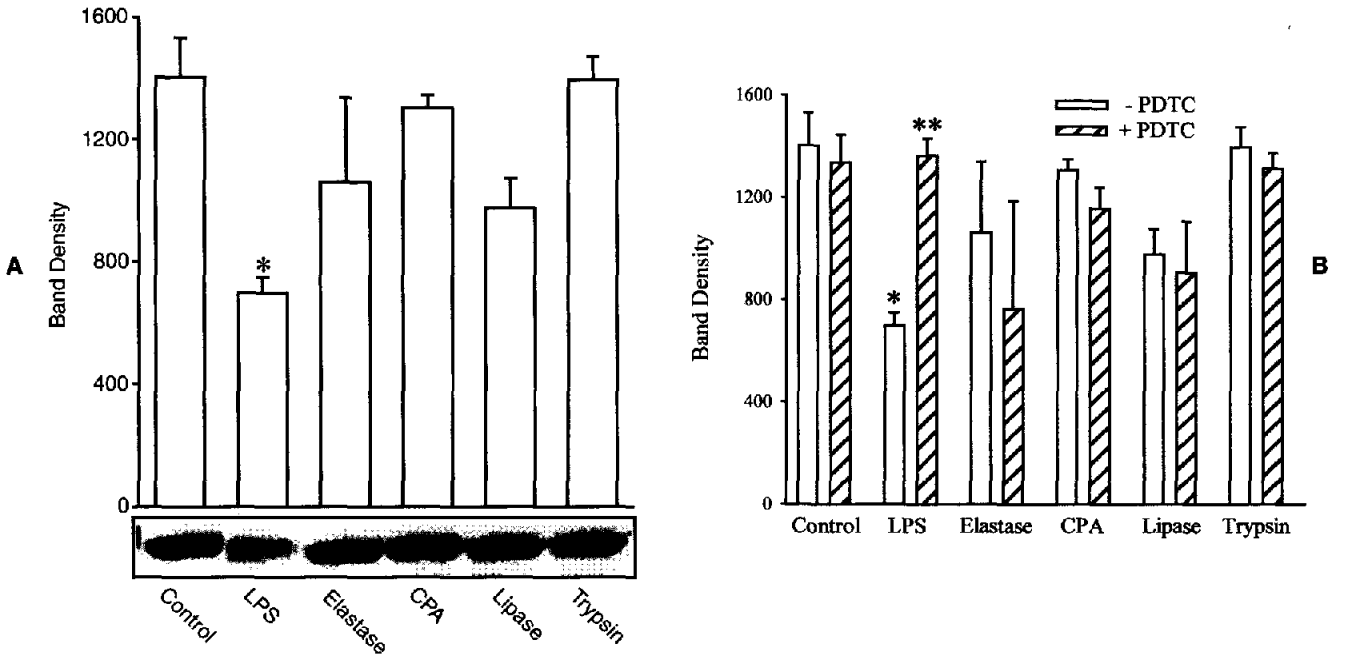

Fig. 1. Cytosolic inhibitory kappa B alpha (IκB-α) protein expression following pancreatic enzyme stimulation. After incubation of the rat alveolar macrophage cell line NR8383 with elastase, carboxypeptidase A *(CPA)*, lipase, trypsin, and lipopolysaccharide (*LPS*, positive control) for 3 hours, IκB-α protein in the cytoplasm was determined by Western blot analysis. **A,** Only LPS induced IκB-α degradation (* = P <0.02 vs. control), whereas elastase, CPA, lipase, and trypsin did not affect IκB-α protein expression. **B,** Pretreatment with the antioxidant pyrrolidine dithiocarbamate *(PDTC)* prevented the degradation of LPS (* = P <0.02 vs. control alone; ** = P <0.05 vs. LPS alone). On the other hand, the protein expression of IκB-α in response to any of the pancreatic enzymes used in this study was not significantly altered by pretreatment with PDTC.

ufacturer's instructions. Samples were run in triplicate and averaged.

Statistical Analysis

Results are expressed as mean ± standard error of the mean. Statistical significance was evaluated using the two-tailed Student's t test (Epistat Services, Richardson, Tex.) with significance assigned to P values less than 0.05 unless otherwise stated.

RESULTS

Cytosolic IκB Degradation in Response to Pancreatic Enzymes

IκB-α Protein. The cytosolic inhibitory protein IκB-α was demonstrated by Western blot analysis to degrade following treatment of NR8383 cells with the LPS (Fig. 1, *A*; P <0.05 vs. control). As expected, pretreatment with the IκB decay inhibitor PDTC prevented degradation of IκB-α in response to LPS (Fig. 1, *B*; P <0.05 vs. LPS alone; P = NS vs. control). However, incubation of this macrophage cell line with pancreatic enzymes previously shown to induce macrophage production of inflammatory mediators including elastase, carboxypeptidase A, and lipase did not induce IκB-α degradation. Trypsin, which did not stimulate macrophages in our prior experiments, also resulted in no significant degradation of IκB-α. In addition, PDTC did not significantly alter the expression of IκB-α protein following exposure to pancreatic enzymes.

IκB-β Protein. As demonstrated with IκB-α, LPS induced the degradation of IκB-β protein in the cytosol of the macrophage cell line NR8383 (Fig. 2, *A*; P <0.0001 vs. control). However, in contrast to IκB-α, IκB-β was significantly degraded following incubation with specific pancreatic enzymes including elastase (P <0.001 vs. control), carboxypeptidase A (P <0.05 vs. control), and lipase (P <0.05 vs. control). Pretreatment of macrophages with PDTC resulted in inhibition of IκB-β degradation by LPS (Fig. 2, *B*; P <0.001 vs. LPS alone), elastase, carboxypeptidase A, and lipase (P <0.05 vs. enzyme alone; P = NS vs. control). Trypsin did not induce degradation of IκB-β and was unaffected by incubation with PDTC.

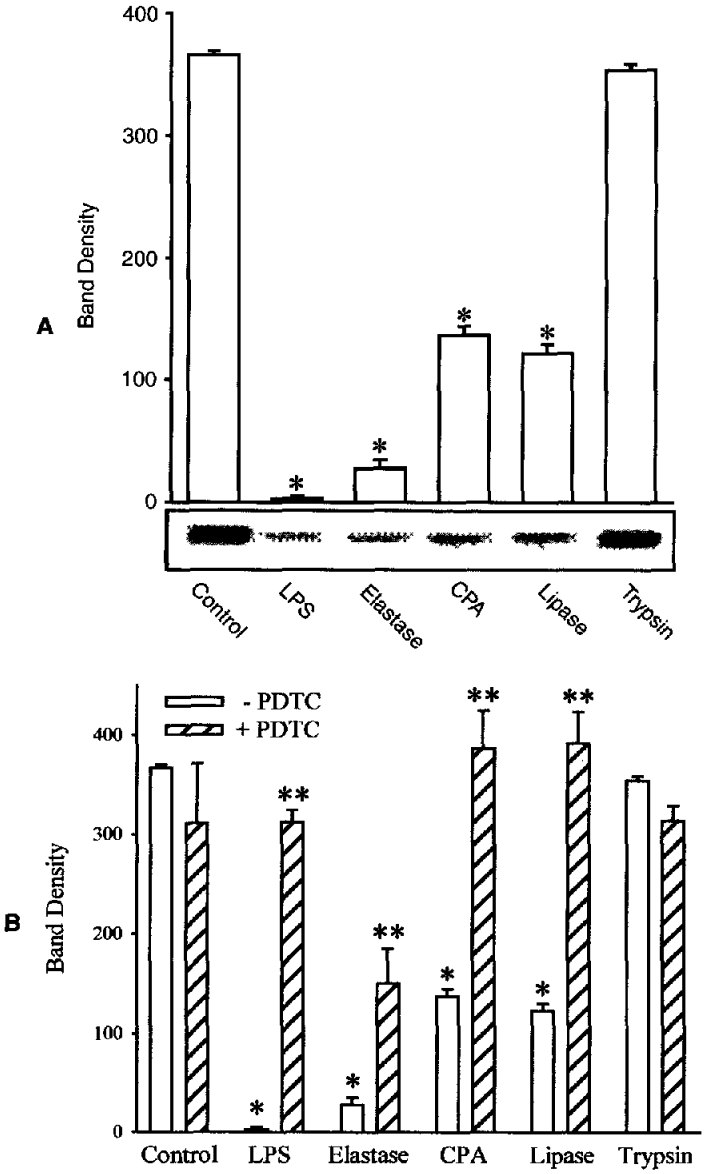

Fig. 2. Cytosolic inhibitory kappa B beta (IκB-β) protein expression following pancreatic enzyme stimulation. Representative bands are demonstrated by Western blot analysis. **A,** In contrast to IκB-α, elastase (* = $P < 0.001$ vs. control), carboxypeptidase A (*CPA;* * = $P < 0.05$ vs. control), and lipase (* = $P < 0.05$ vs. control), as well as lipopolysaccharide (*LPS;* * = $P < 0.0001$ vs. control), induced degradation of IκB-β following exposure of the NR8383 macrophage cell line to these stimulants for 3 hours. Trypsin did not induce IκB-β degradation. **B,** Similarly, pretreatment with the inhibitor of IκB decay pyrrolidine dithiocarbamate *(PDTC)* prevented the degradation of this cytoplasmic inhibitory protein in macrophages incubated with elastase (* = $P < 0.001$ vs. control; ** = $P < 0.05$ vs. elastase alone), CPA (* = $P < 0.05$ vs. control; ** = $P < 0.05$ vs. CPA alone), lipase (* = $P < 0.05$ vs. control; ** = $P < 0.05$ vs. lipase alone), and LPS ($P < 0.0001$ vs. control; ** = $P < 0.001$ vs. LPS alone). IκB-β expression in cells exposed to trypsin was unaffected by PDTC.

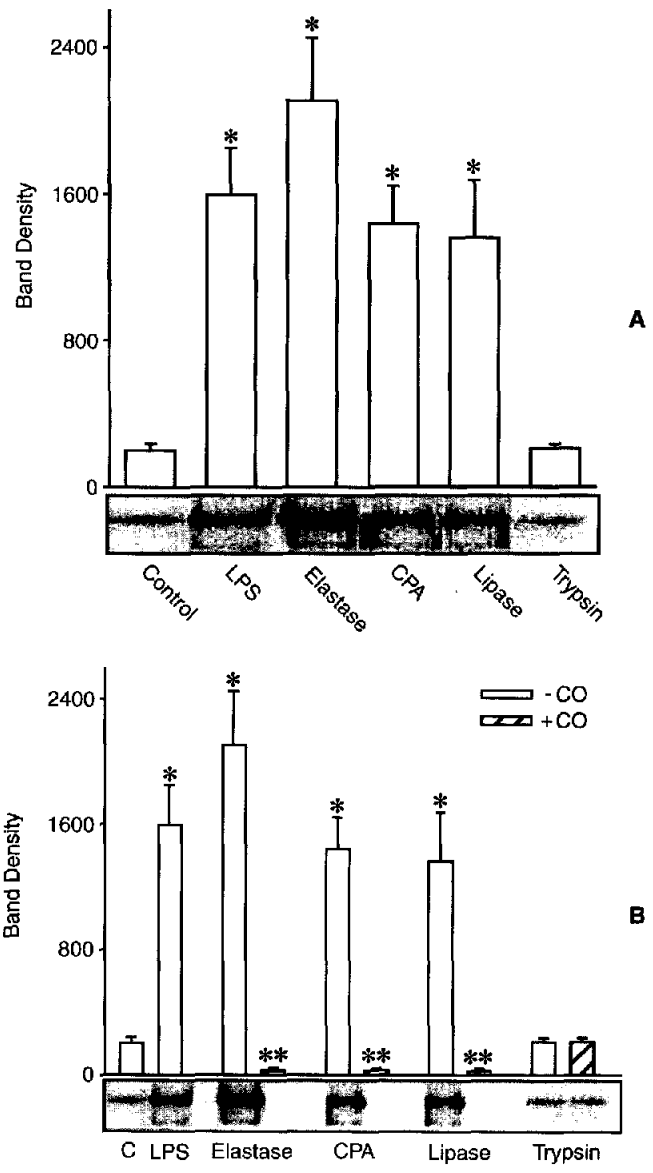

Fig. 3. Binding of nuclear factor kappa B (NF-κB) to nuclear DNA (activation) determined by electrophoretic mobility shift assay in the macrophage cell line NR8383 following exposure to pancreatic enzymes or lipopolysaccharide *(LPS)* for 3 hours. Representative bands are illustrated with corresponding band densities graphed above. **A,** Elastase, carboxypeptidase A *(CPA)*, lipase, and LPS induced NF-κB DNA binding (* = $P < 0.005$ vs. control), whereas trypsin did not. **B,** The absence of DNA binding in the presence of excess unlabeled NF-κB–specific oligonucleotide (competitor oligonucleotide, *CO*) following exposure to elastase, CPA, lipase, and LPS (* = $P < 0.005$ vs. control; ** = $P < 0.001$ vs. stimulant alone; ** = P = NS vs. control) confirmed NF-κB binding specificity. *C* designates control.

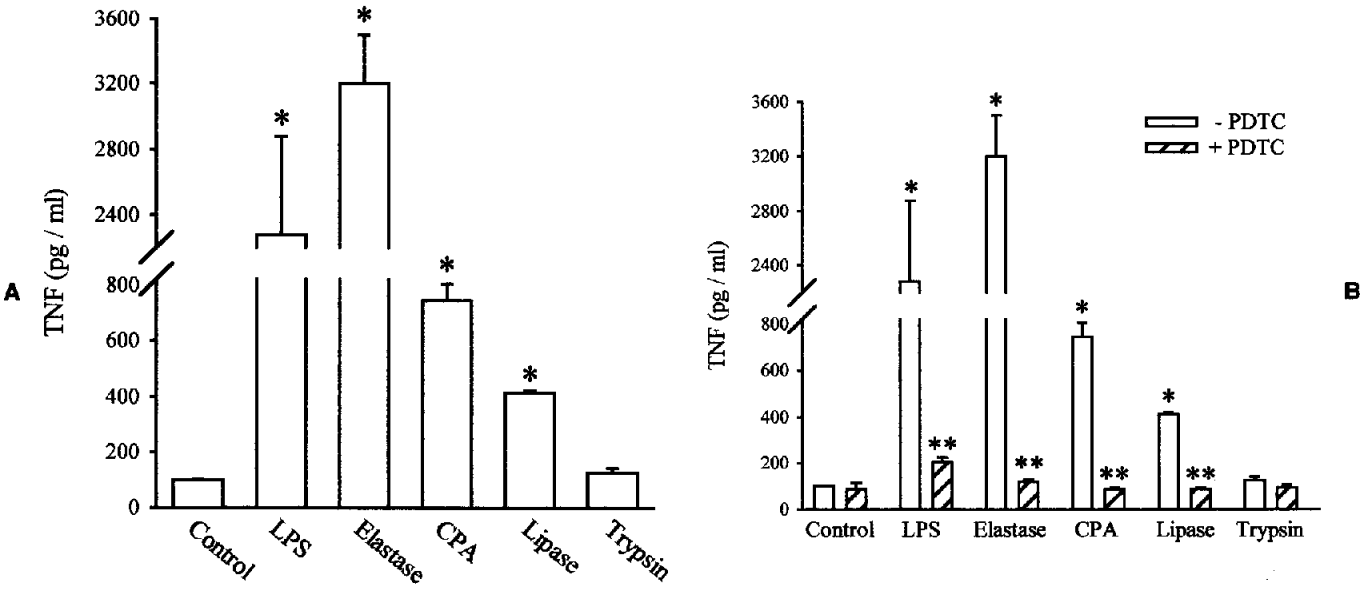

Fig. 4. Tumor necrosis factor-alpha (TNF-α) protein production in response to elastase, carboxypeptidase A *(CPA)*, lipase, trypsin, and lipopolysaccharide *(LPS)*. **A,** Following a 3-hour incubation with elastase, CPA, lipase, and LPS, TNF-α protein significantly increased in the cell culture media. Although CPA and lipase (* = $P < 0.05$ vs. control) did not stimulate macrophages as strongly as elastase (* = $P < 0.0001$ vs. control) and LPS (* = $P < 0.001$ vs. control), the increased TNF-α protein production was statistically significant. **B,** Pretreatment with an inhibitor of IκB degradation pyrrolidine dithiocarbamate *(PDTC)* significantly attenuated the production of TNF-α protein in response to these stimulants (* = $P < 0.05$ vs. control; ** = $P < 0.05$ vs. stimulant alone; ** = P = NS vs. control), confirming the role of IκB and NF-κB in the signal transduction pathway leading to enzyme-stimulated TNF-α production. Trypsin, on the other hand, did not increase TNF-α protein production and was not altered by the inhibition of IκB with PDTC.

Pancreatic Enzyme Induction of NF-κB DNA Binding

Binding of NF-κB to nuclear DNA (activation) was determined by electrophoretic mobility shift assay in the nuclear fraction isolated from the macrophage cell line NR8383 following exposure to LPS or pancreatic enzymes for 3 hours. Consistent with IκB-β degradation results, LPS, elastase, carboxypeptidase A, and lipase-induced NF-κB binding (Fig. 3, *A*; $P < 0.005$ vs. control). Activation of NF-κB was not stimulated by trypsin. The absence of DNA binding in the presence of excess unlabeled NF-κB–specific oligonucleotide (competitor oligonucleotide) following exposure to LPS, elastase, carboxypeptidase A, and lipase confirmed NF-κB–binding specificity (Fig. 3, *B*; $P < 0.001$ vs. treatment; P = NS vs. control).

Pancreatic Enzyme Induction of TNF-α Protein Production

The production of TNF-α protein was determined by ELISA in the cell-free supernate collected following incubation of NR8383 cells with LPS or pancreatic enzymes. As demonstrated in our previous experiments,[10] TNF-α protein production increased following exposure to LPS, elastase, carboxypeptidase A, and lipase (Fig. 4, *A*). Prior work confirmed the upregulation of TNF-α messenger RNA following exposure to these same stimulants.[10] Although carboxypeptidase A and lipase ($P < 0.05$ vs. control) did not stimulate macrophages as strongly as elastase ($P < 0.0001$ vs. control) and LPS ($P < 0.001$ vs. control), the increased TNF-α protein production was statistically significant. Comparable to the lack of IκB-β degradation and NF-κB DNA binding demonstrated following macrophage incubation with trypsin, this pancreatic enzyme did not stimulate macrophage TNF-α protein production. TNF-α protein production was inhibited by treatment with PDTC prior to stimulation with LPS, elastase, carboxypeptidase A, and lipase (Fig. 4, *B*; $P < 0.05$ vs. stimulant; P = NS vs. control).

DISCUSSION

The macrophage plays an integral role in the development of the systemic inflammatory response syndrome, which can occur with severe acute pancre-

atitis. Systemic macrophages throughout the body become activated to produce multiple inflammatory mediators by a signal believed to be released from the pancreas. In previous work our laboratory demonstrated that specific pancreatic enzymes activate human monocytes and a macrophage cell line to produce TNF-α messenger RNA and protein in a time- and dose-dependent manner,[10] implicating these enzymes as the possible missing factor linking local inflammation to systemic illness. In a manner similar to pancreatitis-associated systemic inflammatory response syndrome, the inflammatory response that develops during sepsis is produced by activation of systemic macrophages by endotoxin (LPS) or bacterial products to produce inflammatory mediators. It has been demonstrated that production of cytokines by macrophages in response to LPS requires activation of the transcription factor NF-κB and degradation of IκB.[12,19] Therefore in this study, we aimed to determine whether pancreatic enzyme–stimulated TNF-α production by macrophages involves this signal transduction pathway.

In unstimulated cells, the NF-κB complex is localized to the cytoplasm and is bound to an inhibitory protein, IκB, which exists in multiple isoforms including IκB-α and IκB-β. On stimulation, cytoplasmic NF-κB and IκB dissociate as a result of IκB phosphorylation and subsequent degradation. Paralleling the loss of IκB in the cytoplasm is the translocation of NF-κB into the nucleus where it binds targeted DNA and helps initiate gene transcription.[11] In the current study, specific pancreatic enzymes (elastase, carboxypeptidase A, and lipase) previously shown to induce macrophage production of TNF-α messenger RNA and protein-induced IκB-β degradation and NF-κB activation. Inhibition of IκB phosphorylation with the antioxidant PDTC decreased the degradation of IκB-β and attenuated subsequent TNF-α protein production, confirming the role of this signal transduction pathway in enzyme-stimulated TNF-α production. On the other hand, IκB-α was unaffected by all pancreatic enzymes and PDTC. In addition, a representative enzyme that did not induce the production of TNF-α in prior experiments, trypsin, had no effect on IκB-α, IκB-β, NF-κB, or the production of TNF-α by the macrophage.

The protein IκB-α was the first recognized cytoplasmic inhibitor that retains NF-κB in the cytoplasm through masking of nuclear localization sequences. Subsequently other inhibitory proteins were discovered, namely, IκB-β, IκB-γ, and Bcl-3.[11] IκB-β is structurally similar to IκB-α and has been found to be associated with NF-κB forms in the cytoplasm of various cells. However, where IκB-α is targeted by a variety of stimuli including TNF-α, IL-1β, LPS, and phorbol esters, IκB-β is reportedly targeted by a more limited subset of factors.[20] This offers one possible explanation for the cytoplasmic macrophage degradation of IκB-β and not IκB-α in response to elastase, carboxypeptidase, and lipase. This suggests that IκB-β, not IκB-α, is required for pancreatic enzyme–simulated activation of NF-κB in macrophages.

Timing could also contribute to the differential expression of IκB-α and IκB-β. There is evidence that following NF-κB induction and IκB-α degradation, newly synthesized IκB-α reaccumulates, rapidly enters the nucleus, and actively removes NF-κB from DNA κB binding sites, thereby repressing NF-κB and ensuring that NF-κB will only be present in the nucleus for a limited time.[20] On the other hand, IκB-β has been shown to be degraded more slowly with a delay of hours prior to cytoplasmic reaccumulation.[21] Our experiments were performed at 3 hours based on preliminary studies determining NF-κB nuclear translocation in this macrophage cell line. Possibly IκB-α was rapidly degraded and already resynthesized by the time the cytoplasm was isolated, whereas IκB-β had not yet reaccumulated. Although possible, this explanation is questionable since NF-κB is demonstrated to be retained in the nucleus and IκB-α degraded by LPS at this time point.

Activation of NF-κB and concomitant IκB-β degradation in response to enzyme stimulation strongly suggests a receptor-mediated phenomenon. In LPS-stimulated macrophages, the upstream events leading to IκB degradation and NF-κB activation involve the interaction of LPS with the LPS-binding protein complex and the CD14 membrane receptor[22] protein tyrosine kinase phosphorylation, and subsequent activation of phospholipase C signaling pathways. Elastase, carboxypeptidase A, and lipase may also interact with a receptor complex and thereby eventually induce the nuclear translocation of NF-κB. On the other hand, reactive oxygen intermediates, frequently generated at sites of inflammation, have been demonstrated to stimulate signal transduction pathways leading to NF-κB activation in mesothelial cells[23] and macrophages,[24] independent of cell surface receptors. However, it is unlikely that these reactive oxygen species play a role in our isolated in vitro system. Another mechanism by which stimulation occurs that warrants consideration is lipid peroxidation of membranes. In an endothelial cell line, lipid peroxidation has been shown to lead to NF-κB activation following exposure to TNF-α.[25] Further work exploring the events leading to macrophage IκB-β degradation and activation of NF-κB after stimulation with specific pancreatic enzymes is required to delineate the mechanism by which this process is initiated.

In this study, elastase, carboxypeptidase A, and lipase were shown to induce macrophage TNF-α production in a manner that involves the NF-κB signal transduction pathway, specifically activation of this transcription factor and degradation of IκB-β, not IκB-α. Inhibition of IκB results in decreased TNF-α production indicating the importance of this inhibitory protein and therefore NF-κB in this macrophage activation cascade.

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Discussion

Dr. A. Gukovskaya (Los Angeles, Calif.). IκB-α degradation is usually transient, so in evaluating data at one time point you cannot conclude that it is involved. Do you have the results of your time course experiments?

Dr. C. Jaffray. We chose 3 hours as our time point because we had previously shown NFκB activation at that time. When we did not achieve degradation of IκB-α, we went backward, and in the interest of time I did not present those data. At 15, 30, and 90 minutes, we never had IκB-α degradation. Yet we always had IκB-α degradation in response to LPS so we were pretty confident that we had monitored the appropriate time period.

Dr. Gukovskaya. PDTC is an NFκB inhibitor, but it is also an iron chelator, so in our experiments it produced a lot of side effects. Are you planning to use a different inhibitor to show the same effect?

Dr. Jaffray. Do you mean side effects in vivo or side effects in the sense that cells could be affected in other ways?

Dr. Gukovskaya. Both in vivo and in vitro, PDTC is an iron chelator so it can block enzyme activity if enzyme activity depends on the presence of iron in the media. Thus observed effects can be unrelated to NFκB.

Dr. Jaffray. PDTC has been used routinely before as an IκB inhibitor, but I understand your question.

Dr. M. Callery (Worcester, Mass.). You have shown that as our patients lie ill with pancreatitis, enzymes are being released that can run rampant and stimulate peripheral

macrophages to produce cytokines, and that the control of this process occurs at the level of transcription. You might also conduct some experiments to study the same pathway in neutrophils. We are learning much more about neutrophils as a very important peripheral leukocyte population. I have a few questions about the mechanism that I would like you to comment on. As you showed, this is predicated on IκB degradation. To be degraded, IκB needs to first be phosphorylated. Do you have coupled Western blots showing the phosphorylated form of your IκB? Also, IκB needs to be "ubiquitinated" and subsequently degraded by the 26S proteosome. As suggested by the previous discussant, have you used ubiquitin proteosome inhibition?

My final question takes you upstream. In other work with your colleagues, you have looked at mitogen-activated protein kinases as the predecessor signaling messages indicating what may be happening at the transcriptional level. Can you correlate any of this with your data from the standpoint of P38 mitogen-activated protein kinase or Erk1 or Erk2?

Dr. Jaffray. We agree that neutrophils probably have the same response. Interestingly, it has previously been shown that neutrophils have a specific receptor for elastase. We are currently also looking at macrophages trying to demonstrate an elastase receptor there. Regarding the phosphorylated form of IκB, especially since IκB-α did not correlate, we have gone backward and are now starting to look for the phosphorylated form of IκB-α. We have not done anything with the ubiquitination. As for the kinases, we are currently looking at p38, JNK, and SAPK. We have been stimulating the cells with enzymes and then measuring these kinases intracellularly. So far we have had an increase in p38, SAPK, and ERK and inhibition of p38 blocks cytokine production.

Dr. A. Saluja (Boston, Mass.). I was a bit surprised that you did not achieve much of a response with trypsin. Trypsin can be inactivated fairly quickly, depending on what medium is being used. Is it possible in this instance that trypsin is inactive?

Dr. Jaffray. In all of our experiments we have consistently seen no activation at all with trypsin. Prior to these experiments, we performed in vitro studies in which we looked at TNF-α messenger RNA and protein production, focusing on time courses and dose responses. We have never elicited a response with trypsin at any time or dose. We noted the same thing with IκB-α; in none of the time-course studies did we ever see a response with trypsin in IκB-α, IκB-β, or NF-κB. We have been working on this project over the past year and a half, and we have never gotten a response with trypsin. We have also conducted experiments with and without sera, being concerned about the inhibitors that could exist in sera, and we have had similar results with both.

Dr. M. Sarr (Rochester, Minn.). You have called this a receptor-mediated event. Do you mean that there are receptors for lipase, carboxypeptidase A, and elastase or is this receptor-mediated event a degradative process of the membrane?

Dr. Jaffray. We are specifically looking for a receptor for elastase, primarily because elastase has caused the most marked response. NFk-B activation can occur through lipid peroxidation of membranes, but we have also looked at tyrosine kinases and protein kinase C, trying to find these other signaling messengers in between, and we have been getting positive responses. We really believe that there is a receptor. We are currently in the process of iodinating elastase and will add it to cells and perform Scatchard analysis to see if we can actually determine a binding receptor.

Peripheral Lymphocyte Reduction in Severe Acute Pancreatitis Is Caused by Apoptotic Cell Death

Yoshifumi Takeyama, M.D., Kozo Takase, M.D., Takashi Ueda, M.D., Yuichi Hori, M.D., Masahiro Goshima, M.D., Yoshikazu Kuroda, M.D.

To investigate impairment of cellular immunity in severe acute pancreatitis, alterations of peripheral lymphocytes in acute pancreatitis were examined. In 48 patients with severe acute pancreatitis, the mean peripheral lymphocyte count on admission was 959 ± 105/mm^3, and it was significantly decreased in the patients with subsequent infection (623 ± 90/mm^3) in comparison to those without infection (1084 ± 135/mm^3). According to an analysis of lymphocyte subsets, although both B and T lymphocytes were decreased in peripheral circulation in the patients with infection, it was primarily CD8-positive lymphocytes that decreased in these subsets. Cell cycle analysis of lymphocytes collected from these patients indicated that apoptotic changes occurred after 24 hours' incubation in lymphocytes from patients with *severe* pancreatitis but not in lymphocytes from healthy control subjects. In a rat model of experimental necrotizing pancreatitis, total peripheral lymphocytes and T lymphocytes were significantly decreased 5 hours after induction of pancreatitis. In severe pancreatitis, peripheral lymphocytes are eliminated from systemic circulation possibly as a result of apoptosis. It has been suggested that impairment of cellular immunity due to peripheral lymphocyte apoptosis is linked to the development of subsequent infectious complications in acute pancreatitis. (J GASTROINTEST SURG 2000;4:379-387.)

KEY WORDS: Severe acute pancreatitis, peripheral lymphocyte, apoptosis, infectious complication

Immunologic alterations in severe acute pancreatitis should be closely examined given that infection of devitalized pancreatic and peripancreatic tissues has become the leading cause of death from acute pancreatitis.[1] Although the white blood cell count has been reported by several investigators[2-4] to be of early prognostic value, the peripheral lymphocyte count has not drawn much attention. One exception is the report by Antal et al.[5] who found a significant decrease in the number of peripheral T lymphocytes in patients with acute pancreatitis, which returned to normal after recovery. Moreover, Christophi et al.[6] reported that the absolute lymphocyte count calculated within 48 hours after admission accurately predicted 78% of severe attacks and 86% of mild attacks.

Recently abnormalities in cellular immunity due to apoptosis have been reported by a number of investigators. It is well recognized that apoptosis occurs in lymphoid tissues during sepsis[7-9] or thermal injury.[10] With regard to acute pancreatitis, Curley et al.[11] reported a significant decrease in the proportion of T helper cells and a significant increase in the levels of interleukin-6 and C-reactive protein in severe attacks compared to mild ones. In addition, we recently reported that significant thymic atrophy occurs as a result of apoptosis during severe acute pancreatitis in rats.[12]

Along these lines, it is very possible that the decrease in peripheral lymphocytes during acute pancreatitis is caused by apoptosis. In the present study we hypothesized that a decrease in peripheral lymphocytes on admission correlates with the development of subsequent infectious complications during the course of acute pancreatitis, and that apoptotic cell death is involved in this decrease in peripheral lymphocytes in acute pancreatitis.

In this report we examine the significance of decreased lymphocytes in patients with severe acute pancreatitis and the role of apoptosis in this decrease.

From the First Department of Surgery, Kobe University School of Medicine, Kobe, Japan.
Supported by a Grant-in-Aid for Scientific Research (C) from the Ministry of Education, Science, Sports and Culture of Japan.
Reprint requests: Dr. Yoshifumi Takeyama, First Department of Surgery, Kobe University School of Medicine, 7-5-1 Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan. e-mail:takeyama@med.kobe-u.ac.jp

MATERIAL AND METHODS

AIMV medium and RPMI medium were purchased from Life Technologies, Inc. (Grand Island, N.Y.). Fetal calf serum was obtained from C.S.L. Limited (Victoria, Australia), sodium deoxycholate (DCA) from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), Hoechst 33342 from Molecular Probes (Eugene, Orc.), intramedic polyethylene tubing (PE-10) from Clay Adams, Division of Becton Dickinson & Co. (Parsippany, N.J.), and propidium iodide from Sigma (St. Louis, Mo.). Additional materials and chemicals were obtained from other commercial sources.

