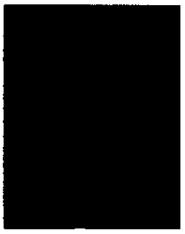
The Challenge of Success



Andrew L. Warshaw, M.D.

The following presentation will consist of personal ruminations, quite literally "what I thought about on my vacation." Having gone through the obligatory routine of considering previous presidential addresses, I have in the end been inspired by a recent book on climbing Mount Everest: the problem of getting to the top is not to be underestimated, the planting of the flag on a summit is a worthy achievement, but the greatest challenges are yet to come. As we stand together on this pinnacle today, let me touch on elements of the successful ascent by surgery, by The Society for Surgery of the Alimentary Tract (SSAT), and by surgical gastroenterology—and consider with you some challenges we face in getting on. I should add that the challenge of preparing this address is the one that caused the most stress. Continuing my analogy, you have greatly honored me by helping me to ascend this podium, and I hope I can get safely back down without frostbite.

I have been blessed with a succession of research fellows and colleagues who have made many important contributions to this Society and to surgical gastroenterology. They have metastasized and continued to prosper around the world. It is part of my job to support them and their followers, but the obstacles are increasing: The current environment of managed care, economic downsizing, decreasing reimbursement, and increasing administrative detail conspire to erode the academic mission by undermining its economic base and leaving too little time for investigation and teaching. Our leaders must stand strong for the importance of our mission or tomorrow's medicine and surgery will have risen no higher than today's level.

As an officer of the SSAT, I have been given the opportunity to help plot the course of the world's most outstanding organization devoted to gastrointestinal surgery, to keep it growing, vibrant, and representative of both academic surgical gastroenterologists and the major component of the practice of 20,000 general surgeons in North America and many more around the world. We truly represent "the guts of surgery." In this context it is of paramount importance to fortify our communication and interaction with medical gastroenterology, endoscopy, and diagnostic and interventional radiology. That scientific and clinical interaction is emblematic of Digestive Disease Week (DDW) but becomes an everyday reality in the evolving nature of care of gastrointestinal disorders.

In 1900, as a boy growing up in Albany, New York, my father decided to become a doctor, and eventually a general surgeon, because he thought it grand that the local physician made house calls in a horse and buggy. How far we have come since those horse-and-buggy days is exemplified by the life of one of my professional fathers, Dr. Claude E. Welch, a surgeon at my institution and president of this Society in 1966 (Fig. 1). Writing in his wonderful autobiography, "A Twentieth Century Surgeon,"[1] Welch (1906-1996) dated his own calling to trips to farms around his native Stanton, Nebraska, with the town veterinarian and to his part-time job tending the local pharmacy. He finished Harvard Medical School in 1932 and trained in surgery at Massachusetts General Hospital, beginning an association that was to last 64 years. In 1937 he was hired to assist Dr. Arthur W. Allen (Fig. 2), one of the master abdominal surgeons of this century, who more than any other person became Dr.

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Reprint requests: Andrew L. Warshaw, M.D., Massachusetts General Hospital, 55 Fruit St., WHT506, Boston, MA 02114-2696.

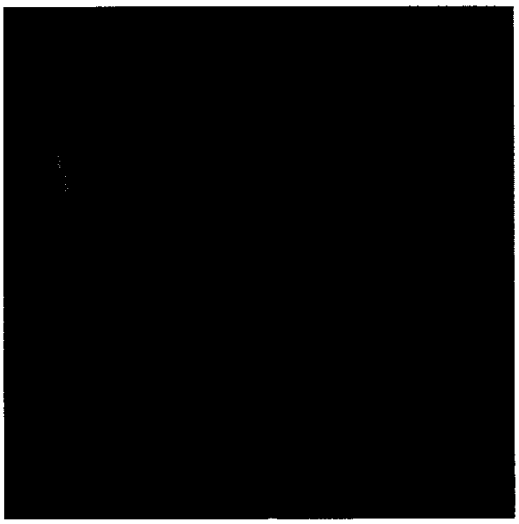


Fig. 1. Claude E. Welch, M.D.

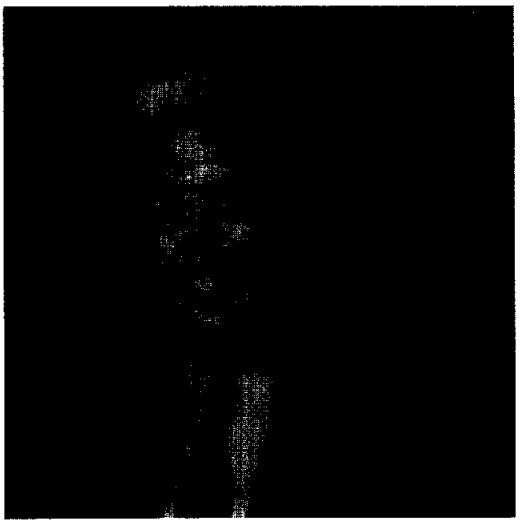


Fig. 2. Arthur W. Allen, M.D.

Fig. 3. Edward D. Churchill, M.D.

Welch's role model and, in the old apprentice system, his principal source of surgical wisdom. In 1942, Dr. Welch went to war in North Africa and Italy as a surgeon with the Sixth General Hospital (the Massachusetts General Hospital unit). Here he came into further contact with another leader in surgery at Massachusetts General Hospital, Dr. Edward D. Churchill (Fig. 3), then the Army's Surgical Consultant in the Mediterranean theater and later the first full-time academic chief of surgery at Massachusetts General Hospital. Dr. Churchill at this time was applying the lessons of military wounds to advances in surgical care that influenced civilian practice for decades, including the use of delayed primary closure for contaminated wounds and colostomy to safeguard complex colon injuries. In Dr. Welch's pantheon Dr. Churchill, the surgical scientist, complemented Dr. Allen, the brilliant surgeon.

Upon his return after the war, Dr. Welch resumed a rapidly growing practice of general surgery. In 1945 he performed the first vein graft at Massachusetts General Hospital, replacing a damaged popliteal artery with a segment of femoral vein. He became known as a bold and skillful surgeon in the abdomen. Like many surgeons of his era, he was fond of making drawings to illustrate his craft (Fig. 4). By his own assessment, his principal contributions were the introduction of catheter duodenostomy to make ulcer surgery safer, the advocacy of more definitive operations for complex cases of intestinal obstruction and fistulas, and the recognition and treatment of "high-output respiratory failure" associated with peritonitis. He said "a surgeon must be able to do many things, but first and foremost he must be able to operate."[1] In 1981 his surgical stature was recognized as he was called to Rome to consult with the surgeons treating Pope John Paul II, who had been shot twice in the abdomen. A letter of thanks from the Pope was one of Dr. Welch's most treasured mementos (Fig. 5).

As a surgical statesman, Dr. Welch, while serving as chairman of the Massachusetts Delegation to the American Medical Association (AMA) House of Delegates in 1968, led the fight for racial equality in all state medical associations. He proposed that state medical societies be expelled from the AMA if they failed to comply with the egalitarian principles of the United States Constitution. The Massachusetts Board of Registration in Medicine, during Dr. Welch's tenure as Chairman in the 1970s, ruled that physicians could not refuse to treat welfare patients just because they were on Medicaid, that women were entitled to full information about the alternative treatments for breast cancer, and that continuing medical education be required for licensure.

In his presidential address to the SSAT in 1966, entitled "The Maintenance of Excellence,"[2] Dr. Welch

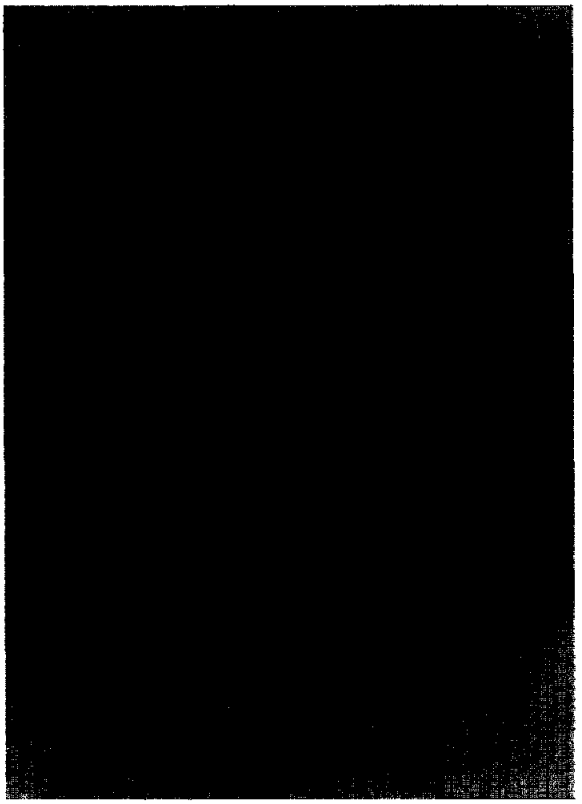
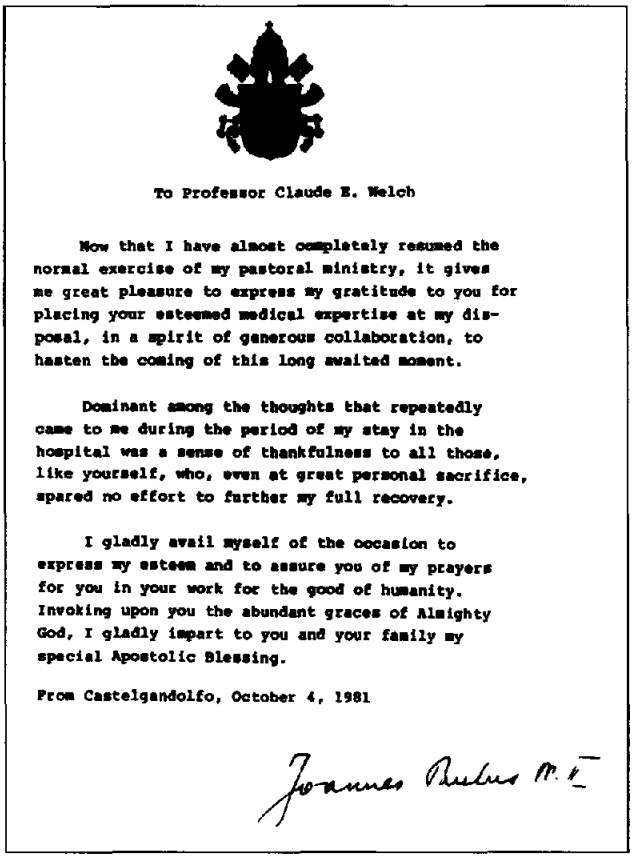


Fig. 4. Welch's drawing of the "Tanner 19" operation for alkaline reflux gastritis.



To Professor Claude E. Welch

Now that I have almost completely resumed the normal exercise of my pastoral ministry, it gives me great pleasure to express my gratitude to you for placing your esteemed medical expertise at my disposal, in a spirit of generous collaboration, to hasten the coming of this long awaited moment.

Dominant among the thoughts that repeatedly came to me during the period of my stay in the hospital was a sense of thankfulness to all those, like yourself, who, even at great personal sacrifice, spared no effort to further my full recovery.

I gladly avail myself of the occasion to express my esteem and to assure you of my prayers for you in your work for the good of humanity. Invoking upon you the abundant graces of Almighty God, I gladly impart to you and your family my special Apostolic Blessing.

From Castelgandolfo, October 4, 1981

Fig. 5. Letter of thanks written to Dr. Claude E. Welch by Pope John Paul II following his recovery from a gunshot wound to the abdomen.

noted that surgeons in the early years of the century concentrated on attaining technical excellence, but in the most recent quarter century the emphasis had shifted to science and research. His message was that staying on top would require organizations like the SSAT to be vitally concerned with continuing education and dissemination of new information "directed widely to all young surgeons with compelling interests in abdominal surgery." Twenty years before the retreat organized by SSAT president Dr. Bernard Langer,[3] Dr. Welch foresaw both the need to expand the SSAT from an elite society and the risks of segregating disease into silos attended only by narrow specialists. He continued his calls for continuing education in his 1977 presidential address to the American Surgical Association.[4] Let us not forget that DDW is one of the greatest continuing medical education "shows" on earth.

Yet Dr. Welch understood that he was a link between the old individualistic practices and the new organization of medicine: "A general surgeon in the time period I was alive . . . often had to make a diagnosis without delay, select an operation fitted to the patient's condition and persevere under conditions that now would be judged primitive and hazardous . . . I regret that a major virtue of surgeons—to rapidly choose a correct course of action and act upon it—has diminished in importance."[1] Nostalgia aside, Dr. Welch recognized that the practice of surgery was rapidly evolving and that new knowledge must be incorporated into the informed care of patients.

During his 50 years of practice, Dr. Welch wrote more than 200 articles and chapters and authored or edited six books. For almost 30 years he contributed authoritative reviews on abdominal surgery to the *New England Journal of Medicine*. As much as any other individual, Dr. Welch embodied the transition from general surgeon to quintessential gastrointestinal surgeon.

That transition and the further maturation of gastrointestinal surgery into surgical gastroenterology is what we are about today. It is the evolution from technique and application of technology to the incorporation of science and partnering with related disciplines. The SSAT program in 1966, the year of Dr. Welch's presidency, contained 24 clinical papers and six experimental studies. Most were retrospective studies of peptic ulcer disease, gallstones, and colon

surgery. Three pioneering papers caught my attention: one on orthotopic allotransplantation of the pancreas by Dr. Richard Lillehei; one on pancreaticogastrostomy by Dr. Jonathan Rhoads; and an experimental study of intestinal blood flow by two cardiac surgeons, one of whom was Dr. Jerry Austen, my predecessor and the namesake of my Chair. This year there are approximately 100 oral and poster presentations including sophisticated studies of gene therapy, transplantation immunology, and molecular and cell biology. The envelope of minimally invasive therapy is constantly being expanded. A whole new world of replacement parts is likely just around the corner through tissue engineering and through induction of tolerance to allow xenotransplantation. Outcomes research is rapidly becoming a major focus for surgeons 100 years after Dr. E. Amory Codman, another surgeon at Massachusetts General Hospital, first incurred the displeasure and resistance of his colleagues by developing a program to evaluate both surgeons and methods of treatment through comparisons of end results. Happily we can now boast of vast reductions in surgical morbidity and mortality. Operations such as pancreaticoduodenectomy, hepatectomy, esophagectomy, and ileoanal pouch reconstructions have become so safe and routine in some hands as to engender calls for regionalization of those complex procedures to centers of expertise.[5,6] The SSAT today finds itself in the somewhat schizophrenic position of trying to balance the interests of the most sophisticated surgical scientists (who speak a language I barely understand), highly specialized clinical surgeons, and the much greater numbers of surgeons who are the principal practitioners of gastrointestinal surgery.[7] This tension between these competing interests is evidenced by the frustration of our Society when its efforts to develop a fellowship mechanism to foster training in gastrointestinal surgery foundered over concerns that such a training program could compromise general surgical residencies and foster critical turf wars in the surgical community. How advanced training interfaces with certification and ultimately credentialing is a conundrum actively being addressed by the American Board of Surgery.[8]

It may seem that I have used the terms gastrointestinal surgery and surgical gastroenterology interchangeably, but that is not my intention. While we struggle internally with our identities, individually and collectively, as general and gastrointestinal surgeons, the field of medicine to which we belong and contribute is broadening, docking with related fields of science and medicine, searching for the next level. Perhaps just as departments of anatomy, biochemistry, and microbiology are becoming nearly indistinguishable, as scientists in each are using similar concepts and tools to probe their particular areas of interest from different entry points, surgeons, gastroenterologists, endoscopists, and radiologists are focusing more on the patient and the needs of disease management and less on a particular technique. Good clinical research is no longer acceptable if it is simply a recital of "how I do it," but requires a comparative standard, often of competing methods. We are and should be metamorphosing from gastrointestinal surgeons into surgical gastroenterologists.

In his address to this Society two years ago, the ethicist Albert Jonsen[9] stated that "surgical competence of the future must be expanded from individual mastery of technique to collaborative deployment and control of the modalities proper to surgery." He pointed out that the etymologic roots of the word competence include both "to compete," to run and stay the course, but also literally "to seek together" as competitors running on the same track, several parties bringing their respective energies and skills to the joint effort. The ethic of collaboration must succeed or supplement the ethic of competence based only on competition. Stated otherwise, real competence can exist only when collaboration is an ethical imperative.

DDW is, of course, an ultimate manifestation of collaboration between the medical and surgical gastroenterology. We meet together and present our findings side by side, and many of our most popular and best-attended sessions are the joint symposia. Oral presentations and posters have been exchanged by the American Gastroenterological Association and the SSAT to be placed on each other's programs. Not coincidentally, our SSAT postgraduate course this year was entitled "Interfaces in Hepatobiliary and Pancreatic Diseases." On a corporate level, the SSAT during the past year has joined with its sister organizations from DDW to become a member of the Federated Societies of Gastroenterology and Hepatology in order to align further our interests and efforts medically, scientifically, and politically. Currently we are contributing to the formation of the International Council for Surgical Gastroenterology, a collaborative initiative of six major surgical organizations whose goal is an enhanced and unified voice for surgery in the world of gastrointestinal tract medicine and science. The name of this organization was chosen after careful deliberation and with the intent that we be understood not as surgical technicians but as surgical clinicians and scientists.

The world of medicine is moving toward new modes of collaboration that include but transcend gastroenterology. There is increasing experimentation with the establishment of disease centers to manage groups of disorders in a multidisciplinary—or perhaps more accurately cross-disciplinary—fashion. Thus we

Table I. Pancreatic cancer: Disease-focused care management program goals

- Accurate selection of patients for cancer-directed treatment, surgical and nonsurgical
- Expedite staging and treatment planning
- Direct patients to the optimal setting, community or tertiary
- Shift resection surgery to the tertiary facilities
- Decrease hospital admissions for palliative treatment or disease complications

Table II. Surgical outcomes: Whipple operation

	Low-volume hospitals[8] (<5/yr)	JHH[8] (1995)	MGH (1997)
Average length of stay (days)	23.6	18.2	13.0
30-Day operative mortality (%)	18.8	0.9	1.7
Cost (per case)	$33,249	$22,379	$18,157

JHH = Johns Hopkins Hospital; MGH = Massachusetts General Hospital.

have oncologic care, neurologic care, cardiac care, musculoskeletal care, and gastrointestinal care. Clinics, hospital floors, and managed care "carve-outs" all are being tested in relation to these concepts. They are based on an approach that to varying degrees looks at complete care of a disease—or a patient—across a continuum of time, place, and caregivers. Programs of disease-focused care management, such as one we at Partners Healthcare developed for pancreatic cancer (Table I), attempt to define a scheme for cost-effective, patient-focused care by the most appropriate provider in the optimal location. Such systems have the potential to build partnerships not only between primary care physicians and specialists, between gastroenterologists and surgeons, but also between community general surgeons and medical center subspecialty surgeons. In our model for management of pancreatic cancer, we assumed that the diagnosis would be made in the community; staging with high-resolution contrast CT and laparoscopy to plan treatment strategy could be accomplished by community physicians and surgeons; palliative treatments such as biliary stent placement and surgical bypasses might preferably be rendered at the community hospital; patients identified as candidates for pancreaticoduodenectomy could be referred to a center at individual discretion. The program is designed to provide the most informed, highest quality, lowest cost care across the network of providers. It also values and validates the roles of both the general surgeon and the subspecialist gastrointestinal surgeon.

Well, here we are on our current pinnacle. As Dr. Welch[10] emphasized in his 1988 Founder's Lecture to this Society, surgery has undergone a profound transition from craft to scientific discipline. General surgery has spawned gastrointestinal surgery, which is now evolving into surgical gastroenterology. Perhaps because of this success we are faced with the danger of losing our identity, our unique viewpoint, and perhaps some of our ability to contribute as disciplines become blurred. Our voice is only one, not the largest, competing to be heard in the DDW council, in funding agencies, and in our hospitals and universities. To ensure the continuing success of the digestive disease matrix, each of its components must have the strength, vitality, and security to be independent contributors, not just participants.

Managed health care methods threaten not only to limit surgical treatment but also to mandate where and by whom it can be delivered. We must continue to stand up for quality of care, not just lower cost. We must develop the evidence to prove that better outcomes bring lower cost. Data indicating this relationship are beginning to appear. For example, the Whipple operation is both safer and less expensive at centers such as Johns Hopkins[6] and Massachusetts General Hospital (Table II).

Changes in health care insurance also threaten to shake us from our academic peaks. Declining reimbursement for surgical services translates into pressure to do more units of work to maintain salaries. The surplus funds that an academic surgical department traditionally generated and used to supplement teaching and research activities have all but disappeared. Start-up and bridge capacity is crippled. Academic surgeons can afford less time for nonremunerative pursuits. Patients are being diverted from high-cost centers with teaching programs, and restrictions are being imposed on the role that surgical residents can play, creating serious problems for technical training. While part of the problem of educating and training gastrointestinal surgeons can theoretically be solved by incorporating community hospitals into a residency network, the logistical and supervisory problems will be great, and the general surgeon outside the academic center must be willing in the near future to assume a far greater role, one of critical importance in training residents. Whether these factors will accelerate the compartmentalization of surgical training into tiers leading to "general general surgeons" and "special general surgeons" (i.e., surgical gastroenterologists) seems quite possible.[11]

Our identity as surgical gastroenterologists is challenged at this very moment of success. The complexity of our discipline has progressed to the point that few surgeons can be the "Renaissance" academics of even the last generation. Science has increasingly become the preserve of the dedicated scientist, whether Ph.D. or M.D. Knowledge is exploding; complex new techniques are being developed daily, and the dilettante cannot hope to remain competent. The danger is that surgeons may become appendages to the process of acquisition of new knowledge, mere providers of tissues to be studied, and hiring a Ph.D. as a front is hardly a satisfying solution. Nonetheless, we will have to partner. More surgeons will devote their investigative energies to outcomes research,[12] but keeping a significant role in basic research will require intellectual collaboration in identifying valid research targets as well as in the conduct of the work. It will not be easy, but failure to find that path will mean we will languish on the summit we have reached.

Collaboration among physicians, surgeons, and scientists interested in the alimentary tract and its appendages must be the key. DDW is a model that should be extended and expanded to our activities during the rest of the year. Centers of gastrointestinal care are in development to provide *consensus instead of consultation*, a seamless continuum of care, increased efficiency, and lower cost. Facilities are being combined. At Massachusetts General Hospital we now have a joint medical-surgical endoscopy unit and a joint liver biliary-pancreas center. We are discussing a medical-surgical gastrointestinal access program and inpatient unit. Such an environment will be a natural vehicle for clinical research, much like the success of cancer centers, and can be a magnet for regionalization of tertiary care. The tools and capabilities for benefiting patients with gastrointestinal disease have improved dramatically in the course of one lifetime. Now is the time to consolidate for next steps up. As Dr. Welch[13] stated, in a retrospective on his experiences, "if one spends his life climbing, he may not go far, but he will never go over the hill."

As surgical gastroenterologists we must be vigorous, intellectually keen, independent in our thinking, cognizant and respectful of alternative views, and collaborative with our sister disciplines of gastroenterology, endoscopy, and radiology. We must not let current success or greed separate us. There are, unfortunately, some among us who place greater value on their own immediate goals than on the greater achievements possible only with synergy. As we happen to be in New Orleans, take notice of the example of the Brennan family who together built a highly successful business comprising such famous restaurants as Commander's Palace and several with the family name. There came a point, unhappily, when even success could not prevent disintegration of the family and the corporate business. Perhaps success itself is naturally entropic, but we must be ever vigilant to focus the mission of the SSAT on climbing to new heights of knowledge, education, and quality of care. No other outcome measure, whether it be in units of size, dollars, or power, is of equal importance, and no other will endure.

Again, to quote Albert Jonsen,[9] "the collaboration of practitioners and researchers from all segments is demanded not only by the constant deconstruction of artificial barriers between the sciences but for the benefit of patients whose conditions can be understood and managed only with a variety of skills and tools."

In the middle of difficulty lies opportunity.
— Albert Einstein —

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Norman Barrett: So Close, Yet 50 Years Away From the Truth

Parakrama Chandrasoma, M.D.
Professor of Pathology,
University of Southern California, Los Angeles, Calif.

Gastroesophageal reflux disease and Barrett's esophagus are assuming increasing importance because of the rising incidence of reflux-induced adenocarcinomas of the lower esophagus and junctional region. A new histology-based pathophysiology is presented that removes much of the present confusion that exists regarding the pathologic changes of reflux disease. Understanding pathophysiology correctly must be the basis of all treatment and prevention of reflux disease and its complications.

NORMAN BARRETT'S PAPERS (1950s)

In the first half of this century, physicians began recording the occurrence of ulcers in the lower esophagus that were associated with what was called gastric mucosa in the tubular esophagus. The perception at the time was that these were peptic ulcers resulting from heterotopic gastric mucosa in the lower esophagus.[1] In 1950, Norman Barrett,[2] recognizing that the answers to this mucosal disease lay in the histologic definition of stomach and esophagus, published the first of his two papers in which he defined the esophagus as that part of the foregut that was lined by squamous epithelium. Since everything distal to the esophagus constituted stomach, the lower part of the tube that was lined by "gastric" mucosa became "tubular stomach," and these patients were deemed to have a congenitally short esophagus.

Barrett's concept must have been intensely criticized over the next 7 years.[3] Many authorities persuaded Barrett that what he called the tubular stomach was, for a variety of reasons (all correct), in fact esophagus. This led to Barrett's second paper[4] on the topic, in 1957, where he retracted his original statement and acceded that this phenomenon represented "columnar-lined esophagus," which came to be called Barrett's esophagus.

The term "columnar-lined esophagus" that Barrett coined is interesting. The fact that he did not call this congenital heterotopic gastric mucosa in the esophagus suggests that he believed either that this was not congenital or that it was not gastric mucosa, or both. If one combines Barrett's 1950 and 1957 statements, it suggests that he is saying that the esophagus, which is normally lined by squamous epithelium, undergoes columnar epithelial transformation.

Norman Barrett pointed us in the right direction, recognizing that the path to understanding this entity lay in the histologic definition of the esophagus and stomach. Fifty years later, we still cannot accomplish this with any accuracy.

TRUTHS AFTER NORMAN BARRETT (1957-1998)

We have learned much about Barrett's columnar-lined esophagus since 1957. In 1959, evidence was presented that Barrett's esophagus was caused by gastroesophageal reflux and was therefore an acquired condition rather than congenital gastric heterotopia in the lower esophagus.[5] This is now a proven fact.

In 1976, Paull et al.[6] elegantly classified Barrett's columnar-lined epithelium into three histologic types based on strictly defined criteria (Table I). The three types of columnar lining in the esophagus as outlined by Paull et al. were junctional, fundic, and specialized. The junctional type is histologically equivalent to cardiac mucosa. (2) The fundic type is a mucosa comprised of glands that contain a mixture of parietal and

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Reprint address: Department of Surgical Pathology, LAC-USC Medical Center, Room 16905, 1200 N. State St., Los Angeles, CA 90033.

