Early Treatment With Antibiotics Reduces the Need for Surgery in Acute Necrotizing Pancreatitis—A Single-Center Randomized Study

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Pancreatic infection is the main indication for surgery and the principal determinant of prognosis in acute necrotizing pancreatitis. Previous studies on the effects of antibiotics have not, however, uniformly demonstrated any reduction in the need for surgery or any decrease in mortality among these patients, although the incidence of pancreatic infections was significantly reduced. This single-center randomized study was designed to compare early vs. delayed imipenem treatment for acute necrotizing pancreatitis. Ninety patients with acute necrotizing pancreatitis (C-reactive protein >150 mg/L, necrosis on CT) were randomized within 48 hours either to a group receiving imipenem (1.0 g plus cilastatin intravenously 3 times a day) or a control group. Not included were those who had been started on antibiotics at the referring clinic, those who were taken directly to the intensive care unit for multiorgan failure, and those who refused antibiotics or might have had adverse reactions. Thirty-two patients were excluded because they were over 70 years of age (not potentionally operable) or for any study violation. There were 25 patients in the imipenem group and 33 patients in the control group. The main end point was the indication for necrosectomy due to infection (i.e., after the initial increase and decrease, there was a second continuous increase in temperature, white blood cell count [>30%] and C-reactive protein [>30%], with other infections ruled out, or bacteria were found on Gram stain of the pancreatic fine-needle aspirate). In the control group, imipenem was started when the operative indication was fulfilled. Conservative treatment was continued for at least 5 days before necrosectomy. The study groups did not differ from each other with regard to sex distribution, patient age, etiology, C-reactive protein concentration, and extent of pancreatic necrosis on CT. Two (8%) of 25 patients in the imipenem group compared to 14 (42%) of 33 in the control group fulfilled the operative indications ($P = 0.003$). Nine patients in the control group responded to delayed antibiotics but five had to undergo surgery. Of those receiving antibiotics, 2 (8%) of 25 in the early antibiotic (imipenem) group needed surgery compared to 5 (36%) of 14 in the delayed antibiotic (control) group ($P = 0.04$). Two (8%) of 25 patients in the imipenem group and 5 (15%) of 13 patients in the control group died (P = NS [no significant difference]). Seven (28%) of 25 in the imipenem group and 25 (76%) of 33 in the control group had major organ complications ($P = 0.0003$). Based on the preceding criteria, early imipenem-cilastatin therapy appears to significantly reduce the need for surgery and the overall number of major organ complications in acute necrotizing pancreatitis, and reduces by half the mortality rate; this is not, however, statistically significant in a series of this size. (J GASTROINTEST SURG 2001;5:113-120.)

KEY WORDS: Acute necrotizing pancreatitis, antibiotics, imipenem, surgery, mortality, morbidity

Various medical treatments for acute pancreatitis have been attempted over the past 40 years. The antibiotics that were used, however, appeared to be ineffective, probably because the preparations studied did not penetrate the pancreas as intended,[1] or the studies consisted mainly of patients with mild edematous pancreatitis, which is seldom found to be associated with bacterial infection.[2-4] In necrotizing pancreatitis, up to 70% of patients develop infected necrosis and this infection is the main determinant of prognosis.[5] During the 1990s, six randomized studies were published on the use of antibiotics in necrotizing

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pancreatitis. Four of the studies showed that early use of antibiotics reduces the pancreatic infection rate but only one of them showed a significant decrease in mortality among patients with acute necrotizing pancreatitis.[6-9] In the fifth study, a smoother clinical course was observed, but there was no reduction in the infection or mortality rate.[10] In the sixth study, imipenem was compared with pefloxacin and was found to be superior in reducing the incidence of infections, the need for surgery, and the mortality rate.[11]

It is widely accepted that infected necrosis is the main indication for surgery in acute necrotizing pancreatitis, whereas sterile necrosis is usually treated nonoperatively.[12] Sometimes nonoperative treatment with antibiotics has been effective even in cases of pancreatic infection.[13] If pancreatic infection is the main determinant of prognosis, if the main indication for surgery is pancreatic infection, and if early antibiotic treatment reduces the pancreatic infection rate, it should follow that early antibiotics would result in a decrease both in the number of patients requiring surgery and the number of deaths among patients with acute necrotizing pancreatitis. Surprisingly, this has not been uniformly demonstrated in previous studies possibly because of the antibiotic regimen used and difficulty in identifying the indications for surgery in multi-institutional studies. This single-center randomized study was designed to compare early vs. delayed imipenem in the treatment of acute necrotizing pancreatitis.

PATIENTS AND METHODS

From September 1995 to May 1999, a total of 90 patients were enrolled in the study. They comprise 9% of the total 1066 episodes of acute pancreatitis occurring during the study period. The population size was calculated with the assumption that reducing by half the prevalence of the main end points (i.e., the need for surgery and death) would yield statistically significant results.

The following inclusion criteria were used: diagnosis of acute pancreatitis based on clinical criteria, an increase in serum amylase activity by at least three times the upper normal range, and CT verification of pancreatitis. The diagnosis of necrotizing pancreatitis was based on a serum C-reactive protein concentration above 150 mg/L during the first 48 hours after admission and identification of necrotic areas in the pancreas with dynamic CT by the radiologist on duty. Not included were those who had been started on antibiotics at the referring clinic, those admitted directly to the intensive care unit because of early multiorgan failure, and those with frequent early need of antibiotics for other reasons. Also excluded were those who refused to participate in the study and those suspected of having a reaction to any of the study drugs. The study protocol was approved by the Ethics Committee of Tampere University Hospital, and written informed consent was obtained from all participants.

Ninety patients were randomized to the imipenem group (imipenem, 1.0 g, plus cilastatin [Tienam, Merck, Sharp & Dohme, The Netherlands] intravenously three times a day) or the control group. The dosage of imipenem-cilastatin was reduced if renal function was affected. After the randomization was complete, 32 of the 90 patients enrolled were excluded. Five were excluded because they were 70 years of age or more and were not considered suitable candidates for necrosectomy because previous studies had shown a poor prognosis in the elderly.[14] In fact, among patients we have seen, of those needing necrosectomy for severe acute necrotizing pancreatitis at the age of 70 or more, none have survived. One patient was excluded because he did not begin his antibiotic treatment as originally scheduled. After the study had been completed, all of the CT scans were re-read without knowing the patient's group or clinical history. Contrast enhancement by less than 30 Hounsfield units was set as the criterion for pancreatic necrosis. Twenty-six patients were excluded because in rereading their CT scans, it was found that this strict criterion for pancreatic necrosis had not been exclusively fulfilled, although severe peripancreatic changes were demonstrated with areas of pancreatic low enhancement. Thus the final analysis involved only 58 patients, with no differences between the imipenem and the control group (Table I).

Nonoperative, full conservative treatment was always attempted first. The three patients with gallstone pancreatitis underwent early endoscopic retrograde cholangiopancreatography. In one patient the common bile duct could not be cannulated, and in two patients stones were detected only in the gallbladder, not in the common bile duct.

The indication for surgery was infected necrosis. The diagnosis of infected necrosis was based on recurrent parallel increases, following an initial decrease, in the inflammation variables (temperature, white blood cell count [+30%], and C-reactive protein concentration [+30%]), after bronchial, urinary, and central line infections were excluded.[13] If the three inflammation variables did not increase in parallel, fine-needle aspirate was obtained under ultrasound or CT guidance from the pancreas for bacterial stain (n = 16). An attempt was made to obtain this specimen without contamination by puncture through the gastrointestinal tract.

In cases where it appeared that the indication for surgery might be, the patient was always reassessed

Table I. Comparison of patients in the two study groups

	Imipenem	Control	
No. of patients	25	33	
Men/Women	23/2	28/5	NS
Age (mean ± SD)	47 ± 8 yr	46 ± 7 yr	NS
Etiology			
Alcohol/Biliary/Other	20/1/4	25/2/6	NS
C-reactive protein (mean ± SD)	211 ± 44	214 ± 41	NS
Glasgow signs[15] (median) range)	3 (range 1-6)	3 (range 1-7)	NS
Pancreatic necrosis on CT			
<30%/30% to 50%/>50%	8/7/10	13/10/10	NS

SD = standard deviation; NS = not significant.

independently by two separate investigators (I.N. and J.S.). Surgery was considered to be indicated only when the two investigators agreed. The operation consisted of necrosectomy with an open packing technique. Of the 16 patients who met the criteria, only those in the imipenem group were treated surgically, whereas those in the control group were now started on imipenem therapy at a dosage similar to that used in the early imipenem group. The effect of this treatment was monitored for 5 days using the three inflammation parameters. If an indication persisted, or if the patient deteriorated rapidly, an operation was performed. If there was even a partial response, and the above-mentioned criteria for the operation were no longer present, the conservative treatment with imipenem was continued. Overall, based on the bacterial cultures, 11 patients needed other antibiotics besides those originally used for this study. Antibiotic treatment was continued until the patient was afebrile, with a normal white blood cell count and a C-reactive protein concentration below 50 mg/L.

Besides the need for surgery, the study parameters included mortality, morbidity, hospital stay, and intensive care unit stay. Analysis of variance or Fisher's exact test was used for statistical analysis, as indicated.

RESULTS

Two (8%) of 25 patients in the imipenem group compared to 14 (42%) of 33 patients in the control group ($P = 0.003$, Fisher's exact test) fulfilled the criteria for surgery. The two patients in the imipenem group were operated on, but the 14 in the control group were started on imipenem. Nine of 14 patients responded and avoided an operation, but the other five had to undergo surgery. Thus of those receiving antibiotics, 2 (8%) of 25 in the early antibiotic (imipenem) group needed surgery compared to 5 (36%) of 14 in the delayed antibiotic (control) group ($P = 0.04$, Fisher's exact test). Because of the study design, all patients were started on the antibiotic at least 3 days before necrosectomy.

Two (8%) of 25 patients in the imipenem group and 5 (15%) of 33 in the control group died (P = NS). Five (36%) of 14 patients in the control group who were started on imipenem later "on demand" died, compared to 2 (8%) of 25 treated early with antibiotic ($P = 0.04$, Fisher's exact test). Six patients died of sepsis syndrome despite multiple operations and changes in antibiotics based on bacterial cultures. One patient died of fulminant metabolic acidosis during hemodialysis for anuria without having undergone surgery.

Six (18%) of 33 patients in the control group and 5 (20%) of 25 patients in the imipenem group (P = NS) required other antibiotics besides those initially used in the study. In the imipenem group these included the following: clindamycin for Staphylococcus catheter sepsis in one patient, ofloxacin for urinary tract infection in one patient, and fluconazole for confirmed (one patient) or suspected (two patients) Candida superinfection. The fifth patient also received vancomycin for staphylococcal contamination at multiple sites. In the control group the following antibiotics were used: piperacillin/tazobactam for eczema possibly caused by imipenem before C-reactive protein returned to normal in one patient, ciprofloxacin for *Yersinia enterocolitica* sepsis in one patient, ciprofloxacin pills to change to oral antibiotic before C-reactive protein normalization in one patient, ceftazidime for infected pseudocyst in two patients, and ceftazidime, vancomycin, and metronidazole for postoperative colonic fistula in one patient.

In 3 of 16 patients undergoing needle aspiration bacteria were found on Gram stain. The Specimen grew staphylococci in one patient, streptococci in one patient, and *E. coli* plus enterococci in one patient. One patient was treated conservatively as previously described. He developed an infected pseudocyst that was later drained percutaneously. The remaining two pa-

Table II. Major organ complications in the two study groups

	Imipenem (n = 25)	Control (n = 33)	
Pancreas			
Pseudocyst	1	5*	NS
Diabetes	0	3	NS
Lung			
ARDS	3	7	NS
Emboli	1	0	NS
Kidney			
Oliguria >1 day	2	8	NS
Hemodialysis	0	2	NS
TOTAL	7 (28%)	25 (76%)	$P = 0.0003$

ARDS = adult respiratory distress syndrome
*Two of the pseudocysts were infected.

tients had the same bacteria isolated from the surgical specimen. In the other five patients treated with necrosectomy, indications for surgery were assessed by means of inflammatory markers. Among these five patients, the culture from the necrotic tissue was negative in one, showed *Yersinia enterocolitica* in one, Staphylococci in one, and *E. coli* in one, and was polymicrobial in one (*E. coli, Streptococcus milleri,* clostridia spp).

Five patients in the imipenem group (20%) compared to 11 in the control group (33%) had major organ complications (P = NS). These complications are shown in Table II. Nine (64%) of the 14 patients in the control group who were started on imipenem later "on demand" developed major complications, compared to 5 (20%) of the 25 who were given early antibiotics ($P = 0.008$, Fisher's exact test).

The mean intensive care unit stay was 8 days (range 0 to 88 days) with no difference between the two groups. The mean intensive care unit stay for the surviving patients was 6 days (range 0 to 88 days) with no difference between groups. The hospital stay was 20 ± 13 days with no difference between groups. The hospital stay in the surviving patients was 21 ± 14 days in the control group and 17 ± 10 days in the imipenem group (P = NS, analysis of variance).

DISCUSSION

The results of a study on surgery, morbidity, and mortality in patients with severe acute necrotizing pancreatitis are largely dependent on patient inclusion criteria and the indications used to determine the need for surgery. At the time the present study was devised, the only published study on the use of antibiotics in severe necrotizing pancreatitis was the Italian multicenter trial, which showed imipenem to reduce the incidence of pancreatic infections but not the need for surgery or the mortality rate.[6] We were also aware of the results of a single-center study from Finland (not yet published at that time), which showed a decrease in mortality with cefuroxime, although the incidence of pancreatic infections was not reduced.[8] Four other trials have since been published.[7,9-11] The inclusion criteria, indications for surgery, and antibiotic preparations used all varied, which could possibly explain the differences in the results.

We included patients with pancreatitis of any etiology, since the cause is not always known at the time the patient is admitted to the hospital. We included only those patients who met the strict criteria for necrosis (<30 Hounsfield units contrast enhancement).[16] This resulted in the exclusion of a number of patients who were randomized without fulfilling this criterion, although the radiologist on duty had also interpreted the disease as necrotizing pancreatitis in these patients. We excluded those patients who needed to be admitted directly to the intensive care unit because of fulminant disease, since these patients are usually thought to need antibiotics for multiple skin penetrations and frequent colonizations by staphylococci.[8] We also excluded those patients who were not operable because of their advanced age. We used 70 years as the age cutoff, since no patient at that age has survived necrosectomy for severe acute necrotizing pancreatitis at our institution. In fact all of our patients under the age of 70 years happened to also be younger than 65.

By now the indications for surgery in acute necrotizing pancreatitis have greatly evolved. We have gradually formulated a strict policy for assessing the indications for surgery in these patients.[13,17] The indications are (1) a second increase in inflammation parameters when urinary, bronchial, and catheter sepsis is excluded or (2) gram-positive culture of fine-nee-

dle aspirate, even with no parallel increase in inflammation parameters. One could argue against the first criterion. However, it has been previously shown to work well in these patients.[13] Also, in the present study, four of five patients operated on following this criterion demonstrated infected necrosis. We have previously demonstrated success using conservative treatment with antibiotics even when the above-mentioned indications for surgery were fulfilled[13]—therefore a 5-day period of antibiotic treatment was required before surgery. Early imipenem appeared to lessen the indications for surgery. Although two thirds of the patients in the control group with the diagnosis of infected necrosis responded to late antibiotics, the rate of surgery was still double that of the early imipenem group.

Imipenem was chosen because of its good penetration and wide coverage of the bacteria found in these patients.[1] We used the highest recommended dosage, which was twice the dosage used in the two previous imipenem studies.[6,11] Recently we found that there is a difference in the pattern of contamination of necrosis in alcoholic and biliary pancreatitis. In biliary pancreatitis the necrosis is earlier contaminated with mostly intestinal-type flora, whereas in alcoholic pancreatitis contamination occurs later with a remarkable proportion showing skin flora in the necrosis.[18] This is in accordance with the observations of Sainio et al.[8] who found staphylococci to be prevalent in the pancreatic necrosis of patients with alcoholic pancreatitis. Imipenem does not necessarily cover all species of staphylococci. In addition, superinfection with fungi may be present. Therefore other antibiotics need to be liberally administered. We administered other antibiotics in one fifth of our patients. Some investigators favor even more liberal use of vancomycin and fluconazole in addition to imipenem in severe acute necrotizing pancreatitis to cover all of the possible microbes.[8]

The mortality rate was 8% in the imipenem group and 15% in the control group. This 50% decrease in mortality was not statistically significant in a series of this size. It is worth noting that overall six of seven patients undergoing surgery died. Others have also recently found increasing mortality in the surgical treatment of acute necrotizing pancreatitis. In a recent study in Italy, all patients who developed infected necrosis despite treatment with imipenem died after necrosectomy.[11] Perhaps the recent encouraging results of conservative treatment have resulted in such lengthy delays in surgery that surgery is not performed until after the point of no return in the development of multiorgan failure in sepsis syndrome. Of the two patients who died in the early imipenem group, one had staphylococci-infected necrosis and the other had metabolic acidosis during hemodialysis with no identifiable infection. Thus we do not know whether it is earlier surgery or improved medical treatment with antibiotics possibly combined with immunomodulation that could finally eliminate virtually all deaths due to this previously devastating disease.

The number of patients having major morbidity was somewhat lower in the imipenem group compared to the control group, but the difference was not statistically significant in a series the size of this one. The total number of complications was reduced by early-onset imipenem therapy, which is in accordance with previous studies on the use of antibiotics in acute severe necrotizing pancreatitis.[6-11] It is impossible to tell which of these organ complications are caused by direct infections of the organs and which are induced purely by the cytokine storm that is triggered by pancreatic tissue necrosis and infection.

The intensive care unit stay and the hospital stay did not differ significantly between the two study groups. This may have been because of wide variations within the two groups. Patients with the most severe pancreatitis early in the course were excluded from this study. The uncomplicated initial course of pancreatitis might also partly explain the short and comparable mean intensive care unit stays in the two study groups.

Of the five study parameters (fulfilling the indications for surgery, mortality, morbidity, intensive care unit stay, and hospital stay), the indications for surgery and the overall number of organ complications could be reduced by early imipenem therapy. The difference in the number of patients undergoing surgery, dying of disease, or developing major organ complications did not reach statistical significance. This was because the number of incidents was less than predicted and some patients had to be withdrawn from the analysis. Statistical power would have been achieved by doubling the number of patients recruited for study. However, this would have prolonged the study a few more years. Meanwhile, more data have accumulated on the benefits of using antibiotics to treat patients with acute necrotizing pancreatitis, largely changing the practice.[19] We believe that the six previous studies[6-11] and the present study in its current scope strongly suggest that early broad-spectrum antibiotics are beneficial in treating patients with severe acute necrotizing pancreatitis. In fact, a meta-analysis of the first four studies showed a significant effect on mortality, suggesting the routine use of antibiotics in severe acute necrotizing pancreatitis.[20] However, none of the currently available studies, including the present one, are double blind. Therefore some believe that questions remain regarding the selection of optimal preparations, the best combina-

tions, and the proper dosages; perhaps yet another large double-blind multicenter study is needed to establish the overall benefit of early antibiotics in the treatment of severe acute necrotizing pancreatitis.

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Discussion

Dr. S. Helton (Chicago, Ill.). First, I took it for granted that all patients who met the criteria for admission to the intensive care unit (ICU) were automatically put on imipenem. Is that true? Second, what criteria did you use for stopping imipenem? What was the average time that patients were kept on the antibiotic regimen?

Dr. I. Nordback. If patients were admitted directly from the emergency room to the ICU, they were started on imipenem. The average time, I believe, was somewhat longer than 2 weeks, and the criteria for stopping were no fever, normal white blood cell count, and C-reactive protein below 50 mg/L.

Dr. D.W. Rattner (Boston, Mass.). I have three questions: (1) What was the interval from the onset of pancreatitis to operation in the patients who had surgery; (2) what was the bacteriology of the patients with infected necrosis and did you see fungal infections; and (3) of the patients in the delayed imipenem (control) group, did any have positive findings on fine-needle aspiration, undergo treatment with imipenem, and avoid surgery altogether?

Dr. Nordback. The median interval to operation was approximately 3 weeks, so we did perform some very late operations. There were some fungal infections—I believe there were three patients who had fungi found in the necrosis. Two patients underwent fine-needle aspiration and had positive cultures; these patients were placed on antibiotics and avoided surgery.

Dr. B. Gloor (Bern, Switzerland). How did you choose the patients whom you referred primarily to the ICU? I would assume that they were the ones who had the most severe disease—could you have somehow excluded them from the study?

Dr. Nordback. I think you are correct. Those patients who had early multiorgan failure that was already apparent when they were admitted to the hospital were excluded from the study, and I think they were the most severe cases.

Dr. Gloor. Can you link that impression to an APACHE score?

Dr. Nordback. No, unfortunately, I do not have APACHE scores for those patients.

Dr. A. Kumar (Delhi, India). Is it true that the patients in your control group were not on any antibiotics before they were switched to imipenem?

Dr. Nordback. That is correct.

Dr. M.G. Sarr (Rochester, Minn.). Two questions are going to arise from a statistical standpoint. First, this was a

prospective study, and I am looking for a power calculation. Did you prospectively select the number of patients you needed to come up with your estimated effect?

Dr. Nordback. Yes we did. Ninety patients were involved in the study according to power calculations. We aimed to reduce the number of patients undergoing surgery by half and achieve statistical significance. Unfortunately we had a higher rate of surgery at our hospital before this study. In this study the patients' need for surgery decreased and the power therefore diminished. Also, we were disappointed that the radiology reports were not sufficient to exclude some patients from the study, so the number of patients ultimately enrolled was decreased in that way. If we could have carried out this study so that we would have reached statistical significance, it would have required twice as many patients and 3 to 4 more years. I think there are now enough data to suggest that patients with severe acute necrotizing pancreatitis need to be started on antibiotics very early. I do not think it is ultimately necessary to conduct such a study anymore.

Dr. Sarr. Unfortunately not everyone in the audience would agree with your latter statement. You showed us that there really was no significant difference when you compared the groups as a whole, yet you selectively extracted those patients who required antibiotics. Is that appropriate?

Dr. Nordback. I used the phrase, "if one wants to compare these two groups," because I thought that someone in the audience might have wished to compare only those patients.

Dr. P. Banks (Boston, Mass.). Did this study start out as a randomized prospective trial or were there some patients who received imipenem and some who did not, and you went from there? Was it a conscious decision early on to treat certain patients with imipenem and use other patients as your control group in a randomized fashion?

Dr. Nordback. This was a randomized trial. Patients were enrolled early in the study, and there was no selection bias.

Dr. Banks. Also, this was a double-blind study so no one knew who was on antibiotics and who was being given sugar water?

Dr. Nordback. This was an open study, not a double-blind study, but we attempted to develop criteria for surgery to avoid selection bias. Also, when the other investigator came to see a patient, he did not know whether the patient was on antibiotics or not.

Dr. Banks. I think you would agree that if it started out as a double-blind study, it would have been helpful in terms of making sure that no one was influenced in any way.

Dr. Nordback. I completely agree.

Dr. Banks. I was a little confused about your indications for surgery. I certainly understand positive fine-needle aspiration, but there were other patients who met other criteria based on white blood cell count and C-reactive protein that made you confident that they probably were infected. It was unclear to me how many patients in the two groups were truly infected at the time of surgery.

Dr. Nordback. There was one patient whose operative specimen showed no bacteria, so that patient was considered to perhaps have sterile necrosis. That patient was one of the five who were operated on based on clinical and biochemical criteria without fine-needle aspiration, so there was one negative case in that respect, but four of five were considered infected according to those criteria. In our previous series, there were 12 patients who were operated on based on these criteria and, similarly, 11 in that series showed bacteria in the necrosis.

Commentary

The paper by Nordback et al. is another contribution to the currently debated topic of early antibiotic treatment in necrotizing pancreatitis. Recent controlled studies have shown these antibiotics to have a positive effect in patients with pancreatic necrosis with regard to both the rate of infected necrosis[1,2] and the severity of the disease.[3] An earlier study from Finland reported a significant reduction in mortality among patients receiving early cefuroxime, with no difference in the incidence of infected necrosis.[4] Up to now this was the only controlled series showing a decrease in mortality. Although none of these series was double blind, today there is a widespread tendency toward early use of antibiotics in severe acute pancreatitis.[5]

In this context, the Nordback study is remarkable in several regards. It compares early antibiotic treatment with imipenem (an antibiotic with proven effectiveness in necrotizing pancreatitis[1,2]) with delayed application when certain criteria for surgical treatment are fulfilled. The study design is based on the authors' clinical experience that prolongation of conservative treatment of necrotizing pancreatitis can be successful even in cases with clear indications for surgical treatment. The study shows a reduction in the number of severe complications as well as a reduction in the need for surgical necrosectomy when imipenem treatment is initiated early.

This study included a selected group of younger patients with pancreatic necrosis proved on contrast-enhanced CT scanning. Thirty-five percent of the patients enrolled in the study were retrospectively excluded because they were too old and therefore not considered candidates for surgical necrosectomy (an experience that we and others[6,7] cannot share), or retrospective analysis of the CT scan was uncertain with regard to the presence of pancreatic necrosis. Surprisingly, initiation of imipenem treatment led to clin-

prospective study, and I am looking for a power calculation. Did you prospectively select the number of patients you needed to come up with your estimated effect?

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Dr. Nordback. I used the phrase, "if one wants to compare these two groups," because I thought that someone in the audience might have wished to compare only those patients.

Dr. P. Banks (Boston, Mass.). Did this study start out as a randomized prospective trial or were there some patients who received imipenem and some who did not, and you went from there? Was it a conscious decision early on to treat certain patients with imipenem and use other patients as your control group in a randomized fashion?

Dr. Nordback. This was a randomized trial. Patients were enrolled early in the study, and there was no selection bias.

Dr. Banks. Also, this was a double-blind study so no one knew who was on antibiotics and who was being given sugar water?

Dr. Nordback. This was an open study, not a double-blind study, but we attempted to develop criteria for surgery to avoid selection bias. Also, when the other investigator came to see a patient, he did not know whether the patient was on antibiotics or not.

Dr. Banks. I think you would agree that if it started out as a double-blind study, it would have been helpful in terms of making sure that no one was influenced in any way.

Dr. Nordback. I completely agree.

Dr. Banks. I was a little confused about your indications for surgery. I certainly understand positive fine-needle aspiration, but there were other patients who met other criteria based on white blood cell count and C-reactive protein that made you confident that they probably were infected. It was unclear to me how many patients in the two groups were truly infected at the time of surgery.

Dr. Nordback. There was one patient whose operative specimen showed no bacteria, so that patient was considered to perhaps have sterile necrosis. That patient was one of the five who were operated on based on clinical and biochemical criteria without fine-needle aspiration, so there was one negative case in that respect, but four of five were considered infected according to those criteria. In our previous series, there were 12 patients who were operated on based on these criteria and, similarly, 11 in that series showed bacteria in the necrosis.

Commentary

The paper by Nordback et al. is another contribution to the currently debated topic of early antibiotic treatment in necrotizing pancreatitis. Recent controlled studies have shown these antibiotics to have a positive effect in patients with pancreatic necrosis with regard to both the rate of infected necrosis[1,2] and the severity of the disease.[3] An earlier study from Finland reported a significant reduction in mortality among patients receiving early cefuroxime, with no difference in the incidence of infected necrosis.[4] Up to now this was the only controlled series showing a decrease in mortality. Although none of these series was double blind, today there is a widespread tendency toward early use of antibiotics in severe acute pancreatitis.[5]

In this context, the Nordback study is remarkable in several regards. It compares early antibiotic treatment with imipenem (an antibiotic with proven effectiveness in necrotizing pancreatitis[1,2]) with delayed application when certain criteria for surgical treatment are fulfilled. The study design is based on the authors' clinical experience that prolongation of conservative treatment of necrotizing pancreatitis can be successful even in cases with clear indications for surgical treatment. The study shows a reduction in the number of severe complications as well as a reduction in the need for surgical necrosectomy when imipenem treatment is initiated early.

This study included a selected group of younger patients with pancreatic necrosis proved on contrast-enhanced CT scanning. Thirty-five percent of the patients enrolled in the study were retrospectively excluded because they were too old and therefore not considered candidates for surgical necrosectomy (an experience that we and others[6,7] cannot share), or retrospective analysis of the CT scan was uncertain with regard to the presence of pancreatic necrosis. Surprisingly, initiation of imipenem treatment led to clin-

ical improvement in most of the patients in the control group. In this report, information that might have explained these remarkable findings was scarce. The authors did not provide data concerning the incidence of pancreatic infection in either the treatment group or the control group since bacterial contamination of pancreatic necrosis by fine-needle puncture was not assessed routinely but only in those cases where the three inflammatory markers did not increase in parallel. Therefore it remains unclear whether the positive effect of imipenem can be attributed to prevention or elimination of pancreatic bacterial infection.

Regardless of the ongoing discussion about whether or not to operate in cases of sterile necrosis, surgical necrosectomy is the accepted standard of treatment in infected pancreatic necrosis, providing acceptable results with regard to morbidity and mortality.[6-9] In this study all but one patient with proven infected necrosis were operated on, and all but one patient undergoing surgery had infected necrosis. Necrosectomy and open packing were associated with a mortality rate of 86% (6 of 7 patients died). It is hard to explain this extraordinarily high rate of death following surgical treatment, but it could be speculated that this might be a consequence of delayed surgical treatment in patients with generalized sepsis.

Nordback et al. provide an additional piece to the puzzle concerning antibiotic treatment in severe acute pancreatitis. We can appreciate the results of this study with its low overall mortality rate of 12% among severely ill patients. However, in our opinion, these findings do not justify prolonged conservative treatment in patients with proven or strongly suspected pancreatic infection nor should they be viewed as an endorsement for indiscriminate antibiotic application in patients with severe acute pancreatitis. The use of antibiotics in this disease is not without risk,[10,11] and it might well be that in the near future, the pendulum will swing away from current propagation of widespread use back to more restricted, selected indications.

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Effect of Preoperative Chemoradiotherapy on Surgical Margin Status of Resected Adenocarcinoma of the Head of the Pancreas

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We examined the effect of preoperative chemoradiotherapy on the ability to obtain pathologically negative resection margins in patients undergoing pancreaticoduodenectomy for adenocarcinoma of the head of the pancreas. Between 1987 and 2000, 100 patients underwent Whipple resection with curative intent for primary adenocarcinoma of the head of the pancreas. Pathologic assessment of six margins (proximal and distal superior mesenteric artery, proximal and distal superior mesenteric vein, pancreas, retroperitoneum, common bile duct, and hepatic artery) was undertaken by either frozen section (pancreas and common duct) or permanent section. A margin was considered positive if tumor was present less than 1 mm from the inked specimen. Margins noted to be positive on frozen section were resected whenever possible. Of the 100 patients treated, 47 (47%) underwent postoperative radiation and chemotherapy (group I) and 53 (53%) received preoperative chemoradiotherapy (group II) with either 5-fluorouracil (32 patients) or gemcitabine (21 patients). Patient demographics and operative parameters were similar in the two groups, with the exception of preoperative tumor size (CT scan), which was greater in group II ($P < 0.001$), and number of previous operations, which was greater in group II ($P < 0.0001$). Statistical analysis of the number of negative surgical margins clear of tumor was performed using Fisher's exact test. All patients (100%) had six margins assessed for microscopic involvement with tumor. In the preoperative therapy group, 5 (7.5%) of 53 patients had more than one positive margin, whereas 21 (44.7%) of 47 patients without preoperative therapy had more than one margin with disease extension ($P < 0.001$). Additionally, only 11 (25.6%) of the 47 patients without preoperative therapy had six negative margins vs. 27 (50.9%) of 53 in the group receiving preoperative therapy ($P = 0.013$). Survival analysis reveals a significant increase in survival in margin-negative patients ($P = 0.02$). Similarly, a strong trend toward improved disease-free and overall survival is seen in patients with a single positive margin vs. multiple margins. Overall, we find a negative impact on survival with an increasing number of positive margins ($P = 0.025$, hazard ratio 1.3). When stratified for individual margin status, survival was decreased in patients with positive superior mesenteric artery ($P = 0.06$) and vein ($P = 0.04$) margins. However, this has not yet resulted in a significant increase in disease-free or overall survival for patients receiving preoperative therapy ($P = 0.07$). (J GASTROINTEST SURG 2001;5:121-130.)

KEY WORDS: Pancreas, adenocarcinoma, neoadjuvant chemotherapy, surgical margins

Adenocarcinoma of the pancreas continues to be a significant source of cancer mortality in the United States, resulting in approximately 28,000 deaths per year.[1] Characterized by vague symptoms, aggressive biology, and difficulties in early diagnosis, pancreatic cancer accounts for 2% of all malignancies but is the fifth leading cause of cancer-related deaths in the United States, with a less than 5% 5-year expected survival. At the time of diagnosis, even patients with small lesions frequently have direct extension into adjacent tissue or lymph node metastases.[2] Additionally, approximately 15% to 20% of patients are considered

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marginally resectable at presentation. Even after resection, predicted 5-year survival rates have yet to surpass 25%, with median survival times of 14 to 19 months.[3,4] Local recurrences in the pancreatic bed alone or in combination with systemic (peritoneal, liver, or lung) metastases are seen in 30% to 50% of patients undergoing presumed curative resection.[5-8] Interest in preoperative chemoradiotherapy has existed since initial reports in 1980 revealed an increase in median survival to 6 to 10 months with 5-fluorouracil (5-FU)–based chemoradiotherapy in patients with locally advanced, nonmetastatic, unresectable disease.[9-11] The addition of adjuvant chemoradiotherapy in resected patients was shown to increase both local control and actuarial 5-year survival.[12-15] These results, along with success in several other cancers, prompted a few institutions to pursue neoadjuvant chemoradiotherapy in patients with isolated pancreatic disease. Theoretical advantages of neoadjuvant therapy include treatment prior to disruption of tumor blood supply, as well as avoidance of a delay in initiating systemic therapy due to postoperative recovery. Indeed, a Gastrointestinal Tumor Study Group trial examining adjuvant chemoradiotherapy revealed a delay of therapy initiation of 10 weeks or more in 24% of patients because of postoperative complications.[14] Several institutional studies have shown similar results.[15,16]

Based on the results of 5-FU–based therapy and, subsequently, gemcitabine-based chemoradiotherapy,[9,17,18] we initiated three trials of neoadjuvant therapy in patients with documented adenocarcinoma of the pancreas. Initial results have been favorable, with improved local-regional control, but without a significant impact on survival when compared to patients receiving postoperative chemoradiotherapy.[19] In light of multiple recent studies showing a negative impact on survival for patients with positive surgical margins,[3,4,7,20] we sought to quantify the local effects of this therapy through a rigorous examination of margins in pancreatic resection specimens. At the Fox Chase Cancer Center, all pancreaticoduodenectomies performed between January 1987 and March 2000 were subjected to rigorous microscopic examination of all six pancreatic margins: superior mesenteric artery (SMA), superior mesenteric vein (SMV), retroperitoneal, pancreatic, common bile duct, and hepatic artery margins. Our series includes all patients with lesions in the head, uncinate process, or neck of the pancreas requiring pancreaticoduodenectomy. Although the examination of margins was done at the time of resection, this is a retrospective analysis of the data collected from those pathology reports. We report our results of these examinations and correlate them to the sequencing of chemoradiotherapy.

MATERIAL AND METHODS

Patient Characteristics

We reviewed the charts of all patients undergoing pancreaticoduodenectomy for adenocarcinoma of the pancreas at our institution between January 1987 and March 2000. Patients with periampullary carcinomas, other pancreatic histopathologic findings, and tumors of the body and tail were excluded. One hundred patients who underwent successful Whipple resection and had complete evaluation of pathologic margins were available for analysis. In this nonrandomized series, 47 patients received postoperative chemoradiotherapy (group I) and 53 patients were treated with neoadjuvant 5-FU–based (32 patients) or gemcitabine-based (21 patients) chemoradiotherapy (group II). All resections and pathologic evaluations were performed at Fox Chase Cancer Center, although the chemoradiotherapy was delivered at a variety of institutions. The characteristics of these 100 patients are described in Table I. There were no significant differences in patient age, sex, number of lymph nodes resected, or length of stay between the two groups. Patients receiving neoadjuvant therapy were more likely to have undergone a prior attempt at resection at a referring institution ($P < 0.0001$) and to have had a larger tumor as measured by preoperative CT scan ($P = 0.001$). Additionally, although the number of lymph nodes retrieved did not differ, there was a significant decrease in the number of involved nodes in the preoperative therapy group.

Criteria for patient assignment to either neoadjuvant or postoperative therapy are outlined in Fig. 1. Those individuals with clearly resectable tumors on preoperative CT scan or those with lesions too small for radiologically guided biopsy received postoperative adjuvant therapy, either gemcitabine or 5-FU based, many as part of Radiation Therapy and Oncology Group (RTOG) Protocol 9704. In those patients with questioned resectability, SMA or SMV invasion, larger lesions, or those judged unresectable at prior exploration, neoadjuvant therapy was instituted. The dosage and/or the drug administered was tailored based on patient proximity to Fox Chase Cancer Center, with full-dose gemcitabine being restricted to our institutional protocol of preoperative gemcitabine and radiation therapy. Of the patients treated, 25 received preoperative mitomycin C and 5-FU, either as part of an in-house pilot study (n = 11) or Eastern Cooperative Oncology Group (ECOG) PD289 (n = 14), and 24 were treated with preoperative gemcitabine/radiation therapy. An additional four patients received 5-FU–based therapy off protocol. Radiation was delivered based on three-dimensional CT planning as previously described.

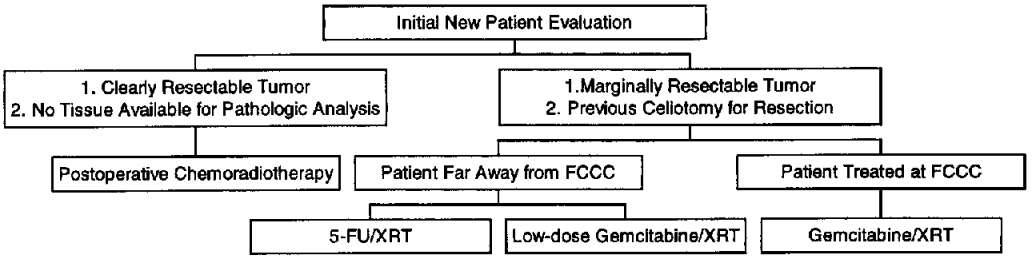

Fig. 1. Decision analysis tree. This patient treatment algorithm is presently being used at our institution. Patients with clearly resectable tumors or those in whom a preoperative tissue diagnosis cannot be obtained undergo resection with postoperative chemoradiotherapy. Patients with marginally resectable tumors or those deemed unresectable at outside institutions are treated with preoperative chemoradiotherapy. At present, the ability of a patient to receive all therapy at Fox Chase Cancer Center *(FCCC)* guides the choice of regimen. Prior to the introduction of gemcitabine, preoperative therapy was exclusively 5-FU based, either as part of RTOG Protocol 97-04 or an in-house protocol predating RTOG 97-04.

Table I. Patient characteristics

	Group I postoperative chemoradiotherapy	Group II preoperative chemoradiotherapy	*P* value
Age			
Median	67 yr	68 yr	NS
Range	41 to 85 yr	41 to 80 yr	
Sex	Male: 34.0%	Male: 45.3%	NS
Preoperative CT size			
Median	2.5 cm	4.0 cm	0.001
Range	2.0 to 5.5 cm	1.5 to 5.0 cm	
No. of lymph nodes			
Median	23.0	15.5	NS
Range	4 to 55	3 to 40	
No. of positive nodes			
Percentage of patients	74%	36%	<0.0001
Range	0 to 10	0 to 12	
Prior attempts at surgery	2	19	<0.0001
Intraoperative vascular resections	6	14	0.072

There were no significant differences with respect to age, sex, overall number of lymph nodes, or number of patients undergoing vascular resections. Patients receiving preoperative therapy were more likely to have fewer positive lymph nodes, larger tumors, and had been declared unresectable at previous laparotomy.
NS = not significant.

Postoperative Therapy

A total of 47 patients underwent primary resection with planned postoperative chemotherapy. Eight patients (17.0%) did not receive adjuvant chemotherapy because of postoperative complications, disease progression, or patient refusal. An additional seven patients are being treated at this time. Thirty-two patients were treated with 5-FU–based chemoradiotherapy either as continuous infusion (n = 17), bolus administration (n = 8), or as part of an in-house protocol (n = 7). Twenty-one patients received maintenance chemotherapy after their postoperative regimen, either 5-FU (n = 14) or gemcitabine (n = 7). The 32 patients receiving radiation therapy had a dose delivery range of 36 to 60 Gy (median 50.4 Gy). All 47 patients undergoing resection for confirmed adenocarcinoma were included in the margin analysis. The survival analyses exclude all patients with early postoperative deaths, whereas those on active treatment are censored at the appropriate date.

Neoadjuvant Chemoradiotherapy

Patients receiving preoperative therapy were those with pathologically confirmed diagnoses of pancreatic malignancy. This group of patients was largely treated

Table II. Specific margin positivity

	SMA	SMV	Retroperitoneum	Pancreas	Common bile duct	Hepatic artery
Postoperative therapy	22	16	19	8	3	1
Preoperative therapy	9	9	9	4	1	0
P value	0.002	0.065	0.014	0.218	0.100	0.470

Each of the six margins was examined for presence of tumor within 1.0 mm of the cut surface on perpendicular sections. There was a significant reduction in the number of patients with positive SMA, SMV, and retroperitoneal margins in the preoperative chemoradiotherapy group. Additionally, in both groups the majority of positive margins were found in the SMA, SMV, and retroperitoneal margins.

as part of in-house trials for locally advanced pancreatic adenocarcinoma. Others were treated off protocol or at other institutions where they received either 5-FU–, gemcitabine-, or paclitaxel-based regimens. All patients received concurrent external-beam radiation therapy in 1.8 Gy fractions to a median total dose of 50.4 Gy, with a planned target volume 3 cm around the gross target volume to 3960 cGy, and then a cone-down to 2.0 cm around the gross tumor volume for the next 1080 cGy. Significant radiographic (tumor shrinkage) or pathologic (tumor necrosis and radiation fibrosis) evidence of a response to induction therapy was documented in all patients.

Definitions of Groups for Comparison

Regardless of the chemotherapeutic agents delivered, patients were divided into two groups for pathologic margin analysis. Group I included all those patients undergoing resection of adenocarcinoma of the head of the pancreas with the intention to deliver postoperative chemoradiotherapy. In the assessment of survival data for this group, those patients who did not receive postoperative therapy were excluded (n = 8). Those patients who died without evidence of disease were censored from survival data at that time. Full survival data were available for 35 patients. Ten patients are without evidence of recurrence, whereas two patients are alive with recurrent disease.

Group II was made up of all patients receiving preoperative therapy (5-FU, gemcitabine, or taxol based). All 53 patients undergoing resection were included in the analysis of pancreatic margins. Two patients were excluded from survival analysis, one each of gemcitabine- and 5-FU–treated patients, because of early postoperative death. Complete survival data were available for 38, with an additional 13 patients alive without evidence of disease.

For determination of the impact of margin positivity on survival, patients were grouped according to the number of margins that were positive out of the six examined. In cases where the impact of a specific margin (i.e., SMA) was examined, all patients with that particular positive margin were assessed, regardless of the status of the additional margins. Therefore some patients were in more than one group on this analysis (Table II).

Intraoperative and Pathologic Specimen Labeling

All patients underwent standard pancreaticoduodenectomy with intraoperative labeling of the proximal and distal resection margins of the SMA and SMV (Fig. 2). The region around the distal SMV and SMA margins has been referred to as the uncinate margin.[16] However, we believe that examination of the entire pancreatic margin along both vessels gives more accurate pathologic data. Frozen-section analysis was done of both the cut pancreatic and common bile duct margins prior to specimen painting in the department of pathology. In instances of positive frozen-section margins, an additional resection was undertaken whenever possible. Specimen marking was completed using five colors to mark the SMA and SMV along their entire respective beds (SMV marking extended up to the portal vein margin beneath the common bile duct margin), as well as the pancreatic, common bile duct, and retroperitoneal resection margins. The retroperitoneal margin is defined as the area from the medial aspect of the duodenal sweep to the lateral aspect of the SMV/portal vein margin. All margins were examined in the plane perpendicular to each of the resection margins, with tumor classified as being at the margin, within 1 mm, or within 2 mm of the cut edge, or negative for tumor. An individual margin was considered positive if tumor was within 1 mm of the margin. Vascular margins were assessed at both the proximal and distal ends and were considered positive if either the proximal or distal end, or both, had tumor. Patients undergoing SMV or portal vein resection for local extension were considered to have a positive SMV margin if tumor was present at the resection margin, not simply intraluminally.

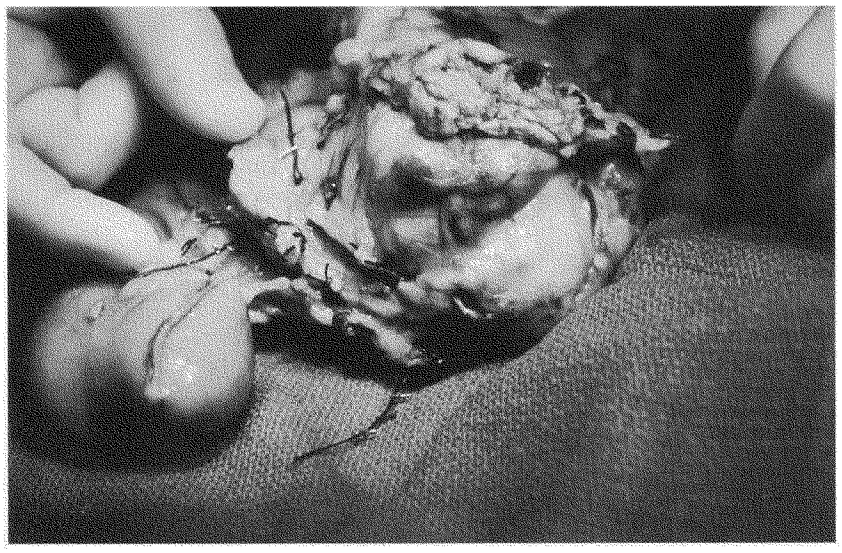

Fig. 2. Intraoperative specimen labeling. Each surgical specimen is marked with clips to show the entire course of the superior mesenteric artery (SMA) and superior mesenteric vein (SMV). The distal SMV and SMA are marked with one and two clips, respectively. Three clips mark the proximal SMV and four mark the SMA.

Statistical Analysis

Age, sex, length of stay, and morbidity and mortality were compared. Complications, including perioperative death, were classified as having occurred during the hospitalization or within 30 days of resection, whichever was longer. Differences between groups were computed by the Wilcoxon test, Kruskal-Wallis test, or Fisher's exact test, where appropriate. Disease-free survival and overall survival define the rates of disease recurrence and cancer-related deaths, respectively, and were analyzed using the method of Kaplan and Meier. Events related to recurrence or survival were measured from the time of tissue biopsy, either at surgery or before preoperative chemoradiation. Survival curves were compared by the log-rank test. Recurrences within the pancreatic resection bed were considered local-regional failures, whereas distant failure was defined as any other recurrences.

RESULTS

Comparative Analyses Among Groups

We compared age and sex in the two groups, in addition to a number of intraoperative and postoperative parameters (see Table I). Although there was a significant increase in estimated blood loss and length of operation in patients receiving neoadjuvant chemotherapy (group II), overall length of hospitalization, number of major complications, and postoperative mortality were not altered (see Table I).