Patients

Forty-eight patients with acute pancreatitis, whose condition was diagnosed as severe pancreatitis within 48 hours after admission, were analyzed retrospectively. The severity of disease was graded according to criteria standardized by the Research Committee for Intractable Diseases of the Pancreas, Japanese Ministry of Health and Welfare, in 1990 (Table I). These criteria were standardized in 1987 using clinical data from the national survey by a committee seeking to assess the state of acute pancreatitis in Japan.[13] Hematologic analyses including total white blood cell and total lymphocyte counts were performed in all patients on admission. In addition, analysis of lymphocyte subsets, such as CD3-positive, CD4-positive, and CD8-positive lymphocytes, were available on admission in 28 of these patients because this analysis was done retrospectively. Patients were divided into two groups based on the development of subsequent infectious complications during the course of pancreatitis. An infectious complication was defined as a positive bacterial culture from blood, ascitic fluid, and/or tissue collected at surgery or biopsy, with or without endotoxemia.

Isolation of Lymphocytes

Based on results obtained from the retrospective studies as described above, peripheral lymphocytes were isolated from four patients with severe pancreatitis on admission and from four healthy volunteers matched for age and sex with the patients. Lymphocytes were isolated using Ficoll-Hypaque medium (Pharmacia, Piscataway, N.J.) according to the manufacturer's protocol and resuspended in AIMV medium. The isolated lymphocytes were examined for apoptosis either immediately or after 24 hours' incubation with AIMV medium in a water-saturated atmosphere of 5% CO_2 in air at 37° C.

Cell Cycle Analysis

In determining the ratio of apoptotic cells using the DNA-binding agent propidium iodide, lymphocytes (1×10^6 cells) were centrifuged at 400 *g* for 5 minutes, and the pellet was resuspended in 1 ml of 70% ethanol. Samples were fixed and stored in a darkroom at −20° C for 24 hours prior to analysis. The fixed cells were then washed by centrifugation and stained with propidium iodide by resuspending them in phosphate-buffered saline containing 50 μg/ml propidium iodide. One-color cell cycle analysis was performed using the FACScalibur flow cytometer (Becton Dickinson Medical, Franklin Lakes, N.J.). Stained cells were excited at 488 nm line by an argon

Table I. Standardized criteria (prognostic factors) for grading the severity of acute pancreatitis

Prognostic factors	Clinical signs	Laboratory data
Factor I	Shock	Base excess ≤−3 mEq
	Dyspnea	Hematocrit ≤30%
	Mental disturbance	Blood urea nitrogen ≥40 mg/dl or
	Severe infection	creatinine ≥2.0 mg/dl
	Hemorrhagic diathesis	
Factor II		Calcium ≤7.5 mg/ml
		Glucose ≥200 mg/ml
		PaO_2 ≥60 mm Hg (room air)
		Lactate dehydrogenase ≥700 IU/L
		Total protein ≤6.0 g/dl
		Prothrombin time ≥15 sec
		Platelets ≤10×10^4/mm^3

If at least one item under the prognostic factor I heading is present, or if more than two items under the prognostic factor II heading are present, the case is considered severe. From the Research Committee for Intractable Diseases of the Pancreas, Japanese Ministry of Health and Welfare, revised 1990.

laser, and emission from the propidium iodide–stained cells was detected with a 620 to 700 nm long-pass filter. Gating, based on side-scatter detection, was applied to eliminate cell debris and doublet formation. No less than 10,000 cells were assessed per sample. A histogram of the gated lymphocytes was then produced using FACSation version 2.1 software (Becton Dickinson Immunocytometry Systems, Franklin Lakes, N.J.).

Nuclear Fragmentation of Lymphocytes

Nuclear fragmentation was examined by fluorescence microscopy under nuclear-staining fluorescent dye (Hoechst 33342). In brief, after 24 hours' incubation, 10 μmol/L Hoechst 33342 was loaded onto the culture medium of the lymphocytes for 10 minutes. The fluorescent dye was then washed using centrifugation, and the washed lymphocytes were resuspended in phosphate-buffered saline. The fluorographs (excitation, 300 to 380 nm; emission, 460 nm) were visualized and photographed.

DNA Extraction and Electrophoresis

After 24 hours' incubation, the lymphocytes were lysed with 0.1 mol/L NaCl, 10 mmol/L Tris-HCl (pH 8.0), 5 mmol/L EDTA in 0.5% sodium dodecyl sulfate, and incubated overnight at 52° C with proteinase K (100 μg/ml). The samples were then extracted with equal volumes of phenol and chloroform (1:1), and the total DNA in the aqueous phase was precipitated with 1/10 volume of 100% ethanol at −20° C overnight. DNA pellets were obtained by centrifugation at 12,000 *g* for 15 minutes, washed with 70% ethanol, air dried, and resuspended in 0.1 ml of 10 mmol/L Tris-HCl (pH 8.0) containing 1 mmol/L EDTA. The samples were treated with DNase-free RNase (10 μ/ml) for 30 minutes at 37° C and subjected to electrophoresis using 1.5% agarose gel containing ethidium bromide. The amount of DNA loaded was 1 μg. The gel was visualized and photographed under ultraviolet transillumination.

Animal Model of Pancreatitis

Male Wistar rats (250 to 280 g) were purchased from Charles River Japan, Inc. (Yokohama, Japan), and the use and care of the animals utilized for this investigation were reviewed and approved by the Institutional Animal Committee of Kobe University School of Medicine. The biliopancreatic ducts of the rats (n = 5) were cannulated right at the opening orifice to the duodenum with a PE-10 tube, and 0.1 ml of 20% DCA was injected under low pressure with the temporary clump of bile duct at the porta hepatis under general diethylether anesthesia. Five control rats underwent a sham operation (anesthesia and laparotomy only). Blood samples were collected from the aorta under general diethylether anesthesia 5 hours after the induction of pancreatitis. Hematologic analyses including total white blood cell and total lymphocyte counts, and subcellular fractionation analyses including CD3-positive lymphocyte count and CD4/CD8 ratio, were performed.

Statistical Analysis

Data were analyzed by parametric analysis of variance and values were expressed as means ± standard error. When differences were found between the groups tested ($P < 0.05$), these were subjected to two-sided parametric multiple comparisons between the control group and all other groups. $P < 0.05$ was considered significant.

RESULTS

Patient Profiles

The profiles of all patients with severe acute pancreatitis who were analyzed are presented in Table II. Among the 48 patients, 13 of them had infectious complications. There were no significant differences in sex, age, or etiology between patients with and without subsequent infectious complications. Details of each patient's infection are presented in Table III. In addition, among the 28 patients whose lymphocyte subset analyses were available on admission, eight had subsequent infectious complications. There were no differences in the profiles of patients with and without infection.

Table II. Patient demographics and disease etiologies

	Total (n = 48)	With infection (n = 13)	Without infection (n = 35)
Age (yr)*	51.4 ± 2.1	56.3 ± 3.9†	49.5 ± 2.5
Female (%)	25	31	23
Etiology			
Alcohol	31	6	25
Gallstones	9	3	6
Idiopathic	4	2	2
Others	4	2	2

*Mean ± standard error of the mean.
†Not statistically different from patients without infection ($P > 0.1$).
Distribution of sex and etiologies was not statistically different between patients with and without infection (Mann-Whitney U test).

Table III. Details of subsequent infection

Patient	Timing (days)	Site	Microbial species
1	13	PPN	EC, EF
2	20	Bile	MRSA
3	22	PPN	EC, EF, PA
4	18	PN	PA
5	28	PN	MRSA, PA
	28	Blood	MRSA
6	23	PPN	EC, EF
7	27	PN	EF, PA
8	37	Ascites	MRSA
9	27	PPN	PA, KP
10	30	Blood	— (endotoxin 22.4 pg/ml)
	32	Ascites	CA
11	25	PPN	AC
12	16	Blood	PA, XM
13	16	Sputum	PA

Timing of detection of infection was expressed in days after onset. PPN = peripancreatic necrosis; PN = pancreatic necrosis; EC = *Enterobacter cloacae;* EF = *Enterococcus faecium;* MRSA = methicillin-resistant *Staphylococcus aureus;* PA = *Pseudomonas aeruginosa;* KP = *Klebsiella pneumoniae;* CA = *Candida albicans;* XM = Xanthomonas maltophilia.

Hematologic Findings in Clinical Pancreatitis

The total peripheral lymphocyte count was 959 ± 105/mm^3, which was below the normal range at our institution. As shown in Fig. 1, *A*, the total white blood cell count in peripheral blood on admission was 170 ± 23 × 10^2/mm^3 and 129 ± 10 × 10^2/mm^3 in patients with and without infectious complications, respectively. Although the white blood cell count was relatively high in those with infectious complications, the difference between the two groups was not statistically significant. On the other hand, the lymphocyte count in peripheral blood on admission was 623 ± 90/mm^3 and 1084 ± 135/mm^3 in patients with and without infectious complications, respectively (Fig. 1, *B*). The difference between these two groups was statistically significant. CD3- and CD8-positive lymphocyte counts were significantly lower in patients with subsequent infectious complications compared to those without such complications. Although the CD4-positive lymphocyte count was also decreased in patients with infectious complications, the difference between the two groups was not statistically significant (Fig. 2). These data suggest that the aforementioned decrease in peripheral lymphocytes, which was mainly the result of a decrease in CD8-positive T lymphocytes, is correlated with the subsequent development of infectious complications.

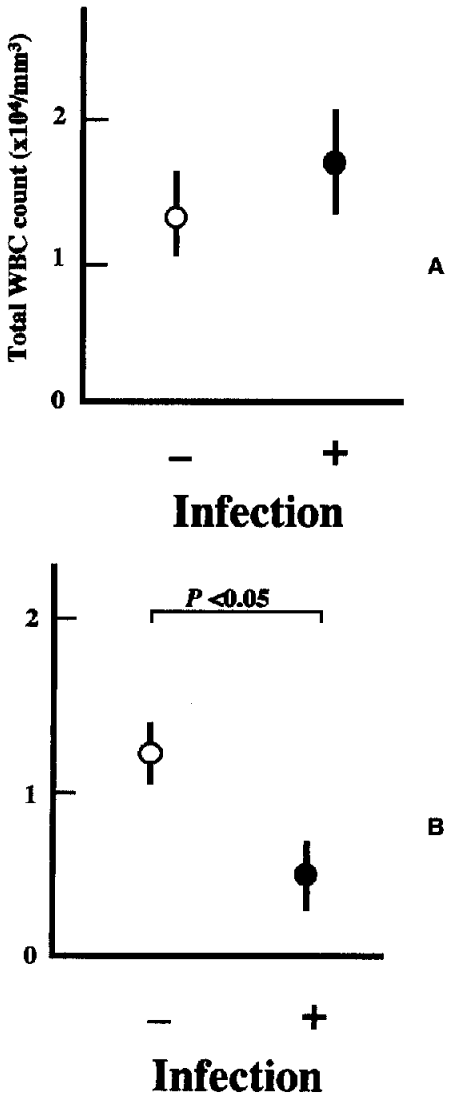

Fig. 1. Hematology of peripheral blood on admission in patients with severe acute pancreatitis. Total white blood cell (WBC) count **(A)** and total lymphocyte count **(B)** in patients with (●, n = 13) and without (○, n = 35) subsequent infectious complications.

Apoptosis of Peripheral Lymphocytes

In light of the preceding results, we focused our attention on the mechanism of lymphocyte reduction in acute pancreatitis and performed cell cycle analysis of lymphocytes in four other patients with severe acute pancreatitis. The average peripheral lymphocyte count was 767 ± 63/mm^3, and one of these four patients subsequently developed an infected pseudocyst. The typical results of cell cycle analysis in one patient and one healthy age- and sex-matched control subject are shown in Fig. 3. In general, cell popula-

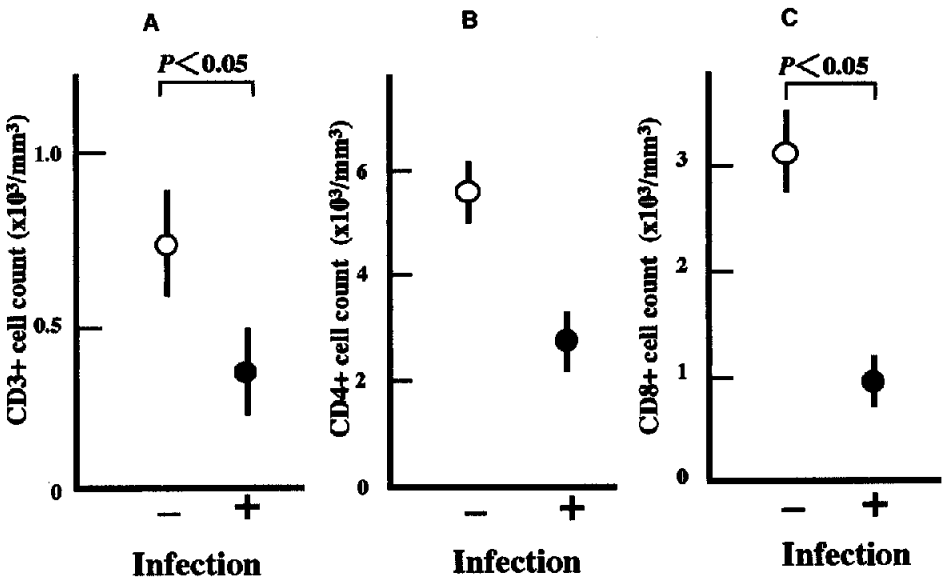

Fig. 2. Analysis of lymphocyte subset on admission in patients with severe acute pancreatitis. CD3-positive (A), CD4-positive (B), and CD8-positive (C) lymphocyte counts in patients with (●, n = 8) and without (○, n = 20) subsequent infectious complications.

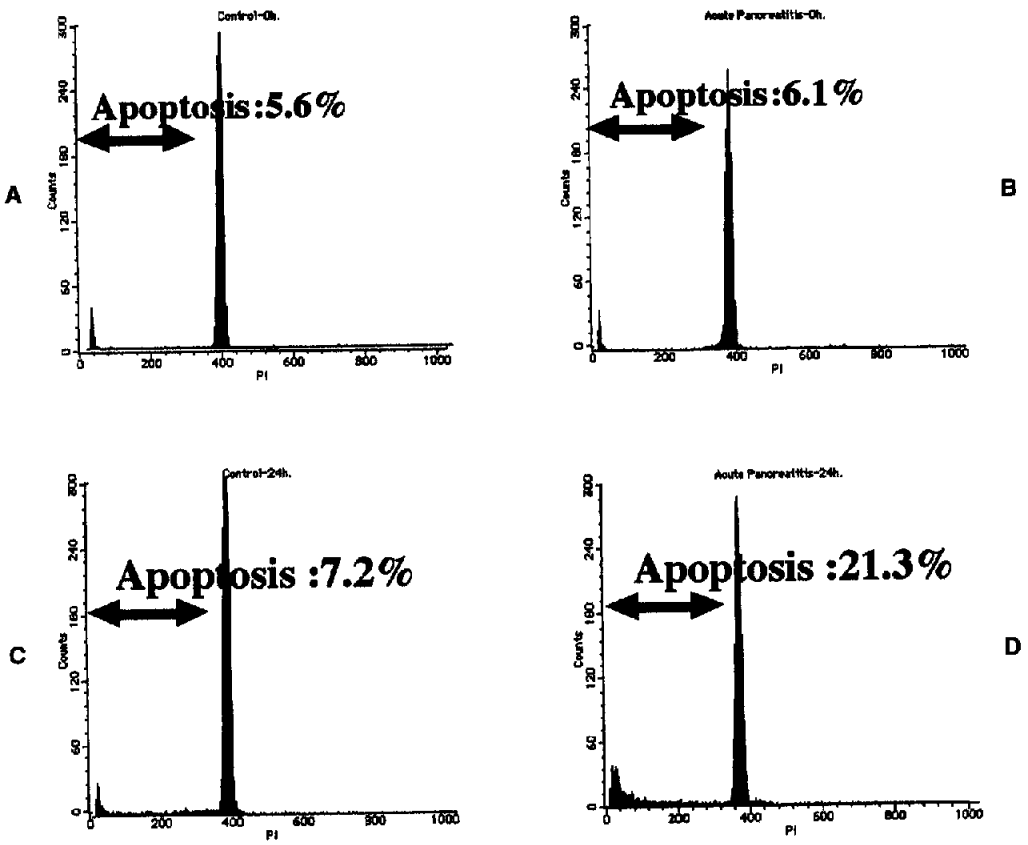

Fig. 3. A typical flow cytometric propidium iodide cell cycle analysis of lymphocytes. Lymphocytes collected from a normal control subject without (A) and with (C) 24 hours' incubation. Lymphocytes collected from a patient with severe acute pancreatitis without (B) and with (D) 24 hours' incubation.

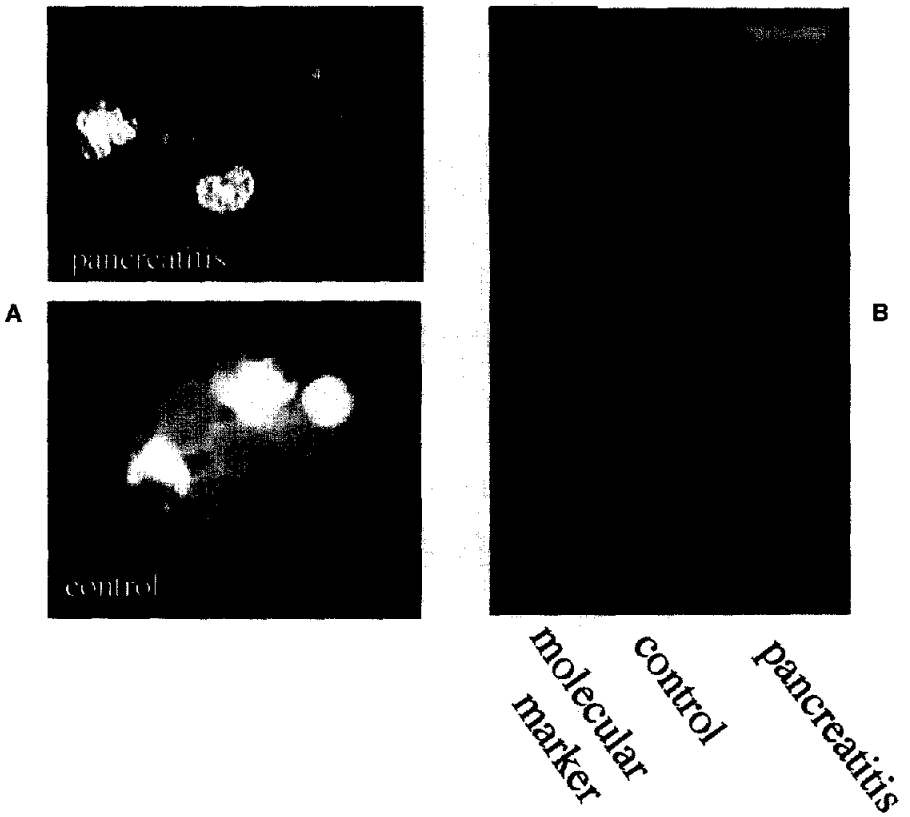

Fig. 4. Nuclear staining and DNA agarose gel electrophoresis of lymphocytes. **A,** Nuclear staining of lymphocytes after 24 hours' incubation. **B,** Agarose gel electrophoresis of DNA extracted from lymphocytes after 24 hours' incubation.

tions containing 2N or 4N DNA represent the cell cycle phase of G1 or G2/M, respectively. Flow cytometric analysis done immediately after isolation of lymphocytes revealed that most of the cells contained 2N DNA. Hypodiploid cells with "sub-G1" DNA content were identified as apoptotic cells, and the ratio of apoptotic cells was less than 10% in both a patient and a control subject, when analyses were carried out immediately after the cells were isolated (Fig. 3, *A* and *B*). However, after 24 hours' incubation, the ratio of apoptotic cells in the lymphocytes from the patient increased more than 20% in contrast to the apoptotic cell ratio in the healthy control subject, which remained below 10% (Fig. 3, *C* and *D*). The apoptotic cell ratios after 24 hours' incubation were 8.3% ± 0.6% and 39.9% ± 12.1% in the lymphocytes from four healthy control subjects and four patients with severe pancreatitis, respectively. This difference is statistically significant. Moreover, apoptosis after the incubation was confirmed by nuclear and DNA fragmentation. Fig. 4, *A* demonstrates nuclear staining of lymphocytes from a patient or a healthy control subject after 24 hours' incubation. In the lymphocytes from the patient, nuclei stained with fluorescent dye exhibited chromatin condensed into crescent-shaped caps adjacent to the nuclear membrane, which is a typical feature of nuclear fragmentation associated with apoptosis (Fig. 4, *A*). In contrast, such features of nuclear fragmentation were not observed at all in the lymphocytes from the normal control subjects, even with the 24 hours' incubation, as is also shown in Fig. 4, *A*. Furthermore, the DNA extracted from the patient's lymphocytes after the incubation period exhibited a stepladder pattern indicating DNA fragmentation; in contrast, there was no stepladder pattern in the DNA from the lymphocytes of the healthy control subject after the same incubation period (Fig. 4, *B*).

Fig. 5 is an example of the chronologic changes in total counts and apoptotic cell ratios of peripheral lymphocytes after the incubation period during the clinical course of a 68-year-old patient with severe pancreatitis. The ratio of apoptotic cells was gradually decreased and the peripheral lymphocyte count

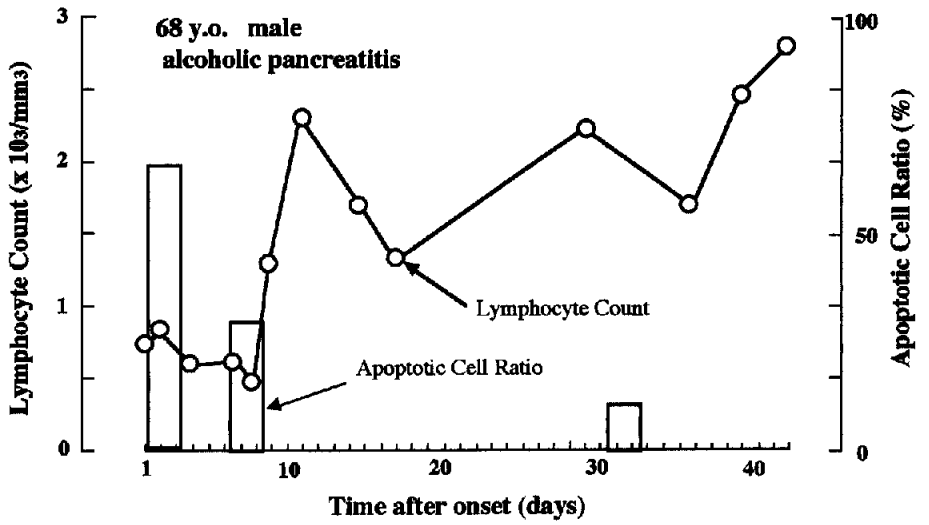

Fig. 5. Chronologic changes in apoptotic cell ratio and cell count of peripheral lymphocytes in a 68-year-old man with severe acute alcoholic pancreatitis.

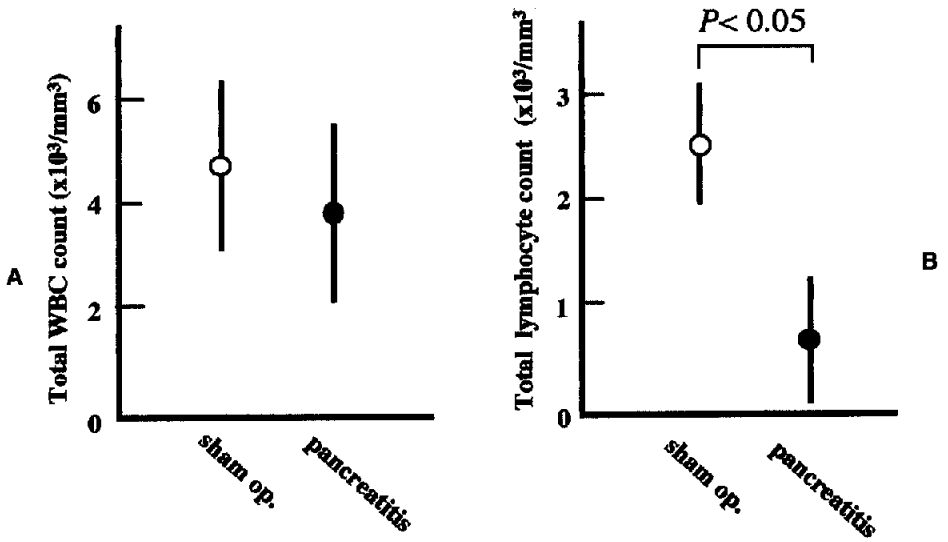

Fig. 6. Hematology of peripheral blood in rats. Total white blood cell (WBC) count **(A)** and total lymphocyte count **(B)** in sham-operated rats (○, n = 5) and rats with pancreatitis (●, n = 5). Samples were taken 5 hours after sham operation or induction of pancreatitis.

was increased during the course of disease in this patient who survived.

Hematologic Findings in Experimental Pancreatitis

In the model of necrotizing pancreatitis used in this analysis, massive necrosis with inter- and intralobular hemorrhage occurred immediately, as previously described.[14] As shown in Fig. 6, a significant decrease in the peripheral lymphocyte count was observed in the rats with pancreatitis, but no alterations in the total white blood cell count were noted. A significant decrease in CD3-positive lymphocytes (T lymphocytes) and a noticeable elevation in the CD4/8 ratio were observed in the rats with pancreatitis compared to the sham-operated rats (Fig. 7).

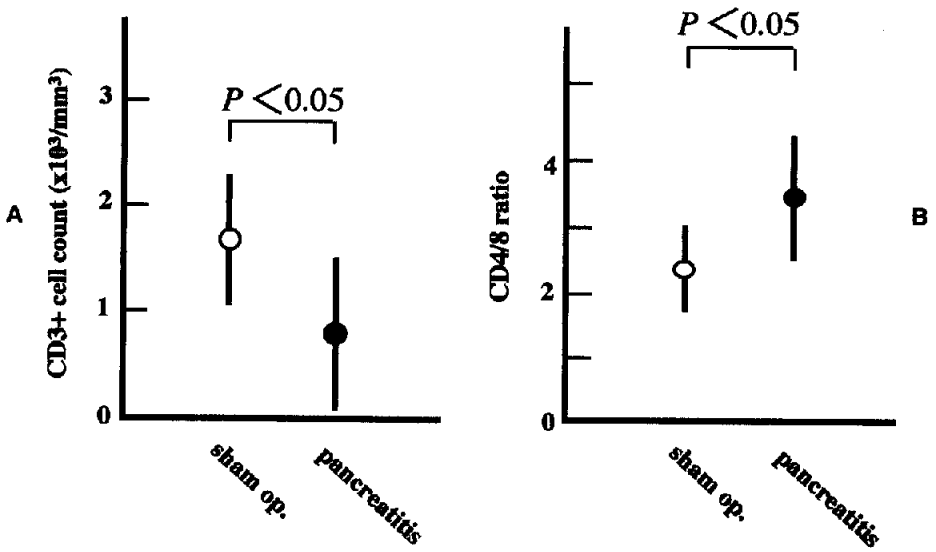

Fig. 7. Analysis of lymphocyte subsets in rats. CD3-positive lymphocyte count **(A)** and CD4-positive/CD8-positive ratio **(B)** in sham-operated rats (○, n = 5) and rats with pancreatitis (●, n = 5). Samples were taken 5 hours after sham operation or induction of pancreatitis.

DISCUSSION

In the present study we have clearly demonstrated that the decrease in peripheral lymphocytes in the early stage of pancreatitis is correlated with the incidence of subsequent infection. Although a high white blood cell count on admission has been adopted as one of the prognostic factors according to some criteria used in grading the severity of this disease,[2-4] the elevation in the white blood cell count was not significant in the patient with infection in our series. One possible explanation for this difference is that the present study was limited to patients with severe pancreatitis. It is conceivable that the elevation in the white blood cell count is representative of the severity of the inflammatory response due to tissue damage rather than impairment of immunologic function.

We have also demonstrated that CD8-positive T lymphocytes were decreased mainly in the patients who had subsequent infection. However, Curley et al.[11] reported that CD4-positive lymphocytes are decreased in patients with acute pancreatitis. We too noted a decrease in CD4-positive lymphocytes in our series, but this decrease was not statistically significant. In fact, Pezzilli et al.[15] reported that on the first day of the study, the number of total and CD4+, CD8+, CD3+ DR−, and CD3− DR+ lymphocytes was significantly lower in patients with acute pancreatitis compared to healthy subjects or patients with nonpancreatic acute abdomen. We assume that the decrease in CD8-positive lymphocytes is more responsible for the development of subsequent infection than the decrease in CD4-positive lymphocytes.

As is clearly shown in this report, apoptotic cell death occurs in lymphocytes collected from patients with severe pancreatitis after 24 hours' incubation, but apoptosis was not detected in freshly isolated lymphocytes. We hypothesize that the lymphocytes undergoing apoptosis are immediately eliminated from systemic circulation and become trapped in the reticuloendothelial system.

There are several possible mechanisms involved in the decrease in peripheral lymphocytes via apoptosis. As Curley et al.[11] pointed out, the decrease in T lymphocytes may be the direct effect of endotoxins. However, in the experimental model used in the present study, endotoxins were not detected in either the peripheral blood or the ascitic fluid within 6 hours.[16]

On the other hand, the cytokines released from monocytes or endothelial cells, such as tumor necrosis factor-α or transforming growth factor-β1 (TGF-β1), can be involved in apoptotic cell death of lymphocytes in acute pancreatitis. Andjeli'c et al.[17] reported that TGF-β1 and intracellular Ca^{2+} mobilization work synergistically in cyclosporin A–induced apoptosis of mature lymphocytes. Recently we have found that macrophage-derived TGF-β1 is partly involved in hepatocyte apoptosis in rats using the same model of pancreatitis. Thus it is possible that TGF-β1 is also involved in inducing apoptosis of peripheral mature lymphocytes. In addition, we cannot ignore Fas ligation, which has been well established as to in-

duce apoptosis in lymphatic cells.[18] Further investigations including the significance of Fas ligation in acute pancreatitis should be performed.

The results obtained herein suggest the usefulness of the peripheral lymphocyte count as a predictor of risk of subsequent infection. To establish a therapeutic strategy to protect the lymphocytes from apoptosis, the molecular mechanism inducing lymphocyte apoptosis in acute pancreatitis should be clarified.

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Somatostatinoma of the Ampulla of Vater in Celiac Sprue

E. James Frick, Jr., M.D., Jeffrey R. Kralstein, M.D., Michael Scarlato, M.D., Herbert C. Hoover, Jr., M.D.

The increased incidence of gastrointestinal lymphoma and adenocarcinoma in patients with celiac sprue is well recognized, with 10% to 15% developing a gastrointestinal malignancy. Somatostatinomas are rare neuroendocrine tumors that occur most commonly within the pancreatic head or duodenum. Although fewer than 100 cases have been reported, somatostatinomas are often associated with multiple endocrine neoplasia-1 syndrome and von Recklinghausen's disease. The unusual case of a 43-year-old woman with celiac sprue in which a somatostatinoma involving the ampulla of Vater was identified and resected is presented. To our knowledge, somatostatinomas have not been previously reported in patients with celiac sprue. (J GASTROINTEST SURG 2000;4:388-391.)

KEY WORDS: Celiac disease, neoplasms, somatostatinoma, Vater's ampulla

Celiac sprue, or gluten-sensitive enteropathy, is a malabsorption disorder of the small intestine. An immune reaction to the gliadin component of dietary gluten (derived from wheat, barley, and rye) results in a loss of small bowel villi and subsequent malabsorption.[1] Celiac sprue is considered a premalignant disease. The most commonly reported neoplasms in patients with celiac sprue are lymphomas and adenocarcinomas of the small intestine, although squamous cell carcinomas of the pharynx and esophagus have also been cited.[2] Somatostatinomas are tumors derived from somatostatin-producing delta cells of the pancreas or endocrine cells (Brunner's glands) of the gastrointestinal tract, and occur most commonly in the pancreatic or peripancreatic region. These rare neuroendocrine tumors may be either functional or nonfunctional and malignant or benign. We report a patient with a somatostatinoma of the ampulla of Vater in conjunction with celiac sprue; to our knowledge, this has never before been reported in the literature.