Table I. Definition and terminology of different types of epithelia in the esophagus and stomach

Type of epithelium (suggested term)	Defining characteristics	Paull et al.[6] term
Squamous epithelium	Stratified squamous epithelium	Not defined
Cardiac mucosa	Mucosa lined by gastric-type surface and foveolar cells with glands containing only mucous cells	Junctional epithelium
Oxynto-cardiac mucosa	Mucosa lined by gastric-type surface and foveolar cells with glands containing a mixture of mucous, oxyntic, and chief cells	Fundic epithelium
Gastric (oxyntic) mucosa	Mucosa lined by gastric-type surface and foveolar cells with glands containing only oxyntic and chief cells	Not defined
Intestinal epithelium	Mucosa with surface, foveolae, and/or glands containing a mixture of mucous cells and goblet cells	Specialized epithelium

mucous cells. This mucosal type is more accurately termed "oxynto-cardiac" because it differs from gastric fundic mucosa, which contains oxyntic glands devoid of mucous cells. The specialized type is a mucosa that contains goblet cells. These three types of columnar lining in the esophagus are easily distinguished histologically from gastric oxyntic mucosa. They represented the three types of Barrett's esophagus (cardiac, fundic, and intestinal) that were recognized until the 1980s. It was also accepted that all of these types of Barrett's esophagus resulted from gastroesophageal reflux.

During the past two decades, it was correctly recognized that the intestinal type of Barrett's esophagus was the only type that predisposed to reflux-associated adenocarcinoma of the esophagus.[7] A sequence of progression of intestinal metaplasia, low-grade dysplasia, high-grade dysplasia, to invasive carcinoma was established. As a result of this, the term Barrett's esophagus became defined by the presence of intestinal metaplasia. Inexplicably the junctional (cardiac) and fundic (oxynto-cardiac) types of Barrett's ceased to be entities associated with reflux. It has been suggested that long segments of nonintestinalized columnar epithelium in the esophagus be termed "columnar-lined esophagus without Barrett's esophagus."[8]

The incidence of reflux-associated adenocarcinoma of the lower esophagus has increased dramatically in the past 30 years and is now the most rapidly increasing type of cancer in the United States.[9] It has also become clear that the epidemiology of adenocarcinoma of the "gastric cardia" is identical to that of lower esophageal adenocarcinoma, and distinct from gastric adenocarcinoma involving the distal stomach.[9] At the same time as the medical community has achieved highly effective control of symptoms and complications of reflux disease with acid suppressant drugs, it is seeing an explosion in the incidence of reflux-associated adenocarcinoma.

CONFUSION AFTER NORMAN BARRETT (1957-1998)

At the present time, much confusion still exists regarding the pathologic changes of reflux. The only function of the pathologist in reflux disease is to diagnose the presence of intestinal (Barrett's) metaplasia, dysplasia, and carcinoma. Accepted histologic criteria for reflux are restricted to changes in the squamous epithelium (basal cell hyperplasia, papillary elongation, intraepithelial eosinophils), which have a very low sensitivity in the diagnosis of reflux as indicated by symptoms, endoscopic features, or 24-hour pH testing. Reflux is the only mucosal disease of the body where pathologic examination plays little or no role in diagnosis or management.

The confusion that exists can be traced logically to one source. We recognize but cannot accurately define normal cardiac mucosa. The concept that the anatomic gastric cardia is normally lined by an epithelium that contains mucous glands is one that has been perpetuated in history. In 1961, Hayward,[10] an influential British surgeon, first pointed out the basic problem with the concept that the gastric cardia was lined by cardiac mucosa. He suggested that the word cardia was a confusing one that was used to describe both the lower esophageal sphincter and the proximal stomach and should be either dropped or used to denote the lower esophageal sphinteric region. He also suggested that the term cardiac mucosa should be replaced by "junctional mucosa." This junctional mucosa must exist, Hayward stated, to prevent digestion of the squamous epithelium from gastric secretions. Hayward quoted no personal observations or data, but his comments must have reflected information existing at the time that there was such a mucous gland containing mucosa in this region in most people. Hayward placed the "normal" junctional mucosa as lining the distal 1 to 2 cm of the esophagus and extending into the stomach. In a line diagram that illus-

Table II. Data from studies attempting to characterize mucosal types at the squamocolumnar junction in patients whose junctional region was perceived as being normal endoscopically

Study	Total patients	CM+	CM−
Öberg et al.[12]	334	246	88 (26%)
Spechler et al.[13]	156	116	40 (26%)

CM = cardiac mucosa.

Table III. Population of the United States, as defined by the characteristics of mucosae in the junctional region

Definition	%
No cardiac mucosa	26-31
Cardiac mucosa <2 cm; no IM	40-50
Cardiac mucosa <2 cm; IM+	15-20
Cardiac mucosa >2 cm; no IM	Rare
Cardiac mucosa >2 cm; IM+	3-5

IM = intestinal metaplasia.

trates his paper (which, interestingly, is credited to Norman Barrett), Hayward clearly illustrates this junctional mucosa as straddling the junctional region (which he calls the cardia).

If, as Hayward suggested, there is a normal 1 to 3 cm of cardiac mucosa in the lower esophagus and proximal stomach, it becomes impossible to interpret biopsies from this region that are lined with glandular epithelium. It is for this reason that the only type of Barrett's esophagus that is universally recognized is the presence of intestinal metaplasia when the endoscopist perceives a columnar lining of the lower esophagus that exceeds 2 to 3 cm in length. This is called long-segment Barrett's esophagus.[8] When intestinal metaplasia is absent in a long segment of columnar-lined esophagus, no diagnosis is possible; Spechler[8] recommends the term "columnar-lined esophagus without Barrett's esophagus"; the relationship of this entity to reflux disease is stated to be not established. When intestinal metaplasia is present and the esophageal columnar lining is less than 2 cm by endoscopy, the term "short-segment Barrett's esophagus" is used. This lacks logic; if a 2 cm length of cardiac mucosa is a normal gastric mucosa, then reflux can have no effect on it and intestinal metaplasia occurring in it does not represent "Barrett's esophagus." It must represent "intestinal metaplasia of the gastric cardia," which is the term used when intestinal metaplasia occurs in cardiac mucosa when no endoscopic abnormality is perceived. According to the present literature, "columnar-lined esophagus without Barrett's esophagus," "short-segment Barrett's esophagus," and "intestinal metaplasia of the gastric cardiac" have no established association with reflux.[8] Short-segment Barrett's esophagus has, however, been recognized to be a precursor of adenocarcinoma.[11]

Confusion would be irrelevant if it had no adverse consequence. However, epidemiologic data strongly suggest that adenocarcinoma occurring in the gastric cardia is identical to adenocarcinoma occurring in the lower esophagus. Both of these types of cancer are increasing similarly in incidence and have identical demographic and risk factors. A common pathogenetic pathway for these two cancers appears likely on epidemiologic grounds.[9]

THE MYTH OF THE NORMAL GASTRIC CARDIA

I would like to present evidence that Hayward's junctional mucosa (and, therefore, gastric cardiac mucosa) is not a normal structure. Certainly we know that Hayward's contention that a junctional mucosa is "necessary to prevent digestion of the squamous epithelium by gastric acid" is not true. The esophagus is protected from gastric juice by the lower esophageal sphincter, which was described in 1956, but whose functional characteristics were defined after Hayward's time.

Two endoscopic studies are available that searched for the presence of cardiac mucosa in people who had normal endoscopic findings (Table II). It is interesting that in both studies, one from each coast of the United States, 26% of patients did not have any cardiac mucosa.[12,13]

We looked at a series of autopsies from Los Angeles County–University of Southern California Medical Center (Chandrasoma P, unpublished data). We selected patients who had no mention of reflux in their medical records and had an evaluable longitudinal section across the gastroesophageal junctional region. We found that 9 of 11 children under 20 years of age did not have a cardiac mucosa. The presence and length of cardiac mucosa increased with age, but there was an 87-year-old patient whose squamous epithelium transitioned directly to oxyntic mucosa. Overall, 21 (31%) of 68 in this patient population did not have evidence of cardiac mucosa.

It is inconceivable that 26% to 31% of people do not have a normal anatomic structure. Our finding that cardiac mucosa appears to be age related with a very low incidence in children strongly suggests that it represents an acquired abnormal mucosa at the junctional region. According to this concept, 26% to 31% of people in the population are "normal" in that they do not have any cardiac mucosa (Table III).

WHAT IS CARDIAC MUCOSA?

Data from our patient population strongly indicate that the abnormality that results in the presence of cardiac mucosa is gastroesophageal reflux. In studies by Öberg et al.[12] and Clark et al.,[14] endoscopically normal patients who had no cardiac mucosa generally had normal 24-hour pH test values. This contrasted with the endoscopically normal group that had cardiac mucosa in whom 24-hour pH testing commonly showed evidence of reflux.

If it is true that the squamous epithelium transitions to gastric fundic mucosa in the junctional region in normal individuals, the pathologic changes in reflux become easy to understand. Gastric oxyntic mucosa is not damaged by gastric contents; esophageal squamous epithelium is affected by refluxed gastric contents. Cardiac mucosa can result only by transformation of squamous epithelium consequent on reflux. The presence of cardiac mucosa at the junction therefore represents the earliest histologic evidence of reflux disease and is recommended as the histologic definition of reflux disease. According to this definition, approximately 70% of the population of the United States have evidence of reflux disease. Approximately 40% to 50% of the population have cardiac mucosa measuring less than 2 cm without intestinal metaplasia. This population is what has up to now been called "normal," but in fact represents patients with histologic evidence of mild reflux.

There is abundant data to indicate that reflux is an extremely common condition. Careful population surveys suggest that heartburn is present in over 50% of the population, and over-the-counter acid suppressive agents are some of the most widely advertised and used drugs.[16] It should not be surprising that histologic features are more sensitive in the diagnosis of reflux disease than symptoms. In 24-hour pH studies on normal volunteers, reflux has been shown to occur in almost all of them.[15] This suggests that a minimum threshold of reflux is necessary to cause transformation of squamous epithelium to cardiac mucosa. This threshold can vary in different individuals; definition of reflux disease is by the histologic demonstration of cardiac mucosa and not by any given quantity of reflux.

The length of cardiac mucosa that is found in a measured series of biopsies correlates with 24-hour pH test results. Our data show that the amount of reflux as measured by pH data varies directly with the length of cardiac mucosa that is present between gastric oxyntic mucosa and esophageal squamous epithelium. That the length of cardiac mucosa present in a patient is an accurate indicator of the severity of reflux has been shown by other investigators.[17]

CHANGES IN CARDIAC MUCOSA IN REFLUX DISEASE

Contrary to Hayward's contention that junctional (cardiac) mucosa represented a mucous-secreting buffer zone that was not damaged by acid gastric contents, it is our observation that cardiac mucosa, when present, is always histologically abnormal. This suggests strongly that cardiac mucosa is not only caused by reflux, but is also damaged by reflux. Cardiac mucosal damage is characterized by chronic inflammation consisting of eosinophils, plasma cells and lymphocytes, elongation and serration of the foveolar region, and hyperplasia of foveolar lining cells. We call these changes "reflux carditis." Most patients with reflux carditis have no evidence of *Helicobacter pylori* gastritis in their distal gastric biopsies. In the study by Öberg et al.,[12] only 11% of patients with carditis had evidence of *H. pylori* infection; in the series by Spechler et al.,[18] only 24 (21%) of the patients with cardiac mucosa had evidence of *H. pylori* infection. When *H. pylori* infection of the stomach is present, there is secondary infection of cardiac mucosa in patients who have concomitant reflux disease. Secondary *H. pylori* infection of carditis increases the amount of inflammation in cardiac mucosa and commonly causes active inflammation with neutrophils.

Cardiac mucosa is the simplest glandular epithelium resulting from glandular transformation of squamous epithelium in reflux disease, with glands containing only mucous cells. Later development of specialized cell types results in cardiac mucosa containing parietal cells (oxynto-cardiac mucosa), Paneth cells, pancreatic cells, and goblet cells (intestinal mucosa). Only the intestinal mucosa progresses to dysplasia and carcinoma. It is relevant to note that intestinal metaplasia appears not to arise in any region of the mucosa that has an admixture of parietal and mucous cells (i.e., in oxynto-cardiac mucosa).

THE UNIVERSITY OF SOUTHERN CALIFORNIA SYSTEM OF BIOPSY INTERPRETATION IN REFLUX DISEASE

If it is accepted that cardiac mucosa is an abnormal mucosa generated from esophageal squamous epithelium as a result of gastroesophageal reflux, it becomes very easy to use biopsies to assess the presence of reflux and its severity with accuracy. Biopsy specimens should be taken as follows: (1) from the distal stomach (to evaluate gastric pathology) in all patients; (2) from the columnar mucosa immediately distal to the squamous epithelium by retrograde and antegrade methods in patients who have no endoscopic abnormality; and (3) at 1 to 2 cm intervals from the upper limit of

Table IV. USC system of biopsy interpretation in reflux disease

Finding	Reflux disease	Risk of intestinal metaplasia	Risk of carcinoma
Oxyntic mucosa only	0	0	0
Oxynto-cardiac mucosa only	0	0	0
Cardiac mucosa <1 cm long; no IM	Mild	Low	0
Cardiac mucosa <1 cm long; IM+	Mild	N/A	Low
Cardiac mucosa 1-2 cm long; no IM	Moderate	Moderate	0
Cardiac mucosa 1-2 cm long; IM+	Moderate	N/A	Moderate
Cardiac mucosa >2 cm long; no IM	Severe	High	0
Cardiac mucosa >2 cm long; IM+	Severe	N/A	High

IM = intestinal metaplasia; N/A = not applicable.
NOTE: The pathologist makes this interpretation *without* the need for any endoscopic information.

Table V. Differences between biopsy interpretation by the USC system and presently accepted criteria

Endoscopic findings	Histologic findings	Accepted diagnosis	USC diagnosis
Normal	Normal squamous + cardiac mucosa	Normal	Mild reflux disease
Normal	Normal squamous + IM in cardiac mucosa	IM of gastric cardia	Mild reflux + reflux-associated IM*
<2 cm CLE	Normal squamous + cardiac mucosa	Normal	Moderate reflux disease
<2 cm CLE	Normal squamous + IM in cardiac mucosa	Short-segment BE	Moderate reflux + reflux-associated IM*
>2 cm CLE	Normal squamous + cardiac mucosa	CLE without BE	Severe reflux disease
>2 cm CLE	Normal squamous + IM in cardiac mucosa	Long-segment BE	Severe reflux + reflux-associated IM*

CLE = columnar-lined esophagus as seen endoscopically; IM = intestinal metaplasia; BE = Barrett's esophagus.
*Intestinal metaplasia is quantitated by serial biopsies in all cases.

gastric rugal folds to the squamous epithelium in patients who have an abnormal columnar-lined esophagus at endoscopy.

Table IV represents possible pathologic findings and what they mean in terms of the presence and severity of reflux according to the University of Southern California (USC) system. Table V contrasts the interpretation of various biopsy findings using the USC system and presently accepted criteria.

Changes of reflux in squamous epithelium are irrelevant. When present, they are almost always accompanied by reflux carditis in adequately biopsied patients.

INTESTINAL METAPLASIA—MUTATIONAL REFLUX DISEASE

Reflux-associated intestinal metaplasia that defines Barrett's esophagus always occurs in reflux carditis. Because cardiac mucosa represents transformed esophageal epithelium and defines reflux disease, the occurrence of intestinal metaplasia in it must represent reflux-associated intestinal metaplasia. It has been our experience that this change occurs in the 1 to 2 cm of the anatomic proximal stomach as well as the tubular lower esophagus in some patients, suggesting that the lower esophageal sphincter may extend down into the anatomic stomach. It is interesting that the distribution of this change as we have found it is identical to the cardia as represented in the diagram by Hayward.[10] If this is so, it would explain why adenocarcinoma of the gastric cardia is epidemiologically associated with reflux. In our study population, intestinal metaplasia involving small lengths of cardiac mucosa at the junctional region is strongly associated with abnormal reflux by 24-hour pH testing.[14]

We recognize intestinal metaplasia as the second phase of reflux disease, most likely representing a mu-

tational change. Genetic abnormalities have been reported in intestinal metaplasia without dysplasia.[19] These include p53 overexpression, 17p allelic deletion, and aneuploidy; these increase in frequency with increasing dysplasia and cancer. Adenocarcinoma associated with reflux probably requires multiple genetic changes.

The initial mutational changes can be recognized only by genetic testing. The first morphologic evidence of mutational change is intestinal metaplasia and the last change is cancer; in between are increasing degrees of dysplasia. It is very likely that there is a time lapse of several years between the occurrence of mutations and their morphologic expression.

The mutagenic agent that causes intestinal metaplasia in reflux-induced cardiac mucosa is known to be a constituent of gastric juice that refluxes into the esophagus. The likelihood of mutation in cardiac mucosa increases with greater length of cardiac mucosa (the majority of patients with cardiac mucosal lengths of greater than 3 cm have intestinal metaplasia) and severity of reflux carditis. The increased cell turnover associated with severe reflux carditis would theoretically result in an increased likelihood of mutations.

Although mutations are more common when cardiac metaplasia is extensive, there is no doubt that intestinal metaplasia occurs with very short segments of cardiac mucosa. It has been reported that 15% to 20% of individuals with normal endoscopic findings show evidence of intestinal metaplasia in the junctional region.[21] Because these patients with short cardiac mucosal lengths have mild, often asymptomatic reflux, their propensity to develop intestinal metaplasia strongly suggests that mutations are not caused by acid in the refluxate.

It is useful to regard reflux disease as representing two etiologically different diseases (Table VI): one caused by acid in the refluxate that correlates with abnormalities on 24-hour pH tests and the other caused by mutagenic agents in the gastric refluxate. There is evidence that mutagenic agents can be produced in the stomach from bile salts entering the stomach by duodenogastric reflux.[22] The production of mutagens appears minimal at extremes of pH (i.e., in a normally acidic stomach and a stomach whose acid suppression is complete as by well-controlled omeprazole therapy) but significant at intermediate gastric pH, as would occur in patients with incomplete and poorly controlled acid suppression, as probably results when over-the-counter acid suppressants are used. If this is so, it would explain why adenocarcinoma is uncommon both during well-controlled omeprazole therapy and after antireflux surgery, but its incidence in the population is increasing.

Table VI. Phases of reflux disease—The reflux adenocarcinoma sequence

Acid-induced (correlates with acid exposure; nonmutational; reversible)
Ulceration and erosion
Squamous epithelial changes: Basal cell hyperplasia and intraepithelial eosinophils
Cardiac transformation of squamous epithelium: Length correlates with severity of acid reflux
Reflux carditis: Foveolar hyperplasia, chronic inflammation with eosinophils
?Reversible, nonmutational intestinal metaplasia
Mutational (etiology unknown; irreversible)
Irreversible mutational intestinal metaplasia: Always occurs in reflux carditis
Dysplasia and adenocarcinoma: Always occurs in intestinal metaplasia

DEFINITION OF REFLUX DISEASE

At the present time, reflux disease has no definition, because no end point for the disease has ever been established. I recommend that we recognize the occurrence of reflux-associated adenocarcinoma as the end point of reflux disease. If this is accepted, reflux disease can be defined by the presence of histologically defined cardiac mucosa which, by being the only mucosa in which intestinal metaplasia occurs, represents the morphologic entry into the sequence whereby reflux results in adenocarcinoma. Patients who have only oxynto-cardiac mucosa at their junction have no reflux disease, because intestinal metaplasia does not occur in oxynto-cardiac mucosa, although it is clear that oxynto-cardiac mucosa is caused by reflux. The conversion of cardiac mucosa to oxynto-cardiac mucosa therefore represents "cure" of reflux disease.

TREATMENT OF REFLUX DISEASE

Acid-suppressive agents are highly effective in treating the acid-induced component of reflux disease, which includes symptomatic relief. Because reflux continues to occur, the mutagen-induced disease is not addressed with acid-suppressive drug therapy. It is therefore not surprising that the mutagenic component of reflux disease, as manifested by intestinal metaplasia, dysplasia, and reflux-associated adenocarcinoma, continues to increase.

Antireflux surgery, when successful, stops gastroesophageal reflux. In our patients who have had postsurgical biopsies, we have observed the following changes: (1) squamous overgrowth on the surface over

cardiac and intestinal mucosa, which decreases the overall surface area of columnar-lined mucosa; (2) oxyntic transformation of cardiac mucosa, which is a morphologic form of "cure" of reflux disease; and (3) regression of features of injury in cardiac mucosa, including a decrease in inflammatory cells and reversal of foveolar hyperplasia. In some cases, intestinal metaplasia has been difficult to find after surgery, but it is not certain whether this represents sampling error, squamous overgrowth, or reversal of intestinal metaplasia. In many cases, particularly those with large areas of intestinal metaplasia, there is no reversal of intestinal metaplasia after surgery for many years. There is also evidence that progression of the mutagenic sequence is halted after successful antireflux surgery because the incidence of adenocarcinoma is markedly reduced.

PREVENTION OF REFLUX-ASSOCIATED ADENOCARCINOMA

The first step toward solving the problem is to recognize the pathogenesis whereby reflux results in adenocarcinoma (the reflux-adenocarcinoma sequence). We believe the sequence to be as follows: (1) acid reflux causes cardiac transformation of squamous epithelium; (2) acid reflux injurs transformed cardiac mucosa, causing reflux carditis with increased cell turnover; (3) intestinal metaplasia occurs in the transformed cardiac mucosa (reflux-associated intestinal metaplasia or Barrett's esophagus), a change that is very likely associated with genetic mutation; and (4) further genetic mutational events occur in the intestinal epithelium leading to low-grade dysplasia, high-grade dysplasia, and invasive carcinoma.

The next step in the solution is to identify the mutagenic agents, but this is likely to take several more years. In the meantime we can achieve something if we recognize that the mutagenic agent is not acid and that it is some constituent of refluxed gastric contents. Suppressing acid, although it is highly effective in removing symptoms of reflux, at best does nothing to prevent the progression to adenocarcinoma. Antireflux surgery, by preventing reflux, will prevent mutations and progression in the reflux-adenocarcinoma sequence.

Prevention of carcinoma is simple in theory. However, a specific sequence of steps is needed. The population should be screened by endoscopy at age 50 years. After all patients at risk are identified (70% to 75% of the population who have cardiac mucosa), antireflux operations would be performed. If the operation is successful, and the operation is performed before the full range of genetic "hits" required for malignancy have occurred, *all reflux-associated adenocarcinoma will be prevented.* A practical, cost-effective solution will need to restrict screening endoscopy to the demographic group most at risk for carcinoma (e.g., white males over the age of 50 years) and limit antireflux surgery to those patients at greatest risk (e.g., with more than 1 cm of cardiac mucosa). Although this number will still be large, the monetary and human benefit resulting from the decrease in adenocarcinoma may make this feasible.

NORMAN BARRETT CAME SO CLOSE TO THE TRUTH

Norman Barrett recognized 50 years ago that the secret to understanding this disease lay in the histologic definition of esophagus and stomach.[2,4] When his statements of 1950 and 1957 are combined, he seems to be saying that the esophagus, which is that part of the foregut lined by squamous epithelium, transforms into columnar-lined esophagus. If, instead of defining the esophagus as that part of the foregut lined by squamous epithelium, he had defined the proximal stomach as that part of the foregut that is lined by oxyntic mucosa, he would have solved the puzzle because all columnar epithelia proximal to oxyntic mucosa would then have been esophageal. Barrett's line drawing in Hayward's paper,[10] which shows cardiac mucosa lining the junctional region, is remarkably accurate.[10] His only failure was in not recognizing that the unsubstantiated but historically established dogma of a normal cardiac mucosa was incorrect.

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Near-Total Completion Gastrectomy for Severe Postvagotomy Gastric Stasis: Analysis of Early and Long-Term Results in 62 Patients

Adrienne W. Forstner-Barthell, B.A., Michel M. Murr, M.D., Sami Nitecki, M.D., Michael Camilleri, M.D., Charlene M. Prather, M.D., Keith A. Kelly, M.D., Michael G. Sarr, M.D.

The aim of this study was to evaluate results of completion gastrectomy for severe postgastrectomy gastric stasis. A total of 51 women and 11 men underwent completion gastrectomy for gastric stasis between 1985 and 1996; follow-up was complete in 98% at 5.4 ± 5 years. All patients had modified Visick scores preoperatively of grade III (37%) or IV (63%). Presentation included combinations of nausea, vomiting, postprandial pain, chronic abdominal pain, and chronic narcotic use. All had undergone prior vagotomy and had a median of four previous gastric operations. Hospital mortality was zero. Complications occurred in 25 patients (40%) and included the following: narcotic withdrawal syndrome (18%), ileus (10%), wound infection (5%), intestinal obstruction (2%), and anastomotic leak (5%). All or most symptoms were relieved in 43% (Visick grade I or II), but 57% of the patients remained in Visick grade III or IV. Nausea, vomiting, and postprandial pain were reduced from 93% to 50%, 79% to 30%, and 58% to 30%, respectively ($P < 0.05$), but chronic pain, diarrhea, and dumping syndrome were not significantly affected. Univariate analysis revealed no preoperative characteristic to be predictive of good outcome. Logistic regression analysis suggested that the combination of nausea, need for total parenteral nutrition, and retained food in the stomach predicted a poor outcome ($P < 0.05$). Completion gastrectomy is successful in 43% of patients. The combination of nausea, need for total parenteral nutrition, and retained food at endoscopy are negative prognostic factors. (J GASTROINTEST SURG 1999;3:15-23.)

KEY WORDS: Gastroparesis, gastric stasis, Roux stasis syndrome, gastric emptying

Reconstruction of gastrointestinal continuity after vagotomy and distal gastrectomy may result in some degree of gastric stasis in 10% to 30% of patients.[1-4] However, chronic gastric stasis severe enough to impair one's ability to maintain adequate oral nutrition is uncommon. Such severe postvagotomy or postgastrectomy gastric stasis manifests clinically as weight loss associated with early satiety, nausea, and vomiting of undigested food in the absence of mechanical obstruction of the gastric outlet or the small bowel. Associated postprandial abdominal pain is also common.[5]

Although many patients achieve relief with the use of prokinetic agents,[5] the few who fail nonoperative treatments are difficult to manage. All too often these patients are subjected to multiple operative revisions of the gastric outlet in an attempt to alleviate the abnormal gastric emptying. Conversions of a gastroduodenostomy to a gastrojejunostomy (or vice versa), "revisions" of the gastrojejunostomy from anterior to posterior or antecolic to retrocolic positions, and finally conversion to a Roux anatomy prove futile. We believe this failure to be the result of delayed emptying due to a paretic gastric remnant—not a "maloriented" or obstructed gastric outlet anastomosis.