Fourteen patients (26.4%) in group II required vascular reconstruction, including seven portal vein/SMV and six hepatic artery procedures, as opposed to six patients (12.8%) who required vascular procedures in group I ($P = 0.072$). In group I there were no postoperative deaths and the overall complication rate was 23.4%. By comparison, 2 of 53 patients in group II experienced postoperative death along with 18 complications (34%). Postoperative complications included sepsis, cholangitis, and myocardial infarction and are listed in Table III.

Local Recurrence

Patients were followed for recurrence with chest radiography and abdominopelvic CT at 6-month intervals and when dictated by symptoms or rising tumor markers (CA 19-9 and CEA). Local recurrence was defined as lesions occurring in the pancreatic bed, with disease of the peritoneum, retroperitoneum, liver, lung, and other organs being defined as distant metastases. No significant patterns of radiologically detected recurrence were noted between the two groups ($P = 0.411$). Group I (excluding patients not receiving postoperative therapy) had two documented local recurrences (5.4%) compared to five in group II (9.4%). These rates are both lower than those in other reported series, probably because they are largely derived from imaging studies rather than autopsies, and often represent the first site of recurrence.

Table III. Postoperative complications

Complications	Postoperative therapy	Preoperative therapy
Wound infection	0	2
Subphrenic abscess	0	1
Cholangitis	1	3 (1)
Adult respiratory distress syndrome	0	1
Ascites	0	4
Gastrointestinal bleeding	0	1
Sepsis	1	2 (1)
Chylous ascites	0	3
Pancreatic fistula	2	0
Gastric outlet obstruction or dysfunction	2 (1)	1
Small bowel obstruction	0	2 (1)
Death	0	2

Complications related to surgery are listed above. There is no significant difference between the two groups. Numbers in parentheses indicate patients requiring reoperation for complications.

Table IV. Number of positive margins

	None	One	Two	3 or more
Postoperative therapy	11	14	13	9
Preoperative therapy	27	21	4	1
P value	0.013	<0.001	0.004	0.004

On evaluation of all six surgical margins, a significant reduction in the number of patients with multiple and single positive margins was seen.

Analysis of Pancreatic Margins

On overall assessment of margin status, among patients in group I 11 (23.4%) were found to have zero positive margins and 14 (29.8%) showed one positive margin. Among the 53 patients in group II, the margin-positive count was zero in 27 (50.9%) and one in 21 (39.6%) (Table IV). Statistical significance was reached for comparisons between the two groups with regard to zero ($P = 0.004$) and one or fewer ($P < 0.001$) positive margins. Nine patients (19.2%) in group I had three or more positive margins versus one (1.9%) in group II ($P = 0.004$).

There was great variability in the specific positive margins observed, both within and between groups I and II (see Table II). A positive SMA margin was noted in 22 (46.8%) of 47 patients in group I, compared to nine patients (17.0%) in group II ($P = 0.002$). There were also significant differences observed ($P = 0.014$) at the retroperitoneal margin between groups I and II, where 19 (40.4%) of 47 patients in group I had positive margins versus 9 (17.0%) of 53 in group II. Similarly, SMV margin-positive rates were greater in group I (16 [34.0%] of 47 patients), than in group II (9 [17.0%] of 53 patients). Statistical significance, however, was not reached ($P = 0.065$). All three of the other margins examined (pancreas, common bile duct, and hepatic artery) had decreased rates of margin positivity in group II in comparison to group I (see Table II).

Although there were nine positive margins at each of three margins (SMA, SMV, and retroperitoneum) in group II, only three patients had a combination of positive SMA and SMV margins, one of whom also had a positive retroperitoneal margin. The remaining patients had one of the three margins positive, alone (n = 18) or with positive pancreatic (n = 1) or common bile duct (n = 1) margins. Overall, these 27 positive margins were found in 23 patients and represent 48% (27 of 32) of the positive margins found in the neoadjuvant therapy group. In group I, SMA, SMV, or retroperitoneal margins were positive in 21, 16, and 19 patients, respectively. In these patients, a positive SMA, SMV, or retroperitoneal margin was associated with an additional positive margin in 45 (80.4%) of 56 patients.

Survival Analysis Based on Margin Status

Survival data were calculated based on both the number of positive margins and specific margin positivity. As previous investigators have shown, there is an improvement in disease-free and overall survival in patients with negative resection margins. A log-rank analysis of Kaplan-Meier disease-free and overall survival is shown in Figs. 3 and 4, respectively, with patients stratified by zero, one, two, or three or more positive margins, regardless of the chemoradiotherapy sequence. Over the range of observed number of positive margins (0 to 4), improved disease-free and overall survival is seen, with tests for equality values of

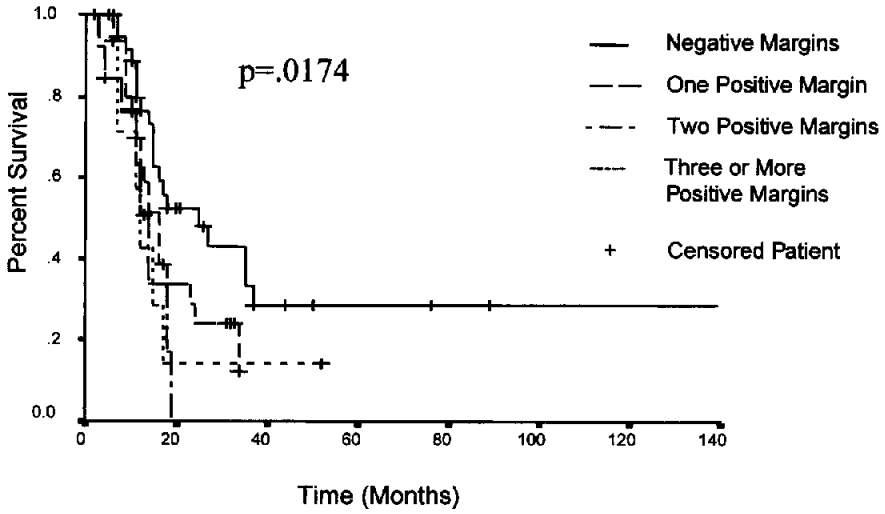

Fig. 3. Disease-free survival for all patients undergoing pancreaticoduodenectomy with respect to number of positive surgical margins.

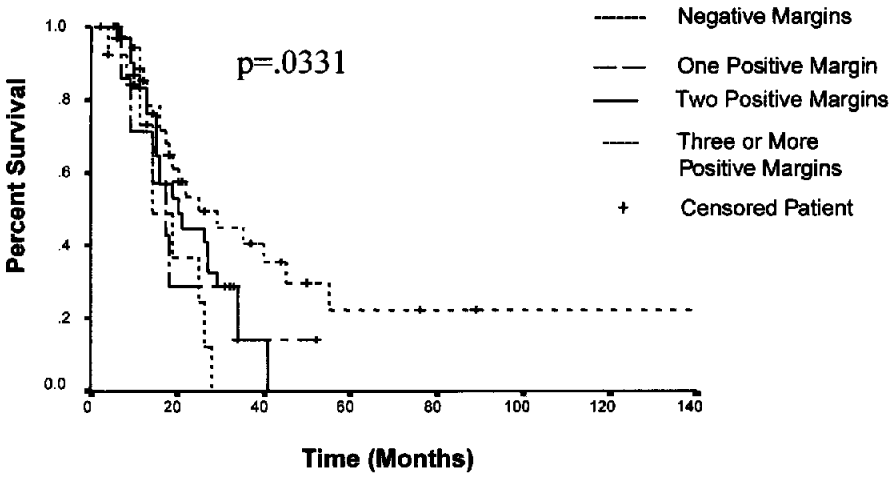

Fig. 4. Overall survival for all patients undergoing pancreaticoduodenectomy with respect to number of positive surgical margins.

$P = 0.04$ and $P = 0.05$, respectively. With regard to number of positive margins, patients with no positive margins showed a significant improvement in disease-free ($P = 0.01$) and overall ($P = 0.02$) survival. There is a trend toward increased disease-free and overall survival ($P = 0.03$ and $P = 0.04$, respectively) in patients with one or fewer positive margins when compared to those with more than one. Statistical significance, however, is not achieved with the use of the Bonferroni correction (significance achieved at $P = 0.02$) for multiple comparisons. When stratified for the sequence of chemoradiotherapy, there is no change in this relationship. However, neither treatment category has sufficient patients to reach statistical significance.

A Cox proportional hazards analysis was performed examining the effects of increasing numbers of positive margins on overall survival. When all patients were considered together (groups I and II), incremental increases in the number of positive margins correlated with decreased survival ($P = 0.0255$, hazard ratio 1.3). We then looked to see if there was an interaction between chemoradiotherapy and the impact of margin positivity. Although the difference between the two treatment groups with regard to the significance of each increase in the number of posi-

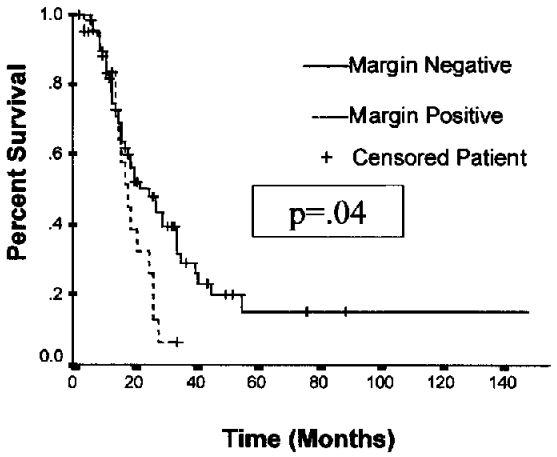

Fig. 5. Overall survival in all patients undergoing pancreaticoduodenectomy with respect to superior mesenteric vein margin status.

tive margins was not statistically significant, increasing the number of positive margins appears to have a stronger negative impact on survival in the postoperative therapy group than in the preoperative therapy group (hazards ratios of 1.68 and 1.10, respectively).

Disease-free and overall survival analyses were based on the presence of a specific positive margin. The presence of a positive SMV margin, alone (n = 11) or in combination with other margins (n = 18), results in a decrease in disease-free ($P = 0.009$) and overall ($P = 0.04$) survival as depicted in Fig. 5. This holds true for SMA-positive patients with regard to disease-free survival ($P = 0.01$), with a trend toward significance in overall survival ($P = 0.06$). In our series, disease-free and overall survival are not affected by the status of the retroperitoneal ($P = 0.517$ and $P = 0.359$) or pancreatic ($P = 0.374$ and $P = 0.448$) margin. When individual margin survival data are stratified for the sequencing of chemoradiotherapy, preoperative vs. postoperative, there is no difference in survival for a given positive margin.

Impact of Superior Mesenteric Vein Resection

A significant difference in the number of SMV resections exists between groups I and II. A trend toward increased disease-free and overall survival was maintained between the two groups when patients undergoing vascular resections were excluded ($P = 0.073$).

DISCUSSION

Neoadjuvant chemoradiotherapy for pancreatic adenocarcinoma has been examined for the treatment of resectable, potentially resectable, and locally advanced tumors at several centers. Reports from Fox Chase Cancer Center,[19] the University of Texas M.D. Anderson Cancer Center,[16] and Stanford University[21] have shown an increase in negative margin resection specimens in patients undergoing preoperative therapy for radiologically resectable and marginally unresectable lesions, respectively. All three institutions have reported a significant reduction in the amount of viable tumor in the resection specimens. As yet, a survival advantage has not been seen for preoperative therapy, but randomized trials have yet to be done. Additionally, patients selected for preoperative chemoradiotherapy usually have more advanced tumors.

The goal of the present study was to see whether preoperative chemoradiotherapy had an effect on surgical margins as compared to no preoperative therapy. A second goal was to see if the presence of multiple positive margins had an impact on survival versus single positive margins. Although previous investigators have concluded that the presence of margin positivity alone is a predictor of poor outcome, this was usually based on analysis of those margins at the retroperitoneum and cut surface of the pancreas only. By examining all six margins, we believe that we are defining a more accurate representation of the true resection margins. Patients receiving preoperative therapy (group II) showed a significant increase in the number of specimens with zero or one positive margin. This correlated with increased disease-free survival and overall survival in patients with zero positive margins and a strong trend toward the same in patients with a single positive margin. This was not a randomized trial, and in fact represents patients from several clinical trials. Thus survival data must not be given too much emphasis. Nonetheless, the presence of fewer tumor-positive resection margins in 53 patients with borderline resectable tumors or those declared inoperable at previous operation is evidence for significant local effects of chemoradiotherapy. The effect of previously being declared unresectable at other institutions had been addressed previously by our group.[22]

Interestingly, when stratified for particular margin positivity, two of the three margins showing signifi-

cant numbers of positive margins, and in which significant effects of preoperative therapy are seen, are those not typically analyzed by pathologists (SMA and SMV). When we reviewed the effect of specific margin positivity on survival, regardless of sequencing of chemoradiation, the presence of tumor at the SMV margin was the only one showing a decreased survival. There was, additionally, a strong trend toward decreased survival in those patients with SMA positive margins. It makes sense that therapy credited with decreasing the rate of local recurrence would show the greatest effect on these two margins, as CT or intraoperative gross involvement of either is a frequent source of patients being judged unresectable. Our study, however, does not show a reduction in local recurrence with preoperative therapy. In fact, in our study, the rate of recurrence is lower in both groups than in previous reports. The reason for this is unclear, but our recurrence rate is based on CT analysis, not autopsy data, and more accurately reflects the site of first recurrence.

Because of the nonrandomized nature of this study, analysis is hazardous. One would expect to see increased survival in a group of patients with better local control of the primary malignancy. In this disease the presence of early, subclinical lymphatic and distant spread is quite common. Thus effects on local disease may not result in better control of systemic recurrence. It is also possible that the trend toward survival improvement with preoperative therapy represents earlier treatment with systemic chemotherapy rather than a significant local effect.

The time required to give the chemoradiotherapy allows for the identification of some patients with metastatic disease while on therapy and thus should increase the survival of those receiving preoperative therapy. However, patients undergoing preoperative therapy are more likely to have larger tumors and to have been deemed unresectable on previous evaluation. Therefore a phase III trial of preoperative versus postoperative chemoradiotherapy is required before we can appreciate the differences in survival effects, if any. Even with such a trial, the advanced stage of this disease at diagnosis, with presumed frequent lymphatic and systemic micrometastases, may make any impact on survival impossible until better systemic chemotherapy is available.

CONCLUSION

We have demonstrated that preoperative chemoradiotherapy results in statistically significant decreases in single and multiple positive margins. The fact that multiple positive margins show further decreases in survival relative to single positive margins indicates that this intensive method of margin examination is indicated in the study of all pancreatic specimens resected in the treatment of pancreatic neoplasms. More accurate and complete staging of these tumors will allow for better prognostication as well as better comparison of treatment regimens.

We thank all of the pathologists at Fox Chase Cancer Center for their diligence in the analysis of these pancreatic specimens. Additionally, we are indebted to Tina Rader, Pathologists' Assistant at Fox Chase Cancer Center, for her excellent and methodical gross dissection of specimens for histopathologic examination.

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Discussion

Dr. M.F. Brennan (New York, N.Y.). To interpret the conclusion that preoperative chemotherapy decreases the positive margin rate, we need to know how many people did go on to resection.

Dr. J.F. Pingpank. In our earlier trials, approximately 50% of patients did not go on to surgical resection. In the most recent gemcitabine trial, approximately 75% have had resections.

Dr. D.W. Rattner (Boston, Mass.). It appeared to me, although you did not present the data by TNM stage, that the neoadjuvant group had more advanced disease to start with, at least as measured by tumor size. Did you analyze your data stage for stage to look for any benefit to neoadjuvant therapy? It seems intuitive that if the two groups did equally well, with one group having more advanced disease than the other, there is, in fact, a benefit to the neoadjuvant therapy.

Dr. Pingpank. These patients are not randomly assigned, so we knew that if there was any bias, it was toward having more advanced disease in the preoperative therapy group. We realize that comparing overall survival in these nonrandomized groups can be erroneous, but our survival curve shows that there is a trend toward increased survival favoring the neoadjuvant group ($P = 0.07$) when the two postoperative deaths in the neoadjuvant group and the eight patients in the postoperative group who did not receive any adjuvant therapy are excluded.

Dr. H.A. Reber (Los Angeles, Calif.). The issue of whether or not neoadjuvant therapy is appropriate for this disease is a critical issue about which many of us are having discussions. Are you planning to conduct a prospective study so that you will answer the question for us definitively?

Dr. Pingpank. Our goal would be to undertake a prospective randomized trial in two comparable groups to assess the effects of preoperative therapy. It will require the participation of the cooperative trial organizations.

CORRECTION

In the January/February 2001 issue of the *Journal of Gastrointestinal Surgery*, in both the Table of Contents (p. 3a) and on page 1, the Pancreas Club Symposium entitled, "Pancreatic Cancer: The Role of Adjuvant Therapies," was erroneously listed under the heading, "2000 SSAT Pancreas Club." The Pancreas Club is an independent, incorporated, nonprofit medical organization which meets at the same time as the SSAT, and there is great overlap of the memberships, but the two organizations are separate. The Editors regret this error.

Angiogenesis Inhibitor TNP-470 Reduces Human Pancreatic Cancer Growth

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In this study we investigated the effects of the angiogenesis inhibitor TNP-470 on human pancreatic cancer cells in vitro and in vivo. The action of TNP-470 on vascular endothelial growth factor (VEGF) was also assessed. In vitro human pancreatic cancer cells (MIAPaCa-2, AsPC-1, and Capan-1), and human umbilical vein endothelial cells (HUVEC) were exposed to increasing concentrations (1 pg/ml to 100 μg/ml) of TNP-470. Cell proliferation was assessed after 3 days by cell count and MTT assay. In vivo, 5×10^6 pancreatic cancer cells were injected subcutaneously into nude mice. Four weeks later, 1 mm^3 fragments of the resulting tumors were implanted into the pancreas of other mice. Animals received either TNP-470 (30 mg/kg every other day) or vehicle subcutaneously for 14 weeks. The volume of the primary tumor and metastatic spread were determined at autopsy. Concentrations of VEGF were determined in serum ($VEGF_S$) and ascites ($VEGF_A$) by enzyme-linked immunosorbent assay. Microvessel density was analyzed by immunohistochemistry in CD31-stained tumor sections. In vitro, proliferation and viability of the human pancreatic cancer cell lines were significantly inhibited at high concentrations of TNP-470 (>1 μg/ml). In contrast, TNP-470 effectively decreased the growth of HUVEC at 100 pg/ml. In vivo, tumor volume and dissemination scores were significantly lower in all three pancreatic cancer cell lines. $VEGF_S$ and $VEGF_A$ were not different between treated groups. Treatment with TNP-470 significantly reduced neoangiogenesis in tumors of all three human pancreatic cancer cell lines: MIAPaCa-2 = 74.8 ± 7.8/0.74 mm^2 vs. 24.8 ± 3.7/0.74 mm^2; AsPC-1 = 65.3 ± 5.0/0.74 mm^2 vs. 26.0 ± 3.4/0.74 mm^2; and Capan-1 = 82.2 ± 5.8/0.74 mm^2 vs. 26.9 ± 2.5/0.74 mm^2 ($P < 0.001$). However, survival was not statistically different between groups. TNP-470 reduced tumor growth and metastatic spread of pancreatic cancer in vivo. This was probably due to the antiproliferative effect of the agent on endothelial cells rather than to the direct inhibition of pancreatic cancer cell growth. TNP-470 activity was not associated with alteration of VEGF secretion. (J GASTROINTEST SURG 2001;5:131-138.)

KEY WORDS: Pancreatic cancer, TNP-470, angiogenesis, endothelial cell, vascular endothelial growth factor

Exocrine pancreatic cancer is now the fifth leading cause of cancer death in the United States, Japan, and Europe, with an overall 5-year survival rate of less than 5%.[1] Even after curative resection, the 5-year survival rates achieved at specialized centers are below 20%, and the majority of patients surviving 5 years will still die of metastatic cancer recurrence.[2] Unfortunately, conventional adjuvant treatments such as radiation therapy and chemotherapy in various combinations have not improved long-term survival after resection.[3] Thus novel treatment strategies directed against this malignancy are being pursued aggressively.

As with all solid tumors, pancreatic cancer depends on the process of neoangiogenesis, the formation of tumor blood vessels, for both local and metastatic growth beyond the size of a few cubic millimeters.[4] Inhibition of neoangiogenesis is a new and attractive

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target for tumor therapy, since it theoretically offers the hope of long-term control of tumor progression. Inhibitors of angiogenesis can be classified into two groups. Factor-specific substances have been developed to neutralize the proangiogenic regulators of neovascularization such as vascular endothelial growth factor (VEGF).[5] Factor-nonspecific inhibitors of neoangiogenesis directly target endothelial cell proliferation. TNP-470 (O-[Chloroacetylcarbamoyl] fumagillol), an analogue of fumagillin derived from *Aspergillus fumigatus*, inhibits neoangiogenesis both in vivo and in vitro as a factor-nonspecific compound.[6] Numerous investigators have reported that TNP-470 inhibits the growth and metastasis of rodent tumors derived from human cancer cell lines. The aims of this study were to assess the effect of TNP-470 on the proliferation of a variety of human pancreatic cancer cell lines in vitro and to evaluate the therapeutic potential of TNP-470 in a clinically relevant orthotopic nude mouse model of human pancreatic cancer.

MATERIAL AND METHODS

Antiangiogenic Drug

TNP-470 was provided by TAP Pharmaceuticals, Inc. (Deerfield, Ill.). Its structure and metabolism have been described previously.[6] For in vitro use, TNP-470 was dissolved in 0.5% ethanol and the respective cell culture medium. For in vivo experiments, TNP-470 was suspended in a vehicle composed of 0.5% ethanol and 5% gum arabic in physiologic saline solution.

Cell Lines and Culture Conditions

The following human pancreatic adenocarcinoma cell lines were obtained from American Type Culture Collection (Rockville, Md.): MIAPaCa-2 (undifferentiated), AsPC-1 (poorly to moderately differentiated), and Capan-1 (moderately to well differentiated). MIAPaCa-2 and Capan-1 cells were cultured in Dulbecco modified Eagle medium (DMEM; Gibco, Grand Island, N.Y.) and AsPC-1 in RPMI-1640 (Gibco). Human umbilical vein endothelial cells (HUVEC) were also purchased from American Type Culture Collection and maintained in RPMI-1640. All media were supplemented with 10% heat-inactivated fetal bovine serum (FBS; Gibco), penicillin G (100 U/ml), and streptomycin (100 μg/ml). The cells were incubated at 37° C in humidified air with 5% (AsPC-1, Capan-1, and HUVEC) or 10% carbon dioxide (MIAPaCa-2). The medium was replaced twice weekly, and cells were maintained by serial passage after trypsinization with 0.1% trypsin.

In Vitro Assessment of Cell Proliferation and Viability

To examine the effect of TNP-470 on in vitro cell proliferation, 2×10^5 cells from each cell line were seeded in six-well culture plates in 2 ml of the respective cell culture medium. The medium was changed the next day (day 1) and TNP-470 was added in the following concentrations: 0, 10 pg/ml, 100 pg/ml, 1 ng/ml, 10 ng/ml, 100 ng/ml, 1 μg/ml, 10 μg/ml, and 100 μg/ml. After 72 hours (day 4), the cells were trypsinized and counted in a standard hemocytometer. Cell viability was assessed by a monotetrazolium (MTT)-based colorimetric assay (Boehringer, Mannheim, Germany) according to the manufacturer's instructions.[7] Briefly, 5×10^3 cells from each cell line were seeded in 96-well culture plates in 200 μl of the respective cell culture medium. The medium was changed the next day (day 1) and TNP-470 was added as described earlier. After 72 hours (day 4), 10 μl of the MTT solution (5 mg/ml) was added to each well. After an additional 4-hour incubation, 100 μl of 10% sodium dodecyl sulfate (SDS) solution was added. The plates were allowed to stand overnight in an incubator (37° C, 5% carbon dioxide). Absorbance at 570 nm, which has been shown to strongly correlate with the number of viable cells, was then determined by means of a microplate enzyme-linked immunosorbent assay reader (Biotek Instruments, Inc., Burlington, Vt.).

Laboratory Animals and Orthotopic Implantation Technique

Four-week-old male nude mice (Crl:NU/NU-*nu*BR), weighing 20 to 22 g, were obtained from Charles River Laboratories (Wilmington, Mass.). The animals were housed in microisolator cages with autoclaved bedding, food, and water. The mice were maintained on a daily 12-hour light/12-hour dark cycle. All experiments were conducted in accordance with the national guidelines for the care and use of laboratory animals, and the experimental protocol was approved by the Chancellor's Animal Research Committee of the University of California, Los Angeles.

Donor nude mice were anesthetized with isoflurane (Metofane; Malinckrodt Veterinary, Mundelein, Ill.) inhalation; 5×10^6 cells from each human pancreatic cancer cell line were injected subcutaneously into the flanks of the animals. The mice were killed by a lethal dose of pentobarbital (0.5 mg/g body weight) after 3 to 4 weeks, when the subcutaneous tumors had reached a size of 1 cm in the largest diameter. The donor tumors were harvested under strict aseptic conditions and minced with a scalpel (No. 11) into small fragments, 1 mm^3 in size.

Tumor recipient nude mice were anesthetized with isoflurane (Metofane) followed by an intraperitoneal injection of sodium pentobarbital (Nembutal; Abbott Laboratories, North Chicago, Ill.; 0.05 mg/g body weight). The animals' abdomens were opened by a midline incision under aseptic conditions at a laminar air flow working bench. Either the duodenal loop with the head of the pancreas or the tail with the spleen was gently exteriorized. Two small tissue pockets were prepared in the pancreatic parenchyma as an implantation bed with a microscissors (RS-5610 Vannas; Roboz, Rockville, Md.). One donor tumor fragment was placed into each pancreatic tissue pocket in such a way that the tumor tissue was completely surrounded by pancreatic parenchyma. The pancreas was relocated into the abdominal cavity, which was then closed in two layers with 5-0 absorbable sutures (Dexon "S"; Davis & Geck, St. Louis, Mo.).

In Vivo Treatment With TNP-470

Sixty animals (20 per pancreatic cancer cell line) were randomly allocated to either a treatment or control group. Treatment with TNP-470 (30 mg/kg subcutaneously every other day) or vehicle was started 3 days after orthotopic tumor implantation. The mice were monitored daily for their clinical condition, weighed weekly, and killed by a lethal dose of sodium pentobarbital (0.5 mg/g body weight) 14 weeks after orthotopic tumor implantation or cell injection. According to the guidelines of the Chancellor's Animal Research Committee of the University of California, Los Angeles, animals had to be killed earlier if one of the following occurred: (1) bulky tumor mass with a visible tumor size >1.5 cm; (2) formation of ascites with visible abdominal distention; or (3) jaundice and/or cachexia associated with significant clinical deterioration of the animal.

All animals underwent autopsy at the end of the observation period. The perpendicular diameters of the primary orthotopic tumor were measured with calipers, and the volume was calculated using the following formula: volume = length × width × depth/2. A dissemination score was developed to assess local tumor infiltration as well as distant metastasis. Local infiltration was determined at the following sites: spleen, stomach, liver (hilum), kidney (hilum), retroperitoneum, diaphragm, mesentery, bowel loops, and abdominal wall. Isolated tumor nodules with no anatomic connection to the primary lesion were judged as distant metastases. The sites of evaluation included the liver, kidney, spleen, lung, diaphragm, mesentery, retroperitoneum, mediastinum, and the suture line. Tumor dissemination was quantified as follows: every manifestation of tumor infiltration or metastasis was credited with one point. Additional points were awarded for massive local infiltration (e.g., including more than half of the circumference of the spleen), multiple metastatic nodules (>1 in parenchymal organs; >10 on the diaphragm, mesentery, or retroperitoneum), and metastatic nodules larger than 50 mm^3. Clinical consequences of the tumor growth were incorporated into this scoring system as follows: formation of ascites (2 points if volume >5 ml); development of jaundice, ileus, and cachexia.

The primary tumor and all sites of potential infiltration or metastasis were harvested, fixed in paraformaldehyde, and embedded in paraffin. Five-micron thin tissue sections were obtained and stained with hematoxylin and eosin for microscopic examination. The sections were reviewed to confirm the findings of the macroscopic dissemination score.

Microvessel Density

Anti-CD31 was used as an endothelial marker to highlight intratumoral microvessels. Human pancreatic cancer xenograft tumors grown in the pancreas of nude mice were immediately fixed in 10% neutral buffered formalin and embedded in paraffin. Tissue sections (3 μ) were deparaffinized and rehydrated, and target retrieval was carried out by autoclaving tissues at 97° C for 30 minutes in 0.01 mol/L citrate buffer (pH 6.0) followed by a 5-minute treatment in 3% hydrogen peroxide solution to block endogenous alkaline phosphatase activity. After blocking slides for 10 minutes, a purified antimouse CD31 (PECAM-1) antibody (BD PharMingen, San Diego, Calif.) was applied in a 1:20 dilution and incubated at 4° C overnight. After thorough rinsing in Tris-buffered saline–Tween solution, slides were incubated with a biotinylated secondary antibody for 20 minutes, followed by a 15-minute incubation with streptavidin-peroxidase. For color development, slides were incubated for 5 minutes in (3,3′-diaminobenzidine tetrahydrochloride (DAB). Microvessel density was quantified as described by Weidner and Folkman.[8] Areas of highest neovascularization were found by scanning the sections at low power (×40 and ×100 total magnification). Individual microvessel counts were made on 10 ×200 fields (0.74 mm^2 per field).

Determination of VEGF Protein Levels in Plasma and Ascites

Blood and ascites (if present) from each animal were collected at autopsy. Then 500 μl of each sample was immediately placed in a tube containing 50 μl of

EDTA and subsequently centrifuged at 2000 rpm for 5 minutes. The samples were stored at −70° C until examination. The amount of VEGF protein in plasma and ascites was determined with a solid-phase enzyme-linked immunosorbent assay kit (Quantikine; R & D Systems, Minneapolis, Minn.) according to the manufacturer's instructions.

Statistical Analysis

Data are presented as mean ± standard error of the mean (SEM). Continuous normally distributed variables were analyzed by Student's *t* test. Differences in survival were analyzed by chi-square test. A P value <0.05 was considered statistically significant.

RESULTS

In Vitro Effects of TNP-470

As shown in previous studies, TNP-470 significantly decreased cell proliferation and viability of HUVEC at concentrations as low as 100 pg/ml (Fig. 1). In contrast, 10^6 times higher concentrations of the drug were necessary to effectively inhibit cell proliferation and viability of the human pancreatic cancer cell lines MIAPaCa-2, AsPC-1, and Capan-1 (see Fig. 1).

Volumes of Primary Tumors

In vivo treatment with TNP-470 significantly reduced the volumes of orthotopic pancreatic tumors in all groups. The size of tumors derived from the undifferentiated MIAPaCa-2 cell line was reduced by approximately half (1847 ± 295 mm³ vs. 3548 ± 299 mm³, P <0.001; Fig. 2, *A*). Treated animals in the AsPC-1 group developed tumors with two thirds the average volume of the control group (918 ± 152 mm³ vs. 1372 ± 132 mm³, P = 0.04; Fig. 2, *B*). Well-differentiated Capan-1 tumors were reduced by approximately 80% by treatment with TNP-470 (261 ± 18 mm³ vs. 1392 ± 144 mm³, P <0.001; Fig. 2, *C*).

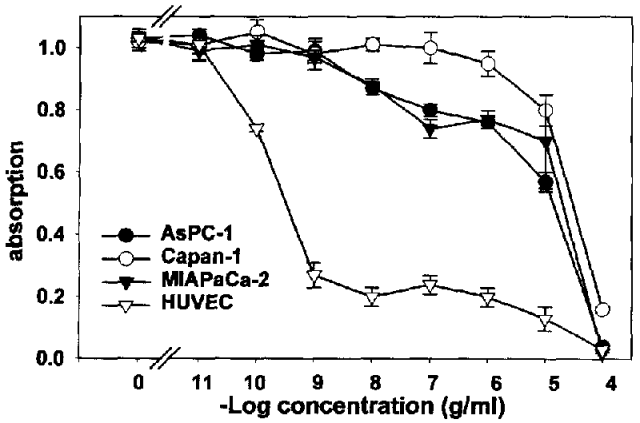

Fig. 1. Effect of TNP-470 on cell proliferation as shown by MTT assay. A dose-dependent effect on human umbilical vein endothelial cells *(HUVEC)* was observed. TNP-470 inhibited all pancreatic cancer cell lines, but this effect was seen at very high doses.

Dissemination Score

Local infiltration and distant metastasis were summarized by a dissemination score. Treatment with TNP-470 resulted in a statistically significant reduction of tumor spread. The score was diminished from 12.9 ± 1.4 points to 7.5 ± 1.5 points in the MIAPaCa-2 group (P = 0.02; Fig. 3, *A*). Control animals with tumors derived from the poorly differentiated AsPC-1 cell line reached the highest score (16.6 ± 1.1 points), and this was reduced to 7.5 ± 1.4 points in treated mice (P <0.001; Fig. 3, *B*). Capan-1 tumors grew less aggressively; treatment reduced the score from 7.1 ± 0.9 points to 2.7 ± 0.6 points (P <0.001; Fig. 3, *C*).

Survival

The aggressive in vivo behavior of MIAPaCa-2 and AsPC-1 tumors was seen with a low 14-week survival in the control groups (30% and 10%, respectively). Treatment with the antiangiogenic drug resulted in a tendency toward increased survival (50% and 40%, respectively; Fig. 4, *A* and *B*), but perhaps because of the limited number of animals in each group (n = 10), this difference was not statistically significant. Animals with Capan-1 tumors did not die within the observation period (Fig. 4, *C*).

VEGF Levels in Plasma and Ascites

Plasma levels of human VEGF produced by the tumor cells were relatively low and comparable in all treatment and control groups (Fig. 5, *A* to *C*). Seventy percent of control animals and 50% of treated mice with MIAPaCa-2 tumors formed ascites. Ascites in the animals with AsPC-1 and Capan-1 tumors was seen in 50% vs. 40% and 50% vs. 30%. VEGF levels in ascites were much higher than in plasma, but there were no marked differences between treatment and control groups (see Fig. 5, *A* to *C*).

Microvessel Density in Primary Tumors

Microvessel density as a parameter of angiogenic activity was significantly enhanced in the untreated primary tumors of all tested pancreatic cancer cell

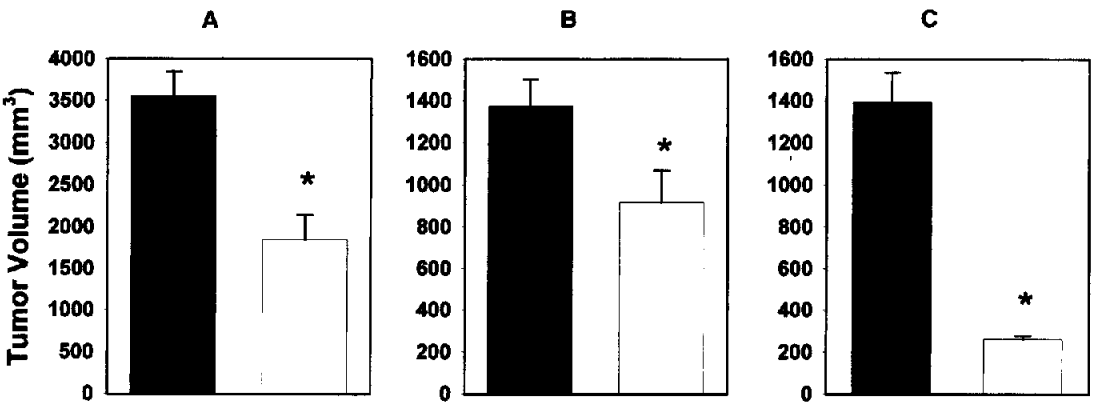

Fig. 2. Tumor volume in TNP-470–treated (□) and untreated (■) animals (n = 10 per group). TNP-470 treatment significantly inhibited local tumor growth in all three cell lines tested (**A**, MIA-PaCa-2; **B**, AsPC-1; **C**, Capan-1).

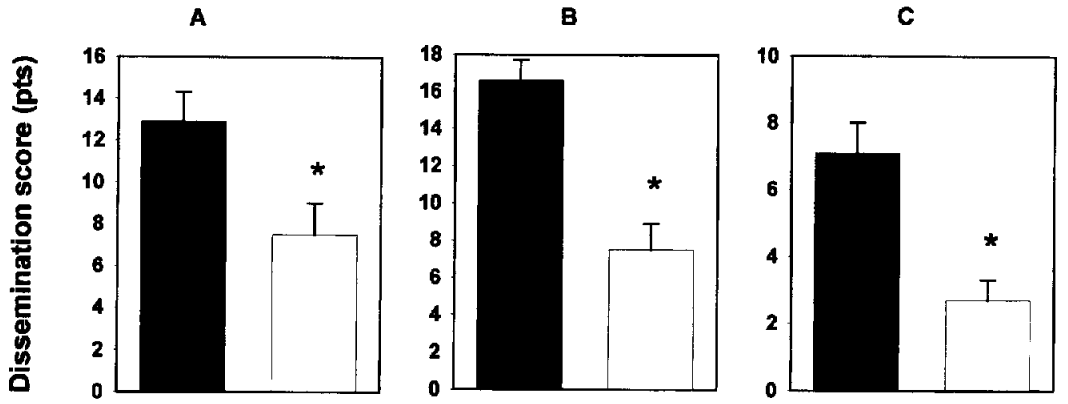

Fig. 3. Dissemination scores in three pancreatic cancer cell lines (**A**, MIAPaCa-2; **B**, AsPC-1; **C**, Capan-1). TNP-470–treated (□) and untreated (■) animals are depicted and clearly demonstrate improved scores in the treated group.

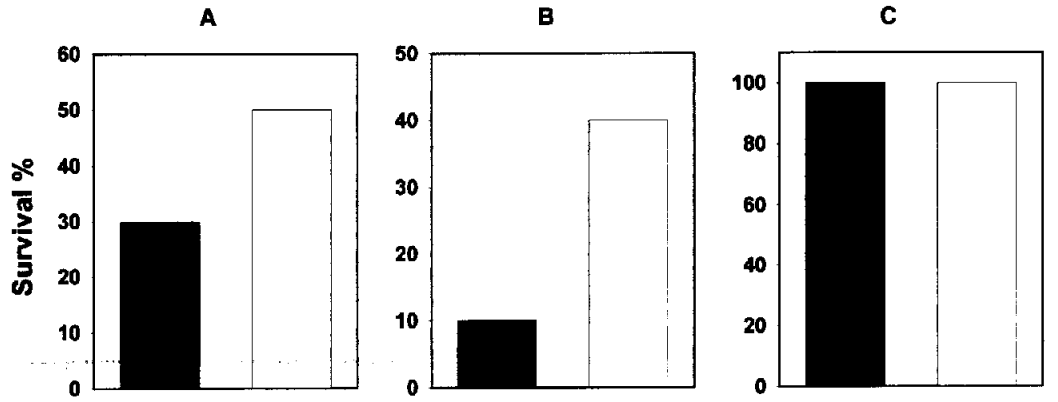

Fig. 4. Survival in TNP-470–treated (□) and untreated (■) animals was not statistically different, although a trend toward improved survival was identified in the MIAPaCa-2 (**A**) and AsPC-1 (**B**) groups at 14 weeks. **C**, Capan-1.

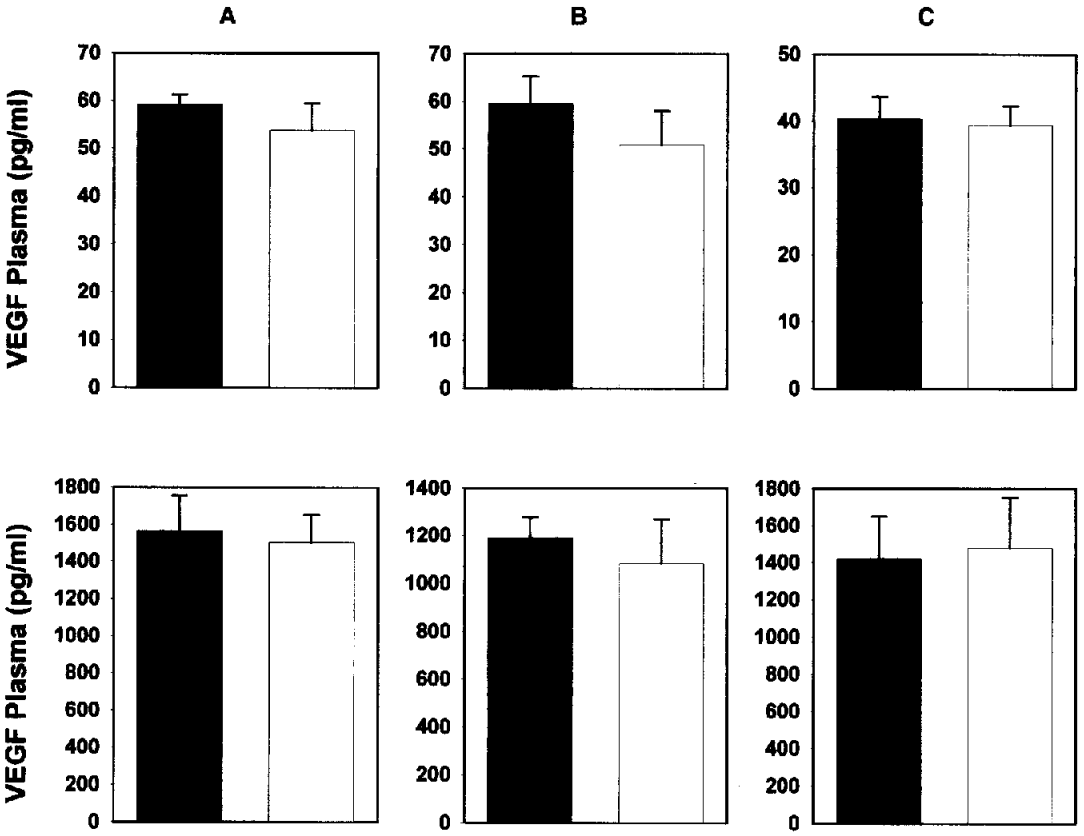

Fig. 5. Vascular endothelial growth factor *(VEGF)* levels in the plasma and ascites as determined by enzyme-linked immunosorbent assay in three pancreatic cancer cell lines (**A**, MIAPaCa-2; **B**, AsPC-1; **C**, Capan-1) in vitro following treatment (□) with TNP-470. TNP-470 appeared to have no effect on VEGF levels.

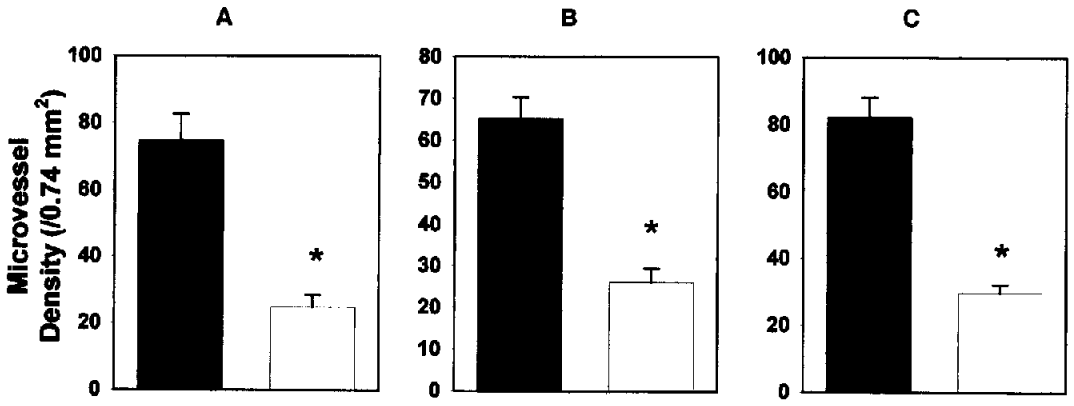

Fig. 6. Microvessel density as determined by CD31 immunostaining. TNP-470–treated (□) animals had significantly fewer microvessels as compared to untreated animals (■) in all three cell lines tested (**A**, MIAPaCa-2; **B**, AsPC-1; **C**, Capan-1).

lines compared to normal exocrine pancreas (MIAPaCa-2 = 74.8 ± 7.8/0.74 mm²; AsPC-1 = 65.3 ± 5.0/0.74 mm²; Capan-1 = 82.2 ± 5.8/0.74 mm²; native pancreas = 15.6 ± 1.5/0.74 mm²; $P < 0.001$). Treatment with TNP-470 significantly reduced neoangiogenesis in tumors of all three human pancreatic cancer cell lines: MIAPaCa-2 = 24.8 ± 3.7/0.74 mm²; AsPC-1 = 26.0 ± 3.4/0.74 mm²; Capan-1 = 26.9 ± 2.5/0.74 mm²; $P < 0.001$ vs. controls; Fig. 6, *A* to *C*.

DISCUSSION

Pancreatic cancer is one of the most lethal human malignancies, and in the United States it is estimated that 29,000 new cases are diagnosed annually.[1] The overall 5-year survival rate is approximately 5%, and most patients, even the minority with resectable disease, die as a result of progression of the primary tumor and the ravages of metastatic disease.[1,2] Because angiogenesis is thought to play a central role in the proliferation of primary pancreatic tumors,[9] and in the systemic spread of this disease, we reasoned that inhibition of angiogenesis might have a significant antitumor effect. Other observations support this. Thus in one study the amount of VEGF, a key angiogenic mediator, in the primary tumor correlated with patient survival in patients with pancreatic cancer.[10,11] Investigators have used antiangiogenic strategies in animal models of pancreatic cancer.[12,13] We recently found that VEGF antisense therapy inhibited tumor growth and improved survival in xenograph models of pancreatic cancer.[14] We also have preliminary evidence to suggest that VEGF antibody treatment shows similar promise.

The agent used in the present study, TNP-470, is an analogue of fumagillin derived from *Aspergillus fumigatus*. This compound was first described in 1990[6] and since then has been extensively studied in a variety of tumors. Although the mechanism of action is only partially understood,[15] there is evidence that the drug directly inhibits endothelial cell proliferation through a cyclin D1 pathway.[16] In addition, TNP-470 upregulates E-selectin, a membrane-bound protein found to be integral to the inhibition of metastatic activity.[17] It is widely believed that TNP-470 does not have a direct effect on VEGF, although one group found that this compound suppressed secreted VEGF in vitro by uterine sarcoma cells.[18]

We investigated the effect of TNP-470 on human pancreatic cancer in vitro and in an orthotopic xenograph model of the disease. TNP-470 in low (physiologic) concentrations inhibited the growth of HUVEC, a finding that confirmed those of previous investigations.[19] However, in each of the pancreatic cancer cell lines tested, similar low concentrations of the drug showed no effect. The growth inhibition that was seen at high concentrations was probably due to the more general toxic effects of the compound. The in vivo data, however, showed a strong inhibition of local tumor growth and metastatic tumor progression. The volumes of the MIAPaCa-1, AsPC-1, and Capan-1 tumors in the treated groups were significantly smaller than those of the control animals. Dissemination scores were also significantly lower in the treated groups and generally were about half the scores of the control groups. Immunohistochemical evaluation for microvessel density with anti-CD31 antibodies demonstrated four to six times the vessel density in untreated tumors compared to normal pancreas (15.6 ± 1.5/0.74 mm²). Treatment with TNP-470 reduced vessel density by approximately one third in treated tumors. This effect on vessel density appeared to be independent of VEGF activity, since serum and ascites VEGF levels were comparable in both control and treated groups.