CASE REPORT

A 43-year-old woman with a history of Hashimoto's thyroiditis presented with poor appetite and fatigue. Nine months earlier a diagnosis of celiac sprue had been made during an evaluation of persistent diarrhea. At that time a small bowel biopsy showed partial villous atrophy, crypt hyperplasia, and patchy lymphocytic, superficial epithelial infiltration. After starting a gluten-free diet, the patient showed improvement in her gastrointestinal symptoms and normalization of antiendomysial levels (>1:64 ELISA units before treatment to 32 ELISA units after treatment) and antigliadin antibody levels (IgG 60 ELISA units before to <7 ELISA units after treatment and IgA of 100 ELISA units before to 3.9 ELISA units after treatment).

Because of her symptoms she underwent an extensive workup including an abdominal CT scan (normal), upper gastrointestinal series with small bowel follow-through (small hiatal hernia and peptic duodenitis), laboratory tests (normal), and esophagogastroduodenoscopy. Endoscopy revealed a 3 cm mass at the ampulla of Vater (Fig. 1). A biopsy was performed, which showed nests of infiltrating epithelial tumor cells with moderate nuclear pleomorphism, abundant pale cytoplasm, and architectural and immunohistochemical patterns compatible with a neuroendocrine tumor.

The patient was referred for resection and subsequently underwent an exploratory laparotomy and transduodenal resection of the tumor. After creating a duodenotomy in the second portion of the duodenum and identifying the mass, a cholecystectomy was performed. A biliary catheter was placed through the cystic duct to help identify the ampulla (Fig. 2). Because the tumor was isolated to the ampulla and there was no evidence of metastatic disease, a localized re-

From the Department of Surgery, Lehigh Valley Hospital, Allentown, Pa.
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Reprint requests: Herbert C. Hoover, Jr., M.D., Chairman, Department of Surgery, Lehigh Valley Hospital, Cedar Crest and I-78, P.O. Box 689, Allentown, PA 18105-1556.

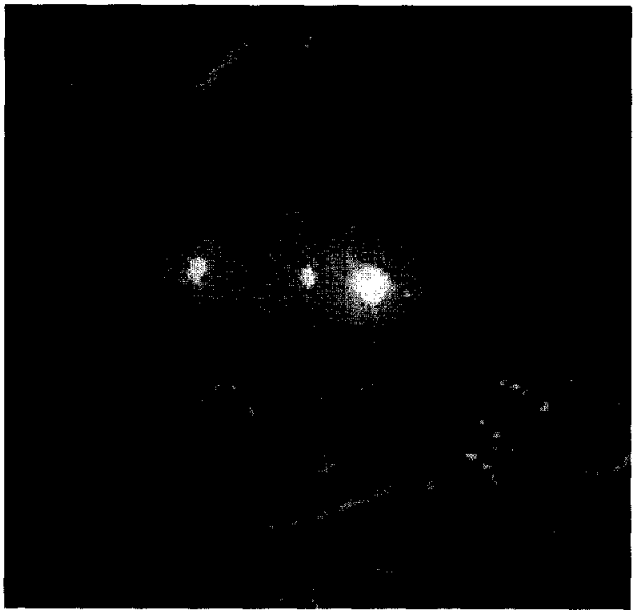

Fig. 1. Endoscopic view of somatostatinoma at the ampulla of Vater.

Fig. 2. Tumor involving the ampulla, seen below the catheter, which was placed through the cystic duct after cholecystectomy.

section was undertaken (Fig. 3). After removal of the tumor and with the aid of the biliary catheter, a choledochoduodenostomy was performed (Fig. 4).

Pathologic examination revealed a 2.4 × 1.7 × 1.7 cm somatostatinoma (Fig. 5). Microscopic examination showed uniform cells in trabecular and packeting patterns with prominent vascularity, spindle and epithelioid tumor cells with "salt and pepper" nuclei, and rare mitoses (Figs. 6 and 7). Immunohistologic staining was negative for smooth muscle actin, neurofilaments, insulin, gastrin, and serotonin; equivocal for S-100; and positive for neuron-specific enolase, chromogranins, and somatostatin. No psammoma bodies were identified.

The patient's postoperative course was uneventful, and she was discharged on the fourth postoperative day. At 6-

Fig. 3. Local resection of the somatostatinoma.

Fig. 4. After the tumor was removed, a choledochoduodenostomy was performed.

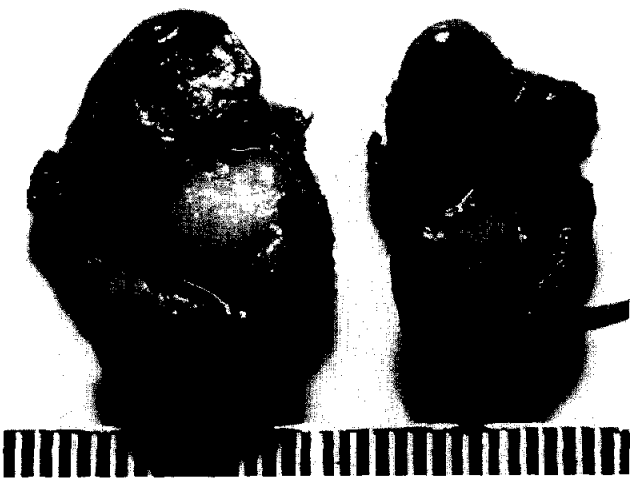

Fig. 5. Cut specimen measuring 2.4 × 1.7 × 1.7 cm.

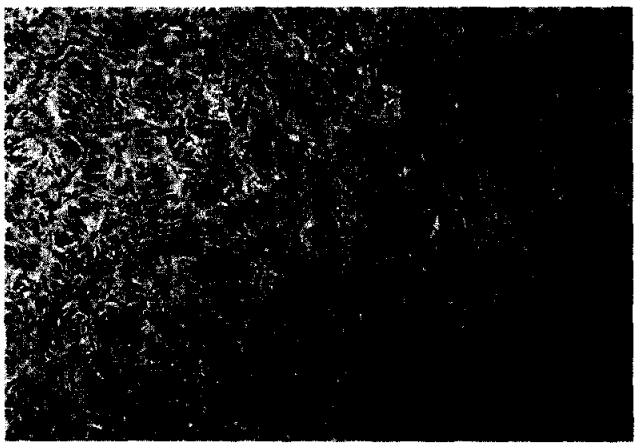

Fig. 6. Microscopic view shows characteristic cells in a trabecular pattern. A packeting pattern with epithelioid cells was present in other areas.

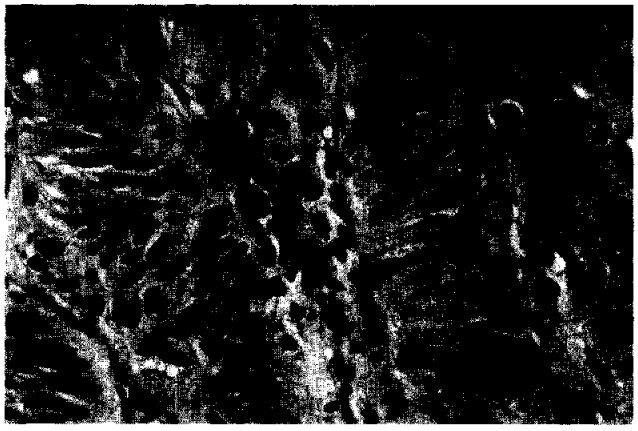

Fig. 7. High-power view of somatostatinoma reveals spindle cells with "salt and pepper" nuclei.

month follow-up, the patient was doing well and no longer had the symptoms of poor appetite and fatigue. A duodenoscopy showed no sign of recurrence of the ampullary tumor. The patient remains on a gluten-free diet.

DISCUSSION

The association between gastrointestinal lymphoma and celiac sprue was first described by Gough et al.[3] in 1962. Prior to this, steatorrhea in patients with lymphoma was often attributed to the malignancy itself. Indeed, some malignant states have been shown to cause small bowel villous atrophy or so-called cancer enteropathy, possibly having been mistaken for the villous atrophy of celiac sprue.[4] Further studies have shown an increased incidence of gastrointestinal lymphoma in patients with celiac sprue.[2,5-9] The jejunum is the site usually involved, although lymphomas have occurred in the ileum, stomach, and colon. Compared to the general population, patients with celiac sprue have a 50- to 100-fold greater risk of developing gastrointestinal lymphoma.[5] Patients with celiac sprue are at risk for developing other malignancies as well, specifically adenocarcinomas of the duodenum and jejunum. Overall, 10% to 15% of patients with celiac sprue will develop a gastrointestinal malignancy.[1] The cause of this tendency toward malignancy is unclear, but it is possibly related to gluten-induced premalignant changes in the villi (numerous mitoses, increased basophilia in the surface epithelium, permeability to carcinogens, and lack of detoxifying enzymes). A significant decrease in cancer morbidity has been shown in patients with celiac sprue who follow a strict gluten-free diet compared to a regular or reduced-gluten diet.[8]

Somatostatinomas constitute less than 1% of all gastrointestinal neuroendocrine tumors. This islet cell tumor was first reported in 1977, and since then less than 100 have been identified. Functional somatostatinomas secrete excessive amounts of somatostatin, a hormone produced in the central nervous system, thyroid gland, pancreas, stomach, and small bowel. This enzyme functions to inhibit gastrointestinal motility, gastrointestinal endocrine and exocrine secretion, and intestinal absorption. A triad of mild diabetes mellitus, cholelithiasis, and diarrhea/steatorrhea characterizes the somatostatinoma or "inhibitory" syndrome. The syndrome was first described in 1979. Additional symptoms have been identified and include dyspepsia, weight loss, anemia, and hypochlorhydria. The diagnosis is established by measuring fasting somatostatin levels, with normal being less than 100 pg/ml. Nonfunctional somatostatinomas tend to be either asymptomatic or present with obstructive symptoms.[10]

Most somatostatinomas (56%) occur in the pancreas, primarily in the head of the pancreas, and are usually functional. Extrapancreatic somatostatinomas (44%) occur in the ampulla of Vater, duodenum, biliary tract, or small bowel. They tend to be nonfunctional (except for biliary tract somatostatinomas, which are functional), present with obstructive symptoms, and are considered less malignant than pancreatic somatostatinomas.[10] Forty to 90% of somatostatinomas are malignant.[10-13] Metastatic spread is primarily to regional lymph nodes or the liver. Multiple endocrine neoplasia-1 syndrome has been identified in 45% of patients with somatostatinomas,[10] and 41% of periampullary somatostatinomas have been associated with von Recklinghausen's disease.[14-16] The presence of psammoma bodies has been considered a hallmark for identifying duodenal somatostatinoma, thus differentiating it from adenocarcinoma and from a

pancreatic (islet cell) origin.[10,14,16-20] However, the psammomatous variant of the gastrointestinal carcinoid has been observed in only 50% of duodenal somatostatinomas, making an explanation for their role in identifying these tumors unclear.[15,21,22]

The treatment for somatostatinomas is surgical excision. Overall, resection for neuroendocrine tumors of the pancreatic and peripancreatic region has yielded good results.[12,16,23,24] The type and extent of resection vary with the location, size, and malignant state of the tumor. Local resection has proved effective for benign somatostatinomas of the ampulla of Vater measuring 2 to 3 cm or less. Larger and malignant tumors of the ampulla generally require pancreatoduodenectomy.[10,16,21]

This case is interesting in that the patient clearly demonstrated symptoms, laboratory test findings, and pathologic evidence consistent with celiac sprue. She showed improvement in her symptoms as well as in her laboratory test values after starting a gluten-free diet. The finding of a somatostatinoma of the ampulla of Vater was accidental, as the symptoms of fatigue and weight loss did not coincide with a functional somatostatinoma. If obtained, a plasma somatostatin level would have most likely been normal, as evidenced by the nonfunctional state of duodenal somatostatinomas in the literature. It is clear from the previous reports, however, that celiac sprue is a premalignant state for gastrointestinal malignancy and warrants further evaluation when patients exhibit constitutional symptoms. The occurrence of a somatostatinoma of the ampulla of Vater in a patient with celiac sprue may be coincidental, as no previous reports of this entity have been published. However, two important aspects of this case may warrant closer inspection: (1) the fact that the anatomic location of malignancies occurring in celiac sprue most often involves the proximal small bowel and (2) the rarity of somatostatinomas and their association with other systemic syndromes.

CONCLUSION

Celiac sprue is a malabsorption disorder characterized by small bowel villous atrophy secondary to an immune response to dietary gluten. Considered a premalignant condition for the development of gastrointestinal lymphoma and adenocarcinoma, but not neuroendocrine tumors, the occurrence of a somatostatinoma of the ampulla of Vater is unique and has not been previously reported in celiac sprue.

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A Randomized Prospective Study of Radially Expanding Trocars in Laparoscopic Surgery

Sunil Bhoyrul, M.D., John Payne, M.D., Bruce Steffes, M.D., Lee Swanstrom, M.D., Lawrence W. Way, M.D.

Trocar injury is one of the most serious and potentially preventable complications of laparoscopic surgery. Use of a blunt rather than a cutting trocar could be expected to lessen the likelihood of this injury. Therefore complications related to laparoscopic port design were studied by comparing conventional cutting trocars with radially expanding (blunt) trocars. A multicenter, prospective, randomized clinical trial was conducted in 250 adult patients undergoing elective laparoscopic procedures at tertiary care centers and community hospitals. The patients were randomly assigned to one of two groups: group C, conventional cutting trocars; or group S, radially expanding trocars. Sixteen surgeons performed 244 elective laparoscopic procedures; six patients were removed from the study. One hundred nineteen patients were assigned to group S and 125 to group C. The groups were similar with regard to age, sex, and type of procedure. The following data were collected: intraoperative complications related to the trocars, abdominal wall bleeding, visceral or vascular injury, other complications, fascial closure, procedure time, trocar site assessment at 4 and 24 hours postoperatively, and visual analog pain scores at 4, 8, 12, and 24 hours postoperatively. Fascial defects from 10 mm or larger trocars in group C were closed; the fascial defects in group S were not closed. The trocar sites were checked for incisional hernias at late follow-up. Mean operating time was not different between the two groups (group S, 92 ± 73 minutes; group C, 100 ± 74 minutes). There were no episodes of intraoperative cannula site bleeding in group S compared with 16 episodes in 13 patients ($P < 0.001$) in group C. Postoperative wound complications were fewer in group S (13 vs. 23; $P < 0.05$). Although the pain scores were generally lower in group S, the differences were not significant. Only 3% of the patients in group S had fascial defects of 10 mm or greater that had to be closed. Within a follow-up period of 6 to 18 months, there have been no incisional hernias in either group. This study shows that radially expanding trocars are safe and effective, and less likely than conventional trocars to result in intraoperative or postoperative complications. The defects created by the radially expanding trocars do not have to be routinely closed. (J GASTROINTEST SURG 2000; 4:392-397.)

KEY WORDS: Trocars, laparoscopic, complications, prospective randomized study

Complications resulting from trocars were reported to occur in 2.8% of gynecologic laparoscopic operations.[1] The most commonly used trocars have tips that cut through the muscle and fascia of the abdominal wall. An alternative is offered by another type of trocar that relies on a needle puncture of the abdominal wall followed by insertion of a blunt radially expanding obturator through the resulting tract. This newer method has the theoretical advantage of producing smaller abdominal wall defects, which may not have to be routinely closed.[2,3] Furthermore, use of a blunt obturator rather than a cutting trocar may be expected to lessen the likelihood of serious vascular or visceral injury.

We performed a multi-institutional, prospective, randomized trial of intraoperative and postoperative complications associated with the use of conventional cutting trocars and radially expanding trocars (*Step*, InnerDyne, Inc., Sunnyvale, Calif.). We measured the incidence of bleeding from the access sites and the occurrence of vascular or visceral injuries and/or postoperative wound complications. We studied the ef-

From the Department of Surgery, University of California, San Francisco, San Francisco, Calif.
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Reprint requests: Lawrence W. Way, M.D., Department of Surgery, University of California, San Francisco, Room S-550, 513 Parnassus Ave., San Francisco, CA 94143-0475. e-mail: lwway@aol.com

fects of not routinely closing the fascial defects created by the radially expanding trocars as opposed to routine closure of defects created by 10 mm or larger cutting trocars. Finally, we recorded the amount of postoperative pain.

MATERIAL AND METHODS

The institutional review boards at each participating facility approved the study before patients were enrolled. All adult patients under the care of the 16 participating laparoscopic general surgeons were invited to participate, and randomization was determined from randomization tables before patient enrollment was begun. Informed consent was obtained before enrollment. There was no financial benefit to the participating patients or surgeons. Assignment of patients to either group C (cutting trocars) or group S (*Step* trocars) was carried out at the time of surgery by drawing consecutive sealed envelopes. Patients and postoperative observers were blinded to the choice of trocar used in the operations. If a patient was randomized to group C, the surgeon used only conventional disposable cutting trocars (United States Surgical Corp., Norwalk, Conn.; Ethicon Endo-Surgery, Cincinnati, Ohio; or Origin Inc., Sunnyvale, Calif.). If a patient was randomized to group S, the surgeon used only the *Step* trocar. All patients were analyzed with the group to which they were originally assigned. Patients with acute inflammatory conditions, such as acute appendicitis or acute cholecystitis, were excluded, because these diseases would confound the postoperative study of pain. Any patient who required conversion to laparotomy was removed from the study, unless the conversion resulted from a trocar-related complication.

Surgical Technique

All participating surgeons used the default method of inserting the first port after establishing a pneumoperitoneum with the use of a standard Veress needle and inserting the device using the blind technique.[4] *Step* trocars were placed by first inserting the Veress needle with its silicone sheath (Figs. 1 to 3). If this was the first access device, the pneumoperitoneum was established by insufflating through the needle, after which the needle was removed, leaving the sheath in place. A tapered blunt obturator with its laparoscopic cannula was inserted through the sheath, stretching the tissues of the abdominal wall to accommodate it. The obturator was removed, and the cannula was left in place. At the end of the procedure, the cannulas were removed under direct laparoscopic vision. Fascial defects created by conventional trocars

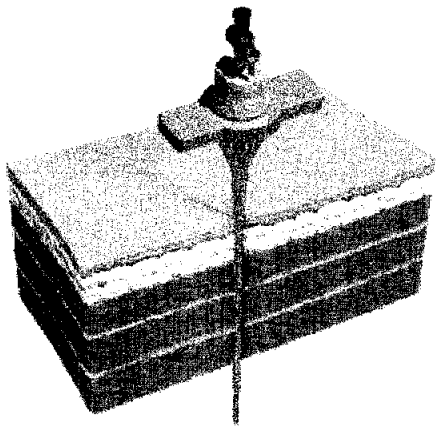

Fig. 1. Access. The peritoneum is insufflated using a Veress needle with a radially expandable sleeve. The needle is then withdrawn leaving the sleeve in place.

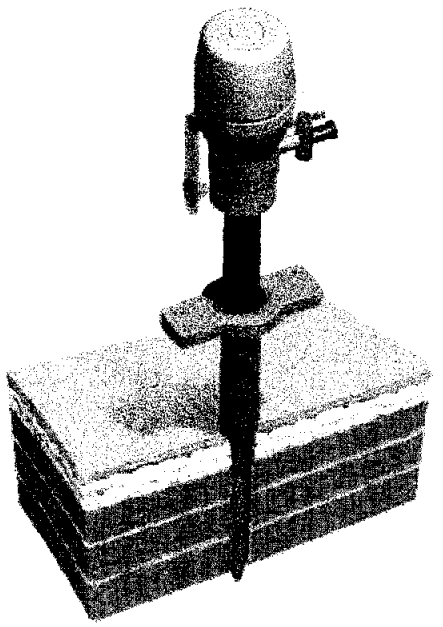

Fig. 2. Dilatation. A tapered blunt obturator is inserted, expanding the sleeve and tissue tract created by the needle. Removal of the obturator leaves a functional laparoscopic cannula in place.

10 mm or greater were closed unless they were too small to be found. Any defect large enough to accommodate the tip of the surgeon's little finger was closed. Defects created by the *Step* trocars were not closed unless they met this criterion. The technique of fascial closure was not predetermined by the study protocol, but it usually entailed placing a single heavy absorbable suture in the external layer of fascia.

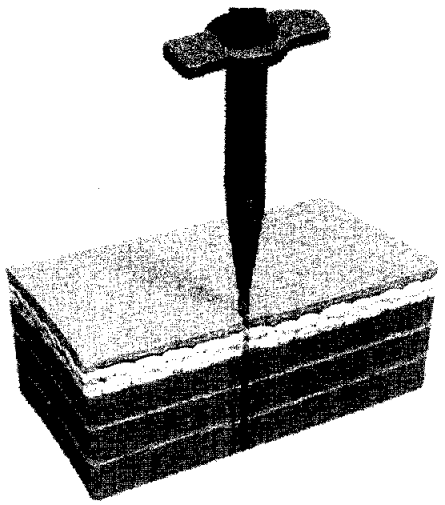

Fig. 3. Cannula removal. After the cannula is removed, a slit-like defect is left through the muscular layers of muscle of the abdominal wall.

Documentation

Immediately after the operation, the surgeon completed a case report form in which the following were recorded: (1) the size of the trocar used; (2) whether a blind (Veress needle) or open technique was used for initial access, and (3) the reason for using the open (Hasson) technique if that was used. The sites of trocar placement were marked on a diagram in the case report form. The surgeon documented any port site bleeding, intra-abdominal vascular or visceral injury, and whether the Veress needle or the obturator had caused the injury. Device-related complications and any corrective measures required to control trocar site bleeding were also recorded. On the diagram used to mark the port sites, the surgeon indicated which fascial defects were closed and the method used (full or partial thickness).

Our "intention to treat" was to close all fascial defects created by conventional trocars 10 mm or greater, but to leave open the fascial defects created by the *Step* trocar, regardless of size. Deviations from this protocol and their reasons were noted. The duration of the operation, from skin incision to skin closure, was also recorded.

Postoperatively a blinded observer (a nurse or physician not involved in the operation) visited the patient and filled in another case report form bearing only the patient's reference number. The observer evaluated the patient 4, 8, 12, and 24 hours after the operation (or until the patient was discharged), and using a 10 cm Visual Analog Scale, asked the patient to judge his or her level of abdominal wall pain 4 and 24 hours after surgery. The observer checked the port site for bruising or persistent bleeding. If the patient had to be returned to the operating room because of access-related complications, this was recorded.

The data were analyzed using Student's *t* test to determine mean values, and a chi-square or Fisher's exact test was used to assess proportions. The calculations were carried out using Microsoft Excel software. Statistical significance was set at $P < 0.05$.

Table I

Procedures performed	Group S	Group C
Cholecystectomy	44 (18%)	42 (17%)
Hernia	31 (13%)	28 (11%)
Fundoplication	30 (12%)	27 (11%)
Colectomy	4 (2%)	9 (4%)
Other*	10 (4%)	19 (8%)

*Other procedures included appendectomy, splenectomy, adrenalectomy, discectomy, and Heller myotomy.

Table II

Intraoperative complications	Group S	Group C	*P* value
Abdominal wall bleeding	0 (0.0%)	13 (10.57%)	0.001*
Visceral injury	1 (0.89%)	1 (0.82%)	0.952
Vascular injury	0 (0.0%)	1 (0.81%)	0.333
Other complications	2 (1.79%)	4 (3.36%)	0.452

*Highly significant.

RESULTS

Two hundred fifty patients were enrolled. Five patients were converted to laparotomy for reasons other than trocar-related complications. One patient, who originally consented to participate and later refused, was removed from the study. The remaining 244 elective laparoscopic procedures (Table I) were performed by 16 surgeons. One hundred nineteen were done using the *Step* trocars (group S) and 125 using conventional trocars (group C). The groups were similar with regard to age, sex, and type of procedure. Mean operating time did not differ between the two groups (group S, 92 ± 73 minutes; group C, 100 ± 74 minutes). There were no episodes of intraoperative port site bleeding in group S compared with 16 episodes in 13 patients in group C ($P < 0.001$) (Table II). There was one visceral (liver) injury in each group ($P = 0.952$). The injury in group S was caused by the Veress needle, whereas the injury in group C was caused by the cutting trocar. There were no vascular injuries in group S, but there was one mesenteric vein laceration in group C ($P = 0.333$). Only 3% of the patients

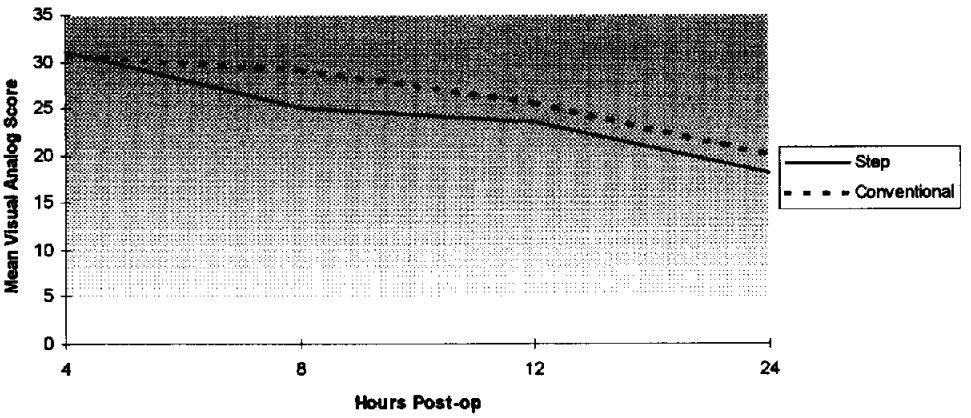

Fig. 4. Pain after laparoscopic surgery, all centers. The differences in pain at 4, 8, 12, and 24 hours after laparoscopic surgery were not significant between groups when data from all centers were pooled.

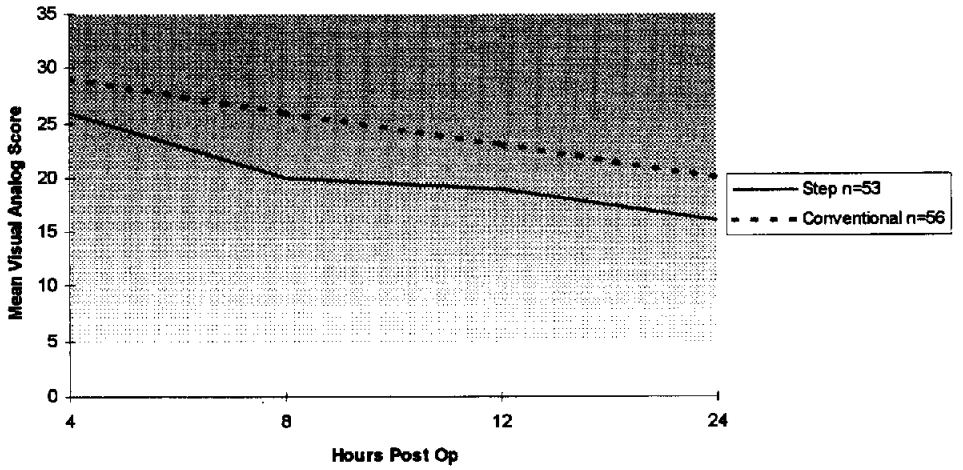

Fig. 5. Pain after laparoscopic surgery, single center. The differences in pain at 4, 8, 12, and 24 hours after laparoscopic surgery were more evident when data from the single largest center were analyzed. Pain was consistently lower in group S *(Step)* when compared to group C *(conventional)*.

Table III

Wound complications	Group S	Group C	*P* value
At 4 hours			
Hematoma	3 (3%)	5 (5%)	0.348
Continued bleeding	14 (13%)	22 (21%)	0.106
TOTAL	17 (16%)	27 (26%)	0.062
At 24 hours			
Hematoma	6 (7%)	15 (20%)	0.016*
Continued bleeding	7 (8%)	8 (11%)	0.652
TOTAL	13 (16%)	23 (30%)	0.028*

*Significant.

in group S had fascial defects of 10 mm or greater that needed to be closed compared with 93% in group C.

The differences in visual analog pain scores were not significant (Fig. 4) when the data from all centers were combined. When the data were stratified for individual centers, however, pain scores were consistently lower in group S, but the differences did not reach statistical significance. Fig. 5, for example, depicts the mean visual analog score for the largest center. The number of patients was too small to stratify for each procedure at each center.

At 24 hours the patients in group C experienced more port site hematomas and total wound complications than patients in group S (Table III). At 4 hours postoperatively, however, local wound complications were not different. During 6 to 18 months of follow-up, there have been no incisional hernias in either group.

DISCUSSION

This study was designed to validate the safety and efficacy of the radially expanding trocars, and to follow up on previous animal studies and retrospective clinical trials in terms of their usefulness.[2-9] The ran-

domized, blinded, prospective design was the first of its kind to be used to evaluate these devices. The multi-institutional aspect of the study corrected for individual variations among surgeons in technique-related complications, but had the disadvantage of incorporating wide variations in operating times and postoperative pain that made these variables difficult to interpret. Capping the study at 250 patients was thought to be reasonable for comparing the safety and efficacy of this device with conventional cutting trocars. Trying to assess the risk of incisional hernia formation was more difficult. This complication has a reported incidence of 1% to 3% in 10 to 12 mm port sites, so determining whether one device is superior to another would require several thousand patients in each arm. It should be noted, however, that none of the investigators has encountered an incisional hernia at a nonclosed *Step* site in the 7 years that this device has been available.

This study shows that among 125 patients who had a wide variety of laparoscopic procedures performed using the *Step* device, there were no serious complications, and the operating times and the amount of postoperative pain appeared to be lower, although not significantly so. A more accurate way to study operating time as a function of the access device may be to observe the time taken to gain access and the time elapsed between completion of the procedure and closure of the incisions. We did not attempt to obtain such measurements retrospectively. The study of operating times was further complicated by the number of sites, surgeons, and procedures performed. Confining the study of procedure time to one institution, one surgeon, and a single procedure could result in a more accurate assessment. The body mass index of the patient may also be an important variable.

In general, pain is difficult to quantify. We studied overall pain rather than port site pain because this was thought to be a more reliable measurement in a multi-institutional setting. Turner[5] studied pain caused by the *Step* trocar, using different kinds of trocars on either side of the abdomen, and recorded pain scores at 1 day, 1 week, and 1 month. Pain was thought to be less where the *Step* trocar had been used, but the assessment was not performed by blinded observers and is therefore of questionable value. Baggish and Lovins[8] found less pain with the *Step* trocar in a small group of patients. In a multi-institutional randomized, prospective, gynecologic study, Feste et al.[9] reported significantly less pain 8, 12, and 24 hours postoperatively in patients in whom the *Step* device was used. Thus the suggestion that pain was not influenced by the choice of trocar may be mainly a function of the study design. Perhaps it would be better to place all patients on the same patient-controlled analgesia protocol and record analgesic use as a function of the other variable.

A difference in the frequency of intraoperative and postoperative port site bleeding was the main finding in this study. There was a 20% incidence of port site bleeding from the conventional cutting trocars, which was most often caused by injury to the superior or inferior epigastric vessels during placement of one of the lateral ports. By comparison, this was rare with the *Step* trocars, because the blunt obturator pushed the blood vessels aside. Our surgeons routinely transilluminated the abdominal wall before inserting a trocar, but this method does not appear to be a reliable way to identify and avoid these vessels, which usually run between the internal oblique and transversus abdominis muscles, and therefore may escape detection.

There was one trocar injury to a mesenteric vein in the conventional group and none in the *Step* group. The reported incidence of less than 1% in some series[10-13] would suggest that the number of patients in this study was too small to assess the superiority of one device over the other. We believe instinctively, however, that a blunt trocar is safer than a cutting trocar.

There was one accidental passage of a Veress needle under direct vision into the liver in group S, and one trocar was placed into the liver in group C. Neither incident resulted in significant bleeding, although the potential for injury was greater with the trocar than with the needle. In a comprehensive report from Finland of 70,607 laparoscopic procedures, it was determined that 41% of recorded visceral injuries were caused by trocars.[13] Soderstrom reviewed 66 cases of bowel injury brought to litigation and found that 90% were caused by sharp trauma during entry.[14]

CONCLUSION

This prospective randomized study verified previous reports that use of a blunt-tipped radially expanding trocar for laparoscopic surgery is safe and effective. Compared with conventional cutting trocars, these devices are less likely to cause abdominal wall bleeding. Furthermore, the defects they create usually do not have to be closed for leaving them open was not followed by incisional hernia formation.

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Anatomic Dilatation of the Cardia and Competence of the Lower Esophageal Sphincter: A Clinical and Experimental Study

Owen Korn, M.D., Attila Csendes, M.D., Patricio Burdiles, M.D., Italo Braghetto, M.D., Hubert J. Stein, M.D.