Because we are a tertiary referral center for patients with motility disorders, our experience with managing patients with disabling chronic gastric stasis has been frustrating. The Mayo experience has suggested results different from those reported in smaller series that claimed relief of symptoms in 60% of 80% of such patients after completion gastrectomy.[1,2,6-8] This

From the Departments of Surgery and Gastroenterology (M.C. and C.M.P.), Mayo Clinic, Rochester, Minn.
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Reprint requests: Michael G. Sarr, M.D., Professor of Surgery, Chair, Division of Gastroenterologic and General Surgery, Gastroenterology Research Unit, Mayo Clinic, 200 First St. S.W., Rochester, MN 55905.

study was undertaken to evaluate the long-term results of near-total completion gastrectomy in 62 consecutive patients with severe, incapacitating, postvagotomy or postgastrectomy gastric stasis.

METHODS

Inclusion Criteria

A search of the medical records for all patients who were diagnosed with gastric stasis and who underwent gastric surgery between 1985 and 1996 at the Mayo Clinic in Rochester, Minnesota, yielded 68 patients who had undergone a near-total completion gastrectomy, most with a classical Roux-en-Y cardiojejunostomy reconstruction. Six patients were excluded because they had less than 6 months of follow-up postoperatively. The diagnosis of gastric stasis was confirmed by a combination of both subjective *and* objective criteria including clinical symptomatology, absence of mechanical obstruction on endoscopy, abnormal gastric emptying on radionuclide studies, and/or abnormal gastroduodenal manometric studies. Each of the 62 patients evaluated fit the clinical definition of "postgastrectomy stasis syndrome" as characterized by postprandial fullness, pain, nausea, and vomiting. None had mechanical obstruction of the gastric outlet or small intestine as verified by either endoscopy or contrast studies performed at the Mayo Clinic. We abstracted data concerning symptoms, nutritional status, diagnostic studies, operation performed, operative morbidity, and outcome.

Follow-Up and Outcome

Follow-up was completed to date in 60 patients using the medical records and a standard questionnaire given via phone interview by one of the authors (A.W.F.B.) (58 patients) or via mail (2 patients). Forty-six patients are still alive. Mean follow-up was 5.4 ± 0.5 years (range 0.7 to 12 years). The incomplete follow-up in the two patients was detailed enough for Visick grading but not for documenting particular symptoms. To measure outcome, patients were classified into four groups using a Visick grading system[9] modified to allow for a more accurate classification of preoperative and postoperative health status. Grade I corresponded to no symptoms. Grade II included patients with intermittent symptoms, with or without continued use of medications, but not affecting lifestyle. Grade III corresponded to continuous nondisabling symptoms refractory to medical therapy, with or without supplemental use of parenteral or enteral nutrition. Grade IV corresponded to severe disabling symptoms, with or without dependence on enteral or parenteral nutrition as the primary source of nutrition. Grade III was not subdivided according to patient satisfaction or dissatisfaction as originally detailed by Visick.[9]

Patient Demographics and Surgical History

The 62 patients included 11 men and 51 women whose mean age was 48 ± 1 years (range 27 to 70 years). They had symptoms of gastric stasis preoperatively for 4 ± 0.5 years (4 months to 18 years), and their weight loss of 12 ± 1 kg showed the extent of the nutritional difficulties. Preoperative symptoms and clinical presentations are noted in Table I. Preexisting relevant medical conditions included hypothyroidism by history and treated by thyroid replacement (15%), connective tissue disorder (8%), and diabetes (2%). In addition, 52% of the patients had a history of narcotic use (duration 8 ± 2 months) or tobacco use (duration 12 ± 2 pack-years) prior to the near-total gastrectomy. Other medications are listed in Table I.

Table I. Clinical presentation of 62 patients with chronic postvagotomy, postgastrectomy stasis syndrome

Characteristics	Preoperative symptoms (% of patients)	Postoperative symptoms* (% of patients)
Nausea	93	50†
Vomiting	79	30†
Postprandial pain	58	30†
Chronic abdominal pain	56	43
Diarrhea	36	42
Dumping syndrome	23	25
Opioid dependence	52	41
NSAID/ASA use	23	5
Prokinetic medications	56	33
Psychotropic medications	37	18

NSAID/ASA = nonsteroidal anti-inflammatory drug/acetylsalicylic acid.
*At the time of latest follow-up; n = 60 (mean follow-up 5.4 years).
†$P < 0.05$ compared to preoperative symptoms.

These patients had undergone a mean of four previous gastric operations (range 1 to 12 per patient). All patients had undergone a previous total abdominal vagotomy and some form of a gastrectomy prior to the near-total gastrectomy. The most commonly reported indication for the initial operation was undocumented "peptic ulcer disease" (87%), which included duodenal/prepyloric ulcers in 25 (40%), gastric ulcers in 17 (28%), and peptic ulcers, site unspecified, in 12 (19%). At the time of their initial gastrectomy, 11 (20%) of the 54 patients with ulcers presented with bleeding, nine (17%) with obstruction, four (7%) with perforation, and the remaining 30 with intractable symptoms. At the time of the near-total completion gastrectomy, the anatomy included 10 patients (16%) with a Billroth I, 19 (31%) with a Billroth II, and 33 (53%) with a Roux-en-Y reconstruction.

Nutritional Status

Almost three fourths of the patients had been hospitalized previously for malnutrition (44/62), and 42% (26/62) were dependent on enteral or parenteral nutrition prior to near-total gastrectomy. Although mean values for hemoglobin and albumin were near the normal limits (12 ± 0.2 g/dl and 3.8 ± 0.1 g/dl, respectively), 45% (28/62) of patients were anemic (hemoglobin <12 g/dl) and 26% (16/62) had a serum albumin concentration of less than 3.5 g/dl.

Diagnostic Studies

Esophagogastroscopy was performed in all patients. Anastomoses were considered patent and nonobstructing if the adult-sized videoscope (external diameter ~10 mm) could be introduced through the anastomosis without resistance. None of the patients had evidence of mechanical obstruction. Results of endoscopy were normal in seven patients but revealed at least one abnormality in 55 patients: bezoars or undigested solid matter in 24 (39%), stomal ulcers or erosions in 29 (53%), and bile in the gastric remnant despite a Roux-en-Y anatomy in four (6%).

Manometric recordings of the gastric remnant and small bowel were obtained in 20 patients. Fourteen (70%) had severe dysmotility in both regions, whereas six (30%) had normal motility patterns. Gastric emptying studies with both radiolabeled liquids and solids were conducted on 19 patients, of whom 15 had markedly slowed emptying of solids (>2 standard deviations of normal), and 10 had rapid initial emptying of liquids. Gastric emptying studies of solid markers alone were conducted in another 15 patients, none of whom had normal emptying ($t_{1/2}$ >240 minutes).[10]

Operation

Near-total completion gastrectomy was performed by resecting the gastric remnant and leaving a 1 to 2 cm remnant of gastric cardia to facilitate construction of an end-to-side cardiojejunal anastomosis. The Roux limb was no more than 50 cm in length. Six patients had an uncut Roux-en-Y limb constructed at their original completion gastrectomy[11] using a double row of staples across the "afferent limb" (Fig. 1). All operations were performed by one of the senior authors (K.A.K. and M.G.S.). Thirty-one patients had concomitant enterostomy tubes placed at the time of operation.

Data Analysis

Data are summarized as means ± standard error of the mean for continuous variables (e.g., age) and percentages for discrete variables (e.g., sex). The association of preoperative characteristics with outcome after gastrectomy was assessed univariately using Fisher's exact test. A multiple logistic regression was used to assess the independent predictive value for several subsets of preoperative characteristics simultaneously (e.g., Roux anatomy, chronic pain, narcotic use, nausea, need for total parenteral nutrition, retained food, etc.). The alpha level for statistical significance was set at 0.05.

RESULTS

There were no operative or in-hospital deaths. One or more complications occurred in 25 patients (40%). Gastrografin contrast studies were performed

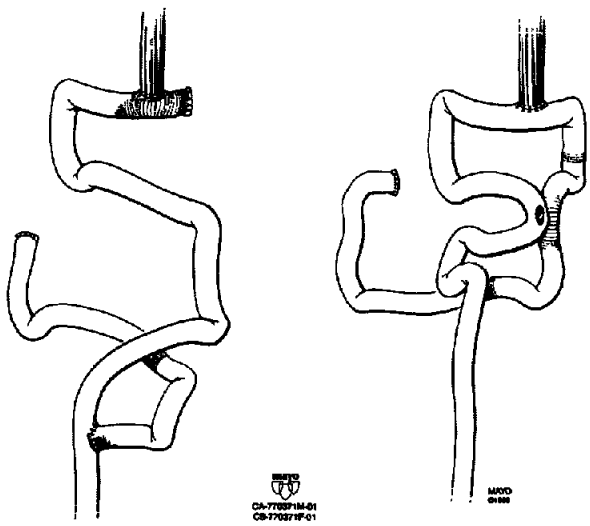

Fig. 1. Near-total completion gastrectomy. Uncut Roux-en-Y jejunostomy is shown on the left.

before oral feedings were initiated; three anastomotic leaks were detected (5%), but only one was clinically significant and required operative intervention. Other complications included prolonged hospitalization for narcotic withdrawal syndrome and pain control (18%), ileus for more than 10 days (10%), wound infection (5%), and one small bowel obstruction (2%). At the time of discharge, 9 (14%) of 62 patients required some form of supplemental enteral or parenteral nutrition.

Outcome at Follow-Up

Detailed follow-up data were obtained for 60 of 62 patients with a length of follow-up of 5.4 ± 0.4 years (range 0.7 to 12 years). Weight at the time of follow-up was 56 ± 2 kg, which was not significantly different from preoperative weight. Subjectively, the majority of the patients believed that the operation had improved their health; only 5 (9%) of 55 believed their condition had worsened, whereas 13 (24%) of 55 thought their health status was unchanged. Symptoms were markedly ameliorated in 27 (43%) of 62 patients. Among the 35 patients who remained symptomatic, nausea (30/60; 50%) vomiting (18/60; 30%), postprandial pain (18/60; 30%), and anorexia (10/60; 17%) were all reduced ($P < 0.05$); however, the symptoms of chronic abdominal pain (26/60; 43%), diarrhea (25/60; 42%), and dumping syndrome (15/60; 25%) were not affected (see Table I).

At the time of follow-up, only 46 patients were alive. Sixteen patients had died as a result of malnutrition (n=3), suicide (n=2), intravenous catheter sepsis (n=2), pneumonia (n=2), liver failure, complications of small bowel transplantation, ovarian cancer, alcoholic pancreatitis (1 case of each), or unknown causes (n=3).

Medications. Detailed information on medication use postoperatively was available for 56 patients. Of these, 22 (41%) and 11 (20%) were using opioid medications or nonsteroidal analgesics for pain relief, respectively; 18 (33%) were still using prokinetic medications, and interestingly five patients (9%) were using H_2 antagonists despite the near-total gastrectomy (see Table I).

Long-Term Morbidity. A subset of patients required subsequent hospitalizations after the near-total gastrectomy for dehydration and malnutrition (23 patients, 172 events), recurrent pancreatitis (7 patients, 21 events), and sepsis from central venous catheters (3 patients, 4 events). Anastomotic strictures developed in 18 patients; 14 were managed with endoscopic dilatation alone (54 events), two required endoscopic dilatation with subsequent operative revision, and four underwent operative revision alone.

Table II. Change in Visick grade after near-total gastrectomy

Visick grade	No. of patients (%)	
	Preoperatively	Postoperatively
I	0 (0)	12 (19)
II	0 (0)	15 (24)
III	23 (37)	8 (13)
IV	39 (63)	27 (44)

Ten patients underwent subsequent operation for episodes of small bowel obstruction. In addition, eight patients required reoperation for insertion of a jejunostomy tube for enteral nutrition, and five patients have required insertion of a chronic intravenous catheter for parenteral feeding to supplement oral intake and caloric needs. Currently 16 patients are maintained on enteral or parenteral nutritional supplementation.

Visick Grade. At the time of follow-up, 29 patients (47%) had improved their Visick scores by a downward shift of at least one grade. Prior to near-total gastrectomy, all patients were classified as Visick grade III (37%) or IV (63%). At follow-up, 27 patients (43%) shifted to grade I or II (Table II); 35 patients (57%) remained in Visick grade III or IV, and three shifted up to grade IV. All patients in Visick grades I and II were subjectively satisfied with their overall health status, whereas 13 patients classified as Visick grade III or IV believed that the near-total gastrectomy had "improved" their overall health. Of the five patients with preexisting connective tissue disorders, only one shifted to Visick grade I, whereas the other four remained in Visick grade III or IV.

Factors Predictive of Poor Outcome. Patients who remained in the same Visick grade (III or IV) or who had shifted to a higher grade at the time of follow-up were considered "failures." On univariate analysis (Table III), sex, postprandial pain, chronic pain, diarrhea, nausea, vomiting, retained food, narcotic use, parenteral nutrition, and preoperative Roux anatomy were found to be of *no* predictive value ($P < 0.05$). As might be expected, the patients considered failures also had more postoperative rehospitalizations for dehydration and/or malnutrition (147 vs. 25 episodes) and more frequently required enteral or parenteral nutritional maintenance (40% vs. 0%) or supplements (8% vs. 0%). Multivariate logistic regression analysis showed that nausea, the preoperative need for total parenteral nutrition (TPN), and retained food at preoperative endoscopy jointly, but not independently, predicted a lower probability of success after gastrectomy for gastric stasis ($P = 0.048$).

Table III. Failures after near-total gastrectomy: Comparison with those who improved*

Preoperative characteristic	% Failures (n = 35 patients)	Improved (n = 27 patients)
Chronic pain	63	48
Narcotic use	60	42
Dumping symptoms	31	11
Roux anatomy	66	37
Retained food on endoscopy	49	27
TPN maintenance	37	15

TPN = total parenteral nutrition.
*Failures = no change or upward shift in Visick grade; improved = downward shift to Visick grade I or II.

DISCUSSION

Chronic severe postvagotomy or postgastrectomy gastric stasis, severe enough to challenge one's ability to maintain adequate nutrition orally, is a disabling problem with inconsistent responses to myriad operative and nonoperative interventions. This study reports the largest series of such patients with the most complete and longest follow-up to date (up to 12 years). Although others have reported a much higher incidence (60% to 80%) of symptomatic relief and palliation,[1,2,6-8] our impression in managing these patients' difficult problems was less favorable. Our tertiary referral practice and our strong interest in clinical and experimental motility disorders has allowed us to develop a large clinical experience. As we suspected, our long-term results showed operative outcomes less encouraging than those from smaller series. We achieved significant objective symptomatic relief in only 43% of the patients treated by near-total completion gastrectomy, that is, patients classified as Visick grade I or II.

Several previous reports of success have claimed much better results.[1,2,6-8] Careful analysis of these reports reveals that clinical improvement was based on subjective assessment by the patients and/or by other vague criteria. In one report the success rate was calculated as 70% based on relief of symptoms, yet only 20 (54%) of 37 patients could be classified as Visick grade I or II.[2] Indeed, although 35 of our 62 patients were classified as "failures" by our criteria (Visick grades III and IV), 13 of these 35 patients reported subjective "improvement" of their health status after near-total completion gastrectomy, five of whom currently depend on TPN to maintain their nutrition. If we were to consider subjective assessment of the quality of life as the primary indicator of success, rather than using what we maintain to be a more rigorous accurate classification system (our modified Visick grade), then 65% of our patients were improved by operative intervention. Although we acknowledge the importance of patient-directed outcomes, we perceive a need to use more stringent, objective, and realistic criteria to label patients as "successes."

Operative results may very well depend on patient selection. Indeed, Eckhauser et al.[1] warned that it was often difficult to accurately diagnose patients with postvagotomy gastroparesis. To that end, we were careful to include only those 62 patients with objective endoscopic, manometric, and/or gastric emptying studies that, along with symptomatology, supported the diagnosis. None of the 62 patients had a mechanical obstruction of the gastric outlet, although 27% had what was termed "anastomotic narrowing" by the endoscopist despite an ability to pass the endoscope into the small bowel—often associated with superficial mucosal erosions. All anastomoses easily admitted the adult-sized endoscope (diameter ~10 cm). Two potential causes of nonmechanical, functional obstruction that might have affected our results were the use of opioid narcotics and, in half of our patients, the so-called Roux stasis syndrome[11-14] (see below). Despite these considerations, all patients had lost weight and were nutritionally challenged preoperatively, necessitating consideration of surgical intervention.

The etiology of *chronic* postgastrectomy gastric stasis remains poorly understood. *All* of our patients had undergone a total abdominal vagotomy with various forms of antrectomy and reconstruction (e.g., Billroth I, Billroth II, and Roux-en-Y gastrojejunostomy). The mean number of previous gastric operations was four. No patient had been treated by gastrectomy without vagotomy. Vagal input modulates the antropyloric trituration mechanism important in emptying of solids; in addition, it mediates receptive relaxation and accommodation of the proximal stomach,[15-17] which regulates the reservoir capacity of the stomach and thereby gastric emptying of liquids. The vast majority of patients tolerate antrectomy, total abdominal vagotomy, and the resultant rapid emptying of liquids very well. Most patients do not develop any abnor-

malities of emptying of solids, and the gastric remnant appears to adapt over time to accommodate larger postprandial volumes. For unknown reasons a small subset of patients develop chronic gastric stasis after vagotomy (with or without antrectomy). In these patients the gastric remnant maintains its tone, fails to exhibit receptive relaxation, and never adapts to accommodate increasing volumes of ingested meals. Moreover, the remnant fails to empty ingested solid food, leading to the development of bezoars and/or vomiting of solid foods ingested hours to days beforehand.

A smaller group of patients with chronic postvagotomy, postgastrectomy gastric stasis may have the so-called "Roux stasis syndrome."[18] This syndrome of early satiety, vomiting, postprandial pain, and/or difficulty eating occurs in the setting of a Roux-en-Y anatomy. Experimental studies suggest that ectopic pacemakers arise in the proximal aspect of the Roux limb leading to orally directed spread of contractions, which results in a functional obstruction to gastric emptying.[16,19,20] Such patients would not be expected to improve after near-total completion gastrectomy if the cause of their gastric stasis syndrome originated in the Roux limb rather than the gastric remnant. Indeed, 53% of our 62 patients had a Roux anatomy prior to completion gastrectomy, and patients with this anatomy had a somewhat lower rate of symptomatic relief after completion gastrectomy. The literature contains conflicting reports on whether the Roux limb or the gastric remnant is the cause of chronic postvagotomy, postgastrectomy gastric stasis.[11-14,17,18] We believe that both mechanisms may coexist. Miedema et al.[21] suggest using a scintigraphic method to evaluate the Roux limb, but this is cumbersome, and few nuclear medicine laboratories perform such studies. The challenge remains how to distinguish them preoperatively.

Another enigmatic problem is how best to treat the patients classified as Visick grade III or IV after completion gastrectomy. Postoperative testing using small bowel manometry or radionuclide tests of gastric emptying must be undertaken with caution and the impact of the information obtained weighed carefully. Several studies have shown that clinical symptoms do not necessarily correlate with abnormal motility patterns or gastric emptying. Moreover, both symptomatic and asymptomatic patients have similar motility patterns.[1,5,21] The potential remedial operations available for a dysfunctional Roux limb are of questionable efficacy.[22] Our previous attempt to use an "uncut Roux limb," as in our successful dog models,[23,24] proved disappointing in clinical application because the occluding double staple line was disrupted in at least one third of patients.[11] Currently we do not recommend this procedure. This operation would be a viable option if a technique to preserve a myoneural bridge devoid of mucosa[23] can be perfected in humans.

Our poor results of near-total gastrectomy raise several questions. Our patients included a high predominance of women (82%), and this skewed gender representation is unexplained. The observation that more than one third of our patients (37%) were using some psychotropic medication preoperatively, along with frequent use of narcotic medications to alleviate abdominal pain long after operation, underscores the possibility of a psychological component or chronic pain syndrome. It remains difficult, however, to separate those patients suffering from a genuine psychological reaction secondary to a chronic disease from those with a primary psychological/psychiatric disorder. The fact that our patients had an average of four gastric operations before referral to our institution may reflect a bias toward the more severe syndromes.

Completion gastrectomy was most successful in relieving preoperative complaints of nausea, vomiting, and postprandial pain but had no significant effect on symptoms of chronic pain. Careful assessment of the type of pain experienced preoperatively is essential, as it may therefore have a strong impact on the projected outcome of completion gastrectomy. This observation is important given the tendency toward opioid dependence as a means of controlling their pain in more than half the patients we treated. The contribution of a chronic syndrome preoperatively and opioid dependence on outcomes has not been made in previous studies of postvagotomy gastric stasis.

Our aim was to determine preoperative factors that were predictive of a good outcome. Univariate analysis failed to identify any characteristic symptom, physical finding, or diagnostic test predictive of a good outcome. Some caution should be exercised in interpreting the value of the multivariate analysis, which suggested that the combination of preoperative TPN, nausea, and retained food was predictive of a poor outcome. Although our experience represents the largest series reported, the subset sample sizes were not sufficiently large to adequately explore all combinations of potentially important preoperative patient characteristics.

Although our results were disappointing, we recommend completion gastrectomy for patients with clear and objective evidence of severe postvagotomy, postgastrectomy gastric stasis who have failed all attempts at nonoperative treatment. Unfortunately the combination of preoperative characteristics in patients presenting with this syndrome do not allow for selection of patients with a greater chance of a good out-

come. Patients who suffer from opioid dependence and/or use psychotropic medications are counseled preoperatively.

In summary, patients with postvagotomy, postgastrectomy gastric stasis pose particularly complex challenges to the gastrointestinal surgeon. Careful evaluation of a functional underlying reason for the clinical symptoms of this stasis syndrome will hopefully improve the success of near-total completion gastrectomy as a palliative measure. The prevalence of preoperative narcotic dependence associated with this disease suggest that a psychological and behavioral approach similar to that in patients with chronic pain should be considered prior to completion gastrectomy, possibly with chemical dependence detoxification preoperatively as well. Completion gastrectomy ameliorates postprandial pain, nausea, and vomiting, but complete or near-total relief of symptoms (Visick grade I or II) can be expected in less than half of patients.

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Discussion

Dr. M. Hocking (Gainesville, Fla.). I compliment the authors on critically analyzing their results, but I would argue that perhaps they were too critical. Although I realize that we need objective data, I do not think we can discount the fact that two thirds of these patients were subjectively improved. The key problem lies in identifying which patients are going to improve. Approximately one third of these patients had preoperative manometry. I would be interested in whether the preoperative manometry was of any predictive value, particularly the small bowel portion of the manometry. My second question has to do with the histories of these patients. In my own review of our patients' histories, a large percentage probably had some underlying motility disorder prior to their initial operation. I wonder if you could comment about that. Finally, there was a relatively high incidence of anastomotic strictures. I was taught

by Dr. Stephen Vogel to leave enough of a cuff of stomach so that a stapled gastroenterostomy could be performed. He prefers to place a double row of staples using the GIA stapler. If we choose to perform an esophagojejunostomy, it is done in a side-to-side fashion with the linear stapler. With the use of these techniques we have had essentially no anastomotic strictures.

Dr. M. Murr (Rochester, Minn.). To be brief, the small bowel manometry did not have any predictive value for outcome after the operation. It is unclear whether these patients had a primary motility disorder that precipitated the first operation, as most of these patients had undergone previous operations at other institutions for "ulcer disease." Interestingly, eight of our patients did not have ulcer disease to start with but were operated on for conditions such as superior mesenteric artery syndrome, gastric bypass, and a potpourri of other gastric disorders that were not related to ulcers at all. Regarding anastomotic strictures, 18 patients had anastomotic strictures that were treated with endoscopic dilatation; six of them required operative revision. I think this has to do with the blood supply to the cardia that had been violated so many times prior to the index operation. We leave a cuff of cardia of approximately 1 to 2 cm to facilitate a hand-sewn, wide-mouthed anastomosis.

Dr. B. Schirmer (Charlottesville, Va.). First, in having looked at this critically, do you think that when a patient reaches Visick grade IV, perhaps we are not dealing with a surgical illness at that point, and maybe it should be considered as much a psychiatric effort as a surgical effort to cure these patients? Second, we have occasionally seen patients who achieve a dramatic reversal of some of their gastroparesis symptoms, even years later. Obviously this is postvagotomy gastroparesis, but do you have any other insight into the etiology having had such a large experience with this? We have occasionally seen patients suddenly have a remarkable reversal of their gastroparesis with pregnancy or other changes.

Dr. Murr. Concerning the etiology of the gastroparesis, all of these patients have had a vagotomy and that was the precipitating factor or one of the factors. We purposely excluded all patients with primary or diabetic gastroparesis in this study. As far as grade IV disease having an underlying psychiatric etiology, that is our concern too. There are no data to support this, but we do try to evaluate our patients preoperatively along those lines.

Dr. R. Hinder (Jacksonville, Fla.). We who have previously reported more favorable results have had a short follow-up. Your paper brings up an important lesson. In reoperative gastric surgery, it is possible to assess results within a year or two, or even three. Did you just notice deterioration with time? I would like to make a few comments; the first pertains to blood supply. In the 18 patients I reported, I very carefully checked them for ischemia at the anastomotic site and was impressed with how good the blood supply was after surgery. I think that nonsteroidal anti-inflammatory drugs (NSAIDs) may play a big part. Many of those who are surreptitious users of these nonsteroidal drugs have poor gastric motility. These pills may lie on the anastomotic site and cause strictures. Finally, you did not mention anything about gastric emptying. In our studies, we looked very carefully at gastric emptying, and most of our patients had gastric stasis. I would be curious to know if you conducted any gastric emptying studies. Was there stasis in the stomach or in the Roux loops? Was improvement related to any improvement in gastric emptying?

Dr. Murr. As far as the duration of follow-up, we have noticed a hiatus in which patients show improvement for up to 2 years after the operation, and after that they begin to have a recurrence of symptoms and problems. Most of our patients have had complete current follow-up and that would explain some of the unfavorable outcomes. Your point about NSAIDs is well taken. Regarding gastric emptying, there were 19 patients who underwent gastric emptying studies with solid and liquid markers. Results in 15 of them were grossly abnormal. Another 15 had solid marker emptying studies, and findings in all of them were also abnormal. Unfortunately, because of the retrospective nature of this study, the reports and records we abstracted did not specify whether the problem was in the stomach or in the Roux limb.

Dr. W. Richards (Nashville, Tenn.). Several years ago your group identified the length of the Roux limb as being a significant factor in patients with gastroparesis and motility problems. What steps, if any, did you take to identify the length of the already constructed Roux limb in 50% of your patients? Does that significantly alter your operative procedure? Do you tailor the Roux limb to a certain length? I assume that all of your patients now, after the total gastrectomy, undergo reconstruction with a Roux limb. How long is that Roux limb, and how important is this in the postoperative condition?

Dr. Murr. The evidence that you are referring to is experimental and refers to the Roux limb having retrograde pacesetter potentials, which may delay emptying of the stomach and/or motility of the Roux limb itself. We do not control the length of the Roux limb primarily, but keep it between 40 and 50 cm. In the patients who had Roux limbs previously, there was no record available of the length of the limb. The mean number of previous operations for our group of patients was four, and they all had different variations with respect to length or position of the Roux limb in the gastric remnant. We do not think this has significant impact on the outcome of the operation.