Although there was a trend in the MIAPaCa-2 and AsPC-1 animals toward improved survival, the difference in these small test groups failed to reach statistical significance. Thus, in the MIAPaCa-2 animals, survival increased from 20% to 50%, and in the AsPC-1 animals survival increased from 10% to 40% at 14 weeks. None of the animals in the Capan-1 groups died since this is a well-differentiated and very slow growing cell line.

Other investigators also have studied the effect of TNP-470 in animal models of pancreatic cancer. Using a mouse model of severe combined immunodeficiency and splenic injection of pancreatic cancer cells, TNP-470 treatment resulted in significantly fewer and smaller liver "metastases."[20] As we found in our study, their in vitro data indicated that this was due to inhibition of endothelial cells rather than cancer cells. Another group combined TNP-470 with cisplatin, which prevented the development of liver metastases in a nude mouse model of pancreatic cancer.[21] However, those investigators found no benefit to TNP-470 treatment alone.

We conclude that TNP-470 appears to have a significant impact on the progression of disease in animal models of pancreatic cancer. The compound already has been tested in several phase I clinical trials, and the only dose-limiting toxicity was reversible neurotoxicity.[22,23] Thus TNP-470 appears to be safe for human use, and its clinical efficacy in pancreatic cancer should be tested promptly.

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Discussion

Dr. M.F. Brennan (New York, N.Y.). In this model you transplant fragments of tumor that are already growing and have recruited endothelial cells. Those endothelial cells presumably are recruited from the original animal that was injected. Do you have evidence that the tumor is able to recruit endothelial cells from the implanted host? In other words, if you performed the experiment using injected cells, would the results be the same?

Dr. H. Hotz. We have not done experiments with injected cells, but we have evaluated this model both with the transplantation technique and the implantation technique, and found basically the same characteristics. The reason we use the implantation technique is to avoid artificial cell spread into the peritoneal cavity with injection and, subsequently early artificial metastasis. We performed histologic investigations of these tumors at an early stage, and noted that these small tumor implants, which are only about 1 mm^3 in size, can actively recruit new capillaries from the host. You can see these capillaries are sprouting in. With antiangiogenic therapy, that process can be diminished.

Dr. J.M. Howard (Toledo, Ohio). How do you explain the effect of the antiangiogenic factor in vitro?

Dr. Hotz. With so-called physiologic doses of this compound, the proliferation of pancreatic cancer cells in vitro cannot be reduced. Unphysiologically high concentrations of TNP are needed to reduce the growth. This is not a specific action of TNP, but a toxic effect of the compound on the pancreatic cancer cells.

Therapy for Pancreatic Cancer With a Recombinant Humanized Anti-HER2 Antibody (Herceptin)

Peter Büchler, M.D., Howard A. Reber, M.D., Manuela C. Büchler, Mendel A. Roth, M.D., Markus W. Büchler, M.D., Helmut Friess, M.D., William H. Isacoff, M.D., Oscar J. Hines, M.D.

The HER2/neu oncogene is overexpressed in human pancreatic cancer, but the clinical significance of that overexpression is uncertain. In the present study we investigated the antitumor efficacy of Herceptin, a new recombinant humanized anti-HER2/neu antibody, which exhibits cytostatic activity on breast and prostate cancer cells that overexpress the HER2 oncogene. That antibody may retard tumor growth in certain patients with those diseases. We quantified HER2 expression in various human pancreatic cancer cell lines and studied the bioactivity of this antibody both in vitro and in vivo. Growth inhibition by Herceptin was observed in vitro in cell lines with high levels of HER2/neu expression. Cell lines with low levels of this protein did not respond significantly to the antibody. In vivo we studied two different pancreatic cancer cell lines in an orthotopic mouse model of the disease. Herceptin treatment suppressed tumor growth in the MIA PaCa-2 tumor cell line, which expressed high levels of HER2/neu. These data suggest that Herceptin treatment of patients with pancreatic cancer who express high levels of the HER2/neu oncogene may be reasonable. (J GASTROINTEST SURG 2001;5:139-146.)

KEY WORDS: Pancreatic cancer, Herceptin, treatment, HER2/neu, animal model

During the past decade, substantial progress has been made in the understanding of basic pathophysiologic mechanisms that are important in the growth of pancreatic cancer.[1-4,5] Recently a variety of genetic alterations have been described for a number of tumors, and overexpression of the erbB-2/HER2 oncogene appears to be important for the regulation of cancer growth in some of them.[6,7] Overexpression of the erbB-2/HER2 oncogene occurs in 20% to 30% of all breast cancer patients, which correlates with a poor prognosis and an undifferentiated tumor phenotype.[7-9] In the case of human pancreatic cancer, more than 50% of the patients have elevated messengerRNA and protein levels of this oncogene.[19] However, in contrast to breast cancer, higher levels of HER2 are found in the better differentiated tumors, and no correlation between elevated HER2 levels and prognosis has been established.[10-12] Thus the significance of the HER2 protein in pancreatic cancer remains to be elucidated. Since it is known that an antibody directed toward the extracellular domain of the HER2 protein (Herceptin) inhibits tumor growth in breast and prostate cancer,[13,14] where a smaller proportion of patients showed HER2 overexpression, we were motivated to investigate the therapeutic efficacy of Herceptin in pancreatic cancer. We examined the effects of Herceptin on a number of human pancreatic cancer cell lines with different levels of HER2 expression in vitro, as well as the growth-suppressive effects of the antibody on pancreatic tumors in an animal model of the disease.

MATERIAL AND METHODS

Compounds

The recombinant humanized monoclonal antibody rhuMAb HER2 (Herceptin) against the extracellular domain of the HER2 receptor and the nonspecific recombinant immunoglobulin G1 (IgG1) antibody were kindly provided by Genentech, Inc. (South San Francisco, Calif.).

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Presented at the Forty-First Annual Meeting of The Society for Surgery of the Alimentary Tract, San Diego, Calif., May 21-24, 2000.
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Cell Lines

Human pancreatic adenocarcinoma cell lines (AsPC-1, Capan-1, HPAF-2, MIA PaCa-2, and PANC-1) were purchased from American Type Culture Collection (ATCC, Rockville, Md.). Cell lines were cultured in Dulbecco modified Eagle medium (MIA PaCa-2, PANC-1) or in RPMI 1640 (AsPC-1, Capan-1, HPAF-2) supplemented with 10% heat-inactivated fetal bovine serum, penicillin G (100 U/ml), and streptomycin (100 μg/ml) (Life Technology). Cells were maintained at 37° C in monolayer culture in a humidified atmosphere containing either 5% CO_2 (AsPC-1, Capan-1, HPAF-2) or 10% CO_2 (MIA PaCa-2, PANC-1). For anchorage-dependent growth assays, 10,000 cells were seeded in six-well plates (Falcon 3046) and grown as a monolayer culture for 48 hours in complete medium. After 48 hours of cell growth, the medium was exchanged and various concentrations of either Herceptin or the nonspecific IgG1 antibody were added.

Western Blot Analysis

Total cell lysates (50 μg/lane) were subjected to 10% sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis (PAGE) and proteins were blotted to nitrocellulose membranes (Amersham Pharmacia Biotech). Equal loading of proteins and complete protein transfer onto nylon membranes were verified by a reversible protein staining using Ponceau red (Sigma, St. Louis, Mo.) for 5 minutes. After blocking in Blotto solution containing 10% low fat dry milk in phosphate-buffered saline, membranes were probed with a monoclonal antibody specific for the human HER2 protein p185 (Oncogene Research, Cambridge, Mass.). Proteins were visualized with peroxidase-coupled secondary antibody using the ECL detection system (Amersham Pharmacia Biotech).

Determination of Cell Viability

To determine cell viability and growth in monolayer cultures, the monotetrazolium (MTT; 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) colorimetric assay[15] was performed using a commercially available assay kit (Boehringer Mannheim, Germany). Briefly, after 48 hours growth in complete cell-specific medium, exponentially growing cells were divided into two portions: to one Herceptin was added at various concentrations (1, 10, and 30 μg/ml) and to the others the IgG1 control antibody was added in the same concentrations as Herceptin. For each determination of cell viability, cells were incubated for 4 hours with the metabolic substrate, tetrazolium salt MTT, at a final concentration of 0.5 mg/ml. Living cells in this assay metabolized tetrazolium to formazan, which precipitates in aqueous solutions. After overnight incubation in a solubilization solution, these formazan salts again dissolve and can be quantified spectrophotometrically. An increase in the number of living cells results in an increase in the total metabolic activity in the sample tested, which is proportional to the amount of formazan formed. Formazan absorbency readings on each sample were performed at 540 nm on a scanning multiwell spectrophotometer (ELISA Reader, Biotek Instruments, Inc. Burlington, Vt.). Control experiments were done with plain medium or medium with corresponding doses of the vehicle. All experiments were performed at least twice in quadruplicate.

Soft Agar Colony-Forming Assay

Anchorage-independent growth assays were done in six-well plates (35 mm; Falcon 3046, Baxter, McGraw Park, Ill.). For each condition triplicates were assayed. A bottom layer of 1 ml cell type–specific medium containing 0.7% agar (Difco, Detroit, Mich.) and 10% fetal bovine serum was poured. After the bottom agar was solidified, 10,000 cells were added in 1 ml of complete culture medium, 0.35% agar, and various concentrations of Herceptin. Cells were incubated in cell type–specific culture conditions and after 14 days, MTT (3-[4,5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide) reagent, which is exclusively metabolized by living cells, was added at a final concentration of 0.5 mg/ml for vital staining. Colonies with more than 20 cells were counted manually.

Laboratory Animals

Five-week-old male nude mice (BALB/cA), weighing 18 to 22 g, were used for subcutaneous and orthotopic tumor implantation. All animals were maintained on a daily 12-hour light/12-hour dark cycle and housed in semisterile microisolator cages with autoclaved bedding, and food and water ad libitum. The experimental protocol was approved by the Chancellor's Animal Research Committee of the University of California, Los Angeles in accordance with the national guidelines for the use and care of laboratory animals.

Tumor Induction in Athymic Nude Mice

The poorly differentiated human pancreatic cancer cell line, MIA PaCa-2, which expressed high levels of HER2, and the more differentiated HPAF-2 cell line, which expressed lower levels of HER2, were used for xenobiotic tumor induction. For subcutaneous tumor formation, MIA PaCa-2 and HPAF-2 tumor cells growing as a monolayer culture, were trypsinized and approximately 10^7 viable cells were in-

jected subcutaneously into the mediodorsal region of a nude mouse. After a 4-week period of subcutaneous tumor growth, one small tumor fragment (~1 mm in diameter) was removed from the subcutaneous tumor and transplanted into the tail of the pancreas of the mice in the two study groups. For orthotopic transplantation, a small (~1 cm) left lateral subcostal laparotomy was performed, and the spleen and the tail of the pancreas were exteriorized. One small piece of tumor was inserted into a small pancreatic parenchymal pocket without any need to further anchor the transplant. After placement of the tumor nodule, the pancreas and the spleen were returned to the abdomen, and the peritoneal incision was closed with a 6-0 Prolene suture. The abdominal wall was closed separately using 5-0 Vicryl sutures. In order to standardize experimental conditions in vivo, all 16 animals for each cell line to be tested were transplanted in the same session with tumor pieces from the same subcutaneous tumor. Afterward, animals bearing orthotopic MIA PaCa-2 (n = 16) or HPAF-2 (n = 16) tumor xenografts were randomly assigned to either the Herceptin-treated group (n = 8) or the sham-treated group (n = 8). After orthotopic tumor transplantation, the mice were inspected daily. The growth rate of the tumor was monitored by abdominal palpation, and the tumor volume was determined after the animal was killed.

Therapeutic Efficacy of Herceptin in Athymic Nude Mice

After 7 weeks of tumor growth, treatment was begun. Herceptin-treated animals received 10 mg/kg Herceptin intraperitoneally three times a week, and the control group received an equal amount of nonspecific IgG1 instead. Treatment was continued until the animals were killed, which was done when clinical signs of excess tumor burden, such as cachexia or ascites with abdominal distention, became evident or the tumor grew larger than 1.5 cm.

Tumor Assessment

All animals underwent detailed autopsies of the thoracic and abdominal cavities. The tumor was measured with a caliper in all three perpendicular dimensions. Tumor volume was calculated as described previously[16] using the following formula: tumor volume = 0.5 × (length × width × depth). Metastatic tumor spread was determined macroscopically in all thoracic, abdominal, retroperitoneal, and pelvic organs, and all suspicious lesions were confirmed by microscopic analysis. Metastatic spread was quantified by counting the different organs that contained metastatic lesions. Thus every point in the metastatic score represented a different organ of metastatic tumor spread.

Statistical Analysis

Experiments were done in quadruplicate and repeated at least two times. Results are expressed as means ± standard error. Statistical significance was determined by Student's t test ($P < 0.05$).

RESULTS

Expression of the HER2 neu Antigen in Human Pancreatic Cancer Cells

The level of HER2 expression was analyzed by Western blot analysis and quantified by laser densitometry. The highest levels of HER2 protein were detected in the undifferentiated human pancreatic cancer cell line MIA PaCa-2. The lowest levels were seen in extracts from HPAF-2 cells, a better differentiated cell line, which expressed only one sixteenth of the HER2 levels found in the MIA PaCa-2 cell line. The well-differentiated Capan-1 cell line had higher levels (~2.5 times) of HER2 than the HPAF-2 cell line (Fig. 1). We confirmed the findings of the Western blot analysis with immunocytochemical grading of HER2 expression. In both analyses, the MIA PaCa-2 cell line showed an intense (+++) signal for HER2, which in the case of immunocytochemistry was clearly seen as cell membrane staining. The HPAF-2 cell line had only low (+) levels of HER2 (Fig. 2).

Effect of Herceptin on Monolayer Cultures of Human Pancreatic Cancer Cells

After an initial growth period of 48 hours in complete culture conditions, the antibody was added at different concentrations (1, 10, and 30 μg/ml). After 24 hours (Fig. 3, *A*) up to 72 hours (Fig. 3, *B*) of anti-

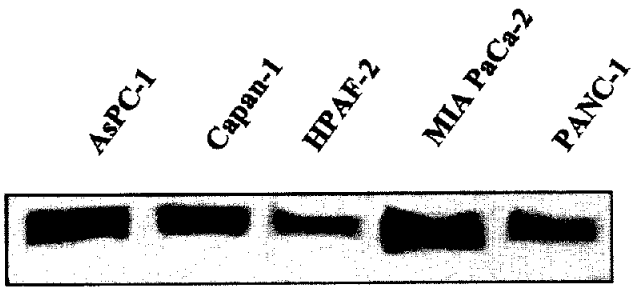

Fig. 1. Expression of HER2 in various human pancreatic cancer cell lines. Human pancreatic cancer cell lines were cultured in complete cell medium. Proteins were extracted and subjected to Western blot analysis. Highest expression of HER2 (~185 kd) was seen in MIA PaCa-2 cells; lowest was found in HPAF-2 cells.

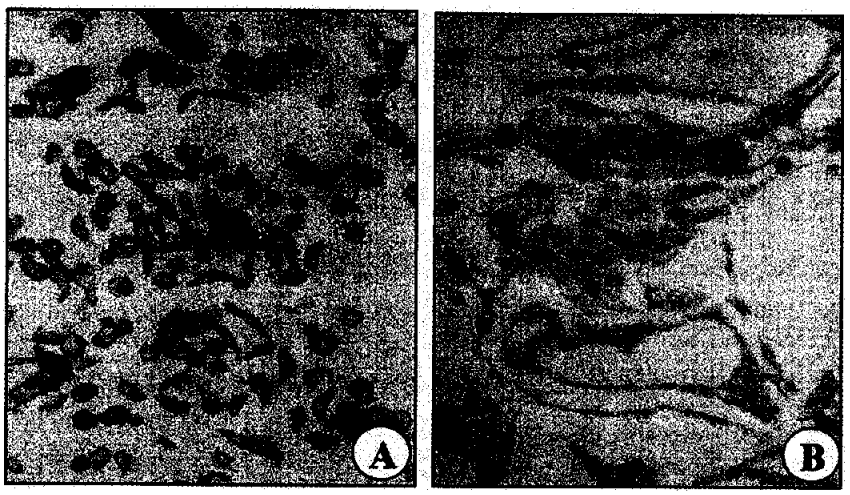

Fig. 2. HER2 cell staining of human pancreatic cancer cells. Human pancreatic cancer cell lines were fixed in paraformaldehyde and embedded in paraffin. Sections (4 μm) were stained with a monoclonal antibody against HER2. A strongly positive signal was seen in the cell membrane of MIA PaCa-2 cells **(A)**, whereas only weak staining was detectable in the HPAF-2 cell line **(B)**.

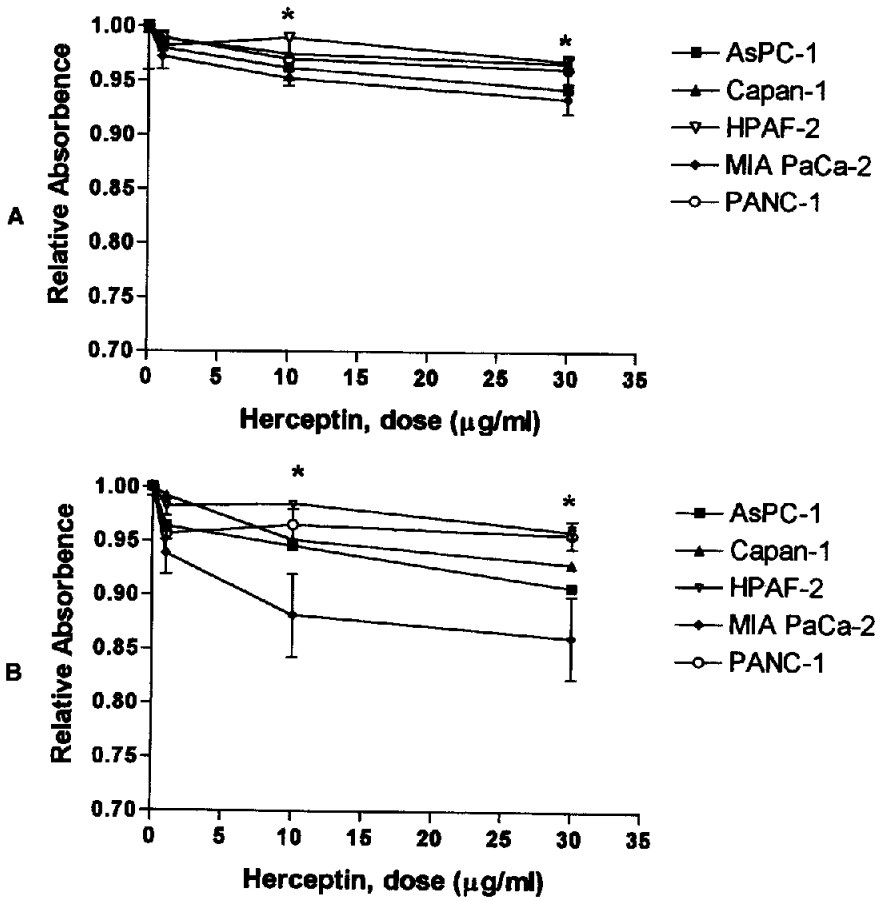

Fig. 3. Effect of Herceptin on cell viability of different human pancreatic cancer cell lines. Cells were cultured for 24 hours **(A)** up to 72 hours **(B)** in complete cell medium in the presence of 1, 10, and 30 μg/ml Herceptin. Cell viability was determined by monotetrozolium assay. Values represent means ± standard error for at least three independent experiments. * = $P < 0.05$ to all indicates that values for Herceptin-treated cells showed less statistical significance than values for untreated cells, except the HPAF-2 cell line.

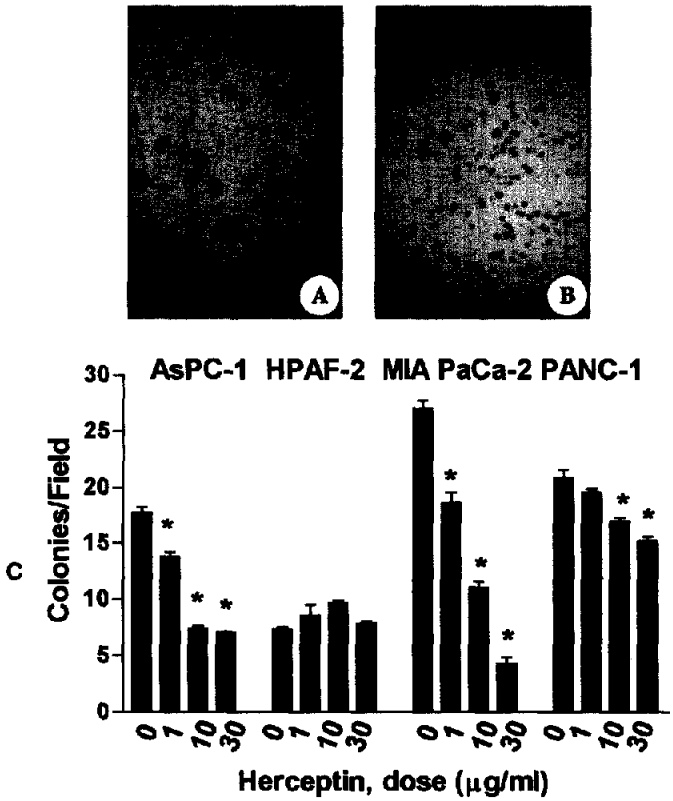

Fig. 4. Effect of Herceptin on anchorage-independent growth of different human pancreatic cancer cell lines. Cells were grown in soft agar containing various doses of Herceptin as described in Methods. Vital colonies were stained with monotetrazolium reagent (**A** and **B**). **A,** MIA PaCa-2 cells were cultured in soft agar containing 10 μg/ml nonspecific IgG1. **B,** The same number of cells were seeded and cultured in the presence of 10 μg/ml Herceptin. **C,** The number of colonies with more than 20 cells was counted. The Capan-1 cell line did not grow in this assay. Values represent means ± standard error for at least three independent experiments. * = $P < 0.05$ indicates that values for Herceptin-treated cells were less statistically significant than values for untreated cells.

body treatment in complete cell culture medium, cells were analyzed with the MTT assay. When cells were assayed after 24 hours, low doses of Herceptin demonstrated inhibited monolayer growth ($P < 0.05$) in all cell lines except the HPAF-2 cell line, yet the overall growth inhibitory effect was minimal, since growth suppression in most cell lines was in the range of only 5% (Fig. 3, *A* and *B*). After 72 hours of antibody treatment, the effect was more pronounced. This was especially apparent in the MIA PaCa-2 cell line where an approximately 15% growth inhibition was seen at the 30 μg/ml dose, in contrast to the other three cell lines (AsPC-1, Capan-1, and PANC-1) where only 5% to 10%, yet statistically significant, growth inhibition was achieved (Fig. 3, *A* and *B*). The overall growth inhibitory efficiency of Herceptin in the MTT assay correlated well with the level of HER2 expression seen in the Western blot analysis (see Figs. 1 and 3, *A* and *B*).

Effect of Herceptin on Anchorage-Independent Growth of Human Pancreatic Cancer Cells

The well-differentiated human pancreatic cancer cell line Capan-1 did not grow in the soft agar assays, whereas the more undifferentiated cell lines grew vigorously. In contrast to the monolayer culture, where only a minimal growth-suppressive effect was detectable, a profound growth inhibitory effect was seen in the anchorage-independent growth assay. This was especially so with the MIA PaCa-2 (Fig. 4, *A* and *B*) and AsPC-1 cell lines that had higher levels of HER2 expression (Fig. 4, *C*). A significant growth inhibition in this assay was already seen with lowest doses of Herceptin, which increased further with higher doses. Growth of the PANC-1 cell line expressing intermediate levels of HER2 was also inhibited, but this required higher doses of Herceptin. In contrast, the HPAF-2 cell

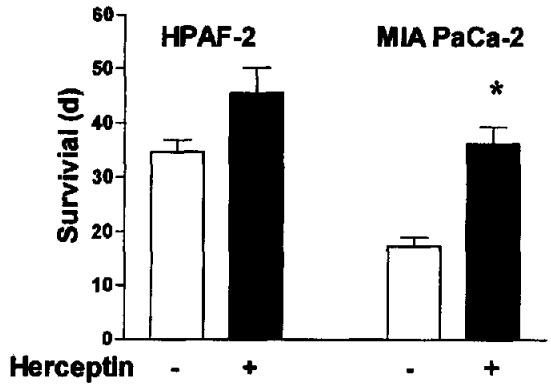

Fig. 5. Effect of Herceptin on survival of animals in an orthotopic model of pancreatic cancer. For induction of tumors two cell lines, HPAF-2 (low HER2 levels) and MIA PaCa-2 (high HER2 levels), were chosen. After 7 weeks of tumor growth, treatment was initiated. Animals received 10 mg/kg Herceptin intraperitoneally (3 times a week) or an equal amount of nonspecific IgG1. Animals were killed once clinical signs of severe tumor disease, such as advanced cachexia, heavy tumor burden, or extensive ascites with distention of the abdominal wall, became apparent. Values represent means ± standard error; n = 8 in each group. * = $P < 0.05$ compared with the sham group.

Table I. Effect of Herceptin treatment in vivo in a highly metastatic orthotopic murine model for pancreatic cancer

	MIA PaCa-2		HPAF-2	
Tumor parameters	Sham	Herceptin	Sham	Herceptin
Tumor volume	3.65 ± 1.7	3.40 ± 1.82	4.03 ± 1.55	3.83 ± 1.23
Metastatic score	3.80 ± 1.1	3.50 ± 0.8	2.1 ± 0.9	2.2 ± 0.8
Ascites production (ml)	2.21 ± 0.85	1.95 ± 1.1	1.3 ± 1.1	1.15 ± 0.95
Animal weight (g)	25.75 ± 2.11	24.55 ± 1.95	26.45 ± 2.21	25.90 ± 2.0

lines did not respond to this treatment in soft agar assays (see Fig. 4, *C*).

Antitumor Effect of Herceptin on Human Pancreatic Cancer Xenografts

After 7 weeks of tumor growth, Herceptin treatment was initiated. Each mouse received 10 mg/kg Herceptin three times a week intraperitoneally. Neither Herceptin nor nonspecific IgG1 antibody administration caused any side effects in the mice. An antitumor effect was seen in both cell lines, but in the case of HPAF-2 the overall survival benefit did not reach statistical significance (Fig. 5). In contrast, survival of mice bearing xenograft tumors derived from the MIA PaCa-2 cell line, which expressed high levels of HER2, was significantly longer than survival of mice in the IgG1-treated control group (see Fig. 5). Herceptin treatment had no effect on animal weight, amount of ascites produced, or number of metastatic lesions (Table I).

DISCUSSION

Although specific ligands of the HER2 receptor remain to be elucidated, recent studies suggest that this protein may serve as a shared coreceptor for multiple stroma-derived growth factors and as a dimerization partner for a number of cell surface receptors (e.g., epidermal growth factor [EGF] receptor and nerve growth factor [NGF] receptor).[17-19] Thus HER2 may possess an intrinsic capacity to enhance signaling by a number of ErbB-stimulating ligands (EGF-like or NGFs), many of which are known to be overexpressed in human pancreatic cancer. This suggests the potential for a central role in growth factor–mediated mitogenesis, a well-described characteristic of pancreatic cancer growth.[2,10,17-19]

In the present study we evaluated the antitumor activity of Herceptin, a humanized monoclonal antibody against the extracellular domain of the HER2 receptor.[20] We reasoned that this agent represented a potential treatment for pancreatic cancer with immediate clinical applicability, since HER2 is overexpressed in more than 50% of patients with this disease.[10,11] Indeed, overexpression of HER2 is observed in only 20% to 30% of patients with breast cancer,[7,21,22] a disease where the therapeutic efficiency of Herceptin has already been demonstrated.[7,14,23,24]

Our in vitro studies characterized expression of HER2 in various human pancreatic cancer cell lines. We used both Western blot analysis and pathologic grading by a pathologist, which represents the clinical standard for staging of HER2 expression in tumor specimens. Among the various cancer cell lines, the MIA PaCa-2 cells showed the highest levels of HER2 protein in the Western blot analysis, which was consistent with the immunocytochemical results. The

HPAF-2 cell line was characterized by the lowest levels of HER2. The AsPC-1, PANC-1, and Capan-1 cell lines expressed intermediate levels of HER2. This is consistent with previously reported observations in human pancreatic cancer specimens where no clear correlation was seen between the degree of tumor differentiation and the level of HER2 expression. This apparent discrepancy may be explained by recalling that the level of HER2 expression itself does not mediate mitogenesis; its dimerization partners must also be present.[17] Then HER2 can enable and augment mitogenesis mediated by a variety of growth factors.[17]

The antitumor efficacy of Herceptin in vitro was evaluated with three different doses of antibody, because previous reports suggested antitumor activity in this dose range in vivo.[13,14,24] Growth studies were done in monolayer cultures and in anchorage-independent growth assays in soft agar, which more closely resembles the actual pattern of tumor growth in vivo. The growth inhibitory effect of Herceptin in soft agar correlated with the expression of HER2, since a strong growth inhibition was seen in MIA PaCa-2, AsPC-1, and PANC-1 cell lines, all of which expressed intermediate to high levels of HER2. In contrast, growth of the HPAF-2 cell line was unchanged with increasing doses of Herceptin. In monolayer experiments, there was also a significant growth inhibition, which was most pronounced in the MIA PaCa-2 cell line (~15%). However, the overall growth inhibitory effect in monolayer cultures was negligible in all other cell lines, where only a 5% to 10% growth inhibitory effect was seen.

To test whether Herceptin treatment was effective in vivo, we used a recently developed murine model for pancreatic cancer, in which tumor xenografts grew in an orthotopic location within the pancreatic parenchyma. For tumor induction in nude mice, we used two different human pancreatic cancer cell lines expressing different HER2 levels. The MIA PaCa-2 cell line expresses high levels, and the HPAF-2 cell line low levels of the protein. To optimize the clinical relevance of this study, Herceptin treatment was initiated after a 7-week period of intrapancreatic tumor growth, when the average diameter of the tumors was 1 cm. By that time some animals had also developed palpable metastatic lesions in the peritoneal cavity. In the case of the MIA PaCa-2 cell line, there was a significant survival benefit in the group treated with Herceptin, which is consistent with similar observations in experimental models of breast and prostate cancer. Survival of animals transplanted with HPAF-2 xenograft tumors was also longer, but perhaps because of the small number of animals tested, that difference was not statistically significant. It would not be surprising if significant inhibition of growth is eventually demonstrated in tumors that express low levels of the HER2 receptor. This could be due to the receptor's complex and poorly understood interactions with other growth factor receptors and their ligands. This would suggest that HER2 may have a central role in the growth of pancreatic cancer and that its inhibition could have a therapeutic benefit in patients whose tumors express even low levels of the protein.

In contrast to clinical protocols for the treatment of breast cancer where Herceptin is usually given once a week, we injected the antibody three times a week. This was done because of the possibility that the nude mice might become immunized to this humanized protein, which could have shortened its half-life in vivo. We did not investigate whether this occurred, but it is reasonable to assume that this would not be an issue if the antibody was used clinically.

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Synthetic Peptide YY Analog Binds to a Cell Membrane Receptor and Delivers Fluorescent Dye to Pancreatic Cancer Cells

Carson D. Liu, M.D., David Kwan, Natalie Simon, David W. McFadden, M.D.

Pancreatic cancer continues to have a dismal prognosis despite multimodality treatment plans. Peptide YY (PYY) is a gut hormone that suppresses pancreatic exocrine and endocrine function. Previous experiments have shown that shortened synthetic PYY(22-36) analog decreases pancreatic cancer cell growth while also decreasing intracellular cyclic adenosine monophosphate. Our purpose was to construct an optimal synthetic PYY analog that binds to pancreatic cancer cells that may be used for imaging and therapy. Biotinylated PYY analogs with lengths ranging from PYY(1-36), PYY(9-36), PYY(14-36), PYY(22-36), and PYY(27-36) were tested with flow cytometry and receptor cross-linking studies to measure cell membrane binding. Growth inhibition studies were also performed using monotetrazolium tests to determine potency of various PYY analogs. Quantitative flow cytometry reveals the highest specific binding of PYY(14-36) to pancreatic cancer cells. Cross-linking studies reveal a receptor on the cell membrane of human pancreatic ductal adenocarcinoma cells. Growth inhibition studies reveal that PYY (14-36) has the highest potency against PANC-1 and MiaPaCa-2 cells. A novel synthetic PYY analog binds to the cell surface of pancreatic cancer cells and has the ability to deliver fluorescent dyes. The strategy of using biotinylated peptides to deliver avidin-dye complexes to cancer cells will allow imaging of pancreatic tumors and delivery of therapeutic agents. (J GASTROINTEST SURG 2001;5:147-152.)

KEY WORDS: Peptide YY, pancreatic cancer, biotin

Pancreatic adenocarcinoma remains one of the most devastating neoplasms of the gastrointestinal tract. Pancreatic cancer is a malignancy that is unresponsive to conventional therapy. More than 85% of patients have metastatic disease when they are first seen. The incidence of pancreatic cancer is 9 per 100,000[1] and has remained steady since 1973.[2] Median survival on diagnosis is 11 months, whereas adjuvant treatment (5-fluorouracil and radiation treatment) with surgical resection (Whipple procedure) has extended life by approximately 9 months.[3] A dismal prognosis is associated with pancreatic adenocarcinoma despite multimodality treatment protocols. Although total pancreatectomy in selected patients offers survival advantages in rare cases, the difference remains negligible.[4] Earlier diagnosis and novel treatment modalities may help to improve survival in patients with pancreatic cancer.

The production of growth factors along with their receptors is a crucial step in triggering growth response in tumor cells.[5] Endogenous gastrointestinal hormones given at pharmacologic doses may provide another mode of adjuvant treatment. Previous works describe the growth-inhibiting properties of the synthetic peptide YY (PYY) analog PYY(22-36) on human pancreatic ductal adenocarcinomas in vitro[6] and

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in vivo.[7] A 2.5-fold difference in human pancreatic cancer mass is seen in nude mice exposed to PYY (22-36) for 2 weeks.[7] Furthermore, a noticeable decrease in the second messenger, cyclic adenosine monophosphate, is observed with the addition of synthetic PYY analog,[7] suggesting alteration of cellular metabolism. Synthetic PYY analogs augment the effects of 5-fluorouracil and leucovorin.[8] A decrease in cell membrane epidermal growth factor receptor protein expression is observed after treatment with PYY analogs and chemotherapy.[8]

We hypothesized that various lengths of PYY analogs would optimally bind to pancreatic cancer cells. Native PYY(1-36), PYY(9-36), PYY(14-36), PYY(22-36), and PYY(27-36) were constructed. These fragments were studied because of three-dimensional crystallographic structure data. Peptide YY has a polyproline type II–like alpha helix in residues 1 to 8, a beta turn in residues 9 to 13, and an alpha helix in residues 14 to 32 with a flexible tail in residues 33 to 36.[9] Biotin was covalently linked to the nonbinding side of the peptide, the amino terminal. The specific binding of biotinylated synthetic PYY to cancer cells would allow delivery of fluorescent dyes to image and possibly treat pancreatic cancer.

MATERIAL AND METHODS

In Vitro Growth of Human Pancreatic Cancer Cells

Human pancreatic ductal adenocarcinoma cell lines MiaPaCa-2 and PANC-1 (American Type Culture Collection, Rockville, Md.) were purchased and grown in Costar T125 flasks (Corning, Inc., Corning, N.Y.). Cells were grown in monolayers in RPMI 1640 medium supplemented with 10% fetal calf serum, 5 ml of 29.2 mg/ml L-glutamine (Irvine Scientific, Santa Ana, Calif.), 25 μg of gentamicin, and 5 ml of penicillin, streptomycin, and fungizone solution (JRH Biosciences, Lenexa, Kan.) at 37° C in a Forma Scientific (Marietta, Ohio) water-jacketed 5% carbon dioxide incubator. All cell lines were detached with 0.25% trypsin (Clonetics, San Diego, Calif.) once or twice a week. Cells were washed by centrifugation at 4° C at 500 *g* for 7 minutes. Viable cells were counted by trypan blue exclusion on a hemocytometer slide.

Monotetrazolium Growth Assays of Biotinylated PYY Analogs

Biotinylated synthetic PYY analogs were constructed by Peninsula Laboratories (Belmont, Calif.) and added to MiaPaCa-2 and PANC-1 human pancreatic adenocarcinoma cells. A total of 30,000 cells were exposed to various concentrations of biotinylated PYY(14-36), bio-PYY(22-36), bio-PYY(27-36), and bio-(9-36) for 12 hours. Concentrations of peptides ranged from 10 nmol/L to 10 pmol/L in a volume of 200 μl with serum-free fortified RPMI 1640. After peptide treatment, monotetrazolium (MTT) assay was performed by the addition of 3-(4,5-dimethylthiazol-2-yl)-2, 5-dephenyltetrazolium bromide (Sigma, St. Louis, Mo.) at a final concentration of 0.5 mg/ml. Cells were incubated with tetrazolium for 3 hours prior to cessation of reaction. Formazon crystals were dissolved with 200 μl of dimethylsulfoxide. MTT assays were read on a Bio-Rad enzyme-linked immunosorbent assay microplate reader (Bio-Rad Laboratories, Inc., Hercules, Calif.) at 550 nm. The MTT assay measures mitochondrial NADH-dependent dehydrogenase activity, and it has been among the most sensitive and reliable methods for quantitating in vitro chemotherapy responses in tumor cells.[10]

Quantitative Flow Cytometry

MiaPaCa-2 and PANC-1 cells (1×10^7) were incubated at 4° C with biotinylated PYY analogs (10 nmol/L, 100 pmol/L) for 30 minutes in cold buffer at 4° C (0.2% albumin and 0.1% sodium azide). Cells were washed twice with phosphate-buffered saline (PBS), spun at 250 *g* for 5 minutes, and incubated with 1.67 μl of phycoerythrin-streptavidin (Caltech Labs, Burlingame, Calif.; stock solution = 150 μg/0.5 ml) in cold buffer for an additional one-half hour. Excess phycoerythrin was washed off twice with PBS and resuspended. Sample tubes were maintained in the dark with aluminum foil wrapping at 4° C and immediately submitted for flow cytometry within 30 minutes.

Flow cytometry was performed at core facilities located at our institution. Quantitative flow cytometry was programmed for phycoerythrin and raw counts were used to determine relative increases in fluorescent detection with escalating doses of biotinylated PYY analogs while maintaining the same concentration of phycoerythrin-streptavidin.

Receptor Cross-Linking Studies

MiaPaCa-2, PANC-1, AR42J, and 3T3 cells were trypsinized and washed with RPMI 1640 serum-free medium. Cells were resuspended at 1.0×10^6 cells/ml and added to synthetic biotinylated PYY analogs at 10 nmol/L per reaction vial for 1 hour at 4° C on a shaker. Cells were resuspended after washing with PBS, pH 8.0, and 1 mmol/L magnesium chloride at 4° C. Water-soluble cross-linking agent, BS3 (Pierce, Rockford, Ill.), was added to cells to form a final concentration of 20 μg/ml. Cells were tumbled with

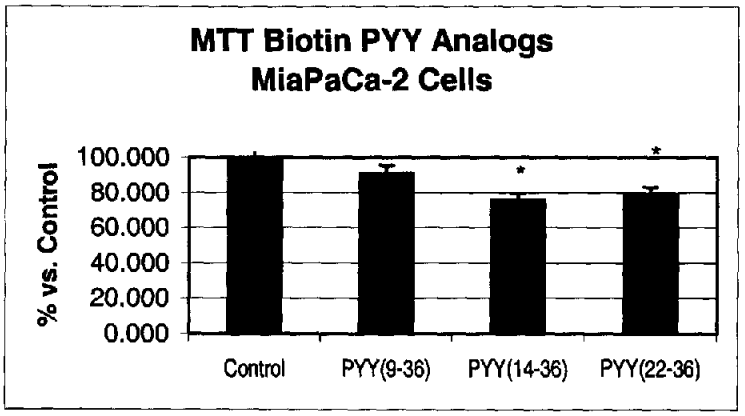

Fig. 1. Growth inhibition of MiaPaCa-2 pancreatic cancer cells exposed to various lengths of biotinylated PYY analogs.

cross-linking buffer for 20 minutes on a rocking platform. One volume of TE buffer (Tris, EDTA, pH 7.4) was added to stop the reaction. Cells were collected with centrifugation at 4° C. Pooled cells were subjected to cell lysis buffer (300 mmol/L NaCl, 50 mmol/L Tris-Cl, pH 7.6, 0.5% Triton X-100 with protease inhibitors, 10 μg/ml leupeptin, 10 μg/ml aprotinin, 1 mmol/L phenylmethylsufonyl fluoride, and 1.8 mg/ml iodoacetamide).

Solubilized cell membranes were separated on a 4% to 12% gradient gel (Bio-Rad Laboratories). Concentrated solubilized cell membranes were added to Laemmli's reducing sample buffer in a ratio of 1:4 (1 ml of 0.5 mol/L Tris-HCl, pH 6.8, 800 μl of glycerol, 1.6 ml of 10% sodium dodecyl sulfate, 400 μl of 2-b-mercaptoethanol, 200 μl of 0.05% bromophenol blue, and 4 ml of distilled water). Electrophoretic separation was performed at 80 volts for 3 hours. Biotinylated molecular weight marker (Pierce) was simultaneously loaded in a different lane to identify the molecular weight of the receptor. Gels were removed from the plastic plates and protein transfer to nylon membrane (Stratagene, Austin, Tex.) was performed overnight at 30 volts. Nylon membranes were probed with streptavidin–horseradish peroxidase provided by ECL kits (Amersham, Buckinghamshire, England). Blots were exposed to Hyperfilm after addition of developing reagents (Amersham).

RESULTS

Growth Inhibition Studies of Biotinylated Peptides

Growth of both MiaPaCa-2 and PANC-1 cell lines were maximally inhibited by biotinylated PYY(14-36) at 10 nmol/L. Biotinylated PYY(27-36) did not have any biologic activity against pancreatic ductal adenocarcinoma cell growth. MiaPaCa-2 cell growth inhibition is depicted in Fig. 1 with the addition of 10 nmol/L bio-PYY(14-36) after a 12-hour exposure time. Growth inhibition is described as the percentage of reduction versus control groups. Control groups received an equivalent amount of PBS (peptide solvent) to mimic the physical disturbance of adding peptide solution in other wells ($N = 12$; $P < 0.05$ by analysis of variance). Biotinylated PYY (14-36) reduces the growth of MiaPaCa-2 by 24% ± 1.2% and PANC-1 cells by 30% ± 1.8% after a 12-hour exposure. MTT studies were performed 72 hours after initiation of exposure to peptide therapy. These studies reveal that biologic activity is maintained with a biotin group covalently linked to the amino terminal. Biotin-PYY(14-36) is the most effective synthetic analog in suppressing pancreatic cancer growth in both cell lines.

Flow Cytometry of Biotinylated Peptide YY With Streptavidin Phycoerythrin

Cell surface binding of human pancreatic cancer cell lines MiaPaCa-2 and PANC-1 was studied in our laboratory by quantitative flow cytometry. Various lengths of biotinylated PYY analogs were studied for their ability to deliver a fluorescent dye, phycoerythrin. Attached to the phycoerythrin is streptavidin. The streptavidin has a high affinity to the biotin group on the PYY analogs. PYY(9-36) did not have significant binding of flow cytometry. PYY(14-36) was effective at lower concentrations than PYY(22-36).

Fig. 2 depicts two different pancreatic ductal adenocarcinoma lines, MiaPaCa-2 and PANC-1, with specific binding up to 47% ± 3% at the higher concentration of 10 nmol PYY(14-36) per 500,000 cancer cells. Quantitative flow cytometry was performed with streptavidin-phycoerythrin as the fluorescent conjugate to biotinylated PYY(14-36). Data are pre-

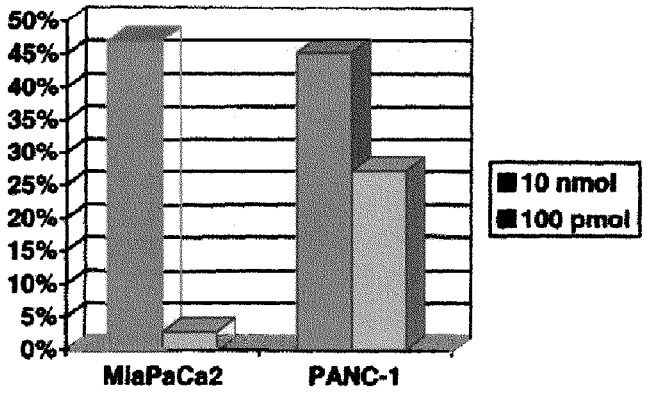

Fig. 2. Percentage of receptor binding of biotinylated PYY-(14-36) as estimated by raw counts obtained by quantitative flow cytometry. PYY(14-36) had the greatest amount of cell membrane binding as detected by an indirect method that uses streptavidin-phycoerythrin to detect the biotinyl group located on the amino terminus of PYY(14-36). No binding is observed in 3T3 fibroblasts, and minimal binding is observed in AR42J cell lines.

sented as the percentage with phycoerythrin fluorescent binding beyond background binding where phycoerythrin-avidin was added without peptide analogs ($N = 6$; $P < 0.05$ by analysis of variance). Histogram shifts are observed when biotinylated PYY(14-36) is added at increasing concentrations. Cancer cells were washed twice with PBS prior to flow cytometry to remove any unbound peptides and washed twice after the addition of streptavidin-phycoerythrin conjugate. Background binding was consistently below 2%. Quantitative flow cytometry proves that biotinylated PYY(14-36) has the ability to bind to the surface of pancreatic cells and allow streptavidin-phycoerythrin to bind to the peptide.

Receptor Cross-Linking Studies

Cross-linking with BS3 (Pierce) was performed with biotinylated PYY(14-36) and pancreatic cancer cells. After incubation with BS3, reactions were stopped and cell membranes were harvested for gel electrophoresis. Standard protein gel separation was performed and revealed a consistent receptor band with biotinylated PYY(14-36) linked to a protein approximating 68 kdal. This band has been found consistently in pancreatic cancer cells and not in fibroblast cell lines when biotinylated PYY(14-36) is added during receptor cross-linking studies (Fig. 3).

Fig. 4 depicts MiaPaCa-2 adenocarcinoma cells pretreated with biotinylated PYY(14-36) and probed with streptavidin-phycoerythrin. Cells were diluted with 50% glycerol and examined with fluorescent microscopy. The photograph depicts fluorescent

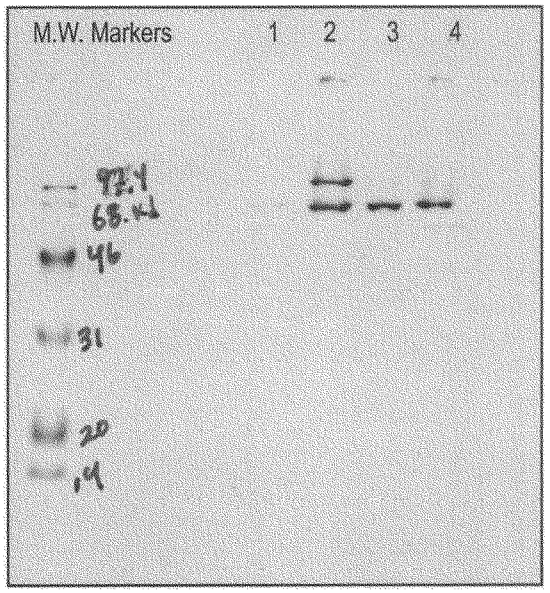

Fig. 3. Cross-linking studies using biotinylated PYY(14-36) and separating the solubilized cell membrane fragments over a protein separating gel. The first lane has biotinylated molecular weight markers. Lane 1 = 3T3 fibroblasts; lane 2 = AR42J pancreatic acinar cancer cells; lane 3 = MiaPaCa-2 ductal adenocarcinoma cells; lane 4 = PANC-1 ductal adenocarcinoma cells.