Anatomic and clinical data suggest that the gastroesophageal junction or cardia in patients with gastroesophageal reflux disease (GERD) may be dilated. We hypothesized that anatomic dilatation of the cardia induces a lower esophageal sphincter dysfunction that may be corrected by narrowing the gastroesophageal junction (i.e., calibration of the cardia). We measured the perimeter of the cardia during surgery in control subjects and patients with GERD and Barrett's esophagus. We then tested our hypothesis in a mechanical model. The model was based on a pig gastroesophageal specimen with perpendicularly placed elastic bands around the cardia simulating the action of the "sling" and "clasp" fibers. "Dilatation" of the cardia was induced by displacing the sling band laterally and decreasing its tension. "Calibration" of the cardia was performed by reapproximation of the sling band toward the esophagus but maintaining the same tension as the dilated model. In the "basal," "dilated," and "calibrated" states, the perimeter of the cardia was noted and rapid mechanized pullback manometry with a water-perfused catheter was performed. The opening pressure was determined, and three-dimensional sphincter pressure images were analyzed. The average cardia perimeter was 6.3 cm in control subjects, 8.9 cm in GERD patients, and 13.8 cm in patients with Barrett's esophagus. The arrangement of the bands in the experimental model generated a manometric high-pressure zone similar to that in the human lower esophageal sphincter. Dilatation of the cardia resulted in a decrease in the resting pressure, length, and vector volume of the high-pressure zone, and reduced the opening pressure. Calibration restored the resting and opening pressure, and normalized the three-dimensional pressure image. In patients with GERD and Barrett's esophagus, the cardia is dilated. Our model supports the hypothesis that lower esophageal sphincter function is compromised by anatomic dilatation of the cardia and can be restored by approximation of the "sling" fibers toward the lesser curvature ("clasp" fibers). This provides evidence for a correlation between gastroesophageal sphincter dysfunction in reflux disease and its correction by antireflux surgery. (J GASTROINTEST SURG 2000;4:398-406.)

KEY WORDS: Gastroesophageal junction, lower esophageal sphincter incompetence, GERD physiopathology, antireflux surgery

Anatomic dilatation of the gastroesophageal junction or cardia in patients with gastroesophageal reflux disease (GERD) has been widely recognized.[1-7] This phenomenon has been termed "patulous cardia,"[6] and its morphologic expression was well described many years ago by Collis.[1] Despite these findings, dilatation of the cardia has never been evaluated or considered as a pathophysiologic factor in GERD and has never been correlated with competence of the lower esophageal sphincter (LES). During the past few years, attention has been focused on other factors related to sphincter competence including resting pressure, overall length, and the abdominal length exposed to positive abdominal pressure.[8] In addition, acute gastric dilatation has been proposed as an explanation for the phenomenon of shortening or "unfolding" of the sphincter.[8] At present, considerable data exist to support the notion that the human gastroesophageal sphincter has its anatomic correlate in the arrangement and architecture of the muscular fibers surrounding the gastroesophageal junction or cardia, that is, the "clasp" fibers and the oblique "sling" fibers.[9-17]

From the Department of Surgery, Clinical Hospital University of Chile, Santiago, Chile (O.K., A.C., P.B., and I.B.), and the Department of Surgery, Technische Universität München, München, Germany (H.J.S.).
Reprint requests: Dr. Attila Csendes, Department of Surgery, Clinical Hospital University of Chile, Santos Dumont 999, Santiago, Chile.

This evidence shows that the sphincter is not a muscular ring but is instead formed by two distinct muscular units.[18] On the basis of these findings, we postulated a mechanism of action for the gastroesophageal sphincter and tested it in an "anatomic" mechanical model.[19]

The purpose of the present study was (1) to measure the perimeter of the gastroesophageal junction or cardia in control subjects and in patients with GERD and Barrett's esophagus, (2) to analyze the potential significance of anatomic dilatation of the cardia on sphincter competence in GERD, and (3) to propose a new concept to explain the effects of the commonly used antireflux operations.

MATERIAL AND METHODS

Clinical Study

The following four groups were included in this prospective evaluation: control subjects, patients with reflux esophagitis, and patients with short-segment or long-segment Barrett's esophagus. The control group included 25 subjects (18 men and 7 women; mean age 37.3 years [range 20 to 64 years]) who had undergone elective surgery for peptic ulcer disease or gallstones. None of them had symptoms of gastroesophageal reflux. Results of preoperative upper endoscopy were normal, and 24-hour pH measurements in 20 of them showed absence of pathologic acid reflux into the esophagus.

The 45 patients with reflux esophagitis (20 men and 25 women; mean age 45.2 years [range 19 to 75 years]) had all been symptomatic for many years and dependent on ranitidine or omeprazol. Preoperative endoscopy showed normal findings in 15 patients and erosive esophagitis in 30. All had undergone antireflux surgery.

The 17 patients with short-segment Barrett's esophagus (8 men and 9 women; mean age 49.5 years [range 26 to 69]) had less than 3 cm of the distal esophagus covered by specialized columnar epithelium with intestinal metaplasia in all of them. All had a lengthy history of gastroesophageal reflux.

The 15 patients with long-segment Barrett's esophagus (mean age 53.8 years [range 26 to 68 years]) all had more than 3 cm of the distal esophagus covered by specialized columnar epithelium with intestinal metaplasia. Eight of them also had peptic ulcers or stricture of the esophagus.

Endoscopic Evaluation. All patients included in the present investigation underwent endoscopic evaluation by means of an Olympus GIF XQ20 endoscope (Olympus Optical Co., Tokyo, Japan). Special care was taken to determine the location of the squamocolumnar junction at the beginning and at the end of the procedure, measuring its distance in centimeters from the incisors[20] and avoiding the "push and pull" effect of the endoscope. The presence of erosion or ulcers was also carefully evaluated. In every case four or more biopsies were done distal to the squamocolumnar junction in order to establish the type of mucosa lining the distal esophagus. In all patients with Barrett's esophagus, intestinal metaplasia was present lining the distal esophagus.

Manometric Evaluation. Manometric studies were performed after a 12-hour fast employing a device with four polyvinyl catheters bonded together in such a way that the side holes were each 3 cm apart from one another (Arndorfer Medical Specialties, Milwaukee, Wis.). The complete details of this procedure have been published elsewhere.[21-26] Three manometric characteristics of the LES were determined: resting pressure, total length, and abdominal length. The existence of a mechanically incompetent sphincter was defined by the presence of one of the following parameters: LES pressure equal to or less than 6 mm Hg, total length equal to or less than 20 mm, and abdominal length equal to or less than 10 mm.[24-26]

Twenty-Four-Hour pH Monitoring. Twenty-four-hour intraesophageal pH monitoring (Digitrapper II, Synectics Medical, Stockholm, Sweden) was performed after a 12-hour fast, placing the electrode 5 cm proximal to the upper limit of the LES.[27,28] Of the six different parameters that were evaluated, the most useful and practical was the percentage of time during which the intraesophageal pH remained below 4, with a normal value being less than 4% in 24 hours (55 minutes).

Twenty-Four-Hour Monitoring of Esophageal Exposure to Duodenal Juice. This procedure has been developed to measure spectrophotometrically the intraesophageal bilirubin concentration. The complete details of this procedure have been published elsewhere.[29-31] The final calculation was based on the percentage of time that bilirubin was measured in the esophagus with an absorbance greater than 0.2, with normal being less than 2% of the time (28 minutes).

Intraoperative Measurements. In all patients except those undergoing cholecystectomy, a selective or highly selective vagotomy was performed as a first step for antireflux surgery. This technique may be performed for several reasons, but the main one is it provides excellent visualization of the esophagogastric muscular junction or cardia, which is delineated by the junction of the longitudinal esophageal muscular fibers and the gastric serosa due to the cleaning of the fat pad that surrounds the gastroesophageal junction. It also preserves the extragastric vagal branches and makes it very easy to perform any type of antireflux surgery. The complete details have been

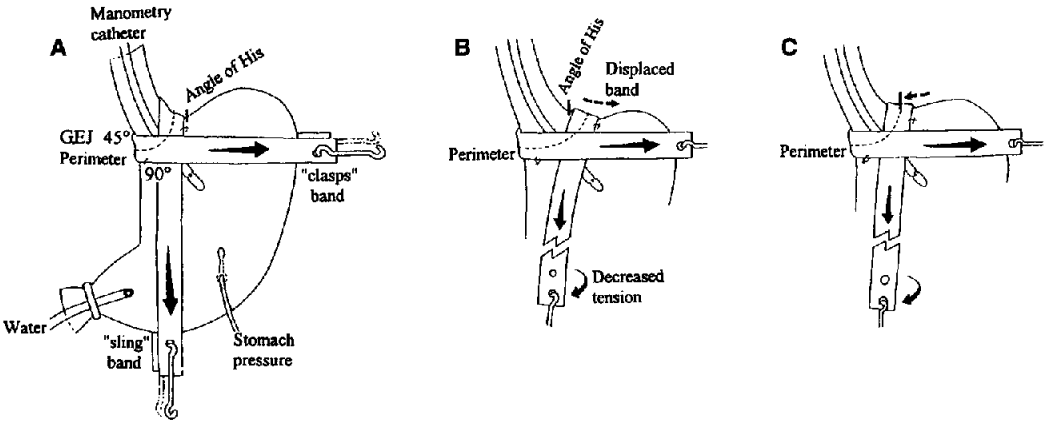

Fig. 1. Schematic aspects of the experimental model and measurements performed in a pig gastroesophageal specimen. **A**, Basal model; **B**, Dilated model; **C**, Calibrated model.

published elsewhere.[21,32] A hiatal hernia, when present, is easily reduced. We have not found a true short esophagus in any of our patients, and in all of our patients the LES and esophagogastric junction were located in the abdomen. The exact perimeter of the cardia was measured by wrapping a 00 silk suture completely around it and then measuring its length in centimeters.

Statistical Analysis. All measurements were subjected to statistical analysis employing the Mann-Whitney U test for unpaired values, with $P < 0.05$ being considered significant.

Experimental Study

Four excised gastroesophageal specimens from pigs, mounted and affixed to a metal basket, were used as an anatomic support for the "external sphincter." A cannula for gastric infusion and a catheter for gastric pressure measurements were introduced into the stomach through stab wounds. The gastroesophageal sphincter was simulated by two elastic bands (each 2 cm wide) placed perpendicularly around the gastroesophageal junction, according to the arrangement of the "clasp" and "sling" fibers. The band that simulated the sling fibers was located above the angle of His,[9] and this point was marked. The angle between the clasp and sling bands was 90 degrees.[9] The obliqueness of the cardia was arbitrarily established at 45 degrees (Fig. 1, *A*). The tension of the bands was adjusted until an adequate balance between the two was reached, being careful to avoid distortion of the zone. The ends of the stretched bands were affixed to the basket walls by small metal hooks. The bands were tied to the gastric wall to prevent displacement during the procedures. After the bands were placed, the perimeter of the gastroesophageal junction was measured around their midpoint.

Two manometric studies were performed in each specimen. The first was accomplished using a water-perfused (1.2 ml/min) single-lumen catheter with four side holes at the same level located at 90-degree angles 2 cm proximal to the distal end. The second study was performed using a continuously perfused (1.2 ml/min/channel) four-channel assembly (5 mm outer diameter) with all side holes located at 90-degree angles 2 cm proximal to the distal end. The catheters were withdrawn from the stomach by rapid mechanized pullback (1.66 cm/sec). Rapid pullback studies were repeated three to four times with both catheters in each specimen. With the pressure data obtained by pullback of four radially oriented pressure transducers, it was possible to construct a three-dimensional image of the high-pressure zone, and to calculate the "sphincter pressure vector volume,"[33] by means of a computer program (UGI Polygram, Gastrosoft, Synectics Medical).

To evaluate the competence of the mechanical model, the stomach was filled with water (180 ml/min), and the intragastric pressure was continuously recorded to the point at which the simulated sphincter opened and retrograde flow occurred (i.e., the opening pressure).[34] All parameters were determined in each of the four specimens, under "normal" conditions or in the "basal" model. Each specimen served as its own control.

Dilatation of the cardia was simulated in each specimen by lengthening the elastic sling band for approximately 1 cm (decreased tension) and moving it away from the cardia toward the fundus (Fig. 1, *B*).

Table I. Manometric, 24-hour pH, and Bilitec studies in control subjects and patients with reflux esophagitis and Barrett's esophagus

	Controls (n = 20)	Reflux esophagitis (n = 45)	Short-segment Barrett's (n = 17)	Long-segment Barrett's (n = 15)
LES pressure (mm Hg)	15.7 ± 6.1	9.1 ± 3.9	6.5 ± 3.1	5.9 ± 2.2
Total length (mm)	38 ± 6	33 ± 8	28 ± 7	31 ± 12
Abdominal length (mm)	20 ± 4	10 ± 6	3 ± 3	4 ± 4
% Time pH <4	2.1 ± 1	9.4 ± 3.2	15.5 ± 10.7	27.3 ± 17.6
% Time bilirubin	—	—	13.3 ± 10.3	20.5 ± 16.1

This resulted in an increase in the perimeter of another 2 cm with respect to the basal perimeter. The tension of the elastic clasp band was not modified. In these "dilated" models, the new perimeters of the gastroesophageal were noted, and all parameters mentioned earlier were determined again in each specimen. Finally, calibration of the cardia was simulated by displacing the elastic sling band toward the esophagus and attaching it at the level of the angle of His, but maintaining the same length as that used in the dilated model, that is, without increasing the tension of the band (Fig. 1, *C*). In these "calibrated" models, the gastroesophageal junction perimeters and the other parameters were determined in each specimen.

RESULTS

Clinical Study

The results of measurements of the perimeters of the cardia are shown in Fig. 2. Control subjects had significantly lower values than patients with reflux esophagitis or Barrett's esophagus ($P < 0.001$). Patients with Barrett's esophagus had significantly larger cardia perimeters than patients with reflux esophagitis ($P < 0.001$), but values did not differ between patients within the Barrett's esophagus groups. Results of functional studies performed in patients with gastroesophageal reflux are shown in Table I. Among control subjects, all parameters were significantly different from those in patients with gastroesophageal reflux or Barrett's esophagus ($P < 0.001$). In patients with Barrett's esophagus, the LES pressure was significantly lower compared to patients with reflux esophagitis ($P < 0.001$). Total sphincter length was similar in all groups, but the abdominal sphincter length was significantly shorter in patients with Barrett's esophagus compared to patients with reflux esophagitis ($P < 0.001$). There were significant differences in acid and duodenal juice exposure of the distal esophagus, which showed a significant increase paralleling the severity indicated by the endoscopic findings ($P < 0.001$).

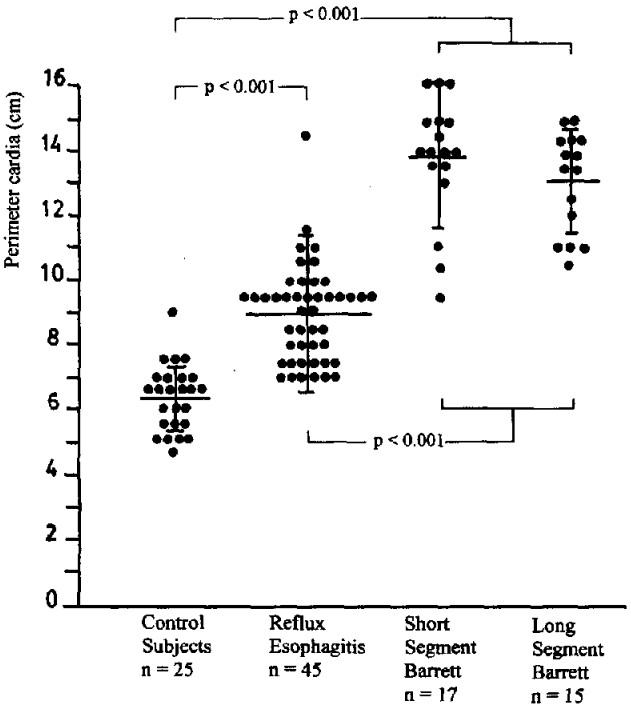

Fig. 2. Individual and mean values for the perimeter of the gastroesophageal muscular junction or cardia in control subjects, patients with reflux esophagitis, and patients with short-segment and long-segment Barrett's esophagus.

Experimental Study

The mechanical model generated a competent high-pressure zone at the gastroesophageal junction with a manometric pattern similar to that of the human LES. Fig. 3 shows the effects of dilatation and calibration of the cardia in each specimen. The competence of the "sphincteric mechanism" correlated with the opening pressure. Its values showed a correlation with the pressure vector volume. The perimeter of the cardia and the values of pressure and length alone were not sufficient to estimate the competence of the sphincter model.

When dilatation of the cardia was simulated by separating the bands, thus increasing the perimeter of the gastroesophageal junction, the sphincteric mechanism was altered. A decrease in the values for pressure, length, pressure vector volume, and opening pressure was observed in all specimens. The "calibration" or reapproximation of the bands resulted in an increase in the resting pressure and opening pressure values compared to the values in the dilated model. In Fig. 4, the pressure tracings and three-dimensional pressure images obtained in the two-band mechanical model under basal, dilated, and calibrated conditions are shown. In the dilated state, the pressure tracing and three-dimensional image are depressed, and both are restored after calibration.

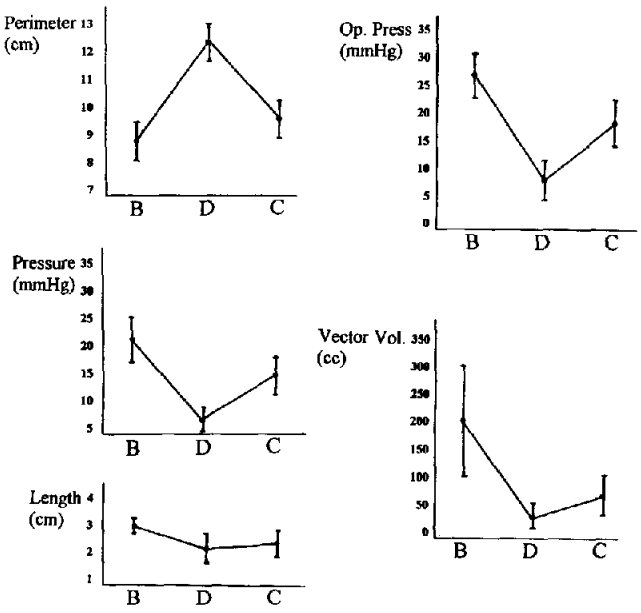

Fig. 3. Effect of dilatation *(D)* and calibration *(C)* of the cardia specimens on the perimeter, pressure, length, opening pressure, and vector volume in the sphincter experimental model. *B*, basal.

DISCUSSION

The results of the present clinical study suggest that the perimeter of the gastroesophageal junction or cardia is significantly greater in patients with severe GERD and Barrett's esophagus, as well as in patients with reflux esophagitis, compared to control subjects. This dilatation is chronic and permanent and seems to be progressive based on the progression of the severity of GERD. It is important to note that this perimeter was measured in subjects in the resting state and on an empty stomach. According to the present anatomic and physiologic studies, there is evidence to suggest that the LES has its anatomic correlate in the arrangement of the so-called muscular "clasp" and oblique "sling" fibers at the gastroesophageal junction.[9-18] Anatomic dilatation of the cardia implies a permanent morphologic change in the gastroesophageal junction, provoked of necessity by an alteration in the architecture or arrangement of the muscular components that shape it. Therefore it is possible to postulate that an alteration in the "disposition" of these fibers will determine the dilatation of the cardia, on the one hand, and the incompetence in the mechanical function of this sphincter on the other. This hypothesis was tested in a mechanical model as an experimental study to support the clinical findings.

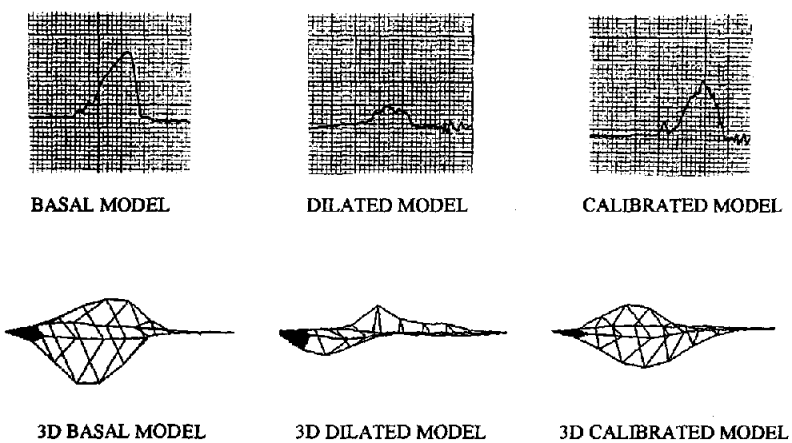

Fig. 4. Pressure tracings and three-dimensional pressure images obtained in the mechanical model under *basal, dilated,* and *calibrated* conditions.

The concept of dysfunction of the gastroesophageal sphincter presented in this work is based on the following: (1) a gastroesophageal sphincter formed by two distinct muscular units—that is, two incomplete rings arranged perpendicularly around the gastroesophageal junction[9-18]; (2) a sphincteric mechanism based on the complementary action of these two muscular units[19]; and (3) the phenomenon of chronic dilatation of the cardia shown clinically, which alters the function of these two components.

The proposed sphincteric mechanism is based on the functional correlation of the regional anatomy and on the fact that the action of a muscular fiber is determined by its arrangement and point of insertion. In brief, the proposed mechanism considers that, in order to achieve contact between the walls of the gastroesophageal junction, it is necessary that the greater curvature "descend and advance medially," by virtue of the oblique sling fibers, to the "encounter" with the lesser curvature, which only can move in a transverse direction toward the center, in that it is limited by the shortness of the semicircular clasp fibers.[19] Fig. 5, which is a schematic representation of the normal condition, demonstrates the arrangement of the fibers around the gastroesophageal junction (see Fig. 5, *A*) in a model of the oblique sling fiber and its theoretical force vector (see Fig. 5, *B*). The contact zone (mucosal seal) is reached at the intersection of the "displacement areas" of each curvature (see Fig. 5, *C*). This results in closure of the sphincter with adequate length, pressure, and competence (see Fig. 5, *D*).

The cause of anatomic dilatation of the cardia in patients with GERD is unknown, but the phenomenon has been verified objectively by our group and other investigators[1,5,6] and is confirmed in the present clinical study. Moreover, there was a direct correlation between the severity of GERD and a greater perimeter of the cardia.

In addition, it has been shown experimentally that patients with reflux esophagitis exhibit different sphincter pressure-diameter curves from those in healthy volunteers. In these experiments, incompetent sphincters had a lower pressure at all diameters, with pressure gradually increasing with larger probe diameters. This mechanical dysfunction can be caused by a dilated cardia.[35] In addition, calibration of the cardia is an essential part of each of the current antireflux surgical techniques. Its importance was noted many years ago by Larrain[2] and has been repeatedly acknowledged[7,36] since it allows application of the law of La Place. However, this concept is valid only if the cardia is dilated in patients with GERD. Finally, it has been shown experimentally that sectioning or removal of the sling fibers results in a loss of the so-called cardiac notch, and the angle of His becomes more rounded and obtuse.[37,38]

The dilatation implies elongation of the muscular fibers, and alterations in its angulation and arrangement. A schematic view of this phenomenon and its consequences is shown in Fig. 6. The angle of the cardia changes (see Fig. 6, *A*), and the oblique sling fibers are separated, elongated, and angulated modifying their force vector (length-tension properties) (see Fig. 6, *B*). The greater curvature is more distant and by displacing for closure, the "displacement" is now no more coincidental with that of the lesser curvature (see Fig. 6, *C*). The contact area (mucosal seal) is smaller and the pressure zone is shortened. Thus the closing pressure is impaired (see Fig. 6, *D*); in other words, a mechanically defective sphincter results.

The values of pressure and length of the sphincteric area are not enough to represent this sphincter and to estimate its competence. This has been shown in patients with low values for these parameters who

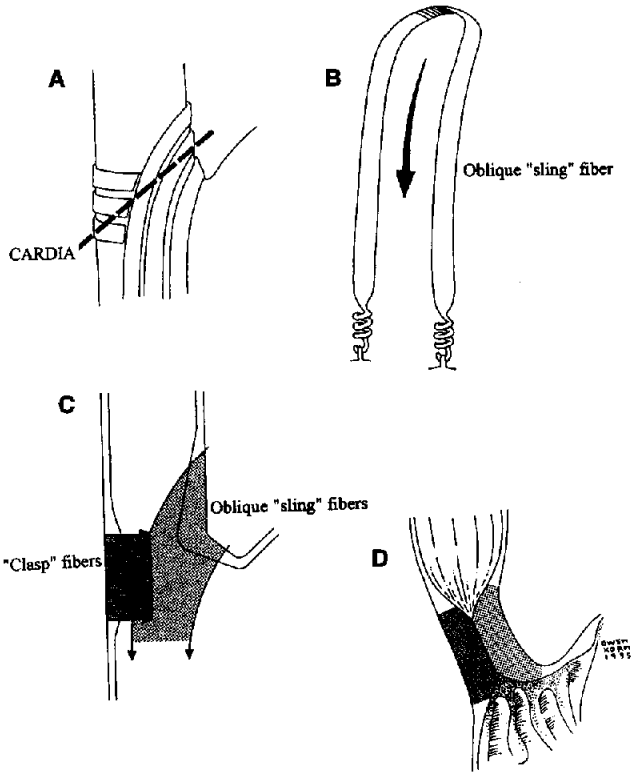

Fig. 5. Schematic representation of the proposed mechanism of action of the gastroesophageal sphincter in the normal state. **A,** Arrangement of the muscular fibers around the gastroesophageal junction. **B,** An oblique "sling" fiber and its force vector. **C,** The mucosal seal is reached at the intersection of the "displacement areas" of each curvature. **D,** Sphincter closure area with normal length, pressure, and competence.

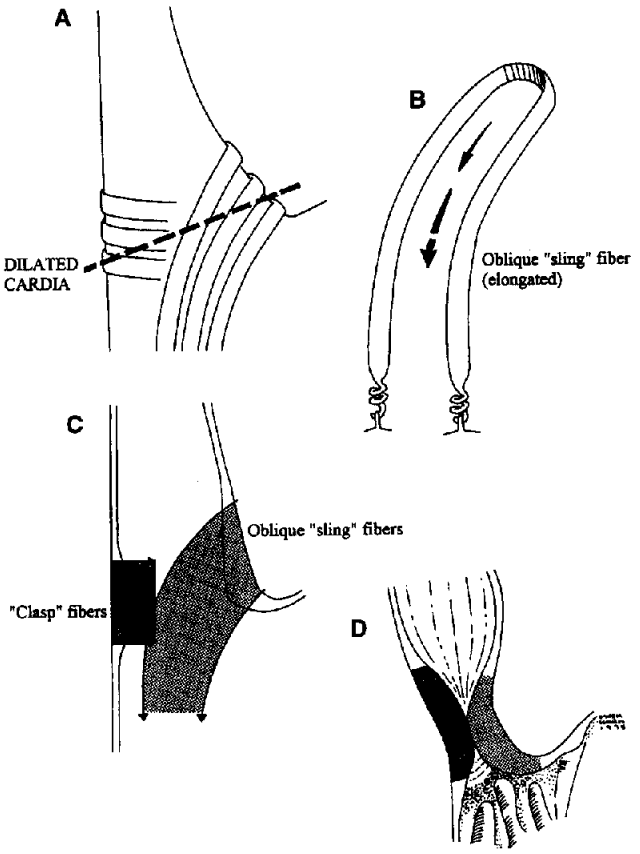

Fig. 6. Schematic diagram of the proposed gastroesophageal sphincter dysfunction when the cardia is anatomically dilated. **A,** The oblique angle of the cardia changes, and the angle of His becomes obtuse. **B,** The "sling" fibers are elongated and angulated modifying their length-tension properties. **C,** The mucosal seal is smaller and the pressure zone is shortened. **D,** The sphincter is mechanically incompetent.

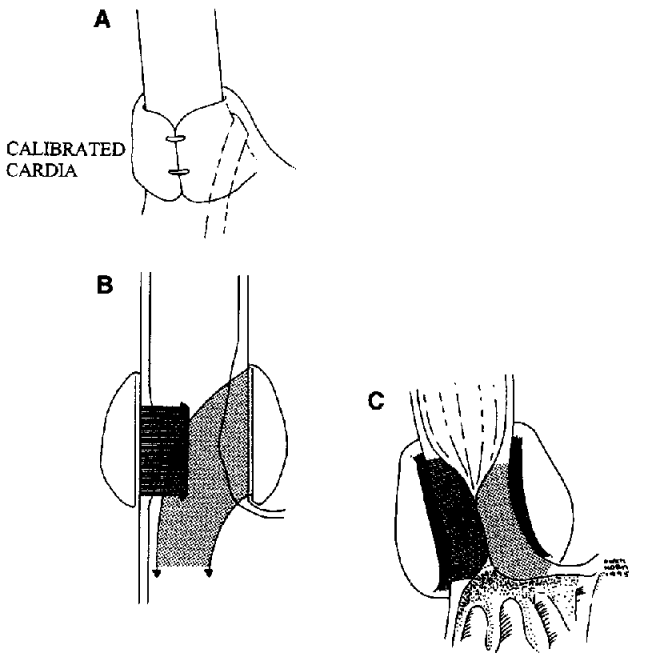

Fig. 7. Schematic diagram of the mechanism of surgical correction by means of calibration of the cardia. **A,** Fundoplication results in approximation ("calibration") of the greater curvature toward the lesser curvature. **B,** The coincidence and size of the mucosal seal are improved. **C,** Sphincter competence is restored.

still could have a competent sphincter.[24] In addition, in our patients, there was some overlap between the perimeter of the cardia in patients with esophagitis and the control subjects (see Fig. 2). These findings can be explained by the fact that the architecture of each cardia is unique. The impact of an equal increase in the perimeter is not the same in any two cardias, which start from different basal perimeters; thus an increase of 1 cm in a basal perimeter of 5 cm can be more serious than an increase of 1 cm in a cardia that has a normal perimeter of 7 cm.

The anatomic dilatation of the cardia is not the origin of the GERD.[39] However, a dilated cardia represents the point of no return, that is, the point at which the LES becomes mechanically defective. At this point in the disease, medical therapy is no longer useful, and surgical intervention offers the only hope for a possible cure. The "ideal" repair would be to restore the lost anatomy. Beyond the known fact of a direct muscular action of the gastric wrap with total fundoplication,[36] this fundoplication also produces a calibration of the cardia, which results in approximation of the greater curvature toward the lesser curvature. This can correct the "displacement areas," thereby improving the coincidence of the mucosal seal and the size of the contact zone (Fig. 7). In addition, this mechanism could improve the intrinsic condition of the fibers (length-tension properties). It is necessary to emphasize that, based on these concepts, calibration of the cardia does more than simply provide a "squeeze" of the orifice and allow for the application of the law of La Place.[7,36] The gastroesophageal junction is more than a flaccid rubber tube, and its calibration must mean the reapproximation of the two sphincteric components in order to recover and to achieve a better interplay between them. Thus under the best of circumstances, it is possible to reestablish the concidence of the mucosal seal and the competence of the sphincter, to improve the closing pressure and the length of the pressure area, but all of these do not always occur. This is a very important point because it could explain why classic antireflux surgery (Nissen fundoplication or any other procedure) can fail in a significant number of patients with

long-segment and complicated Barrett's esophagus, as has been reported recently.[40]

These concepts provide a new vision of the gastroesophageal sphincter and its dysfunction, and open new ways to approach the pathogenesis and treatment of GERD. In addition, permanent dilatation of the cardia is a constant anatomic finding, which must also be considered in any future analysis of the factors that determine normal gastroesophageal competence.

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Percutaneous Replacement Jejunostomy After Esophagogastrectomy

Malcolm V. Brock, M.D., Anthony C. Venbrux, M.D., Richard F. Heitmiller, M.D.

A surgically placed jejunostomy tube is a safe and effective means of delivering nutritional support for the postesophagogastrectomy patient. We have previously described a method that permits percutaneous replacement of surgically placed jejunostomy feeding tubes, and now present our results with the use of this technique in 350 consecutive esophagogastrectomy patients. Replacement jejunostomy was required in 17 patients (4.9%). All patients had successful percutaneous jejunostomy replacement. There were no procedural complications or deaths. The timing of feeding tube replacement following esophagogastrectomy was predictive of the indication. Before 16 weeks, the indication for feeding tube replacement was intubation and inability to eat (1 patient) or anorexia with weight loss and dehydration (7 patients). At or after 16 weeks, the indications for feeding tube replacement were all related to symptoms resulting from recurrent carcinoma. We conclude that the technique of percutaneous jejunostomy allows the surgeon tremendous flexibility in the management of the postesophagogastrectomy patient as it preserves the advantages of an adjuvant surgically placed feeding tube over the lifetime of the patient. The technique is safe, and the success rate is excellent. (J GASTROINTEST SURG 2000;4:407-410.)