Dr. T. DeMeester (Los Angeles, Calif.). Have you been able to get patients off of narcotics before surgery? Do you try? How many continue to use narcotics? Second, do you think that some of your bad results are related to the presence of reflux afterward? Do you make any effort to evaluate reflux following gastrectomy?

Dr. Murr. Approximately 52% of the patients have used narcotics, and 41% continue to use narcotics postoperatively. These patients did not routinely undergo any detoxification program preoperatively. We do not perform any reflux studies postoperatively.

Dr. J. Fischer (Cincinnati, Ohio). You have made a very good case for a policy of watchful waiting, making sure there is no stricture, and if patients are using narcotics, probably nonintervention. Do you understand why these

people end up on narcotics? It seems to me that you think the reason is gastric distention, or maybe some stasis. I have been struck by the predominance of women among the patients I treat, and I have been struck by the fact that they are really addicted or habituated to narcotics. Once that happens, I agree with you that total gastrectomy is probably not appropriate.

Dr. Murr. Your comments parallel our findings. The preponderance of women is unexplained. The use of narcotics is probably multifactorial and perhaps underlies a psychological factor. We do not perform a total gastrectomy in any patient who have just recently developed gastroparesis, but reserve this procedure for those with end-stage disease who have had this problem for many years.

Effect of Microscopic Resection Line Disease on Gastric Cancer Survival

Steve H. Kim, M.D., Martin S. Karpeh, M.D., David S. Klimstra, M.D., Denis Leung, M.D., Murray F. Brennan, M.D.

To study the effect of residual microscopic resection line disease in gastric cancer, we compared 47 patients with positive margins to 572 patients who underwent R0 resections using a multivariate analysis of factors affecting outcome. Although the presence of positive margins was a significant and independent predictor of outcome for the entire group (N = 619), this factor lost significance in patients who had undergone D2 or D3 lymph node dissections (N = 466). Subset analysis within the D2/D3 group determined that this finding was limited mainly to those patients with >5 positive nodes (N = 189). The survival of patients who had ≤5 positive nodes (N = 277) was significantly worsened by a microscopically involved margin. Supporting this observation, intraoperative reexcision of microscopic disease based on frozen section analysis resulted in a significant improvement in overall survival in patients with ≤5 positive nodes but not in those with >5 positive nodes. We conclude that the significance of a positive microscopic margin in gastric cancer is dependent on the extent of disease. This factor is not predictive of outcome in patients who have undergone complete gross resection and have pathologically proved advanced nodal disease. Thus the goal in these cases should be an R0 resection when feasible but with the realization that the presence of ≥5 positive nodes (N2 disease according to the 1997 American Joint Committee on Cancer criteria) will mainly determine outcome and not microscopic residual cancer at the margin. (J GASTROINTEST SURG 1999;3:24-33.)

KEY WORDS: Gastric, adenocarcinoma, recurrence, gastrectomy

Several reports have espoused the detrimental effect of residual microscopic disease at the surgical margins on survival in patients with gastric cancer.[1-13] In an early report from this institution, Papachristou et al.[2] noted a histologically positive margin in 20% of 350 patients undergoing resection for gastric cancer. They suggested, however, that most of these patients (mainly those with stage III and IV disease) would die of distant metastases before the effect of residual microscopic disease at the margin could become manifest. Thus re-resection was not advocated. With this in mind, the goal of the present study was to define selection criteria for the aggressive treatment of patients with histologic disease at the resection margins who did not also have distant metastases. Accordingly, we performed a retrospective analysis of prospectively collected data from patients with stage II and III gastric cancer operated on at our institution during a 12-year period. Specifically, we wished to determine those stages at which a positive margin was no longer a significant predictor of diminished survival by entering known factors of poor prognosis into a multivariate analysis with margin status as a covariate. Our aim was to see if appropriate management of the microscopically positive margin could be based on factors related to the degree of locoregional spread of the primary lesion.

MATERIAL AND METHODS

Clinicopathologic Factors

Data on patient demographics, tumor characteristics, operations, TNM stage, number of involved nodes, margin status, and patient survival were ob-

From the Departments of Surgery (S.H.K., M.S.K., and M.F.B.), Pathology (D.S.K.), and Biostatistics (D.L.), Memorial Sloan-Kettering Cancer Center, New York, N.Y.

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Reprint requests: Martin S. Karpeh, M.D., Department of Surgery, Memorial Sloan-Kettering Cancer Center, 1275 York Ave., New York, NY 10021.

tained from the Prospective Database on Gastric Cancer at Memorial Sloan-Kettering Cancer Center. Primary tumor site was described as either the gastroesophageal junction or proximal one third (defined as "proximal" lesions), or the middle one third or antrum/pylorus (defined as "distal" lesions), or involving the entire stomach. Overall survival was recorded from the time of the operation to the time of death from disease. Pathologic staging was in accordance with the 1992 American Joint Committee on Cancer (AJCC) criteria.[14] Patients with stage IV disease were excluded from the analysis as were patients who had minimal stage I disease.

Operation

All patients in this study had their primary tumors resected at Memorial Sloan-Kettering Cancer Center between July 1985 and March 1997. All operations were performed for curvative intent. Operative procedures were defined as follows: esophagogastrectomy = resection of the thoracic esophagus with anastomosis to the stomach in the chest or neck; proximal gastrectomy = resection of the proximal stomach with intra-abdominal esophageal-gastric anastomosis; distal gastrectomy = resection of any portion of the distal stomach while still leaving a cuff of stomach behind; and total gastrectomy = removal of the entire stomach and proximal duodenum with esophagojejunal reconstruction. A positive margin was defined as disease present at the line of luminal transection in the mucosa, submucosa, and/or adventitia or serosa.

Survival Analysis

Survival analysis was by the method of Kaplan and Meier.[15] Factors affecting survival were analyzed by log-rank testing (univariate analysis) and the Cox regression model (multivariate analysis).[16] Chi-square analysis was used to examine correlations between clinicopathologic factors.

RESULTS

Patient and Tumor Characteristics

There were a total of 619 patients in the study with 47 patients having positive margins (7.6%). Anatomic sites of the involved margins are shown in Table I. The esophagus was the most common site of residual disease at the proximal margin, whereas the duodenum was the most frequent distal site. Patient and tumor characteristics are summarized in Table II. For this study, only patients with AJCC stage II and III disease were examined so as to specifically examine lesions that were locally advanced and had a higher risk of a positive margin but were without known metastatic disease. There were no differences in the sex ratio or median age between the positive and negative margin subsets. However, microscopic resection line disease was associated with more advanced cancers as determined by AJCC stage, tumor (T) and node (N) stage, and the median number of positive nodes. There was a significantly higher fraction of patients with lesions of Lauren's intestinal histotype in the negative margin subset. No significant differences were noted in the relative percentages of other histotypes. There was also no difference in the ratio of proximal to distal lesions, although there was a significantly higher percentage of whole stomach lesions in the group with positive margins (13% vs. 2%). This may have partly accounted for the higher rate of total gastrectomy in this subset (36% vs. 18%) (Table III). The incidences of other operations were the same in the two groups. However, D2 or D3 lymphadenectomies were more frequently used in the patients with negative margins, although there was no statistical difference in the total number of nodes removed. Patients with positive margins were also more likely to

Table I. Site of microscopically positive surgical margins (N = 47)

	Anatomic site of positive margin		
Positive margin(s)	**Esophagus**	**Stomach**	**Duodenum**
Proximal only (N = 23)	20	3	
Distal only (N = 15)		7	8
Proximal and distal (N = 9)	Proximal ◄— 2 —► Distal		
	Proximal ◄——— 4 ———► Distal		
		Proximal ◄— 3 —► Distal	

Table II. Patient and tumor factors

	Positive margins (N = 47)	Negative margins (N = 572)	*P* value
Sex			0.79
Male	34 (72%)	403 (70%)	
Female	13 (28%)	169 (30%)	
Median age (yr)	64.2	62.4	0.35
Primary tumor site			
Gastroesophageal junction	15 (32%)	248 (43%)	
Upper third	8 (17%)	84 (15%)	
Middle third	6 (13%)	97 (17%)	
Antrum/pylorus	12 (25%)	129 (23%)	
Whole stomach	6 (13%)	14 (2%)	<0.01*
AJCC stage			<0.05†
II	7 (15%)	181 (32%)	
IIIA	16 (34%)	269 (47%)	
IIIB	24 (51%)	122 (21%)	
Tumor stage			<0.01‡
T1	1 (2%)	7 (1%)	
T2	2 (4%)	126 (22%)	
T3	44 (94%)	426 (75%)	
T4	0	13 (2%)	
Node stage			
N0	5 (11%)	96 (17%)	
N1	16 (34%)	313 (55%)	<0.01§
N2	26 (55%)	163 (28%)	
Median number of involved nodes	10.0	5.5	<0.01
Lauren's histotype			
Intestinal	14 (30%)	303 (53%)	<0.01
Mixed	6 (13%)	47 (8%)	0.20
Diffuse	22 (47%)	207 (36%)	0.15
Indeterminate	2 (4%)	5 (1%)	0.09
Unknown	3 (6%)	10 (2%)	

AJCC = American Joint Committee on Cancer.
*Whole stomach lesions vs. other sites.
†Stage II vs. III and stage IIIA vs. IIIB.
‡T1/T2 lesions vs. T3/T4 lesions.
§N1 vs. N2 lesions.

have had chemotherapy, either preoperatively or within 6 months of surgery postoperatively. There was no significant difference in the use of radiation therapy between the two groups.

Survival Analysis

Univariate analysis demonstrated that sex, T stage, N stage, margin status, and type of lymphadenectomy (Figs. 1 to 5) were all significant factors predicting overall survival after gastrectomy in the entire population of 619 patients. The absolute number of positive lymph nodes was also significantly associated with survival when entered as a continuous variable. Factors not associated with survival were age (as a continuous variable; $P = 0.36$) and Lauren's histotype (intestinal vs. mixed vs. diffuse; $P = 0.065$). Although chemotherapy was more frequently used in the group with positive margins, it did not have a significant effect on overall survival ($P = 0.17$). The significant nontreatment variables as well as the type of lymphadenectomy were evaluated using the Cox model of proportional hazards, and all were found to be independently associated with poor survival (Table IV).

However, we wished to know whether a positive margin would continue to have a negative prognostic effect in the group of patients who had undergone complete nodal staging (i.e., those who had D2 or D3 lymphadenectomies). Although residual disease at the resection line continued to be associated with worse survival in this subset by log-rank testing (Fig. 6), this factor lost significance on multivariate analysis (Table V). We suspected that this was due to the more accu-

Table III. Treatment-related factors

	Positive margins (N = 47)	Negative margins (N = 572)	*P* value
Surgical procedure			
Esophagogastrectomy	16 (34%)	256 (45%)	
Proximal gastrectomy	3 (6%)	48 (8%)	
Distal gastrectomy	11 (24%)	168 (29%)	
Total gastrectomy	17 (36%)	100 (18%)	<0.01*
Lymphadenectomy			<0.01
Less than D2	20 (43%)	133 (23%)	
D2 or greater	27 (57%)	439 (77%)	
Median number of nodes removed	21	23	0.12
Radiation therapy			0.18
No	40 (85%)	524 (92%)	
Yes	7 (15%)	48 (8%)	
Chemotherapy†			<0.001
No	27 (57%)	458 (80%)	
Yes	20 (43%)	114 (20%)	

*Total gastrectomy vs. other operations.
†Postoperative within 6 months of surgery or preoperative (neoadjuvant).

Table IV. Results of multivariate analysis of prognostic factors associated with survival in entire study population (N = 619)

Factor	*P* value
No. of positive nodes	0.0001
N stage (1992 AJCC criteria)	0.0001
Type of lymph node dissection (less than D2 vs. D2 or greater)	0.0001
T stage	0.0004
Surgical margins	0.003
Sex	0.05

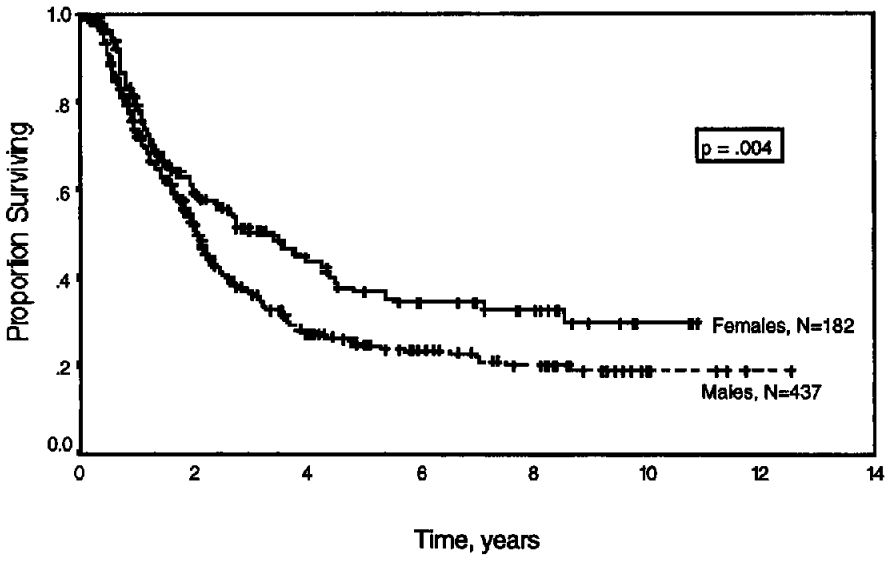

Fig. 1. Overall survival by sex for the entire study population (N = 619).

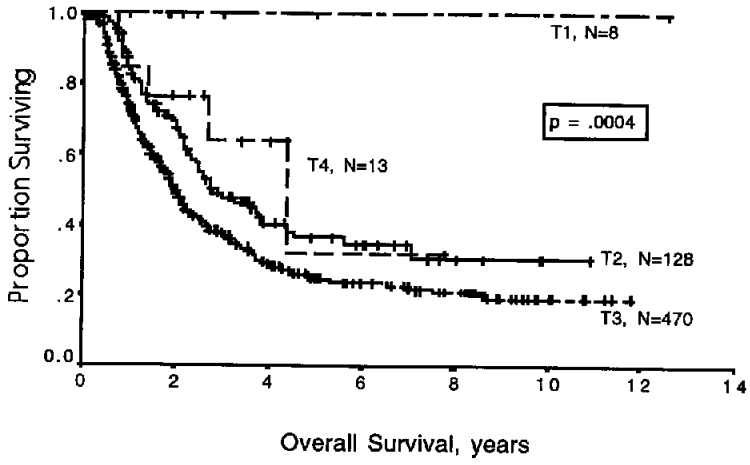

Fig. 2. Overall survival by T stage for the entire study population (N = 619).

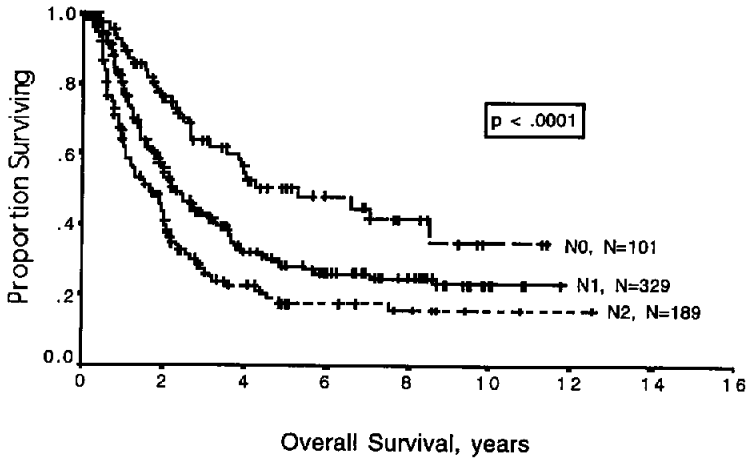

Fig. 3. Overall survival by N stage for the entire study population (N = 619).

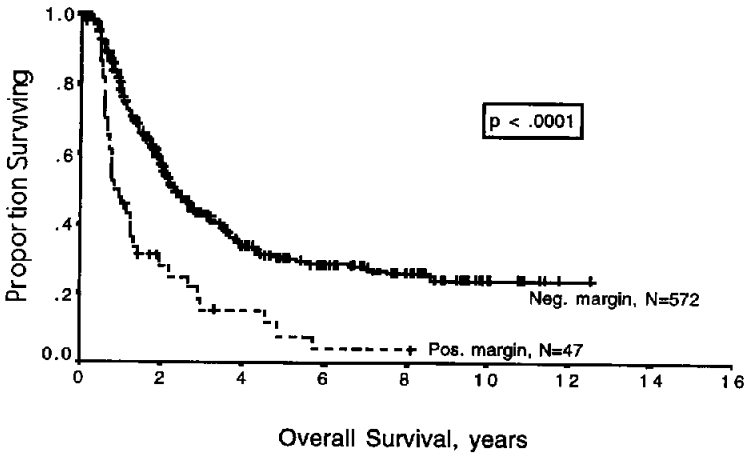

Fig. 4. Overall survival by margin status for the entire study population (N = 619).

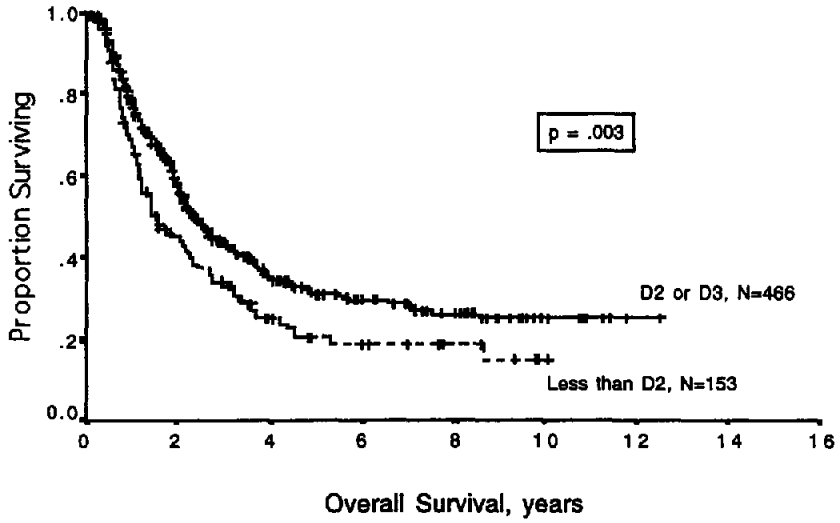

Fig. 5. Overall survival by extent of lymphadenectomy for the entire study population (N = 619).

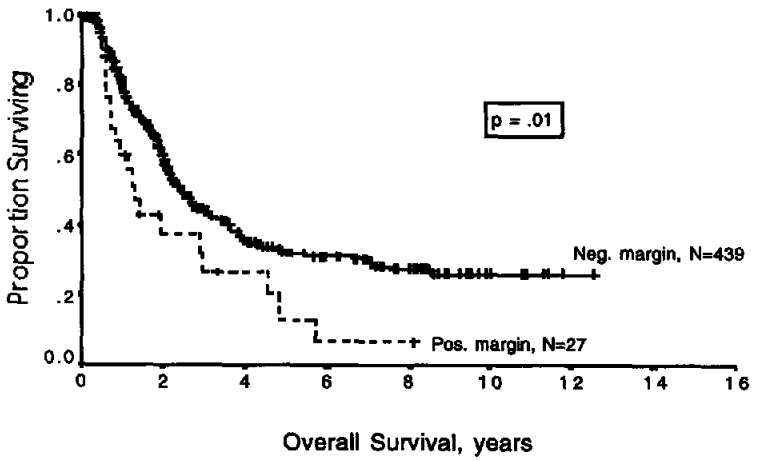

Fig. 6. Effect of positive surgical margins on survival in patients undergoing D2 lymphadenectomy (N = 466).

rate nodal staging in this group of patients. To clarify the relationship between nodal status and the effect of positive margins, a multivariate analysis of survival was performed using the median number of positive nodes in this group (N = 5) as a reference point. For patients with more than five positive nodes, there was no association between residual cancer at the surgical margin and final outcome (Fig. 7). However, those patients with lesser degrees of nodal involvement (≤5 positive nodes) had significantly improved survival if residual resection line disease was absent (Fig. 8 and Table V). This was true on both univariate and multivariate analyses.

Subsequently we compared survival between those patients who had a positive frozen section that was re-excised to a negative margin (N = 26) and those who were left with residual resection line disease (N = 27). There were no differences in final outcome between the two groups overall or in the subset of patients who had more than five positive nodes (Fig. 9). However, if ≤5 nodes were involved, there was a significant improvement in survival associated with intraoperative reexcision of the positive margin (Fig. 10). There were no significant differences in patient- and tumor-related factors between the two groups represented in Fig. 9 (Table VI).

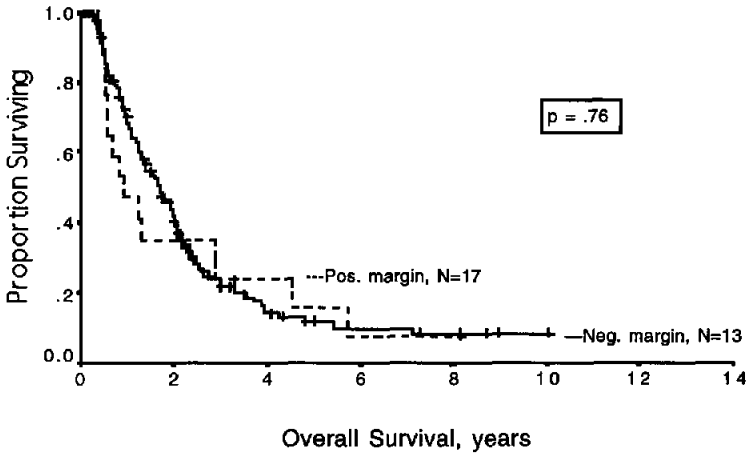

Fig. 7. Effect of positive margins on survival in patients undergoing D2 lymphadenectomy and with >5 positive nodes.

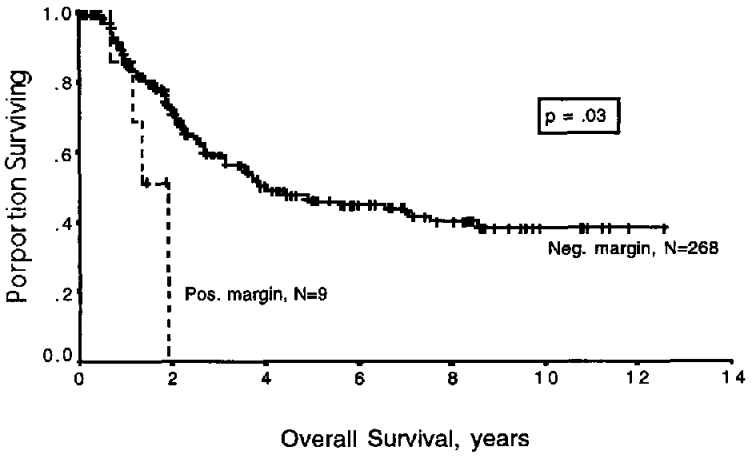

Fig. 8. Effect of positive margins on survival in patients undergoing D2 lymphadenectomy and with ≤5 positive nodes.

Table V. Results of multivariate analysis of prognostic factors associated with survival

Factor	*P* value
Patients undergoing D2 or D3 lymphadenectomies (N = 466)	
No. of positive nodes	<0.0001
N stage (1992 AJCC criteria)	0.0006
T stage	0.0015
Sex	0.04
Surgical margins	0.60
Patients with >5 positive nodes (N = 189)	
T stage	0.005
No. of positive nodes	0.015
Sex	0.02
N stage (1992 AJCC criteria)	0.07
Surgical margins	0.39
Patients with ≤5 positive nodes (N = 277)	
No. of positive nodes	0.01
Surgical margins	0.03
T stage	0.12
Sex	0.30
N stage (1992 AJCC criteria)	0.99

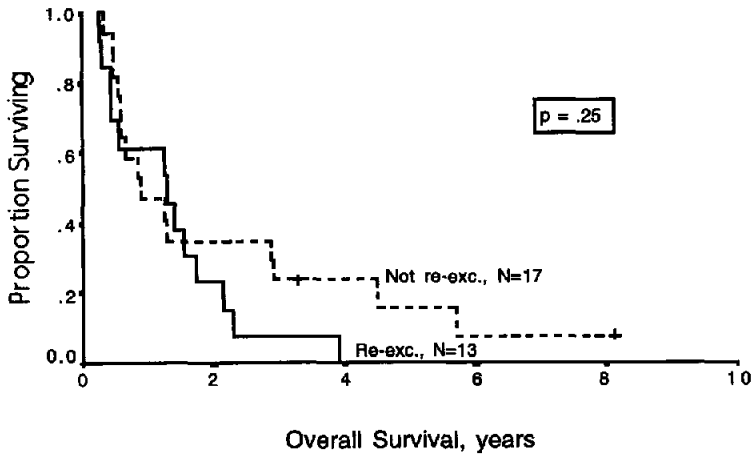

Fig. 9. Effect on survival of intraoperative reexcision of a positive margin based on frozen section analysis in patients with >5 positive nodes.

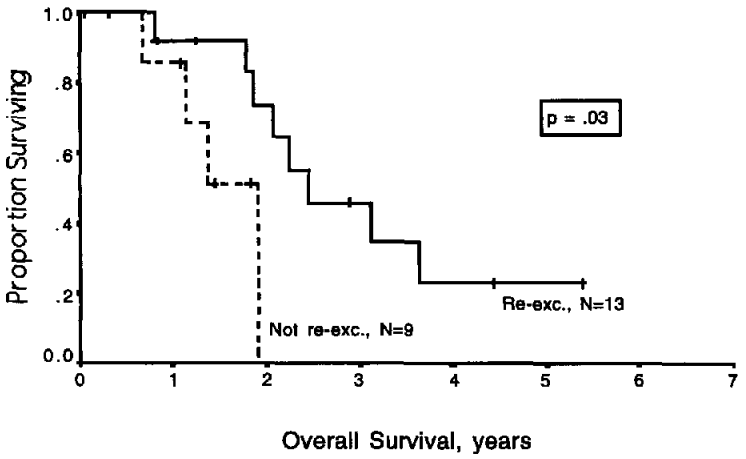

Fig. 10. Effect on survival of intraoperative reexcision of a positive margin based on frozen section analysis in patients with ≤5 positive nodes.

Table VI. Comparison of factors between patients with ≤5 positive nodes who underwent reexcision of disease on frozen section (N = 13) vs. those who did not (N = 9)

Factor	Reexcision (N = 13)	No reexcision (N = 9)	*P* value
Sex			NS
Male	11 (85%)	5 (56%)	
Female	2 (15%)	4 (44%)	
Median age (yr)	57.1	62.6	NS
Tumor site			
Proximal	11 (85%)	4 (44%)	0.06
Distal	2 (15%)	5 (56%)	
T stage			
T1	0	1 (11%)	NS
T2	3 (23%)	1 (11%)	
T3	10 (77%)	7 (78%)	
Nodal status			
Negative	1 (8%)	2 (22%)	NS
Positive	12 (92%)	7 (78%)	
Median number of positive nodes	2.2	1.8	NS

NS = not significant.