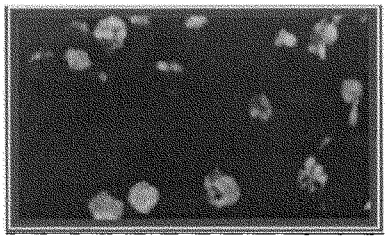

Fig. 4. Fluorescent microscopy of MiaPaCa-2 cells with biotinylated PYY(14-36) and streptavidin-phycoerythrin. Similar fluorescence is observed in PANC-1 cell lines and no fluorescence is observed with AR42J and 3T3 cell lines during microscopy.

cells after vigorous washing of excess streptavidin-phycoerythrin from cells.

DISCUSSION

The initial finding that a shortened PYY analog had growth-suppressing effects was surprising. Previous receptor binding studies have revealed no binding of native PYY to cancer cells, but binding does exist with the shortened PYY(22-36) and PYY(14-36). We have described additional evidence and techniques proving that an improved analog of PYY, biotinylated PYY(14-36), has the ability to

bind to pancreatic cancer cells and deliver fluorescent dye attached to streptavidin. The implications of this streptavidin-biotin indirect model will allow delivery of other fluorescent agents that are more toxic when photoactivated, or other radioactive compounds that may be used for imaging or treatment against metastatic disease. Previous studies by our group have shown that a significant decrease is observed during in vivo administration of synthetic shortened PYY analogs when administered over 2 to 4 weeks.[7] Prolonged exposure to the synthetic PYY analogs will geometrically increase the growth suppression. Pancreatic cancer cells were exposed to peptides for 12 hours and assayed at 72 hours after exposure to peptides. These growth suppressions are remarkable after a short period of exposure to synthetic peptides.

The ability to isolate a novel receptor may be helpful in designing newer antibodies or receptor antagonists to decrease growth of pancreatic cancer. The single band on pancreatic ductal adenocarcinoma cell lines may represent a novel receptor on pancreatic cancer cells. Although verification of this receptor will have to be performed in freshly harvested pancreatic cancer tissue, the ability to localize this single band on tissue culture cell lines is encouraging. Of interest is the ability to isolate a second band on AR42J pancreatic acinar cell lines. The heavier protein band may represent a secondary binding site on acinar cell lines as compared to ductal adenocarcinoma cell lines. The difference may also be explained by the difference in species.

From our previous studies using PYY(22-36), we have seen dramatic decreases in pancreatic cancer cell growth and concomitant decreases in intracellular cyclic adenosine monophosphate.[7] Y receptors have been extensively studied since the sequencing of the first subclass in 1992, and five different Y subtypes have been described to date.[11] All Y-subtype receptors seem to maintain the seven transmembrane regions and their association with G proteins. It remains to be determined whether PYY(14-36) binds to a novel version of Y-subtype receptors or whether this is a new receptor altogether. It would be feasible that this receptor is novel and does not exist in the Y subtype because of our inability to prove binding of native PYY or NPY(3-36) in our previous work. These two peptides represent ligands for Y1- and Y2-subtype receptors, respectively.

The ability to isolate a new receptor may allow us to clone the receptor and use the expression of this receptor as a prognostic indicator for patients with pancreatic cancer. The quantitative expression of growth receptors has been studied in breast cancer with estrogen, epidermal growth factor, and Her-2-neu. We have not quantified the number of receptors with biotinylated PYY(14-36). In our previous work we did calculate 27,000 receptors per cell by Scatchard analysis.[7] Because PYY(14-36) has a higher affinity for pancreatic cancer cells than PYY(22-36), competitive receptor studies will have to be repeated with radioactive iodine-125. It is difficult to quantify the number of receptors by flow cytometry because variable numbers of ligands bind to each cell. Furthermore, the expression of this PYY(14-36) receptor may exist in other malignancies in addition to pancreatic cancer. The advantage of using a shortened synthetic analog is its toxicity is minimal because it is a shortened analog of an endogenous gut hormone, PYY, and its ability to uniquely bind to cancer cells. The shortened peptide is more specific than the previously described PYY(22-36), and it has the ability to deliver more ligands to the cell surface as observed in flow cytometry, receptor cross-linking studies, and fluorescent microscopy.

The ability to decrease pancreatic cancer cell growth and bind to a unique cell membrane protein may prove useful in imaging and treating pancreatic cancer. We have shown the ability to deliver a nontoxic agent, phycoerythrin, by using an indirect method of binding a synthetic analog of PYY to cancer cells followed by streptavidin-phycoerythrin. The extremely high affinity of streptavidin to biotin is useful in vitro, but further studies will have to be performed in vivo. Naturally occurring biotin may interfere with this model of dye delivery.

CONCLUSION

Biotinylated PYY(14-36) decreases pancreatic cancer cell growth while also binding to a specific cell membrane receptor. The implication of discovering a new receptor on pancreatic cancer cells will be useful for future therapy and detection. If a specific receptor is solely expressed on pancreatic cancer cells, earlier detection of pancreatic cancer may be feasible. Eighty-five percent of patients have unresectable pancreatic cancer when initially seen. If more of these patients were diagnosed earlier, survival after surgical resection and chemotherapy might be improved.

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Discussion

Dr. S.B. Archer (Boston, Mass.). Why do you think the shortened version of PYY works so much better with this receptor versus the full length? Are you planning to do any in vivo studies to follow up on these findings?

Dr. C. Liu. The full length must have a three-dimensional conformity that does not bind with cancer cells. I do not know whether or not this is the case only in tissue culture cells, but we do know that when just two amino acids are taken off the end tumors of PYY, it changes the entire fold of the peptide. It starts to unfold. We are in the process of using a mouse model in which we orthotopically grow human cancer in the pancreas as well as in the liver and see if we can image the cells.

Long-Term Prospective Assessment of Functional Results After Proctectomy With Coloanal Anastomosis

Alessandro Fichera, M.D., Fabrizio Michelassi, M.D.

The objective of this study was to prospectively assess the long-term functional results after restorative proctectomy with coloanal anastomosis for rectal cancer. Thirty consecutive patients (18 males; mean age 59.6 ± 9.8 years, range 40 to 75 years) underwent proctectomy with coloanal anastomosis for rectal cancer between January 1990 and March 1997. Cancers were located between 5 and 12 cm from the anal verge. Differences existed in the administration of adjuvant therapy and in the kind of anastomotic reconstruction. An 8 cm colonic J-pouch was fashioned in 11 patients. The coloanal anastomosis was protected by a diverting loop ileostomy in 22 patients. All patients were evaluated using a prospective patient-completed protocol to record daily bowel activity over a 1-week period at 3, 6, and 12 months, and yearly thereafter. Mean follow-up extends to 55.5 ± 27 months (range 7 to 117 months). There were no perioperative deaths. Four patients (13.3%) developed a clinically evident anastomotic dehiscence. Overall, stool frequency decreased from 4.4 ± 2.5 bowel movements per day at 3 months to 3.0 ± 2.8 bowel movements per day at 5 years. Patients with a J-pouch had a lower stool frequency in comparison to patients with an end-to-end coloanal anastomosis during the entire study period (from 3.2 ± 2.2 vs. 3.9 ± 2.7 bowel movements per day at 6 months to 2.8 ± 1.9 vs. 3.4 ± 4.0 bowel movements per day at 5 years; no statistical significance). The percentage of continent patients increased from 50% at 6 months to 75% at 5 years; the percentage of patients with incontinence for solid stool and with frequent incontinence (≥7 episodes per week) decreased from 35.7% at 6 months to 12.5% at 5 years. The influence of the type of anastomosis, dehiscence, protective stoma, J-pouch, radiation therapy, and gender was evaluated with univariate analysis. Although there was no statistically significant correlation between any of these variables and the development of incontinence, when incontinence occurred, a history of anastomotic dehiscence increased the number of episodes of incontinence per week and the percentage of episodes of incontinence for solid stools at 6 months, 2 years, and 5 years ($P < 0.05$ and $P < 0.001$, respectively); the use of preoperative radiation therapy increased the number of episodes of incontinence per week at 6 months, 1 year, 2 years, and 5 years ($P < 0.01$) and the percentage of episodes of incontinence for solid stools at 3 and 6 months and 1 and 2 years ($P < 0.04$); and the presence of a J-pouch increased the number of episodes of incontinence per week at 1 and 2 years ($P < 0.03$ and 0.005, respectively) and the percentage of episodes of incontinence for solid stools at 2, 3, and 4 years ($P < 0.05$). These data suggest that the functional results after proctectomy with coloanal anastomosis improve at least over the course of the first 5 postoperative years. Furthermore, when incontinence develops, its severity is made worse by the occurrence of an anastomotic dehiscence, the use of preoperative radiation therapy, and the presence of a J-pouch. (J GASTROINTEST SURG 2001;5:153-157.)

KEY WORDS: Rectal cancer, coloanal anastomosis, anal continence

Functional results after proctectomy with coloanal anastomosis have been studied prospectively by several investigators.[1-5] Although all of these studies have demonstrated an improvement in functional results with time, none has provided an analysis extending for longer than the first 24 postoperative months. This article details the long-term (up to 5 years) functional results obtained in a group of 30 consecutive patients who underwent restorative proctectomy with coloanal anastomosis for

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rectal cancer at The University of Chicago by a single surgeon.

MATERIAL AND METHODS

Thirty consecutive patients (18 males; mean age 59.6 ± 9.8 years, range 40 to 75 years) who underwent a restorative proctectomy with coloanal anastomosis for rectal cancer (stage I, n = 13; stage II, n = 4; stage III, n = 9; stage IV, n = 3; no evidence of disease in one patient after preoperative chemoradiation therapy) were entered into this prospective study between January 1990 and March 1997 (mean follow-up 55.5 ± 27 months, range 7 to 117 months). The lower edge of the rectal cancer was located between 5 and 12 cm from the anal verge as measured on proctosigmoidoscopic examination. The operative phases of the proctectomy were identical in all 30 patients. Specifically the splenic flexure was completely mobilized, a total mesenteric excision was performed, and the descending colon was used for the reconstruction in all patients. Differences existed in the administration of adjuvant therapy (preoperative radiation therapy in 12 patients, postoperative radiation therapy in six patients, and pre- and postoperative therapy in one patient) and in the kind of anastomotic reconstruction (hand-sewn anastomosis in six patients, stapled anastomosis in 24 patients). An 8 cm colonic J-pouch was fashioned in 11 patients by folding the descending colon onto itself and creating a side-to-side anastomosis with a linear stapler. The coloanal anastomosis was protected by a diverting loop ileostomy in 22 patients. In these patients, the diverting stoma was closed after 4.5 ± 2.8 months (range 1.5 to 13 months).

A questionnaire[6] was mailed to patients at 3, 6, and 12 months, and yearly thereafter. The protocol consisted of a 7-day diary to record oral intake, sleep patterns, bowel activity, and continence daily for 1 week. Following a detailed legend, patients were able to chart the consistency of each bowel movement and grade the severity of eventual episodes of fecal incontinence. A pictorial 24-hour clock allowed patients to record the time of each bowel movement and episode of incontinence.

Two to 3 weeks after receiving the protocol, patients came to our outpatient clinic where the protocol was evaluated by the treating surgeon (F.M.). Answers that appeared to be unclear or inconsistent with previous answers were clarified; bowel activity and episodes of incontinence were averaged over 7 days and were expressed with 24-hour mean values. Diet and medications were reviewed, and suggestions were made to improve functional results. A complete physical examination was performed with particular attention given to a digital examination aimed at assessing the status of the coloanal anastomosis, the anal canal, the eventual colonic J-pouch, and the sphincter mechanism.

Data were entered into an IBM PC/II computer and analyzed by an independent observer (A.F.). Results were expressed as mean ± standard deviation (SD) or as the percentage of patients at each specific time interval. The absolute number of patients available at each successive follow-up interval gradually decreased because of differences in length of follow-up, death, conversion to a permanent stoma, or patients' failure to return their questionnaires and/or maintain their scheduled follow-up visits at specific intervals. The absolute number of patients observed at each time interval is specified in Tables I to V.

Table I. Frequency of daily bowel movements

Time	No. of patients	No. of bowel movements	Range/day
3 mo	11	4.4 ± 2.5	2-10
6 mo	15	3.6 ± 2.4	1-7
1 yr	15	3.2 ± 1.9	1-7
2 yr	12	3.6 ± 2.1	1-7
3 yr	10	3.9 ± 2.4	1-6
4 yr	8	3.0 ± 2.2	1-5
5 yr	9	3.0 ± 2.8	1-8

RESULTS

There were no perioperative deaths. Four patients (13.3%) developed a clinically evident anastomotic dehiscence. In the course of this study, two patients underwent conversion of the coloanal anastomosis to an end colostomy: one for a tight stenosis after postoperative radiation therapy and another because of a recurrence of cancer in the pelvic region.

Frequency of bowel movements at various intervals is detailed in Table I. Patients had a mean (± SD) of 4.4 ± 2.5 (range 2 to 10) bowel movements per 24 hours at 3 months. Fecal frequency decreased to 3.2 ± 1.95 (range 1 to 7) bowel movements per 24 hours at 1 year. By 5 years, patients had a mean of 3 ± 2.8 (range 1 to 8) bowel movements per 24 hours. Twenty-two patients (73%) experienced difficulty with bowel movements, varying in severity from clustering to the need to self-administer enemas to initiate defecation. Patients with a J-pouch had lower stool frequency in comparison to patients with a straight coloanal anastomosis (Table II) during the entire study period, from 3.2 ± 2.2 vs. 3.9 ± 2.7 bowel

Table II. Effect of J-pouch reservoir on frequency of bowel movements

	Pouch		No pouch	
Time	No. of patients	No. of bowel movements ± SD	No. of patients	No. of bowel movements ± SD
3 mo	5	3.6 ± 1.5	5	5.5 ± 3.3
6 mo	7	3.2 ± 2.2	8	3.9 ± 2.7
1 yr	7	3.1 ± 1.5	8	3.4 ± 2.4
2 yr	4	3.7 ± 1.0	8	3.6 ± 2.5
3 yr	3	2.6 ± 1.2	7	4.4 ± 2.7
4 yr	4	1.8 ± 1.6	4	2.9 ± 2.8
5 yr	5	2.8 ± 1.9	4	3.4 ± 4.0

Table III. Rate of complete continence

Time	No. of patients	Percent
3 mo	10	60
6 mo	14	50
1 yr	16	50
2 yr	14	50
3 yr	10	60
4 yr	8	50
5 yr	8	75

Table IV. Rate of incontinence for solid stool

Time	No. of patients	Percent
3 mo	10	40
6 mo	14	35.7
1 yr	15	20
2 yr	14	28.6
3 yr	9	11.1
4 yr	7	14.3
5 yr	8	12.5

Table V. Frequency of incontinence

Time	No. of patients	1-2 episodes/wk (%)	3-6 episodes/wk (%)	≥7 episodes/wk (%)
3 mo	10	10	10	20
6 mo	14	7.1	7.1	35.7
1 yr	16	6.2	6.2	37.5
2 yr	14	14.3	7.1	28.6
3 yr	10	10	10	20
4 yr	8	12.5	25	12.5
5 yr	8	12.5	0	12.5

movements per day at 6 months to 2.8 ± 1.9 vs. 3.4 ± 4.0 bowel movements per day at 5 years (no statistical significance).

Percentage of complete continence, occurrence of incontinence for solid stools over time, and frequency of episodes of incontinence are shown in Tables III to V. Complete continence was experienced by 60% and 50% of patients at 3 and 6 months, respectively; by 5 years 75% of patients were completely continent. Forty percent and 35.7% of patients experienced incontinence for solid stools at 3 and 6 months, respectively; by 5 years only 12.5% of patients were incontinent for solid stool. The percentage of incontinent patients who had frequent episodes of fecal soilage (>7 per week) decreased from 35.7% at 6 months to 12.5% at 5 years.

The influence of the type of anastomosis (hand-sewn 6; stapled 24), anastomotic dehiscence (yes 4; no 26), protective stoma (yes 22; no 8), colonic J-pouch (yes 11; no 19), and radiation therapy (preoperative 12; postoperative 6), and sex (18 males, 12 females) on continence was evaluated by means of univariate analysis. Although there were no statistically significant correlations between any of these variables and the development of incontinence, when incontinence occurred, a history of anastomotic de-

hiscence, the use of preoperative radiation therapy, and the presence of a colonic J-pouch made it more severe. The occurrence of anastomotic dehiscence increased the number of episodes of incontinence per week ($P < 0.05$) and the percentage of episodes of incontinence for solid stool at 6 months, 2 years, and 5 years ($P < 0.01$). The use of preoperative radiation therapy increased the number of episodes of incontinence per week at 6 months and 1, 2, and 5 years ($P < 0.01$) and the percentage of episodes of incontinence for solid stool at 3 and 6 months and 1 and 2 years ($P < 0.04$). Patients with a J-pouch had an increased number of episodes of incontinence per week at 1 and 2 years ($P < 0.03$) and an increased percentage of episodes of incontinence for solid stools at 2, 3, and 4 years ($P < 0.05$).

DISCUSSION

In this study we describe the long-term functional results following restorative proctocolectomy with coloanal anastomosis for rectal cancer. The patients enrolled in the study all had rectal cancer that was located between 5 and 12 cm from the anal verge. Although it may be surprising to see patients with rectal cancer as high as 12 cm from the anal verge in this particular study, it must be remembered that the level at which intestinal continuity is restored after surgical resection of a rectal adenocarcinoma depends on several variables. One of these variables is represented by the ease with which the clearance around and distal to the tumor can be achieved by the operating surgeon and by the ease of the transection of the rectal mesentery. Occasionally, in the obese patient with a narrow pelvis, even lesions at 12 cm are better approached with a complete resection of the rectum and a coloanal anastomosis at the level of the pelvic floor. Obviously, most patients with a tumor between 8 and 12 cm do not require a complete rectal excision and therefore do not undergo a coloanal anastomosis. Yet, for reasons described earlier, patients with rectal cancers as high as 12 cm from the anal verge have been included in this study as well as other previous studies on coloanal anastomosis.[4,5]

To minimize patients' inaccuracies and physicians' subjectivity, the long-term functional results were assessed prospectively through periodic administration of patient-completed daily diaries. The outcome of our study suggests that functional results after proctectomy with coloanal anastomosis continue to improve over the first 5 years after surgery. Specifically, the frequency of bowel movements decreases, the percentage of patients who are fully continent increases and, when fecal incontinence develops after the surgical procedure, its severity, measured both as the number of episodes of incontinence per week as well as the percentage of incontinence for solid stool, decreases over time.

Our data on the frequency of bowel movements are in agreement with those reported by others. Lazorthes et al.[5] performed clinical assessments in 40 patients after proctectomy and coloanal anastomosis. Patients with a straight coloanal anastomosis had 5.2, 4.5, and 3.6 bowel movements per 24 hours at 3, 12, and 24 months, respectively, after closure of the protective stoma. Lazorthes et al.[5] concluded that a significant reduction in the frequency of defecation continues for at least 2 years. Our data on the frequency of bowel movements during the first 24 months parallels the experience of Lazorthes et al.[5]; in addition, our observations suggest that the improvement continues at least for the first 5 years postoperatively.

Our data on the beneficial effects of a colonic J-pouch on stool frequency mirror data from several prospective randomized studies.[1,2,4,5] The lower stool frequency obtained with a colonic J-pouch is maintained for the entire observation period. Yet, caution should be used in interpreting these data as no statistically significant differences are apparent, probably because of the small size of our patient cohort and, more important, the selection of patients for the colonic J-pouch followed no randomized design.

Three fourths of our patients continued to be fully continent at 5 years, a 50% improvement when compared with the 6-month continence rate. In addition, the percentage of patients who were incontinent for solid stools dropped from 35.7% to 12.5%. A similar decrease was evident in the percentage of patients with frequent ($\geq$7 per week) episodes of incontinence. Other investigators have noted an increase in the number of completely continent patients over time. Benoist et al.[7] in a retrospective study of 129 patients after coloanal or colorectal anastomosis, noted that the percentage of patients who were completely continent increased from 60% at 1 year to 75% at 3 years. Lazorthes et al.,[8] in a prospective study aimed at comparing clinical results between small and large colonic J-pouches following coloanal anastomosis, reported that 50% and 65% of patients were completely continent at 3 and 24 months, respectively. They also noted that the percentage of patients who were incontinent for solid stool decreased with time.

As our study validates these previous observations and extends them to 5 years, it is appropriate to offer possible explanations for this improvement over time. The reasons may be multifactorial and may include localized factors (such as a decrease in postoperative edema and improvement in resting anal sphincter tone) and generalized factors (such as physical recovery from the stress of surgery and, often, adjuvant

therapy, dietary modifications, and the use of medication to control bowel activity).

Although our study offers no statistically significant correlation between any of the pre- or perioperative variables and the development of incontinence, when incontinence develops, it seems to be of much greater severity if it follows anastomotic dehiscence, preoperative radiation therapy, or construction of a J-pouch. The reasons for these correlations are not clear and may be different for each of these variables. The fibrosis that occurs after secondary healing of an anastomotic dehiscence may have a negative effect on the sphincter mechanism; preoperative radiation therapy has been shown to decrease the anal sphincter resting tone; and the capacity of a J-pouch increases the volume of stools necessary for a patient to feel the need to defecate and, consequently, may place patients with impaired continence at higher risk for soiling by the mere presence of stool in the pouch.

CONCLUSION

We have reported our long-term functional results after restorative proctectomy with coloanal anastomosis for rectal cancer. Following complete rectal excision, a coloanal reconstruction offers good functional results that improve over time. This knowledge helps to offer a realistic and positive long-term view of the functional results that patients may be able to achieve with an appropriate period of recovery.

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Fibrin Glue for All Anal Fistulas

Stephen M. Sentovich, M.D.

The aim of this study was to determine if a new sphincter muscle–sparing technique that uses fibrin glue was effective in closing all types of anal fistulas. All patients with anal fistulas who were seen by a single surgeon over a 2-year period were treated with fibrin glue. Six to 8 weeks after a seton was placed in the fistula tract, either autologous fibrin glue or commercially available fibrin sealant was used to close the fistula tract. Twenty patients were treated with a mean follow-up of 10 months. Etiology of the anal fistulas was as follows: cryptoglandular in 13, Crohn's disease in four, and miscellaneous in three. Fibrin glue closure of the anal fistula was successful initially in 15 patients (75%) and was successful after a second treatment in two additional patients, for an overall fibrin glue fistula closure rate of 85% (17 of 20). Functional results have remained excellent with no patient reporting any change in continence after treatment. Fibrin glue is simple and effective treatment for all anal fistulas with excellent functional results. (J GASTROINTEST SURG 2001;5:158-161.)

KEY WORDS: Anal fistula, fibrin glue, Crohn's disease

The goal of surgical treatment of an anal fistula is to heal the fistula tract with the lowest rate of recurrence and still maintain continence. Standard surgical treatment of anal fistulas is anal fistulotomy, which involves laying open the fistula tract and any associated sphincter muscle. Despite being properly performed, fistulotomy wounds can have prolonged healing times and can result in contour defects around the anus.[1] In addition, Lunniss et al.[2] have shown that even minimal division of the anal sphincter muscle during fistulotomy can be associated with changes in fecal continence.[2] Given these results, investigators have sought simpler sphincter muscle–sparing techniques to treat anal fistulas.

Recently the use of tissue adhesives or sealants in surgery has increased because of improved autologous and commercially available products. In 1993 Abel et al.[3] first reported the treatment of rectovaginal and complex anal fistulas with autologous fibrin glue. In patients with complicated Crohn's or HIV-related anal fistulas, they reported a fistula closure rate of 60%. Venkatesh and Ramanujam[4] reported similar results in a small group of patients with recurrent anal fistulas. Using an improved autologous fibrin glue, Cintron et al.[5] recently reported a fistula closure rate of 85%. Although these first three studies had reported promising results, two involved only a small number of patients, all three used autologous fibrin glue, which can be technically demanding to prepare, and in all three studies only selected patients were treated.

Approximately 2 years ago, we began treating all patients with anal fistulas with either autologous fibrin glue or the recently available commercial fibrin sealant. The aim of the current study was to determine if this relatively new sphincter muscle–sparing technique that uses fibrin glue is effective in closing all types of anal fistulas.

PATIENTS AND METHODS

All patients with anal fistulas who were seen by a single surgeon over a 2-year period were treated with fibrin glue. After an initial evaluation in the office that confirmed the presence of a fistula and documented the degree of bowel control, all patients were scheduled for surgery.

At the first operation, the fistula tract and internal opening were identified. Any associated abscess was drained. The fistula tract was debrided and biopsy specimens were sent for histologic examination. At the internal opening, the offending gland was destroyed

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with electrocautery. Finally, a vessel loop was placed in the tract and tied to itself with a 3-0 silk suture to create a loop seton drain. Postoperatively all patients were seen in the office at 2 and 4 weeks, at which time healing around the vessel loop seton was assessed. When all inflammation had resolved and the external and internal openings of the fistula tract had healed around the seton, patients were scheduled for their second operation.

In preparation for the second operation, all patients underwent mechanical bowel cleansing. At the second operation, the type of fistula and length of the fistula tract were noted. The fistula tract was debrided with a curette and the internal opening closed with a 3-0 polyglactin suture. Fibrin glue was then instilled via the external opening to fill the tract (less than 4 ml). Postoperatively patients received pain medication and stool softeners and were instructed to avoid vigorous physical activity for 1 week. Sitz baths were prohibited for fear of dislodging the fibrin glue plug. All patients were seen in the office at regular intervals postoperatively (usually 2 and 6 weeks).

Fibrin Glue

Patients were treated with either autologous fibrin glue or fibrin sealant (Tisseel, Baxter Healthcare Corp., Deerfield, Ill.). Fibrin sealant was unavailable for the first four patients in this series. All other patients were given a choice of either the autologous fibrin glue or commercial fibrin sealant. Patients who elected to have autologous fibrin glue donated 1 unit of blood prior to their second operation from which autologous cryoprecipitate was made. At the time of the second operation, the thawed cryoprecipitate was drawn into a syringe and connected via a Y-connector to a second syringe with thrombin (Thrombinar, Jones Medical Industries, St. Louis, Mo.) at 1000 units/ml. Simultaneous administration of the cryoprecipitate and thrombin via the Y-connector creates the fibrin glue that is placed into the fistula tract. For most of our patients, blood donation was not necessary because they had elected to be treated with fibrin sealant. The fibrin sealant (Tisseel) was prepared according to the manufacturer's directions using a similar two-syringe and Y-connector technique for the creation and administration of the sealant.

RESULTS

Over a 2-year period, 20 consecutive patients have been treated with fibrin glue. The mean age of these patients was 43 years (range 28 to 71 years) and included 16 men and four women. The average length of the fistula tracts was 2.6 cm (range 0.7 to 9 cm). None of the patients reported any pretreatment fecal incontinence.

At the first operation, all patients had a seton placed in the fistula tract and any associated abscess was drained. This operation lasted less than 30 minutes and was performed as an outpatient procedure. After 6 to 8 weeks, all patients had no evidence of sepsis and had healed around their vessel loop seton. Only five patients voiced minor complaints (perianal drainage and irritation) regarding the seton, but there were no other complications related to this first operation.

The second operation, at which time the seton was removed and fibrin glue placed to close the fistula tract, also lasted less that 30 minutes and was performed as an outpatient procedure. Some patients complained of some drainage for the first 1 to 2 weeks after the second operation, but this resolved once their fistulas had completely closed. There were no septic complications or other problems related to this second operation in any of the patients.

The types of fistulas and their treatment are shown in Table I. Thirteen (65%) were cryptoglandular in origin, four (20%) were due to Crohn's disease, and three (15%) were due to miscellaneous causes. Two patients had two separate fistula tracts treated: one of these

Table I. Type of fistula, treatment, and results with fibrin glue

Fistula type	Treatment	Results
Cryptoglandular (n = 13)	Fibrin sealant (n = 8)	Healed
	Autologous (n = 4)	Healed
	Fibrin sealant twice (n = 1)	Failed
Crohn's disease (n = 4)	Fibrin sealant (n = 2)	Healed
	Autologous/sealant (n = 1)	Healed
	Fibrin sealant (n = 1)	Failed
Miscellaneous (n = 3)		
Surgery/x-ray therapy	Autologous/sealant (n = 1)	Healed
Surgery/fissure	Fibrin sealant (n = 1)	Failed
HIV	Fibrin sealant (n = 1)	Healed

patients was in the cryptoglandular group and the other was in the group with Crohn's disease. Both fistulas healed in both patients. Fourteen patients (70%) were treated with fibrin sealant alone and four (20%) with autologous fibrin glue alone. Two (10%) were treated with autologous fibrin glue followed by fibrin sealant.

After treatment with autologous fibrin glue or fibrin sealant, 15 (75%) of the anal fistulas had closed. Two patients whose fistulas had failed to close after autologous fibrin glue were retreated with fibrin sealant. Fibrin sealant successfully closed the fistula tract in both patients. After a mean follow-up of 10 months, the overall success rate for fibrin glue closure of anal fistulas was 85%.

Fibrin glue failed to close the fistula in three patients. One patient had a recurrent cryptoglandular fistula after two previous fistulotomies and seton placement. He had significant scarring and contour defects around the anus from his previous surgery. After two unsuccessful attempts with fibrin sealant, the patient's fistula was successfully closed with a rectal advancement flap. The second patient to have unsuccessful fibrin glue closure of his fistula tract had a long extrasphincteric ileal pouch–anal fistula 8 years after total proctocolectomy and ileal pouch–anal anastomosis for ulcerative colitis. This patient failed a single attempt at fibrin glue fistula closure, was diagnosed with Crohn's disease, and eventually was converted to an end ileostomy. The third patient to have unsuccessful fibrin glue closure of his fistula had originally undergone hemorrhoidectomy for bleeding hemorrhoids. Soon after this operation, a sphincterotomy for anal fissure was performed. He eventually came to our clinic for evaluation of chronic rectal pain, and an anal fistula originating from a chronic anal fissure was diagnosed. The short fistula tract was treated with a seton for less than 4 weeks prior to unsuccessful fibrin glue closure. The patient refused a second treatment with fibrin glue and underwent subcutaneous fistulotomy that did successfully resolve his symptoms.

Preoperatively, none of the patients complained of any degree of fecal incontinence. After treatment, all patients were evaluated for signs or symptoms of fecal incontinence. None of the patients reported any change in continence following treatment with fibrin glue.

DISCUSSION

In this study we report successful closure of anal fistulas by means of a new sphincter muscle–sparing technique that uses fibrin glue. Our overall success rate was 85%. There were no septic complications as a result of this treatment, and no patient had any change in continence. Although the mechanism of fibrin glue closure of anal fistulas has not been studied, presumably fibrin glue closes anal fistulas by temporarily plugging the fistula tract and providing a framework for fibroblast ingrowth. With this framework, fibrosis and permanent fistula tract closure occurs.

Our results compare favorably to those achieved by Abel et al.[3] and Venkatesh and Ramanujam,[4] both of whom reported a fistula closure rate of only 60%. This difference, however, may be explained by their patient selection (only complex or recurrent fistulas were studied by Abel et al. and Venkatesh and Ramanujam), their use of autologous fibrin glue (compared to primarily fibrin sealant in our study) and/or their one-stage operative technique (compared to our two-stage technique). It is certainly possible that the success rate for treating complex and recurrent anal fistulas is, indeed, lower. In addition, it is also possible that their one-stage technique and their exclusive use of autologous fibrin glue that has a lower fibrinogen concentration and may not form as strong a plug as the commercial fibrin sealant also contributed to their lower success rate. Certainly additional investigations are warranted to resolve these issues.

Our overall results, however, wear no better than those reported by Cintron et al.,[5] who also reported an overall fibrin glue fistula closure rate of 85%. Cintron's study differed from ours in many ways. First, we treated all patients with anal fistulas with fibrin glue, and their patients may have been selected for fibrin glue treatment. Second, all of their patients were treated with autologous fibrin glue, whereas most of our patients were treated with fibrin sealant. Third, their patients were treated in a single operation with no bowel preparation rather than a two-stage operation as we did. Of note, they did not report any septic complications as a result of their one-stage technique, but certainly a relatively small infection within the fistula tract could dislodge the fibrin glue plug and result in treatment failure that would be very difficult to recognize clinically. Finally, the mean follow-up in Cintron's study was 3 months, whereas in our study the mean follow-up was 10 months. Following fibrin glue closure, recurrent/persistent fistula may not be detected within 3 months, which would imply that the 85% success rate reported by Cintron et al. might diminish somewhat with time. At this point in time, it is impossible to determine which of the preceding variables is most important in the successful closure of anal fistulas with fibrin glue. We can say, however, that regardless of which technique or type of fibrin glue is used, fibrin glue does successfully close most anal fistulas with no reported complications. Thus fibrin glue closure can be used for all anal fistulas.

Despite all of our success using fibrin glue to close anal fistulas, we were not successful in closing the fistulas of three of our patients. Two of these patients had quite unusual fistulas, which in all likelihood contributed to our failure to achieve closure with fibrin glue. One of these patients had undergone two previous fistulotomies with seton placement and had significant scarring and contour deformity around the anus. We suspect that the degree of scarring and fibrosis in combination with a relatively short fistula tract resulted in expulsion of the fibrin glue plug. The other patient had a complex ileal pouch–anal fistula in the setting of Crohn's disease. The final patient to have unsuccessful fibrin glue closure had undergone previous surgery (hemorrhoidectomy and sphincterotomy) and had a relatively short fistula tract to a chronic anal fissure. This person was impatient for his second-stage operation, which was performed less than 4 weeks after placement of the seton. We suspect that persistent sepsis in the tract in combination with a relatively short fistula tract contributed to the expulsion of the fibrin glue plug and failure of the fistula tract to close in this case.

CONCLUSION

We report an 85% success rate in closing anal fistulas with a sphincter muscle-sparing technique using fibrin glue. Future investigations are necessary to determine the most effective fibrin glue type and operative technique to improve on these results.

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Discussion

Dr. S.W. Ashley (Boston, Mass.). It seems that there is a group of patients who have very simple fistulas with very little muscle involvement for whom this treatment is unnecessarily complex.

Dr. S.M. Sentovich. I would agree that a simple subcutaneous fistula or one that has very little muscle involved is not likely to result in any of the potential complications of fistulotomy. In terms of complexity, I set this up as two small operations plus the bowel preparation because I was concerned about sepsis with insertion of glue into a tract, and I wanted to eliminate that possibility. Also, we did not have any patients who developed subsequent abscesses. In the future, we will simplify this procedure and perhaps be able to use fibrin glue in the office setting, but I have not yet tried this.

Dr. M. Sailer (Wurzburg, Germany). This is a very intriguing method of treating fistulas. What actually happens? Obviously this is only a temporary closure. Does the fibrin glue then get replaced by normal tissue?

Dr. Sentovich. We suspect that the glue provides a framework or lattice within which fibroblast ingrowth can occur, and then the tract becomes permanently sealed. Certainly, long-term results on these patients would be important to confirm that.

Dr. T.M. Young-Fadok (Rochester, Minn.). Do you place setons in all of your patients who present with abscesses? A number of patients, as you know, will eventually heal without fistulas anyway. Second, if a patient has a fistula, do you still use a two-part procedure, first placing the seton to clean out the tract and later bringing the patient back, or would you treat the fistulas primarily with the fibrin glue?

Dr. Sentovich. Primary abscesses are simply drained. All of our patients had fistulas, and I think there were three who seemed to have a small abscess associated superficially that was drained at the time of the first operation. So these are patients with fistulas not abscesses. I have been using a two-stage procedure for everyone because of my concern about possible sepsis, and I wanted simply to instill the glue into a tract.

Dr. Victor H. Finch (Chicago, Ill.). Your follow-up, as I gathered, was 7 months on average. I realize that most of the fistulas will recur within 7 months, but do you have any laboratory data to show that the repair will hold up for a longer period of time?

Dr. Sentovich. I do not.

Dr. Herbert R. Freund. (Jerusalem, Israel). Do you inject more than one fistula at the same time, or do you inject multiple fistulas?

Dr. Sentovich. There were two patients in this series who had two fistulas.

Contribution of Intraoperative Enteroscopy in the Management of Obscure Gastrointestinal Bleeding

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Obscure gastrointestinal bleeding remains a significant diagnostic challenge. Our aims were (1) to determine the efficacy of intraoperative enteroscopy (IOE) in identifying lesions responsible for obscure gastrointestinal bleeding and (2) to determine the outcome of patients after treatment of these lesions. We retrospectively reviewed all patients who underwent IOE for obscure gastrointestinal bleeding from 1992 to 1998. Patients were divided into those with overt and those with occult gastrointestinal bleeding. Follow-up was complete in 67 patients (96%), with a median of 32 months (range 1 to 91 months). Seventy patients (52 overt and 18 occult) underwent IOE after extensive preoperative evaluation. Median duration of bleeding was 12 months, requiring a median of 14 blood transfusions. Risk factors for bleeding were identified in 46 patients (61%). A lesion was identified and treated in 52 patients (74%)—39 in the overt group and 13 in the occult group. Lesions identified were vascular (54%), ulcerations (31%), tumors (11%), and small bowel diverticula (4%). Overall, 35 patients (52%) were found to have one or more lesions at IOE that were treated surgically and had no further bleeding. IOE, through a mid–small bowel enterotomy, has low morbidity and is effective in that it identified a treatable lesion in 74% of patients, which led to cure of bleeding in 52%. (J GASTROINTEST SURG 2001;5:162-167.)

KEY WORDS: Intraoperative, enteroscopy, endoscopy, obscure gastrointestinal bleeding

Identifying the source of obscure gastrointestinal bleeding presents a formidable diagnostic challenge. Recently the American Gastroenterological Association defined obscure gastrointestinal bleeding as bleeding of unknown origin that persists or recurs after a negative colonoscopy and/or upper endoscopy.[1] Obscure bleeding can be further classified as *overt*, manifested by passage of visible blood, or *occult*, with positive fecal occult blood testing and iron deficiency anemia. Based on this definition of obscure gastrointestinal bleeding, approximately 5% of all gastrointestinal bleeding would be placed into this category. Push enteroscopy, often called extended upper endoscopy because it visualizes 60 to 125 cm of the proximal jejunum, is able to identify lesions in up to 75% of these patients.[2-5] Additional diagnostic procedures such as visceral angiography, radionuclide bleeding scans, and small bowel contrast studies can also identify the source of bleeding in another fraction of these patients with obscure gastrointestinal bleeding. Thus obscure bleeding after all nonsurgical methods of detection are exhausted probably represents approximately 1% of all patients with gastrointestinal bleeding. The "gold standard" for diagnosing the source of bleeding in this highly select, intensely evaluated group of patients is intraoperative enteroscopy (IOE) performed at the time of exploratory celiotomy. Several series have demonstrated the efficacy of IOE in identifying a lesion responsible for obscure gastrointestinal bleeding in 70% to 100% of patients.[5-9] However, diagnosis and treatment of these lesions cures bleeding in only 40% of these patients in our previous experience.[6]

Using a large series of patients from a single institution, our aims were (1) to determine the current efficacy of IOE in finding lesions responsible for ob-

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scure bleeding, and (2) to determine the outcome of these patients after treatment of these lesions.

METHODS

We retrospectively reviewed the records of all patients who underwent IOE for obscure gastrointestinal bleeding from January 1992 to September 1998. All patients underwent extensive preoperative evaluation using an array of diagnostic tests including esophagogastroduodenoscopy (EGD), colonoscopy, push enteroscopy (extended upper endoscopy), Sonde enteroscopy, small bowel contrast studies, radionuclide bleeding scans, visceral angiography, and/or computed tomography. Preoperative data were evaluated to ascertain patients' risk factors for bleeding, significant comorbid conditions, prior hospitalizations for bleeding, blood transfusion requirements, and diagnostic studies used. IOE was performed under the direction of a staff surgeon and a staff gastroenterologist. Pathologic evaluation was performed on all surgical specimens. Patients were divided into two groups based on their patterns of bleeding: those with overt bleeding and those with occult bleeding. These groups were compared with respect to identification of lesions by IOE, lesion pathology, and outcome. Patient follow-up information was obtained through clinic visits or telephone contact, which included history of postoperative complications, recurrent bleeding, subsequent hospitalizations, and need for further blood transfusions.

RESULTS

Patient Group

A total of 70 patients underwent IOE after extensive preoperative evaluation during this period for obscure gastrointestinal bleeding. The 38 women and 32 men had a median age of 68 years (range 13 to 86 years). Median follow-up was 32 months (range 1 to 91 months) and follow-up was complete in 67 patients (96%).

Risk factors for bleeding were identified in 43 patients (61%). These risk factors included daily use of coumadin or nonsteroidal anti-inflammatory agents, chronic renal failure, liver insufficiency, blood dyscrasias, and inherited disorders of arteriovenous malformations (Table I). Additionally, 10 patients (14%) had a known malignancy prior to evaluation for gastrointestinal bleeding, including colorectal cancer in two, and prostate, breast, bladder, endometrial, pancreatic islet cell, renal, lymphoma, and chronic lymphocytic leukemia in one patient each. Significant medical comorbidity was noted in 40 patients (57%).

The median duration of bleeding prior to IOE was 12 months (range 0.1 to 360 months). Patients had a median of two prior hospitalizations (range 0 to 12) and 14 blood transfusions (range 0 to 300) prior to IOE. Additionally, 14 patients had undergone laparotomy for bleeding before undergoing IOE at our institution.

Preoperative gastrointestinal bleeding presented as melena in 35 (50%), hematochezia in 17 (24%), and iron deficiency anemia with Hemoccult-positive stools in 18 (26%). Patients were divided into those with overt bleeding (52 patients) and those with occult bleeding (18 patients).

Preoperative diagnostic evaluation included EGD and colonoscopy at our institution in all patients. In 47 (67%) and 34 (49%) patients, respectively, EGD and colonoscopy were performed more than once. Additional procedures used included push enteroscopy, small bowel contrast studies, visceral angiography, computed tomography, radionuclide bleeding scans, and Sonde enteroscopy (Table II).

Technique of Intraoperative Enteroscopy

At celiotomy, using a midline incision, adhesiolysis was performed as necessary, and all patients underwent a meticulous surgical exploration of the abdomen to exclude abnormalities. If a source was identified on gross exploration, the patient was excluded from analysis and

Table I. Risk factors for gastrointestinal bleeding

Risk factor	Overt (52 patients)	Occult (18 patients)	Total (%)
Coumadin	13	3	23
Nonsteroidal anti-inflammatory drug use	12	3	21
Renal failure	7	0	10
Liver insufficiency	3	0	4
Blood dyscrasia	2	0	3
Osler-Weber-Rendu disease	1	1	3
Blue rubber bleb nevus	0	1	1
Systemic vasculitis	0	1	1

Table II. Preoperative diagnostic evaluation for gastrointestinal bleeding (N = 70)

Method	No. of patients (%)
Esophagogastroduodenoscopy	70 (100)
Colonoscopy	70 (100)
Push enteroscopy	57 (81)
Small bowel contrast	47 (67)
Visceral angiography	47 (67)
Abdominal computed tomography	46 (66)
Radionuclide bleeding scan	39 (56)
Sonde enteroscopy	18 (26)

Table III. Lesions identified at intraoperative enteroscopy (n = 52)

Lesion	Overt (39 patients)	Occult (13 patients)	Total (%)
Vascular	18	10	54
Ulcer/erosion	14	2	31
Tumor	5	1	11
Diverticulum	2	0	4

was not included in this series. If no source was identified, IOE was performed using two teams consisting of the surgeon and endoscopist. The surgeon introduced the endoscope through a mid–small bowel enterotomy in 66 patients (94%). In four patients (6%) the endoscope was inserted via a transoral and/or transanal route. The surgical team helped advance the endoscope while examining the bowel externally using the transilluminating light from the endoscope. Additionally, an air-trapping technique was used for better mucosal visualizations as follows: the surgeon isolates a 20 to 25 cm segment of intestine by gentle occlusion of the distal aspect allowing air insufflation of the limited segment. This maneuver avoids excessive air insufflation and more importantly enables a full, meticulous examination of the entire mucosal surface. After inspection, the segment is released, and the segmental isolation continues in an anterograde fashion. In all patients, the endoscopist examined the mucosa in an anterograde fashion before advancing the endoscope past the segment to be examined. The type of endoscope used was a colonoscope in 47, an enteroscope in 21, a pediatric colonoscope in one, and a Sonde enteroscope in one. Three patients initially underwent an attempted laparoscopic-assisted transoral IOE, but the inability to pass the endoscope through the entire length of bowel, and adequately and reliably view the entire mucosal surface, necessitated conversion to an open method in two. The third patient had a reasonably successful Sonde enteroscopy to the distal ileum.

Findings at Intraoperative Enteroscopy

Overall, a lesion was found in 52 patients (74%), with 39 (75%) in the overt group and 13 (72%) in the occult group. Lesions were identified in the jejunum in 27 (52%) and the ileum in 16 (31%). The remainder of the lesions were located in the duodenum (n = 5) and colon (n = 4). All patients with an identified source underwent resection (n = 49) or oversewing (n = 3) of the lesions. Four "blind" resections (partial colectomies) were performed after negative IOE in patients with a suspicious colonic site either clinically or by imaging preoperatively.

Histologic evaluation was performed of all resected specimens. Vascular ectatic abnormalities were the most common lesions identified and comprised a greater proportion of lesions in patients with occult bleeding (Table III). Ulcerative lesions comprised a third of the lesions found in the group with overt bleeding, and were caused by cytomegalovirus infection, Crohn's disease, nonsteroidal anti-inflammatory drugs, radiation enteritis, or vasculitis, or were of unknown etiology. Other lesions such as tumors or jejunal diverticula were found less frequently.

Morbidity/Mortality

Intraoperative complications attributed to IOE occurred in two patients (3%). In one patient a significant mucosal tear was noted during enteroscopy that required oversewing. In another patient with extensive intestinal and mesenteric angiomatosis, mesenteric bleeding occurred during IOE probably from mesenteric traction, necessitating a segmental resection of the small intestine, which included both the pathologic specimen and additional uninvolved bowel.

In-hospital or 30-day postoperative death occurred in four patients (6%). Three of these patients had ongoing bleeding and died of multisystem organ failure on postoperative days 1, 14, and 20. Another patient with multisystem organ failure prior to IOE had persistent organ failure postoperatively with no evidence of bleeding.

Postoperative complications occurred in 18 patients (26%), which included prolonged postoperative ileus (≥7 days postoperatively, n = 11), transient atrial fibrillation (n = 3), and wound infection, nasogastric tube trauma requiring transfusion, myocardial infarction, and respiratory failure requiring temporary mechanical ventilation (1 patient each). One patient was noted to have deep venous thrombosis 2 months after discharge.

Success of Intraoperative Enteroscopy

Follow-up information was obtained from 67 of the 70 patients. Of the 52 patients who had a lesion identified by IOE and treated surgically, 49 were available for follow-up. No further bleeding occurred in 35 (72%); bleeding persisted in 10 (20%) and recurred (more than 1 year postoperatively) in four (8%). No differences in cure were noted between patients with overt or occult bleeding. Of the 18 patients in whom no site of bleeding was detected, the four who underwent "blind" partial colectomies were cured of further bleeding. Among the 14 others, rebleeding occurred in eight (57%). Overall, 45 (65%) of the 67 patients no longer had bleeding. Excluding those with cure after "blind" resection or spontaneous resolution, 35 patients (52%) were found to have one or more lesions at IOE that were resected or oversewn and had no further gastrointestinal bleeding.