KEY WORDS: Esophagectomy, feeding tube, jejunostomy

A surgically placed jejunostomy tube is a safe and effective means of delivering nutritional support to the postesophagogastrectomy patient. We have previously described a method that permits *percutaneous* replacement of a surgically placed jejunostomy feeding tube,[1,2] and we now have 8 years' experience with this technique. The purpose of this report is to present our results with the use of percutaneous replacement jejunostomy in patients who had undergone esophagogastretomy.

METHODS

It is the practice of one of us (R.F.H.) to surgically place a jejunostomy tube in all patients undergoing esophagogastrectomy for benign or malignant disease. Our ongoing patient care pathway calls for removal of the jejunostomy tube prior to discharge, unless the patient is found to have swallowing abnormalities (i.e., aspiration) noted on postoperative fluoroscopic video pharyngoesophagography. Beginning in 1992, the intubated loop of jejunum was marked with metal hemoclips to permit percutaneous jejunostomy replacement using a technique described below.

All patients following esophagogastrectomy for carcinoma or Barrett's esophagus with high-grade dysplasia who later required percutaneous replacement jejunostomy were identified through the procedure records of the Cardiovascular Diagnostic Laboratory at The Johns Hopkins Hospital from November 1992 through March 1999. All replacement jejunostomy tubes were inserted under the supervision of us (A.C.V.) using a percutaneous technique that has previously been reported.[1,2]

Briefly, at the time of esophagogastrectomy, a surgical jejunostomy is placed into the proximal jejunum using the Witzel or Stamm technique. A 14 F red rubber catheter is used as the jejunostomy tube. The intubated loop of jejunum is tacked to the anterior abdominal wall in the left upper quadrant using four tacking sutures of 4-0 silk. Each tacking stitch is "marked" with a metal hemoclip as illustrated in Fig. 1. To prevent clip migration, the clip is incorporated into the knot as it is tied. Five centimeters distal to the intubation site, three additional 4-0 silk

From the Departments of Surgery (M.V.B. and R.F.H.) and Radiology (A.C.V.), The Johns Hopkins Medical Institutions, Baltimore, Md.
Supported by the Evelyn Glick Fund for Thoracic Surgery.
Reprint requests: Richard F. Heitmiller, M.D., Osler 624, Johns Hopkins Hospital, Baltimore, MD 21287.

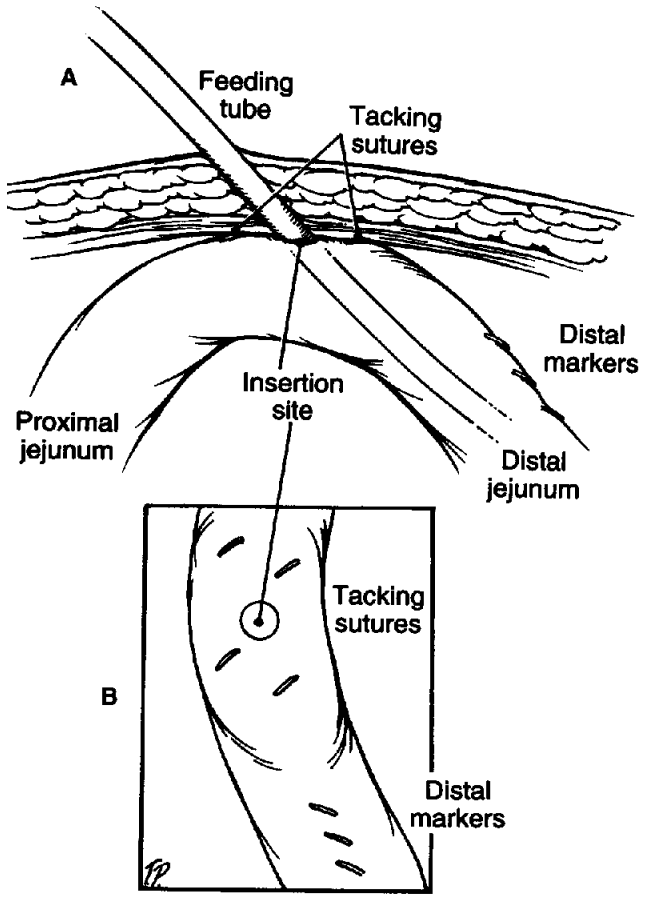

Fig. 1. Technique of preparing intubated loop of jejunum for insertion of replacement feeding tube. **A,** Side view; **B** *(inset),* top view.

sutures are placed, 1 cm apart from one another, and each suture is "marked" with a metal hemoclip as well. The four tacking sutures at the intubation site serve as the radiographic target for percutaneous tube replacement, and the distal sutures identify the direction of the distal bowel. After the original jejunostomy tube is removed and the tract is healed, the tube may be replaced at any time percutaneously in the catheterization laboratory.

With the patient under local anesthesia with intravenous sedation, a standard angiographic needle or a micropuncture needle set (Cook Surgical, Bloomington, Ind.; cost $178.20) is directed at the surgically placed metal clip target under fluoroscopic guidance. Injection of air and a small amount of angiographic contrast medium confirms intraluminal needle placement. A 260 cm length, 0.038 inch diameter Coons interventional guidewire provided in the Carey-Alzate-Coons gastrojejunostomy set (Cook Surgical) was passed through the intubating needle and directed into the distal bowel. The intubating needle was removed and the guidewire directed into the more distal jejunum. Once the guidewire was advanced sufficiently, the guidewire tract was sequentially dilated and then a 10.2 F 100 cm long Carey-Alzate-Coons gastrojejunostomy tube was placed and its position confirmed fluoroscopically. A self-retaining Malecot retention lock is used to secure the tube in place. After 24 hours, the tube is used for enteral feeding.

For those patients identified as undergoing percutaneous replacement jejunostomy, The Johns Hopkins medical records were reviewed to determine patient characteristics, indications for the initial esophagogastrectomy, time interval from removal of the original jejunostomy tube to the date of tube reinsertion, indications for tube replacement, procedural success rate and complications, and length of time that the feeding tube was subsequently used. The percentage of postesophagogastrectomy patients who need percutaneous replacement jejunostomy was determined by comparing the number of patients requiring replacement jejunostomy with the total number of esophagectomies performed during the study interval.

RESULTS

Seventeen patients required percutaneous replacement jejunostomy following esophagogastrectomy. Patient demographics reflect the fact that the primary diagnosis for the majority of patients was esophageal carcinoma. There were four women and 13 men whose mean age was 66 years (range 58 to 78 years). Esophagogastrectomy technique was as follows: transhiatal in 11 patients, left thoracoabdominal in two patients, and three-incision (right thoracotomy, midline laparotomy, and left cervical) in four patients. Indications for esophagogastrectomy included esophageal carcinoma in 15 patients, gastric cancer in one patient, and Barrett's esophagus with high-grade dysplasia in one patient. The indications for jejunostomy tube replacement were anorexia (8 patients) with dehydration and weight loss severe enough to require readmission to the hospital for intravenous hydration, intubation for postoperative aspiration pneumonia (one patient), and recurrent carcinoma with dysphagia or anorexia with weight loss (8 patients).

Seventeen replacement jejunostomies were identified out of a total of 350 esophagogastrectomies that were performed during the study period. Therefore the jejunostomy reinsertion rate was 17 of 350 or 4.9%. Replacement of the jejunostomy tube was successful in all patients. There were no deaths and no procedural complications. The length of time for which the reinserted jejunostomy tube was used was

Table I. Elapsed time between initial surgery and replacement of jejunostomy feeding tube

Patient no.	Time to jejunostomy replacement (wk)	Indications for replacement
1	2	Intubation/NPO
2	2	Anorexia
3	2	Anorexia
4	3	Anorexia
5	6	Anorexia
6	10	Anorexia
7	13	Anorexia
8	15	Anorexia
9	16	Recurrent cancer
10	16	Recurrent cancer
11	18	Recurrent cancer
12	19	Recurrent cancer
13	24	Recurrent cancer
14	30	Recurrent cancer
15	50	Recurrent cancer
16	131	Recurrent cancer
17	156	Recurrent cancer

NPO = nothing by mouth; indicates a patient who is unable to take food or liquids by mouth.

not recorded. In patients with recurrent carcinoma, the feeding tube was left in place for the remainder of the patient's life. For those patients in which the jejunostomy tube was ultimately removed, the shortest time of enteral feeding was 3 weeks.

The time and indication for jejunostomy tube replacement is shown in Table I. The indication for feeding tube replacement can be predicted as a function of time. Prior to 16 weeks, the indication for tube replacement were anorexia (8 patients) or intubation and inability to eat (1 patient). At 16 weeks or greater, the indication for jejunostomy tube replacement was always symptoms related to recurrent carcinoma.

DISCUSSION

The data regarding postesophagectomy enteral feedings are conflicting, although most reports support the use of an adjunctive feeding tube. Watters et al.[3] documented impaired respiratory mechanics and decreased mobility of patients recovering from esophagectomy or pancreaticoduodenectomy. On the other hand, Baigrie et al.[4] found enteral feedings to be safe and superior to parenteral nutritional support after esophagectomy, and Roy and DeMeester[5] have recommended jejunostomy tube feedings as an important aspect of supportive care to reduce postesophagectomy mortality. Based on their experience with 523 esophagectomy patients, Gerndt and Orringer[6] have advocated tube jejunostomy as an adjunct to esophagectomy for both benign and malignant pathology. Our experience confirms the experience of others demonstrating the utility of surgically placed jejunostomy tubes in patients undergoing esophagectomy. In addition to the theoretical benefits of routine *supplemental* nutritional support, adjunctive jejunostomy provides enteral access in the event that a postesophagectomy patient is unable to tolerate oral alimentation because of anastomotic leakage, aspiration, anorexia, postoperative complications resulting in endotracheal intubation, or recurrent cancer. In these patients, nutritional support is essential and potentially lifesaving. Options for postesophagectomy enteral access include surgically placed jejunosotomy and nasojejunal tubes. There are advocates for both methods. Comparison of the two techniques with regard to cost, replacement rates, occlusion incidence, success for long-term use, and patient satisfaction has not been studied prospectively. Our bias has been to use surgical jejunostomy tubes as an adjunct to esophagectomy.

The safety of surgically placed jejunostomy tubes in esophagogastrectomy patients is well established.[4-7] Gerndt and Orringer[6] reported major complications in only 11 (2.1%) of 523 patients. Complications included small bowel obstruction, intubation site abscess, bowel leakage, and catheter dislodgment. The prevalence of complications was similar for patients with benign and malignant disease. There were no deaths in their large series.

Although the data support the use of adjunctive jejunostomy, recommendations for the timing of feeding tube removal are not well established. We have found that esophagectomy patients are usually anxious to have all tubes removed as soon as possible following surgery. On the other hand, once the jejunostomy tube is removed, the tract rapidly closes and ready enteral access is lost. We have previously reported a surgical technique in which the jejunal intubation site is marked radiographically for percutaneous replacement jejunostomy. The technique is illustrated in Fig. 1. A replacement jejunostomy tube may be inserted at any time after esophagogastrectomy under fluoroscopic radiographic guidance. The procedure does not require endoscopy or general anesthesia and may be repeated. Because this technique permits feeding tube replacement, our postesophagectomy guidelines[8] call for removal of the jejunostomy tube prior to discharge in patients without complications.

In this series, 17 (4.9%) of 350 esophagogastrectomy patients required replacement jejunostomy. Only 1% (4 patients) of the jejunostomy tubes were replaced in the first postoperative month. Therefore a policy of leaving all surgically placed jejunostomy

tubes in patients as a prophylactic measure, even for 1 month, would not replace the need for this technique and would needlessly subject 99% of patients to prolonged feeding tube care. We believe that these results support our patient care pathway in which the original jejunostomy tube is removed prior to discharge in patients without complications. Our data do not allow us to predict who will need early jejunostomy replacement.

The timing of feeding tube replacement following esophagogastrectomy was predictive of the indication. Prior to 16 weeks, the indication for feeding tube replacement was intubation and inability to eat (1 patient) and anorexia with weight loss and dehydration (7 patients). At or after 16 weeks, the indications were all related to symptoms from recurrent carcinoma.

The technique of percutaneous jejunostomy is safe and effective. In this series the feeding tube was replaced successfully in all patients without complications. It has been our practice to observe patients overnight before restarting enteral feedings and discharging patients from the hospital. With greater experience it may be possible to replace the feeding tube on an outpatient basis.

Other techniques for jejunostomy tube replacement have been reported including direct percutaneous insertion,[9-11] percutaneous endoscopic jejunostomy,[12-14] and laparoscopic jejunostomy.[15-17] Reichle et al.[2] have reported that attempting replacement jejunostomy without the marking hemoclips is problematic. They found that the healed jejunostomy skin mark is not a reliable marker for localization of the fixed jejunal loop. In one of their patients, the skin mark and jejunal loop were several centimeters apart. In addition, without the hemoclips it can be difficult and time consuming to identify the direction of the distal bowel. The other two techniques require endoscopy or general anesthesia, respectively, which increases the time, cost, and complexity compared with the approach described in this report. We believe that the safety and effectiveness of the percutaneous approach described in this report make it superior to other options for feeding tube replacement.

In summary, the technique of percutaneous jejunostomy allows the surgeon tremendous flexibility in the management of the postesophagogastrectomy patient as it preserves the advantages of an adjuvant surgically placed feeding tube over the lifetime of the patient. The technique is safe, and the success rate is excellent.

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Management of Esophageal Perforation After Pneumatic Dilation for Achalasia

David R. Hunt, M.D., Vanessa L. Wills, M.B.B.S., Beatrix Weiss, M.B.B.S., John O. Jorgensen, M.S., David J. DeCarle, M.B.B.S., Ian J. Cook, M.D.

Current management of esophageal perforation after pneumatic dilation for achalasia is thoracotomy and repair with myotomy. This study aims to assess the outcome of patients managed by laparotomy, and the role of laparoscopic repair. The study was carried out by means of retrospective case review and prospective follow-up with a symptom questionnaire. Results were compared with results in patients undergoing elective Heller myotomy. Over a 20-year period, 445 dilations for achalasia were performed in 371 patients. There were 10 esophageal perforations. Nine patients were referred for surgery and were successfully managed with a transabdominal repair. Laparoscopic repair was attempted in four patients but was successful in only one because of the perforation site. After a mean follow-up of 5.4 years, grade 1 or 2 Visick scores were recorded in all patients. Residual symptoms of dysphagia occurred in 67% in the emergency group and 88% in the elective group. There was an increased incidence of heartburn compared to elective myotomy. Early operation after perforation provides good results for treatment of achalasia. Mild dysphagia persists and there is an increasing sensation of heartburn. The site of perforation is typically posterolateral, which makes laparoscopic repair difficult. (J GASTROINTEST SURG 2000;4:411-415.)

KEY WORDS: Achalasia, laparoscopy, myotomy, perforation, pneumatic dilation

Pneumatic esophageal dilation for achalasia results in esophageal perforation in 2% to 6% of procedures.[1] Although esophageal perforation can be treated conservatively,[2] immediate operation reliably provides good short- and long-term results,[3-6] whereas failed conservative management results in a sick patient and a difficult salvage surgical procedure. Most perforations have been approached through a left thoracotomy[3-6] and have been managed by primary repair and a longitudinal myotomy on the uninjured side, with or without fundoplication. Elective myotomy for achalasia is being done increasingly through an abdominal approach with laparoscopy.[7,8] This study presents the results and long-term follow-up from a series of patients referred for surgical management after sustaining esophageal perforation from pneumatic dilation for achalasia. All patients were managed with an abdominal approach to the esophageal perforation, and more recently laparoscopic repair of the perforation has been attempted. The aims were to assess the outcome after an abdominal approach to the esophageal perforations and the role of laparoscopy in the repair of these perforations. Long-term outcome was also compared to that in patients undergoing elective laparoscopic myotomy and fundoplication.

MATERIAL AND METHODS

Since 1980, a total of 445 pneumatic dilations for achalasia have been done in 371 patients. Since 1990, dilation has been performed with a Microvasive Rigiflex pneumatic balloon (Boston Scientific Corp., Boston, Mass.). The balloon diameter used for the first dilation was 30 or 35 mm. For repeat dilations either a 35 or 40 mm balloon was used. Before 1990, a Rider-Moeller pneumatic bag was used. After gastroscopy and placement of a guidewire, the balloon is positioned with the aid of fluoroscopy and inflated to a pressure of 350 mm Hg for 15 seconds. Until 1993, a routine chest x-ray examination was ordered after

From the Departments of Upper Gastrointestinal Surgery (D.R.H., V.L.W., B.W., and J.O.J.) and Gastroenterology (D.J.D. and I.J.C.), St. George Hospital, Sydney, Australia.
Reprint requests: Dr. D. Hunt, St. George Upper Gastrointestinal Surgical Unit, Level 5, Suite 1, St. George Private Medical Centre, 1 South St., Kogarah 2217, Sydney, Australia.

the procedure, and a contrast study was done only for clinical suspicion of a leak. Patients were observed overnight. Because of a missed perforation in 1993, a Gastrografin swallow is now performed routinely and patients are discharged home after 4 hours if they are well.

Patients with a Gastrografin swallow showing evidence of an extramural leak were referred for early surgery. Initially laparotomy was performed with esophageal mobilization, esophageal repair, and fundoplication. The esophagus was repaired by closure of the mucosa-submucosa with absorbable sutures. A longitudinal myotomy was done by extending the muscle tear, both proximally and distally, onto the upper centimeter of stomach. The type of fundoplication depended on the site of the tear and was used to cover the mucosal repair if possible. In four recent cases, repair, myotomy, and fundoplication were attempted laparoscopically.

Patients were followed up prospectively using a standard questionnaire designed to measure postoperative symptoms. The intensity of dysphagia, regurgitation, heartburn, and chest pain were each scored from 0 to 3. The frequency of these symptoms was also assessed and the score for intensity and frequency multiplied for each symptom to give a score out of 9.[9] Therefore a score of 0 indicates no symptoms and a score of 9 indicates severe symptoms occurring daily. Patient satisfaction following the procedure was also assessed by a modified Visick score. Patient satisfaction was graded on a scale of 1 to 4 as follows: 1 = patient considered the operation a complete success; 2 = much improvement but some residual problems; 3 = minor improvement but major residual problems; and 4 = no improvement or worse than preoperatively.

Postoperative symptoms were compared with those in 43 patients who had undergone elective Heller myotomy and who had been followed up for at least 12 months postoperatively. Prospective follow-up data had been collected from these patients since 1992. Almost all of these patients underwent laparoscopic Heller myotomy and Nissen fundoplication. Inferences regarding differences between symptom scores of patients in the emergency and elective groups were made by means of the Wilcoxon rank-sum test with consideration given to the small sample size in the group with perforation. Comparison of the incidence of symptoms between the groups was done using a two-tailed Fisher's exact test.

RESULTS

Ten patients sustained esophageal perforations after pneumatic dilation for achalasia between 1980 and 1999. During this period, 445 esophageal dilations for achalasia were performed for a perforation incidence of 2.2%. Prior to routine Gastrografin swallow, one male patient was discharged and was seen at another hospital with a perforation; this patient subsequently died of mediastinitis. This study reports the results in the nine patients referred to the surgical unit. There were three male and six female patients who had a mean age of 53.4 years (range 36 to 76 years). The perforation occurred on the first dilation in four patients, the second in four patients, and the third in one patient. Symptoms of perforation included chest pain in six patients, epigastric pain in three patients, and pain at both sites in one patient. With the exception of one patient, all patients reported symptoms at the completion of the procedure. Gastrografin swallow tests were performed in all patients, and eight of nine were positive for a leak. The patient whose scan was normal had a small perforation and was operated on because of worsening clinical signs. Accurate localization of the site of the leak (i.e., anterior, posterior, or lateral) occurred in only five of eight studies. Eight patients were taken for operation as soon as possible after the diagnosis. The mean time to operation for these patients was 11.8 hours. The exception was an elderly patient with coexisting cardiac disease. Conservative treatment was attempted after Gastrografin swallow revealed a small, contained mediastinal collection. A repeat swallow test at 48 hours showed a persistent leak with a larger collection, and it was decided to abandon nonoperative treatment.

Details of the operative findings and type of operation performed are shown in Table I. All perforations were situated in the lower third of the esophagus, usually just proximal to the hiatus. It was thought that the dilation had completely disrupted the muscle across the lower esophageal sphincter in only two cases. The type of fundoplication performed varied according to the site of the perforation. The first patient had a long fundoplication as was standard practice in 1981, but subsequent Nissen fundoplications have been short and floppy and have involved division of the short gastric vessels. All patients had an abdominal drain placed at the site of the mediastinal collection.

Laparoscopic repair was attempted in four patients; however, it was successful in only one patient with an anterior perforation. The other patients required conversion to laparotomy after laparoscopic mobilization of the short gastric vessels and the esophagus because the site of the perforation could not be adequately visualized.

Postoperatively two patients had possible small, contained leaks on Gastrografin swallow, although there was no evidence of sepsis or drainage from the mediastinum. Repeat swallow test 1 week later showed no evidence of a leak. Two patients also had pleural ef-

Table I. Operative findings

Operation	Free vs. contained	Site of leak	Length (cm)	Laparoscopy attempted	Type of fundoplication	Laparoscopy successful
1	C	Posterior	6.0	No	Long Nissen	
2	Free (pleura)	Anterior	2.0	No	Short Nissen	
3	C	Anterior	2.0	No	Anterior	
4	C	Left lateral	2.5	Yes	Toupet	No
5	C	Anterior	0.7	No	Anterior	
6	C	Posterior	2.0	Yes	Short Nissen	No
7	C	Anterior	2.5	Yes	Short Nissen	Yes
8	C	Left lateral	2.5	Yes	Short Nissen	No
9	C	Left lateral	0.2	No	Anterior	

C = contained mediastinal collection.

Table II. Comparison of outcome in patients operated electively

	Perforated	Elective	*P* value
N	9	43	
Mean age ± SD (yr)	53.4 ± 10.7	46.4 ± 19.4	NS
Mean follow-up (yr)	5.4	2.3	
Mean score ± SEM			
Dysphagia	1.8 ± 2	2.8 ± 2.3	NS
Heartburn	1.7 ± 1.1	1.6 ± 2.5	NS
% With symptoms			
Dysphagia	67	88	0.046
Heartburn	78	44	0.001
Chest pain	44	42	NS
Regurgitation	22	35	NS
Severe dysphagia (score >5)	1/9	5/43	NS
Visick grade 1 or 2	9/9	25/31	NS
Medication for reflux	4/9	10/43	NS

SEM = standard error of the mean; SD = standard deviation; NS = not significant.

fusions requiring chest drainage. One patient had a peroneal vein thrombosis. The mean postoperative length of stay was 14 days (range 10 to 25 days). There were no postoperative deaths. No patient has required further surgery or endoscopic intervention for esophageal symptoms.

Patients have been followed for a mean of 5.4 years. Three patients report complete satisfaction after the procedure with the remainder reporting significant improvement in symptoms but some residual symptoms including mild dysphagia (6 patients) and heartburn (7 patients). Four patients are currently taking H_2 antagonists with relief of heartburn symptoms, although objective evidence of reflux with pH studies or endoscopy was not obtained. A comparison of frequency of symptoms, and symptom scores, with patients undergoing elective laparoscopic Heller myotomy with follow-up of 1 year or more, is presented in Table II. Although a high proportion of both groups report postoperative symptoms, these are minor and are significantly improved from preoperative symptoms. The mean preoperative dysphagia score for all patients was 7.3, which is significantly different from the postoperative score ($P < 0.001$). The mean preoperative score for heartburn was 1.5, which is no different from the postoperative score ($P = 0.3$).

DISCUSSION

This series demonstrates a good outcome in the nine patients who underwent urgent surgical intervention after sustaining esophageal perforations from pneumatic dilation for achalasia. Esophageal repair, myotomy, and fundoplication were all performed transabdominally demonstrating the validity of this approach in the emergency situation. With a large experience of elective laparoscopic Heller myotomy and antireflux surgery, laparoscopic repair was attempted but was successful in only one of four procedures. The major reason for the failure of this approach was the location of the esophageal perforation. Posterior and lateral perforations could not be adequately visualized despite esophageal mobilization. An anterior perforation was successfully repaired laparoscopically. Two cases of laparoscopic repair are reported in the literature.[10] The site of perforation in these cases was posterior and lateral. The use of a 30-degree endoscope, and ultrasonic shears, which have been recently adopted by our unit, enhances mobility and visibility significantly. These technical modifications may have permitted laparoscopic repair in the cases previously converted.

Although four of nine patients in this series had anterior perforations, this could not be reliably predicted from the preoperative Gastrografin swallow. It may be worthwhile for surgeons with a large esopha-

geal laparoscopic experience to perform preliminary laparoscopy and mobilization. In cases of high posterior perforation, the procedure may require conversion to an open operation so that the esophagus can be rotated and the defect closed accurately. Two cases have been reported where the perforation was repaired at thoracoscopy, although fundoplication and myotomy were not performed.[11]

The perforation is most commonly thought to occur at the left lateral site,[4,12] similar to that which occurs with spontaneous esophageal rupture because of the attenuation of muscular fibers in this region.[13] As shown in this series, the length of the tear is usually less than 6 cm.[6] Tears have also occurred in the neck[14,15] and midesophagus[14]; therefore results of post-dilation Gastrografin swallow should be carefully examined. These extensive and multiple tears have been reported following dilation with high-compliance pneumatic bags, which apply greater force to the proximal esophagus.[16] Low-compliance balloon dilators, such as the Rigiflex dilator used for most cases in this series, maintain their shape and rupture longitudinally in the event of excessive inflation, limiting their force to the site of narrowing.

Nonoperative management has been used following perforation. Cases may present with radiographic findings of a small leak in the absence of symptoms.[15] This may sometimes be a mucosal outpouching or dissection in the esophageal wall rather than a contained leak.[2] Closure of a small perforation within 2 hours of injury with endoscopic clips has been reported,[17] and conservative management consisting of nothing by mouth, parenteral nutrition, and intravenous antibiotics has been reported in a small number of cases.[2,18-20] None of these patients is reported to have required subsequent myotomy or dilation for recurrent symptoms, but the longest follow-up available in these series is 4 years,[20] with most patients followed up for less than 1 year.[2,17-20] The safety of repeat dilation after conservative management of a mediastinal leak has not been demonstrated, and surgical myotomy would likely be technically challenging.

It has been suggested that myotomy is not necessary during operative repair of a perforation[15] because a sufficiently long myotomy has been achieved with the balloon dilation and perforation, therefore closure of the mucosal-submucosal defect is adequate. None of the small number of patients treated without a myotomy has required a subsequent procedure,[15] although once again the follow-up was limited[15] or results not available.[21] Pricolo et al.[15] report that all of the perforations in their series were across the lower esophageal sphincter. This is in contrast to the findings in our series where the perforation seldom extended to below the hiatus. If myotomy is not done, there is a risk of an unrelieved distal obstruction, potentially complicating healing of the esophageal repair.[22] Some surgeons also prefer to close both muscle and mucosa at the site of the perforation,[4] in which case contralateral myotomy is necessary.

The need for a fundoplication after esophageal myotomy is an issue that has been debated in the literature regarding elective surgery. Most surgeons who use an abdominal approach routinely perform a fundoplication to prevent the postoperative gastroesophageal reflux that occurs in up to 22% of cases.[1] The choice of which antireflux procedure was used in this series depended on the site of the perforation, with the fundus used as a patch for the esophageal repair. Although Nissen fundoplication is used after elective myotomy, partial fundoplication was used in four of nine emergency cases. The effect of different methods of partial fundoplication, performed after elective myotomy, was compared by Raiser et al.[23] in a retrospective, nonrandomized study. Dor (anterior) fundoplication and Toupet (posterior) fundoplication were compared. Complaints of "reflux" were experienced after anterior fundoplication in 57% of patients compared to 27% after the posterior fundoplication. However, pH testing failed to show any pathologic reflux in either group. Other investigators have reported good long-term results after myotomy and Dor fundoplication with only an 8.6% incidence of reflux on pH testing.[24] The association of anterior fundoplication with postoperative symptoms of heartburn may partly explain the higher rate of clinical heartburn seen in the patients undergoing emergency repair in this series, as anterior fundoplication was used more frequently in this group. The higher incidence of dysphagia demonstrated in the patients undergoing elective myotomy may reflect the use of Nissen fundoplication in the elective group as it is suggested that Nissen fundoplication may contribute to worsening dysphagia in long-term follow-up.[25] However, the groups are not directly comparable because of differences in the underlying severity of achalasia prior to surgery with several patients in the elective group subsequently requiring esophagectomy for megaesophagus. The groups also had different durations of follow-up. Otherwise a comparison of long-term symptom scores shows minimal differences between the elective and perforated groups. Although many patients have residual symptoms, these are mild and usually improved from preoperative values. Other series examining patients undergoing elective or emergency procedures have also shown no difference in outcome.[3,6] A direct comparison with results of pneumatic dilation was not made in this study. Previous data from this unit assessed long-term symptoms after pneumatic dilation in 105 patients.[26] Mean fol-

low-up was 6 years. At follow-up 77% had dysphagia, 60% had regurgitation, and 61% had heartburn with 46% taking medication for this.

CONCLUSION

Patients having a perforation after pneumatic dilation for achalasia should expect a good outcome. They have been fasting and should have a residue-free esophagus prior to the dilation, thus minimizing septic complications, provided the perforation is recognized early. This series demonstrates that early operation with closure, myotomy, and fundoplication should provide an uncomplicated recovery for most patients. It also deals with the risk of failed dilation managed conservatively. Long-term results are comparable to results in patients undergoing elective procedure.

It is feasible to approach perforations in the distal third of the esophagus through the abdomen. Although laparoscopic repair is possible, the perforation must be adequately visualized. If the perforation is posterior, an accurate repair may not be possible laparoscopically.

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Prevention of Mucosal Atrophy: Role of Glutamine and Caspases in Apoptosis in Intestinal Epithelial Cells

Harry T. Papaconstantinou, M.D., Dai H. Chung, M.D., Weiping Zhang, M.D., Naseem H. Ansari, Ph.D., Mark R. Hellmich, Ph.D., Courtney M. Townsend, Jr., M.D., Tien C. Ko, M.D.

Glutamine starvation induces apoptosis in enterocytes; therefore glutamine is important in the maintenance of gut mucosal homeostasis. However, the molecular mechanisms are unknown. The caspase family of proteases constitutes the molecular machinery that drives apoptosis. Caspases are selectively activated in a stimulus-specific and tissue-specific fashion. The aims of this study were to (1) identify specific caspases activated by glutamine starvation and (2) determine whether a general caspase inhibitor blocks glutamine starvation–induced apoptosis in intestinal epithelial cells. Rat intestinal epithelial (RIE-1) cells were deprived of glutamine. Specific caspase activation was measured using fluorogenic substrate assay. Apoptosis was quantified by DNA fragmentation and Hoechst nuclear staining. Glutamine starvation of RIE-1 cells resulted in the time-dependent activation of caspases 3 (10 hours) and 2 (18 hours), and the induction of DNA fragmentation (12 hours). Caspases 1 and 8 remained inactive. ZVAD-fluoromethyl ketone, a general caspase inhibitor, completely blocked glutamine starvation–induced caspase activation, DNA fragmentation, and nuclear condensation. These results indicate that glutamine starvation selectively activates specific caspases, which leads to the induction of apoptosis in RIE-1 cells. Furthermore, inhibition of caspase activity blocked the induction of apoptosis, suggesting that caspases are potential molecular targets to attenuate apoptotic responses in the gut. (J GASTROINTEST SURG 2000;4:416-423.)