DISCUSSION

The incidence of residual microscopic resection line disease varies from 5% to 20% of resections for gastric cancer and is usually associated with "late-stage" disease. These findings were confirmed in our analysis. The incidence of this event was 7.6% in the present study and, by most criteria, patients with positive margins had more advanced disease than their counterparts who had undergone R0 resections (see Table II). There was a higher rate of D2/D3 lymphadenectomies in the latter group, although the total number of lymph nodes resected was not significantly different (see Table III). Since locoregionally advanced disease was found more frequently in patients with residual disease, this was likely secondary to surgical bias against an extensive lymph node dissection in these cases. Not surprisingly, radiation and chemotherapy were more often used in the group with positive margins, although neither treatment improved their survival.

Several large studies have demonstrated that microscopic resection line disease has deleterious consequences and is independently associated with poor prognosis.[4,5,9,11] In 1984 a report from the British Stomach Cancer Group found that this event resulted in worsening of the prognosis of patients with stage II and III disease such that it was the same as for patients with stage IV disease.[4] However, the study population was not well characterized in terms of N stage. More recently Gall et al.[10] echoed the earlier findings of Papachristou et al.[2] Among 87 patients undergoing resection for distal esophageal or gastric cancer, 10 patients (11.5%) had a microscopically positive proximal margin, and all of them had undergone palliative resection for advanced disease.[10] The majority were classified as T4 and/or N2, and three were M1. Median survival was only 8 months. The authors believed that microscopic residual disease at the resection line was inconsequential in patients with late-stage disease and advocated a conservative approach to resection, without the need for histologic clearance. In a larger more detailed study, pathologic analysis of 699 patients entered into the Dutch Gastric Cancer Trial revealed 41 patients with positive microscopic margins (6%).[11] Although this factor was independently associated with poor survival for the entire population and in those with involved nodes, subset analysis demonstrated a loss of predictive value in patients with T3 or T4 lesions or stage III or IV disease. Furthermore, nodal status was only defined as positive or negative without detailing the levels involved with disease.

Despite the preceding studies, the microscopically positive margin often poses a management dilemma for the surgeon operating on a patient with gastric cancer. For instance, reexcision of a positive proximal margin to histologically normal tissue may necessitate extension into the chest and/or a return to the operating room, relatively morbid event in the elderly population of patients with gastric adenocarcinoma. If their prognosis was defined mainly by factors related to locoregionally advanced diseased and not by microscopic disease at the surgical margin, the need for more extensive resections or re-resections would be obviated in many patients. Therefore our goal was to define strict staging criteria for the management of microscopic residual cancer at the resection line in patients without M1 disease.

Based on the preceding results, we would make the following recommendations. Patients who have undergone complete nodal staging and who have involvement of more than five lymph nodes or have N2 disease according to the new 1997 AJCC criteria[17] need undergo only gross clearance at the resection line, since their outcome will mainly be determined by whether they have locoregionally advanced disease. However, all efforts should be made to avoid a positive microscopic margin in patients with ≤ 5 positive nodes. Clearly, the two main points of difficulty will occur because margins that appear negative on frozen section may be deemed positive on permanent sections and because nodal status is often difficult to assess intraoperatively. We believe that if the patient is medically fit for reoperation, reexcision of residual resection line disease is indicated if the patient has ≤ 5 positive nodes. Although the numbers are small ($N = 6$ and $N = 20$, respectively), neither radiation nor chemotherapy was effective in treating this residual disease in our study population.

CONCLUSION

Based on the preceding results, we would make the following recommendations. When operating for curative intent, every effort should be made to obtain a frozen section on the proximal and distal margins. If confronted with a positive margin intraoperatively, the surgeon should consider the extent of gross nodal involvement in deciding how far to proceed in order to obtain a negative margin. When confronted with a positive margin on permanent section, we believe that margin should be treated if the patient has five positive nodes or less. This study does not address the best treatment for this situation; however, six patients were treated with radiation therapy and 20 patients received subsequent chemotherapy for a positive margin in the setting of less than five positive nodes. Neither treatment appeared effective in treating the residual disease in these patients.

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Discussion

Dr. L. Way (San Francisco, Calif.). In patients who had positive margins, what was the subsequent course of the disease? Did they die of recurrence at the margins? We conducted a study some years ago, and it seemed that death in the patients who had positive margins was not necessarily due to the margin disease.

Dr. S. Kim. The numbers were small, but the proportion of patients having a locoregional recurrence as opposed to a distant recurrence was not significantly different.

Dr. T. DeMeester (Los Angeles, Calif.). I always find it interesting that the number of positive nodes that seems to be significant is somewhere around five, both with gastric cancer and esophageal cancer and that area in the mediastinum. This seems to be a critical zone and as reports come in from various places, we are seeing that lymphadenectomy for patients with node disease of this nature is beneficial. Now the problem is, how do you tell? How do you determine this type of disease before surgery and when you are in the operating room? Obviously, as you have pointed out, if there is a great deal of advanced disease, the "horse" is "out of the barn" and it is not necessary to put forth that final extra effort to obtain an exact microscopic margin. But how do you know when you are in that situation? Obviously there are going to be some cases where this is very clear, but how does this transmit into practice? Are you going to consider some alternative, such as performing a much more limited operation, and what are the indications for using that technique when there is advanced disease? What will be your guidelines? Second, did you look at the ratio of lymph nodes because that would give you a little better idea how extensive the lymph node dissection would be. There are many who believe that the ratio is important because it is a measurement of the adequacy of the lymph node dissection as well as positivity.

Dr. Kim. I would like to answer the second question first. In the patients who underwent D2/D3 dissections, the median number of nodes removed was 26. The median number of positive nodes in that group was five. Therefore the ratio would be approximately 1:5. In answer to your first question, I think that every effort should be made to try to avoid a positive margin in any tumor that is considered resectable, and that requires liberal use of intraoperative frozen sections. As you mentioned, there are cases where nodal disease is seen to such an extent that there will certainly be more than five positive nodes and that will make it easier to make a decision. I think this will not be as helpful intraoperatively as postoperatively in terms of decision making, and obviously the surgeon will need to see the pathology report before any decisions can be made.

Dr. Way. As I now recollect from our previous study, the positive margin usually was something of a surprise to the surgeon and was unintended. In some cases the line of transection had to be close to the tumor for practical reasons, but in other cases the tumor seemed to have spread a much greater distance from the gross margins than is ordinarily expected. We were coming to the notion that this relatively extensive type of infiltration indicated an incurable tumor, and whether or not the margin was positive would not influence the outcome in those cases. Thus do you have any data regarding the distance of the line of transection from the gross margin of the tumor and how that correlated with outcome?

Dr. Kim. We tried to look at that. Unfortunately the pathology records are very inconsistent in terms of recording how far the margin was from the tumor, so it was impossible to collect that data for all 619 patients. I would agree that, in certain patients, a positive margin is more an indication of advanced disease rather than an independent prognostic factor in and of itself. We think this is true in those patients with more than five positive nodes.

Influence of p53 on Herpes Simplex Virus Type 1 Vectors for Cancer Gene Therapy

Sam S. Yoon, M.D., Nancy M. Carroll, M.D., E. Antonio Chiocca, M.D., Ph.D., Kenneth K. Tanabe, M.D.

Herpes simplex virus type 1 (HSV 1) vectors are under investigation for use in gene therapy for colorectal cancer liver metastases. Approximately 60% of colorectal cancers possess p53 mutations, and p53 mutations can cause tumor cell resistance to radiation therapy and chemotherapy. p53 is also known to colocalize with at least one HSV 1 protein and influence HSV 1 gene expression. The purpose of this study was to determine if the loss or mutation of p53 in tumor cells alters the cytotoxicity of HSV 1 vectors. HSV 1 vector-mediated in vitro cytotoxicity assays were performed using stable transfectants of SAOS-2-LM2 cells and WiDr cells that express no p53, wild-type p53, mutant p53, or both wild-type p53 and mutant p53. All stable transfectants were equally susceptible to HSV 1 vector cytotoxicity, and cell lines with mutant p53 were not resistant to HSV 1 vectors. These results provide additional rationale for the application of HSV 1 vector gene therapy for colorectal cancer. (J GASTROINTEST SURG 1999;3:34-38.)

KEY WORDS: HSV 1, p53, colorectal cancer, gene therapy

Colorectal cancer is the fourth leading cause of newly diagnosed cancers and the second most common cause of cancer death.[1] Up to 25% of patients with colorectal cancer have synchronous hepatic metastases, and up to 50% of patients develop metachronous liver metastases.[2] A small percentage of patients with limited liver metastases are potentially resectable for cure. However, currently available treatments for patients with unresectable liver metastases offer essentially no hope for cure.[3]

Our laboratory has been interested in the use of herpes simplex virus type 1 (HSV 1) vectors to treat liver metastases from colorectal cancer.[4-7] We previously demonstrated that an HSV 1 vector, hrR3, effectively destroys several colon carcinoma cell lines in vitro and significantly inhibits growth of the human colon carcinoma cell line HT29 in the flanks of nude mice. However, there is some heterogeneity between different colon carcinoma cell lines in susceptibility to HSV 1 vectors in vitro.[7] Approximately 60% of colon carcinomas possess mutations in the p53 gene,[8] and these mutations may affect the efficacy of HSV 1 vectors used in cancer gene therapy.

Much is already known about the normal role of the p53 tumor suppressor gene as well as its role in oncogenesis and cancer progression. The human p53 gene encodes 393 amino acids, which have been divided into four domains: (1) a transcriptional activation domain; (2) a sequence-specific DNA-binding domain; (3) a tetramerization domain; and (4) a DNA-binding activation domain. p53 functions to integrate cellular responses to stress. Signals such as DNA damage and hypoxia lead to higher p53 protein levels through increased half-life and possibly increased translation. Following p53 upregulation, p53 mediates several downstream events through transcriptional activation and protein-protein interactions that culminate in either cell cycle arrest or apoptosis. p53 mutations in tumor cells strongly select for p53 proteins that fail to bind DNA in a sequence-specific fashion and thus alter its ability to induce cell cycle arrest or apoptosis.[9,10]

From the Division of Surgical Oncology, Department of Surgery (S.S.Y., N.M.C., and K.K.T.), and the Neurosurgery Service (E.A.C.), Massachusetts General Hospital, and Harvard Medical School, Boston, Mass.
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Reprint requests: Kenneth K. Tanabe, M.D., Division of Surgical Oncology, Massachusetts General Hospital, Cox 626, 100 Blossom St., Boston, MA 02114.

In addition to its role as a transcription factor, p53 interacts with several cellular proteins as well as some viral proteins. The adenoviral protein E1B 55K binds the transcriptional activation domain of p53 and blocks its transcription factor activity.[11,12] The human papilloma virus E6 protein binds to p53 and targets it for proteolysis.[13] The SV40 large T antigen inactivates p53.[9] As for p53 interactions with HSV 1, Wilcock and Lane[14] demonstrated that following HSV 1 infection of cells, p53 co-localizes with the HSV 1 protein ICP8 along with other cellular proteins. Yuan et al.[15] and Subler et al.[16] demonstrated that wild-type p53 inhibits expression of the HSV 1 genes thymidine kinase and UL9, whereas certain mutations in p53 release this inhibition significantly.

Mutations in p53 are already known to influence tumor cell sensitivity to radiation therapy and chemotherapy.[17] In anticipation of using HSV 1 vectors in the treatment of colorectal carcinoma liver metastases, this study investigates whether the cytotoxicity of HSV 1 vectors is affected by host cell p53 expression. We demonstrate in a variety of cancer cell line stable transfectants that express no p53, wild-type p53, mutant p53, or both wild-type p53 and mutant p53 that loss or mutation of p53 does not alter HSV 1 vector cytotoxicity.

MATERIAL AND METHODS

Cell Lines and Antibodies

The African Green Monkey kidney cell line Vero was obtained from the American Type Culture Collection (Rockville, Md.). The cell lines SAOS-2-LM2, SAOS-2-LM2 $p53^{wt}$ 1, SAOS-2-LM2 $p53^{wt}$ 2, SAOS-2-LM2 $p53^{mut}$ 24, SAOS-2-LM2 $p53^{mut}$ 143, and SAOS-2-LM2 $p53^{mut}$ 175 were generously provided by Dr. Robert Radinsky (M.D. Anderson Cancer Center, Houston, Tex.). SAOS-2-LM2 is a metastatic variant of the human osteogenic sarcoma cell line SAOS-2. It was established from a lung metastasis from SAOS-2 cells injected intravenously into nude mice.[18] SAOS-2-LM2 $p53^{wt}$ 1 and SAOS-2-LM2 $p53^{wt}$ 2 are stably transfected with plasmid pC53-SN3 containing cDNA for wild-type p53 driven by the cytomegalovirus (CMV) promoter. SAOS-2-LM2 $p53^{mut}$ 24, SAOS-2-LM2 $p53^{mut}$ 143, and SAOS-2-LM2 $p53^{mut}$ 175 are stably transfected with plasmids pC53-SCX3-24, −143, and −175 containing cDNA encoding p53 with missense mutations at codon 24, 143, and 175, respectively, driven by the CMV promoter.[19] The cell lines WiDr PL and WiDr B were generously provided by Dr. Rei Takahashi (Osaka University, Osaka, Japan).[20] These two cell lines were derived from the human colon carcinoma cell line WiDr and are stably transfected with either a control plasmid pCDM8/neo (Invitrogen, San Diego, Calif.) or pCDM8-p53/neo (containing cDNA for wild-type p53 gene driven by the CMV promoter), respectively.

Vero cells were maintained in Dulbecco's modified Eagle's medium (DMEM) with 1:1 Hamm's F-12 supplement (F-12) and 8% (volume/volume) fetal calf serum (FCS). SAOS-2-LM2 cells were maintained in DMEM with 10% FCS. SAOS-2-LM2 stable transfectants were maintained in DMEM with 10% FCS and 250 μg/ml G418. WiDr stable transfectants were maintained in DMEM with 10% FCS and 200 μg/ml G418.

Viral Vectors

KOS is a wild-type strain of HSV 1. The HSV 1 vector hrR3 was generously provided by Dr. Sandra Weller (University of Connecticut Health Center, Farmington, Conn.). hrR3 is a recombinant HSV 1 vector derived from KOS. It has the *Escherichia coli* lacZ reporter gene inserted into the ribonucleotide reductase gene locus.[21] hrR3 and KOS were grown in Vero cells and titered as previously described.[22]

Virus-Mediated In Vitro Cytotoxicity Assay

Virus-mediated cytotoxicity was determined as previously described.[6] Briefly, 5000 cells per well were plated onto 96-well plates and grown for 36 hours. The medium was replaced with 50 μl DMEM/F-12 containing hrR3 or KOS at multiplicities of infection (MOI, number of plaque-forming units per cell) ranging from 0.0001 to 10. Cells were incubated at 37° C for 45 minutes with gentle shaking every 15 minutes. Fifty microliters of DMEM/F-12 with 16% FCS, 200 U/ml penicillin, and 200 μg/ml streptomycin were then added, and cells were incubated at 37° C. After 6 days, the medium was replaced with 50 μl RPMI 1640 without phenol red containing 0.5 mg/ml 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT, Sigma Chemical Co., St. Louis, Mo.), and cells were incubated for 2 hours at 37° C to allow formazan crystals to form. The medium was replaced with 50 μl dimethylsulfoxide (DMSO), and the plates were shaken vigorously. The optical density (OD) of each well was measured using an automatic plate reader (Anthos HTS, Anthos Labtes Instruments, Salzburg, Austria) at a wavelength of 550 nm with a reference wavelength of 650 nm. Percentage of cell survival was determined by dividing the $OD_{550/650}$ of hrR3-infected cells by the $OD_{550/650}$ of mock-infected cells. All experiments were performed in quadruplicate.

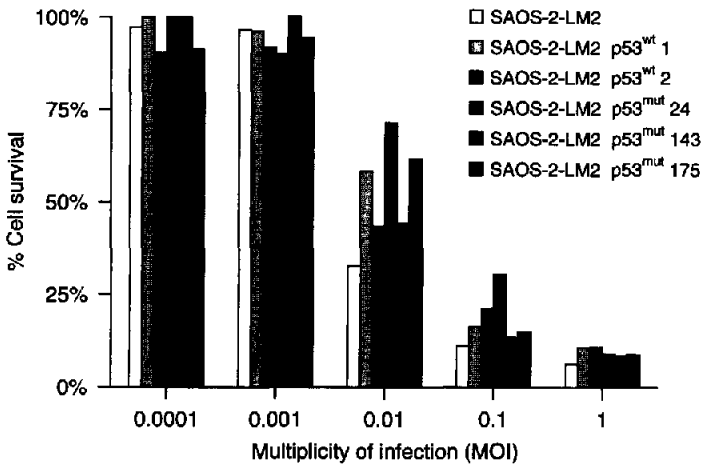

Fig. 1. hrR3-mediated cytotoxicity in SAOS-2-LM2 stable transfectants. SAOS-2-LM2 cell lines were plated onto 96-well plates and infected with hrR3 at various multiplicities of infection. Six days after infection, the number of surviving cells was quantitated by MTT assay (see Methods). Percentage of cell survival was determined compared to that of mock-infected cells.

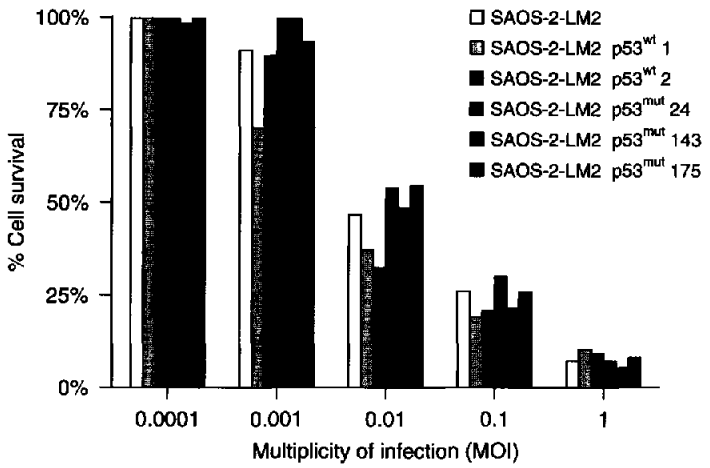

Fig. 2. KOS-mediated cytotoxicity in SAOS-2-LM2 stable transfectants. SAOS-2-LM2 cell lines were infected with KOS at various multiplicities of infection, and percentage of cell survival was determined compared to that of mock-infected cells.

RESULTS

Loss or Mutation of p53 Does Not Affect hrR3 and KOS-Mediated Cytotoxicity in SAOS-2-LM2 Cells

To determine whether changes in host-cell p53 expression alter HSV 1 vector cytotoxicity, SAOS-2-LM2 cells, which normally express no p53, and stable transfectants, which express wild-type p53 (SAOS-2-LM2 p53^{wt} 1 and SAOS-2-LM2 p53^{wt} 2) or mutant p53 (SAOS-2-LM2 p53^{mut} 24, SAOS-2-LM2 p53^{mut} 143, and SAOS-2-LM2 p53^{mut} 175), were subjected to in vitro cytotoxicity assays using hrR3 and KOS. As shown in Fig. 1, host-cell p53 status does not significantly alter hrR3-mediated cytotoxicity. The MOI causing 50% cell survival is approximately 0.01 for all cell lines, and the majority of cells are destroyed in all cell lines at an MOI of 1.0. Similarly, host cell p53 status does not significantly alter KOS-mediated cytotoxicity (Fig. 2). Again, the MOI causing 50% cell survival is approximately 0.01 regardless of whether the cell line expresses no p53, wild-type p53, or mutant p53.

Expression of Wild-Type p53 Does Not Alter hrR3 and KOS-Mediated Cytotoxicity in WiDr Cells

To determine whether p53 status may possibly affect HSV 1 vector cytotoxicity in a human colon carcinoma cell line, stable transfectants of the WiDr cell

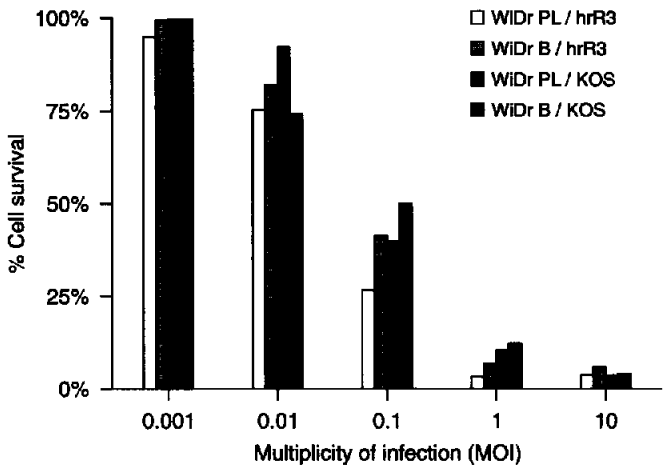

Fig. 3. hrR3 and KOS-mediated cytotoxicity in WiDr stable transfectants. WiDr PL and WiDr B cell lines were infected with hrR3 and KOS at various multiplicities of infection, and percentage of cell survival was determined compared to that of mock-infected cells.

line were studied. WiDr cells normally express a mutant p53 with a missense mutation in the DNA binding region at codon 273.[23] WiDr PL is stably transfected with a control vector, and WiDr B is stably transfected with a vector containing wild-type p53 driven by the CMV promoter so that it expresses both the aforementioned mutant p53 and wild-type p53. WiDr PL and WiDr B were subjected to an in vitro cytotoxicity assay using hrR3 and KOS. As shown in Fig. 3, stable transfection of wild-type p53 into WiDr B cells does not alter their susceptibility to hrR3-mediated or KOS-mediated cytotoxicity as compared to WiDr PL cells. Approximately 50% of both cell lines are destroyed by both hrR3 and KOS at an MOI of 0.1 and nearly 100% of both cell lines are destroyed by both hrR3 and KOS at an MOI of 1.0.

DISCUSSION

The SAOS-2-LM2 cell line does not express any p53, and various stable transfectants derived from SAOS-2-LM2 express either wild-type or mutant p53. All of these cells lines are equally susceptible to the HSV 1 vectors hrR3 and KOS. Similarly the WiDr PL cell line, which expresses a mutant p53, and WiDr B, which expresses both mutant p53 and wild-type p53, are equally susceptible to HSV 1 vector cytotoxicity.

p53 status does affect susceptibility of certain tumor cells to other types of viral vectors. A mutant adenovirus that does not express the E1B 55K protein can lyse p53-deficient tumor cells but not cells with functional p53.[24] As mentioned previously, p53 does interact with several viral proteins, including at least one HSV 1 protein, and affects the expression of HSV 1 genes. However, it does not appear that the influence of p53 on HSV 1 proteins and gene expression has an impact on HSV 1 vector-mediated cytotoxicity.

We previously demonstrated that there are differences between various colon carcinoma cell lines in susceptibility to hrR3-mediated cytotoxicity. The human colon carcinoma cell line SW620 is nearly completely destroyed at an MOI of 0.01, whereas the human colon carcinoma cell line WiDr requires an MOI of 1.0 for similar cell destruction.[7] This study examines only one possible explanation for this difference, host cell p53 status. Variability in HSV 1 vector susceptibility could be due to differences between cell lines in their accommodation of one or more of the following steps in the HSV 1 replication cycle: viral adsorption and entry, viral capsid transport to the nucleus, viral gene expression, viral protein translation, viral DNA replication and packaging, and viral particle formation.[25]

CONCLUSION

Although p53 mutations are prevalent in a majority of colorectal carcinomas and may lead to resistance to radiation therapy and chemotherapy, cancer cells with no p53 or p53 mutations are as susceptible to HSV 1 vectors as cancer cells with wild-type p53. These results provide additional rationale for application of HSV 1 vector gene therapy for colorectal carcinomas.

We thank Dr. Robert Radinsky and Dr. Rei Takahashi for providing the SAOS-2-LM2 and WiDr stable transfectants, respectively. We are also grateful to Dr. Hiroshi Eto and Dr. Seung Ho Choi for their technical advice and helpful discussion.

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Discussion

Dr. S. Leach (Nashville, Tenn.). What is the mechanism of cytotoxicity induced by this virus in human tumor cell lines? Even though there does not appear to be a correlation between p53 status and viral sensitivity, is it possible that there are different mechanisms of cytotoxicity in the different cell lines according to p53 status? Did the p53 wild-type cells undergo apoptosis? Did the p53 mutant cells die as a result of an alternative mechanism? Second, if p53 status is not the basis for sensitivity to HSV, could you suggest alternative mechanisms that would explain such divergent sensitivity?

Dr. S. Yoon. This study did not investigate the mechanism of HSV 1 vector–mediated cell death but rather quantitated the number of surviving cells by a colorimetric assay. The primary mechanism of cell death, however, is most likely viral replication and cell lysis. All cell lines and stable transfectants used in this study exhibited cytopathic effects when viewed under a microscope. We are currently investigating whether HSV 1 vectors induce apoptotic cell death in these cells. In terms of what may be the mechanism for different sensitivities to HSV 1 cytotoxicity between different colon carcinoma cell lines, I do not have an answer for you. There are many steps in the HSV 1 life cycle, and differences likely exist between cell lines in allowing these steps to occur. We are beginning a functional cloning assay to identify genes that may confer resistance to HSV 1 vectors.

Role of Alpha- and Beta-Calcitonin Gene-Related Peptide in Postoperative Small Bowel Ileus

Mark E. Freeman, M.D., Guozhang Cheng, M.D., Michael P. Hocking, M.D.

Ablation of α-calcitonin gene-related polypeptide (CGRP) containing neurons with the afferent neurotoxin capsaicin improves postoperative foregut transit in a rodent model. Similarly, administration of a selective α-CGRP antibody or $hCGRP_{(8-37)}$, a CGRP receptor antagonist, improves postoperative gastric emptying. Unlike the stomach, which contains only α-CGRP, the small bowel additionally contains β-CGRP. The role of the latter in postoperative small bowel transit is unknown. The purpose of this study was to evaluate the effect of an α-CGRP antibody and $hCGRP_{(8-37)}$ on postoperative small bowel transit. Male Sprague-Dawley rats underwent placement of duodenal catheters and were randomly assigned to 1 of 11 groups. Four groups were pretreated with 1% capsaicin. One week later, all animals underwent standardized laparotomy following administration of a control antibody or the α-CGRP monoclonal antibody, or during infusion of $hCGRP_{(8-37)}$ at varying doses. Small bowel transit was measured 25 minutes postoperatively. The α-CGRP antibody sped postoperative transit when given alone or in combination with capsaicin. In contrast, animals treated with $hCGRP_{(8-37)}$ showed no significant improvement in postoperative transit, and the beneficial effect of capsaicin was blocked. Unlike their similar effects on postoperative gastric emptying, we found that $hCGRP_{(8-37)}$ and the α-CGRP antibody had differing effects on postoperative small bowel transit. The reason for this is unknown but may be related to their differing specificities for α- and β-CGRP. (J GASTROINTEST SURG 1999;3:39-43.)