DISCUSSION

Identifying the source of obscure gastrointestinal bleeding can be arduous, expensive, and occasionally impossible with current diagnostic methods. With the current technologic improvements in endoscopic instruments and techniques, those patients with true obscure bleeding represent a highly select group and present a diagnostic and therapeutic dilemma. Previous reports indicated that obscure gastrointestinal bleeding consisted of 5% of all patients with gastrointestinal bleeding.[10] However, with improvement in both the capabilities and availability of push enteroscopy (extended upper endoscopy), this technique is becoming a "standard" in the evaluation of patients with obscure gastrointestinal bleeding. Push enteroscopy has been reported to identify a suspicious lesion in up to 75% of patients with obscure gastrointestinal bleeding.[3-5] Other diagnostic methods such as Sonde enteroscopy, small bowel contrast studies, visceral angiography, radionuclide bleeding scans, and computed tomography may also identify the source in an additional subset of these patients. Thus it is likely that approximately only 1% of patients with gastrointestinal bleeding have an unidentified source of bleeding after this extensive, nonsurgical array of diagnostic procedures. IOE is the gold standard diagnostic method for this highly select group of patients, and several studies have documented the efficacy of IOE in identifying a lesion responsible for bleeding, ranging from 70% to 100%.[5-9] Identification and treatment of these lesions found on IOE, however, does not always equate with cure of further bleeding.[6-8]

Comparisons of the efficacy of IOE in identifying a lesion or the outcome after treatment of this lesion are fraught with hazard. This problem originates from a high variability in defining obscure bleeding, the extent and sophistication of preoperative diagnostic workup, characteristics of the pattern of bleeding, differing IOE techniques, and extent of mucosal visualization.

A source in patients with *occult* gastrointestinal blood loss is often difficult to identify, even after extensive diagnostic workup, and can remain elusive in up to 52%.[1] Conversely, a source in patients with *overt* bleeding, especially if it is acute and severe, is more likely to be identified, because multiple diagnostic methods designed to reveal a site of active bleeding can be employed if the bleeding is more apparent or active. In this study of obscure gastrointestinal bleeding, IOE was able to identify a potential source of bleeding with similar efficacy in patients with either occult or overt bleeding, demonstrating the efficacy of IOE.

The reported techniques of IOE also vary in several potentially important respects such as the approach to intra-abdominal access (laparotomy vs. laparoscopy), the endoscope used, and the technique of endoscope insertion. We prefer a midline celiotomy for surgical assistance in IOE. Many of these patients have had multiple prior surgeries, and adhesiolysis is often required. This approach also allows greater care in telescoping the intestine and controlling the mesenteric stretching. Further, in our initial limited experience with laparoscopic assistance, the procedure is more time consuming and has a high failure rate in allowing a full and more important, an adequate intestinal visualization, requiring conversion to an open laparotomy. The endoscope used in IOE varies both in the literature and in our current experience. A sterile colonoscope was most commonly used in our series; however, the use of the push enteroscope is increasing. We were unable to identify any significant difference in complications using one endoscope over another. It has been proposed that the use of a Sonde enteroscope be used given the small radius of curvature and "potential" for decreased complications.[11] However, with the use of a colonoscope or push enteroscope, we had only two complications related to IOE, and it is our opinion that the greater field of view and the therapeutic capability afforded by these instruments precludes the use of the Sonde enteroscope. Moreover, the rate of complications may have more to do with the method of insertion than the scope used. We have changed our method of insertion over the past decade from that of a transoral or transanal endoscopic access to almost exclusively a direct access via a mid–small bowel enterotomy. This technique has several potential advantages. Using a transoral or

transanal method of insertion requires excessive pleating or telescoping of the bowel onto the endoscope, as much of the endoscope traverses areas previously evaluated such as the esophagus, stomach, colon, or rectum. This leaves less endoscope length to traverse the small bowel and necessitates excessive telescoping, causing complications such as mucosal or serosal tears with an incidence as high as 52%.[7-9,11-14] Conversely, the mid–small bowel enterotomy divides the small intestine into two shorter segments to traverse with less telescoping of the bowel and less associated mesenteric stretching. An additional potential advantage of using the enterotomy insertion technique is its ability to view the gastrointestinal tract in a "reverse" fashion. In this study, nine (17%) of the lesions identified were in the duodenum or colon, which one would think should have been detected by the extensive, multiple endoscopic evaluations prior to IOE. Viewing from a reverse fashion (duodenum "retrograde" and the colon "prograde," via the jejunum) may allow visualization of lesions on intestinal folds or other areas that are difficult to evaluate from the opposite direction; this possibility, however, remains to be substantiated. Although the enterotomy has the advantage of avoiding "intestinal dead space" by avoiding traversing the esophagus, stomach, colon, and rectum, we recommend visualization of the entire gastrointestinal tract in the event a lesion is not identified in the small intestine and is typically easily performed through a single enterotomy. This is supported by the number of lesions identified outside the small intestine in this study and others.[11,15] We also believe that the enterotomy method allows a more complete and controlled visualization of the entire small intestinal mucosa. In patients with extensive adhesions or areas of extreme fixation, more than one enterotomy can be used to maximize mucosal inspection. In no patient did technical failure result in limitation to visualize the entire mucosal surface of the small intestine. Only in a small number of patients with unsuspected lesions such as jejunal diverticula was the remainder of the small intestine not fully visualized based on clinical decision. Transoral and transanal methods are less likely to achieve full small intestinal visualization, with many reports describing failure to reach the terminal ileum in a significant number.[9,13,14]

This study represents the largest series of patients undergoing IOE for obscure gastrointestinal bleeding after extensive preoperative diagnostic evaluation. Using an enterotomy technique of insertion, complication rates were minimal, and full visualization of the small intestine was attained. The efficacy of 74% in finding a lesion is likely less sensitive than in some previous reports; however, currently patients are undergoing more extensive preoperative evaluation with push enteroscopy, excluding many of the patients included in earlier studies. In addition, our patients include those who have no recognizable source of bleeding after both an extensive, exhaustive preoperative evaluation *and* after intra-abdominal exploration at the time of celiotomy. We have previously described our experience with IOE using a transoral/transanal access method,[6] but we believe that an enterotomy technique affords a more complete, controlled examination of the small bowel mucosa and has fewer complications. Despite reports of potential complications of enterotomy, we have not observed a single complication directly related to the enterotomy in more than 70 patients with the use of this method. We propose that this is a safer and more effective method for visualizing all of the intestinal mucosa with minimal morbidity.

IOE, through a mid–small bowel enterotomy, identifies a treatable lesion in 74% of patients, leading to cure of bleeding in 52%. Thus IOE is an effective tool in the management of these highly select patients with obscure gastrointestinal bleeding when all nonsurgical methods of detection have failed.

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Discussion

Dr. B. Dunkin (Baltimore, Md.). I have had some experience managing these patients with laparoscopically assisted enteroscopy and have been impressed that I would have found most of the lesions using laparoscopic exploration alone. I am skeptical about what enteroscopy is adding to the exploration. I was wondering if you could comment a bit more about your technique and speculate as to whether these lesions that have been seen during enteroscopy would have been seen with exploration alone.

Dr. M.L. Kendrick. These were very highly selected patients who had undergone a complete and thorough surgical exploration of the abdomen prior to the intraoperative enteroscopy. All patients with identifiable lesions at the time of celiotomy were excluded from this study. So these represent not only those in whom nonsurgical methods have failed to identify the source, but also a failure of intraoperative exploration of the abdomen.

We perform a mid–small bowel enterotomy following abdominal exploration, and the scope is inserted by the surgeon. Usually the gastroenterologist is maneuvering the scope and watching the monitor. The surgeon watches the monitor but also observes the serosal surface of the bowel using the transilluminating light as it traverses the bowel. Additionally, we use an air-trapping method in which the surgeon occludes the intestine 20 to 25 cm from the scope with either fingers or a large bowel clamp, and the endoscopist insufflates that segment of bowel so that the entire mucosa is visualized in an anterograde fashion until the scope reaches the point of occlusion. The next 20 to 25 cm is then visualized in the same fashion and so on.

Dr. V.H. Fink (Chicago Ill.). Do you believe that Sonde enteroscopy—total enteroscopy prior to surgery—is of any value to the surgeon? Does it cut down on time? Does it result in increased accuracy? Second, you mentioned ulceration as the second most common cause. What was the etiology of the ulceration?

Dr. Kendrick. Sonde enteroscopy may be a valuable aid in certain patients, particularly those with known diffuse arteriovenous malformations in whom you may suspect that there are multiple lesions throughout the extent of the bowel, which would preclude any surgical treatment or resection. Beyond that, we have had only limited success with Sonde enteroscopy in visualizing much more distally than is possible with our push enteroscopes.

Those ulcerations had multiple different etiologies. Some were due to ischemia, and some were in immunosuppressed patients with cytomegalovirus infections. There were some that we suspected were caused by nonsteroidal anti-inflammatory drugs or other medications, and some radiation-induced erosions.

Dr. R. Hodin (Boston, Mass.). What types of resections were performed? I assume there was some preoperative test such as a bleeding scan that suggested there was a problem? I have a question concerning technique. I believe you examined the bowel antegrade. My experience suggests that inserting the scope, then pulling it back to see something small, is not going to work very well. Finally, what is your experience with laparoscopy?

Dr. Kendrick. The resections were all partial colectomies. Actually, in one of the patients a bleeding scan suggested the presence of something on the right side; however, it was unclear whether that was pooling or whether there was a definitive lesion. Preoperatively, this patient was known to have diverticulosis throughout the colon; however, on intraoperative enteroscopy there was an area in the right colon that was suggestive of recent hemorrhage.

Technically, we use the air-trapping method with the insufflation. All the folds flatten, and maximal mucosal visualization is achieved. Further, you look as the scope goes in, avoiding confounding variables such as endoscope trauma.

We have very limited experience with laparoscopy. In this series it was used in only three patients and two had to be converted to an open procedure because of failure to adequately visualize all of the small bowel mucosa. In the open technique, we have more control over enteric traction for exposure.

Dr. B. Bass (Baltimore, Md.). Your results confirm the difficult nature of dealing with these patients. Have you had a chance to characterize those who have had rebleeding? Did the rebleeding occur early or late after your surgery? If it was late, is it just that more angiodysplasias are developing over a course of years?

Dr. Kendrick. The majority of the failures continue to bleed postoperatively. If a lesion is not found or if a lesion is found that we thought was the source and the patient has rebleeding, usually it is fairly soon after surgery. There were some patients, however, who did not have rebleeding for more than a year. In that subset, although the source is unclear because they have not undergone an additional intraoperative enteroscopy, we suspect recurrent small bowel arteriovenous malformations. Most of those patients who did not have rebleeding were patients who had arteriovenous malformations on intraoperative enteroscopy.

Segmental Living Related Small Bowel Transplantation in Adults

Luca Cicalese, M.D., Cristiana Rastellini, M.D., Pierpaolo Sileri, M.D., Herand Abcarian, M.D., Enrico Benedetti, M.D.

The advent of small bowel transplantation has provided selected patients with chronic intestinal irreversible failure with a physiologic alternative to total parenteral nutrition. Recently a standardized technique for living related small bowel transplantation (LR-SBTx) has been developed. Three patients with short bowel syndrome underwent LR-SBTx at our institution. All donors were ABO compatible with a good human leukocyte antigen match. A segment of 180 to 200 cm of ileum was harvested and transplanted with its vascular pedicle constituted by the ileocolic artery and vein. The grafts were transplanted with a short cold and warm ischemia time. The immunosuppression regimen consisted of oral FK-506, prednisone, and intravenous induction with atgam. Serial biopsies of the intestinal grafts were performed to evaluate rejection or viral infections. The postoperative course was uneventful for all donors. All of the recipients are currently alive and well. Two of three patients are off total parenteral nutrition and tolerating an oral diet with no limitations on daily activity. In the third patient, the graft was removed 6 weeks after transplantation. At the time of enterectomy, no technical or immunologic complications were documented. Absorption tests for D-xylose and fecal fat studies were performed showing functional adaptation of the segmental graft. All biopsies were negative for acute rejection. A well-matched segmental ileal graft from a living donor can provide complete rehabilitation for patients with short bowel syndrome. Our initial experience suggests that the risk of acute rejection and infection is greatly reduced compared to cadaveric bowel transplantation. Further clinical application of this procedure is warranted. (J GASTROINTEST SURG 2001;5:168-173.)

KEY WORDS: Intestinal transplantation, human, living related donor, outcome

Regardless of the etiology, irreversible intestinal failure is the condition in which absorption of fluid and nutrients from the small bowel is not adequate to sustain life. Until recently, long-term total parenteral nutrition (TPN) was the only possible alternative for keeping patients with intestinal failure alive. Unfortunately TPN is associated with serious complications such as line sepsis, venous thrombosis, hepatic dysfunction, and cirrhosis.[1] As a consequence, survival of patients on long-term TPN for nonmalignant intestinal failure has been shown to be as low as 49% at 5 years.[2] Furthermore, the quality of life of patients on TPN is suboptimal since they often do not tolerate an oral diet and are limited in their activity during the infusions. Additionally, TPN is associated with high costs. In 1992, in the United States, the estimated cost per patient per year was approximately $100,000 for supplies only, not including home nursing care, physician fees, laboratory costs, or expenses related to the treatment of TPN-related complications.[3]

Small bowel transplantation (SBTx) represents the physiologic alternative to TPN, and it has recently become a valid therapeutic option for patients with intestinal failure, with a 5-year graft survival rate close to 70% in some centers.[4] However, the widespread application of this procedure is still limited by the relatively high rate of complications.

Infections, surgical complications, acute rejection, graft-versus-host disease, and post-transplant lymphoproliferative disorder are all observed following

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SBTx with a higher incidence if compared to the transplantation of other organs.[5] At the present time, infective complications are responsible for the majority of graft and patient loss in the centers with the larger experience.[6] These may be, at least in part, the consequence of the peculiarity of this graft, which contains gut-associated lymphoid tissue and potentially pathogenic enteric flora. Furthermore, in these patients the existing disease and the relative malnutrition could predispose them to infective complications. Additionally, other factors associated with SBTx, such as laparotomy, preservation injury, abnormal motility, lymphatic disruption, deviate systemic venous drainage, antibiotic therapy, and the potent immunosuppressive agents, could all be implicated in the development of infection in these patients.[7-9]

Most intestinal transplants have been performed using whole small bowel grafts obtained from cadaveric donors.[10] At our institution, we have recently initiated an intestinal transplantation program using segmental small bowel grafts obtained from living related donors (LR-SBTx). This procedure offers several advantages over cadaveric SBTx such as a better tissue matching with consequently reduced immunosuppression and insignificant cold ischemia time with improved graft quality. Because LR-SBTx is an elective operation, donor bowel preparation and recipient medical conditions can be optimized. Furthermore, since the donor is a healthy individual, hemodynamic instability and consequent preharvesting graft hypoperfusion in the donor are obviated.[11] All of these benefits allow easier management of LR-SBTx recipients while providing an adequate segment of bowel to support physiologic alimentation. The main disadvantage remains the risk for the donor, which includes early surgical complications of bowel resection as well as potential long-term impairment of intestinal absorption. Other potentially detrimental factors include the functional limitation related to the need to use a shorter graft and the increased risk of vascular thrombosis related to the smaller vascular pedicle. We report our experience in three patients with irreversible intestinal failure who underwent LR-SBTx at our institution.

PATIENTS AND METHODS

Between April 1998 and January 2000, three patients (2 men and 1 woman, 27, 29, and 46 years of age, respectively) with irreversible intestinal failure due to short bowel syndrome underwent LR-SBTx at the University of Illinois at Chicago (Table I). The cause of short bowel syndrome was post-traumatic in two patients and superior mesenteric artery thrombosis in one. All of the recipients were on TPN from 5 to 13 months preoperatively and had experienced more than one episode of bacterial or fungal infection. Incipient cholestasis (total bilirubin greater than 3 mg/dl) was present in all. All donors and recipients were ABO compatible. Human leukocyte matching was as follows: six antigens, sister to brother; three antigens, father to son; and five antigens, son to mother. Donor-recipient cytomegalovirus status was negative to negative in one case and positive to positive in the other two. Donor-recipient Epstein-Barr virus status was positive to positive in all cases. Preoperative angiography of the superior mesenteric artery was performed in the donors to study the vascular supply to the cecum, ileocecal valve, and terminal ileum, and to exclude any vascular abnormalities. The donor bowel was mechanically prepared and sterilized with antibiotic prior to graft procurement.

The donor and recipient operations have been described elsewhere.[12] A segment of ileum, 180 to 200 cm, was resected 15 cm from the ileocecal valve, which was preserved in all donors (Fig. 1). The length of the graft obtained was decided in relation to the total length of the donor ileum and was measured carefully to guarantee a proper residual segment in the donor. The donor ileum was primarily reanastomosed in an end-to-end fashion using 4-0 polyglyconate for the mucosal layer and 4-0 polypropylene for the seromuscular layer. The graft was transplanted suturing the ileocolic vessels in an end-to-side fashion to the aorta and inferior vena cava using 6-0 polypropylene with a cold ischemia time of less than 10 minutes and a warm ischemia time of 30 to 40 minutes. The intestinal continuity was immediately reestablished proximally anastomosing the graft to the recipient's intestinal stump (duodenum in all three patients) and

Table I. Summary of patients

Sex	Age (yr)	Cause of irreversible failure	TPN prior to SBTx (mo)	HLA matching	Complications	Follow-up (mo)
Male	27	Trauma	5	6 antigens	—	27
Male	29	Trauma	13	3 antigens	—	14
Female	46	SMA thrombosis	7	5 antigens	Acute pancreatitis Graft removed (6 wk)	11

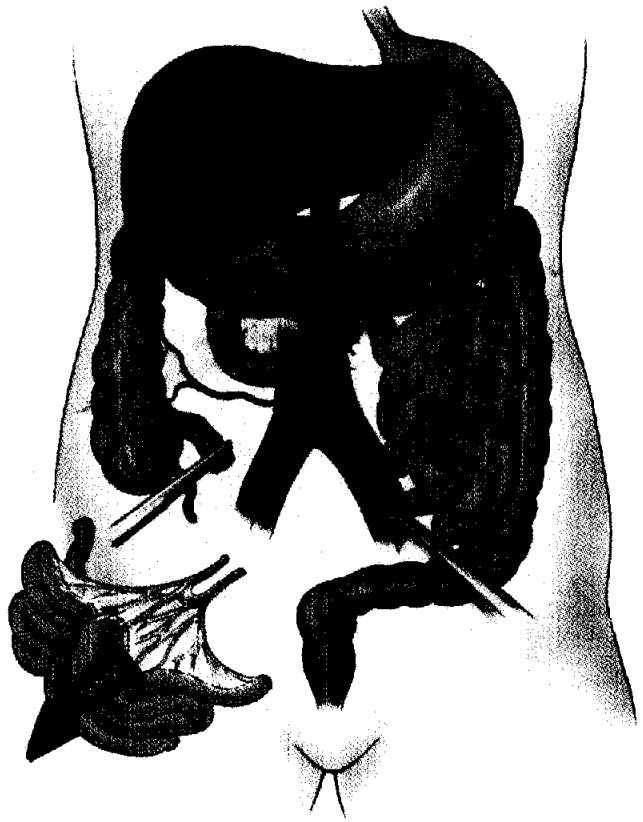

Fig. 1. Donor operation. A 200 cm segment of ileum, starting 12 cm from the ileocecal valve and moving proximally, was measured and resected. The graft was vascularized by the ileocolic vessels transected below the origin of the right colic artery. Intestinal continuity was immediately reestablished with a side-to-side intestinal anastomosis.

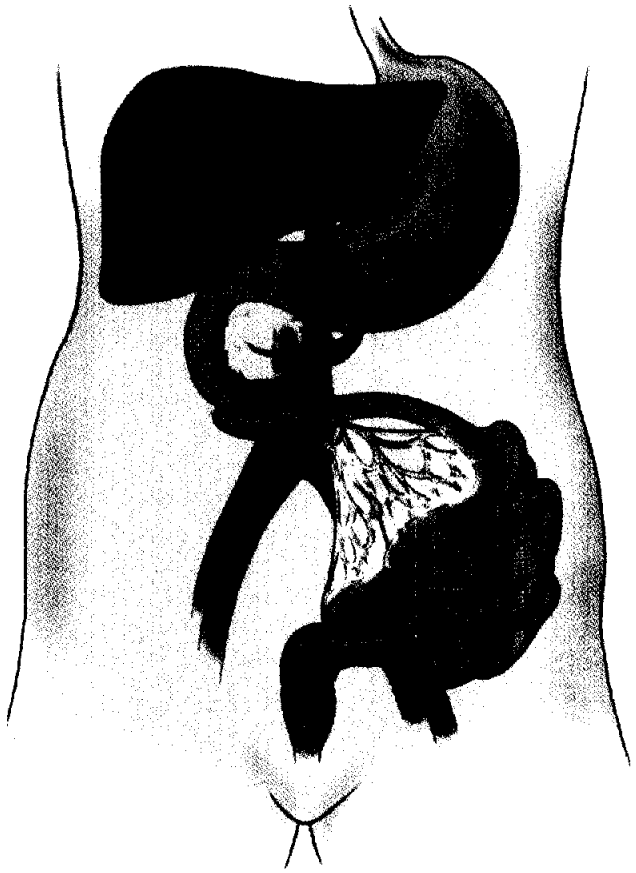

Fig. 2. Recipient operation. The graft ileocolic vessels were anastomosed end to side to the aorta and vena cava. Intestinal continuity was restored anastomosing the proximal end of the ileal graft side to side with the second portion of the recipient's duodenum, while the distal end was anastomosed side to side to the sigmoid colon. A temporary loop ileostomy, 10 cm proximal to the ileosigmoidostomy, was performed to monitor the output and to use as a port for graft biopsy.

distally to the sigmoid colon in all patients, using similar suture materials as in the donor (Fig. 2). This was accomplished using 4-0 polyglyconate for the mucosal layer and 4-0 polypropylene for the seromuscular layer. A temporary distal loop ileostomy was performed in all patients to monitor graft output and to perform endoscopic surveillance and biopsies.

A short course of perioperative antibiotic prophylaxis with vancomycin (1 g intravenously before surgery) and piperacillin (3 g intravenously six or eight times a day, depending on renal function, for 3 days) was used in all recipients, while antiviral prophylaxis was achieved using perioperative ganciclovir (5 mg/kg intravenously every 12 hours for 14 days) followed by high-dose acyclovir (800 mg by mouth four times a day for 3 months). Immunosuppression consisted of oral tacrolimus, prednisone, and intravenous induction with atgam until therapeutic blood levels of tacrolimus were reached. Blood, stool, urine, sputum, and peritoneal fluids were collected as part of routine surveillance or when infections were clinically suspected, and cultures were performed using standard microbiologic techniques. Serial biopsies (weekly for the first postoperative month, then based on clinical indications) of the intestinal grafts were performed to evaluate rejection or viral infections.

RESULTS

All donors had an uneventful recovery. During the first 2 months, they had occasional diarrhea that was easily controlled with symptomatic therapy. All donors maintained a stable weight and serial testing for vitamin B_{12} absorption demonstrated values within

the normal range beginning immediately after the procedure.

All recipients are currently alive with a follow-up to 21 months. TPN was discontinued postoperatively and oral feeding was initiated by the first post-transplant month since a persistent delay in gastric emptying was observed secondary to the prolonged previous inadequacy of oral intake. In two of three patients, transplantation has been successful with the patients on an oral diet and having good graft function at 21 and 9 months, respectively, after the transplant. Their loop ileostomies were closed 6 months after the transplant. They are currently off TPN and back on an oral diet, enjoying regular activity, and both have returned to their baseline weight. Fat-soluble vitamin levels and Schilling tests normalized within 1 month after the transplant. Fat absorption, documented by fecal fat studies, were markedly abnormal 1 month after the transplant but normalized within 6 months. The D-xylose absorption test was also abnormal early after the transplant but progressively improved to reach normal values by 6 and 9 months after the transplant. The serum albumin levels increased steadily, normalized by the third month after the transplant, and have remained normal to date in both patients.

In the third patient, the graft was removed 6 weeks after the transplant for diffuse ischemic injury probably related to intravenous octreotide administration for severe pancreatitis. Examination of the graft after removal failed to show technical, immunologic, or infectious complications.

No bacterial infections were observed during the post-transplant follow-up period in any of the three patients. In one patient, incidental positivity on routine urine cultures was observed for *Klebsiella* in the absence of clinical manifestations of urinary tract infection on two different occasions. The patient was given oral antibiotic prophylaxis. The second patient developed cytomegalovirus enteritis 4 months after LR-SBTx. The cytomegalovirus donor-recipient status was positive to positive, and he was successfully treated with intravenous ganciclovir. Graft biopsies failed to show post-transplant preservation injury and remained negative for acute rejection in all of the patients.

DISCUSSION

In the past 10 years, advances in surgical technique, better patient care, and improved immunosuppression therapy contributed to improve the outcome of SBTx. A recent report from the International Intestinal Transplant Registry stated that more than 270 intestinal transplants had been performed worldwide up to February 1997. Since 1994 a linear rate of increment has been witnessed.[10] Unfortunately the survival rate for this transplant is still lower compared with transplantation of other solid organs. Infective complications are the most common cause of death and graft loss after SBTx accounts for up to 69% of patient loss in some centers.[13] Also, viral infections or reinfections (mainly cytomegalovirus and Epstein-Barr virus) occur more frequently after SBTx than after any other organ transplantation, probably as a consequence of the need for more rigorous immunosuppression. In fact, acute rejection of intestinal grafts is frequent and its early diagnosis is difficult. Acute rejection can be associated with translocation of enteric microorganisms, whereas a simultaneous need for higher immunosuppression can further increase the risk of infections and makes management of these patients particularly difficult. Furthermore, the fear of acute rejection leads most surgeons to use potent immunosuppression, which also increases considerably the risk of post-transplant lymphoproliferative disorder in patients undergoing SBTx. Post-transplant lymphoproliferative disorder, a B-cell lymphoma, occurs in up to 30% of SBTx recipients and is associated with a mortality rate of approximately 50% in children.[14] In addition, not all patients who undergo successful SBTx achieve the ultimate goal of reestablishing adequate absorption of an oral diet. In fact, 77% of the patients with intestinal grafts are off TPN, whereas others still require long-term intravenous fluid supplementation.[10] Our strategy of using segmental bowel grafts from living related donors can, in our opinion, improve the outcome of SBTx. Our approach of using segmental LR-SBTx is based on the rationale that intestinal grafts are extremely sensitive to preservation injury. In a previous analysis of 50 pediatric SBTx recipients, it was shown that the length of graft preservation was the most significant factor in inducing bacterial translocation.[15] Such injury cannot be avoided since hemodynamic instability of the donor and subsequent splanchnic hypoperfusion can trigger ischemic damage even before the intestine is procured. Furthermore, specific preservation solutions designed for intestinal grafts are not yet available.

Other factors still limiting the outcome of SBTx are, in our opinion, acute rejection and the potent immunosuppression used with all its dangerous consequences. The strategy of using living related donors implies optimal donor-recipient human leukocyte antigen matching and reduced immunosuppression. An added advantage of LR-SBTx is the reduction in the waiting time for transplantation with the benefit of reduced TPN requirements and, consequently, a lower risk of long-term TPN-associated complications. The transplantation can be carried out expeditiously in an elective fashion and preoperative donor

and recipient intestinal preparation could also prevent local and systemic infections by gut-derived organisms. The use of segmental intestinal grafts is also helpful in these patients because the abdominal cavity is small due to massive bowel resection, loss of abdominal wall, or severe intra-abdominal adhesions as a result of the numerous laparotomies.

Naturally there are also some disadvantages to LR-SBTx. These include the surgical risks for the donor, the technical difficulty in using smaller vessels for the vascular anastomoses, and the possibility that the relatively short graft might not provide adequate absorption. However, the surgical risk associated with elective small bowel resection and primary anastomoses is low if performed by experienced surgeons.

Furthermore, according to the available literature,[12,16-19] it does not appear that the donor will suffer long-term absorption problems with ileal resection limited to approximately 200 cm. In our experience, mild occasional diarrhea was observed only in the early postoperative period and was well controlled with medical therapy with no evidence of vitamin B_{12} absorption deficit or weight loss. Also, acute rejection was not observed in our series. We believe that this is related to the optimal HLA match, which also allowed us to use a less aggressive immunosuppression regimen.

To date, only a few cases of LR-SBTx have been described.[12,16-19] Our findings suggest that LR-SBTx is associated with a low rate of infections. We believe that this may be due to optimal graft decontamination in the living donor, a relatively short cold ischemia time, significantly reduced immunosuppression, and shorter pretransplant TPN administration accounting for a reduced susceptibility to infection in the recipient.

From this analysis, it is also evident that the segment of intestinal graft used can undergo a progressive and relatively rapid functional adaptation and a 200 cm ileal graft can fully support the nutritional requirements of an active young adult. Despite the limited experience with LR-SBTx, we believe this procedure could offer several advantages compared to cadaveric SBTx in patients with chronic irreversible intestinal failure and should be considered for further clinical application and studies.

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Discussion

Dr. B.M. Jaffe (New Orleans, La.). I am an advocate of living related transplantation, but I think it is unreasonable to leave readers with the impression that this type of transplant is not associated with rejection. Our first patient had two mild bouts of rejection that were easily treated, and the transplant was perfectly successful with a very good functional result. Our second patient, despite a good match from his mother, had multiple bouts of rejection, which ultimately became very difficult to treat. He had a good match and with good transplant care still had multiple bouts of rejection.

Dr. L. Cicalese. I did not want to give the wrong impression that with this strategy the risk of rejection is completely eliminated, but it is drastically reduced compared to what is seen with cadaveric transplants. Most of the larger centers performing small bowel transplantations from cadaveric donors are showing up to 100% rejection in their transplant recipients. We can still see rejection with this particular combination, but I think the risk is lower.

Dr. J.S. Thompson (Omaha, Neb.). The mortality rate for those on the waiting list for cadaveric transplantation is still fairly high, so this is an important effort. Your follow-up is fairly short and there can be problems long term. I am not sure you gain as much as we would hope to by matching these patients ahead of time. It is important that every center not have just one modality but rather be able to offer different approaches for treating these patients properly. We have not been enthusiastic about the living related approach at our institution because we do not believe there will be immunologic benefits and there is also the risk of donor complications in these patients. Do you have any evidence that your 200 cm remnant adapts or improves its function over time? That would be another potential advantage over whole intestinal transplants, where there does not appear to be any adaptation. There may be further growth or improvement with these grafts.

Dr. Cicalese. We achieved success in two patients cases. In one patient, we used a 200 cm segment and in the other we used a 180 cm segment, and this small difference actually accounted for the longer period of intravenous fluid hydration that was necessary for the first few months after transplantation. Adaptation is going to be important. We did not use glutamine or other nutrients to encourage it, but it is something we are planning to do in the future, if necessary.

Dr. R.M. Craig (Chicago, Ill.). I am interested in why you only use ileum for the donor. With the loss of 200 cm of ileum, what kind of problems did you encounter in your donors? What type of screening do you do for your donors?

Dr. Cicalese. This is a very important point because with living related transplants, there is always the ethical issue of putting another individual at risk. For this operation, the reason we selected the ileum was basically the technical advantages. The vascular pedicle that can be obtained by using the ileum is larger and it is a single vessel. In the donors in our series, we have not seen any major difficulties. The only problem was diarrhea during the first month, which resolved by itself; we had to prescribe some medications during the first few weeks and then this was no longer necessary. The donors are all doing well now. In regard to screening of the donors, we basically selected healthy donors and we did obtain angiograms. We eliminated some of the potential donors because they had abnormalities of the vasculature or severe atherosclerosis. We also did screenings for cytomegalovirus and other viruses.

Upregulation of Ornithine Decarboxylase mRNA Expression in Barrett's Esophagus and Barrett's-Associated Adenocarcinoma

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The Barrett's multistage process is characterized histopathologically by progression from Barrett's intestinal metaplasia to Barrett's esophagus with dysplasia and ultimately adenocarcinoma. Understanding the cellular and molecular events in this multistage process may contribute to improved diagnosis and treatment. Ornithine decarboxylase (ODC) is the first enzyme in the biosynthesis of polyamines. Elevated ODC activity has been found to be associated with progression during Barrett's esophagus, but the regulation of ODC gene expression in the development of Barrett's-associated adenocarcinoma has not been reported. The aim of this study was to assess the prevalence and timing of ODC mRNA expression in the Barrett's metaplasia-dysplasia-adenocarcinoma sequence. ODC mRNA expression levels, relative to the stably expressed internal reference gene β-actin, were measured using a quantitative reverse transcription-polymerase chain reaction (RT-PCR) method (ABI 7700 Sequence Detector System) in 104 specimens from 19 patients with Barrett's esophagus without carcinoma and 22 patients with Barrett's-associated adenocarcinoma. The median ODC mRNA expression levels were significantly increased in Barrett's esophagus tissues compared to matched normal tissues in patients without adenocarcinoma of the esophagus ($P = 0.002$; Wilcoxon test). A significant progressive increase in ODC mRNA expression was detectable through the stages of the metaplasia-dysplasia-carcinoma sequence in patients with Barrett's-associated adenocarcinoma ($r = 0.719$; $P \leq 0.001$; Spearman's rho test). These findings show that upregulation of ODC mRNA expression is an early event in the development and progression of Barrett's-associated adenocarcinoma of the esophagus, and they suggest that high ODC mRNA expression levels may be a clinically useful biomarker for the detection of occult adenocarcinoma. (J GASTROINTEST SURG 2001;5:174-182.)

KEY WORDS: Ornithine decarboxylase, ODC, Barrett's esophagus, esophageal neoplasia, esophageal adenocarcinoma

Barrett's esophagus is defined as the replacement of normal squamous esophageal epithelium by a metaplastic columnar epithelium.[1,2] It arises in up to 12% of patients with chronic gastroesophageal reflux disease.[3] Compared to the general population, patients with Barrett's esophagus have a 30- to 125-fold greater risk of developing esophageal adenocarcinoma,[4] and Barrett's esophagus is therefore considered to be a premalignant condition. The development of adenocarcinoma appears to occur via a multistep process, recognized histologically as a metaplasia-dysplasia-carcinoma sequence.[5] Over the past two

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decades, the incidence of this cancer has increased at a dramatic rate in Western countries.[6,7] Survival rates for this malignancy are poor because patients are not usually diagnosed at an early, curable stage.[8]

Regular surveillance endoscopy with systematic biopsy of Barrett's epithelium is widely used to identify patients with an increased risk of cancer.[9] So far, the best predictor of carcinoma is the histopathologic detection of high-grade dysplasia. Patients with high-grade dysplasia have a significant risk of occult adenocarcinoma or the rapid development of adenocarcinoma. Approximately 50% of patients with a maximum diagnosis of high-grade dysplasia who underwent esophagectomy have had invasive adenocarcinomas found in the resection specimen.[10] Unfortunately, areas of high-grade dysplasia or adenocarcinoma can go undetected at endoscopy because of sampling error, and there can be considerable interobserver disagreement in the histopathologic grading of dysplasia.[11] Identification of biomarkers that are significantly associated with each Barrett's stage and with an increased risk of progression to cancer would therefore greatly benefit the selection of patients with this disease who should undergo surgical resection or other interventions.

Ornithine decarboxylase (ODC) is the first enzyme in the biosynthesis of polyamines,[12] which are essential for cell proliferation[13,14] and cell regeneration.[15,16] The expression of ODC is transiently increased on stimulation by growth factors[17-19] but becomes constitutively activated during cell transformation induced by a variety of factors including viral infection and oncogene activation.[20-25] Aberrant regulation of ODC has been reported to play an important role in neoplastic transformation and tumor growth.[26,27] Enzyme assay studies reported elevated levels of ODC activity in human cancers including colorectal cancers, gastric cancers, and squamous cell carcinomas of the esophagus.[28-30] Elevated ODC activity has been found to be associated with progression during Barrett's esophagus.[31-33] ODC activity has been studied as an intermediate biomarker of cancer risk in Barrett's esophagus and clinical intervention trials using chemopreventive agents including the ODC inhibitor alpha-difluoromethylornithine (DFMO).[34-36] Although tissue-specific decreases in polyamine contents in Barrett's esophagus[35,36] and growth inhibition in metaplastic cell lines[32] were detectable after DFMO treatment, there was no statistically significant effect of DFMO on ODC activity.[35,36]

The studies discussed above measured ODC activity, and no published studies have reported on ODC mRNA expression levels in Barrett's esophagus and Barrett's-associated adenocarcinoma. We undertook this study to assess the prevalence and timing of ODC mRNA gene expression in the Barrett's metaplasia-dysplasia-adenocarcinoma sequence.

MATERIAL AND METHODS

Tissue Samples

One hundred fourteen tissue samples obtained at endoscopy and operation from 19 patients with Barrett's esophagus without adenocarcinoma (BE group), 22 patients with Barrett's-associated adenocarcinoma of the esophagus (EA group), and 10 patients with no evidence of Barrett's esophagus or chronic reflux disease (control group) were collected and immediately frozen in liquid nitrogen. There were 33 men and 18 women, who had a mean age of 52.2 years (range 24 to 79 years). Endoscopic biopsies were obtained according to a protocol that required biopsy at 2 cm intervals from each quadrant (anterior, posterior, and right and left lateral positions) of the visible length of Barrett's mucosa and an additional biopsy from the normal-appearing squamous mucosa of the esophagus. Normal esophagus biopsies were taken at least 4 cm proximal to the macroscopically abnormal epithelium. Part of the specimen or an adjacent specimen was fixed in formalin and paraffin for histopathologic examination by pathologists considered experts in Barrett's disease.

Specimens were classified as intestinal metaplasia if intestinal metaplasia but no dysplasia or cancer was present. Specimens were classified as dysplastic if either low-grade dysplasia or high-grade dysplasia was present. Dysplastic tissues were not divided into high-grade and low-grade groups because areas of low-grade and high-grade dysplasia were commonly present in the same specimen.

Barrett's intestinal metaplasia (n = 16), Barrett's dysplasia (n = 3), and matching normal squamous esophagus mucosa specimens (n = 19) were analyzed in the group without cancer (BE group). Adenocarcinoma (n = 22) and matching normal squamous esophagus mucosa (n = 22), as well as Barrett's intestinal metaplasia (n = 5) and Barrett's dysplasia (n = 17) tissues adjacent to adenocarcinoma, were analyzed in the group with esophageal adenocarcinoma (EA group).

RNA Extraction and cDNA Synthesis

Total RNA was isolated by a single-step guanidinium isothiocyanate method using the QuickPrep Micro mRNA Purification Kit (Amersham Pharmacia Biotech, Inc., Piscataway, N.J.) according to the manufacturer's instructions. Following RNA isolation, cDNA was prepared from each sample as previously described.[37]

Table I. Ornithine decarboxylase PCR primers and probes

Forward primer: ODC-1257F [21bp]
Sequence: TGTTGCTGCTGCCTCTACGTT
Reverse primer: ODC-1392 [23bp]
Sequence: GCTGGCATCCTGTTCCTCTACTT
TaqMan probe: ODC-1316T [23bp]
Sequence: CATGAGTTCCCACGCAGGCCCTG

bp = base pairs.

Polymerase Chain Reaction Quantification and mRNA Expression

Quantitation of ODC cDNA and an internal reference gene (β-actin) was done using a fluorescence-based real-time detection method (ABI PRISM 7700 Sequence Detection System [TaqMan], Perkin-Elmer Applied Biosystems, Foster City, Calif.) as previously described.[38,39] In brief, this method uses a dual-labeled fluorogenic oligonucleotide probe that anneals specifically within the forward and reverse primers. Laser stimulation within the capped wells containing the reaction mixture causes emission of a 3′ quencher dye (TAMRA) until the probe is cleaved by the 5′ to 3′ nuclease activity of the DNA polymerase during polymerase chain reaction (PCR) extension, causing release of a 5′ reporter dye (6FAM). Production of an amplicon thus causes emission of a fluorescent signal that is detected by the TaqMan charge-coupled device (CCD) detection camera, and the amount of signal produced at a threshold cycle within the purely exponential phase of the PCR reaction reflects the starting copy number of the sequence of interest. Comparison of the starting copy number of the sequence of interest with the starting copy number of the reference gene provides a relative gene expression level.

The PCR reaction mixture consisted of 600 nmol/L of each primer (Table I), 200 nmol/L probe (see Table I), 5 U AmpliTaq Gold Polymerase, 200 μmol/L each dATP, dCTP, and dGTP, 400 μmol/L dUTP, 5.5 mmol/L $MgCl_2$, 1 U AmpErase uracil N-glycosylase, and 1 × TaqMan Buffer A containing a reference dye, to a final volume of 25 μl (all reagents from Perkin-Elmer Applied Biosystems. Cycling conditions were 50° C for 2 minutes, 95° C for 10 minutes, followed by 40 cycles at 95° C for 15 seconds and 60° C for 1 minute.

Statistical Analysis

TaqMan analyses yield values that are expressed as ratios between two absolute measurements (gene of interest/internal reference gene). ODC expression levels in adenocarcinoma, Barrett's dysplasia, intestinal metaplasia, and normal squamous esophagus tissues were compared using the Kruskal-Wallis test to show significant differences in expressions within all histopathologic groups. Associations between two variables were tested by using either the Wilcoxon signed-rank test or the Mann-Whitney U test. The Spearman rho test was used to analyze the progressive increase in ODC expression in the multistage process of Barrett's carcinogenesis. Differences between two groups were considered significant at confidence levels greater than 95% ($P < 0.05$). Analyses were carried out using the SPSS software package (SPSS, Chicago, Ill.).

RESULTS

ODC mRNA expression was readily detectable by reverse transcription–polymerase chain reaction (RT-PCR) in all 114 samples analyzed. Relative ODC mRNA expression (ODC × 100/β-actin) was generally upregulated in both premalignant and malignant Barrett's tissues, as shown by the finding that ODC expression was higher in pathologic compared to normal esophageal tissues in almost all patients. Twenty-one (95.5%) of the 22 patients with Barrett's adenocarcinoma had higher ODC mRNA expression in the cancer specimens compared to matching normal squamous esophagus specimens, and 17 (89.5%) of the 19 patients with a maximum diagnosis of Barrett's esophagus had higher ODC mRNA expression in the Barrett's mucosa compared to paired normal squamous mucosa.

The median values and ranges of ODC mRNA expression in tissues from the 22 patients with adenocarcinoma of the esophagus and the 19 patients with Barrett's esophagus without cancer are shown in Table II. As shown in Table II and Figs. 1 and 2, ODC mRNA expression was significantly upregulated in Barrett's esophagus tissues in patients with and without adenocarcinoma of the esophagus. Fig. 1 shows that the median ODC mRNA expression level was significantly higher in Barrett's esophagus tissues compared to paired normal tissues in patients without cancer ($P = 0.002$; Wilcoxon test). Fig. 2 shows that ODC expression levels increased progressively and significantly in histopathologically worse tissue types in patients with adenocarcinoma, with an increase from normal squamous esophagus mucosa to Barrett's esophagus adjacent to cancer, and from Barrett's esophagus adjacent to cancer to cancer (Spearman rank test, $r = 0.719$, $P < 0.001$). Elevated ODC mRNA expression was detectable in adenocarcinomas compared to adjacent Barrett's esophagus tissues in 21 (95.5%) of the 22 cancer patients.

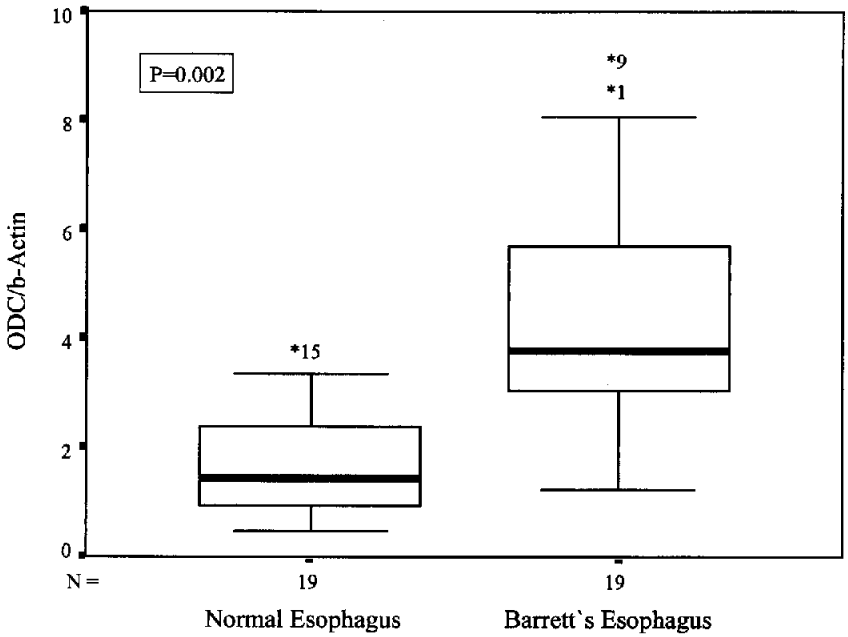

Fig. 1. Box and whisker plots of relative ODC expression levels for normal esophageal tissue and Barrett's tissue from patients with Barrett's esophagus without evidence of cancer. Boxes show the twenty-fifth and seventy-fifth percentile (interquartile) ranges. Median values are shown as a horizontal bar within each box. Whiskers show levels outside the twenty-fifth and seventy-fifth percentiles but exclude far outlying values, which are shown above the boxes.

Table II. Comparison of ornithine decarboxylase mRNA expression levels in different Barrett's esophagus tissues

Pathology	n	ODC expression		Significance
		Median	Range	
Adenocarcinoma group	22			
Adenocarcinoma		4.77	0.80-28.70	$r = 0.719$; $P \leq 0.001$*
Barrett's esophagus (intestinal metaplasia, dysplasia)		2.44	0.30-30.98	
Normal esophagus		1.02	0.02-4.73	
Barrett's group	19			
Barrett's esophagus (intestinal metaplasia, dysplasia)		3.76	1.22-48.08	$P = 0.002$†
Normal esophagus		1.43	0.48-8.94	
Control group	10			
Normal esophagus		1.26	0.54-5.69	

*Spearman's rho test.
†Wilcoxon test.

There were no significant differences in the ODC expression levels between Barrett's esophagus tissues from patients with cancer compared to patients without detectable cancer. To investigate a possible "field effect" in patients with adenocarcinoma of the esophagus, we compared the ODC expression levels in normal squamous esophagus specimens of the EA group, BE group, and control group. No significant differences were detectable between these groups (see Table II; Fig. 3).

To search for further differences in the ODC mRNA expression between patients with and without adenocarcinoma of the esophagus, we calculated the ratio of ODC expression in the highest grade pathologic lesion for each patient to the expression in the matching normal esophagus tissue from that patient.