KEY WORDS: Glutamine, intestinal mucosa, apoptosis, caspases

Glutamine is the primary metabolic fuel for the enterocyte[1] and an important nutrient for gut mucosal integrity and health.[2,3] Clinical conditions including trauma, sepsis, and prolonged use of total parenteral nutrition are often associated with glutamine depletion and gut mucosal atrophy. Gut mucosal homeostasis is achieved through a balance between cell proliferation located in the crypts of Lieberkühn and cell elimination by apoptosis occurring in both the crypt and villus compartments.[4,5] The normal small bowel mucosa is a dynamic epithelium that renews itself every 3 to 8 days. A decrease in enterocyte proliferation and/or an increase in apoptosis would result in gut mucosal atrophy. We have reported that glutamine starvation induces apoptosis[6] and blocks mitogen-stimulated proliferation[7] in intestinal epithelial cells, suggesting that glutamine is an important regulator of gut mucosal homeostasis. The molecular mechanism by which glutamine starvation induces apoptosis is not known.

Apoptosis can be triggered by many stimuli, which then activate the apoptotic execution machinery.[8] Signals that induce apoptosis in the enterocyte include glutamine deprivation,[6] detachment from the villus,[9]

From the Departments of Surgery (H.T.P., D.H.C., M.R.H., C.M.T. and T.C.K.), Physiology (M.R.H.), and Human Biological Chemistry and Genetics (W.Z., N.H.A., and T.C.K.), The University of Texas Medical Branch, Galveston, Tex.

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Reprint requests: Tien C. Ko, M.D., Department of Surgery, The University of Texas Medical Branch, 301 University Blvd., Galveston, TX 77555-0542.

chemotherapeutic agents, and ionizing radiation.[10] These stimuli activate a family of *c*ysteine *asp*artate prote*ases* (caspases), which constitute the molecular machinery responsible for the execution phase of apoptosis.[11] Thirteen mammalian caspases have been identified and can be further classified into the following three subfamilies based on their sequence homology: the ICE subfamily (caspase 1, caspase 4, and caspase 5); the CPP32 subfamily (caspase 3, caspase 6, caspase 7, caspase 8, and caspase 10); and the ICH-1 subfamily (caspase 2 and caspase 9).[12,13] Not much is known about the newly discovered caspases 11, 12, and 13. Each caspase is synthesized and sequestered intracellularly as proenzymes, and these are activated by proteolytic cleavage.[11] Caspases transduce and augment the initial apoptotic signal by activating other caspases in a cascade fashion. Initiator caspases include caspase 8 (activated in the Fas-mediated pathway) and caspase 9 (activated by cytochrome c redistribution to the cytoplasm), and function to amplify the initial stimulus by activating downstream effector caspases (i.e., caspases 1, 2, and 3).[8,11,14] Activated effector caspases cleave key substrates that result in cell cycle arrest, impaired DNA repair mechanisms, detachment from surrounding cells, disassembly of structural components of the nuclear and cytoplasmic membrane, and DNA fragmentation.[8,11] Therefore activated caspases are responsible for the biochemical and morphologic characteristics of apoptotic cells including DNA fragmentation and nuclear condensation. The central role of caspases in the apoptotic process may indicate that these proteases are potential therapeutic targets to attenuate apoptotic responses to a variety of stimuli. General caspase inhibitors prevent apoptosis in myocardiocytes following ischemia/reperfusion injury[15] and in lymphoid cells following irradiation or staurosporin treatment.[16] The specific caspase cascade activated by glutamine starvation and whether caspase inhibitors can block glutamine starvation–induced apoptosis in intestinal epithelial cells is not known.

The purpose of this study was to identify specific caspases activated by glutamine starvation in intestinal epithelial cells, and to determine whether inhibition of caspase activation prevents apoptosis. Rat intestinal epithelial (RIE-1) cells were used because they are an established, well-characterized, nontumorigenic intestinal epithelial cell line[17] and are a useful in vitro model to study the effects of growth factors and nutrients on cell proliferation[5] and apoptosis[6] in the gut. We showed that glutamine starvation resulted in the time-dependent selective activation of caspases 2 and 3. Furthermore, inhibition of caspase activation blocked glutamine starvation–induced apoptosis in RIE-1 cells.

MATERIAL AND METHODS

Tissue Culture

RIE-1 cells were a generous gift from Dr. Kenneth D. Brown (Cambridge Research Station, Babraham, Cambridge, U.K.). Cells were maintained as monolayer cultures in glutamine-free Dulbecco's modified Eagle medium (DMEM; Mediatech Inc., Herndon, Va.) supplemented with 1 mmol/L glutamine (Life Technologies, Grand Island, N.Y.) and 5% dialyzed fetal bovine serum (Life Technologies), and were incubated at 37°C in a humidified atmosphere containing 5% carbon dioxide. All experiments were performed on cells between passages 10 and 24. Cells were seeded at 4500 to 9000 cells/cm^2 in 150 mm plates for caspase activity assay, Western blot analysis, and Cell Death Detection^{PLUS} assay (CDD+; Boehringer-Mannheim, Mannheim, Germany) or 100 mm plates for Hoechst nuclear staining. Twenty-four hours after plating, cells were washed twice with Dulbecco's phosphate-buffered saline (Mediatech Inc.), incubated with fresh serum containing DMEM with or without 1 mmol/L glutamine, and simultaneously treated every 12 hours with 0 to 100 μmol/L ZVAD-fluoromethyl ketone (ZVAD-FMK; Bachem, Torrance, Calif.), a general caspase inhibitor.

Caspase Activity Assay

Specific caspase activation was determined from cytoplasmic cell lysates, which were isolated as described by Sarin et al.[18] with modifications. Briefly, cells were collected by trypsinization, and the total cell number was determined by means of a model Zf Coulter counter (Coulter Electronics, Hialeah, Fla.). Cells were incubated for 20 minutes at 4° C in ICE lysis buffer (1×10^6 cells in 200 μl of 50 mmol/L Hepes buffer, pH 7.5, 10% sucrose, and 0.1% Triton X-100). After centrifugation at 10,000 *g* for 10 minutes at 4° C, supernate was collected and dithiothreitol (10 mmol/L) was added. Caspase-specific fluorogenic substrates (amino-trifluoromethylcoumarin [AFC]) for caspase 1 (Ac-YVAD-AFC), caspase 2 (Ac-VDVAD-AFC), caspase 3 (Ac-DEVD-AFC), and caspase 8 (Ac-IETD-AFC) were purchased from Enzyme Systems Products (Livermore, Calif.). Fluoromethyl ketone protease inhibitors for caspase 2 (Z-VDVAD-FMK), caspase 3 (Z-DEVD-FMK), and caspase 8 (Z-IETD-FMK) were also purchased from Enzyme Systems Products, whereas caspase 1 inhibitor (Z-YVAD-FMK) and a general caspase inhibitor (ZVAD-FMK) were purchased from Bachem. Total caspase activity was determined for each sample using 100 μl of cell lysate incubated with caspase-specific fluorogenic substrates at a final concentration of 50 μmol/L for 1 hour at room temperature. Reaction

was stopped by diluting with 900 μl of phosphate-buffered saline solution. Nonspecific caspase activity was determined by preincubating cell lysate with caspase-specific substrate inhibitor at a final concentration of 5 to 20 μmol/L for 15 minutes before the addition of caspase-specific substrate. Sample fluorescence was measured at excitation 400 nm and emission 505 nm using the F-4500 fluorescence spectrophotometer (Hitachi Instruments Inc., San Jose, Calif.). Fluorescence was converted to concentration of free AFC (μmol/L) using a standard curve of known concentrations (0 to 2 mmol/L AFC). Specific caspase activation was determined by subtracting nonspecific caspase activity from total activity for each caspase.

DNA Fragmentation Assay

DNA fragmentation was quantified by the CDD+ assay, which is based on an enzyme-linked immunosorbent assay (ELISA) using antibodies directed against DNA-associated nucleosomes. Cells were collected, lysed, and analyzed for DNA fragmentation as per manufacturer's protocol. Light absorbance was measured at 405 to 490 nm on the E_{MAX} precision microplate reader (Molecular Devices Corp., Sunnyvale, Calif.). Light absorbance from cell-free lysis buffer was used as background and subtracted from each group.

Western Blot Analysis

Cells were harvested, then lysed in NP-40 lysis buffer (50 mmol/L Tris-HCl, pH 7.4, 150 mmol/L NaCl, and 0.5% NP-40), as previously described.[19] Protein extracts (50 μg) were separated by the Nu-PAGE Bis-Tris gel electrophoresis system (Novex, San Diego, Calif.) and electrotransferred to PVDF membrane (Bio-Rad, Hercules, Calif.). The membrane was blocked with 5% milk in 0.05% Tween-20 in tris-balanced saline (150 mmol/L Tris, 10 mmol/L NaCl, and pH 7.4) overnight and incubated for 2 hours at room temperature with indicated primary antibody. Anti-caspase 1 and anti-caspase 8 monoclonal antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, Calif.). The membranes were washed three times with 0.05% Tween-20–tris-buffered saline and then incubated for 2 hours with appropriate horseradish peroxidase–conjugated secondary antibody (Santa Cruz Biotechnology). Membranes were washed and incubated with chemiluminescent substrate (Amersham Life Science, Arlington Heights, Il.). The membranes were then exposed to film (Eastman Kodak Company, Rochester, N.Y.) and visualized on the Lynx Densitometry System (Applied Imaging, Santa Clara, Calif.).

Hoechst Staining

Cells were fixed and stained with Hoechst 33258 stain as previously described,[6] and examined under a fluorescence microscope at 365 nm. Photographs were obtained with Kodak Ektachrome color slide film. The percentage of apoptotic cells was determined by counting at least 1000 cells per plate in triplicate.

Statistical Analysis

Data are represented as mean ± standard error of the mean (SEM). One-way analysis of variance was used with the Tukey multiple-comparison test, and the differences were considered significant at $P < 0.01$. Each experiment was repeated two to three times with similar results.

RESULTS

Selective Activation of Caspases by Glutamine Starvation in RIE-1 Cells

Caspases are the molecular machinery that drives apoptosis, and are responsible for the morphologic and biochemical characteristics of apoptotic cells.[8,11] Activation of specific caspases is dependent on tissue type and apoptotic stimulus. We have reported that glutamine starvation induces apoptosis in intestinal epithelial cells by measuring an increase in DNA fragmentation and nuclear condensation.[6] To investigate specific caspase activation and the relationship to DNA fragmentation in glutamine-starved intestinal epithelial cells, RIE-1 cells were treated for 0 to 24 hours with or without 1 mmol/L glutamine. Caspase activity was determined by caspase-specific fluorogenic substrate assay. Because of the complexity of caspase cascades, we examined the activities of caspases 1, 2, 3, and 8 because they represent major caspases of each subfamily and key components of specific apoptotic caspase cascades. Glutamine starvation resulted in the activation of caspase 3 starting at 10 hours, with maximum activation at 18 hours (Fig. 1, *A*). Caspase 2 was subsequently activated starting at 18 hours (Fig. 1, *B*). Glutamine starvation did not affect caspase 1 and caspase 8 activities up to 24 hours (Fig. 1, *C* and *D*). The induction of DNA fragmentation started at 12 hours after glutamine withdrawal, reaching maximal induction at 18 hours (Fig. 1, *E*).

Caspases 1 and 8 are expressed in human intestinal epithelium and lymphoid tissue.[20-22] Since caspases 1 and 8 are not activated by glutamine starvation in RIE-1 cells, we determined whether these caspases are expressed in their inactive procaspase forms by Western blot analysis. A lymphoid cell line, Jurkat, served as a positive control. Fig. 2 shows that RIE-1 and Jurkat cells express both procaspases 1 and 8. These data suggest that execution of glutamine starvation–induced

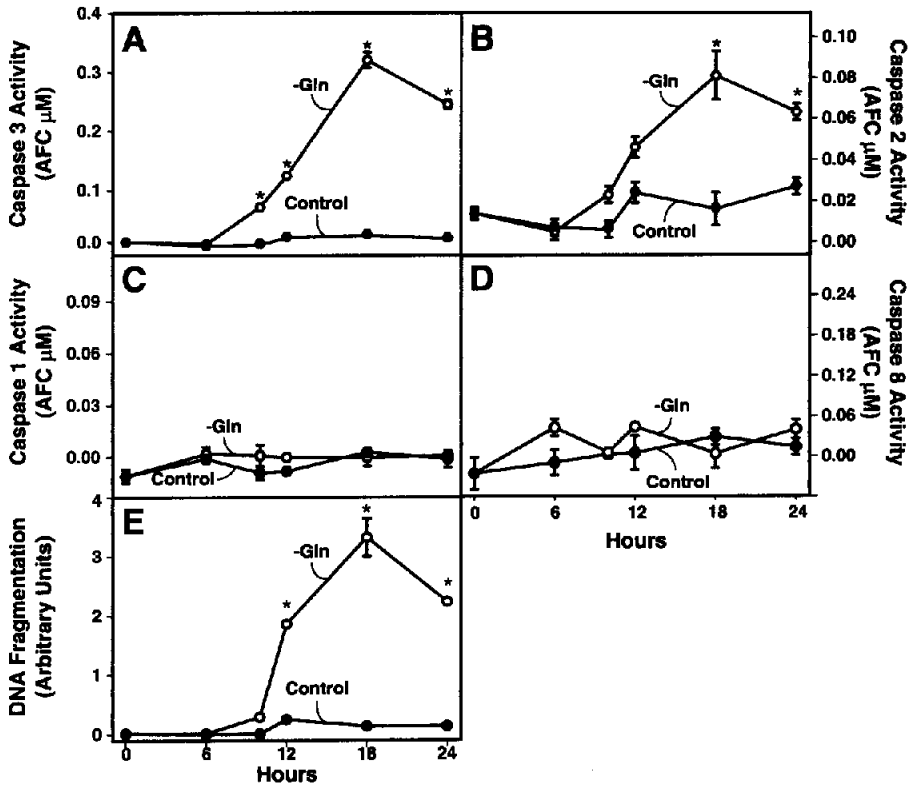

Fig. 1. Time course of caspase activation and DNA fragmentation in glutamine-starved RIE-1 cells. Cells were incubated for 0 to 24 hours with or without 1 mmol/L glutamine *(Gln)*. Cell lysates were added to caspase-specific fluorogenic substrates and analyzed on a fluorometer for activities of caspase 3 **(A)**, caspase 2 **(B)**, caspase 1 **(C)**, and caspase 8 **(D)**. DNA fragmentation was quantified by CDD+ ELISA assay **(E)**. All results are expressed as mean ± SEM. (* = $P < 0.01$ vs. control; n = 3.)

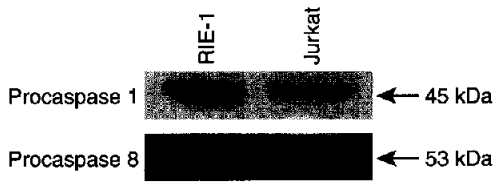

Fig. 2. Procaspases 1 and 8 expression in RIE-1 cells. Total protein isolates from untreated RIE-1 and Jurkat cells were analyzed for procaspase 1 (45 kDa) and procaspase 8 (53 kDa) expression by Western blot analysis. Jurkat cells served as a positive control.

apoptosis in RIE-1 cells is mediated by the activation of specific caspases and that the caspase cascade activated by glutamine starvation is selective.

Caspase Activation Is Required for Glutamine Starvation–Induced Apoptosis in RIE-1 Cells

The central role of caspases in apoptosis suggests that they are potential therapeutic targets to attenuate apoptotic responses to a variety of stimuli. To determine whether caspase inhibitors could block glutamine starvation–induced apoptosis, RIE-1 cells were incubated for 24 hours with or without 1 mmol/L glutamine and simultaneously treated every 12 hours with 0 to 100 μmol/L ZVAD-FMK. We found that ZVAD-FMK blocked glutamine starvation–induced DNA fragmentation in a dose-dependent manner, with maximum inhibition at 80 μmol/L (Fig. 3). Next we examined whether ZVAD-FMK blocked glutamine starvation–induced caspase activation, as well as nuclear condensation, a morphologic characteristic of apoptotic cells. Treatment of glutamine-starved cells with ZVAD-FMK (80 μmol/L) prevented activation of caspase 2 and caspase 3 (Fig. 4, *A* and *B*), and blocked DNA fragmentation (Fig. 4, *C*). To confirm these results, we determined the presence of nuclear condensation by Hoechst nuclear stain. As seen in Fig. 5, glutamine withdrawal resulted in an eightfold increase in nuclear condensation (white arrows) and a decrease in cell number per high-power field. Simultaneous treatment of glutamine-starved cells with ZVAD-FMK prevented nuclear condensation but did not reverse the decrease in cell number. Collectively these data indicate that inhibition of caspase activa-

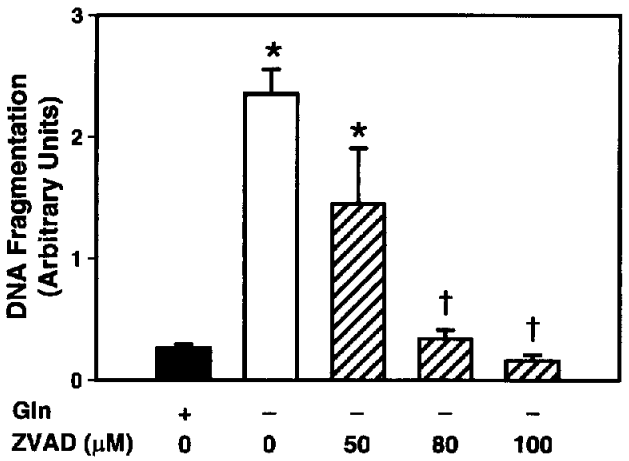

Fig. 3. Dose-dependent effects of a general caspase inhibitor on glutamine starvation–induced DNA fragmentation in RIE-1 cells. Cells were incubated for 24 hours with or without 1 mmol/L glutamine *(Gln)* and simultaneously treated with 0 to 100 μmol/L ZVAD-FMK every 12 hours. Cytoplasmic DNA was isolated, and DNA fragmentation was quantified by CDD + ELISA assay. Results are expressed as mean ± SEM. (* = P <0.01 vs. 1 mmol/L Gln alone [closed bar]; † = P <0.01 vs. −Gln alone [open bar]; n = 3.)

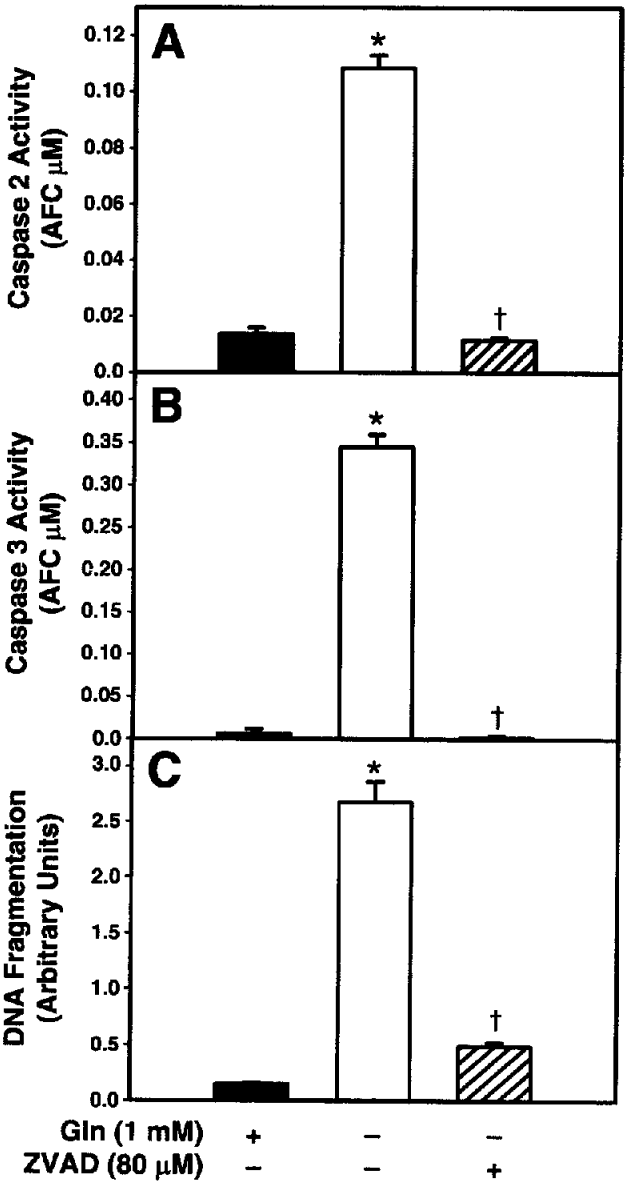

Fig. 4. Effect of ZVAD-FMK on glutamine starvation–induced caspase activation and DNA fragmentation. RIE-1 cells were incubated for 24 hours with or without 1 mmol/L glutamine *(Gln)*, and simultaneously treated with or without 80 μmol/L ZVAD-FMK every 12 hours. Cell lysates were added to caspase-specific fluorogenic substrates, and analyzed on a fluorometer for caspase 2 activity **(A)** and caspase 3 activity **(B)**. DNA fragmentation was quantified by CDD+ ELISA assay **(C)**. Results are expressed as mean ± SEM. (* = P <0.01 vs. +Gln [closed bar]; † = P <0.01 vs. −Gln [open bar]; n = 3.)

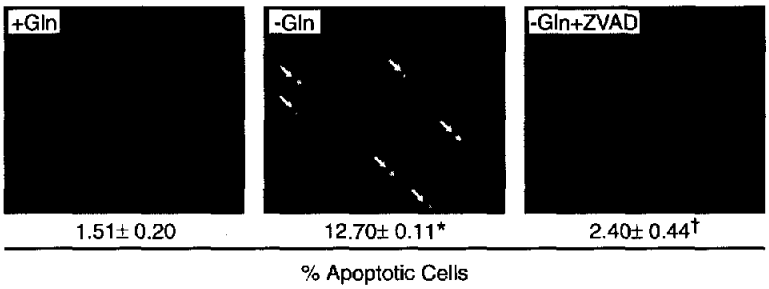

Fig. 5. Effect of ZVAD-FMK on glutamine starvation–induced nuclear condensation. RIE-1 cells were incubated for 24 hours with or without glutamine *(Gln)* and simultaneously treated with or without 80 μmol/L ZVAD-FMK every 12 hours. Cells were fixed, stained with Hoechst 33258 nuclear stain, and visualized under fluorescence microscope at 365 nm wavelength. Nuclei were photographed at 400× magnification. Arrows point to condensed nuclei characteristic of apoptosis. Percentage of apoptotic cells was determined by counting a minimum of 1000 cells per plate. Results are expressed as mean ± SEM. (* = $P < 0.01$ vs. +Gln; † = $P < 0.01$ vs. −Gln; n = 3.)

tion prevents glutamine starvation–induced apoptosis in RIE-1 cells.

DISCUSSION

We found that glutamine starvation in RIE-1 cells results in the sequential and selective activation of caspase 3 beginning at 10 hours and caspase 2 beginning at 18 hours. Activation of caspase 3 preceded the induction of DNA fragmentation, which was increased at 12 hours. Caspase 1 and caspase 8 were not activated, although their inactive precursors were expressed in RIE-1 cells. Inhibition of glutamine starvation–induced caspase activity using ZVAD-FMK blocked DNA fragmentation and nuclear condensation.

Clinical conditions resulting in glutamine-depleted states are associated with gut mucosal atrophy.[2,3] We have reported that glutamine starvation induces apoptosis in intestinal epithelial cells,[6] suggesting that an increase in apoptosis may be one of the mechanisms involved in gut atrophy that is associated with glutamine depletion. Different apoptotic signaling pathways activate the molecular execution machinery known as caspases, which are responsible for the morphologic and biochemical features of apoptotic cells.[8,11] Caspase activation depends on tissue type and apoptotic-inducing insult. Our studies indicate that glutamine starvation selectively activates specific caspases in intestinal epithelial cells. We showed that caspase 3 activation preceded both DNA fragmentation and caspase 2 activation. Caspase 3, a member of the CPP32 subfamily, is the most common caspase activated by various apoptotic stimuli. It is responsible for disabling many key substrates, including focal adhesion kinase, and for activating DNA fragmentation factor.[23] Histologic studies demonstrated expression of caspase 3 in the intestinal epithelium.[24] Caspase 3 is rapidly activated during detachment-induced cell death in enterocytes isolated from the human intestine.[9] The early activation of caspase 3 in glutamine starvation–induced apoptosis in RIE-1 cells indicates that caspase 3 is an upstream effector caspase and further suggests that caspase 3 modulates the induction of DNA fragmentation after glutamine depletion. Little is known about the substrates and function of caspase 2, a member of the ICH-1 subfamily. However, recent reports indicate that caspase 2 is activated by caspase 3 and that specific inhibition of caspase 2 did not prevent the induction of DNA fragmentation.[25] Our results indicate that caspase 2 activation occurs subsequent to activation of caspase 3, suggesting that caspase 2 may be a substrate of caspase 3 in glutamine starvation–induced apoptosis in RIE-1 cells.

We have shown that procaspases 1 and 8 are expressed (see Fig. 2) but not activated by glutamine starvation in RIE-1 cells (see Fig. 1). Both caspases are involved in Fas ligand–induced apoptosis.[11] Ligand binding to the Fas receptor activates caspase 8 through an intracellular adapter protein called the Fas-activated death domain.[26] Caspase 8 then activates downstream effector caspases including caspase 1. Fas receptor is expressed in the human intestinal epithelium, making the gut susceptible to Fas ligand–induced apoptosis.[20] Our data suggest that glutamine starvation uses a different caspase cascade to activate apoptosis other than that of the Fas-mediated apoptotic pathway.

It has been shown that caspase 3 activation in detachment-induced cell death in human intestinal epithelial cells occurs within 1 hour.[9] Others have shown that various stimuli, including ultraviolet irradiation and staurosporin treatment, result in the acti-

vation of caspase 3 as early as 3 hours.[16] In our studies we found caspase 3 to be activated at 10 hours after glutamine withdrawal. This delay in caspase 3 activation is likely due to the time required to deplete the intracellular glutamine pool until a threshold level is reached for activating the apoptotic signal. This is supported by our previous findings that glutamine starvation induces apoptosis in a dose-dependent fashion, with the induction of DNA fragmentation below 0.2 mmol/L.[6] In vivo, plasma glutamine concentrations range from 0.42 to 0.68 mmol/L.[3,27] In catabolic states, both intracellular and plasma concentrations of glutamine can decrease by 30% and are associated with gut mucosal atrophy.[3] Collectively these data suggest that there is a critical threshold of intracellular glutamine levels in enterocytes below which caspases are activated and apoptosis is induced.

The mitochondrion has been shown to play a key role in the induction of apoptosis.[11,14,16] Cytochrome c is a component of the electron transport chain located in the mitochondrial membrane and is an important factor for the synthesis of adenosine triphosphate. Cytochrome c is released into the cytoplasm in response to specific apoptotic stimuli and serves as an upstream signal leading to activation of caspase 9.[14] Glutamine is the primary metabolic fuel for the intestinal epithelium.[1] We speculate that glutamine starvation of enterocytes may cause mitochondrial damage, leading to the activation of caspase 9 through the cytochrome c–mediated pathway; however, further studies are necessary to determine whether caspase 9 or cytochrome c is involved in the apoptotic response to glutamine starvation.

Caspase inhibitors are known to attenuate the apoptotic response associated with ischemia/reperfusion injury in the heart[15] and with irradiation or staurosporin treatment in lymphoid cells.[16] We propose that caspase inhibitors may also block the apoptotic response associated with glutamine starvation in the gut. Considering the complexity of caspase cascades and our limited knowledge of the exact functions of each caspase activated by glutamine depletion in the gut, we chose a general caspase inhibitor, ZVAD-FMK, which inactivates all known caspases. Our studies indicate that caspase inhibition blocked glutamine starvation–induced apoptosis in intestinal epithelial cells as determined by DNA fragmentation and nuclear condensation. However, the caspase inhibitor did not prevent the decrease in cell number associated with glutamine starvation. Cell number is a reflection of cell proliferation and cell elimination by apoptosis, and glutamine starvation affects both processes.[6,7] Our current study suggests that caspases do not mediate the cell cycle arrest seen in glutamine-starved intestinal epithelial cells. Although proliferation may not be restored, our results suggest that ZVAD-FMK should protect the gut mucosa from the apoptotic response to glutamine depletion. Therefore caspase inhibitors may have therapeutic benefits in glutamine-depleted clinical conditions by maintaining gut epithelial integrity and gut barrier function, thereby protecting critically ill patients from malabsorption and gut-associated sepsis.

In summary, we have shown that glutamine starvation induces apoptosis through the selective activation of specific caspases. Caspase 2 and caspase 3 are activated in a time-dependent manner. Inhibition of caspase activation prevented glutamine starvation–induced apoptosis. These results indicate that glutamine is an important survival factor for the gut epithelium by suppressing caspase activation and suggest that caspases are potential therapeutic targets to attenuate apoptotic responses in the gut epithelium.

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Discussion

Dr. B. Warner (Cincinnati, Ohio). Is this an effect of nutrient withdrawal or is this specific for glutamine? Are there other factors that induce apoptotic cell death when they are not present in the medium? Second, can you induce apoptosis in this model with other mediators, and can it be prevented by feeding glutamine?

Dr. H. Papaconstantinou. We have looked at two different amino acid deprivations, one being glutamine starvation and the other cysteine starvation. Elimination of either of these amino acids induces apoptosis. Methionine starvation, in addition to the cysteine starvation, induces more of a necrotic picture. We have not looked at any other amino acids. We have examined apoptosis with methotrexate in these cells, and our preliminary data show that supplemental glutamine does not prevent apoptosis.

Dr. T. Gadacz (Augusta, Ga.). There is a time delay of 10 hours before you start seeing the effects. What do you think is going on in that interval?

Dr. Papaconstantinou. With glutamine withdrawal, a threshold level of glutamine must be achieved in order to induce apoptosis. Critically ill patients who are glutamine depleted have a decrease in glutamine to nearly 30% to 40% of normal circulating levels, so it is probably a time delay caused by that depletion.

Dr. J. Fischer (Cincinnati, Ohio). Glutamine may serve as a substrate, but it also serves as an energy source as you know. And I wonder whether what you are seeing is deprivation of energy or deprivation of protein substrate? This could be approached by using beta-hydroxybutyrate or acetoacetate which, at least in vitro, may substitute for the energy source for these cells. Have you tried either of those?

Dr. Papaconstantinou. We have not, but the medium that we use has a high glucose content. We are pursuing the role of glutamine as an energy source in the enterocyte, and we hypothesize that glutamine depletion may be detrimental to the mitochondria. The mitochondria are a central regulator of apoptosis and caspase activation.

Dr. R. Bell (Seattle, Wash.). I would like to ask a question about the strategy of using apoptosis inhibitors in a therapeutic manner. In the field in which I work, in cancer, we like cells to die; we do not like to keep them alive when they are damaged. Is it an appropriate therapeutic goal to keep cells alive that want to enter an apoptotic pathway? What evidence is there that doing so would result in well-functioning cells? For example, did you try adding back glutamine after 10 or 12 hours to see if that restored perfectly functional normal cells even after caspase levels had risen?

Dr. Papaconstantinou. Indeed, the last thing you would want to do in cancer patients is counterbalance the effects of chemotherapeutic agents on the neoplastic cells. But there have been studies that have shown that there are differences in caspase activation with the same stimuli according to tissue type. The key would be to identify specific upstream caspases that might be selectively activated in the noncancerous cells as compared to the tumor cells, and apply specific caspase inhibitors to protect the noncancerous cells. It would definitely be interesting to see if adding back glutamine at 10 hours would restore normal function.

Teflon Buttress Inhibits Recanalization of Uncut Stapled Bowel

William S. Richardson, M.D., Hadar Spivak, M.D., James E. Hudson, MED, Mark A. Budacz, MSEE, John G. Hunter, M.D.

The uncut Roux limb operation is designed to have the benefits of a Roux limb but still have electrical continuity from proximal to distal bowel, thus eliminating the risk of Roux stasis syndrome. The main complication has been recanalization of the uncut staple line leading to bile reflux. This study aims to employ a new technique, which will not allow recanalization of an uncut staple line but will not interfere with normal bowel myoelectric activity. Fourteen mongrel dogs, 25 to 35 kg, underwent a midline laparotomy under general anesthesia. An uncut staple line was placed 25 cm from the ligament of Treitz. In seven animals an uncut staple line alone was placed, and in the other seven animals the bowel was stapled between a sandwich of Teflon reinforcing strips such that the staples were held on both sides of the bowel by the Teflon. A jejunojejunostomy was placed 6 cm proximal to the staple line. Insulated bipolar electrical leads were placed around the staple line. After the electrical leads were monitored 2 days to 3 months postoperatively for bowel myoelectric activity, the animals were killed and the operative sites inspected. No animal suffered morbidity or mortality from the procedure. All seven unreinforced staple lines recanalized and all seven reinforced staple lines remained competent. The duodenal pacemaker potentials were transmitted through the staple line in five animals (3 controls and 2 with Teflon reinforcement) within 1 week postoperatively. The uncut staple line does not reliably transmit the duodenal pacemaker potentials. The staple line does not recanalize when it is reinforced with a permanent material, increasing the utility of the "uncut" Roux limb operation. (J GASTROINTEST SURG 2000;4:424-429.)