KEY WORDS: Small intestine, ileus, calcitonin, gene-related peptide

Postoperative ileus, defined as an inhibition of propulsive bowel motility resulting from surgery, is responsible for significant postoperative pain, nausea, and vomiting.[1,2] Obviously this entity is not new. Postoperative ileus was described in 1872, and as early as the 1930s reports were published on the effects of drug therapy on ileus.[3] What is new are concerns regarding the length of hospital stays, and particularly attempts to decrease the average length of stay. Postoperative ileus prolongs surgical recovery, requiring the patient to remain hospitalized for additional days of care.[4] It has been estimated that health care costs secondary to postoperative ileus exceed 750 million dollars per year in the United States.[4] No effective clinical therapy has been developed to shorten the duration of postoperative ileus, and our understanding of the pathophysiology of this process remains rudimentary.[2]

Recent studies have shown that the neuropeptide calcitonin gene-related peptide (CGRP) inhibits gastric motility following surgery, and that blockade of CGRP receptors with the CGRP receptor antagonist $hCGRP_{(8-37)}$,[5] or neutralization of CGRP with a monoclonal antibody, improves postoperative gastric motility and emptying in rodents.[6,7] CGRP is located in visceral sensory nerve fibers arising from the gut. Ablation of these fibers with the sensory neurotoxin capsaicin has been shown to improve foregut transit in a rodent model.[7] It is postulated that these visceral afferent neurons are stimulated during abdominal surgery, with the release of CGRP causing inhibition of gastrointestinal transit (directly or via reflex pathways).[8,9] We theorized that blockade of CGRP would improve postoperative small bowel transit in a manner similar to its effect on postoperative gastric emptying.

The purpose of this study was to evaluate the effect of the CGRP receptor antagonist $hCGRP_{(8-37)}$, and the α-CGRP monoclonal antibody No. 4901,[10] on postoperative small bowel transit in a rodent model of postoperative ileus.

From the Department of Surgery, VA Medical Center and the University of Florida, Gainesville, Fla.
Presented at the Thirty-Ninth Annual Meeting of The Society for Surgery of the Alimentary Tract, New Orleans, La., May 17-20, 1998.
A portion of this study was published as an extended abstract in *Surgical Forum* 47:188-189, 1998.
Reprint requests: Michael P. Hocking, M.D., Box 100286, University of Florida Health Science Center, Gainesville, FL 32610.

MATERIAL AND METHODS

Animals

Male Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, Ind.), weighing 275 to 300 g, were randomly assigned to 1 of 11 groups (Fig. 1). The animals were deprived of food but not water for 18 hours prior to surgery. All studies were approved by the Subcommittee on Animal Care at the Gainesville Veterans Administration Medical Center, and conducted in accordance with federal guidelines.

Intestinal Transit Studies

Polyethylene catheters were placed in the duodenum through a midline incision, with the rats under ketamine anesthesia (75 mg/kg intraperitoneally, Parke-Davis, Morris Plains, N.J.), as described by Weisbrodt et al.[11] At this time four groups were randomly assigned to also receive capsaicin treatment to ablate sensory afferent fibers to the foregut, as described by Plourde et al.[7] Briefly, parafilm was placed around the celiac and superior mesenteric ganglia, and a pledget of cotton wool soaked with capsaicin (1%) and vehicle (10% Tween 80, 90% olive oil) was maintained around the ganglia for a total of 30 minutes. Additional drops of capsaicin were applied at 10-minute intervals but did not exceed a total volume of 0.1 ml (1 mg/animal). After treatment, the area was thoroughly rinsed with sterile saline solution. The remaining seven groups underwent the same protocol utilizing a pledget soaked in vehicle only.

Six days later the animals were again anesthetized with ketamine, and a polyethylene external jugular catheter was placed to provide intravenous access. The catheters were filled with heparinized saline, occluded with a knot, and exteriorized at the base of the neck. The animals were allowed to recover overnight.

The following day the animals underwent standardized laparotomy under thiopental (40 mg/kg intraperitoneally) anesthesia. The laparotomy consisted of a midline incision, manipulation of the stomach, small bowel, and colon for 1 minute each, evisceration of the small bowel for 5 minutes, and abdominal closure.

$hCGRP_{(8-37)}$

Control animals received an infusion of 0.1% bovine serum albumen (BSA) (300 μl/hr for 90 minutes) via the jugular catheter, starting 30 minutes prior to laparotomy, while study animals received $hCGRP_{(8-37)}$ (Sigma Chemical, St. Louis, Mo.) at four doses, with the infusion also starting 30 minutes prior to laparotomy. The very-low-dose hCGRP group received a 0.05 nmol intravenous bolus of $hCGRP_{(8-37)}$ followed by a continuous infusion of 0.1 nmol/hr for 90 minutes, whereas animals in the low-dose group received a 0.5 nmol bolus followed by an infusion of 1.0 nmol/hr. The medium-dose animals received a 5.0 nmol bolus of $hCGRP_{(8-37)}$ followed by an infusion of 10 nmol/hr for 90 minutes. Finally, animals in the high-dose group received a 50 nmol bolus followed by an infusion of 100 nmol/hr. Two separate groups of rats pretreated with capsaicin received 0.1% BSA vehicle or the medium dose of $hCGRP_{(8-37)}$ as outlined above.

α-CGRP Antibody No. 4901

Control animals received KLH monoclonal antibody (2 mg in 0.5 ml saline) as an intravenous bolus 30 minutes prior to laparotomy. The α-CGRP antibody group received purified α-CGRP monoclonal antibody No. 4901 (2 mg in 0.5 ml saline) preoperatively in a similar manner. (Both the control and α-CGRP antibodies were supplied by Dr. Helen Wong, UCLA, Los Angeles, Calif.[10]) Capsaicin control animals received KLH antibody (2 mg in 0.5 ml saline), while the capsaicin/α-CGRP antibody group received the purified α-CGRP monoclonal antibody No. 4901 (2 mg in 0.5 ml saline) as an intravenous bolus.

Fifteen minutes later, a 0.2 ml bolus of $Na^{51}CrO_4$ (ICN Radiochemicals, Irvine, Calif.) in saline was instilled via the duodenal cannula. The animals were killed with sodium pentobarbital (50 mg/kg intravenously, Abbott Laboratories, N. Chicago, Ill.) 25 minutes following the administration of $Na^{51}CrO_4$. The small bowel was then carefully ligated, excised, and divided into 10 equal segments. The radioactivity in each segment was determined with a gamma counter (Beckman Gamma 5500, Beckman Instruments, Inc., Irvine, Calif.), and expressed as the percentage of activity per segment. Small bowel transit was then calculated as the geometric center of radiolabel distribution. The geometric center is equal to the sum of the fraction of radioactivity per segment multiplied by the segment number and represents the median of radiolabel transit. The geometric center has previously been shown to accurately represent small bowel transit,[12] with higher values reflecting faster transit.

Statistical Analysis

All data are expressed as means ± standard error of the mean. The 95% confidence interval was chosen as the level of significance. Statistical analysis was per-

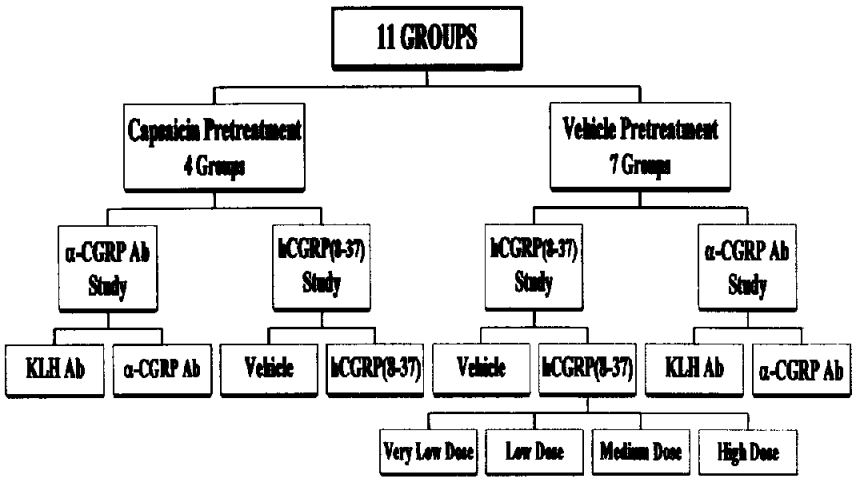

Fig. 1. Study groups.

Table I. Effect of $hCGRP_{(8-37)}$ on postoperative small bowel transit

Group	Bolus (nmol)	Infusion (nmol/hr)	Geometric center
Control	—	—	2.44 ± 0.24
Very low dose	0.05	0.1	2.25 ± 0.21
Low dose	0.5	1.0	2.24 ± 0.13
Medium dose	5.0	10.0	2.29 ± 0.21
High dose	50.0	100	1.90 ± 0.14
Capsaicin alone	—	—	*3.67 ± 0.12
Capsaicin + medium dose	5.0	10.0	2.06 ± 0.19

*$P < 0.001$ vs. all other groups.

formed by one-way analysis of variance to determine significant differences between multiple groups, followed by Tukey's post hoc test, using a statistical software program (Epistat Services, Richardson, Tex.).

RESULTS

hCGRP

The geometric center of radiolabel transit was 2.44 ± 0.24 in the non–capsaicin-pretreated control animals (Table I). None of the four non–capsaicin-pretreated groups given varying doses of $hCGRP_{(8-37)}$ showed any significant alteration in postoperative small bowel transit. Pretreatment with capsaicin alone resulted in a significant improvement in early postoperative small bowel transit ($P < 0.001$ compared to all other groups), with a geometric center of 3.67 ± 0.12. When capsaicin-pretreated animals were given medium-dose hCGRP, however, this improvement in postoperative small bowel transit was lost, with a geometric center of 2.06 ± 0.19.

α-CGRP Monoclonal Antibody No. 4901

The geometric center of the non–capsaicin-pretreated control animals given the nonspecific KLH antibody was 1.92 ± 0.19 (Fig. 2), compared to the non–capsaicin-pretreated animals given the α-CGRP monoclonal antibody, which had a significantly improved geometric center of 3.16 ± 0.15. Unlike the animals given $hCGRP_{(8-37)}$, there was no loss of the beneficial effect of capsaicin pretreatment on postoperative small bowel transit when the animals were given the α-CGRP monoclonal antibody with a geometric center of 2.63 ± 0.28.

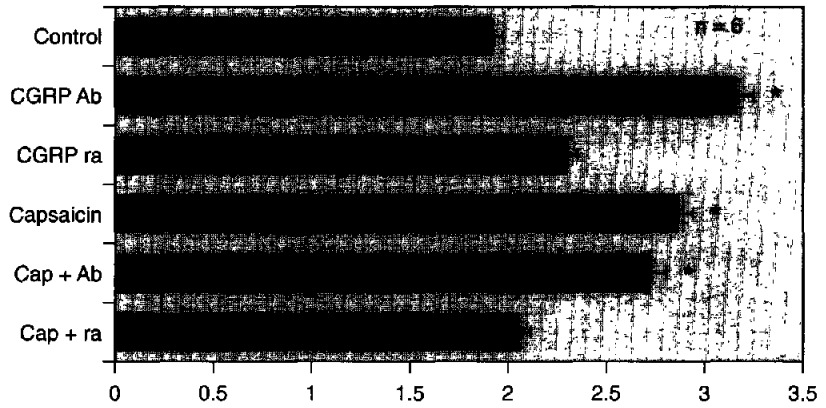

Fig. 2. Effect of α-CGRP antibody on postoperative small bowel transit. Geometric center (means ± standard error of the mean). *=P <0.05 vs. control (analysis of variance).

DISCUSSION

In this study we found a significant improvement in postoperative small bowel transit following the administration of a selective α-CGRP antibody, similar to its beneficial effect on postoperative gastric emptying in previous studies in rodents.[6,7] Although these studies showed improved postoperative gastric emptying and motility with both the antibody and the CGRP receptor antagonist, $hCGRP_{(8-37)}$,[6,7] we were unable to demonstrate improved postoperative small bowel transit following treatment with the receptor antagonist. The reason for these conflicting results is unknown. A possible explanation may be related to differences in the distribution of CGRP subtypes in the gut.

CGRP is present in two forms in the gastrointestinal tract: α-CGRP, which is located within extrinsic afferent neurons throughout the gut, and β-CGRP, which is found within myenteric neurons in the small bowel and colon but not in the stomach.[13,14] In addition to these two forms of the neuropeptide, two CGRP receptors have been identified: CGRP-1 and CGRP-2.[15,16] α-CGRP appears to preferentially bind to CGRP-1 receptors, whereas β-CGRP appears to bind most avidly to CGRP-2 receptors.[17] $hCGRP_{(8-37)}$ is known to preferentially bind CGRP-1 receptors, but also binds with a 10- to 50-fold lower affinity for CGRP-2 receptors.[16,18] It may, therefore, potentially block the actions of both α- and β-CGRP, depending on the dose used. The recently described α-CGRP monoclonal antibody has a higher affinity for α-CGRP, with a median effective dose for α-CGRP and β-CGRP of 350 and 4000 pg/ml, respectively, and therefore would appear to be more selective for the action of α-CGRP than $hCGRP_{(8-37)}$.[10]

Unfortunately no selective β-CGRP antagonist exists. However, α-CGRP can be eliminated from the foregut by pretreating animals with the afferent neurotoxin capsaicin, which selectively destroys sensory neurons containing α-CGRP, without altering β-CGRP within myenteric neurons of the small bowel.[19-21] A prior study had shown that ablation of capsaicin-sensitive nerves resulted in improved postoperative foregut transit.[7] Pretreatment of our animals by local application of capsaicin to the celiac and superior mesenteric ganglia did result in a significant improvement in postoperative small bowel transit. Fascinatingly, however, when capsaicin-pretreated animals were given $hCGRP_{(8-37)}$, this beneficial effect was lost. This effect was not seen with the antibody. The reason for the blocking action of $hCGRP_{(8-37)}$ on the prokinetic effect of capsaicin is unknown. One explanation is that $hCGRP_{(8-37)}$ may have an agonist effect on CGRP-1 receptors as a result of denervation hypersensitivity following ablation of α-CGRP–containing neurons. However, this would not explain the lack of benefit of $hCGRP_{(8-37)}$ on postoperative small bowel transit in non–capsaicin-pretreated animals, as we used the same dose that improved postoperative gastric motility in a previous study.[6] We believe a more likely explanation lies in the difference in specificity of $hCGRP_{(8-37)}$ and the α-CGRP antibody. $HCGRP_{(8-37)}$, at the doses used, may not only have blocked CGRP-1 receptors for α-CGRP but also blocked CGRP-2 receptors for β-CGRP located within the small bowel myenteric plexus. As the stomach does not contain β-CGRP,[19-21] and by inference CGRP-2 receptors, this effect would not be expected in the stomach. If the lack of benefit of $hCGRP_{(8-37)}$ is due to blockade of both CGRP-1 and CGRP-2 receptors, this would suggest that these receptors, and by implication α- and β-CGRP, have opposing effects on postoperative small bowel transit. Confirmation of this theory must await the development of a selective

β-CGRP antibody or CGRP-2 receptor antagonist. The fact that an inhibitory neuropeptide such as β-CGRP may have a beneficial effect on postoperative ileus is not without precedent, as the inhibitory peptide somatostatin has been shown experimentally to decrease the length of postoperative ileus.[22]

CONCLUSION

In summary, similar to previous studies on postoperative gastric emptying and motility, administration of the α-CGRP monoclonal antibody No. 4901 and selective ablation of α-CGRP with the afferent neurotoxin capsaicin improved small bowel transit in a rodent model of postoperative ileus, supporting a role for α-CGRP in the pathophysiology of postoperative small bowel ileus. In contrast, the CGRP receptor antagonist, $hCGRP_{(8-37)}$, did not improve postoperative small bowel transit and, furthermore, blocked the beneficial effect of capsaicin pretreatment. The mechanism of this effect is unknown, but we postulate that $hCGRP_{(8-37)}$ may block CGRP-2 receptors for β-CGRP within the small bowel myenteric plexus.

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Laparoscopic Cholecystectomy as a "True" Outpatient Procedure: Initial Experience in 130 Consecutive Patients

Keith D. Lillemoe, M.D., John W. Lin, M.Eng., Mark A. Talamini, M.D., Charles J. Yeo, M.D., Douglas S. Snyder, M.D., Stephen D. Parker, M.D.

Laparoscopic cholecystectomy has received nearly universal acceptance and is currently considered the "gold standard" for the treatment of cholelithiasis. Many centers have employed "short-stay" units or "23-hour admissions" for postoperative observation following laparoscopic cholecystectomy. The practice of early discharge as "true" outpatients following this procedure has not been well defined. A retrospective analysis of 130 consecutive patients undergoing laparoscopic cholecystectomy in an outpatient surgery unit was performed. A follow-up telephone survey was carried out of patients who successfully completed the procedure as outpatients. One hundred thirty patients underwent outpatient laparoscopic cholecystectomy. The patient population consisted of 78% women, with an age range of 17 to 76 years (mean age 47.1 years). Symptomatic gallstone disease was the indication for laparoscopic cholecystectomy in 92% of the patients. All patients underwent successful completion of laparoscopic cholecystectomy with no conversions to an open procedure. The mean length of operation was 75 ± 23 minutes (range 25 to 147 minutes). The mean length of stay in the postanesthesia care unit (PACU) ranged from 95 to 460 minutes with a mean length of stay of 200 ± 79 minutes. A total of eight patients (6.2%) were admitted to the hospital directly from the PACU in the immediate postoperative period. Six of these eight patients were discharged on the first postoperative day. Following discharge from the PACU, an additional six patients (4.6%) required hospital admission. Three of these six patients were discharged after a single day of hospitalization. Ninety-eight of 116 eligible patients were available for follow-up telephone evaluation. The outpatient experience was rated as good by 75.5% of the patients, fair by 22.5%, and poor by 2%. In retrospect, 20.4% of the patients stated that they would have preferred an inpatient to an outpatient procedure. Laparoscopic cholecystectomy can be performed as a true outpatient procedure with patients discharged to home within hours of completion of the procedure. Less than 10% of patients will fail this protocol and another 5% of the patients may require hospitalization after returning to their homes. (J GASTROINTEST SURG 1999;3:44-49.)

KEY WORDS: Laparoscopic cholecystectomy, outpatient surgery, gallstones

The introduction of laparoscopic cholecystectomy in the late 1980s dramatically changed the management of symptomatic gallbladder disease.[1] In less than a decade, the procedure has received near-universal acceptance and is currently considered the "gold standard" for treatment of cholelithiasis.[2] The advantages of laparoscopic cholecystectomy over open cholecystectomy have been well defined in a number of prospective, randomized studies. These advantages include earlier return of bowel function, less postoperative pain, shorter hospital stay, more rapid return to full activity, and decreased overall costs.[3-8]

Although the operation itself has remained essentially unchanged since its introduction, there has been significant evolution in the manner in which patients undergoing laparoscopic cholecystectomy are managed.[9] In its early application, all patients were hospitalized overnight following the procedure. More recently there has been a shift from hospital admission to the use of "short-stay units" or "23-hour admis-

From the Departments of Surgery (K.D.L., J.W.L., M.A.T., and C.J.Y.) and Anesthesiology (D.S.S. and S.D.P.), The Johns Hopkins Medical Institutions, Baltimore, Md.

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Reprint requests: Keith D. Lillemoe, M.D., Professor and Vice-Chairman, Department of Surgery, Johns Hopkins Hospital, Blalock 679, 600 N. Wolfe St., Baltimore, MD 21287-4679.

sions," in hopes of providing an economic advantage to the laparoscopic approach. The performance of laparoscopic cholecystectomy as a "true" outpatient procedure with a short postoperative stay, however, has not been well defined, and this practice is not universally applied. It is the goal of this report to review the experience with short-stay outpatient laparoscopic cholecystectomy at an academic health center.

METHODS

During the 25-month period between April 1996 and April 1998 inclusive, 130 patients underwent laparoscopic cholecystectomy at The Johns Hopkins Outpatient Center. These outpatient operating rooms are part of a separate facility immediately adjacent to The Johns Hopkins Hospital and therefore have access for immediate hospital admission if needed for complications and/or other indications for admission. Patients were selected for outpatient laparoscopic cholecystectomy based on their surgeon's preference and the patient's willingness to accept the outpatient procedure. The only established requirement for performing the procedure on an outpatient basis was the availability of a responsible adult to be with the patient at home on the night following the procedure. Patients underwent standard preoperative evaluation and instruction by the attending surgeon and nursing personnel concerning outpatient surgery in general, and laparoscopic cholecystectomy specifically.

Laparoscopic cholecystectomy was performed using a standard four-trocar technique that employed 12 and 5 mm trocars and carbon dioxide insufflation. All procedures were performed by the attending staff of The Johns Hopkins Hospital with surgical house staff participating, as appropriate, based on their experience and the nature of the case.

All patients received the 5-hydroxytryptamine type 3 antagonist ondansetron (4 mg) prior to the induction of general anesthesia for antinausea prophylaxis. Midazolam (2 mg) was given as monitors were applied in the operating room. Induction of anesthesia was accomplished with propofol and muscle relaxation with mivacurium. Following endotracheal intubation, patients were maintained on an infusion of propofol and mivacurium. Patients were mechanically ventilated with a 70%/30% mixture of nitrous oxide and oxygen. An orogastric tube and urinary catheter were placed after endotracheal intubation and were removed at the completion of the procedure. Fentanyl (100 to 250 μg) was given intraoperatively as was ketorolac, 15 or 30 mg. Bupivicaine (0.5%) was sprayed into the gallbladder bed and between the right hemidiaphragm and the liver following removal of the gallbladder. Trocar sites were also infiltrated with bupivicaine. Neuromuscular blockade was not reversed. Propofol and mivacurium infusions were discontinued following removal of the gallbladder.

Patients were extubated in the operating room and taken to the postanesthesia care unit (PACU) and monitored per established protocols. While patients were in the PACU, nurses made assessments of pain and nausea/vomiting on a scheduled basis. Pain was controlled with small (25 μg) doses of fentanyl. Rescue antiemetic therapy in the PACU consisted of additional doses of ondansetron (4 mg), metroclopramide (10 mg), or promethazine (12 to 25 mg) at the discretion of the anesthesiologist. Patients were discharged when they were able to meet standard discharge criteria (i.e., adequate pain control, ability to stand, ambulate, void, and tolerate oral liquids).

Patients were given instructions to contact their attending surgeon if they developed fever, chills, evidence of bile drainage from the incision, significant nausea and/or vomiting, or abdominal pain. Patients were routinely contacted on the first postoperative day by the outpatient nursing staff, but home visiting nurses were not used. Patients were reevaluated as needed for symptoms or routinely at approximately 3 to 4 weeks postoperatively by their attending surgeon.

To assess their opinion of short-stay outpatient laparoscopic cholecystectomy, patients were contacted by an independent observer and asked a series of questions concerning their satisfaction with the outpatient procedure. Patients were asked if any problems occurred during the immediate postoperative period, and their opinion concerning whether they would choose to have this procedure performed on an outpatient basis again, knowing the experience.

Data are expressed as the mean ± the standard deviation. Statistics were performed using a STATA package (STATA Corp., College Station, Tex.). Clinical significance was reached with a P value of <0.05 using Pearson's chi-square for the yes/no variables and the two-tailed t test for continuous variables.

RESULTS

During the 25-month period, 130 patients underwent outpatient laparoscopic cholecystectomy. During the same time period, 279 patients underwent laparoscopic cholecystectomy as hospital inpatients (120 with a 1 night hospital stay). The patient population consisted of 102 women (78%) with an age range of 17 to 76 years (mean age 47.1 years). The American Society of Anesthesiologists (ASA) classification of the patients found that 15% were ASA I, 72% were ASA II, and 13% were ASA III.[10]

The indication for a cholecystectomy was symptomatic gallstone disease in 92% of patients. Six

percent of these patients had a history of gallstone pancreatitis. Chronic acalculous cholecystitis or biliary dyskinesia was the indication for cholecystectomy in the remaining 8% of the patients. Twenty-four percent of the patients had had previous abdominal surgery and 14% of patients were considered obese, defined as more than 25% above ideal body weight.

The duration of the laparoscopic cholecystectomy ranged from 25 to 147 minutes. The mean length of operation was 75 ± 23 minutes. Intraoperative fluorocholangiography was performed in 7.7% of patients. In 5.3% of patients an umbilical hernia repair was performed at the time of closure of the supraumbilical trocar site. A needle biopsy of the liver was performed in 2.3% of patients. In two patients (1.5%) closed suction drains were placed because of concern for bleeding.

No patient underwent conversion to an open cholecystectomy. The single intraoperative complication noted was bleeding from a lateral trocar site, which required placement of mattress sutures and a closed suction drain, which also led to hospital admission for observation.

The length of stay in the PACU ranged from 95 to 460 minutes with a mean length of stay of 200 ± 79 minutes. The only postoperative complication noted in the PACU was a patient in whom prolonged muscle relaxation prohibited extubation. The patient required prolonged mechanical ventilation in the recovery room until relaxation was reversed. The patient was then extubated but was admitted to the hospital for overnight observation. She was eventually proven to have a cholinesterase deficiency.

A total of eight patients (6.2%) were admitted to The Johns Hopkins Hospital from the PACU in the immediate postoperative period (Table I). The indications for admission included suspected common bile duct stones in three patients, observation for bleeding in two patients, and abdominal pain and shortness of breath with the eventual diagnosis of a cystic duct leak in one patient. One patient was admitted for protracted nausea and vomiting. The final admission was the patient with cholinesterase deficiency who was admitted for postoperative observation.

Six of the eight patients admitted were discharged on the first postoperative day. Two of the patients had endoscopic retrograde cholangiopancreatography performed on the day after admission, with one patient discharged the same day after a normal examination and the other discharged on the next day (postoperative day 2). The single patient with the cystic duct leak required percutaneous transhepatic biliary drainage and eventual laparotomy for drainage of an infected biloma, with a total hospital stay of 29 days.

Following discharge from the PACU, an additional six patients (4.6%) required hospital admission after they returned home. The indication for admission in three patients was protracted nausea and vomiting. In one other patient significant abdominal pain led to admission. These four patients were admitted on days 1, 1, 3, and 4, respectively, following laparoscopic cholecystectomy. In all four patients these symptoms developed shortly after the patients returned home following laparoscopic cholecystectomy, and therefore these patients may have benefitted from immediate hospital admission. The other two patients were admitted with a late cystic duct leak (postoperative day 30) and pancreatitis (postoperative day 32). The length of stay for patients admitted following discharge ranged from 1 to 6 days, with three patients discharged after a single day of hospitalization. Only the patient with a cystic duct leak required an invasive procedure, which was the placement of an endoscopic stent to control the leak and percutaneous drainage of a biloma.

Table I. Indications for hospital admission

Indication	No.	(%)
From PACU		
Total admissions	8	(6.2)
Suspected common bile duct stone	3	(2.3)
Observation for bleeding	2	(1.5)
Cystic duct leak	1	(0.8)
Cholinesterase deficiency	1	(0.8)
Nausea/vomiting	1	(0.8)
From home		
Total admissions	6	(4.6)
Nausea and vomiting	3	(2.3)
Abdominal pain	1	(0.8)
Cystic duct leak	1	(0.8)
Pancreatitis	1	(0.8)

PACU = postanesthesia care unit.