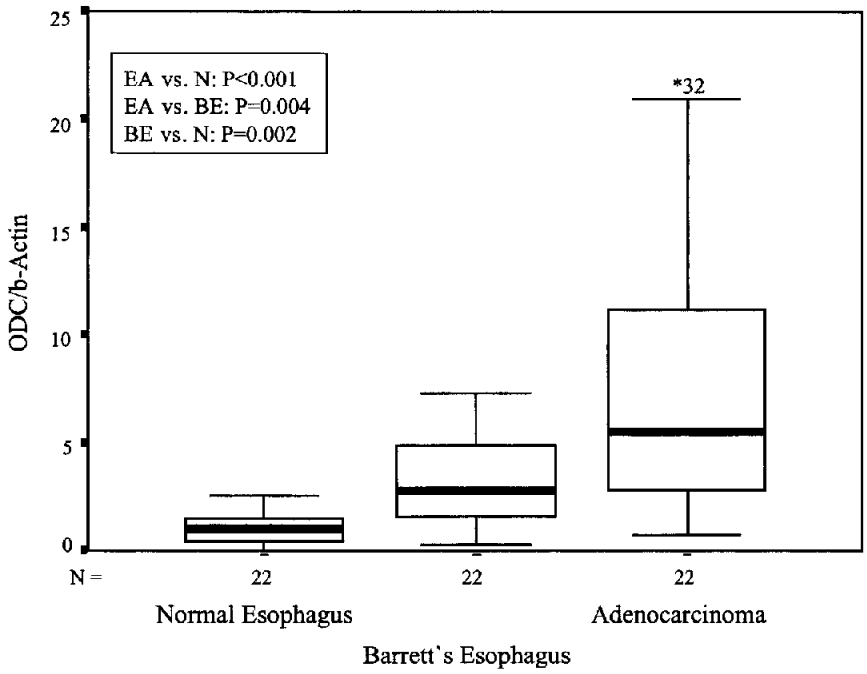

Fig. 2. Box and whisker plots of relative ODC expression levels from specimens of normal esophagus, Barrett's esophagus, and adenocarcinoma of the esophagus from patients with cancer. ODC gene expression levels at different histopathologic stages were compared using the Mann-Whitney U test.

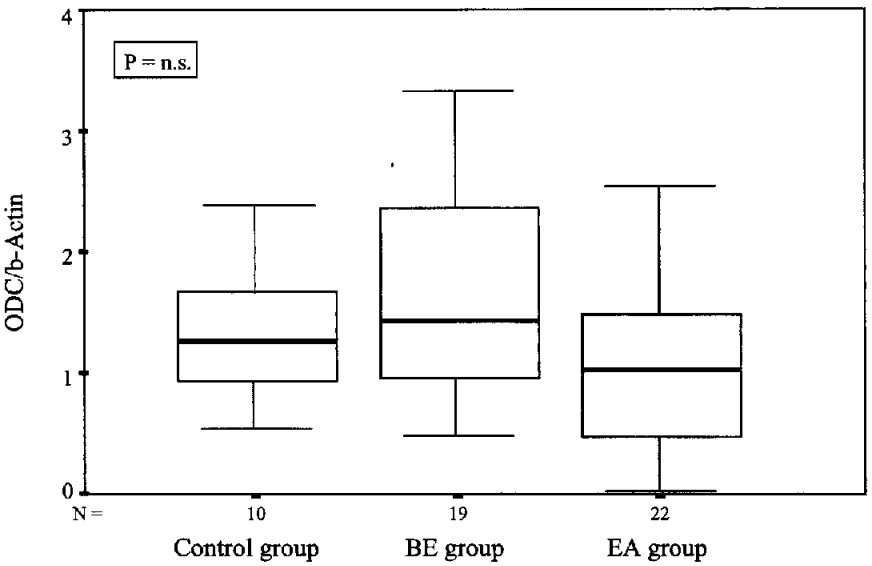

Fig. 3. Box and whisker plots of relative ODC expression levels of normal squamous esophagus tissues from healthy patients without evidence of Barrett's esophagus or chronic reflux disease and patients with Barrett's esophagus and adenocarcinoma of the esophagus.

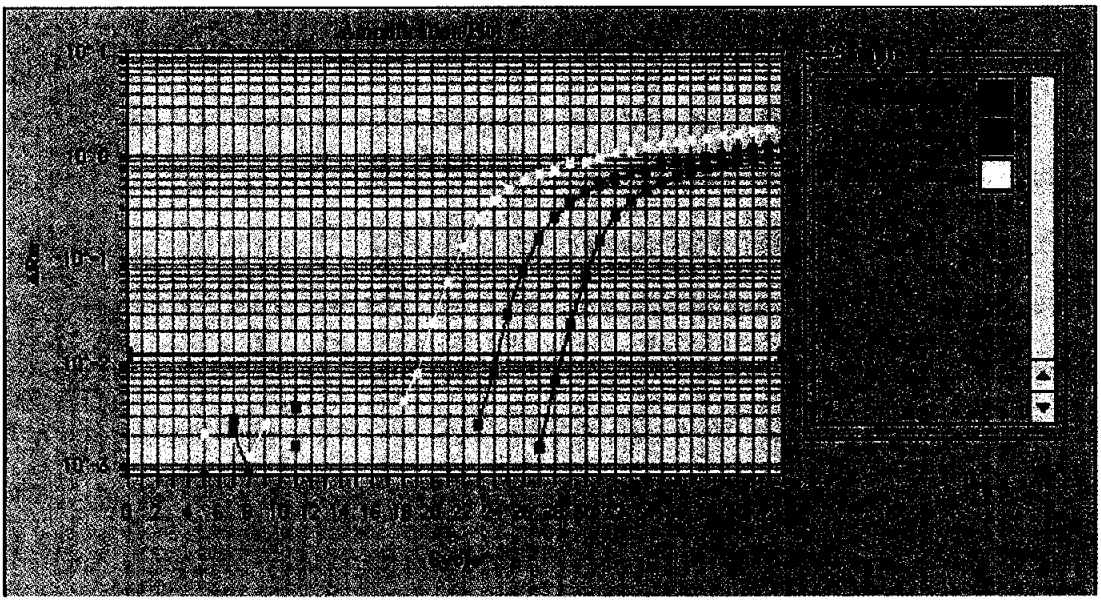

Fig. 4. ABI 7700 Sequence Detection System. Ornithine decarboxylase PCR reaction curves for normal, Barrett's esophagus, and cancer tissues from a patient with esophageal adenocarcinoma. The plots show sample analysis for ODC mRNA expression in normal squamous esophagus mucosa (red panel), Barrett's esophagus (green panel), and adenocarcinoma (yellow panel) in a patient with Barrett's adenocarcinoma. *ΔRn* represents the change of the fluorescence signal of the reporter at each cycle. The point at which the tracings cross the horizontal threshold line correlates with the amount of starting cDNA in the samples.

The median ratio of ODC expression in the adenocarcinoma compared to normal squamous tissues (EA/N) in the patients with cancer (median 5.87, range 0.96 to 224.5) was significantly higher than the median ratio in Barrett's esophagus/normal squamous tissue (BE/N) in patients without cancer (median 2.60, range 0.56 to 20.65; $P = 0.043$). The PCR reaction curves of a typical patient in whom the ODC expression was highest in adenocarcinoma, intermediate in Barrett's esophagus, and lowest in normal squamous esophagus are shown in Fig. 4.

DISCUSSION

These results show that upregulation of ODC mRNA expression is present in Barrett's esophagus and associated esophageal adenocarcinomas. ODC expression was increased even in premalignant Barrett's metaplasia tissues, indicating that induction of the expression of this gene is an early event in the Barrett's multistage process. There was considerable variation in ODC mRNA expression levels in tissues at each Barrett's stage, but analysis of the grouped results for each stage showed that there was a significant increase in the median ODC mRNA expression in Barrett's esophagus tissues compared to normal tissue in patients without cancer. Furthermore, there was a significant progressive elevation of ODC through the stages of Barrett's esophagus to carcinoma in patients with adenocarcinoma and adjacent Barrett's epithelium.

Our findings complement the results of previous studies in which increased ODC enzyme activity was found in Barrett's esophagus tissues. Garewal et al.[32,33] showed elevated ODC enzyme activity in metaplastic and dysplastic Barrett's epithelium. Elevated ODC activity has been shown to be associated with the development of dysplasia and adenocarcinoma in Barrett's esophagus and has been suggested to be a useful biochemical marker for increased cancer risk.[40] So far, ODC mRNA expression has only been investigated in squamous cell carcinoma of the esophagus. Mafune et al.[41] showed higher ODC mRNA levels in squamous cell carcinomas compared to normal esophagus tissues in approximately 90% of patients evaluated.

The finding that very high levels of ODC mRNA expression ratios were found only in patients with cancer indicates that measurement of ODC expression might be a clinically useful biomarker for the early detection of malignancy in patients with Barrett's esophagus. However, some patients with cancer had low ODC expression levels, so the predictive power of a low or moderately elevated ODC expression level is limited. We anticipate that analysis of a larger data set might lead to the identification of precise threshold levels above which the likelihood that tumor is present can be estimated with some accuracy.

ODC mRNA expression levels were not significantly different in the group of histologically normal squamous esophagus tissues from patients with cancer compared to the group without cancer and the normal control group without evidence of Barrett's esophagus and chronic reflux disease. This observation differs from our previous studies in which we reported significantly altered expression of telomerase reverse transcriptase (hTRT) and retinoic acid receptors (RARs) in normal squamous esophagus tissues from patients with adenocarcinoma of the esophagus compared to normal esophagus epithelium from patients without cancer.[37,42] This suggested that an oncogenic field effect was not detectable for ODC mRNA expression in our study.

The lack of a significant difference in ODC expression between normal mucosa in patients with cancer and those without cancer prompted us to calculate the pathologic:normal tissue ODC ratio for each patient. This method of analysis showed that the ODC expression ratio for cancer tissues (adenocarcinoma/normal esophagus) was significantly higher than the ratio for Barrett's esophagus tissues from patients without cancer (Barrett's/normal esophagus). Although this result is not surprising in view of our other results, it was reassuring that this method of analysis produced similarly significant results to those obtained by comparing median values for groups of histopathologically similar tissues. This method of analysis was used by Mafune et al.[41] in their study of patients with esophageal squamous cell carcinoma. In that study, a high ODC mRNA cancer:normal tissue expression ratio was correlated significantly with TNM staging, vascular vessel invasion, and worse survival.[41] However, we cannot comment on associations between ODC gene expression and clinical features in our patient group because of the relatively short follow-up period after tissue procurement.

Our finding that ODC is increasingly upregulated in the development of Barrett's-associated adenocarcinoma suggests that this is a tumorigenesis-associated effect and not simply a function of generalized inflammation. It implies that induction of ODC expression may be involved in the maintenance of a malignant phenotype. The hypothesis that ODC is involved in human neoplastic transformation is supported by data obtained in tumors of other organ systems, such as colon cancer,[29] squamous cell carcinoma of the esophagus,[29,41] and gastric carcinoma.[29] Auvinen et al.[26] indicated that aberrant expression of ODC is not just a coincident response to transformation but a critical factor contributing to oncogenesis. Clifford et al.[43] reported that ODC overexpression by itself is not sufficient to induce tumors in normal cells but that increased expression of ODC enhances tumor progression in premalignant cells. Therefore the increasing overexpression of ODC during the progression of Barrett's adenocarcinoma suggests that this gene may play an important role in the development of adenocarcinoma in Barrett's epithelium.

CONCLUSION

Upregulation of ODC mRNA expression is an early event in the Barrett's multistage process. ODC mRNA expression was significantly higher in both adenocarcinoma and Barrett's esophagus tissues compared to normal squamous esophagus mucosa. There was a progressive, significant increase in ODC expression levels in progressively worse histopathologic stage specimens in patients with Barrett's-associated adenocarcinomas. In contrast to enzyme activity measurements, gene expression can be determined in diagnostic biopsies. Therefore ODC gene expression quantification may be a clinically useful marker for the presence of malignancy in patients with Barrett's esophagus.

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Discussion

Dr. M.G. Patti (San Francisco, Calif.). Based on the data you presented, how do you plan to proceed from here?

Dr. J. Brabender. We are planning to, first of all, determine the ODC mRNA expression in a larger study population, and of course we would like to analyze a panel of other genes and maybe detect a specific pattern.

Dr. C.A. Pellegrini (Seattle, Wash.). Among the patients with Barrett's esophagus but without cancer, were there any with high-grade dysplasia? Are there any differences in the ODC mRNA expression in relation to the type of dysplasia?

Dr. Brabender. Because of the relatively small number of patients, we were not able to detect any statistically significant difference between these groups, but we are now analyzing larger study populations, and we hope that we can detect differences between these groups.

Dr. R.H. Bell, Jr. (Chicago, Ill.). It seems to me that you are suggesting you could use the appearance of ODC upregulation as a sign of impending risk for cancer, and I wonder whether you have considered the possibility that patients who develop cancer have a field defect in which ODC is upregulated, and that patients who are not ever going to develop cancer probably will not ever change their ODC.

Dr. Brabender. We were not able to detect this type of field defect for ODC. We detected the field defect for other genes, for instance, telomerase, but not ODC.

Dr. V.H. Finch (Chicago, Ill.). Do you think that the ODC gave earlier information than would be obtained by doing serial biopsies and studying the histology? Did you have any idea which came first—the rise in ODC or changes in the histology?

Dr. Brabender. I do not think that ODC by itself can do that, but I think that a panel of genes can be additive to endoscopic surveillance and to the histology. I cannot say which changed first, the histology or the ODC.

Pharyngeal pH Monitoring in 222 Patients With Suspected Laryngeal Reflux

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To determine the existence of and characterize gastroesophagopharyngeal reflux in patients with symptoms of airway irritation, we monitored pharyngeal pH over a 24-hour period in 222 consecutive patients. Pharyngeal reflux was defined as a drop in pH to less than 4 at the pharyngeal sensor, which occurred simultaneously with acidification of the distal esophagus. Patients were divided into two groups: those with pharyngeal reflux (PR+) and those without (PR−). The Mann-Whitney U test and Student's *t* test were used to assess intergroup comparisons. Episodes of pharyngeal reflux (range 1 to 36, average 4.4) were identified in 90 PR+ patients (40%). No pharyngeal reflux was identified in the remaining 132 patients (PR−). Episodes of pharyngeal reflux were rapidly cleared (average duration 1.5 minutes), and occurred while in the upright position in 77 (86%) of 90 patients and while in the supine position in 11 (12%) of 90 patients. Twenty-three patients (25%) experienced symptoms in association with an episode of pharyngeal reflux. In the distal esophagus, the percentage of time the pH was below 4 during the upright position and the total percentage of time the pH was below 4 were greater in PR+ patients (6.4% and 5.8%, respectively) when compared to PR− patients (2.6% and 2.6%, respectively). Laryngoscopic findings did not distinguish PR+ from PR− patients. Pharyngeal reflux occurs most commonly in the upright position and can be identified in more than 40% of patients thought to have acid-induced laryngeal symptoms. Even though these episodes are short lived and rapidly cleared, symptoms occur concomitantly in 25% of patients with proven pharyngeal reflux. Patients with laryngeal symptoms and documented pharyngeal reflux have greater amounts of esophageal reflux when compared to patients with laryngeal symptoms and no demonstrable pharyngeal reflux. (J GASTROINTEST SURG 2001;5:183-191.)

KEY WORDS: GERD, reflux, pH, larynx, pharynx, hoarseness, cough, extraesophageal reflux

Symptoms of chronic cough, hoarseness, and throat clearing are often caused by smoking, voice abuse, and postnasal drip. It has recently been postulated that regurgitation of gastric contents above the upper esophageal sphincter with subsequent spillage into the airway may be an important factor in the genesis of these symptoms. Indirect evidence, such as the presence of abnormal gastroesophageal reflux[1,2] and improvement of symptoms with acid suppression therapy, supports this etiology of laryngeal injury. For example, the response of laryngeal symptoms to proton pump inhibitor therapy and life-style modification has been reported to be as high as 85% after 12 weeks of therapy in one study.[3] On the other hand, Kibblewhite and Morrison[4] found no difference between placebo and H_2 blockers after 2 weeks of therapy in patients with cervical symptoms thought to be related to reflux.[4]

A more accurate, objective method of diagnosing reflux laryngitis would be helpful in directing management and predicting treatment response. Laryngoscopy may reveal erythema, nodularity, ulceration, granuloma, or leukoplakia in patients with pharyngeal reflux,[5,6] but these changes are not pathognomonic of reflux. Indeed, even in patients suspected of having acid-induced vocal cord nodules, as many as 36% may not have detectable acid exposure in the pharynx.[7]

The "gold standard" for identifying and measuring gastroesophageal reflux within the esophagus is

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24-hour pH monitoring. Direct monitoring of laryngeal pH to determine the presence of reflux in patients with airway symptoms is impractical.[8] We hypothesized that measuring pharyngeal pH with a system that was similar to that used to measure pH in the esophagus might act as a proxy for laryngeal acid exposure. The ability of a pharyngeal pH sensor to accurately measure acid exposure has been questioned because it may desiccate, which would interfere with pH measurements. Williams et al.,[9] in a recent publication, developed specific criteria to help eliminate erroneous data from pharyngeal pH monitoring. These include simultaneous esophageal and pharyngeal acidification, a drop in the pharyngeal pH to less than 4.0, and excluding meals from the period of analysis.

Although some investigators believe that any amount of pharyngeal reflux is abnormal,[10] others have documented occasional pharyngeal reflux in as many as 20% of healthy subjects.[9] To date, the degree of pharyngeal reflux required to initiate laryngeal irritation, the prevalence of pharyngeal reflux in patients with symptoms indicative of laryngeal irritation, and the severity of gastroesophageal reflux in patients with pharyngeal reflux are not well established. The objective of this study was to measure pharyngeal pH in a large number of patients with laryngeal symptoms to identify the following: (1) the frequency with which pharyngeal acid exposure occurred, (2) the characteristics of these episodes, (3) the extent of esophageal reflux in these patients, and (4) the correlation of pharyngeal reflux with laryngoscopic findings.

MATERIAL AND METHODS

Ten volunteers underwent pharyngeal pH monitoring with approval from the Human Subjects Review Committee. The volunteers had no esophageal or extraesophageal symptoms of gastroesophageal reflux. These data were used to establish normal values for episodes of pharyngeal reflux.

We studied 222 consecutive patients (136 women and 86 men; mean age 52 years, range 20 to 85 years) clinically suspected of having laryngeal or pulmonary symptoms induced by gastroesophageal reflux. Symptoms and results of esophageal physiologic testing were entered prospectively into the Swallowing Center database. Patients with laryngeal symptoms were evaluated with a full head and neck examination including flexible laryngoscopy and videostroboscopy.

Symptoms

Symptoms were rated on a frequency scale ranging from 0 to 4 as follows: 0 = never; 1 = once per month; 2 = once per week; 3 = once per day; and 4 = several times per day. Any frequency that fell between two numbers was upgraded to the higher of the two numbers. Twenty-two symptoms were queried: 11 gastrointestinal (heartburn, regurgitation, abdominal pain, belching, dysphagia to liquids and solids, bloating, nausea, chest pain, odynophagia, globus) and 11 extraesophageal (coughing, hoarseness, wheezing, laryngitis, aspiration, choking, dyspnea, sore throat, asthma, bronchitis, pneumonia).

Manometry

A water-perfused eight-channel catheter (four radial ports at the same level and four separated by 5 cm intervals) was used to assess esophageal pressures with the patient in the supine position. The lower esophageal sphincter (LES) was examined with the four radial ports. A station pull-through measurement of the LES pressure determined the characteristics of the sphincter. The LES pressure was averaged over a series of three respiratory cycles. The peristaltic pump of the esophageal body was assessed over a minimum of 10 episodes of deglutition with 5 ml aliquots of water. A single port was used to evaluate the upper esophageal sphincter (UES) location, pressure, and relaxation.

pH Monitoring

All acid suppression therapy was stopped 5 to 7 days prior to testing. A four-sensor solid-state pH catheter was placed with the proximal sensor 1.5 to 2.0 cm above the UES as determined by manometry. The remaining three sensors were spaced at 5 cm intervals along the catheter; thus the most distal sensor was always located 13 cm below the UES. pH measurements were sampled every 8 seconds in a recorder (Medtronic, Inc., Minneapolis, Minn.) worn by the patient for a 24-hour period. Symptom diaries were maintained by the patients during the observation period. Data analysis was performed by a software program, which reported events (number and duration of reflux episodes) and calculated acid exposure times over the course of the study. Data collected during and 1 hour after meals were not analyzed because ingested foods have variable effects on acid detection in the pharynx.[9]

A drop in the pH of the pharyngeal sensor was considered a "pharyngeal reflux episode" only if the following occurred: (1) pH dropped below 4; (2) pH fell more than one unit; and (3) the drop in pH was accompanied by a simultaneous drop in the esophageal pH to below 4. Every episode of pharyngeal acidification that met these criteria was considered to be pathologic. Pharyngeal episodes of acidification that

did not occur with esophageal acidification were not categorized as esophagopharyngeal reflux. All tracings were individually reviewed, rather than relying on the computer interpretation, to determine those that had the characteristics cited above and were thus considered true pharyngeal reflux. Based on the presence of one or more such episodes, patients were labeled as having pharyngeal reflux (PR+) or not having pharyngeal reflux (PR−).

Distal acid exposure times less than a pH of 4 (normal <4%) were generated from the caudad sensor. Although standard sensor placement in the distal esophagus would be 5 cm above the LES at our center, the distal sensor in this study varied from 5 to 14 cm above the LES because of the fixed position we kept for the pharyngeal sensor. Thus the distal sensor was, in all cases, either at or above the standard position.

Laryngoscopy/Endoscopy

Laryngoscopy and videostroboscopy was performed with a flexible endoscope and topical anesthetic by one of two laryngologists (A.H. or N.M.). The larynx was examined during both quiet respiration and free phonation. The description of the findings was categorized for the purpose of this report into one of four broad classes as follows: no abnormal findings, inflammatory changes (erythema, edema, vocal cord or interarytenoid thickening, and nodularity), physiologic changes (anteroposterior compression, bowing, and vocal cord paresis), and structural changes (posterior vocal cord granuloma, subglottic stenosis, interarytenoid pachydermia, and leukoplakia/carcinoma).

Esophagoscopy was performed using sedation, topical anesthetic, and a flexible endoscope. The presence of a hiatal hernia, esophageal mucosal injury, stricture, and Barrett's esophagus was noted.

Statistical Analysis

Symptoms were compared between the PR− and PR+ groups using a Mann-Whitney U test. Continuous data generated from manometry and pH monitoring were analyzed using Student's t test. Pearson correlation coefficients were calculated to establish relationships between episodes of pharyngeal reflux and other parameters. Significance was accepted for $P < 0.05$.

VOLUNTEER RESULTS

Of the 10 volunteers studied, nine had no pharyngeal reflux. One volunteer had a single episode of pharyngeal acid exposure. Thus the mean frequency of pharyngeal reflux according to our criteria was 0.1 ± 0.32. The average distal esophageal acid exposure time in the volunteer group was 1.5% ± 0.25%.

PATIENT RESULTS

Pharyngeal reflux was present in 90 patients (40%) and absent in the remaining 132 patients (60%).

Symptoms

Of 22 symptoms queried, the five most commonly reported are shown in Table I. Coughing and hoarseness were reported more frequently than other respiratory and gastrointestinal symptoms. Heartburn was found to be more severe in PR+ patients ($P < 0.05$). No statistically significant differences were demonstrated for any of the other symptoms.

Twenty-three of the PR+ patients (25%) experienced symptoms during an episode of pharyngeal reflux: cough in 19 patients, hoarseness in two, heartburn in one, and belching in one.

Manometry

The characteristics of the UES were the same in both groups of patients (Table II). The LES pressures in the PR− and PR+ groups were 17.0 ± 9.5 mm Hg and 15.0 ± 7.7 mm Hg, respectively ($P = 0.15$). All other parameters of esophageal motility were similar between the two groups (see Table II).

Table I. Average symptom frequencies (0-4) in patients suspected of having pharyngeal reflux

Symptom	Total group (n = 222)	PR− (n = 132)	PR+ (n = 90)
Cough	2.3	2.3	2.3
Hoarseness	1.9	2.0	1.7
Dyspnea	1.5	1.5	1.4
Belching	1.4	1.4	1.2
Heartburn	1.3	1.0	1.5*
Wheezing	1.2	1.2	1.2

PR− = patients without pharyngeal reflux on pH monitoring; PR+ = patients with pharyngeal reflux on pH monitoring.
*$P < 0.05$ compared to PR− patients.

Table II. Manometric findings in patients with and without pharyngeal reflux

Manometric parameter	PR− (SD)	PR+ (SD)	*P* value
UES pressure (mm Hg)	84 (60)	80 (53)	0.68
UES relaxation (%)	98 (6.7)	99 (2.2)	0.19
Peristalsis with deglutition (%)	94 (16)	96 (9.7)	0.32
Proximal esophageal amplitudes (mm Hg)	59 (21)	56 (22)	0.36
Distal esophageal amplitudes (mm Hg)	90 (41)	89 (40)	0.74
LES pressure (mm Hg)	17 (9.5)	15 (7.7)	0.15
LES relaxation (%)	100 (2.2)	97 (17)	0.07
LES relaxation duration (sec)	4.7 (1.9)	4.6 (2.0)	0.80

UES = upper esophageal sphincter; LES = lower esophageal sphincter; SD = standard deviation.

Table III. Distal esophageal acid exposure in patients with and without pharyngeal reflux

Distal esophageal pH parameter	PR− (SD)	PR+ (SD)	*P* value
Episodes of reflux	46 (61)	100 (90)	<0.0001
Episodes of reflux longer than 5 min	0.82 (1.5)	2.4 (3.4)	<0.0001
Longest episode of reflux (min)	9.5 (21)	13 (21)	<0.0001
Total acid exposure time (%)	2.6 (3.8)	5.8 (6.8)	<0.0001
Upright acid exposure time (%)	2.6 (3.8)	6.5 (6.6)	<0.0001
Supine acid exposure time (%)	2.3 (5.5)	4.4 (9.4)	0.07

SD = standard deviation.

Pharyngeal pH Monitoring

The mean number of episodes of pharyngeal reflux in the PR+ group was 4.4 (range 1 to 36). Seventy-five percent of all PR+ patients had five or fewer episodes of pharyngeal reflux. Eleven patients (12%) had more than eight episodes of pharyngeal acidification (Fig. 1). The average duration of individual events of pharyngeal acid exposure was 1.5 minutes (range 0.5 to 5 minutes).

Seventy-seven (86%) of 90 patients experienced pharyngeal reflux only in the upright position, 11 (12%) had pharyngeal reflux in the supine position, and two (2%) had pharyngeal reflux in both the upright and supine positions. Patients with isolated supine pharyngeal reflux also had more supine esophageal reflux compared to patients with other types of pharyngeal reflux (11% vs. 2.7%, $P = 0.001$).

The pH probe and the software used to analyze data detected "acid" in the pharynx more frequently than was observed according to the manual analysis criteria presented earlier. The average number of episodes of acid reflux in the pharynx was reported to be 44 in the PR+ group and 19 in the PR− group. Because these episodes were not accompanied by a simultaneous drop in esophageal pH, most computer-identified episodes of pharyngeal reflux were discounted.

Esophageal pH Monitoring

Patients with pharyngeal reflux had abnormal acid exposure in the distal esophagus (determined 5 to 14 cm above the LES) when compared to patients without pharyngeal reflux (Table III). The number of episodes of acidification (pH <4) of the distal esophagus was 100 ± 90 for the PR+ group and 46 ± 61 for the PR− group ($P < 0.0001$). The acid exposure time (percentage of time the pH was <4) was higher in the PR+ group, 5.8% ± 6.8% vs. 2.3% ± 5.5% ($P = 0.0001$). The duration of acid reflux in PR+ patients was greater when measured by episodes of reflux lasting more than 5 minutes (2.4 vs. 0.8) and the longest episode of reflux (13 minutes vs. 9.5 minutes) when compared to PR− patients.

Upright esophageal reflux occurred 6.4% ± 6.6% of the time in PR+ patients compared to 2.6% ± 3.8% in PR− patients ($P < 0.0001$). Supine reflux had a tendency toward a higher prevalence among PR+ patients but did not reach statistical significance (4.4% ± 9.4% vs. 2.3% ± 5.5%; $P = 0.07$).

Correlation

The total number of episodes of pharyngeal reflux had a positive correlation with the symptom of heart-

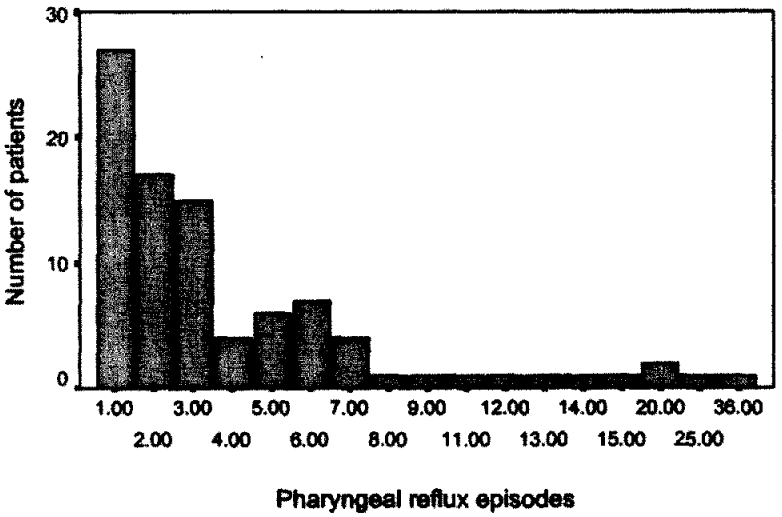

Fig. 1. Bar graph demonstrating the number of PR+ patients with increasing frequencies of true pharyngeal reflux.

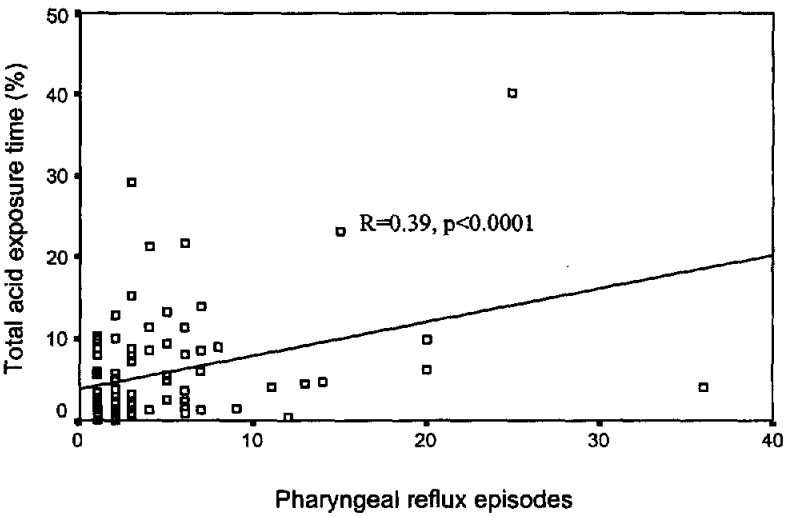

Fig. 2. Scatter plot showing the correlation between distal esophageal acidification and frequency of pharyngeal reflux in PR+ patients.

burn ($r = 0.14$, $P = 0.04$), with prolonged episodes of acid reflux in the esophagus ($r = 0.34$, $P < 0.0001$), and with the total percentage of acid exposure in the distal esophagus ($r = 0.39$, $P < 0.0001$) (Fig. 2). Other symptoms and manometry parameters did not show significant correlationship with episodes of reflux in the pharynx.

Laryngoscopy

Laryngoscopy was completed in 95 patients (38 PR+ and 57 PR−) undergoing pharyngeal pH monitoring. Only three patients were found to have normal laryngoscopic examinations. The most common findings in the remaining 92 patients were inflammatory changes of edema, erythema, and interarytenoid thickening. No differences in the presence or degree of inflammatory changes were noted between the PR+ and PR− groups. The most common physiologic abnormality at laryngoscopy was anteroposterior compression, which occurred in 28% of patients. Again, no difference was noted between the PR+ and PR− groups. Anatomic abnormalities including granulomas, cysts, stenosis, and carcinoma were found in

10% of patients. There was a tendency toward a difference in the presence of subglottic stenosis ($P = 0.17$, two-tailed) for the PR+ group; however, no other differences were detected.

Esophagoscopy

Twenty-five patients (16 PR− and 9 PR+) underwent endoscopic examination at the request of their referring physician. Seven PR− patients (44%) had esophagitis, two (12%) had hiatal hernias, and one had Barrett's esophagus. Six PR+ patients (67%) had esophagitis and two (22%) had hiatal hernias. No strictures were identified.

DISCUSSION

Our study shows that acid reflux into the pharynx occurs in a substantial proportion of patients with laryngeal and pulmonary symptoms and that pharyngeal pH monitoring accurately identifies these patients. Furthermore, patients with laryngeal symptoms and pharyngeal reflux appear to be different from those with the same clinical presentation and no reflux based on their symptoms, esophageal function, and laryngoscopic findings.

Symptoms

Most of the patients in this study were referred to our laboratory by an otolaryngologist or a pulmonologist to help determine whether or not abnormal reflux might be the cause of their symptoms. As such, their symptoms were primarily laryngopharyngeal (dysphonia, globus pharyngeus, sore throat, hoarseness) or tracheobronchial (cough, choking, asthma, pneumonia). Many also complained of heartburn and other typical symptoms of reflux. Therefore it is no surprise that the extraesophageal symptoms did not distinguish PR− patients from PR+ patients.

However, the average heartburn score (1.0) for PR− patients indicated that these patients had the symptom of heartburn once a month. The PR+ patients with a score of 1.5 would have been upgraded to a frequency of once per week. This difference was the only significant discriminating symptom in all 22 patients monitored. That heartburn was a distinguishing feature is supported by our finding that PR+ patients had larger amounts of esophageal reflux. The fact that, in these patients, reflux extended all the way to the pharynx strongly suggests that laryngeal symptoms were the result of aspiration of refluxed material; however, it is beyond the scope of this study to determine which patients had symptoms induced by pharyngeal reflux.

Esophageal pH Monitoring

Abnormal esophageal acid exposure was more common and severe among patients with pharyngeal reflux in almost all measured parameters. It is important to understand that because our study required that the uppermost sensor of the pH probe be placed in the pharynx, above the UES sphincter, the position of the lowermost probe varied considerably, from 5 to 14 cm above the LES. Thus what we called "distal esophageal acid exposure" is clearly an underestimation of true distal esophageal acid exposure (defined as that 5 cm above the manometrically detected LES). Therefore "distal esophageal acid exposure" in this study is likely to be lower than that in other studies in patients with laryngeal injury. Even though there was considerable variation in the position of our lowermost sensor, this is unlikely to have influenced comparisons between PR+ and PR− patients given the large number in each group and given that the probe position was exactly the same (and without knowledge of ultimate pharyngeal reflux status) in all 222 patients studied.

Patients with pharyngeal reflux had more upright reflux than those without pharyngeal reflux. The association of a more severe type of upright reflux with pharyngeal reflux was a surprise to us, but the finding supports the fact that the majority (85%) of pharyngeal reflux occurred in the upright position.

PR+ patients had clear evidence of decreased acid clearance from the esophagus; they had a greater number of episodes lasting longer than 5 minutes and longer episodes of reflux. This defective clearance was not explained by the manometric data; we found no difference in any of the parameters related to peristalsis between the PR+ and PR− patients (see Table II). However, esophageal acid clearance involves more elements than those studied with traditional stationary manometry. For example, it depends on the conscious or unconscious perception of reflux and the initiation of neuromuscular activity to strip the esophagus, which are elements that we did not measure. Indeed, the conscious sensation of acid present in the esophagus is known to vary among patients and may account for differences between the two groups. Because we only measured peristaltic waves induced by deglutition, our study could not assess the efficacy of a secondary wave or the time between acidification and secondary peristalsis. Either explanation (lack of detection or lack of secondary peristaltic activity) can account for the differences in esophageal acid clearance. Whether poor clearance, with subsequent pooling of acid in the esophagus, contributes to pharyngeal reflux is unclear; however, the correlation between increased episodes of prolonged esophageal acidification and pharyngeal reflux is supported by our data.

Pharyngeal pH Monitoring

Criteria for identifying pharyngeal reflux are still being defined. Previous investigators have recommended exclusion of episodes that occurred during meals, a drop in pharyngeal pH to less than 4.0, and simultaneous acidification of the esophagus.[7,9-13] Some have imposed more stringent criteria with the hope of eliminating spurious data, for example, a change in pH of more than two units,[9] eliminating a brief postprandial period,[11] and excluding episodes of belching.[7] Because we wanted to make sure that what we identified as pharyngeal reflux was indeed a reflection of acid in this location, we chose to follow strict criteria that required the following: (1) a change in pharyngeal pH of more than 1 point; (2) a drop below a pH of 4; (3) a simultaneous decrease in esophageal pH; and (4) exclusion of meals and a 1-hour postprandial period from analysis. This process required that we obtain all readings directly from the tracing, discarding a number of changes in pharyngeal pH that the computer software detected as acidification of the pharynx. Using this method of detecting pharyngeal acid exposure, only one episode of pharyngeal reflux was identified in the volunteer group. Even if two standard deviations are added to the mean, the expected normal frequency of pharyngeal reflux in asymptomatic subjects is less than one. Therefore we assumed that even one episode of pharyngeal reflux was pathologic.

In PR+ patients the mean number of reflux episodes counted by the computer was 44 (range 1 to 317) and the mean number identified using our criteria was 4.4 (range 1 to 36). This is in agreement with others who have found that 92% of pH changes in the pharynx are artifact.[9] Thus our study may well have underestimated the true incidence of acid reflux, but this was essential if we wanted to be confident that patients identified as having pharyngeal reflux did in fact have gastroesophagopharyngeal reflux and were not a consequence of misinterpretation of the pH tracing.

The characteristics of pharyngeal reflux identified in our study confirm what others have found. Even in patients with pharyngeal reflux, the episodes are infrequent and of short duration when compared to episodes of esophageal reflux. The majority of reflux episodes occur in the upright position.

Laryngeal Findings

The fact that 92 (97%) of 95 patients had abnormal findings on laryngoscopy is not surprising since the majority of these patients were being evaluated for extraesophageal symptoms. On the other hand, the various findings at laryngoscopy (inflammatory, physiologic, anatomic) had a similar prevalence among PR+ and PR− patients. This may be explained by the nonspecific nature of the laryngeal changes, such that smoking, voice abuse, or postnasal drip may present with the same findings as those induced by reflux and provides further evidence favoring the potential diagnostic use of pharyngeal pH. Alternatively some of the PR− patients may have had infrequent bouts of pharyngeal reflux not detected during the monitoring period but still sufficient to cause laryngeal injury. Indeed, since neither laryngeal symptoms nor endoscopic findings appear to be helpful in distinguishing patients who may be aspirating acid into the airway, direct measurement of pharyngeal pH may be the only tool currently available to make that diagnosis. This study, however, cannot answer the question of whether only PR+ patients are aspirating acid. As previously indicated, our system was aimed at identifying "true" episodes of pharyngeal reflux and thus it may have underestimated the true incidence. The study does suggest, however, that PR+ patients should probably be regarded as those at higher risk of having associated reflux as the cause of laryngeal irritation.

CONCLUSION

This study shows that up to 40% of patients with symptoms of laryngeal reflux will have detectable acid reflux in the pharynx. Episodes of pharyngeal reflux are brief, are associated with acidification of the distal esophagus, and occur most commonly while the patient is in the upright position. One fourth of these patients will experience symptoms in association with an episode of pharyngeal reflux. Patients with detectable pharyngeal reflux are more likely to have heartburn and abnormal esophageal acid exposure, particularly in the upright position. Since neither laryngeal symptoms nor laryngoscopic examination can currently distinguish between patients with acid-induced injury and other types of laryngeal injury, direct measurement of pharyngeal reflux may play an important role in diagnosis and appropriate treatment for patients with symptoms of laryngeal irritation.

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Discussion

Dr. M.G. Patti (San Francisco, Calif.). This study attempts to shed light on patients with extraesophageal symptoms. Regarding the symptoms, you did not find any difference in the incidence of respiratory symptoms. You found that heartburn was most frequent among the patients with pharyngeal reflux. This is not surprising because you were comparing patients who had no reflux with a normal DeMeester score to patients who did have reflux.

With regard to the patients who were found to have pharyngeal reflux, we look at the reflux score, there is a very high standard deviation, which means some of the patients had an abnormal distal exposure but some patients had a normal distal exposure. Did you find among these two groups a difference in LES pressure or motility of the esophageal body? What was the incidence of the esophagitis and the degree?

Finally, focusing again on the PR+ patients, how would you treat the patient who has pharyngeal reflux but normal esophageal acid exposure and the patient who has positive pharyngeal reflux with abnormal acid exposure? When does medical therapy play a role and when should we consider fundoplication?

Dr. T. Eubanks. I agree that respiratory symptoms are not going to serve to distinguish these patients because they all present with respiratory symptoms. There was no difference in motility between the patients with and without pharyngeal reflux. There is some evidence in the literature that patients with reflux-induced asthma have worse esophageal motility, but we did not find that to be the case in this study.

In the abstract we submitted, you will note that the LES pressure in PR− patients was 17, and in the PR+ patients it was 14. Now that might be a statistical difference, but we all know that it is not a clinical difference. As we have accrued more patients, that difference has disappeared.

I do not think we have an answer yet concerning treatment, because we do not have sufficient normative data for pharyngeal acid exposure. Some believe that any episode of pharyngeal acidification is abnormal, even one. But there are others who have seen occasional pharyngeal acidification in control patients without symptoms up to 20% of the time. If I saw patients with extraesophageal symptoms, I would send them for pharyngeal pH monitoring. If they had pharyngeal acid exposure, I would prescribe a trial of high-dose proton pump inhibitor and allow 3 months on that dose before bringing them back for laryngoscopy. If their symptoms had not improved, I might perform pharyngeal pH studies while they were on the medication to document that they had acid suppression. I do not know if surgery is indicated for these patients because we cannot be confident that pharyngeal pH monitoring identifies those patients who would benefit from an operation.

Dr. J.G. Hunter (Atlanta, Ga.). I think you have shown that all posterior laryngitis is not pharyngeal reflux. You have just mentioned that there are no normative data. Dr. DeMeester very nicely showed what the norms were for esophageal pH monitoring years ago. Is there anyone who has developed these norms for pharyngeal pH monitoring, and how do we obtain these data?

The second question is, do you have any postoperative data? Does a laparoscopic Nissen procedure ablate this pharyngeal reflux in your patients? Finally, is the subpharyngeal lead, the lead in dual-channel pH monitoring that is placed directly below the cricopharyngeus, an effective surrogate for a pharyngeal lead. Obviously the technical problem of lead desiccation will not occur in a subpharyngeal lead.

Dr. Eubanks. As I said, we do not have normative data. This is going to be difficult to establish because, in contradistinction to esophageal episodes of acidification, which occur in high numbers in patients with reflux and even relatively frequently in asymptomatic patients, episodes of pharyngeal acidification are so few that it is going to take a

large number of patients who do not have symptoms to establish normalcy. As I have stated, investigators who have studied small numbers of control patients have documented pharyngeal reflux up to 16 or 17 times in a single patient, and in up to 20% of control patients.

Dr. S. Horgan (Chicago, Ill.). Did patients who had abnormal pharyngeal exposure respond better to medical treatment than those who had normal pharyngeal exposure?

Dr. Eubanks. They did. Their symptoms improved as did the number of episodes. Because we carried out the pH monitoring while these patients were on medication, the number of reflux episodes decreased.

Dr. T.R. DeMeester (Los Angeles, Calif.). One of the pathophysiologic mechanisms in this disease is a jet phenomenon, in which reflux is actually jetted up into the pharynx. That may explain why these episodes occur more often in the upright position, when the jet is most apt to occur, than during the night when the patient is supine. It also may explain why this jet phenomenon seems to be more critical in patients who have a normal valve, and there were patients with normal valves in both groups. Because hiatal hernia sets the stage anatomically for this jet phenomenon to occur, have you looked at the number of patients in your study who had hiatal hernias?

It is known that reflux can be jetted up into the upper esophagus without a resulting drop in pH in the lower esophagus. Surprising as it may seem, that has indeed been reported and pretty convincingly so. So why do you require a drop in pH at all levels in order to consider it a positive result? That may explain why you only achieved a 40% positive result in your patients.

Finally, you do show increased exposure to acid in the patients who had positive results. How do you know that the increased exposure to acid wasn't due to some functional problem in the body of the esophagus and clearance, even though you did not measure it in the motility data?

Dr. Eubanks. It would be easy for us to look at the incidence of hiatal hernia in this population because all of these data are kept in a prospective database, including the results of upper gastrointestinal studies and upper endoscopy. With a normal LES, we could certainly look at that. I am not sure that we would see significantly more hiatal hernias in these patients than in the normal population, however, but we will review this possibility.

You asked why we require acidification in the distal esophagus in order to call it pharyngeal acidification. Practitioners in this field have taken to requiring this because the pH probe in the pharynx can dry out and can be affected by decreased salivary production. To eliminate this spurious data and avoid an overly sensitive test, we have adopted this policy, and I guess we have ignored the jetting phenomenon.

Dr. D.O. Castell (Philadelphia, Pa.). Relative to hypopharyngeal monitoring, there are normative data being developed. We examined 20 normal individuals and showed that approximately one third of them will have at least one episode of documented hypopharyngeal reflux—that is, distal acid going up into the proximal esophagus. So I would think you might want to select out that large group of patients who had one episode that you referred to as abnormal. You might get different results because that is within the normal range.

A Decision Analysis of the Optimal Initial Approach to Achalasia: Laparoscopic Heller Myotomy With Partial Fundoplication, Thoracoscopic Heller Myotomy, Pneumatic Dilatation, or Botulinum Toxin Injection

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In the absence of randomized controlled trials that directly compare all of the modern methods of managing achalasia, decision analysis may help determine the optimal treatment strategy. Four strategies for the initial management of achalasia were compared using the following decision model: (1) laparoscopic Heller myotomy and partial fundoplication; (2) pneumatic dilatation; (3) botulinum toxin injection; and (4) thoracoscopic Heller myotomy. Probabilities of clinical events and utilities of health states were estimated using review of the medical literature and patient interviews. A recursive decision tree (Markov model) was used to simulate all the important outcomes of each initial treatment option, allowing for complications, relapses over time, and transitions between strategies when appropriate. After 10 years, laparoscopic Heller myotomy with partial fundoplication was associated with the longest quality-adjusted survival (quality-adjusted life years [QALY] = 7.41). The difference between this strategy and either pneumatic dilatation or botulinum toxin injection was small. Thoracoscopic Heller myotomy was associated with the poorest quality-adjusted survival (QALY = 7.15). Pneumatic dilatation was the favored strategy when the effectiveness of laparoscopic surgery at relieving dysphagia was less than 89.7%, the operative mortality risk was greater than 0.7%, or the probability of reflux after pneumatic dilatation was less than 19%. In a decision model, laparoscopic Heller myotomy with partial fundoplication is at least as effective as endoscopic approaches for managing achalasia symptoms. However, the differences are small enough that patient preferences and local expertise should be taken into consideration when tailoring a treatment plan for an individual patient. (J GASTROINTEST SURG 2001;5:192-205.)