KEY WORDS: Roux stasis syndrome, postgastrectomy syndrome, Roux-en-Y, uncut Roux limb

The "uncut" Roux limb is a new operation designed to lower the rate of postgastrectomy Roux limb stasis. After gastric reconstruction, 20% of patients develop chronic abdominal complaints called postgastrectomy syndromes: early satiety, dumping, bile gastritis, afferent loop, efferent limb obstruction, gastric atony, postvagotomy diarrhea, anemia, and metabolic bone disease.[1] All reconstruction techniques lower the risk for certain postgastrectomy syndromes. The uncut Roux limb combines the best aspects of several of these operations but has been compromised by recanalization of the bowel through the uncut staple line allowing bile reflux.

After gastrectomy, in the Billroth I reconstruction the gastric remnant is anastomosed to the duodenum. The Billroth I procedure has the lowest morbidity of the possible reconstructions but is often impossible to perform because of the pathology. In Billroth II reconstruction with a Roux limb, the gastric remnant is anastomosed to a loop of jejunum. This operation is easier to perform in most cases but has a higher incidence of postgastrectomy syndromes such as alkaline reflux gastritis and dumping than does Billroth I reconstruction. The jejunum is bisected just past the ligament of Treitz. The free proximal end is anastomosed 40 cm distal to the free distal end, which is anastomosed to the gastric remnant. Roux-en-Y gastrojejunostomy has a lower rate of alkaline reflux than does Billroth II reconstruction, but up to 30% of patients develop the Roux stasis syndrome (postprandial

From the Department of Surgery (W.S.R.), Ochsner Clinic and Alton Ochsner Medical Foundation, New Orleans, La.; and the Department of Surgery (H.S., J.E.H., M.A.B., and J.G.H.), Emory University School of Medicine, Atlanta, Ga.
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Reprint requests: William S. Richardson, M.D., Ochsner Medical Institutions, 1514 Jefferson Highway, New Orleans, LA 70121.

nausea, vomiting, and abdominal pain)[2] because of slower gastric emptying and Roux limb stasis secondary to aberrant peristalsis in the Roux limb.[3] When the smal bowel is bisected, the distal end is disconnected from the duodenal pacemaker, and an ectopic pacemaker starts somewhere distal to the point of transection creating bidirectional pacemaking potentials from that point.

In the uncut Roux limb procedure, a loop of proximal jejunum is anastomosed to the gastric remnant (Fig. 1).[4] A jejunojejunostomy is created 45 cm distal to the gastrojejunostomy on the efferent limb of the loop. An uncut four-row staple line is placed just distal to the jejunojejunostomy on the afferent limb of the loop. Surprisingly, an uncut staple line allows transmission of normal duodenal pacemaker potentials, and gastric emptying is improved over simple Roux reconstruction.[5] The uncut Roux limb operation lowers the risk of stomal ulcers because the jejunum is used for continuity and keeps myoelectric continuity with the duodenal pacemaker through the uncut staple line, lowering the risk of Roux stasis. However, in a human study, 36% of patients had alkaline reflux gastritis or esophagitis in follow-up after gastrectomy and uncut Roux limb reconstruction. In all of these patients, recanalization through the uncut staple line was demonstrated.[6] In an animal study, all animals recanalized their uncut Roux limbs by 3 months.[7]

The bowel recanalizes because the staples migrate through the bowel wall and the mucosa heals. The aim of this study is to report a safe method for preventing recanalization of the uncut staple line that still allows propagation of duodenal pacemaker activity.

METHODS

Procedures and subsequent animal care were undertaken according to the guidelines of the Animal Care and Use Committee of Emory University Hospital. After 14 female mongrel dogs (25 to 35 kg) were given general anesthesia, an uncut staple line (TL4 55, Ethicon Endo-Surgery, Cincinnati, Ohio) fitted with a thick tissue cartridge (Proximate, TRT 55, Ethicon Endo-Surgery) was placed across the bowel 25 cm from the ligament of Treitz (Fig. 2). In seven animals, the stapler was covered with a Teflon product (Seamguard, W.L. Gore & Associates, Inc., Flagstaff, Ariz.), which fits on the stapler like pants legs and is 0.4 mm thick. Firing the stapler left a strip of Teflon stapled to both sides of the bowel, and the excess was stripped or cut away. In the other seven animals, this material was omitted. Six centimeters proximal and distal to the staple line, a two-layered jejunojejunos-

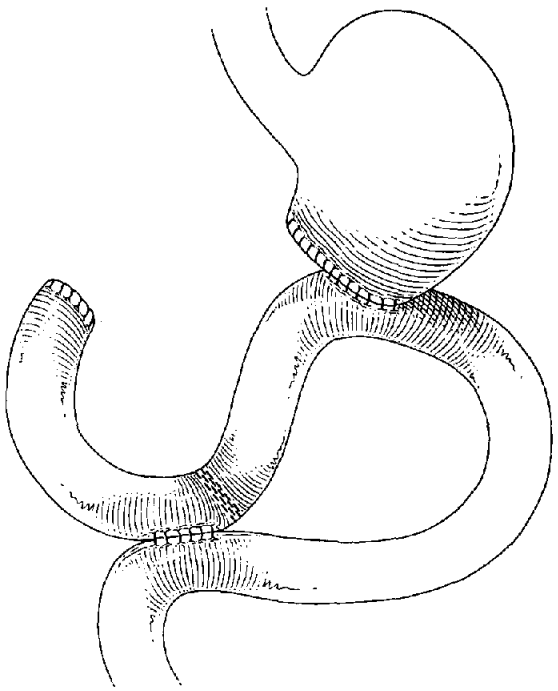

Fig. 1. Diagram of "Uncut" Roux limb reconstruction after partial gastrectomy showing loop gastrojejunostomy with proximal staple line and enteroenterostomy.

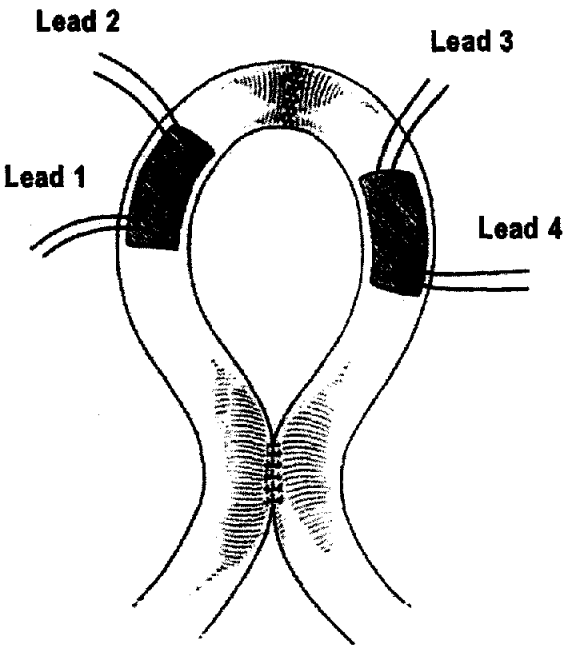

Fig. 2. Mock-up of the experiment. A staple line is placed on the loop of the small bowel with pacemaker leads 1 through 4 placed proximally and distally; the enteroenterostomy is just outside of the pacemaker leads.

Table I. Results

Animal No.	Complications	Conduction	Necropsy findings
Control group: Unreinforced staple line			
1	Insulation patch seroma	Aberrant, anad	Patent staple line
2	Insulation patch abscess	Aberrant, anad	Patent staple line
3	None	Normal	Patent staple line
4	None	Normal	Patent staple line
5	None	Normal	Patent staple line
6	None	Aberrant, orad	Patent staple line
7*	None	Aberrant, orad	Patent staple line
Experimental group: Teflon-reinforced staple line			
1	Fistula at insulation patch	Normal	Intact staple line
2	Insulation patch abscess	Normal	Intact staple line
3	Insulation patch abscess	Aberrant, anad	Intact staple line
4	None	Aberrant, anad	Intact staple line
5	None	Aberrant, anad	Intact staple line
6	None	Aberrant, anad	Intact staple line
7*	None	Aberrant, orad	Intact staple line

*Animal No. 7 in both the control and experimental groups were tested early on.

tomy was placed. Thus this procedure was performed on a loop of small bowel and no gastroenterostomy was performed. Four sets of bipolar electrical leads (TPW 32, Ethicon Endo-Surgery) were placed surrounding the staple line. They were 2 cm apart except over the staple line where they were 4 cm apart. The ends of the electrodes were placed through the serosa of the bowel and covered with a Teflon patch (Preclude Pericardial Membrane, W.L. Gore & Associates) to insulate them from adjacent loops of bowel except for the first animal, who had a Prolene patch (Ethicon Endo-Surgery) placed. The free ends were brought out through the flanks of the animals. The animals were fasted overnight and the electrical leads were monitored for bowel myoelectric activity using an amplifier and pen recorder (Linear Recorder Mark VII, Western Graphtec, Irvine, Calif.; Graphtec 2214 digital storage oscilloscope, Tektronix, Beaverton, Ore.) within a week of operation. All leads were cut below skin level after monitoring. Nine animals lost duodenal pacemaker conduction through the uncut staple line. At 1.5 months, two animals were killed because of time constraints. Three months postoperatively, the remaining seven animals underwent reoperation, and the electrical leads were replaced in the same positions proximal and distal to the staple line. This time the leads were brought out directly through the abdominal wall. Electrical activity was remonitored within 1 week of operation as previously described. The remaining 12 animals were killed 3 months after the initial operation. The operative sites were harvested from all 14 animals, and methylene blue solution was placed in the bowel to check for leaks through the staple line. The bowel was inspected for gross recanalization. The specimens were placed in formalin, and hematoxylin and eosin slides were prepared for histologic evaluation.

RESULTS

All animals tolerated the procedures well and ate normally within 24 hours. No complications required medical treatment before planned death.

Pacemaker potential readings were taken in phase III of the migrating myoelectric complex and occurred at a rate of 18 to 19 beats per minute proximal to the staple line (Table I). Normal duodenal pacemaker potentials transmitted through the uncut staple line in five animals: two in the Teflon-reinforced staple line group and three in the nonreinforced group. The potential propagates from lead 1 to lead 4 sequentially at 18 to 19 beats per minute (Fig. 3). In the nine remaining animals, aberrant conduction occurred distal to the staple line (Fig. 4). Potentials occurred at a rate of 18 to 19 beats per minute in leads 1 and 2 sequentially, but in leads 3 and 4 distal to the staple line the potential rate was 15 beats per minute and the distal potential in lead 4 occurs prior to the potential in lead 3. Two animals were killed at 1.5 months because of time constraints and were not retested after the first postoperative week. At 3 months postoperatively, seven animals that previously had no duodenal pacemaker transmission distal to the staple line still had aberrant transmission, but all the action potentials had converted from orad to anad conduction (Fig. 5). The pacemaker potentials in

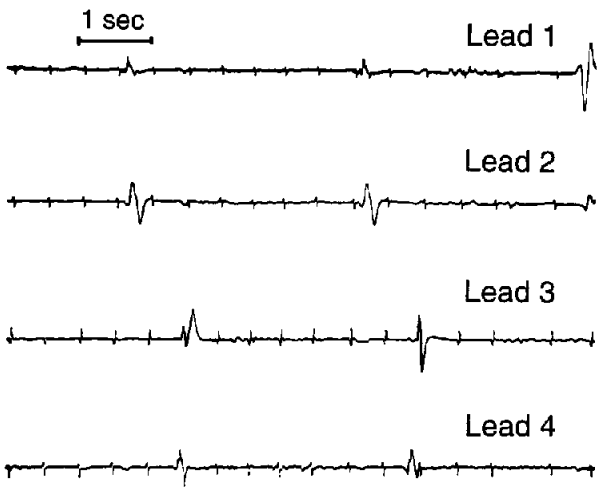

Fig. 3. Pacemaker potential activity from sequentially placed serosal leads in the bowel wall. The animal had an uncut staple line placed between leads 2 and 3. This shows normal conduction of pacemaker activity through the staple line.

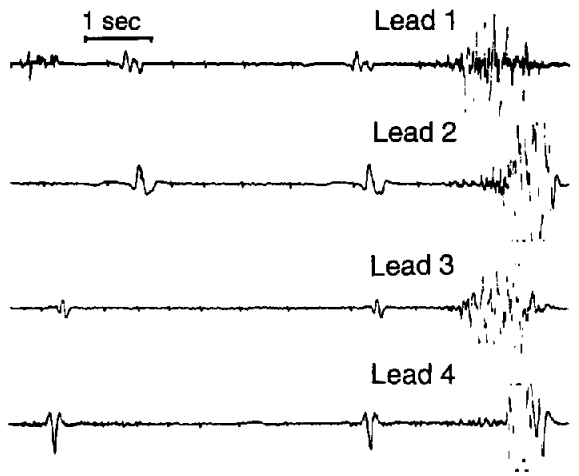

Fig. 4. Pacemaker potential activity from sequentially placed leads. A staple line was placed between leads 2 and 3. Recordings from leads 3 and 4 show a slower rate than leads 1 and 2 and orad conduction. This recording was made within 2 days of operation.

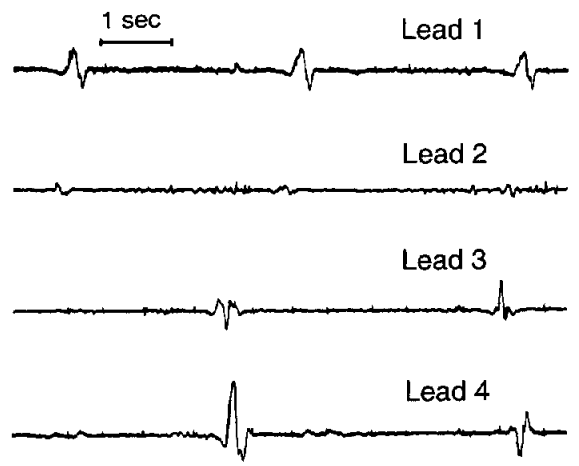

Fig. 5. Pacemaker potential activity from sequentially placed leads. A staple line was placed between leads 2 and 3. This recording was taken 3 months after staple line placement. Recordings from leads 3 and 4 show a slower rate than leads 1 and 2, but orad conduction is now arad.

Fig. 6. Microscopic examination of tissue surrounding the Teflon patch showing a mild fibrotic reaction and no recanalization.

leads 1 and 2 occurred at a rate of 18 to 19 beats per minute and fired sequentially as they had before. Potentials in leads 3 and 4 occurred at a rate of 15 to 16 beats per minute, but now lead 3 pulsations occurred before lead 4.

At necropsy, the operative site was inspected and the Teflon staple line reinforcement patches were covered with omentum or adjacent bowel that was easily dissected free. There was no evidence of chronic inflammation or abscess around these patches. However, over the Teflon electrical wire insulation patches there were abscesses on one animal operated on only once and two animals that had been operated on for replacement of electrical leads. We expect that this occurred because the wires brought out directly through the abdominal wall allowed bacteria to enter. There was also one seroma over a Teflon insulation patch. One fistula occurred between small bowel loops at the site of a Prolene insulation patch.

All nonreinforced staple lines were recanalized in their entirety including those in the animals killed at 1.5 months. All Teflon-reinforced staple lines were competent, and no methylene blue solution could traverse the area. All staple lines through Teflon patches remained in their immediate postoperative configuration. All of the enteroenterostomies were patent.

Microscopic examination revealed no recanalization in the reinforced group and little tissue reaction to the Teflon (Fig. 6). Only a mild fibrotic reaction was seen on the hematoxylin and eosin preparations.

DISCUSSION

The uncut Roux limb operation prevents the Roux stasis syndrome by allowing normal duodenal pacemaker activity to traverse an uncut staple line, thus creating a physiologic Roux limb. In this study, recanalization was prevented by stapling a permanent material, Teflon, to both sides of the bowel. Each staple traverses one Teflon patch, then both sides of the bowel, and then the other Teflon patch, permanently holding it in place. This strategy worked in all animals.

Our findings of incomplete electrical conduction were different from those of other investigators.[5,6] Duodenal pacemaking activity traversed only five of nine staple lines. In the event that the restoration of enteric electrical activity required healing after staple line placement, we remonitored myoelectric activity in seven animals 3 months after the initial operation. Initial orad aberrant conduction distal to the staple line had changed direction, but the staple line remained a barrier to electrical conduction. What this means in terms of small bowel transit time was not tested.

Aberrant conduction was not due to the Teflon reinforcement patches, which could have caused more crush injury because of the bulk added to the staple line. This material is 0.4 mm thick and the staples allow 2.5 mm of thickness. Of the five animals that had duodenal pacemaker activity traverse, the staple line in two had Teflon reinforcement patches. In the remaining nine animals with aberrant distal myoelectric activity, five had Teflon reinforcement and four did not. The loss of conduction across staple lines that we

observed may be stapler dependent, and the staplers we used may have caused too much crush injury. We have no other logical explanation for the observed differences between this study and others.

Teflon was used because it is inert and causes only mild tissue reaction. We expected that it would have a low fistula and abscess rate. In our series of Nissen fundoplications, we have been using Teflon pledgets when closing the crura under tension and have had no such complications.[7] Teflon patches have been used for inguinal hernias with few complications.[8,9]

The use of Teflon on small bowel has not been extensively studied. There was no gross or microscopic evidence of abscess or fistula formation on the Teflon staple line reinforcement patches, but the Teflon insulation patches likely became infected because of transmission of bacteria through the skin along the wires. The minor inflammation consisted of a fibrous reaction.

These results indicate that this method is a good adjunct to the uncut Roux limb operation that will not allow recanalization of the bowel. However, in our experience, myoelectric activity was not transmitted through the staple line in all animals and therefore normal peristalsis may not be maintained. Nonetheless, normal conduction was present across more than 33% of staple lines, and anad conduction was reestablished in the remainder of the staple lines 3 months postoperatively. These encouraging results warrant further investigation in clinical trials.

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Effect of the Distal Remnant on Ileal Adaptation

Jon S. Thompson, M.D., Debra C. Ferguson, B.S., S.R.S.

The ileum has a greater adaptive capacity than the jejunum after intestinal resection, which may be, in part, related to increased exposure to luminal contents and intrinsic properties of the ileum. However, the intestinal remnant might contribute to this adaptive response as well. Our aim was to determine the effect of the distal intestinal remnant on ileal adaptation when the ileum is proximal in the intestinal tract. Twenty-one Lewis rats were included in the study. One group (n = 7) served as unoperated control subjects, the second group (n = 7) underwent transposition of the jejunum and ileum, and the third group (n = 7) underwent 50% proximal resection with syngeneic transplantation of the ileum. Nutritional status and structural adaptation were studied at 14 days. Animals in both the transposition and transplant groups initially lost weight but weights returned to above preoperative levels at 14 days. Food intake, stool weight, and serum albumin levels were similar in these two groups. Intestinal weight and diameter were similar in the proximal end of the ileal segment in the two study groups and were significantly increased compared to control values (0.26 ± 0.04 and 0.31 ± 0.02 vs. 0.10 ± 0.0 g/cm and 8.4 ± 0.5 and 9.1 ± 0.8 vs. 4.9 ± 0.3 mm; $P < 0.05$). Intestinal weight and diameter of the distal end of the ileal segment were greater than those values in unoperated control animals but were greatest in the ileal transplant group (0.15 ± 0.1 and 0.24 ± 0.03 vs. 0.07 ± 0.01 g/cm and 5.6 ± 1.1 and 8.7 ± 0.6 vs. 4.3 ± 0.2 mm; $P < 0.05$). Villus height and crypt depth were similar in both the proximal and distal ends of the ileal segments in the two study groups and were significantly increased compared to control values (642 ± 75 and 720 ± 15 vs. 411 ± 24 proximal and 443 ± 49 and 500 ± 46 vs. 343 ± 22 μm distal, $P < 0.05$; 223 ± 34 and 244 ± 33 vs. 173 ± 20 proximal and 192 ± 28 and 209 ± 18 vs. 144 ± 26 μm distal, $P < 0.05$). Proximal placement of the ileum by either transposition or transplantation results in structural adaptation. This occurs to a similar extent whether the distal remnant is jejunum or ileum. Thus increased exposure to luminal contents and intrinsic properties appear to be the important factors in the adaptive capability of the ileum when the ileum is the proximal portion of the intestinal tract. (J GASTROINTEST SURG 2000; 4:430-434.)

KEY WORDS: Intestinal transplantation, intestinal adaptation

After intestinal resection, the ileum has a greater adaptive capacity than the jejunum.[1-3] Several factors might be responsible for this. One factor is an increased exposure of the ileum to nutrients and pancreaticobiliary secretions after proximal resection.[4-7] Intrinsic features of the intestinal remnant might contribute to this difference as well.[5] This concept is supported by the observation that proximal resection stimulates hyperplasia in the ileum to a greater extent than either proximal intestinal bypass or transposition of the ampulla just proximal to the ileum.[8] Furthermore, we recently observed that replacing jejunum with transplanted ileum resulted in a better functional outcome than replacing jejunum with transplanted jejunum.[9] These transplanted segments were exposed to the same luminal environment as well as any regulatory effects of the intact ileum. Thus any differences observed should reflect the intrinsic response of the transplanted segment. Finally, the nature of the remaining intestinal remnant might also be an important factor. The aim of this study was to compare the effect of altering the distal intestinal remnant on intestinal structure after proximal placement of the ileum in the gastrointestinal tract.

From the Department of Surgery, University of Nebraska Medical Center and Surgical Service, Omaha VA Medical Center, Omaha, Neb.
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Reprint requests: Jon S. Thompson, M.D., Department of Surgery, University of Nebraska Medical Center, 983280 Nebraska Medical Center, Omaha, NE 68198-3280.

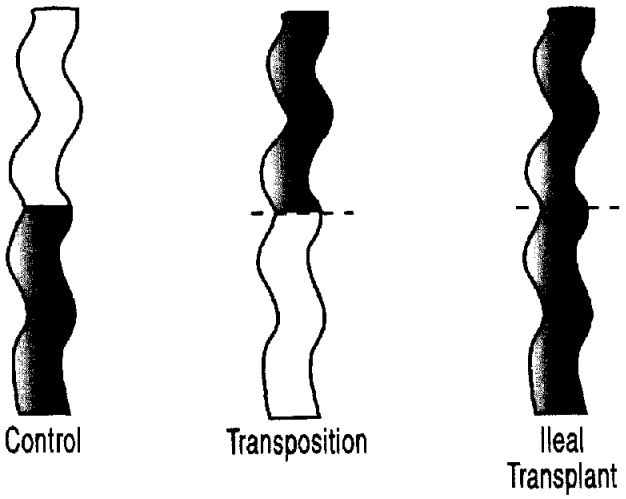

Fig. 1. Diagrammatic representation of the surgical models.

METHODS

Twenty-one Lewis rats (>250 g) were included in the study (Fig. 1). Seven animals served as unoperated control subjects. A second group (n = 7) underwent transposition of the jejunum and ileum. The third group (n = 7) underwent a 50% proximal resection with simultaneous isotransplantation of ileum. The ileal isografts were harvested from donor Lewis rats and placed in continuity with the recipient distal remnant. All animals received a powdered rodent diet (Teklad ED 604 rodent chow, Teklad, Madison, Wis.) ad libitum and were killed 2 weeks after operation. Nutritional status was evaluated by food intake, body weight, and serum albumin levels. Structural adaptation was estimated by measuring gut diameter and weight and by microscopic morphometry. Functional adaptation was assessed by measurement of stool weight. Mucosal proliferation was estimated by the crypt cell production rate.

Operative Procedures

The animals were fasted for 12 hours prior to operation and were anesthetized with inhalation metofane. They received Bicillin, 30,000 units intramuscularly, and 2 ml lactated Ringer's solution intravenously prior to operation. In the transposition group, the intestine was divided just distal to the ligament of Treitz, at the midpoint, and just proximal to the ileocolonic junction. The proximal and distal segments were transposed and reanastomosed with 6-0 silk. In the donors, the superior mesenteric artery and vein were dissected distal to the pancreas with enough side branches ligated to provide suitable pedicle length. These vessels were ligated proximal to the last branches feeding the graft and the graft was excised. The distal half (ileum) of the harvested graft was transplanted. The infrarenal aorta and inferior vena cava of each recipient were mobilized circumferentially and clamped. The superior mesentric artery and vein grafts were anastomosed end to side with 10-0 monofilament nylon to the recipient aorta and inferior vena cava, respectively. The proximal half of the native bowel was resected, and the transplanted bowel was anastomosed end to end in continuity with native bowel with 6-0 silk.

Nutritional Studies

Animals were weighed preoperatively and then weekly after operation. Food intake was quantitated in grams. Stool was collected in a metabolic cage and weighed daily. Serum albumin levels were measured in a clinical laboratory using an automated technique at sacrifice.

Structural Adaptation

Intestinal diameter and weight were determined on segments from the proximal and distal portions of the transposition and transplanted ileal segments. The thickness of the various intestinal wall components was measured on formalin-fixed specimens stained with hematoxylin and eosin. Ten fields were quantitated around the circumference of the specimen with an ocular micrometer and mean values calculated. Villus height and crypt depth were measured in 10 consecutive axially oriented villi and crypts.

Table I. Comparison of nutritional status

	Control	Transposition	Ileal transplant
Body weight (% preoperative)			
7 days	—	91 ± 2	91 ± 4
14 days	—	100 ± 3*	108 ± 2*†
Serum albumin (g/dl)			
14 days	3.1 ± 0.1	2.5 ± 0.1‡	2.6 ± 0.1‡
Food intake (g/day)	22.9 ± 0.8	20.3 ± 0.8‡	19.1 ± 0.9‡
Stool weight (g/day)	5.1 ± 0.2	3.9 ± 0.1‡	4.1 ± 0.2‡

*P <0.05 vs. 14 days.
†P <0.05 vs. transposition.
‡P <0.05 vs. control.

Table II. Comparison of intestinal weight and diameter

	Control jejunum	Control ileum	Transposition	Ileal transplant
Intestinal weight (g/cm)				
Proximal	0.11 ± 0.1	0.10 ± 0.0	0.26 ± 0.04*	0.31 ± 0.02*
Distal	0.10 ± 0.1	0.07 ± 0.01	0.15 ± 0.01*	0.24 ± 0.3*†
Intestinal diameter (mm)				
Proximal	4.7 ± 0.2	4.9 ± 0.3	8.4 ± 0.5*	9.1 ± 0.8*
Distal	4.9 ± 0.3	4.3 ± 0.2	5.6 ± 1.1*	8.7 ± 0.6*†

*P <0.05 vs. controls.
†P <0.05 vs. transposition.

Crypt cell production rate was determined using a metaphase arrest technique with vincristine sulfate.[10] Vincristine, 0.25 mg intraperitoneally, was injected approximately 2 hours prior to sacrifice. The mucosal samples were fixed in Carnoy's fixative, hydrolyzed in acid at 60° C for 5 minutes, and stained with Schiff's reagent. The crypts were dissected free with a dissecting microscope. Samples of crypts were transferred to a glass slide in 15% glacial acetic acid and squashed for determination of the number of metaphases per crypt in 10 crypts. Crypt cell production rate was calculated assuming a linear accumulation for 2 hours.

Data are expressed as means ± standard error of the mean. Comparisons between groups were made using analysis of variance with the Bonferonni correction as appropriate. Statistical significance was ascribed to P values <0.05.

RESULTS

Body weight as a percentage of preoperative weight decreased by 9% 7 days after transposition and transplantation (Table I). Body weight increased at 14 days and was significantly greater in the transplant group compared to the transposition group. Serum albumin levels were significantly lower in the transplant and transposition groups at 14 days compared to control values. Mean daily food intake and stool weight were similar after both transplantation and transposition but were decreased approximately 15% compared to control values.

Intestinal weight and diameter of the proximal ileal segment increased to a similar extent in the transposition and transplant groups compared to values for unoperated jejunum and ileum. Both parameters increased to a greater extent in the distal ileal segment of the transplant group compared to transposition values (Table II). The proximally placed ileal segments in both groups were shortened when measured at 14 days (86% ± 7% vs. 93% ± 12% initial, transposition, and transplant; P = not significant).

Thicknesses of mucosa, submucosa, muscle, and the total intestinal wall were similar in the transposition and transplant groups in both the proximal and distal ileal segments (Table III). These values were significantly greater than those of control jejunum and ileum. Villus height and crypt depth were similar in both proximal and distal ileal segments and were also significantly greater than control values (Table IV). There were no significant differences in crypt cell production rates among the different study groups.

Table III. Comparison of intestinal wall components

	Control jejunum	Control ileum	Transposition	Ileal transplant
Mucosal thickness (μm)				
Proximal	0.75 ± 0.04	0.59 ± 0.03	1.00 ± 17*	0.97 ± 0.17*
Distal	0.60 ± 0.03	0.45 ± 0.05	0.70 ± 0.08*	0.74 ± 0.12
Submucosal thickness (μm)				
Proximal	0.02 ± 0.00	0.02 ± 0.00	0.04 ± 0.01*	0.03 ± 0.00*
Distal	0.03 ± 0.00	0.02 ± 0.00	0.04 ± 0.00*	0.03 ± 0.00*
Muscle thickness (μm)				
Proximal	0.08 ± 0.0	0.08 ± 0.01	0.17 ± 0.05*	0.19 ± 0.03*
Distal	0.08 ± 0.01	1.0 ± 0.01	0.21 ± 0.08*	0.24 ± 0.09*
Wall thickness (μm)				
Proximal	0.85 ± 0.05	0.69 ± 0.07	1.22 ± 0.21*	1.18 ± 0.17*
Distal	0.71 ± 0.05	0.56 ± 0.05	0.95 ± 0.08*	1.00 ± 0.12*

*P <0.05 vs. controls.

Table IV. Comparison of mucosal parameters

	Control jejunum	Control ileum	Transposition	Ileal transplant
Villus height (μm)				
Proximal	523 ± 78	411 ± 24	642 ± 75*	720 ± 15*
Distal	414 ± 48	343 ± 22	443 ± 49*	500 ± 46*
Crypt depth (mm)				
Proximal	169 ± 22	173 ± 20	223 ± 34*	244 ± 65*
Distal	153 ± 10	144 ± 26	192 ± 28*	209 ± 8*
CCPR (cells/hr)				
Proximal	7.8 ± 0.9	7.7 ± 0.5	7.4 ± 1.2	7.9 ± 1.1
Distal	7.6 ± 1.0	7.8 ± 1.2	7.7 ± 0.5	7.4 ± 1.9

CCPR = crypt cell production rate.
*P <0.05 vs. controls.

DISCUSSION

Ileal segments placed proximally in the intestinal tract, for example, after proximal resection, bypass, or transposition, undergo structural adaptation. We speculated that the nature of the distal remnant might influence adaptation of this proximal segment of intestine. There are regional differences in the small intestine of several characteristics including structure, absorptive function, motility, and endocrine function.[3] Differences in endocrine function, in particular, might be expected to influence the adaptive response. In the present study, the nature of the distal remnant (i.e., jejunum vs. ileum) did not influence this adaptive response.

In transposition models, the ileum achieves a villus height similar to that of the jejunum when interposed proximally, whereas the jejunum loses villus height when moved distally in the gastrointestinal tract.[4-7] This may reflect changes in exposure to luminal nutrients and secretions. A similar adaptive response was seen in the proximal ileal segment in the present study as intestinal weight, diameter, and wall thickness exceeded values in the normal jejunum and ileum. However, certain regional properties do not appear to be influenced by transposition of intestinal segments. Aiken et al.[5] found no change in the region-specific expression of enteroendocrine cell populations or their products. Wang et al.[11] made a similar observation.

There are several potential differences between the transposition and transplant groups. One difference between these two groups is the systemic venous drainage of transplanted segments. This might affect the response of these segments compared to portal venous drainage of the transposed ileum. Denervation and ischemia/reperfusion injury could also affect the transplanted ileum. However, results of our previous studies suggest that these factors do not have significant effects on intestinal structure.[9]

Functional adaptation was not specifically evaluated in the present study. Dowling and Booth[6] found that transposed ileum had a marked increase in glucose absorptive capacity. However, this probably re-

flected increased intestinal mass rather than a change in cellular function. The greater weight gain in the transplanted group in our study is consistent with improved absorption in these animals, but there was no difference in stool weight or serum albumin levels. Furthermore, the weight gain in both groups was less than the 14% increase observed in animals undergoing transection alone.[9]

The adaptive response in the ileum included the muscle as well as the mucosal layer. Thus growth of all layers of the bowel wall appears to be stimulated by proximal exposure to luminal contents.