To assess patient satisfaction following outpatient laparoscopic cholecystectomy, an independent observer attempted to contact by telephone all patients who successfully underwent laparoscopic cholecystectomy without hospital admission. Ninety-eight (84.5%) of 116 patients were contacted. In response to a standard series of questions, 75.5% of patients rated their experiences as good, 22.5% as fair, and 2% as poor. Poor pain control after hospital discharge was cited by 13.3% of patients, and 12.2% reported significant nausea and vomiting.

In retrospect, 20 (20.4%) of the 98 patients interviewed stated that they would have preferred an inpatient to an outpatient experience. Only 2 of these 20 patients, however, described their outpatient experi-

Table II. Comparison of patients admitted vs. not admitted following outpatient laparoscopic cholecystectomy

	Admitted	Not admitted	*P* value
No. of patients	14	116	NS
Age (yr)	49.6 ± 16.9	47.3 ± 13.9	NS
Females (%)	64	80	NS
Gallstones (%)	100	91	NS
ASA class			
I	14	15	NS
II	71	72	NS
III	14	12	NS
Operative time (min)	90 ± 24	73 ± 22	<0.05
PACU time (min)	249 ± 129	195 ± 70	NS
Drain placed (%)	100	0	<0.05

ASA = American Society of Anesthesiologists; PACU = postanesthesia care unit; NS = not significant.

ence as being poor. The reasons for preferring an inpatient procedure included significant anxiety in 35% of these patients, postoperative pain in 30%, and the feeling of being a burden to their family in 5%.

To determine if any factors such as patient demographics, length of the operative procedure, PACU stay, or operative factors contributed to a greater likelihood of admission, a statistical comparison was performed between those patients admitted (n = 14) vs. those patients not admitted (n = 116) following outpatient laparoscopic cholecystectomy (Table II). There was no difference in age, sex, indication for surgery, ASA class, or length of PACU stay between those patients admitted and those not admitted. The operative procedure lasted significantly longer (90 ± 24 minutes vs. 73 ± 22 minutes; $P < 0.05$) in those patients admitted to the hospital following laparoscopic cholecystectomy. In addition, both patients in whom a drain was placed for postoperative observation for bleeding were admitted.

DISCUSSION

Currently, laparoscopic cholecystectomy is almost universally applied and is considered by most to be the "gold standard" for the treatment of symptomatic gallbladder disease. In 1992, without the availability or large controlled trials, the National Institutes of Health Consensus Development Conference concluded that laparoscopic cholecystectomy "provides a safe and effective treatment for most patients with symptomatic gallstones. Indeed it appears to have become the procedure of choice for many of these patients."[11] Although recently, with improved equipment, there has been enthusiasm for a new laparoscopic approach using smaller (2 mm) trocars,[12,13] the operative technique has changed little since its introduction in this country.[14] Despite this, there has been significant evolution in patient management. Initially all patients were hospitalized at least overnight following the procedure. More recently there has been a shift from hospital admission to the use of "short-stay units" or "23-hour admissions" in an attempt to further decrease costs associated with the procedure.

In recent years a number of series have reported the performance of "true" outpatient laparoscopic cholecystectomy, in which the patients were discharged within hours of completion of the procedure. One of the largest experiences with this practice has been reported by Voitk.[15,16] In both an initial experience with 100 patients[15] and a follow-up series of 273 patients,[16] 94% and 95% of procedures, respectively, were successfully completed in the outpatient setting. Furthermore, this author analyzed a subgroup of patients over the age of 70 years with an ASA classification of III or greater.[17] In this series of 87 high-risk patients, 61 (70%) underwent successfully completion as outpatients, with no complications or readmissions. Nine patients were admitted simply as a "precaution." Only one of these patients demonstrated a need for admission, suggesting that the other eight patients could have also been managed as outpatients.

Similar experiences have been reported from other centers. Narain and DeMaria[18] reported that 58 (97%) of 60 patients were successfully discharged after an average stay of 3 hours. Three additional patients (5%), however, did require readmission after discharge. Lam et al.[19] discharged 97% of patients with a mean recovery period of 6.6 hours, whereas Mjaland et al.[20] discharged 94% of 200 patients between 4 and 8 hours after completion of the procedure. In the latter series, however, another 8% were readmitted after discharge. Mjaland et al.[20] also completed a patient satisfaction questionnaire and reported that 95% of the patients felt that their procedure had been an "excellent experience." Finally, in an early report in which cost analysis was provided, Farha et al.[21] described their initial experience with 55 patients. Fifty of these patients (90%) were discharged from their freestanding outpatient surgery center. The average facility charge for laparoscopic cholecystectomy in their series was $2300 as compared to the average cost over the similar time period of $6500 at the community hospital.

Despite these data, there has been limited application of "true" laparoscopic cholecystectomy in this country. Furthermore, the role of this procedure in an academic health care setting has also not been well defined. Results from our series are similar to those reported in the literature. In the current series, 93.8% of patients were successfully discharged from the

PACU with a mean stay of 200 ± 79 minutes. Although another six patients (4.6%) did require subsequent hospitalization after they returned home, in only four of these patients was the admission related to symptoms that were temporally related to the cholecystectomy and may have benefitted from immediate hospital admission. Of the 14 patients eventually admitted to the hospital, eight required only one day of hospitalization. Although two patients developed complications of bile leakage, and another patient developed symptoms of acute pancreatitis, there was no evidence that performance of the procedure in the outpatient setting had a negative impact on their outcome.

The total of 130 patients who had the procedure performed in the outpatient setting at this institution represented only 32% of laparoscopic cholecystectomies performed at our institution during this time period. However, of the 279 patients who were treated as inpatients, only 120 were discharged after a single night's stay. This finding suggests that of those patients deemed suitable for an outpatient procedure, more than 60% of them employed this option. The selection of patients for outpatient laparoscopic cholecystectomy in this series was based on the surgeon's personal preference and selection criteria. Specifically, the criteria for each individual surgeon varied significantly with one surgeon (K.D.L.) performing 69% (110 of 160 patients) as outpatients.

Although cost analysis data were not obtained in this analysis, current costs for laparoscopic cholecystectomy in The Johns Hopkins Outpatient Center ranges from $2100 to $2500, as compared to a single overnight stay at The Johns Hopkins Hospital for which costs typically range from $4000 to $5500. Therefore there appears to be a significant cost advantage to performing this procedure on an outpatient basis.

This series also made an attempt to determine the patients' perspective of the laparoscopic cholecystectomy performed as an outpatient procedure. An attempt was made to contact all patients in which the procedure was successfully completed as an outpatient. Of those patients responding, 75% rated their experience as good and only 2% of the patients rated their experience as poor. The patients were also asked if in retrospect they were given the choice of having the procedure performed as an inpatient versus an outpatient, almost 80% of patients felt that they preferred the outpatient setting. These data suggest that in addition to potentially providing a cost savings, outpatient laparoscopic cholecystectomy is well received by patients.

Finally, a limited analysis was performed to compare the factors associated with the need for hospital admission. Factors such as patient demographics, ASA class, indication for surgery, or length of PACU stay did not predict admission. The operative procedure, however, did last significantly longer (90 ± 24 minutes vs. 73 ± 22 minutes) in those patients who were admitted to the hospital following laparoscopic cholecystectomy.

CONCLUSION

This series demonstrates that laparoscopic cholecystectomy at an academic health center can be successfully completed as a true outpatient procedure in more than 90% of patients. Late hospital admission may be required in less than 5% of patients after discharge to home; however, there appears to be no adverse effect to early outpatient discharge. These data suggest that laparoscopic cholecystectomy can be routinely applied to selected patients undergoing laparoscopic cholecystectomy at an academic medical center.

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Discussion

Dr. J. Ponsky (Cleveland, Ohio). It may seem trivial, but in terms of the cost, true outpatient cholecystectomy is significant. I have been performing this procedure for almost 2½ years. Prior to that, I could not get any of my patients to go home, even though I tried to educate them. The use of ondansetron preoperatively, in combination with ketorolac just before the operation is completed, has made all the difference. Our anesthesiologists now, in reviewing the literature, believe that droperidol, which is less expensive than ondansetron, is equally effective. Have you used droperidol and do you think it is equally effective?

Dr. K. Lillemoe. We have no significant experience in the outpatient setting with this agent. Our patients do follow a standard anesthetic protocol designed to facilitate early discharge and I do have to give significant credit to that team. Our patients are counseled before surgery about the expectations, but there is no routine nursing follow-up. I feel it is really a team effort to get them home and comfortable.

Dr. N. Soper (St. Louis, Mo.). Your study shows that outpatient cholecystectomy can be done safely in an academic health center. I think we have to ask why we are doing this, though, and the obvious answer is the cost difference. What is the cost difference at your institution for true outpatient cholecystectomy with sometimes long periods in the PACU compared to cholecystectomy with an admission overnight? How does this compare to your inpatient or 23-hour admissions? How does your outpatient group compare to patients admitted overnight in terms of demographics and patient satisfaction? Finally, you did not identify any factors preoperatively that would indicate who would be admitted and who would not. So what are the relative contrandications to outpatient cholecystectomy and to whom do you not recommend outpatient laparoscopic cholecystectomy?

Dr. Lillemoe. Because of the retrospective nature of this analysis, we were not able to obtain as much data concerning cost as we would have liked. We were able to generate some information about current cost. The range for a true outpatient procedure with recovery room discharge, at our institution, is about $2500 to $3200. A one-day overnight stay at our institution is approximately $4000 to $4500. So the savings are roughly $1000 to $1500 per patient. We do not have comparison demographic or clinical data for patients who were admitted, but I do know the number admitted. During the same time period, we performed 279 inpatient laparoscopic cholecystectomies, of which 120 were one-night stays or patients who potentially could have been treated as outpatients. The utilization of the outpatient center varies from surgeon to surgeon. In my own practice, I have treated more than three quarters of my patients as outpatients. My criteria include anyone whom I think can tolerate a routine general anesthetic and who has the appropriate support mechanisms at home. The patient must not live a great distance from Johns Hopkins, unless he or she is willing to stay in a hotel. Other surgeons have their own criteria. I personally avoid patients with significant cardiac disease, who may need postoperative monitoring. Cirrhosis and significant previous upper abdominal surgery with a high risk of conversion are also indications that I would probably plan to admit a patient. My threshold may be a little lower for outpatient procedures than some of my colleagues, and certainly one must feel comfortable doing this.

Dr. M. McDonald (Allentown, Pa.). We have been discharging our patients within 24 hours for several years now, but I wonder if we are "cutting our nose to spite our face." With laparoscopic spine surgeries, insurance companies are now refusing to pay for overnight stays. I wonder whether it is appropriate for discharge planners and insurance companies to pressure us to get these patients out on the same day? They are doing this now and I feel that we are bringing this pressure on ourselves by bragging about same-day discharges.

Dr. Lillemoe. That is certainly a difficult problem. The reason for doing outpatient surgery is not just cost conservation. I think many patients are simply more comfortable at home. Many of my patients come in with the hope of going home, and in general they have been very happy with the outpatient procedure. On the other hand, I think we have to maintain a low threshold for admitting the patient if we feel it is indicated. I do not know of any insurance company that will deny an admission if it is decided in the recovery room that a patient cannot go home. If you have a facility that can allow you to make the decision whether to admit or send a patient home postoperatively as opposed to preoperatively, you have the option at that time to do what is best for each individual. What can a society such as The Society for Surgery of the Alimentary Tract do? Hopefully practice guidelines will be helpful.

Early Laparoscopic Cholecystectomy for Acute Cholecystitis: A Safe Procedure

Peter C. Willsher, F.R.A.C.S., Juan-Ramon Sanabria, M.D., Steven Gallinger, M.D., Ljubo Rossi, M.D., Steven Strasberg, M.D., Demetrius E.M. Litwin, M.D.

Acute cholecystitis is increasingly managed by laparoscopic cholecystectomy. Some reports have shown conversion and complication rates that are increased in comparison to elective laparoscopic cholecystectomy. This study reviews the combined experience of two hospitals where the intention was to perform early laparoscopic cholecystectomy for acute cholecystitis. A total of 152 cases of laparoscopic cholecystectomy for acute cholecystitis (evidence of acute inflammation clinically and pathologically) were identified. Conversion to open cholecystectomy was required in 14 cases (9%) in the total series. Laparoscopic cholecystectomy was performed within 2 days of admission in 76% (115 of 152) of patients. Conversion was significantly less likely in patients undergoing laparoscopic cholecystectomy within 2 days of admission (4 of 115) compared to those undergoing surgery beyond 2 days (10 of 37; $P < 0.0001$). Eleven patients (7%) had postoperative complications; however, there were no cases of injury to the biliary system and no perioperative deaths. This series shows that laparoscopic cholecystectomy can be performed safely in patients with acute cholecystitis and suggests that early laparoscopic cholecystectomy is preferable to delaying surgery. Although the conversion rate to open surgery is higher than for elective cholecystectomy, the majority of patients (91%) still derive the well-recognized benefits of laparoscopic cholecystectomy. Early laparoscopic cholecystectomy is an acceptable approach to acute cholecystitis for the experienced laparoscopic surgeon. (J GASTROINTEST SURG 1999;3:50-53.)

KEY WORDS: Laparoscopic cholecystectomy, acute cholecystitis

In the early days of the "laparoscopic cholecystectomy era," acute cholecystitis was considered a contraindication to the laparoscopic approach.[1] However, with increasing experience, a number of reports show that this is no longer the case.[2] These series tend to show conversion and complication rates that are increased in comparison to those for elective laparoscopic cholecystectomy. Of particular concern are reports with an increased incidence of common bile duct injury.[2,3] The study reported herein represents the combined experience of two Canadian hospitals where the approach to acute cholecystitis emulates that of open surgery (i.e., early laparoscopic cholecystectomy). It has recently been suggested[2] that based on current data, this approach may not yet be considered safe. Since we have aimed to manage acute cholecystitis with early laparoscopic cholecystectomy for a number of years, this series is presented to examine whether this policy can be successfully and safely advocated.

METHODS

Patient Population

One hundred fifty-two cases of acute cholecystitis were identified from a prospectively accrued database of 2449 patients undergoing laparoscopic cholecystectomy. These procedures were carried out by the authors at two institutions between May 1990 and December 1994. Patient demographic data were available from the database, and information regarding outcome was obtained by review of hospital and office records.

Only patients with a confirmed diagnosis of acute cholecystitis were included in the study, and this was based on evidence of acute inflammation of the gall-

From Mount Sinai Hospital, Toronto, Ontario, Canada (P.C.W., J.-R.S., S.G., S.S., and D.E.M.L.), and Saskatoon City Hospital, Saskatoon, Saskatchewan, Canada (L.R. and D.E.M.L.).
Reprint requests: Dr. Peter Willsher, Department of General Surgery, Royal Perth Hospital, Box X2213 GPO, Perth 6001 WA, Australia.

Table I. Rate of conversion from laparoscopic to open cholecystectomy for each individual surgeon, performed at different intervals from presentation

Interval from presentation	Surgeon A	Surgeon B	Surgeon C	Surgeon D	Total
Within 2 days	2/12 (17%)	0/5 (0%)	2/76 (2.6%)	0/22 (0%)	4/115 (3.5%)
3 days to 6 weeks	7/13 (54%)	1/1 (100%)	0/6 (0%)	0/8 (0%)	5/22 (23%)
>6 weeks	2/5 (40%)	0/1 (0%)	—	0/3 (0%)	2/9 (22%)
TOTAL	11/30 (37%)	1/7 (14%)	2/82 (2.4%)	0/33 (0%)	14/152 (9%)

bladder, both clinically (right hypochondrial tenderness and fever) and pathologically.

Statistical Analyses

Analyses were carried out using SPSS (Windows) Data Analysis Program. Fisher's exact test was used for comparison of proportions. Student's two-tailed *t* test was used to compare continuous data. Degrees of freedom were n-1 for all analyses.

Table II. Postoperative complications in laparoscopic or converted cholecystectomy

Complication	No. of cases
Intra-abdominal abscess	3
Pulmonary or cardiac complication	3
Postoperative intra-abdominal bleeding	1
Paralytic ileus	2
Wound infection	1
Urinary retention	1

RESULTS

The one hundred fifty-two cases comprised 84 females and 68 males. Mean age was 54 years (range 18 to 86 years). Mean follow-up is now 36 months. Conversion to open cholecystectomy was required in 14 cases (9%). This compares with our conversion rate of 3% for patients having laparoscopic cholecystectomy for indications other than acute cholecystitis.

For the acute series the reasons for conversion were adhesions/difficult dissection in eight cases, uncertainty about the anatomy in three cases, and concern regarding cholecystoduodenal fistula in one case. In two cases conversion was carried out to allow open exploration of the common bile duct. These latter cases occurred before the use of laparoscopic choledochotomy. Overall there were 10 patients with choledocholithiasis; the stones were removed by open choledochotomy in two cases, laparoscopic choledochotomy in four, and postoperative endoscopic retrograde cholangiopancreatography in four. The majority of patients (115 [76%] of 152) had surgery within 2 days of admission. Nine operations (6%) were performed 6 weeks after diagnosis. The delay in surgery was most commonly due to patient preference, and two patients were severely ill and were initially treated by percutaneous cholecystostomy. These latter two individuals were readmitted for interval laparoscopic cholecystectomy.

The rate of conversion to open cholecystectomy was significantly lower in patients undergoing laparoscopic cholecystectomy within 2 days of admission (4 of 115) compared to those beyond two days (10 of 37; $P < 0.0001$). Table I shows that the conversion rates differed among operating surgeons ranging from 0% to 37%. The mean hospital stay was significantly shorter in patients undergoing early laparoscopic cholecystectomy (3.7 days) compared to delayed laparoscopic cholecystectomy (5.6 days; $P = 0.003$). There were no perioperative deaths, and complications were seen in 11 patients (7%) (Table II). CT-guided drainage was used to successfully treat cases of intra-abdominal abscess. The patient with postoperative bleeding required a two-unit transfusion. There were no cases of injury to the common bile duct.

DISCUSSION

Acute cholecystitis is a common condition and prior to laparoscopic cholecystectomy, most cases were managed by early cholecystectomy.[4] This approach evolved with the recognition that the advantages of interval cholecystectomy (usually after 6 weeks) were outweighed by the disadvantages, particularly failure of conservative therapy, recurrence of acute cholecystitis before interval surgery, and the need for two hospital admissions. Our group (and others)[5] believes that the principle of early surgery should also apply to laparoscopic cholecystectomy. In 1990, soon after laparoscopic cholecystectomy was introduced in our hospitals, we began to approach all patients with acute cholecystitis with the aim of performing early laparoscopic cholecystectomy on the first admission.

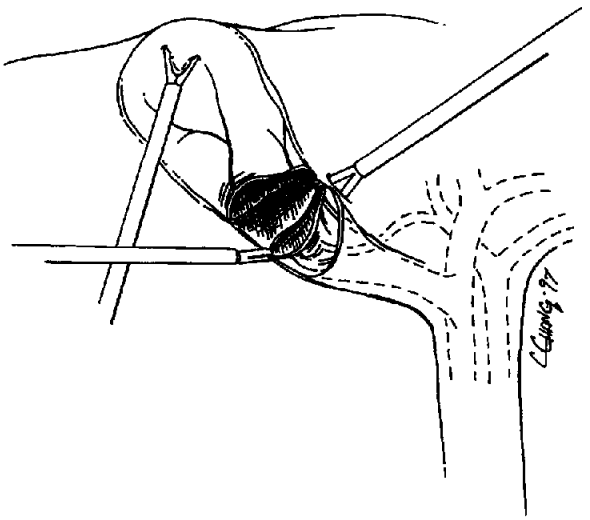

Fig. 1. Retrograde dissection of Calot's triangle. Cephalad retraction on the fundus of the gallbladder is maintained, while the body of the gallbladder is divided well beyond Calot's triangle. The divided stump of the gallbladder can be retracted either caudally or laterally to allow the retrograde approach to Calot's triangle.

Surgical Technique

There is no doubt that laparoscopic cholecystectomy for acute cholecystitis can be a difficult procedure because of the presence of edema and inflammation.[6] Although these cases generally were approached as for elective cholecystectomy, with increasing experience it became evident that a willingness to modify the surgical technique was advantageous in completing the procedure laparoscopically. Since the gallbladder is often tensely distended, early decompression facilitates the ability to grasp and manipulate the gallbladder. We have found that the simplest method is to directly puncture the gallbladder with the 5 mm trocar and aspirate the contents via this portal. Alternatively, a large-bore intravenous catheter and 50 cc syringe were also used. In this manner, infected contents can be suctioned and irrigated. This makes cephalad retraction of the gallbladder easier. The edematous and thickened gallbladder wall is stiff, and difficulty is often encountered in elevating Hartmann's pouch to provide adequate exposure of Calot's triangle. We have found that achieving this exposure is one of the most important factors for safe dissection and completion of such cases.[2] To assist this retraction we have frequently employed the gallbladder "claw" passed through the subcostal operating port (which is replaced with a 10 mm port for this purpose).[3] Further exposure may be necessary by caudad retraction of the duodenum or omentum via an additional left-sided abdominal 5 mm port. We believe there should be a low threshold for inserting additional ports.

Dissection in the region of an inflamed gallbladder is often associated with minor bleeding, which is troublesome in that it obscures the view of the operative field. We have found the use of the blunt-ended suction/irrigator as a dissector to be helpful, particularly when continuous irrigation is used. This technique of "hydrodissection" not only keeps the operative field clear but takes advantage of the natural tendency for edematous tissue planes to open.[7] Furthermore, we consider the careful use of a blunt-ended instrument to be safer in comparison to cautery or sharp dissection in these cases.

Where dissection of Calot's triangle is particularly difficult, in selected cases we have used retrograde dissection of the gallbladder (as would be employed in open surgery). This can be performed using the "fundus first" technique. We have also used an alternative technique (Fig. 1). To allow continued cephalad retraction of the gallbladder fundus during this technique, it is necessary to divide the gallbladder well beyond Calot's triangle (Fig. 1). This maintains exposure but allows the structures in Calot's triangle to be approached in a retrograde manner (see Fig. 1). We emphasize that this approach does not prevent major duct injury and should therefore be employed with caution.

Once Calot's triangle is dissected, the anatomy may then be confirmed by operative cholangiography—although this should not substitute for careful dissection. The cystic duct is often edematous and acute cholecystitis has been associated with an increased risk of cystic duct stump leak, accounting for 47% of leaks in a recent large series.[8] The authors of this latter report suggest that the use of a pretied loop may be more secure than clips in such cases; however, use of this method did not prevent stump leakage. Another alternative is to directly oversew the cystic duct stump. To avoid spillage of contents from a friable or fragmented gallbladder, an extraction bag can be used for removal of the gallbladder via a suitably extended incision.

Clinical Outcome

The overall conversion rate to open cholecystectomy of 9% compares favorably to other series of acute cholecystitis where conversion rates vary between 4% and 35%.[2] As expected, this is higher than what is considered usual for elective laparoscopic cholecystectomy.[9] The most common reasons for conversion were difficulty in carrying out the dissection and uncertainty about the anatomy, both of which having been previously described.[3,10]

The complication rate in our series (Table II) is similar to those in previous reports.[2] It has been suggested that complications are less frequent following laparoscopic cholecystectomy for acute cholecystitis in comparison to open acute cholecystectomy because of reduced pulmonary problems.[10,11] Of note, however, is that common bile duct injury has been reported in up to 5.5% of cases.[2] This complication was not seen in our series and the majority of recent series have also achieved this outcome.[2,12,13] It would seem likely that this is a result of both meticulous attention to identification of the anatomy before any structures are divided and an appropriate threshold for conversion to open surgery. Before dividing the cystic duct we ensure that the following specific criteria are met: (1) Calot's triangle must be widely opened along the gallbladder bed; (2) the presumed cystic duct must be seen passing as a funnel into the gallbladder; and (3) a *single* tubular structure must be seen at the site for application of the most proximal clip.

Our policy of undertaking early laparoscopic cholecystectomy was successful in most cases (see Table I). Short delays in laparoscopic cholecystectomy were often due to either a delay in presentation or organization/availability of operating time. Longer delays were usually due to patient choice, except for the two patients treated by tube cholecystostomy. These latter two patients both had successful interval laparoscopic cholecystectomy. However, early laparoscopic cholecystectomy (<2 days) was associated with a significantly shorter hospital stay than when laparoscopic cholecystectomy was delayed. These findings have been reported in previous studies.[3,10,13,14] In our series this finding is likely explained by the different conversion rates. Of interest, this study shows that conversion rates varied widely among surgeons (see Table I). Although this difference may be attributed to the case-mix, it would seem more likely that it relates to different thresholds for conversion. This latter factor is likely to be a reflection of the surgeon's experience. Nevertheless, no surgeon had any cases of common bile duct injury, suggesting that conversion was carried out appropriately. Table I shows that for Surgeon D, laparoscopic cholecystectomy was performed successfully regardless of the interval between presentation and surgery. In comparison, Surgeon A had higher conversion rates at all intervals from presentation. This latter observation highlights the significant surgeon variability in both early and delayed laparoscopic cholecystectomy. However, the 3.5% conversion rate for early laparoscopic cholecystectomy (see Table I) shows this approach can be performed safely and successfully in the vast majority of cases of acute cholecystitis.

CONCLUSION

The outcome of the patients in this study provides further evidence that laparoscopic cholecystectomy can be performed safely for acute cholecystitis without death or significant morbidity. Although the conversion rate is higher than expected for elective laparoscopic cholecystectomy, the majority of patients (91%) receive the recognized benefits of laparoscopic cholecystectomy. Furthermore, our experience shows that early laparoscopic cholecystectomy is possible in most patients with acute cholecystitis, and may be preferable to interval cholecystectomy. Early laparoscopic cholecystectomy is therefore an acceptable approach to acute cholecystitis for the experienced laparoscopic surgeon.

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Selective Decreases in Levels of mRNA Encoding a Water Channel (AQP3) in Ileal Mucosa After Ileostomy in the Rat

M. Jennifer Purdy, M.D., Robert R. Cima, M.D., Marc A. Doble, B.A., Michael A. Klein, M.D., Michael J. Zinner, M.D., David I. Soybel, M.D.

Water channels (aquaporins) provide pathways for water permeation in a variety of epithelia. Aquaporin-3 (AQP3) has been localized to the basolateral membranes of epithelial cells in the small intestine, but mechanisms that regulate its expression and function have not been explored. To determine whether luminal content may influence intestinal AQP3 gene expression, adult Sprague-Dawley rats underwent sham laparotomy (N = 11) or loop ileostomy (N = 9) and were killed 8 days after procedures. Northern blot analysis was used to measure messenger RNA (mRNA) levels for AQP3 and the Na^+/K^+ ATPase, a housekeeping transporter that regulates cellular levels of Na^+ and K^+. At sacrifice, histologic examination revealed only minimal changes in mucosal morphology. In sham animals, Na/K mRNA levels increased moderately in distal regions of the small intestine. Ileostomy did not alter these levels in any region. In contrast, in sham animals, AQP3 mRNA levels increased along the length of the intestine and were markedly higher in the distal ileum. Diversion of luminal contents decreased AQP3 mRNA levels in the postileostomy region by 30% to 50%. These findings indicate regional variations in expression of the AQP3 water channel in mucosa of the small intestine. In addition, they suggest that AQP3 gene expression may depend on the presence of luminal contents. (J GASTROINTEST SURG 1999;3:54-60.)