KEY WORDS: Esophageal achalasia, surgical procedures, minimally invasive, balloon dilatation, botulinum toxin type A, decision support techniques

Achalasia is an idiopathic disorder of esophageal motor function characterized by impaired lower esophageal sphincter relaxation and diminished esophageal body peristalsis. Typically, affected patients have progressive dysphagia to solids and liquids, chest pain, and weight loss. The natural history of achalasia is progression to end-stage esophageal dilatation and dysfunction, which may ultimately only be manageable with esophagectomy. Therefore treatment of achalasia is directed toward relieving dysphagia symptoms and preventing irreversible esophageal damage.[1,2]

A number of different interventions have been used to treat achalasia. Pharmacologic therapy with nitrates and calcium channel blockers is relatively ineffective, and is associated with adverse effects and tolerance that limit long-term use.[3] Endoscopic injection of botulinum toxin into the lower esophageal sphincter

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improves symptoms in a proportion of patients, but the effect is usually temporary, and multiple repeat injections may be required.[4] Pneumatic dilatation of the lower esophageal sphincter has a longer duration of effect than botulinum toxin injection, but is associated with a small risk of esophageal perforation, chest pain, and subsequent gastroesophageal reflux.[5] Heller myotomy, with or without a partial fundoplication, is the "gold standard" surgical treatment of achalasia.[6] Surgical myotomy can now be accomplished using laparoscopic or thoracoscopic approaches, eliminating much of the morbidity, mortality, and cost associated with open surgical procedures.[7]

Because achalasia is so uncommon (estimates of incidence range from 0.03 to 1.1 per 100,000 population),[8] few trials comparing different achalasia treatments contain enough study subjects to make valid inferences regarding treatment effectiveness. Furthermore, few centers have a large experience in managing achalasia, and referral centers tend to favor one approach. As a result, there are presently advocates for botulinum toxin injection, pneumatic dilatation, laparoscopic Heller myotomy with partial fundoplication, and thoracoscopic Heller myotomy as the primary treatment of achalasia.

Decision analysis is particularly useful for determining the optimal treatment for a condition such as achalasia, where there are several effective treatment strategies that have not been compared "head-to-head" in large, well-designed randomized controlled trials. We used a decision analysis model to determine the best initial approach to idiopathic esophageal achalasia in a typical patient, and performed sensitivity analyses to determine how variations in patient and treatment characteristics affect the treatment choice.

MATERIAL AND METHODS

Decision Model

Decision analysis is a modeling technique that is used to simulate all the components of a clinical decision-making process. All of the relevant treatment strategies for the selected clinical problem, and all of the consequences arising from the initial choice of strategy, are mapped out in a decision tree. Probabilities of chance events that result from an initial treatment decision are quantified as precisely as possible using the best available evidence from the medical literature. When the outcome of a decision model is quality of life, the quality of life associated with different health states is frequently represented in a model by utility values ranging from 0 (dead) to 1.0 (perfect health). The initial treatment strategy that results, on average, in the longest quality-adjusted life expectancy (the product of the duration of survival in a particular health state and the utility of the health state, usually expressed in terms of quality-adjusted life years [QALY]) is identified by "folding back" the decision tree. Typically, the effect of uncertainty in the estimated variables is explored using sensitivity analyses.[9-14]

We constructed such a decision tree to compare four strategies for managing achalasia. All of the important consequences of following each strategy were included in the model. The simulation evaluated the experience of a hypothetical cohort of patients followed over a 10-year period. To allow for symptom relapses over time, and transitions between strategies and health states when appropriate, we used a recursive (Markov) model[15] with a cycle length of 1 month. During each cycle, patients either underwent a procedure or occupied one of the following chronic health states: well (no dysphagia or reflux), post esophagectomy, gastroesophageal reflux, dysphagia, and dead. Each month, patients accrued quality-of-life experience that varied according to the health state occupied. The cohort simulation continued until a specified number of cycles had elapsed (e.g., 120 cycles for a 10-year period), or the entire cohort had died. The baseline monthly probability of dying was taken from published tables of vital statistics for the 1992 United States population.[16] The base-case analysis evaluated the optimal initial treatment strategy for a 45-year-old white male with typical symptoms, appropriate clinical and physiologic findings of achalasia, and no important comorbid diseases.

Structure of the Decision Tree and Model Assumptions

The decision tree we constructed simulated a four-armed clinical trial. All "patients" in the simulated cohort began in one of the four initial strategies: laparoscopic Heller myotomy with partial fundoplication, pneumatic dilatation, botulinum toxin injection, or thoracoscopic Heller myotomy. Transitions between strategies were allowed when appropriate. The same chronic health states were used for patients in each treatment strategy. Probabilities of similar events differed according to the treatment strategy (for example, the probability of response and relapse was different for laparoscopic surgery, pneumatic dilatation, and botulinum toxin injection).

In the laparoscopic Heller myotomy with partial fundoplication strategy, patients had a risk of conversion to open surgery, and had a different risk of operative death and operative complications depending on whether or not a procedure was completed laparoscopically. Following surgery, patients entered one of three chronic health states: well, residual symptoms

(dysphagia), or gastroesophageal reflux (either symptomatic or asymptomatic). In each of the chronic health states, there was a small monthly age-specific probability of death. Patients in the well state were allowed to relapse over time (we assumed that late recurrent symptoms were always related to the onset or progression of gastroesophageal reflux), in which case they transitioned into the reflux state. Patients in the reflux state either recycled within this state (with persistent symptoms), died, or required an esophagectomy (due to stricture formation). We assumed that gastroesophageal reflux that developed following treatment did not improve spontaneously. Patients in the dysphagia state could either remain in this state with persistent symptoms, die, or require esophagectomy. Esophagectomy was associated with a risk of death and operative complications. Patients who survived esophagectomy entered the postesophagectomy state, where they remained until they died or the simulation terminated. The chronic health states and the transitions allowed in the model following laparoscopic surgery are illustrated in Fig. 1.

Patients who began the simulation with pneumatic dilatation could suffer an esophageal perforation, which was managed medically or with open Heller myotomy and partial fundoplication. If uncomplicated, the pneumatic dilatation may not have relieved symptoms, in which case patients underwent laparoscopic surgery. In patients in whom dysphagia was improved, there was a risk of developing symptomatic or asymptomatic gastroesophageal reflux. Patients with reflux entered the reflux chronic health state as described above. Patients who were well without reflux following pneumatic dilatation were allowed to relapse over time, and were allowed to undergo up to two additional dilatations. We assumed that all patients with persistent or recurrent symptoms after three dilatations went on to laparoscopic surgery.

The botulinum toxin injection tree had a similar structure to the pneumatic dilatation tree, except that we assumed that this treatment did not induce gastroesophageal reflux, and we allowed for patients to have an unlimited number of repeat injections for recurrent symptoms. Patients who no longer responded to injection underwent laparoscopic surgery. We assumed that the outcomes for surgery following botulinum toxin injection or pneumatic dilatation were the same as those for primary surgery. The thoracoscopic Heller myotomy strategy was similar in structure to the laparoscopic surgery strategy described earlier, ex-

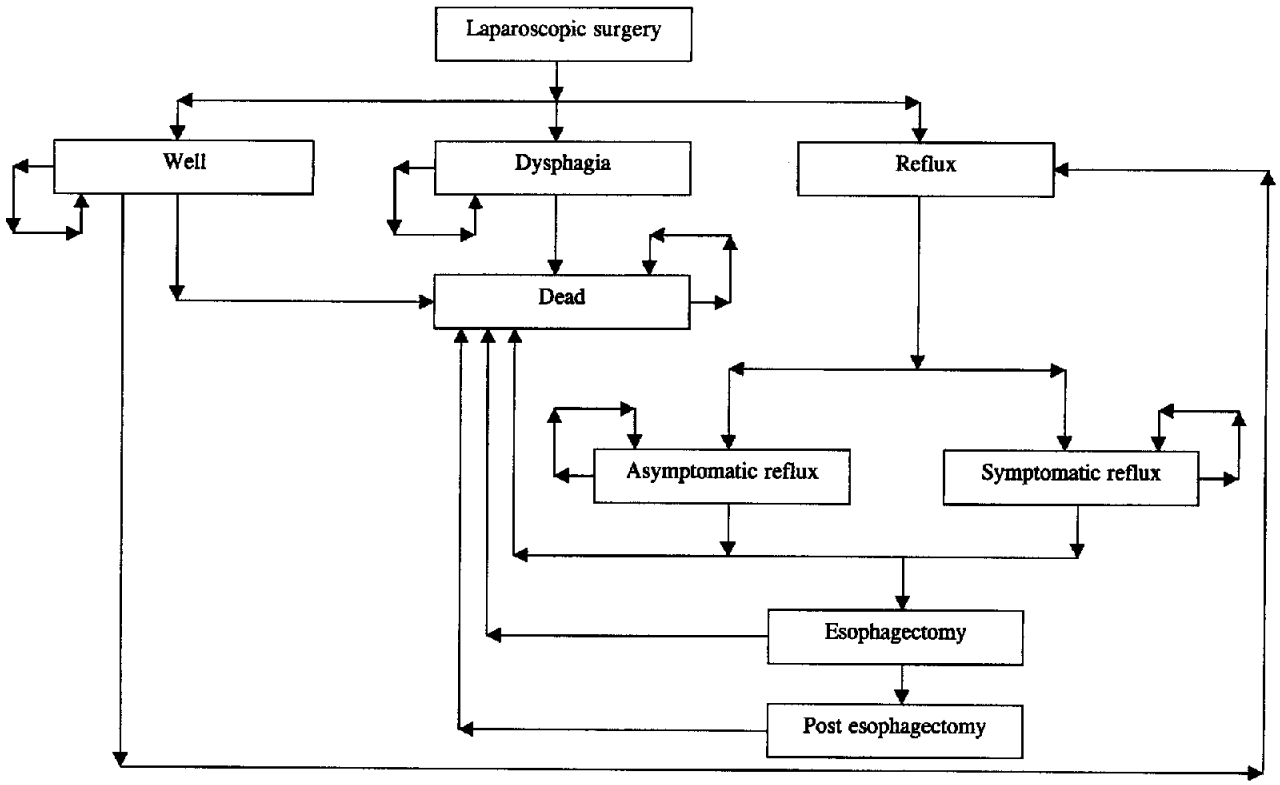

Fig. 1. State transition diagram for the laparoscopic Heller myotomy and partial fundoplication strategy. Boxes denote chronic health states and arrows indicate transitions that are allowed between health states in the model. State transition diagrams for the other strategies have a similar structure.

cept that conversions to open surgery were conversions to thoracotomy.

Estimation of Probabilities

We obtained estimates of event probabilities from published articles. We used the 95% confidence interval (CI) around each estimate to represent the plausible range of values. If an appropriate CI was not reported, we calculated an approximate 95% CI using the standard error of a proportion given by:

$$\sqrt{\frac{p(1-p)}{n}}$$

Confidence limits for proportions were truncated at 0 or 1.0 if they overlapped these values. For survival-type data, the approximate standard error of the event-free proportion at a given time was calculated by substituting the number of individuals who had failed and the number who were still at risk for n.

Botulinum Toxin Injection. Pasricha et al.[4] reported the results of botulinum toxin injection in 31 patients aged 19 to 85 years with clinical and manometric findings of achalasia. Effectiveness was evaluated using a clinical scoring method to determine symptomatic response. Seventeen patients responded to one injection and did not relapse within 2 to 3 months, for a probability of symptom relief of 55% (95% CI = 37% to 72%). Of 15 patients who had a subsequent injection, nine responded (60%; 95% CI = 35% to 85%). Ninety-five percent of all responders eventually relapsed over a median follow-up period of 2.4 years. The median time to recurrence was 16 months (95% CI = 8% to 34%). In the absence of empiric data on the median time to repeat treatment following the recurrence of symptoms, we estimated this to be 3 months and allowed the monthly transition probability to range from 0 to 1.0 in sensitivity analyses.

Pneumatic Dilatation. The probability of esophageal perforation from pneumatic dilatation, the probability of requiring surgery to manage an esophageal perforation, and the probability of a successful pneumatic dilatation were taken from a study by Panaccione et al.[17] As part of decision analysis comparing the cost of pneumatic dilatation and botulinum toxin injection for the treatment of achalasia, these investigators described their experience with 131 pneumatic dilatations in 114 patients. Five of the 131 dilatations resulted in an esophageal perforation (3.8%; 95% CI = 0.5% to 7.1%), and all perforations occurred with the use of the 35 mm balloon. All of the perforations were managed by surgery in this series. In our decision model we assumed that all perforations would require repair with open surgery, but we allowed this value to vary from 40% to 100% in sensitivity analyses to account for the fact that some perforations may be managed successfully using nonoperative means. After initial treatment, 68 of 75 patients were improved (91%; 95% CI = 84% to 97%).

Parkman et al.[18] reported detailed symptom follow-up in patients treated by pneumatic dilatation. Long-term information on symptoms was available for 109 patients (74%) who completed the follow-up questionnaire, after a mean duration of 4.7 years. According to a life table of event-free survival, approximately 60% of patients remained symptom free after 48 months of follow-up, with 58 patients still at risk. Using these data, we estimated the median event-free "survival" in our model to be 66 months (95% CI = 48% to 94%).

The probability of pathologic reflux following pneumatic dilatation, and the proportion of patients with reflux who are symptomatic, was taken from a prospective study of pneumatic dilatation and transthoracic Heller myotomy in patients with achalasia confirmed by esophageal manometry. Shoenut et al.[19] prospectively studied 17 previously untreated patients who had pneumatic dilatation. Following pneumatic dilatation, 6 of the 17 patients had a pH <4.0 more than 6% of the total time on 24-hour pH monitoring (35%; 95% CI = 13% to 58%). Of 12 patients who developed pathologic reflux after either pneumatic dilatation or myotomy, four were symptomatic (33%; 95% CI = 7% to 60%).

Laparoscopic Heller Myotomy With Partial Fundoplication. We used representative published studies[6,7,20-22] to estimate the probabilities of operative death, operative complications, and conversion to open surgery for laparoscopic and open Heller myotomy and partial fundoplication (Table I). The probability of symptom relief following laparoscopic Heller myotomy with partial fundoplication was derived from the combined results of two series.[20,21] Of 49 patients who had either a Dor or a Toupet partial fundoplication in addition to a Heller myotomy, 36 experienced subsequent dysphagia rarely or never (94%; 95% CI = 87% to 100%). The probability of postoperative gastroesophageal reflux was estimated from two studies. Anselmino et al.[23] found that 2 of 35 patients studied 1 year after a laparoscopic Heller-Dor procedure had abnormal 24-hour pH exposure, and Swanstrom and Pennings[20] did not detect pathologic reflux in any of nine patients who had a laparoscopic Heller-Toupet procedure. The pooled probability of gastroesophageal reflux from these two studies was 4.5% (95% CI = 0% to 11%).

Because late results of laparoscopic surgery for achalasia have not yet been reported, we used studies of open Heller myotomy and partial fundoplication

Table I. Probabilities and failure times of adverse events

Variable	Estimate	Range	Reference
Botulinum toxin injection			
Symptom relief, initial treatment	55%	37%-72%	4
Symptom relief, subsequent treatment	60%	35%-85%	4
Median time to recurrence (mo)	16	8-34	4
Pneumatic dilatation			
Perforation	3.8%	0.5%-7.1%	17
Needing surgical repair of perforation	100%	40%-100%	17
Symptom relief	91%	84%-97%	18
Reflux*	35%	13%-58%	19
Median time to recurrence (mo)	66	48-94	18
Laparoscopic Heller myotomy with partial fundoplication			
Operative death, laparoscopic procedure	0	0-1%†	20,21
Operative death, laparotomy	0	0-5%†	6
Conversion to open procedure	5.9%	0-17%	7
Operative complication, laparoscopic procedure	5.8%	0-13%	20,21
Operative complication, laparotomy	8.3%	0-16%	22
Symptom relief	94%	87%-100%	20,21
Immediate reflux*	4.5%	0-11%	20,23
Median time to development of late reflux (mo)‡	158	87-370	24
Median time to esophagectomy (mo)	120§	60-180†	
Thoracoscopic Heller myotomy			
Operative death, thoracoscopic procedure	0	0-0.01†	
Operative death, thoracotomy	2.7%	0-8.1%	25
Conversion to thoracotomy	5.7%	0-13.4%	26
Operative complication, thoracoscopic procedure	12.1%	0-23.0%	26
Operative complication, thoracotomy	8.6%	4.6%-12.7%	27
Symptom relief	85.7%	74.1%-97.3%	26
Immediate reflux*	60%	29.6%-90.4%	26
Median time to development of late reflux (mo)‡	120	89-168	28
Esophagectomy			
Operative death	5.4%	0-12.7%	29
Operative complication	32.4%	17.3%-47.5%	29
Miscellaneous			
Duration of simulation (yr)	10	1-20	
Patient age (yr)	45	35-65	
Patient sex	Male	Both sexes	
Proportion symptomatic with reflux	33%	7%-60%	19

*Reflux was defined as abnormal esophageal acid exposure on 24-hour pH monitoring.
†95% CI determined by opinion.
‡These estimates were derived from studies of open surgical procedures.
§Opinion.

to estimate the long-term risk of developing recurrent symptoms (reflux or dysphagia) and requiring esophagectomy. Malthaner et al.[24] studied 22 patients who underwent transthoracic Heller myotomy and Belsey fundoplication, and were followed for at least 10 years. Forty-one percent of patients had developed symptoms of reflux by 10 years. From these data we estimated the median time to development of symptoms to be 158 months (95% CI = 87% to 370%). We could not find reliable data on the rate of progression to esophagectomy among patients with reflux following achalasia surgery. Three of 22 patients studied by Malthaner et al.[24] required esophagectomy, at times ranging from 7 to 23 years following their initial procedure. We assumed that 50% of patients with reflux following achalasia treatment would end up needing an esophagectomy by 5 years (120 months; 95% CI = 60% to 180%).

Thoracoscopic Heller Myotomy. The probabilities of operative death and complications for thoraco-

scopic Heller myotomy and open thoracotomy from representative studies[25-27] are summarized in Table I. In a series of 35 Heller myotomies that were initially approached thoracoscopically, the probability of conversion to thoracotomy reported by Patti et al.[26] was 12.1% (95% CI = 0% to 23%). When a complete Heller myotomy was performed, the probability of symptomatic improvement following thoracoscopic surgery was 85.7% (95% CI = 74.1% to 97.3%).[26] However, the probability of postoperative reflux was high. Of 10 patients who underwent 24-hour pH monitoring after surgery, six had abnormal acid exposure in their distal esophagus (60%; 95% CI = 29.6% to 90.4%).[26] The probability of developing late reflux following a transthoracic Heller myotomy was estimated from a study of patients who had open thoracotomy reported by Jara et al.[28] Forty-eight percent of 145 patients had developed reflux after 10 years, with 41 patients remaining under observation. We therefore used 10 years as the median time to develop late reflux following a transthoracic Heller myotomy (120 months; 95% CI = 89% to 168%).

Esophagectomy. In a review of 37 patients with achalasia who underwent esophagectomy, the operative mortality rate was 5.4% (95% CI = 0% to 12.7%) and the probability of serious operative complications was 32.4% (95% CI = 17.3% to 47.5%).[29]

Estimation of Utilities

No studies have reported utility measures specific to achalasia. However, a cost-utility analysis of treatment of peptic esophageal stricture by Stal et al.[30] estimated a utility of 0.90 for dysphagia, the predominant symptom of achalasia. We elicited utilities for achalasia in two patients using the standard gamble technique. The patients were a 35-year-old man and a 40-year-old woman, both of whom had typical radiologic and manometric findings of achalasia, and were in the hospital awaiting surgery. One patient had been treated with botulinum toxin during the previous month. Both patients had dysphagia to liquids and solids as well as weight loss, and both reported profound limitation of their social activities. Both patients' utility for achalasia was 0.80. We used the value 0.80 to estimate the utility for achalasia symptoms, and tested the range 0.70 to 0.90 in sensitivity analyses.

Heudebert et al.[31] estimated a utility value for "severe esophagitis" of 0.82 using a consensus of expert opinion. Ethiopia et al.[32] performed a battery of quality-of-life assessments in 40 patients with a clinical diagnosis of gastroesophageal reflux disease (of varying severity). The mean standard-gamble utility was 0.90. We used this value for the base-case estimate of the utility for gastroesophageal reflux disease, and tested the range 0.80 to 0.99. Provenzale et al.[33] used the time tradeoff technique to elicit a utility for the postesophagectomy state in a decision analysis of strategies for managing Barrett's esophagus. We used the reported value of 0.97 to represent the chronic impairment in quality of life that occurs following an esophagectomy.

Utilities for short-term health states such as operative and endoscopic procedures were derived by decomposing a 1-month (30-day) cycle for each such health state into a number of discrete health states, and weighting the utility for each of these "substates" by the duration of time spent in that state. We estimated that the utility for a day on which a surgical or endoscopic procedure was performed was 0, a day in an intensive care unit (ICU) was 0, a day spent in the hospital on a regular-care ward was 0.6, a day spent out of the hospital but not in the usual state of health was 0.8, and a day in the usual state of health was 1.0. We used a "typical" hospital course for each procedure to estimate the final quality adjustment factor. All utilities used in the model are summarized in Table II.

Analytic Considerations

The cycle length for all Markov models was 1 month. We assumed that event rates were constant over time. A constant probability of failure applied over multiple cycles results in a declining exponential function, which has been used frequently to represent failure patterns in medical decision models.[34,35] The monthly probability (P) of a chance event was derived from published data on the event rate using the relationship $P = 1 - e^{-rt}$, where e is the base of the natural logarithm, and r is the event rate in the specified time t.[15] When studies reported recurrence-free "survival" data on the effectiveness of an intervention, the average hazard was calculated from the median time to recurrence. Utilities were discounted at 5% per year to account for the increased value that individuals place on their immediate health compared to considerations of future health states.

All modeling and analyses were carried out on a personal computer using a software package designed for medical decision-making applications (DATA 3.5, TreeAge Software, Inc., Williamstown, Mass.). In addition to the base-case analysis, one-way sensitivity analyses were performed on all estimated utilities and probabilities over their plausible range. Multiway sensitivity analyses were performed selectively on combinations of variables that had clinical importance.

Table II. Utilities of health states included in the decision model

Health state	Estimate	Range	Reference
Chronic health states			
Well	1.0	—	
Reflux	0.90	0.80-0.99	32
Achalasia	0.80*	0.70-0.90	
Post esophagectomy	0.97†	0.80-0.99	33
Dead	0	—	
Temporary health states‡			
Botulinum toxin injection	0.97	0.90-0.99	
Pneumatic dilatation	0.97	0.90-0.99	
Laparoscopic Heller myotomy, fundoplication	0.86	0.60-0.95	
Thoracoscopic Heller myotomy	0.85	0.60-0.95	
Laparotomy	0.73	0-0.80	
Thoracotomy	0.71	0-0.80	
Esophagectomy	0.67	0-0.80	
Disutilities			
Esophageal perforation	0.10§	0.05-0.30	44
Operative complication	0.20‖	0.10-0.30	

*Standard gamble technique.
†Time tradeoff technique.
‡Decomposed method of determining utilities for short-term health states associated with interventions (see text for explanation).
§Decision analysis on the use of transesophageal echocardiography.
‖Opinion.

RESULTS

Base Case

The base-case scenario we evaluated was of a 45-year-old white male with typical symptoms and classic physiologic findings of achalasia. Over a 10-year period (120 one-month cycles), with future utility values discounted by 5% per year, the laparoscopic Heller myotomy with partial fundoplication strategy was associated with a quality-adjusted survival of 7.41 QALY, compared to 7.36 QALY for pneumatic dilatation, 7.33 QALY for botulinum toxin injection, and 7.15 QALY for thoracoscopic Heller myotomy (Table III). The distribution of health states among a cohort of patients managed with the four treatment strategies after 5 and 10 years is depicted in Fig. 2.

Table III. Results of base-case analysis

Strategy	Quality-adjusted survival*
Laparoscopic Heller myotomy and partial fundoplication	7.41
Pneumatic dilatation	7.36
Botulinum toxin injection	7.33
Thoracoscopic Heller myotomy	7.15

*QALY (quality-adjusted life years).

One-Way Sensitivity Analyses

Single-variable sensitivity analyses were performed on each estimated quantity included in the model, for the range of values specified in Tables I and II. Although modeling parameters such as the time horizon of the simulation and the rate of discounting had a large impact on the expected value of each strategy in terms of QALY, none of these parameters altered the optimal choice of treatment strategy. The main results of the model were also robust to variations in patient age, sex, or race.

Variation in four of the estimated clinical input variables altered the choice of optimal treatment. Laparoscopic Heller myotomy with partial fundoplication is the optimal strategy when the probability of postoperative relief of dysphagia is greater than 89.7% (Fig. 3) and when the probability of operative death is less than 0.7% (Fig. 4). When either of these conditions is not true, the pneumatic dilatation strategy becomes the optimal initial treatment choice. Variation in the probability of gastroesophageal reflux following pneumatic dilatation also influenced the results of the model. If the probability of abnormal 24-hour pH testing following pneumatic dilatation is 19% or less, then the pneumatic dilatation strategy is associated with greater quality-adjusted survival than the laparoscopic surgery strategy (Fig. 5). The final

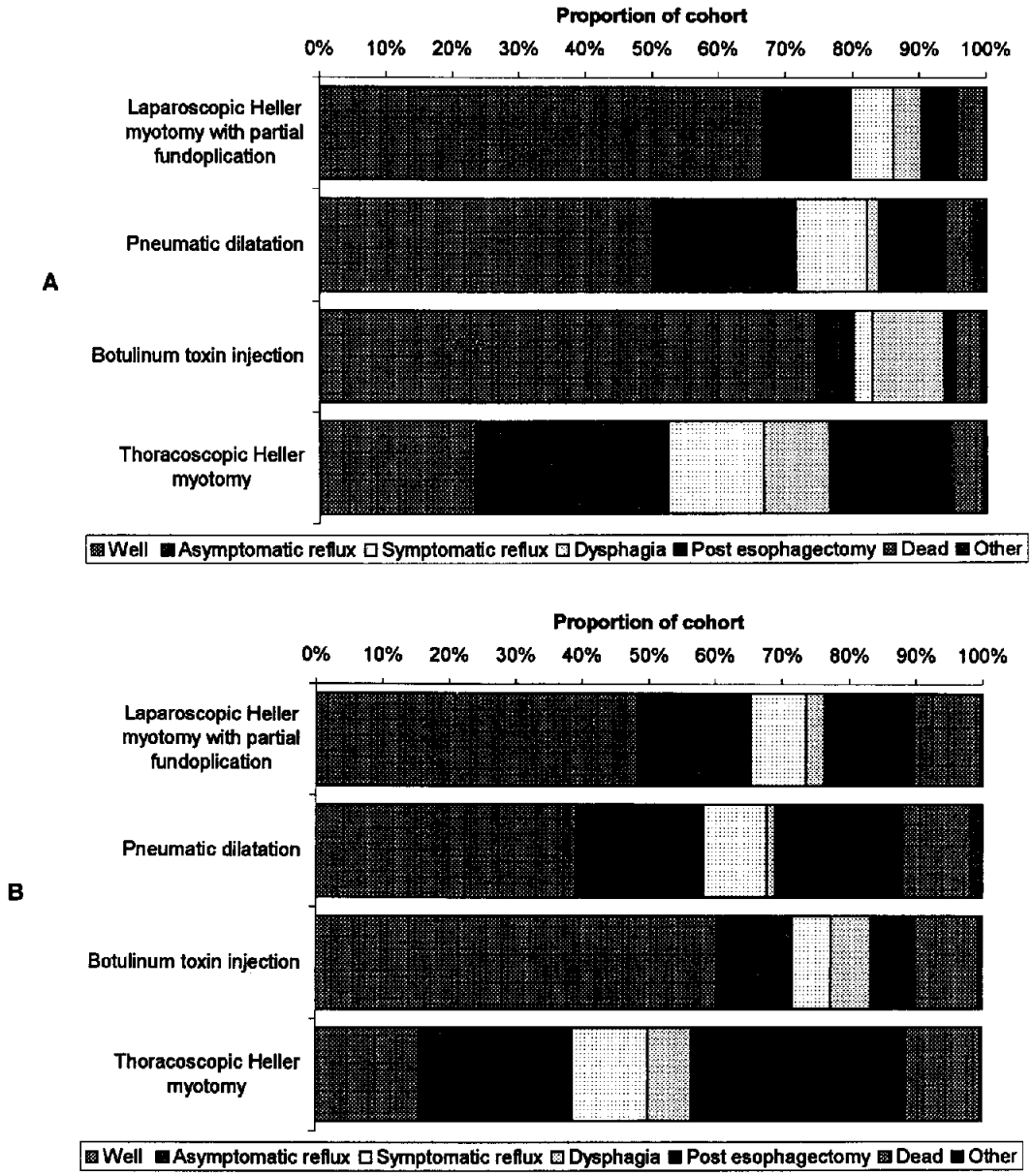

Fig. 2. Distribution of health states after **(A)** 5 year and **(B)** 10 years.

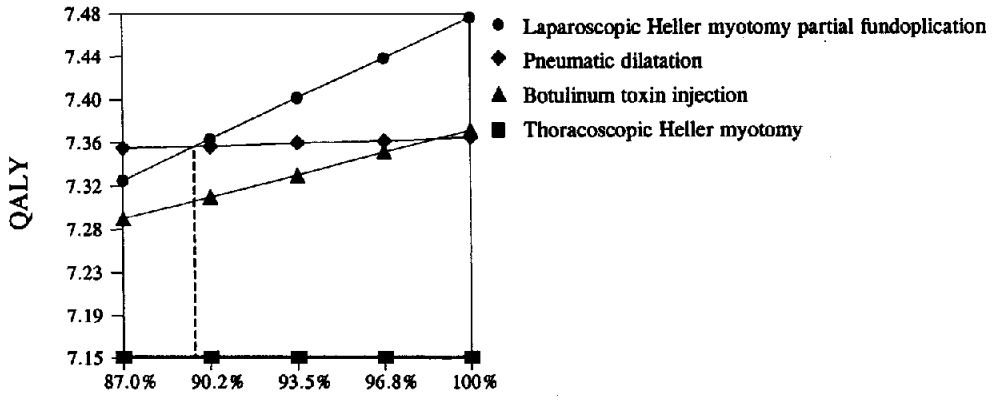

Fig. 3. One-way sensitivity analysis on the effectiveness of laparoscopic Heller myotomy and partial fundoplication improving dysphagia. The effect of variation in the effectiveness of laparoscopic surgery on quality-adjusted survival for all four strategies is shown. There is a threshold at 89.7% (dashed vertical line). The pneumatic dilatation strategy is associated with greater quality-adjusted survival when the effectiveness of surgery is below this threshold, and the laparoscopic surgery strategy is favored when the effectiveness of surgery is above this threshold. QALY = quality-adjusted life years.

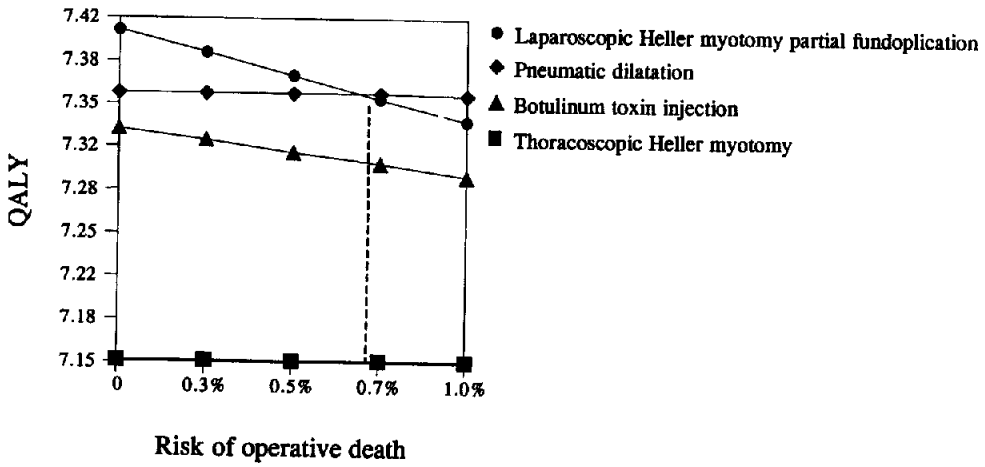

Fig. 4. One-way sensitivity analysis on the risk of operative death from laparoscopic Heller myotomy and partial fundoplication. The effect of variation in the risk of operative death from laparoscopic surgery on quality-adjusted survival for all four strategies is shown. There is a threshold at 0.7% (dashed vertical line). The pneumatic dilatation strategy is associated with greater quality-adjusted survival when the risk of operative mortality is above this threshold, and the laparoscopic surgery strategy is favored when the risk of operative mortality is below this threshold. QALY = quality-adjusted life years.

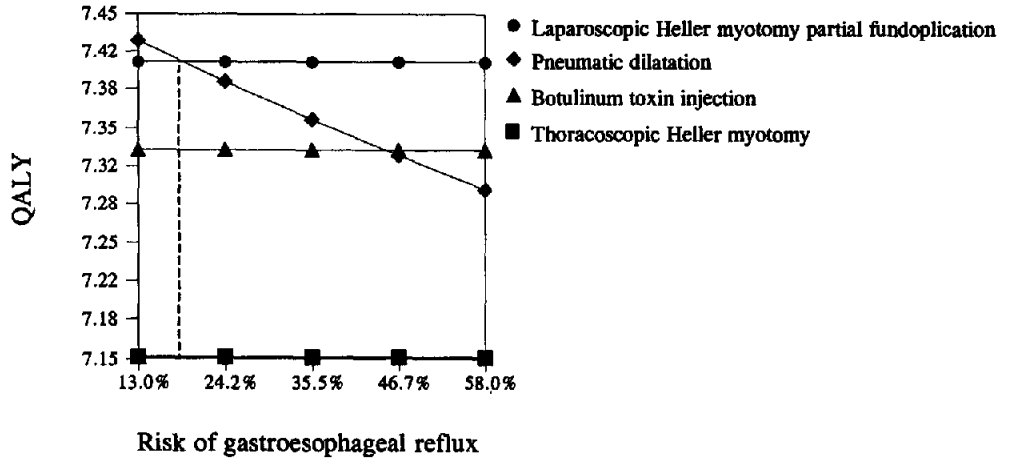

Fig. 5. One-way sensitivity analysis on the risk of gastroesophageal reflux following pneumatic dilatation. The effect of variation in the risk of gastroesophageal reflux following pneumatic dilatation on quality-adjusted survival for all four strategies is shown. There is a threshold at 19.0% (dashed vertical line). The pneumatic dilatation strategy is associated with greater quality-adjusted survival when the risk of gastroesophageal reflux is below this threshold, and the laparoscopic surgery strategy is favored when the risk of gastroesophageal reflux is above this threshold. QALY = quality-adjusted life years.

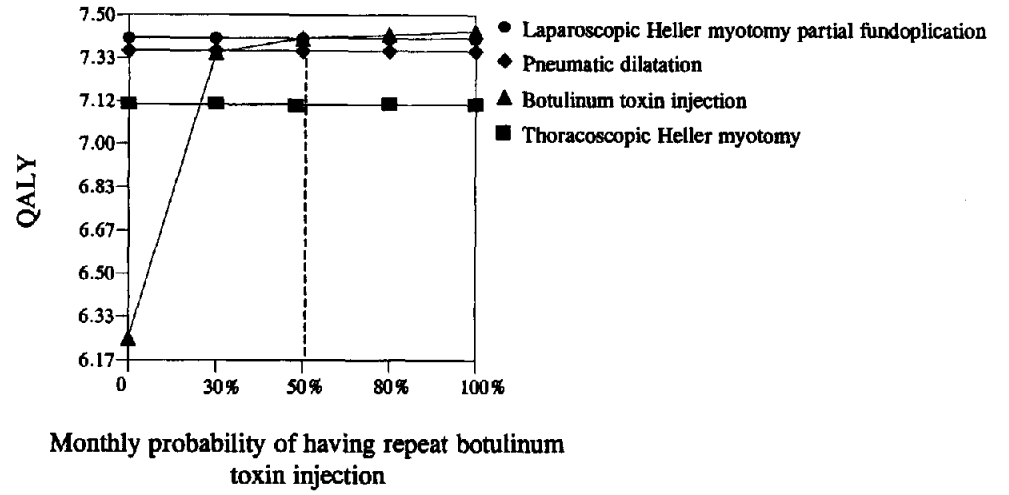

Fig. 6. One-way sensitivity analysis on the monthly probability of having a repeat injection of botulinum toxin for patients with recurrent dysphagia following a successful injection. The effect of variation in the monthly probability of having a repeat botulinum toxin injection on quality-adjusted survival for all four strategies is shown. There is a threshold at 50.0% (dashed vertical line). The botulinum toxin injection strategy is associated with greater quality-adjusted survival when the monthly probability of having a repeat botulinum toxin injection is above this threshold, and the laparoscopic surgery strategy is favored when the monthly probability of having a repeat botulinum toxin injection is below this threshold. QALY = quality-adjusted life years.

sensitive variable was the median time to repeat botulinum toxin injection following symptomatic relapse. We estimated this to be 3 months. However, the optimal treatment choice was sensitive to this variable, with the botulinum toxin injection strategy being favored when the time to repeat injection following recurrence of dysphagia was very short (Fig. 6).

DISCUSSION

Physicians are frequently required to choose between different therapeutic options for treating a patient's disease. Too frequently such decisions are made based on habit, outdated information, or lack of awareness of more appropriate options. Decision analysis is a tool that creates a clinically realistic model of the ther-

apeutic options for a particular disease entity. Decision analysis is particularly useful for deciding on the best initial treatment for achalasia, as it is a potentially devastating disease with widely differing, but mostly successful, treatment options.

In our decision analysis evaluating quality of life following the treatment of achalasia using one of four different initial treatment strategies, we found that initial treatment of all patients with laparoscopic Heller myotomy and partial fundoplication was associated with the longest quality-adjusted survival. However, there was not a large difference between treatment strategies. After 10 years, laparoscopic surgery resulted in an average of 18 more quality-adjusted life days than pneumatic dilatation, which in turn was associated with 11 more quality-adjusted life days than botulinum toxin injection. Furthermore, the choice of optimal treatment strategy was sensitive to values within the plausible range of variables as important as the effectiveness of laparoscopic surgery, the probability of operative death with laparoscopic surgery, and the probability of developing gastroesophageal reflux following pneumatic dilatation.

Compared to other studies measuring gains in life expectancy from treating established diseases, this difference in quality-adjusted survival is quite small.[36] Most gains in life expectancy from treating established diseases that have been considered clinically important are typically several months or greater.[36] The small difference in quality-adjusted survival that we demonstrated in this analysis suggests that in terms of effectiveness, laparoscopic surgery, pneumatic dilatation, and botulinum toxin injection as *initial* treatment options for a patient with achalasia are relatively equal if performed with a high level of expertise. The small difference also means that even small variations in the "quality" of a procedure may alter the advantage of one procedure over another. For example, if the operative mortality risk for laparoscopic Heller myotomy exceeds our estimate, this would become a less desirable initial strategy if pneumatic dilatation had equal or better results than what is reported in the literature.

An apparent contradiction exists between the prevailing opinion of those who currently manage patients with achalasia and the findings of this analysis. There is uniform consensus among medical and surgical gastroenterologists that surgery is more effective than pneumatic dilatation in improving achalasia symptoms, and that both are superior to botulinum toxin injection. We suggest that it makes little difference whether patients are treated *initially* with laparoscopic surgery, pneumatic dilatation, or botulinum toxin injection. How can these two views be reconciled?

In fact, these positions are not mutually exclusive. Our model accounted for the fact that patients who do not respond to a particular procedure may be successfully managed with a different procedure, and that patients who initially respond and fail over time may be re-treated successfully using the same procedure. Therefore patients in our model who did not respond to endoscopic therapy or who failed over time were "salvaged" by surgery, and their aggregate quality-of-life experience did not differ significantly from patients who were treated initially with laparoscopic surgery. This concept of a patient's clinical course, where several different treatment modalities may be used at different times and in different situations, is what often occurs in actual practice. Clinical researchers frequently consider decision analyses to fall under the category of "outcomes" or "effectiveness" research, since they study what would happen to typical patients treated in realistic clinical settings, as opposed to the narrow, limited context of a randomized controlled trial.

We did not find any other published decision analyses that compared minimally invasive surgery, pneumatic dilatation, and botulinum toxin injection for the treatment of achalasia. Panaccione et al.[17] reported a decision analysis that compared the cost of pneumatic dilatation and botulinum toxin injection. They found that pneumatic dilatation was less costly than botulinum toxin injection over a 10-year period, primarily because of the increased costs incurred by the multiple repeat injections of botulinum toxin that are typically required. This decision analysis did not evaluate quality of life and did not consider any strategies based on primary surgical treatment.

There are several limitations to our methods that should be emphasized. To create any working decision model, several simplifying assumptions must be made, which undoubtedly do not capture all the subtleties of clinical practices. In building our final decision tree, we did evaluate several alternative models, and the use of different assumptions did not substantially alter our main results. Our assumption of a constant failure rate over time, and the declining exponential function of treatment relapse, probably does not mirror the exact failure patterns following each treatment strategy. Unfortunately, precise estimates of time-dependent failure rates following achalasia treatments do not exist. The assumption of a constant failure rate to model complex biologic phenomena has been used in other decision analyses.[31,33]

We also assumed that having one intervention did not affect the effectiveness of a subsequent procedure. In our model, patients who have laparoscopic Heller myotomy and partial fundoplication following one or

more injections of botulinum toxin had the same postoperative outcomes as patients having surgery who never had botulinum toxin injection. This assumption may not be accurate for several reasons. Some surgeons believe that a previous pneumatic dilatation or botulinum toxin injection makes a subsequent Heller myotomy more difficult, and increases the risk of intraoperative esophageal perforation, although studies by expert surgeons did not show worse outcomes for these patients.[37,38] In addition, patients who present for surgery after failing previous treatments may have more severe disease, such as end-stage esophageal dilatation or complications, than unselected patients having surgery. In our model we used the same surgical outcomes for laparoscopic surgery, when it was performed as a "salvage" procedure after failure of endoscopic treatments, as for primary laparoscopic surgery. Because we would expect patients who failed previous attempts at endoscopic treatment to have worse outcomes than unselected patients, this assumption would bias the model in favor of pneumatic dilatation and botulinum toxin injection.

We did not explicitly consider differences in procedure technique that may impact the effectiveness of different modalities. Prospective studies have not demonstrated important differences in treatment outcomes based on differences in the technique of pneumatic dilatation.[39-41] Our estimates of probabilities and utilities may have been subject to measurement error. Several probabilities were derived from retrospective clinical studies with relatively weak designs, and were subject to several sources of bias and confounding. Unfortunately, we could not find high-quality data from well-designed randomized controlled trials that could be used in our model. We did, however, perform sensitivity analyses for all clinical inputs on a wide range of possible values, and relatively few variables had treatment thresholds within their plausible range. Our estimates of utilities are also subject to several sources of error. The utility value for achalasia was derived from a small sample. Large samples would be difficult to obtain because of the rarity of the disease. Finally, our model did not allow for interactions between patient characteristics and the type of intervention on treatment effectiveness. For example, it has been suggested that the effectiveness of pneumatic dilatation differs depending on a patient's age.[42]

Our analysis highlights several interesting issues that are relevant to the evaluation of therapy for achalasia. When treatments vary not only in their effectiveness, but also in their duration of effect or time to effect, it may be inadequate to represent treatment effect simply by measuring the proportion of patients who are well after a defined period of time. Rather, a more informative measure of effectiveness is the time spent feeling well during the study period. In our model, the distribution of health states after 5 and 10 years showed that more patients treated initially with botulinum toxin were asymptomatic than those treated initially with laparoscopic surgery. Laparoscopic surgery still resulted in greater aggregate quality-of-life experience, since it caused a more rapid relief of symptoms.

Another phenomenon of interest is the heterogeneous nature of the outcomes of treatment for achalasia. An intervention for achalasia may result not only in symptom relief or symptom persistence, but may also cause other important consequences, such as gastroesophageal reflux or esophageal perforation. Future trials of treatments for achalasia should take into consideration the multidimensional nature of treatment outcomes. It may be best to evaluate treatment effectiveness in terms of a global measure of health-related quality of life, instead of the usual clinical outcome measures such as changes in individual symptoms or alterations in physiologic or radiographic findings.

An advantage of decision analysis is the ability to identify key deficiencies in clinical understanding that should direct further research. We identified three clinical variables that were very influential in determining the optimal treatment strategy: the effectiveness of laparoscopic surgery in improving symptoms of achalasia, the risk of operative death from laparoscopic surgery, and the probability of developing abnormal gastroesophageal reflux following pneumatic dilatation. Future prospective studies should focus on better quantifying the probabilities of these outcomes.

There are several reasons why we did not evaluate the costs of the different treatment strategies. Costs and practices vary from center to center, and an accurate accounting of relative costs usually requires that all cost data be obtained from the same institution.[43] It would be uncommon, however, for a single center to have enough experience in each of the treatment modalities we evaluated to generate meaningful cost comparisons. Finally, achalasia is extremely rare, affecting between one and 10 patients per million in 1 year.[8] We believe that the implications for health resource utilization of choosing between treatments for a disease as uncommon as achalasia are far less pressing than for more prevalent conditions.[44]

CONCLUSION

In a decision analysis comparing the quality of life in patients with achalasia, initial treatment with lapa-

roscopic Heller myotomy and partial fundoplication was at least as effective as pneumatic dilatation and botulinum toxin injection, although the difference in effectiveness between these strategies was small. Thoracoscopic Heller myotomy was associated with poorer quality-adjusted survival than the other three strategies. There does not appear to be a single best strategy for the initial treatment of achalasia, and variations in patient preferences and local expertise among gastrointestinal surgeons and gastroenterologists should be taken into account in tailoring a treatment plan. Unless the outcomes of thoracoscopic Heller myotomy can be improved, this strategy appears to be the least effective initial treatment for achalasia according to currently available data.

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Discussion

Dr. M.G. Patti (San Francisco, Calif.). I think this is a very interesting model that you are presenting, but why don't we talk about patients rather than a model? Let me go over the treatment modalities. Botulinum toxin does not work. I do not know what type of data you are getting with your model, but there are data from Pasricha et al.[4] showing that with a follow-up of 2.4 years, only 30% of patients are free of dysphagia. In addition, these patients need multiple injections. In addition, your type of treatment algorithm does not really work because some patients develop severe damage to the esophagus because of botulinum toxin. I very strongly believe that botulinum toxin should be used only in elderly patients who are not candidates for either pneumatic dilatation or surgery. With regard to surgery, we use the laparoscopic Heller myotomy as our preferred treatment; we find the results of laparoscopic Heller myotomy in terms of relief of dysphagia and patient satisfaction to be excellent because in most patients with reflux, it is asymptomatic reflux. Therefore, to put this report into perspective, it must be emphasized that there are presently enough data so that rather than using a decision analysis of this type, we should look at the results of real clinical studies.

Dr. D. Urbach. In regard to the data from Pasricha et al.,[4] we actually used their published data and took a survival analysis approach using a Kaplan-Meier type of method; what we were looking for was the median time to failure, so that may explain some of the differences in the actual value that you were using. As far as the effectiveness of surgery after a previous injection of botulinum toxin, we did find some studies that clearly made the point that surgery appears to be much more difficult after botulinum toxin injection. However, there is no clear evidence that patient outcomes are worse with surgery after botulinum toxin compared to surgery in patients who have never had injections. Finally, to address your question about the fact that not all patients with reflux are symptomatic, we actually did incorporate them into the model. However, we did not show every single parameter that we entered and all of the possible pathways, but we did allow that not all patients with abnormal 24-hour pathologic reflux after surgery were symptomatic, and in sensitivity analyses, it really did not matter much. I would also like to add that the way this model works is that patients are not necessarily penalized for failing a therapy such as pneumatic dilatation or botulinum toxin if they eventually have surgery, which can then be quite an effective treatment, because what we are doing is simply scoring their quality-of-life experience over a prolonged period of time. As long as you manage to keep patients as asymptomatic as possible for the duration of that period, they fare equally well no matter how you achieve this.