Interestingly, crypt cell production rates were similar in all three groups of animals 14 days after operation. Chu et al.[13] found that proliferation decreased after 21 days. Rijke et al.[4] found that proliferative activity was greater in transposed ileum up to 30 days after operation, although the relative size of the proliferative cell compartment was similar. Thus they found that increased villus height was secondary to an actual increase in the number of proliferating crypt cells rather than any change in the enterocyte migration rate. Based on the similar rate of cell migration in their study, they suggested that enterocyte life span was not altered in the transposed ileum. Adaptation of the proximally placed ileum appears to have occurred more rapidly in the present study.

Chu et al.[13] reported that transposing the terminal 25% of the small intestine to various proximal locations caused a proliferative response that was greater with more proximal transposition. Furthermore, the transposed ileum caused a proliferative response in the duodenum and pancreas, suggesting a possible humoral mechanism. Chu et al.[13] found an increase in serum neurotensin in the transposed animals. However, the expression of the gene encoding neurotensin is minimally altered by transposition.[11] Interestingly, in the present study the denervation of the transplanted ileum did not alter this proliferative response related to proximal placement of the ileum.

Proximal placement of the ileum by either transposition or transplantation results in structural adaptation. This adaptive response occurs to a similar extent whether the distal remnant is jejunum or ileum. Thus increased exposure to luminal contents and intrinsic properties appear to be the important factors in the adaptive capability of the ileum when the ileum is the proximal portion of the intestinal tract.

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Effects of Glutamine Isomers on Human (Caco-2) Intestinal Epithelial Proliferation, Strain-Responsiveness, and Differentiation

Mark Murnin, Ph.D., Atul Kumar, M.D., Guang di Li, M.D., Mark Brown, Bauer E. Sumpio, M.D., Ph.D., Marc D. Basson, M.D., Ph.D.

Enteral feeding with small amounts to stimulate bowel motility, and glutamine supplementation, which provides nutrients selectively used by intestinal epithelial cells, might preserve the gut mucosa during fasting. We evaluated the effects of the interaction between mechanical strain and glutamine supplementation in human Caco-2 intestinal epithelial cells, and pursued the finding of equivalent effects of L- and D-glutamine in Caco-2, HT-29, and primary malignant human colonocytes. Caco-2 cells were subjected to repetitive strain in media containing 2 mmol/L of L-glutamine and media supplemented with L- or D-glutamine. Proliferation was determined by automated cell counting. Differentiation and cellular production of L-glutamine were determined spectroscopically. Rhythmic deformation stimulated Caco-2 proliferation in a frequency-dependent manner. Maximal stimulation occurred at 10 cpm, consistent with in vivo frequencies of peristalsis and villous motility. Deformation at 10 cpm and L-glutamine supplementation from 2 to 5 mmol/L concentrations independently stimulated Caco-2 proliferation; the combination further increased proliferation. D-Glutamine supplementation yielded similar results, although with lesser potency. Furthermore, both L- and D-glutamine equivalently reduced Caco-2 dipeptidyl dipeptidase activity. The effects of each isoform were blocked by 1 to 3 mmol/L acivicin, a selective antagonist of glutamine metabolism. Indeed Caco-2 and HT-29 cells and primary malignant colonocytes each metabolized D-glutamine to L-glutamine. Glutamine supplementation in fasting patients might prove synergistic with stimulation of bowel motility by non-nutritive feeding, whereas tissue-specific variations in D-glutamine metabolism might facilitate selective nutripharmaceutical targeting of the gut mucosa. (J GASTROINTEST SURG 2000;4: 435-442.)

KEY WORDS: Deformation, glutamine, intestine, mucosa

Although modern methods of parenteral nutrition permit the nutritional support of fasting patients, preservation of the intestinal mucosa remains a major problem in such patients. Parenteral or enteral glutamine dipeptide supplementation has recently been used in a variety of settings[1-8] in an effort to provide the small and large intestinal mucosa with a stable form of glutamine, a primary metabolic fuel for enterocytes,[9] although not always with successful results.[10,11] We[12,13] have recently described the stimulation of human intestinal epithelial cell proliferation by rhythmic mechanical deformation of Caco-2 monolayers at frequencies and amplitudes consistent with that which occurs during peristalsis[14] or villous motility.[15] These results suggest that repetitive mechanical strain during normal gut function may be mitogenic for intestinal epithelial cells.

Although techniques for stimulating repetitive mechanical mucosal strain in vivo are less well char-

From the Departments of Surgery, Yale University (M.M., G.d.L., B.E.S., and M.D.B.), and Connecticut VA Health Care System (M.B., B.E.S., and M.D.B.), and the Department of Medicine, Bridgeport Hospital (A.K.), New Haven, Conn.
Supported in part by a VA Merit Award (Dr. Basson).
Reprint requests: Dr. Marc Basson, Department of Surgery, Yale University School of Medicine, 333 Cedar St., P.O. Box 208062, New Haven, CT 06520-8062. e-mail: marc.basson@yale.edu

acterized than those for parenteral glutamine supplementation, diverse possibilities exist in this regard. Minimally nutritive enteral feeding, in which an elemental diet is delivered to the apical surface of the gut mucosa in amounts too small to have a significant impact on overall nutritional requirements, has been used to avoid cholestasis.[16-18] The presentation of nutrients to the gut mucosa also stimulates both intestinal and villous motility,[15,19] and thus promotes repetitive mechanical strain of the gut mucosa. Pharmacologic and electrical methods of stimulating intestinal and/or villous motility have also been described.[20,21] The present study was performed in a cell culture model and may not necessarily illuminate the effects on the gut mucosa of such techniques. Nevertheless, the human Caco-2 intestinal epithelial cell is a well-characterized model for the study of many aspects of intestinal mucosal biology in general,[22,23] as well as for the effects of mechanical strain and glutamine in particular.[12,13,24]

In the present study we therefore sought to examine the potential for synergy between glutamine supplementation and repetitive deformation in the stimulation of intestinal epithelial cell proliferation, using the Caco-2 cell monolayer as a model system. We have previously described the stimulation of proliferation by glutamine supplementation in these cells, with maximal effects being observed at 5 to 7 mmol/L concentrations.[24] In this study we characterized the frequency dependence of the mitogenic effects of repetitive mechanical strain and then evaluated the effects of combining a maximally effective strain frequency with maximal glutamine supplementation. In addition, we observed that both the L- and D-isomers of glutamine had similar effects, and we then sought to further explain this observation. As we had previously[24] demonstrated that L-glutamine supplementation resulted in a decrease in Caco-2 dipeptidyl dipeptidase specific activity, a common marker of intestinal epithelial differentiation, we compared the effects of the glutamine isomers on Caco-2 dipeptidyl dipeptidase specific activity. Finding of equivalence in this effect as well prompted us to evaluate whether acivicin, an antagonist of L-glutamine metabolism that inhibits amido transfer from L-glutamine, could block the effect of D-glutamine. We then further sought to determine whether Caco-2 cells, HT-29 cells (a second human colon cancer cell line), and primary malignant cells isolated from surgically resected human colon cancers were capable of increasing cytosolic and extracellular L-glutamine concentrations in response to D-glutamine supplementation.

METHODS

Cells

The Caco-2 cells used for these studies represent a clonal subpopulation of this established cell line selected for enterocytic differentiation.[25] Cells were maintained at 37° C in 5% CO_2 in Dulbecco modified Eagle medium (DMEM) with 10% fetal calf serum, 10 μg/ml transferrin (Boehringer Mannheim, Indianapolis, Ind.), 2 mmol/L glutamine, 1 mmol/L pyruvate, 10 mmol/L HEPES, 100 U/ml penicillin G, and 0.1 mg/ml streptomycin. For experimental studies of the effects of mechanical deformation, the cells were cultured for at least 4 days after seeding onto the culture plates to allow for the development of cell-matrix adhesion and the development of a confluent monolayer. The HT-29 cells used for these studies were obtained from American Type Culture Collection and were maintained at 37° C in 5% CO_2 in 90% McCoy's 5a medium with 10% fetal bovine serum. Primary human colon cancer tissue was harvested from surgically resected tumors, washed in phosphate-buffered saline supplemented with 2 mmol/L dithiothreitol, and minced in 37° C, oxygenated DMEM containing 100 units/ml penicillin/streptomycin until a uniform cell suspension was achieved. The cells were then washed until visibly clean, at least five times, in several volumes of warm, oxygenated DMEM and maintained in DMEM without phenol red (Sigma, St. Louis, Mo.) supplemented with 10% fetal bovine serum, 10 mmol/L HEPES, penicillin G (1000 U/ml), and streptomycin (100 g/ml).

Rhythmic Strain

The strain unit consists of a vacuum manifold regulated by computer-controlled solenoid valves. Cells are cultured on flexible-bottomed culture plates precoated with type I collagen (FlexI, Flexcel, McKeesport, Pa.). The computer-regulated valves apply a precise vacuum to the system, deforming the culture plate bottoms to a known percentage elongation, which we have previously demonstrated to be transmitted to adherent Caco-2 cells.[13] When the vacuum is released, the plate bottoms return to their original conformation. Thus the magnitude, duration, and frequency of the applied force can be regulated by the computer software. Force analysis of the strain on the membrane during stretch at various vacuum levels has been calculated mathematically by finite element analysis[26,27] and empirically by measuring with a micrometer the distance between concentric circles (radial strain) or diameter axes (axial strain) marked on

the membrane. Very little axial deformation is observed, so the force on the attached cells is primarily in one axis. Application of a −20 kPa vacuum to the membrane produces a deformation pattern ranging from 0% at the membrane center to 24% at the periphery (average strain, 10%).[28,29]

Proliferation Studies

For proliferation studies, cells were seeded onto the collagen-coated membranes at an initial density of 50,000/cm^2 and allowed to adhere for at least 24 hours prior to study. Monolayers were then either subjected to repetitive strain for 72 hours as described above or maintained in the same incubator under static (control) conditions. Thus all of the proliferation studies described here were performed similarly, with an initial 24-hour adhesion, followed by 3 days under either static or repetitively strained conditions, with or without supplemental glutamine. The cells from each monolayer were then resuspended into single cell suspensions with absolute cell numbers being determined electronically by an automated cell counter (Coulter Electronics, Ltd., Luton, England).

Dipeptidyl Dipeptidase Specific Activity Assay

Using methodology previously described in detail elsewhere,[24] we lysed the cells in lysing buffer (D-PBS, 0.5% Triton X-100, and 0.35 mol/L NaCl) at 4° C, performed a preliminary bicinchoninic acid assay in triplicate according to the manufacturer's instructions (Pierce, Rockford, Ill.) to quantitate protein in the cell lysates, and then diluted the lysates with lysing buffer to equal protein concentration. The protein-matched aliquots of cell lysates were assayed in triplicate for dipeptidyl dipeptidase specific activity by enzymatic cleavage of ala-p-nitroanilide. Briefly, 100 μl of 1.4 mmol/L ala-p-nitroanilide was added to 100 μl of cell lysate and 350 μl of 114 mmol/L Tris buffer, pH 8.0. The solutions were incubated for 6 hours at 37° C, and the resulting reaction product was quantitated spectrophotometrically at 405 nm using an automated enzyme-linked immunosorbent assay reader (Whittaker Bioproducts EIA 400AT Reader, Walkersville, Md.). All reactions were performed in parallel with assays of serial dilutions of an enzymatic standard (Sigma, St. Louis, Mo.), and dipeptidyl dipeptidase (DPDD) specific activities for the cell lysate samples were calculated by linear interpolation against the standards curves thus generated. All assays were performed within the linear range of the assay.

Acivicin Experiments

Acivicin was obtained from Sigma. For acivicin inhibition studies, acivicin was solubilized as a stock solution of 50 mg/ml (280 mmol/L) in 1.0 N HCl, as per the manufacturer's suggested protocol, and then diluted into Caco-2 cell culture medium containing 2 mmol/L of L-glutamine alone or supplemented with either 3 mmol/L of D-glutamine or an additional 3 mmol/L of L-glutamine to yield cell culture media with final acivicin concentrations of 1 μmol/L and 3 μmol/L, which were then used to treat Caco-2 cells for 24 hours prior to cell lysis and DPDD assay as described earlier. To control for any potential effects of the HCl vehicle used for the acivicin dilution, control cells were similarly treated with 1.0 N HCl in identical concentrations to that used to achieve the higher final acivicin concentration.

Glutamine Conversion Assay

For assays of D-glutamine to L-glutamine conversion, cells were plated on bacteriologic plastic dishes (Falcon, Oxnard, Calif.) precoated with type I collagen, and allowed to grow to confluence in standard culture medium from which phenol red had been deleted prior to D-glutamine treatment. Cells were then treated for 6, 12, 18, and 24 hours, respectively, with 30 mmol/L of D-glutamine. The samples were then centrifuged at 3000 rpm for 5 minutes. The supernate was removed and assayed for L-glutamine. The cells remaining in the centrifuge tube were lysed in phosphate-buffered saline supplemented with 9 mmol/L calcium and 4.9 mmol/L magnesium, containing 0.5% Triton X-100 and 0.35 mol/L NaCl on ice for 1 hour. After centrifugation, the supernates were assayed for L-glutamine. L-Glutamine assays were performed using a Gln-2 kit (Sigma), which is a quantitative colorimetric assay specifically designed for use in a cell culture system and is based on the reductive deamination of L-glutamine.

Statistical Analysis

Ninety-five percent confidence was set a priori as the limit required for statistical significance. For proliferation studies, all data were normalized to the mean of control values, and data from at least three similar experiments were pooled for statistical analysis by Wilcoxon signed-rank test. The DPDD assay

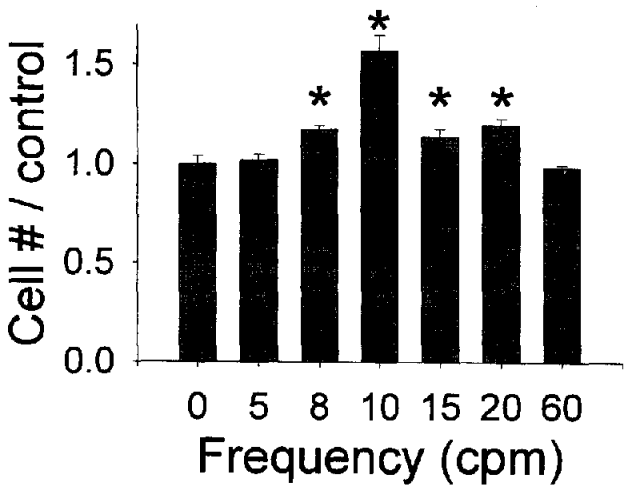

Fig. 1. Frequency-dependent effects of rhythmic deformation. Repetitive deformation at an average 10% strain stimulates Caco-2 proliferation in a dose-responsive manner (mean ± standard error; * = $P < 0.01$).

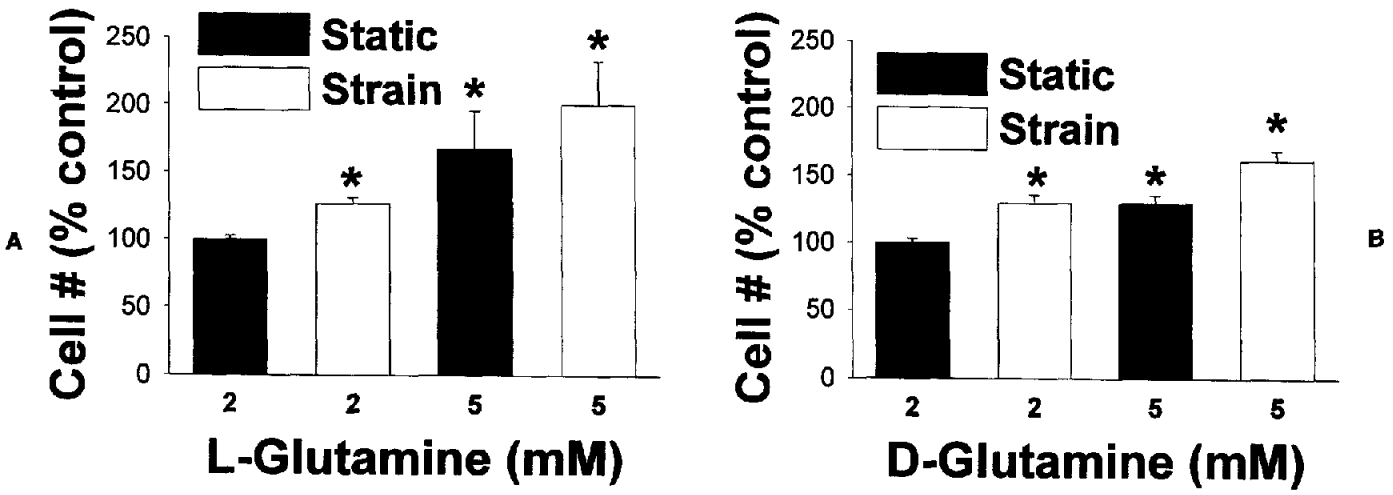

Fig. 2. Synergistic stimulation of Caco-2 proliferation between L-glutamine supplementation (A) or D-glutamine supplementation (B) and rhythmic strain (open bars) as compared with cells in static monolayers (shaded bars) (mean ± standard error; * = $P < 0.005$).

and glutamine conversion assay data presented here represent at least one of three similar experiments and were analyzed by unpaired *t* test. All data are depicted graphically here as mean ± standard error.

RESULTS

In initial experiments, we found that rhythmic deformation at an average 10% strain resulted in a frequency-dependent stimulation of Caco-2 proliferation, which became statistically significant at 8 cycles per minute and was maximal at 10 cycles per minute. Higher strain frequencies resulted in a decreased mitogenic effect as compared with 10 cycles per minute, although some stimulation was still observed as compared with control (static) monolayers ($n \geq 24$ pooled from at least four similar experiments, $P < 0.01$; Fig. 1).

Both rhythmic deformation at an average 10% strain at a frequency of 10 cycles per minute and L-glutamine supplementation increased to 5 mmol/L stimulated Caco-2 proliferation, increasing the absolute cell number to 126% ± 5% and 167% ± 29%, respectively, that observed in control monolayers, which were maintained on a static (undeformed) membrane in medium containing 2 mmol/L of L-glutamine (Fig. 2, *A*; $n \geq 21$, pooled from 11 similar experiments, $P < 0.005$ for each). Furthermore, when glutamine

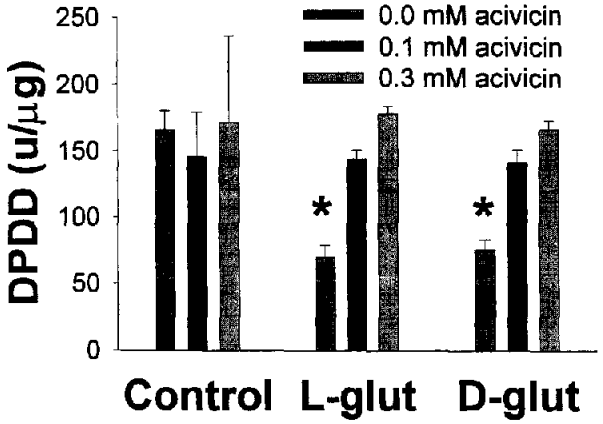

Fig. 3. Effects of L-glutamine *(L-glut)* or D-glutamine *(D-glut)* isomers and the L-glutamine antagonist acivicin on Caco-2 DPDD specific activity (mean ± standard error; * = P <0.005).

supplementation was combined with rhythmic deformation, a substantial additional increase in proliferation was observed to a total of 201% ± 33% that of control values (see Fig. 2, *A*; P <0.001).

Our next series of studies used the D-isomer of glutamine. In these experiments, rhythmic deformation in medium containing 2 mmol/L of D-glutamine again stimulated Caco-2 proliferation, increasing absolute cell numbers to 130% ± 6% that of static monolayer. This effect was similar to that obtained when rhythmic strain was applied to the Caco-2 monolayer maintained in medium containing 2 mmol/L of L-glutamine. Increasing the concentration of D-glutamine supplementation to 5 mmol/L also stimulated Caco-2 proliferation to 129% ± 6% that of control monolayers, which were cultured under static conditions in 2 mmol/L of D-glutamine (Fig. 2, *B*; n ≥9, pooled from three similar experiments, P <0.005 for each). As for the L-glutamine studies, a further mitogenic effect was observed when D-glutamine supplementation was combined with rhythmic strain (P <0.001). Since the summation of the individual effects of L-glutamine supplementation and strain would have predicted a 94% increase (vs. the 101% observed increase) and the summation of the individual effects of D-glutamine supplementation and strain would have predicted a 59% increase (vs. the 61% observed increase), it seems likely that the effects of strain and glutamine supplementation are additive.

Supplementation with D- or L-glutamine to a 5 mmol/L total glutamine concentration also decreased Caco-2 DPDD specific activity similarly (by 57% ± 5% and 54% ± 5%, respectively, n = 4 from one of four similar experiments, P <0.005; Fig. 3). Acivicin (0.1 to 0.3 mmol/L, an antagonist of glutamine metabolism, did not significantly alter basal DPDD specific activity in Caco-2 cells cultured in control medium (media containing 2 mmol/L of L-glutamine) but blocked the decrease in DPDD specific activity associated with either L-glutamine or D-glutamine supplementation.

The similarity of effects of L-glutamine and D-glutamine and the ability of acivicin to block the effects of each led us to speculate that Caco-2 cells might be converting D-glutamine to L-glutamine. We tested this hypothesis by treating Caco-2 cells with glutamine-deficient culture medium supplemented with 30 mmol/L of D-glutamine and measuring L-glutamine concentrations, both secreted by the cells into the deficient medium and in cell lysates, over time. Indeed we found that Caco-2 cells cultured in the presence of D-glutamine appeared able to synthesize L-glutamine, exhibiting time-dependent increasing concentrations of L-glutamine in both cell lysates and culture medium. In one typical experiment, the L-glutamine concentration measured in the culture medium was increased from 0.16 ± 0.003 mmol/L to 4.15 ± 0.02 mmol/L within 18 hours after the start of the experiment. The L-glutamine concentrations measured in the cytosolic fraction of Caco-2 lysates also increased from 0.34 ± 0.01 to 1.85 ± 0.02 mmol/L over the same time period (P <0.001, n = 3 from one of three similar experiments; Fig. 4, *A*).

In parallel studies we also documented similar time-dependent increases in L-glutamine concentrations when human colonic HT-29 cells (Fig. 4, *B*) and primary cells isolated from human colon cancers (Fig. 4, *C*) were supplemented with D-glutamine. The HT-29 cells resembled Caco-2 cells in that L-glutamine concentrations in the cell culture medium were substantially increased compared to those of the cytosol. The tumor cells exhibited a substantial increase in cytosolic L-glutamine concentrations compared to those secreted into the L-glutamine–deficient culture medium.

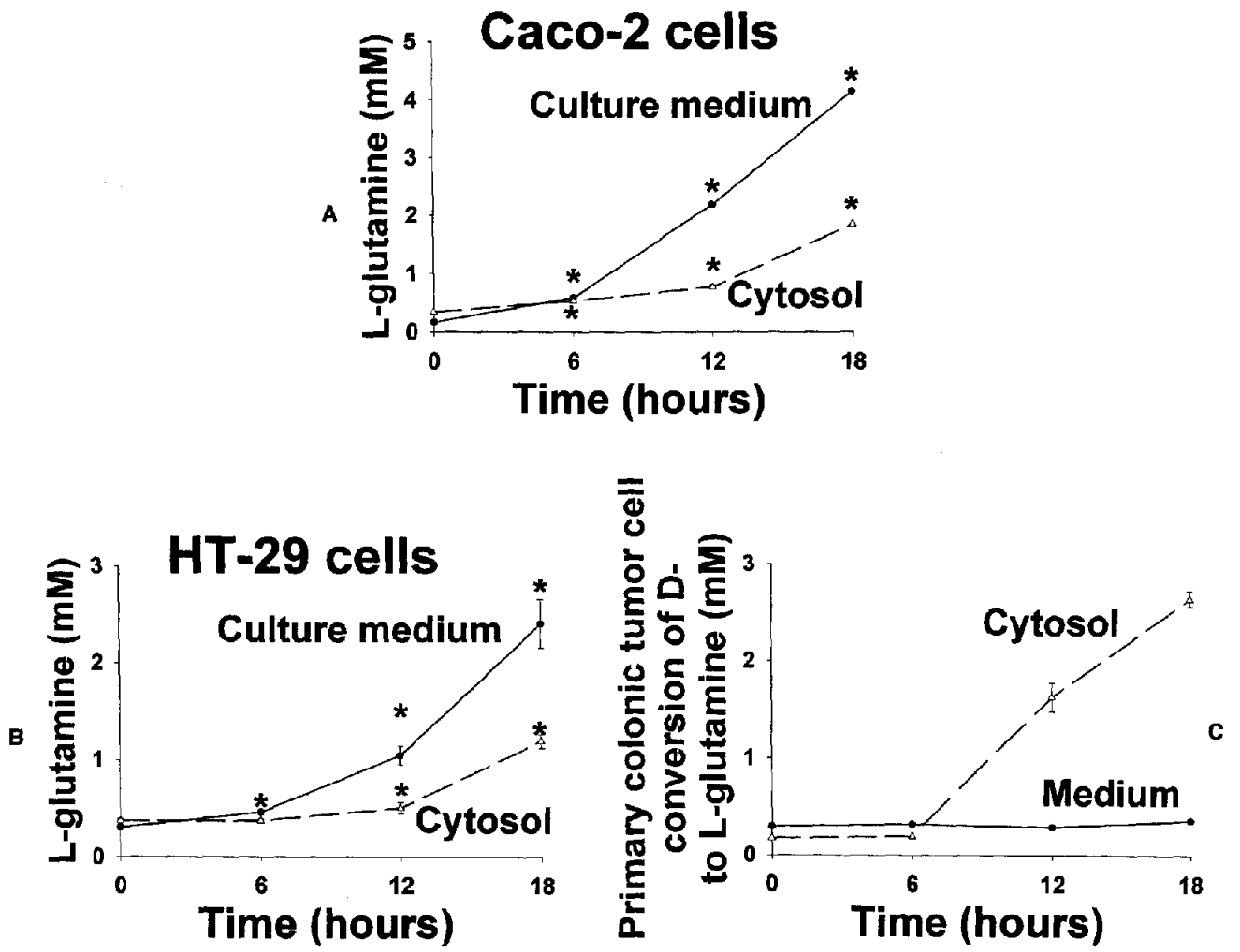

Fig. 4. Increases in measured L-glutamine concentrations in both culture medium (solid line) and cytosolic fractions of the cell lysates (dashed line) in Caco-2 cells **(A)**, HT-29 cells **(B)**, and primary malignant human colonocytes **(C)** treated with excess D-glutamine (mean ± standard error; $P < 0.005$).

DISCUSSION

Both rhythmic deformation and glutamine supplementation appear mitogenic for human intestinal Caco-2 cells, consistent with our previous observations.[12,13,24] Although it is difficult to model in vitro the complex deformations to which the bowel mucosa is subjected during peristalsis, villous motility, and passage of luminal contents, a frequency of 10 cycles per minute and an average strain of 10% would seem conservatively within the parameters of the strain patterns to which the mucosa is subjected in vivo.[12,14,15] The stimulation of mucosal deformation by non-nutritive feeding in the fasting patient may be an unexpected benefit of a therapy typically directed primarily at avoiding cholestasis.[16-18] This study raises the possibility, at least based on extrapolation from an in vitro model, that the mitogenic effects of mucosal strain may be independent of glutamine concentrations and that glutamine supplementation may have synergistic effects with increasing bowel motility since combining these two stimuli yielded substantially more stimulation than maximal stimulation by either modality alone.

Interestingly, we observed that L-glutamine and D-glutamine supplementation yielded similar effects. We previously reported that both the levo- and dextrorotatory isomers of glutamine are mitogenic for Caco-2 cells, and demonstrated that the effects of each of these glutamine isomers significantly exceeded the mitogenic effects of the levoisomers of glutamic acid, glycine, or asparagine.[24] This study extends these findings to the mitogenic effects of combinations of rhythmic strain and glutamine supplementation as well as the tendency of L-glutamine to inhibit Caco-2

brush border enzyme expression.[24] The physiologic significance of this direct decrease in brush border specific activity by glutamine supplementation remains to be established, but it represents a useful marker of glutamine effects.

If D-glutamine also modulates the Caco-2 phenotype, then what could be the mechanism of the effect of this dextrorotatory isomer? Acivicin [(alpha-S, 5S)-alpha-amino-3-chloro-4,5-dihydro-5-isoxazoleacetic acid; AT-125; NSC-163501] is an amino acid analog derived from fermentation by *Streptomyces serviceus*, which inhibits growth in several mouse tumor models. Its antitumor activity is probably mediated through the inhibition of enzymes catalyzing amido transfer from L-glutamine, especially those in the de novo pathways of purine and pyrimidine biosynthesis. The concentrations used in this study are consistent with those previously used in other cell culture models.[30-32] The inhibition of the effects of both D-glutamine and L-glutamine supplementation by acivicin suggested the possibility that a common pathway was involved and, in particular, that each was being metabolized by the Caco-2 cells. Indeed we were able to demonstrate in Caco-2 cells, as well as in a second human colonic intestinal epithelial line and also in primary colonic tumor cells, that D-glutamine supplementation resulted in increasing L-glutamine concentrations over time. We used a high (30 mmol/L) concentration of D-glutamine in these studies to maximize our chances of detecting rapid conversion using an assay of limited sensitivity. Whether this increase in L-glutamine concentrations represents the direct activity of an amino acid racemase, which converts amino acid optical isomers,[33] catabolism of D-glutamine to L-glutamine and subsequent synthesis of L-glutamine, or induction of L-glutamine synthetic enzymes of D-glutamine awaits further exploration. Although many biological systems preferentially use L-isomeric amino acids,[34] preferential interaction of some enzymes with D-isomeric amino acids has previously been described.[35]

The mechanism of the effects observed here awaits further study. However, these results suggest that glutamine and rhythmic strain are likely to stimulate human Caco-2 intestinal epithelial cell proliferation by independent mechanisms and that the mitogenic effect of D-glutamine reflects an autocrine feedback loop in which D-glutamine–treated cells synthesize L-glutamine, which then stimulates its own proliferation. Extrapolation from an in vitro cell culture model to the clinical situation must always be cautious.[36] Nevertheless, to the extent to which Caco-2 cells in culture represent a reasonable model for intestinal epithelial biology in vivo, these observations suggest that glutamine supplementation in fasting patients may be synergistic with the stimulation of bowel motility by non-nutritive feeding or by pharmacologic or electrical stimulation. Furthermore, tissue-specific variations in glutamine metabolism[37-39] might facilitate selective nutripharmaceutical targeting of the gut mucosa in the future.

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Minimally Invasive Therapies Made Possible by New Imaging Technology

MODERATORS: *L. William Traverso, M.D. (SSAT Representative), Virginia Mason Medical Center, Seattle, Wash., and Dan Deziel, M.D. (SAGES Representative), Rush Presbyterian St. Lukes Medical Center, Chicago, Ill.*

Once the technological advancements for imaging had been described at the SSAT/ASGE Joint Symposium for CT, MRI, ultrasound, and PET scanning, participants were able to listen to a more expanded description of how a variety of new imaging technologies have allowed new minimally invasive diagnoses or therapies for common bile duct stones, colorectal cancer, and esophageal diseases. Each of the authors was asked to provide a handout that was distributed to the audience. Later they were asked to rewrite their summaries for submission to JOURNAL OF GASTROINTESTINAL SURGERY. The subsections on common bile duct stones, colorectal cancer, esophageal diseases were then assigned to a Pa Care Committee member to expand and edit the tribution while working with the authors. Encl are these lecture summaries. We wish to thanl following persistent and hardworking editors:

Steven M. Strasberg, M.D., St. Louis, M Common bile duct stones

Lee Swanstrom, M.D., Portland, Ore.—Color cancer

Lee Sillin, M.D., Los Angeles, Calif.—Esoph: diseases

Presented at Digestive Disease Week, Orlando, Fla., May 16-19, 1999.