Dr. D.W. Rattner (Boston, Mass.). In this model, you are allowed to fail with Botox injections, you are allowed to fail with pneumatic dilatation, and ultimately it is the operation that works. So I would say that quality probably is not the appropriate measure to analyze. It is not the correct end point; the end point should be relief of dysphagia.

Dr. V.H. Finch (Chicago, Ill.). Regarding the use of dilatation, as we reviewed the national data, we found that at one time there was a 2% to 5% mortality rate throughout the United States. Our mortality rate was approximately 1.8% with Dr. Charles Whines performing the procedure. The chairman of our department of medicine would go crazy each time the mortality report was shown, and unless you achieve better dilatation than we did—and we had an expert doing it—I would think we should avoid dilatation. Our results with Botox have been quite good. I think that fairly good results have been achieved at Johns Hopkins as well.

Dr. Urbach. As far as dilatation is concerned, in our model patients who had a perforation as a result of dilatation went on to have open surgery as opposed to laparoscopic surgery and had a slightly increased risk of death relevant to their having undergone laparotomy, if that helps answer the question.

Rationale for the Combination of Cryoablation With Surgical Resection of Hepatic Tumors

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Only 5% to 10% of metastatic and primary liver tumors are amenable to surgical resection. Hepatic cryoablation has increased the number of patients who are suitable for curative treatment. The aim of this study was to evaluate survival and intrahepatic recurrence in patients treated with cryoablation and resection. From June 1994 to July 1999, thirty-eight surgically unresectable patients underwent a total of 42 cryoablative procedures for 65 malignant hepatic lesions. Twenty patients underwent cryoablation alone, and 18 patients were treated with a combination of resection and cryoablation, with a minimum of 18 months' follow-up. The 38 patients had the following malignancies: primary hepatocellular carcinoma (n = 8) and metastases from colorectal cancer (n = 21), neuroendocrine tumors (n = 3), ovarian cancer (n = 3), leiomyosarcoma (n = 1), testicular cancer (n = 1), and endometrial cancer (n = 1). Patients were evaluated preoperatively with spiral CT scans and intraoperatively with ultrasound examinations for lesion location and cryoprobe guidance. Local recurrence was detected by CT. Major complications included bleeding in three patients and acute renal failure, transient liver insufficiency, and postoperative pneumonia in one patient each. Two patients (5%) died during the early postoperative interval; mean hospital stay was 7.1 days. Median follow-up was 28 months (range 18 to 51 months). Overall survival according to Kaplan-Meier analysis was 82%, 65%, and 54% at 12, 24, and 48 months, respectively. Forty-eight-month survival was not significantly different between those patients undergoing cryoablation alone (64%) and those treated with a combination of resection and cryoablation (42%). Disease-free survival at 45 months was 36% for patients undergoing cryoablation plus resection compared to 25% for those undergoing cryoablation alone. Local recurrences were detected at five cryosurgical sites, for a rate of 12% overall (5 of 42), 11% (2 of 18) for patients in the cryoablation plus resection group, and 12% (3 of 24) for those in the cryoablation alone group. For patients with colorectal metastases, survival was 70% at 30 months compared to 33% for hepatocellular cancer and 66% for other types of tumors. Patients with tumors larger than 5 cm or numbering more than three did not have significantly decreased survival. Cryoablation of hepatic tumors is a safe and effective treatment for some patients not amenable to resection. The combination of cryoablation and resection results in survival comparable to that achieved with cryoablation alone. (J GASTROINTEST SURG 2001;5:206-213.)

KEY WORDS: Cryoablation, hepatic neoplasms, hepatectomy

Surgical resection remains the "gold standard" for treatment of patients with metastatic and primary liver tumors. However, only 5% to 10% are amenable to resection.[1,2] Approximately 60,000 new cases of hepatic colorectal metastases and about 18,000 new cases of primary hepatocellular carcinoma are diagnosed each year in the United States.[3,4] Thus many patients could potentially benefit from an alternative therapy such as cryoablation. With no treatment, the median survival of patients with liver malignancy is less than 1 year,[5] whereas patients with resectable disease have a 5-year survival rate of 20% to 50%.[6,7] Hepatic cryotherapy is increasingly recognized as a safe and effective alternative therapy for patients who are not suitable candidates for conventional hepatic resection. In fact, 5-year survival rates for patients treated with hepatic cryoablation have been reported to be as high as 15% to 50%.[8-11]

The use of cryoablation in combination with hepatic resection expands the use of potentially curative procedures to bilobar disease that is not readily amenable to standard surgical resection. The concept

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of combining the two procedures is appealing. Patients who might not survive an extensive hepatic resection may tolerate a lesser resection plus cryoablation, thus maximizing the remaining viable liver tissue and decreasing the overall morbidity and mortality of the procedure. In addition, patients who are not candidates for a cryoablative procedure alone, because of bilobar disease, tumor number, or anatomic location might be amenable to a combined procedure. Only one series in the literature demonstrates the feasibility of this approach, but it included only seven patients and did not report any long-term survival data.[12] Herein we report our experience in 38 patients with a variety of hepatic malignancies who underwent hepatic cryoablation either alone or in combination with resection.

MATERIAL AND METHODS

Patients

From July 1994 to July 1998, a total of 38 patients underwent 42 cryoablative procedures at the University of Wisconsin Hospital and Clinics. All of these patients were unresectable by standard liver resection techniques. The mean age was 61 ± 12 years (range 33 to 68 years), and there were 22 men (58%) and 16 women (42%). Neoplasms treated included primary hepatocellular carcinoma (n = 8) and metastases from colorectal carcinoma (n = 21), neuroendocrine tumors (n = 3), ovarian cancer (n = 3), leiomyosarcoma (n = 1), testicular cancer (n = 1), and endometrial cancer (n = 1).

Technique

Preoperative arterial and portal phase helical CT scans were performed in all patients to evaluate the extent of hepatic disease and to rule out any other metastatic foci. Patients underwent laparotomy through a bilateral subcostal incision. A complete surgical exploration of the abdomen was performed to exclude any extrahepatic disease. In the event of abnormal extrahepatic tissue or suspicious lymph nodes, biopsies were done for frozen tissue analysis. Intraoperative ultrasound was performed using a dedicated high-frequency 7.0 MHz T-shaped transducer (550-2000, Aloka, Inc., Wallingford, Conn.) to delineate all hepatic lesions. In patients undergoing a combined resection and cryoablative procedure, the resection was performed first, followed by cryoablation of the remaining lesions. Cryoablation was performed under intraoperative ultrasound guidance to confirm the location of all lesions and the relationship to major biliary and vascular structures. An 18-gauge, Teflon-coated needle was inserted into the tumor followed by a cryoprobe using the Seldinger technique. Selection of probe size was determined based on the required iceball size that would be necessary to create a 1 cm margin around the tumor. Either liquid nitrogen or argon gas units were used in this series (Cryomedical Sciences, Inc., Rockford, Md., and EndoCare, Inc., Irvine, Calif.). Cryogen was infused through the probes, creating a temperature below 160° C at the tip. Two 10-minute freeze cycles with an intervening 5-minute thaw were performed on each lesion, under direct visualization using intraoperative ultrasound, with the intent of entirely covering the lesion and a 1 cm margin. Multiple cryoprobes were placed in a single lesion when necessary to achieve this margin.

Statistical analysis

Actuarial survival was calculated according to the Kaplan-Meier method. Values are expressed as mean ± standard deviation. Variables were compared using Fisher's exact test (two-sided) or the rank-sum test when appropriate. A P value <0.05 was considered significant. Prognostic factors for survival were examined by univariate and multivariate analysis using log-rank and Cox regression models, respectively.

RESULTS

Sixty-five lesions were treated either with cryoablation alone (cryo-alone; 20 patients) or in combination with surgical resection (cryo-resection; 18 patients). Most patients (87%) had three or fewer hepatic tumors, and no patient had more than 12. The median diameter of the cryoablated metastasis was 3.1 cm with a range of 1 to 13 cm. The majority of patients (74%) had lesions smaller than 5 cm. Table I compares the characteristics of the two patient populations. The cryo-resection group tended to have larger tumors and had a greater number of metastases than the cryo-alone group. The majority of patients in both groups had metastatic colorectal adenocarcinoma.

The reasons for performing a cryoablative procedure instead of surgical resection alone are shown in Table II. All patients who were considered resectable underwent standard surgical resection alone. Involvement of major vascular structures was an indication for cryoablation when tumors involved the inferior vena cava, portal vein, or hepatic veins and could not be resected with an adequate margin. In addition, cryoablation alone was performed whenever possible. For the majority of patients in the cryo-resection group, cryoablation was added to surgical resection in patients who had unresectable bilobar disease, usually with large (>10 cm) or multiple diffuse lesions resected in one lobe followed by cryoablation of lesions

Table I. Comparison of features in patients undergoing cryoablation alone vs. cryoablation plus resection (n = 38)

	Cryo-alone (n = 20)	Cryo-resection (n = 18)
No. of metastases		
1	15	0
2	3	10
3	1	4
>3	1	4
	(median = 1)	(median = 2)
Diameter of largest lesion	1.3 – 6.3 cm	1.0 – 13.0 cm
	(mean 3.2 ± 1.6 cm)	(mean 6.5 ± 5.2 cm)
Tumor type		
Colorectal metastases	8	13
Hepatocellular	7	1
Other metastases	5	4

Table II. Contraindications to standard surgical resection in both the cryoablation alone and cryoablation plus resection groups (n = 38)

	Cryo-alone (n = 20)	Cryo-resection (n = 18)
Involving major vascular structure	8 (40%)	3 (17%)
Centrally located tumor	4 (20%)	2 (10%)
Bilobar lesions	2 (10%)	10 (56%)
Insufficient liver reserve/poor risk	6 (30%)	3 (17%)

Table III. Liver resections performed in the cryoablation plus resection group (n = 18)

Type of resection	No. of patients
Segmental resection	12 (67%)
Right lobectomy	3 (25%)
Left lateral segmentectomy	3 (25%)
Left lobectomy	2 (17%)
Right lateral segmentectomy (VI, VII)	2 (17%)
Extended right lobectomy	1 (8%)
Right anterior segmentectomy (V, VIII)	1 (8%)
Wedge resection	6 (33%)

in the opposite lobe. Patients were not excluded from the study because of the number of metastases, per se. The type of liver resection performed in the cryo-resection group is shown in Table III. The majority of patients had segmental or lobar resections (67%), whereas six patients (33%) had wedge resections. Two patients (11%) in the cryo-resection group had positive resection margins at final histopathologic analysis.

Complications

The overall complication rate was 16%. The complication rate was 10% for the cryo-alone group and 22% for the cryo-resection group (Table IV). The mean hospital stay was 7.9 ± 7.3 days for patients in the cryo-alone group and 7.3 ± 2.6 days for those in the cryo-resection group. There was one perioperative death in each group. The patient in the cryo-alone group who died had a 10 cm colorectal metastasis at the confluence of the hepatic veins, compressing all three vessels. Five cryoprobes were required to ablate it, but postoperative bleeding led to cardiovascular collapse and death on postoperative day 2. The early death in the cryo-resection group occurred in a patient with alcoholic cirrhosis who underwent resection of a left lobe hepatoma and cryoablation of a smaller lesion in the right lobe. This patient developed postoperative bleeding and died on postoperative day 1. Both of these deaths occurred early on in our experience with cryoablation.

Survival

After a mean follow-up of 28.2 ± 11 months (range 18 to 51 months), 13 patients (34%) show no evidence of disease, 10 patients (26%) are alive with recurrent disease, 13 patients (34%) have died of their disease, and two patients (5%) have died of other causes. Table V shows the breakdown for these categories comparing the cryo-alone group with the cryo-resection sub-

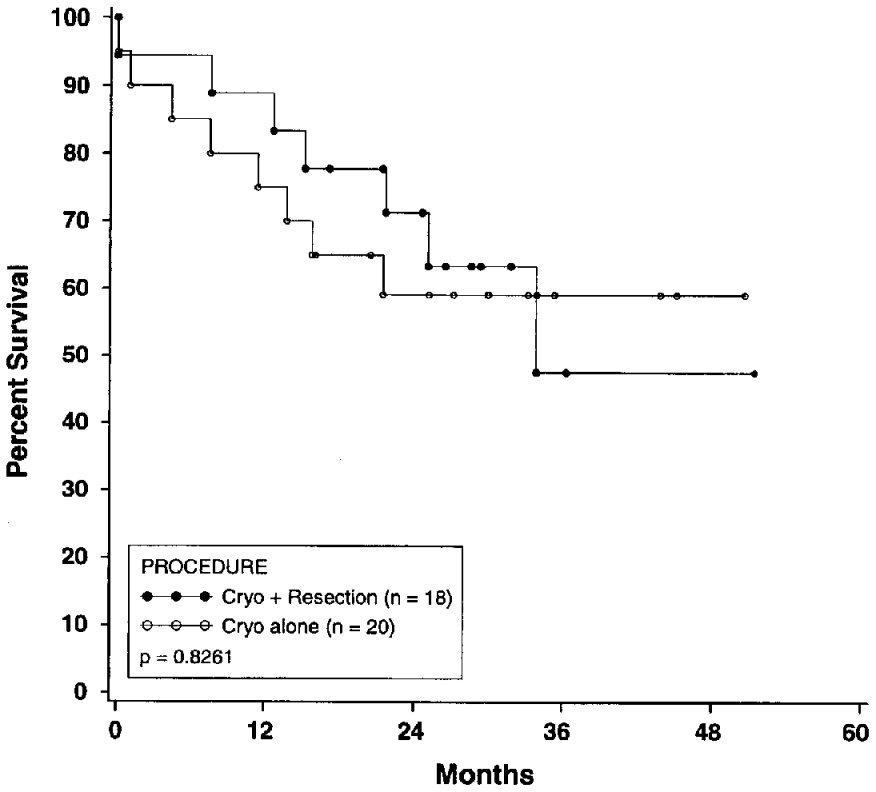

Fig. 1. Overall survival following cryoablation alone (n = 20; ○) and cryoablation plus resection (n = 18; ●) ($P = 0.82$).

Table IV. Complications and mortality for cryoablation alone vs. cryoablation plus resection

	Cryo-alone (n = 20)	Cryo-resection (n = 18)	
Bleeding	1	2	
Acute renal failure		1	
Liver insufficiency		1	
Pneumonia	1		
Total	10%	22%	$P = 0.30$
Perioperative mortality	1 (5%)	1 (6%)	$P = 0.94$

P values calculated by chi-square analysis are not significant.

set. The 12-, 24-, and 48-month survival rates for all 38 patients are 82%, 65%, and 54%, respectively. Forty-eight-month survival rates for the cryo-alone and cryo-resection groups are 64% and 42%, respectively ($P = 0.83$; Fig. 1). Disease-free survival at 48 months was 36% for cryo-resection patients compared to 25% for cryo-alone patients ($P = 0.49$; Fig. 2). Survival curves for patients according to tumor type are shown in Fig. 3. For patients with colorectal metastases, survival was 70% at 30 months compared to 33% for hepatocellular cancer and 66% for other types of tumors ($P = 0.10$).

Table V. Survival and disease status of cryoablation alone vs. cryoablation plus resection

	Cryo-alone (n = 20)	Cryo-resection (n = 18)
No evidence of disease	7 (35%)	6 (33%)
Alive with disease	5 (25%)	5 (28%)
Died of disease	7 (35%)	6 (33%)
Died of other causes	1 (5%)	1 (6%)
Survival		
12 mo	75%	83%
24 mo	60%	71%
48 mo	60%	47%

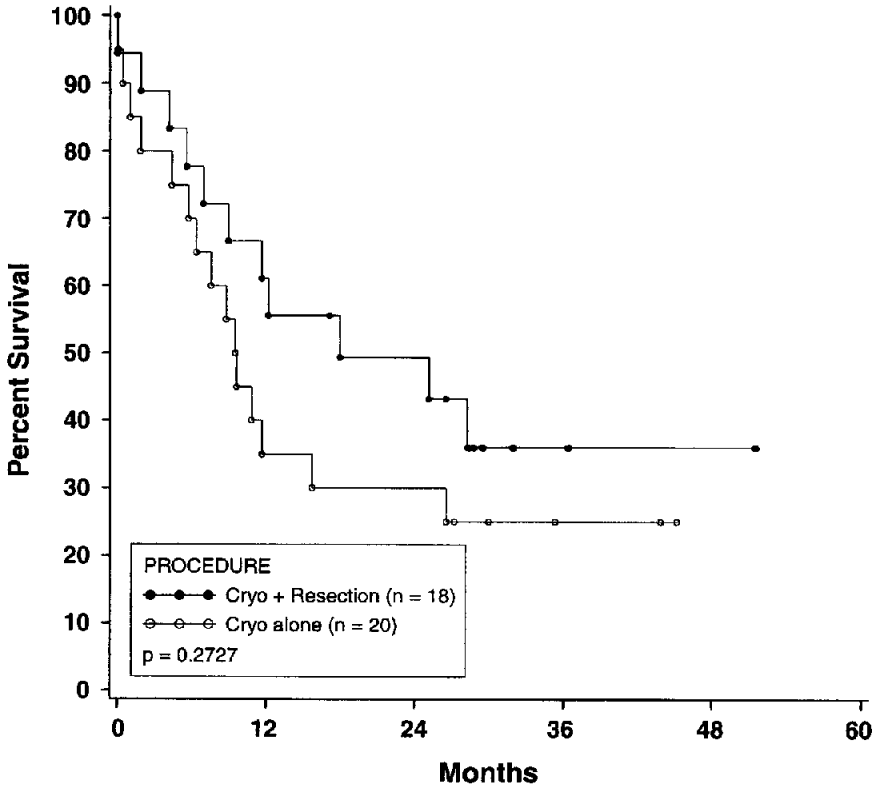

Fig. 2. Disease-specific survival following cryoablation alone (n = 20; ○) and cryoablation plus resection (n = 18; ●) ($P = 0.27$).

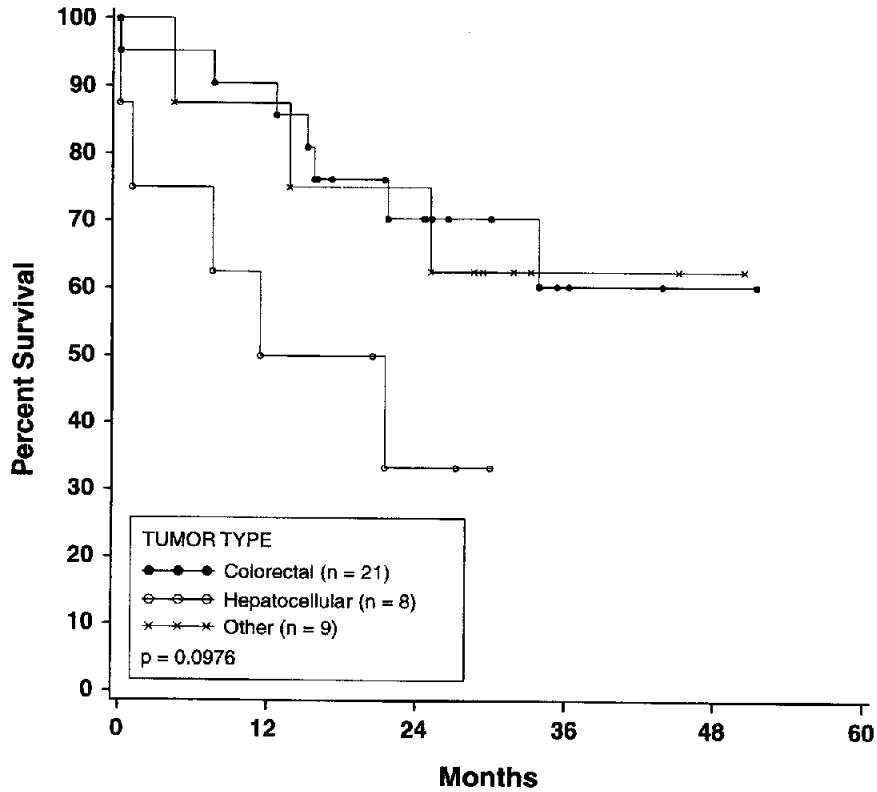

Fig. 3. Survival in patients according to tumor type: Colorectal (●), hepatocellular (○), and other tumor types (X) ($P = 0.10$).

The overall local recurrence rate in the cryoablative sites was 12%. The local recurrence rate per lesion was 9%. The local recurrence rates were similar in the cryo-alone (12%) and cryo-resection (11%) groups. Of note, there were two patients in the cryo-resection group who had a recurrence at the resected margin, separate from the cryoablative site, for a surgical resection local recurrence rate of 11%.

Age, tumor type, number of liver metastases, and size of liver metastases had no demonstrable impact on survival. Patients who had local recurrences were three times more likely to die of their disease.

DISCUSSION

The present study evaluates the effect of cryoablation of hepatic malignancy when used in conjunction with a resective procedure and compares this approach to cryoablation alone. Surgical resection has long been the treatment of choice for patients with liver malignancy. Resection is safe and effective[6,13]; however, if tumors are present near the hepatic hilum or within both lobes of the liver, patients will often be deemed unresectable. The combination of cryoablation with a resective procedure permits the preservation of as much functioning hepatic tissue as possible, reducing the scope of resection. For example, a patient with multiple lesions in the right lobe and a solitary lesion in the left lobe would not be a good candidate for either cryoablation alone or a standard resection. However, the combination of a right hepatic lobectomy and cryoablation of the lesion in the left lobe could potentially result in long-term survival. The preservation of hepatic parenchyma is especially relevant in the setting of hepatocellular carcinoma and a cirrhotic liver, where patients are often excluded from resection because of limited hepatic functional reserve.

The use of cold temperature to treat tumors was first described by Arnott in 1945.[14] The first use of cryotherapy for ablation of liver tumors was described by Cooper[15] in 1963. The addition of intraoperative ultrasound was a crucial advancement in the technique, allowing for more accurate monitoring of cryotherapy, as well as the ability to freeze deep parenchymal tumors.[16-18] The mechanism by which cold temperatures induce necrosis of tumor cells is through localized freezing of tissue as well as microvascular thrombosis of surrounding vessels.[19-21]

Cryoablation of liver tumors effectively controls local hepatic disease.[22,23] Early studies demonstrated a 5-year survival of 62% in 24 patients with hepatic colorectal metastases who underwent cryoablation after being deemed unresectable.[10] Cryoablation has been compared to resection in one prospective randomized trial.[9] A higher survival rate was seen in the patients treated with cryosurgery than those treated with standard resection (44% vs. 36% at 5 years and 19% vs. 8% at 10 years). Other groups have reported 24-month survival rates of 63% and 52% for 59 patients with hepatocellular carcinoma and hepatic colorectal metastases, respectively. The incidence of local recurrence has varied widely from the 44% in a cohort of patients with colorectal metastases[24] to the 10% to 15% rates reported in other series.[10,25,26]

In the present study the survival rate in the cryo-alone group was slightly higher than that in the cryo-resection group, although this difference is not statistically significant. The cryo-resection group tended to have multiple hepatic lesions of greater size, which necessitated the combined procedure. Interestingly, 39 patients who had conventional resections for hepatic malignancy during the same time period at our institution had a 4-year survival rate of 68%, which was not statistically different from the 59% and 51% 4-year survival rates seen in the cryo-only and cryo-resection groups, respectively. This is despite a bias toward longer survival in the patients undergoing standard resections, who in general had less extensive disease and better performance status. To examine the effect that multiple pathologic diagnoses may have played, both overall and disease-free survival rates were analyzed according to tumor type, and no statistical differences were seen ($P = 0.10$ and 0.50, respectively). It is difficult to draw definitive conclusions comparing the groups because of the inhomogeneous patient populations, but the actuarial survival rates of patients undergoing cryoablation in this study are similar to, if not better than, those of historical controls for standard resection. The suggestion is not that cryoablation is equivalent to surgical resection, but rather that it is a viable option for patients who are considered unresectable. Cryoablation of hepatic tumors represents the only therapy other than standard resection that has been demonstrated to provide long-term survival, albeit mostly in smaller, nonrandomized trials. Other methods of treatment for unresectable hepatic malignancies, such as radiofrequency ablation, chemoembolization, and percutaneous ethanol injections, have generally higher local recurrence rates than we report for cryoablation.[27]

Survival was analyzed according to tumor type, and we found no statistical difference between patients with colorectal, hepatocellular, or other types of tumors. However, there did appear to be a trend toward decreased survival in the group with hepatocellular carcinoma, although this population of patients was small ($n = 8$), making it difficult to adequately assess this subset of patients. Additionally, only one patient was found in the combined cryo-resection group, and this patient was also one of our early operative deaths. The small number of patients with hepatomas com-

pared to colorectal metastases (n = 21) in this study is in line with the greater than three times higher incidence of colorectal metastases in the US compared to hepatocellular carcinoma. Others have specifically investigated cryoablation in hepatocellular carcinoma, and in larger series of more than 50 patients, 5-year survival rates of 27% to 64% have been reported.[28,29]

Prevention of local recurrence of tumor at the ablation site is a critical indicator of the success of cryoablative therapy. A local recurrence in a cryoablated lesion would be the equivalent of a positive margin on a resected liver specimen, which has been demonstrated to have a negative impact on survival.[6] One published report of cryoablation of hepatic tumors found that incomplete cryoablation and a lesion size of less than 3 cm were adverse prognostic indicators.[24] Others have found that the percentage of hepatic replacement by tumor and the presence of extrahepatic disease had a negative impact on survival.[30] In our series we did not find the size of lesions, number of lesions, or tumor type to be prognostic factors. Those patients who did have local recurrences were three times more likely to die of their disease, however. Our local recurrence rate was equivalent to the 10% to 20% reported by others,[1,23] and represents an aggressive policy of detecting recurrences on serial CT scans and performing repeat biopsies and repeat recryoablation in lesions that recur locally. Local recurrence rates within the combined procedure group were equivalent for surgical resection (11%) and cryoablation (11%). Although this is a small series and represents our initial experience with cryoablation, the data indicate that the size of the lesion and the number of lesions may not be contraindications to cryoablation or cryo-resection.

From a technical standpoint, cryoablation is performed in conjunction with a radiologist, who uses intraoperative ultrasound to guide probe placement within the tumor and monitor iceball size. Intraoperative ultrasound allows for two-dimensional imaging of probe placement; however, we employ a transverse puncture technique where the transducer is held perpendicular to the probe to allow for a three-dimensional perspective. In addition, we often use multiple probes to allow overlapping iceballs to completely freeze tumors in addition to reducing microvascular blood flow to the tumor, although we have learned that cryoablating lesions larger than 10 cm greatly increases the incidence of liver cracking and hemorrhage. Cryoablation at our institution is used only by a limited number of surgeons and radiologists who have a specific interest in and expertise with the technique.

The current report represents our early experience and demonstrates that a combined resection and cryoablative procedure can be performed with minimal morbidity and mortality and may result in long-term survival. Since the completion of this trial, we have performed approximately 50 additional cryoablative procedures with no further deaths. By providing an effective therapy for patients who are unresectable and not suitable candidates for cryoablation alone, the combination of cryoablation with resection expands the pool of patients with potentially curable liver disease. This combination is as effective as cryoablation alone and does not adversely affect the local recurrence rate or the incidence of postprocedure complications.

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Discussion

Dr. A. Bilchek (Los Angeles, Calif.). This is a very heterogeneous group of patients, and I am not sure how much we can make of the survival data. How did you select the patients with noncolorectal metastases to undergo surgery? Second, how did chemotherapy factor into this? Did any patients with colorectal metastases receive regional chemotherapy?

Dr. C.H. Cha. None of our patients had regional chemotherapy. That is something we are currently investigating at our institution. Any patient who is considered resectable with colorectal metastases or any other type of metastases underwent a standard resection, so all of the patients in our series were considered unresectable. In regard to the heterogeneous nature of the patients, there was no significant effect on survival by tumor type.

Special Problems in Minimally Invasive Surgery of the Foregut: Part I

COURSE COCHAIRS: L. William Traverso, M.D. (SSAT Representative), Virginia Mason Medical Center, Seattle, Wash.; and Bruce Schirmer, M.D. (SAGES Representative), University of Virginia Health Sciences Center, Charlottesville, Va.
MODERATORS: Bruce Schirmer, M.D., University of Virginia Health Sciences Center, Charlottesville, Va.; Carlos A. Pellegrini, M.D., University of Washington Medical Center, Seattle, Wash.; and Tom R. DeMeester, M.D., University of Southern California Medical Center, Los Angeles, Calif.

Introduction

The focus of this year's annual SSAT/SAGES Joint Symposium was on special problems in laparoscopic foregut surgery. The symposium chose to focus not on the routine antireflux operation performed for gastroesophageal reflux disease (GERD), but instead on those operations and procedures used in the treatment of achalasia, recurrent GERD, paraesophageal hernia, and other less frequently occurring conditions. These situations, however, require an increased amount of expertise to successfully manage patients both perioperatively and operatively. A panel of such experts was assembled to provide those attending this symposium with the benefit of their collected experience with these problems. Finally, but chronologically first, the symposium included a section on new tools for the gastrointestinal surgeon, focusing on hemostatic tools and ultrasound, both of which are important to the gastrointestinal surgeon operating in the upper abdomen. Summaries of these presentations follow. Each individual work was edited by the moderators of the session, whose names are listed above. A unique feature of the moderators' role in this year's session was their summary comments concerning the area and the presentations to help put the information into perspective.

The Course Cochairs wish to thank the authors for their excellent presentations and summaries, as well as the moderators for their editing efforts. Finally, I wish to thank Dr. Traverso for doing the lion's share of the organizing of this symposium.

New Tools or Devices for the Gastrointestinal Surgeon

The two articles encompassing this topic address two new types of technology that the gastrointestinal surgeon likely has encountered only within the past decade. There are also likely a significant number of gastrointestinal surgeons who have no experience with several of the new technologies discussed. Clearly these tools are important for the current practice of gastrointestinal surgery, particularly minimally invasive gastrointestinal surgery.

Dr. Fried's article discusses three different types of devices for achieving hemostasis during both open and laparoscopic surgery. Although bipolar coagulation has been available for many years, general and gastrointestinal surgeons have not adopted this tool efficiently into their practices in general. The ultrasonic scalpel has become a standard tool in the practices of many laparoscopic general surgeons within just a few years. It can be used as an effective dissecting instrument as well as for rapidly and efficiently dividing tissue containing small to medium-sized vessels. More recently introduced, the LigaSure sealing system provides the maximum in hemostatic properties of the three technologies, but is not rapid to use. It behooves gastrointestinal surgeons, particularly those performing minimally invasive gastrointestinal surgery, to become familiar with all three of these technologies, as all have a role in various operations. However, knowledge of their properties and experience should also result in a limitation of the use of

Presented at the Forty-First Annual Meeting of The Society for Surgery of the Alimentary Tract, San Diego, Calif., May 21-24, 2000.

only one energy source for hemostasis during a procedure, thus minimizing any increase in the cost of these new technologies over standard monopolar cautery, which is still the most popular modality used.

The article by Dr. Soper on laparoscopic ultrasound succinctly describes both the essentials of ultrasound technology, as well as indications for and results achieved with laparoscopic ultrasound in the current practice of minimally invasive gastrointestinal surgery. Any surgeon who practices any form of minimally invasive oncologic abdominal surgery should have, by now, realized the benefit of this technology in staging and assessing various tumors of the abdominal viscera. This includes hepatic scanning to screen for liver metastases during laparoscopic-assisted colon resection. Dr. Soper also presents a convincing case for incorporating ultrasound as a complementary procedure to fluoroscopy to scan the common bile duct for stones during laparoscopic cholecystectomy. Although the technology may seem daunting to novice surgeons, and the cost may be a deterrent to others, the applicability of ultrasound to other realms of their practice and the relatively rapid learning curve for learning normal operative anatomy are more convincing reasons why all practicing gastrointestinal surgeons should learn to use this important technology. Ultrasound is an important diagnostic and therapeutic tool that is used routinely by surgeons in Europe and Asia, and should no longer be considered by surgeons in America to be strictly within the realm of our radiologist colleagues.

Bruce Schirmer, M.D.

Hemostatic Tools for the Gastrointestinal Surgeon: Ultrasonic Coagulator vs. Bipolar Ligation

Gerald M. Fried, M.D.

Gastrointestinal surgeons have traditionally relied on several different tools to control bleeding during surgery. These include vessel ligation, hemostatic clips, and monopolar electrosurgery. These tools have provided reasonably reliable and secure means of controlling bleeding. In some areas of the abdomen, or during laparoscopic approaches to gastrointestinal disease, traditional means of securing hemostasis may be technically difficult, unreliable, or inefficient. It is in these circumstances where novel energy sources are particularly useful in providing secure hemostasis. Two of the more exciting new technologies include the ultrasonic coagulator and bipolar devices that weld the vessel wall closed or bipolar devices with incorporated cutting blades.

Traditional Hemostatic Options

Vascular control with ties or sutures provides secure hemostasis and is a part of every surgeon's armamentarium. The limitation of these "low-tech" devices is that they are difficult to use in laparoscopic surgery and in areas of the abdomen such as deep in the pelvis or up under the diaphragm. In these regions visualization is difficult and it may be hard to place the knot carefully. Hemostatic clips are quick and easy to apply, but they are less secure and can be easily dislodged as dissection continues. For every vessel controlled by ligatures, multiple steps are required; these include application of hemostatic clamps, placement of ligatures for proximal and distal control, tying of the ligatures, dividing the vessel or tissue, and then cutting the sutures. For clips, this requires at least three steps—clip proximally, clip distally, and divide.

Monopolar electrosurgical devices are attractive since in most cases the tissue can be coagulated and divided in a single action. This energy source can be conducted through a variety of instruments of varying shapes designed for specific needs. Despite the convenience of this technology, it is associated with certain risks related to electrical burns, and the hemostatic security is unreliable. It is certainly not appropriate for medium-sized or large vessels.

New Technologies: Goals

The goals of any new technology to achieve hemostasis are to improve safety and efficiency, and to provide greater security of hemostasis than currently available methods. In addition, such instruments should work effectively in areas where exposure is limited. In these areas, control of active bleeding is particularly difficult, so the goal is to prevent bleeding by achieving secure hemostasis. The advent of laparoscopy has provided additional challenges to both the surgeon and the engineer.

Properties of the Ideal Hemostatic Tool

An ideal hemostatic device could be used to grasp, dissect, coagulate, and divide tissue. It would be ergonomically comfortable and easy to operate, and would provide feedback to the surgeon when hemostasis has been secured. It would cause little collateral damage as a result of the energy imparted to tissue. It would also be available in a variety of configurations for both open and laparoscopic surgery. Unfortunately, no such single device is currently available. Surgeons must choose between a variety of technologies depending on the properties most important in the conduct of a particular procedure.

Ultrasonic Coagulator Technology in Surgery

The ultrasonic coagulator (Harmonic Scalpel LaparoSonic Coagulating Shears, Ethicon Endo-Surgery, Inc., Cincinnati, Ohio; AutoSonix Ultra Shears, United States Surgical, Norwalk, Conn.) is a device that provides hemostasis during surgery using ultrasonic energy. It allows grasping, coagulation of

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small to medium-sized vessels, and cutting in a single device. Because it uses ultrasonic energy rather than electrical energy to achieve its effects, leakage of electrical current is not a risk with this technology. Handpieces are available for both open and laparoscopic surgery in a scissors-like and a hooked blade configuration. The laparoscopic handpieces are available in 10 mm and 5 mm diameters.

Electrical energy from the ultrasonic coagulator generator is converted to mechanical energy at the handpiece. One blade vibrates at 55,500 Hz. The excursion of the moving blade can be controlled and varies from 50 to 100 microns per stroke. The blade motion couples with tissue protein. This causes the protein hydrogen bonds to break and the protein to disorganize. The resultant protein coagulum seals small coapted vessels. Secondary heat produced by the vibrating protein results in deeper coagulation and seals larger coapted vessels.

There are several advantages of ultrasonic coagulator technology in surgery. These include precise cutting and coagulation, minimal lateral tissue damage, no smoke, and no electrical energy being transferred to or through the patient. This device provides reliable hemostasis for vessels up to 3 mm in diameter. The effects of the ultrasonic coagulator on tissue depend on the power setting, the sharpness of the blade edge, the grip force, and the tissue tension or torque. Thus there is a definite learning curve associated with this device and the effectiveness is somewhat operator dependent.

In summary, the ultrasonic coagulator is a convenient grasping and dissecting tool that can coagulate and divide tissue with good hemostasis for small to medium-sized vessels. Appropriate instruction on its use is essential to provide reliable hemostasis.

LigaSure Bipolar Vessel Sealing System

The LigaSure Vessel Sealing System (Valleylab, Boulder, Colo.) is a hemostatic device that compresses the vessel and uses bipolar energy to seal the vessel. It provides an audible signal when the vessel is reliably sealed. In addition, there is a visible area where the vessel wall is sealed. This area is often translucent and marks the area where the vessel can be divided.

The principle of the LigaSure is that bipolar energy is used with instant electronic feedback control. The heat generated by the bipolar energy melts the collagen and elastin of the vessel wall, which then reforms as a sealed vessel. This differs from traditional bipolar devices because it does not depend on thrombus to obliterate the lumen of the vessel.

The clinical benefits of the LigaSure include the ability to reliably control blood vessels up to 7 mm in diameter without the need to dissect vessels out of tissue bundles. There is little lateral spread of heat or electrical energy. There is both visual and auditory confirmation that hemostasis has been secured before the vessel is divided. This "automation" implies that the learning curve is short and it is less operator dependent than other hemostatic tools. It is an ideal tool to control vessels in the mesentery of the small or large bowel. Currently it is the most effective energy source to reliably control medium to large vessels (up to 7 mm).

The LigaSure device does not divide tissue. A separate instrument must be used to cut tissue once the LigaSure has sealed the vessels. This extra step may diminish the efficiency of the device somewhat, especially in laparoscopic surgery, unless scissors are used through a separate trocar.

Bipolar Cutting Instruments

Bipolar coagulation has been available for many years and has been widely used by gynecologists, neurosurgeons, and other surgical specialists. Despite this, gastrointestinal surgeons have underutilized this technology. The principle of bipolar electrosurgery is that current passes between electrodes rather than through the patient. It uses two parallel poles or electrodes in proximity. Low voltages are effective, and hemostasis is very good. Lateral thermal spread is minimal and little smoke is created. Bipolar electrosurgery avoids the hazards of monopolar devices, because energy does not travel through the patient. Traditional bipolar coagulators do not divide tissue.

Bipolar cutting instruments have been developed to take advantage of the hemostatic properties of bipolar energy (BiCOAG Bipolar Cutting Forceps, Everest Medical, Minneapolis, Minn.). These devices incorporate a retractable cutting blade between the poles of the bipolar coagulator electrodes. Once the tissue between the jaws of the instrument has been coagulated, the cutting blade is advanced manually to divide the tissue. The design of the bipolar cutting device is somewhat limited. Currently the bipolar cutting devices are available with only straight grasping jaws and are primarily intended for laparoscopic surgery. The device is available in 5 mm diameter. It provides good hemostasis, but its design is not ideal for dissection and only fair for grasping tissue. There is no clear feedback when hemostasis has been achieved. The surgeon must rely on indirect cues, so there is a learning curve before surgeons are comfortable dividing tissues without opening the forceps and inspecting the coagulated tissue. Laparoscopic bipolar scissors are also available, but these are best applied to division of tissue where control of small vessels is necessary.

Comparison of the Three Devices

Little objective data are available for comparing the three hemostatic devices. Each has its own benefits and drawbacks. In purely personal subjective experience, the best hemostasis is achieved with bipolar devices. The LigaSure provides the reliability of auditory and visible evidence of hemostasis, but lacks a cutting component. It has the shortest learning curve. The bipolar cutting device is the least expensive. It provides very good hemostasis. It is efficient for coagulating and cutting but its design is somewhat awkward for dissecting and grasping, and there is no clear indication of when hemostasis has been reliably achieved. The ultrasonic coagulator is currently the most versatile instrument. It is well designed for dissection and grasping. It provides reasonable hemostasis but is limited to reliably controlling vessels up to 3 mm diameter.

One randomized controlled trial compared the use of the harmonic scalpel and the bipolar cutting forceps to divide the short gastric vessels in 86 patients during antireflux surgery.[1] Outcomes evaluated were operative time, bleeding, complications, equipment problems, and surgeon satisfaction. No significant differences were measured. The cost per case was less with the bipolar cutting forceps.

The harmonic scalpel was evaluated in comparison with monopolar electrosurgery for mucosal proctocolectomy and ileal J-pouch anal anastomosis in 74 patients with ulcerative colitis or polyposis.[2] The operative time and blood loss were significantly reduced in the group using the harmonic scalpel.

Summary

Excellent hemostatic tools are now available for the gastrointestinal surgeon. Each technology has its benefits and indications. Knowledge of these technologies can improve efficiency and outcomes for both open and laparoscopic procedures. There is a learning curve associated with the use of these devices, and proper training and understanding of the technology will result in safer and more effective results.

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Laparoscopic Ultrasound for the Gastrointestinal Surgeon

Nathaniel J. Soper, M.D.

Ultrasound Physics and Instrumentation

Ultrasonography may be performed using both extracorporeal and intracorporeal techniques. Miniaturization of ultrasound transducers opened the way for the development of both flexible endoscopic and laparoscopic imaging methods. This union of endoscopic and ultrasound technology has expanded the potential for clinical application of ultrasonography, which may grow in importance as laparoscopic surgical techniques expand.

Ultrasound consists of mechanical sound waves that propagate through a medium. Medical ultrasound waves oscillate at frequencies ranging from 1 to 30 megahertz (MHz-millions of cycles per second). The velocity of ultrasound waves depends on the medium in which they are propagated. Soft tissue of the human body (e.g., water) is an excellent medium for ultrasound transmission.

Ultrasound images are produced using the pulse-echo principle of sonar and radar. A transducer transmits and then receives the ultrasound waves (pulses) reflected back to it. During passage through tissue, some of the ultrasound pulse is lost (attenuated) by absorption and scattering. A portion of the energy (called an "echo") is reflected back to the transducer, which measures the time since transmission and the amplitude of the received echo. Energy attenuation is dependent on tissue impedance, a factor related primarily to tissue density. An interface is the boundary between two media with differing impedances. Ultrasound waves are either transmitted through, reflected, or refracted at an interface.

The amplitude of the ultrasound echo received back by the transducer is represented by shades of gray on a video monitor. Specular echoes arise from large reflectors in the body and have large amplitudes. Diffuse echoes originate from small point reflectors and have low amplitudes due to scatter of the ultrasound pulse. Specular echoes define boundaries of large organ masses. Focal disease (stones, cysts, surgical clips) creates echo amplitudes higher (hyperechoic) or lower (hypoechoic) than the surrounding tissue.

The ultrasound transducer produces a planar, two-dimensional image that may be transverse, longitudinal, or oblique in orientation. The active element inside a transducer is a piezoelectric disk. Electric voltages are converted into changes in the disk thickness resulting in ultrasound waves. When an echo is received back at the disk, changes in disk conformation caused by the incoming echo produce electrical voltage changes that are proportional to the pressure amplitude of the echo. Modern ultrasound transducers are capable of producing 30 frames (images) per second and can thus present anatomy in "real time."

Several transducer configurations are used for clinical real-time ultrasonography. The simplest is the mechanical sector scanner in which a single element is oscillated to and fro. Alternatively, several elements can be mounted on a wheel that rotates around an axis. Such transducers produce a field that is sector (pie) shaped with a limited field of view. Multiple transducers may also be configured in longitudinal arrays of single elements. These linear-array transducers use groups of neighboring elements to produce parallel beams by firing and receiving sequentially along the array producing a rectangular image. Bending the transducer array onto a convex surface increases the field of view. Phased-array transducers consist of transducer heads constructed with a number of elements placed side by side. Slight time delays, or phase differences, allow for control of the direction of the resultant ultrasound beam. A small stationary transducer head may thus be used to view a large anatomic area or to avoid some anatomic acoustic obstructions.

Small parts scanners produce images of limited depth for visualization of specific structures. Limited requirements for depth penetration permit the use of higher frequencies, which enhances resolution and image detail. Intraoperative ultrasonography places transducers in close proximity to the area of interest. Such scanners are designed to incorporate the benefits associated with small parts scanners.

Most medical ultrasound techniques employ the same method of collecting anatomic information. Vari-

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ation is found only in the way the acoustic beam is directed into the body or in the presentation of the signals on a display. Real-time, or B-mode imaging, is a method of displaying the amplitude of an echo by varying the brightness of a dot on an image to correspond to the echo strength. B-mode imaging is the most common display technique used clinically. Real-time processing of images is possible because of the high speed of ultrasound transmission in soft tissue. More than 25 images may be generated per second. Below this rate of processing, the image will appear to flicker. B-mode imaging allows the observation of motion of organs within the body and allows the scan plane to be changed quickly in order to scan through anatomy.

Doppler ultrasound measures the dependence of the observed frequency of sound reflected to the transducer on the motion of the source of the reflection. The observed frequency of the sound reflected from flowing fluid, such as blood, is higher if the blood is moving toward the receiver than when flow is moving away. Two transducer elements are necessary for Doppler imaging. One continuously generates ultrasound waves that a second one receives. The combination of Doppler ultrasound and B-mode ultrasound scanning is called duplex imaging. Regions of blood flow are depicted either by changes in audible tone of pulses or by "color-flow imaging" techniques with red representing movement of blood toward the probe and blue representing movement away from the probe.

Ultrasound is a useful, inexpensive, and safe imaging method. The accuracy and utility of extracorporeal, flexible endoscopic intraluminal, and conventional intraoperative ultrasound examinations performed at laparotomy have been established. New applications of intraoperative ultrasonography at laparoscopy and thoracoscopy are currently being evaluated. Laparoscopic ultrasound (LUS) can be used to supplement or obviate cholangiography during cholecystectomy, to more accurately stage patients undergoing laparoscopic evaluation for malignant disorders, and for direct tissue sampling and treatment such as cryotherapy. The application of ultrasound to laparoscopy—a surface imaging modality—should extend the abilities of surgeons during laparoscopic operations. LUS allows the laparoscopic surgeon to "see beyond the visible surface." Also, by using a transducer that is placed directly on the area of interest, a higher frequency transducer with less penetration but greater resolution can be used. Therefore LUS should be more accurate than ultrasonography performed across the body surfaces.

LUS can be used for many purposes. It is particularly helpful for assessing the parenchyma of solid organs (such as the liver, spleen, kidney, or adrenal) and assessing retroperitoneal structures, and may be useful for identifying abnormalities in the walls of hollow viscera. However, we have found the greatest utility of LUS to be for screening the common bile duct during laparoscopic cholecystectomy and, combined with staging laparoscopy, to improve the staging of patients with hepatobiliary or pancreatic cancers prior to laparotomy.

For assessing the common bile duct during laparoscopic cholecystectomy, LUS visualizes the duct in virtually all patients in 10 minutes less time than cholangiography, at a savings of $150. Sensitivity for demonstrating ductal stones is greater for ultrasound than for cholangiography, and dissection-pertinent ductal anatomy is well assessed. The "learning curve" for LUS assessment of the common bile duct encompasses approximately 20 cases. However, detailed anatomic information, particularly of the proximal ducts, is better obtained by cholangiography. In our hands, the combination of LUS and staging laparoscopy decreased the incidence of nontherapeutic laparotomies for patients with hepatobiliary or pancreatic malignancies to approximately 4%.

The American College of Surgeons has realized the importance of ultrasound to practicing general surgeons and recommends exposure to ultrasound techniques and imaging to all surgical trainees. The American College of Surgeons currently offers a course in basic ultrasound (physics and technology), as well as "advanced" modules including one for abdominal and laparoscopic imaging.

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