KEY WORDS: Water channels, aquaporins, intestinal mucosa, ileal mucosa, ion transport

Aquaporins are a recently described family of water-permeable channels located in cell membranes of a number of epithelial cell types. At least six isoforms have been identified in mammalian tissues.[1-8] Aquaporin-1 (AQP1) is found in a variety of tissues. Its expression is not considered prominent in the intestine, but it has been detected in crypt cells but not the absorptive cells of the colon. AQP2 is the vasopressin-sensitive water channel of the renal collecting duct; its localization has been predominantly apical. AQP3 has been localized to the basolateral membranes of epithelial cells in the small intestine and colon. AQP4 has been identified in the brain and, most recently, in gastric glands. AQP5 has been identified in salivary glands. One other, designated AQP8, has recently been identified[8] in hepatocytes, exocrine pancreas, and absorptive villus cells in the colon.

Of the isoforms cloned and characterized thus far, only AQP3 has been detected with certainty in the mucosa of the small intestine. However, its physiologic role in the intestine remains relatively unexplored. The basolateral localization[4,5] of AQP3 suggests that it may play a role in maintaining cell volume and osmotic equilibrium of the intestinal epithelium, especially during high rates of salt and solute absorption from the lumen. This study was undertaken to (1) evaluate regional differences in gene expression of AQP3 in the small intestine of the rat and (2) determine whether the presence or absence of luminal contents influences AQP3 expression in the distal

From the Department of Surgery (M.J.P., R.R.C., M.A.D., M.J.Z., and D.I.S.), Brigham and Women's Hospital, and the Departments of Surgery (M.J.P., R.R.C., M.A.D., M.J.Z., and D.I.S.) and Pathology (M.A.K.), West Roxbury Veterans Administration Medical Center, Harvard Medical School, Boston, Mass.

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Reprints requests: David I. Soybel, M.D., Department of Surgery/112, West Roxbury Veterans Administration Medical Center, 1400 VFW Parkway, West Roxbury, MA 02132. E-mail: dmsoybel@bics.bwh.harvard.edu

ileum, a region of the alimentary tract that plays a critical role in salt and water absorption.

MATERIAL AND METHODS

Animal Model

The study protocol was approved by the Harvard Medical Area Standing Committee on Animals at Harvard Medical School, Boston, Massachusetts. Twenty adult, 4- to 6-month-old, male Sprague-Dawley rats had operative procedures performed. Eleven animals randomly underwent a sham laparotomy. The remaining nine animals underwent creation of a loop ileostomy modified from the procedure described previously.[9,10] All animals underwent similar preoperative and postoperative care. This entailed food deprivation 24 hours before and 24 hours after the operation. All animals had access to water prior to the surgery, and were allowed both water and 0.25 normal saline solution ad libitum after the surgery. Animals were anesthetized using a combination of ketamine (40 mg/kg) and xylazine (5 mg/kg) administered intramuscularly. After induction of anesthesia, a 22-gauge, 1-inch intravenous catheter was placed in the tail vein, and a syringe was attached to provide intravenous fluid during the operative and postoperative recovery period. All animals received a total of 3.0 ml in 0.25 ml boluses of normal saline prior to removal of the intravenous cannula. Body temperature was monitored with a rectal thermistor. During all operations the animal's body temperature was maintained above 35° C by use of a warming blanket. Postoperative pain control was achieved by subcutaneous injection of Buprenex (Buprenorphine HCl, 0.1 mg/kg) administered 4 hours after the animals were fully recovered from the anesthetic and then again 12 hours after that injection. Additional doses were given only if animal's behavior indicated discomfort. Postoperatively, animal weights were recorded daily. Ileostomy animals were given a bath on each postoperative day to prevent skin injury from the caustic ileostomy effluent.

All operations were performed under sterile conditions. After the induction of anesthesia, the animals' abdomens were shaved and cleansed with 95% ethanol. In both sham and ileostomy animals, the abdomen was entered through a 4 cm midline incision. In the sham laporatory animals (N = 11), once the abdomen was entered, the distal small intestine and cecum were identified. The distal small intestine and cecum were then brought out of the midline incision and draped with warm saline-soaked gauze. Ten minutes after exteriorization, the distal small intestine and cecum were returned to the abdominal cavity. The midline incision was then closed with a running 4-0 Vicryl suture. The skin edges were approximated and closed with animal skin staples. In ileostomy animals (N = 9), after the abdomen was entered, a 4 to 6 mm circular stoma was created in the abdominal musculature and skin approximately 1.0 cm to the right of the midline incision. A loop of distal ileum 5.0 cm proximal to the ileocecal valve was delivered through the stoma. The loop of ileum was opened along the antimesenteric border for a distance of 1.0 cm. The free edges of this loop were matured to the abdominal musculature and skin with interrupted 4.0 silk sutures. Prior to closure of the abdomen, both afferent and efferent limbs of the loop ileostomy were examined for patency by infusion of warm saline solution through a 22-gauge intravenous catheter. The abdominal cavity was closed as previously described.

Tissue Preparation for mRNA

On postoperative day 8, animals were killed by a pentobarbitol overdose (100 mg/kg). Intestinal tissues were harvested and their mucosa was separated rapidly from the underlying muscularis by sharp dissection.[10,11] The mucosal tissue was frozen and stored in liquid nitrogen until processed for mRNA. Messenger RNA from the samples was isolated by RNAzol B (Tel-test, Inc., Friendswood, Tex.). This isolation kit was slightly modified by the addition of the following steps. After the RNA pellet was resuspended in diethyl pyrocarbonate (DEPC) water, chloroform and isoamyl alcohol were added in a 2:1:1 ratio, vortexed for 30 seconds, and then centrifuged at 14,000 rpm. The supernate was washed with a 1:1 ratio of chloroform isoamyl alcohol. The supernate was then added to a mixture of NaOAc, pH 5.2, and two volumes of 100% and allowed to precipitate overnight at −80° C. The solution was centrifuged and the pellet was washed in 70% ethanol followed by resuspension in DEPC-treated water. The RNA samples were then stored at −80° C until use. The following tissues were harvested from ileostomy animals: duodenum, midjejunum (5 cm segment), ileum proximal to the stoma (5 cm segment), and ileum distal to the stoma (5 cm segment). Corresponding segments of intestine were harvested from the sham animals.

Northern Blot Analysis

Total RNA (10 μg per lane) was run on a 1% agarose gel containing 0.63% formaldehyde staining of 18 S and 28 S ribosomal RNA. The RNA was then transferred to nylon membranes and underwent ultraviolet cross-linking. The membranes were prewashed at 50° C in a NaCl, sodium dodecyl sulfate (SDS) solution for 30 minutes, then prehybridized

for 2 hours at 42° C in a NaCl, formamide, and dextran sulfate solution. The membranes were hybridized overnight with 10^6 counts × min^{-1} × ml^{-1} ^{32}P-labeled cDNA probe for (1) AQP3 (2200 base pairs, transmembrane domain, provided by William Harris, Ph.D., Children's Hospital, Boston, Mass.) and (2) alpha subunit of the ouabain-sensitive Na/K ATPase (3500 base pairs, provided by Eric Delpire, Ph.D., Vanderbilt University School of Medicine). The membranes were washed twice at low stringency (room temperature, 300 mmol/L NaCl) and once at higher stringency (65° C, 30 mmol/L NaCl). The membranes were then exposed to x-ray film (Reflexion NEF; Du Pont Company, Boston, Mass.) at −80° C for 1 to 3 days. Quantification of relative mRNA abundance was performed via scanning densitometry (Microtek Image Scanner, Microtek International, Inc., Hsinchu, Taiwan; Image 1.49 software from National Institutes of Health, Bethesda, Md.). Membranes were allowed several weeks between probings so as not to require stripping. All membranes were probed with ^{32}P-labeled cDNA encod-ing the housekeeping enzyme, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), to provide additional confirmation of the equivalence of gel loading.[10,11]

Histologic Examination

From two ileostomy and two control animals, tissue samples were obtained from each region of the small intestine (duodenum, jejunum, prestoma ileum, poststoma ileum) submitted for fixation in 10% formalin. Embedding was performed in standard fashion. Serial thick sections were cut and stained with hematoxylin and eosin. Photomicrographs were obtained at ×250.

Statistical Analysis

Data were recorded and analyzed by means of a standard software statistical package (Excel, Microsoft Corp., Redmond, Wash.). Comparisons of measurements between experimental groups underwent analysis of variance (ANOVA) for multiple measurements.

RESULTS

Animal Response to Ileostomy

Animal weight was monitored daily throughout the study. As described previously, there was no significant difference between the two operative groups prior to the operations.[10,11] The sham group had a preoperative weight of 468 ± 11 gm and ileostomy animals weighed 445 ± 14 gm preoperatively (no significant difference by unpaired t test). Postoperatively the sham animals had an initial weight loss but regained weight by the end of the first postoperative week. By the time of sacrifice the sham laporatomy animals had had a slight weight gain (476 ± 11 g, $P < 0.03$ compared to preoperative weights by paired t test) above their preoperative weight. Ileostomy animals had severe postoperative diarrhea from postoperative day 2 to approximately day 8. During this period they lost nearly 30% of their body weight (336 ± 17 g, $P < 0.000001$ compared to preoperative weight by paired t test) but maintained normal, healthy behavior.

Gross Mucosal Appearance and Histologic Findings

At the time of sacrifice the stomach and intestines of each animal were inspected. In sham animals the stomach typically appeared full of solid food. Variable amounts of succus entericus were visible in the small intestine. The cecum and colon were invariably full of semisolid fecal matter. In the ileostomy group the stomach was typically enlarged, with a mildly dilated lumen. The small intestine proximal to the ileostomy was mildly dilated. The ileum distal to the stoma, the cecum, and the colon were devoid of luminal contents. In the ileostomy group, after opening of the stomach and intestines, just prior to harvest of mucosal scrapings, inspection revealed dilatation of the lumen along all regions proximal to the stoma. It was difficult to discern atrophy or hypertrophy of the mucosa compared to sham animals. Distal to the ileostomy, the lumen of the ileum was markedly decreased but the mucosa itself was not noticeably atrophied compared to sham animals.

Histologic sections, stained with hematoxylin and eosin, were obtained from the different regions of the small intestine in two animals. In both groups the size of the lumen was clearly smaller in the ileum distal to the stoma. With respect to mucosal architecture, there was little visible difference between sham-operated and control animals. Specifically, in the segments of ileum proximal and distal to the stoma (Fig. 1), there were no observable differences in the villus/crypt height ratio or density of goblet cells. There was slight flattening of the villi tips in defunctionalized areas, and a slight increase in the number of intraepithelial lymphocytes in comparison to the corresponding terminal ileum in control animals.

Small Intestine Mucosal mRNA Levels of AQP3 and Na/K

Fig. 2 shows, for normal (sham-operated) animals, the relative abundance of mucosal mRNA levels for the water channel, AQP3, and Na^+/K^+ ATPase in dif-

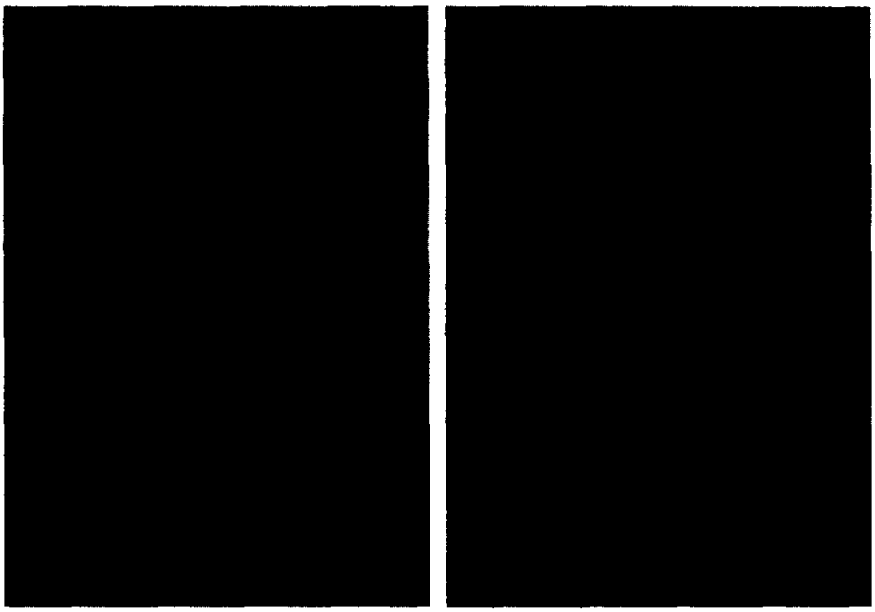

Fig. 1. Histologic evaluation of ileum proximal **(A)** and distal **(B)** to the ileostomy (paraffin-embedded sections stained with hematoxylin and eosin). Slight flattening of villi was observed in the postileostomy region, with some infiltration of lymphocytes into the epithelium. No obvious differences in villus/crypt height ratio, columnar morphology of enterocytes, or Paneth cell hyperplasia were noted in either region compared to control sections (not shown). Horizontal bar indicates length of 100 μmol/L. (Original magnification ×250.)

ferent regions of the intestine. This figure also summarizes measurements of mRNA levels encoding the housekeeping enzyme, GAPDH, used to control for equivalence of loading. In normal animals these levels were minimally altered, if at all, along the length of the small intestine, indicating that the amount of GAPDH mRNA does not change substantially in different regions. The equivalence of these levels in different regions thus made it possible to evaluate whether the abundance of AQP3 and Na/K mRNA varied along the length of the intestine.

The figure thus shows that, in the sham-operated animals, mean levels of mRNA encoding for AQP3 increased along the length of the small intestine. In the terminal 5 cm of the ileum, AQP3 mRNA levels were almost three times those observed in the duodenum and between 50% and 100% greater than the levels observed in the more proximal 5 cm of the ileum. Levels of Na/K ATPase also increased along the length of the small intestine, rising to levels in the terminal ileum averaging 50% greater than those observed in the duodenum. Comparing the most distal 5 cm of ileum with the proximal 5 cm segment of ileum, these levels were not significantly different.

Fig. 3 summarizes differences in AQP3 mRNA levels observed between sham-operated animals and those that had undergone ileostomy. In the duodenum, midjejunum, and prestoma regions, there were

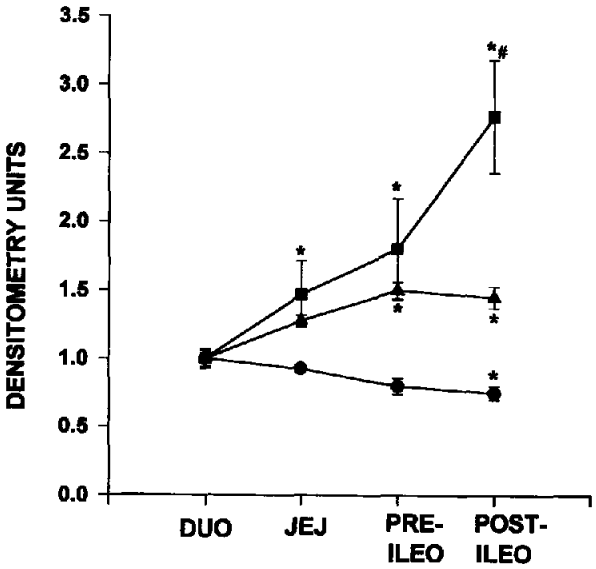

Fig. 2. Regional variations in levels of mRNA encoding for AQP3 (■), Na/K (▲), or GAPDH (●). Densitometry levels were normalized to GAPDH levels, then compared to an arbitrary value of 1.00, assigned to the levels detected in duodenal mucosa. Results are expressed as mean ± standard error of the mean. * = $P < 0.001$ compared to duodenal levels by ANOVA for multiple comparisons; # = $P < 0.001$ compared to levels in the preileostomy segment.

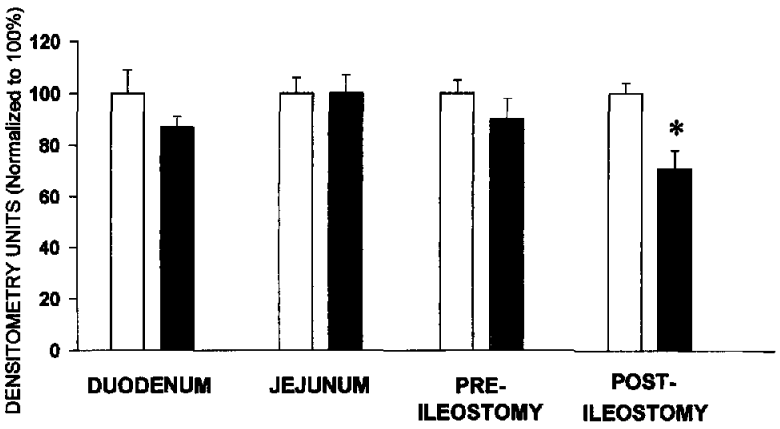

Fig. 3. Comparison of AQP3 mRNA levels in each region of the small intestine in control animal *(open bars)* and ileostomy animals *(cross-batched bars)*. In each region, AQP3 mRNA levels in ileostomy animals were normalized to GAPDH mRNA levels, then normalized to an arbitrary value of 100%, assigned to levels in control animals. Results are expressed as mean ± standard error of the mean. * = $P < 0.001$ compared to duodenal levels by ANOVA for multiple comparisons.

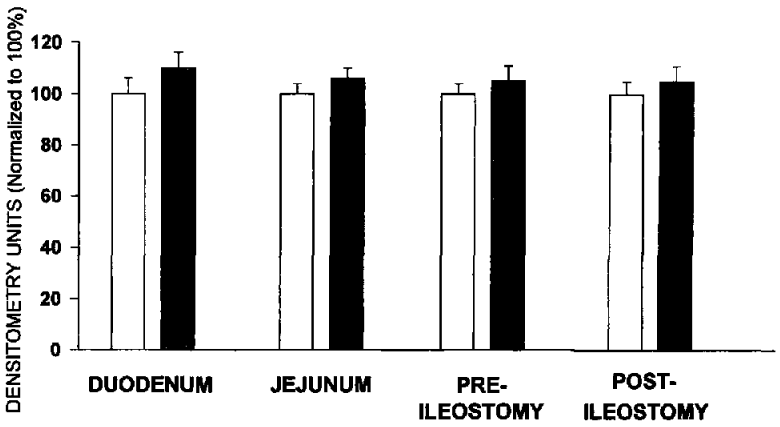

Fig. 4. Comparison of levels of mRNA encoding for Na^+/K^+ ATPase in each region of the small intestine in control animals *(open bars)* and ileostomy animals *(cross-hatched bars)*. In each region, Na/K mRNA levels in ileostomy animals were normalized to GAPDH mRNA levels, then normalized to an arbitrary value of 100%, assigned to levels in control animals. Results are expressed as mean ± standard error of the mean.

no significant differences between the two groups. In the poststoma region, however, there was a significant decrease in AQP3 mRNA levels in ileostomy animals compared to sham-operated animals. Fig. 4 summarizes measurements of levels of mRNA encoding for the alpha subunit of the Na/K ATPase in sham-operated and ileostomy animals. Ileostomy did not elicit any changes in these Na/K mRNA levels, compared to sham-operated animals, in any region of the small intestine.

DISCUSSION

There has been considerable investigation of the neurohumoral mechanisms that regulate mucosal growth and function in the distal small intestine.[12-15] Relatively little is known about how specific components of ileal succus influence expression of ion and fluid transporters involved in salt and water absorption in the distal small intestine. Inasmuch as the aquaporin family of water channels has only recently been described, there is, to our knowledge, no available information on luminal factors that regulate their expression or activity. In this study we created a loop ileostomy model in the rat to evaluate early responses of the mucosa to deprivation of luminal content. Gross inspection and histologic studies indicate that hypertrophy of the bowel proximal and atrophy of the mucosa distal to the stoma had not yet occurred. Key histologic indices for loss of absorption capacity (con-

ical villus morphology, villus/crypt ratio, columnar morphology of villus cells) had changed minimally, if at all. Thus changes observed in mRNA levels of the different transporters could not easily be attributed to loss of villus or enterocyte numbers or architecture.

A key finding in our study was that levels of mRNA for AQP3 appeared to increase along the length of the small intestine, so that the highest levels were observed in the ileum just proximal to the cecum. Remarkably AQP3 mRNA levels were almost twofold higher in the most distal 5 cm ileal mucosa compared to the 5 cm region of ileum just proximal. Deprivation of luminal contents by diversion decreased mRNA levels of AQP3 by more than 30%, which is equivalent to reducing these levels to those observed in the upstream regions of the ileum. These findings are consistent with classic observations.[15,16] that higher rates of solute-driven water transport are observed as the succus moves distally in the small intestine. These findings also suggest that at some level AQP3 expression may be regulated by the content or volume of what is in the lumen.

These observations also offer the possibility that the transcellular cellular pathway may be more important for transmucosal water absorption than has been suspected previously. Prevailing notions have suggested that the paracellular pathway is most important for water permeation in all regions of the intestine.[15,17] Key observations to support the paracellular route as predominant include studies of electrophysiologic behavior and morphology of cells and intercellular junctions during exposure to solutes of varying size, tonicity, and reflection coefficients.[17,18] For this traditionally accepted hypothesis to be seriously questioned, however, an apical water channel must be identified in the absorptive cells of the intestinal villus, and this has not yet occurred. AQP3 has been localized to the basolateral membranes of the intestinal epithelial cells. If an apical water channel were to be identified, AQP3 might then be postulated to play a role in vectorial transport of water from the apical to the basolateral cell membrane. Alternatively, if the dominant route of water permeation is paracellular, AQP3 would be postulated to play a role in intracellular volume and osmotic regulation, particularly during high rates of salt or solute absorption from the lumen. The studies reported herein cannot address the relative importance of water absorption through membrane channels versus pathways through tight junctions and desmosomes. However, they do suggest that experimental models based on the terminal ileum may be appropriate for understanding luminal and neurohumoral factors that regulate expression of water channels in intestinal epithelial cells and their importance relative to the paracellular pathway.

An increase in the magnitude of mRNA levels for the Na^+/K^+ ATPase was also observed along the length of the intestine, but was not as dramatic as that observed for AQP3. Perhaps this is not so surprising since active Na^+-dependent transport processes for many different kinds of solutes (NaCl, Na-glucose, Na-bile salt) are present all along the length of the small intestine, regardless of whether the mucosa is active in secretion (proximal) or absorption (distal). The failure of diversion to significantly alter these mRNA levels is also consistent with the role of the Na^+/K^+ ATPase, fundamentally as a "housekeeping" transporter for maintaining cellular [Na^+] and [K^+].[15] The dependence of numerous transport and membrane electrical properties on these concentrations makes it unlikely that they would have been affected by any shift in neurohumoral or luminal conditions. However, the fact that they were not altered so early after ileostomy does not rule out the possibility of downregulation in a more chronic phase after mucosal atrophy has appeared. Studies are underway in our laboratory to compare changes in transporter gene expression during early and later phases after ileal diversion.

In summary, we have shown that construction of a distal small intestine loop ileostomy can alter gene expression of a newly described water channel, AQP3, in the distally diverted ileum. At an internal of 8 days after the procedure, the diversion has not substantially altered mucosal morphology or gene expression of the housekeeping enzyme GAPDH or the housekeeping transporter Na^+/K^+ ATPase. Loop ileostomy in the rat may serve as a useful model for studying the luminal and neurohumoral feedback mechanisms of transporter expression and function in the distal small intestine. The model may also provide insight into the physiologic adaptations that occur in disease processes affecting the ileum or after ileostomy in humans.

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Role of Angiography and Embolization for Massive Gastroduodenal Hemorrhage

R. Matthew Walsh, M.D., Paul Anain, M.D., Michael Geisinger, M.D., David Vogt, M.D., James Mayes, M.D., Sharon Grundfest-Broniatowski, M.D., J. Michael Henderson, M.D.

The role of mesenteric angiography and embolization for massive gastroduodenal bleeding is unclear. We reviewed the records of patients who underwent angiography for acute, nonmalignant, and nonvariceal gastric or duodenal hemorrhage that was documented but not controlled by endoscopy. Fifty patients were identified over a 7-year period ending in March 1998. Only 17 patients (34%) were originally admitted to the hospital with gastrointestinal bleeding. All required treatment in the intensive care unit (mean 15 days) with a mean APACHE III score of 79 (29% predicted hospital mortality), and 32 (64%) had organ failure. A mean of 2.1 endoscopies were performed to locate the source of acute duodenal bleeding in 37 (74%) and gastric bleeding in 13 (26%). An average of 24.3 units of packed red blood cells were transfused per patient. Twenty-five patients (50%) were found to have active bleeding at angiography; all were treated by embolization as were 22 who underwent empiric embolization. Twenty-six patients (52%) were successfully treated by embolization and thus spared imminent surgery. Multiple variables were compared between those who were successfully treated by embolization and those considered failures. Time to angiography was considerably shorter (2.5 vs. 5.8 days, $P < 0.017$) and fewer total units of packed red blood cells were used (14.6 vs. 34, $P < 0.003$) in those who were successfully treated. There was also a strong trend toward using fewer units of packed red blood cells for transfusion prior to angiography (11.2 vs. 17.1, $P < 0.08$). No differences were found that could be attributed to gastric vs. duodenal sources, number of comorbid diseases, organ failure, APACHE score, age, or whether active bleeding was found at angiography. A total of 20 patients (40%) died including 9 of 17 patients operated on in an attempt to salvage angiographic failure. In summary, angiographic embolization should be performed early in the course of bleeding in otherwise critically ill patients. (J GASTROINTEST SURG 1999;3:61-66.)

KEY WORDS: Angiography, embolization, gastrointestinal bleeding, peptic ulcer disease

The bleeding complications of peptic ulcer disease remain a challenging problem in terms of avoiding potentially preventable death. Although up to 80% of patients with acute gastrointestinal bleeding will stop spontaneously, hemorrhage represents the most common complication of peptic ulcer disease requiring emergent operation.[1-3] Endoscopy is valuable and accurate in determining the site and nature of upper gastrointestinal bleeding.[4] Therapeutic endoscopy may aid in achieving hemostasis, but rebleeding requiring further treatment occurs in 11% to 25%.[5-7] Factors associated with rebleeding and death from acute bleeding include age greater than 60 years, ulcers larger than 1 cm, hemodynamic instability with an ongoing transfusion requirement, and active or stigmata of recent bleeding at endoscopy.[1,7,8] Surgical intervention for acute nonvariceal gastroduodenal hemorrhage is usually an expeditious and gratifying endeavor. Required in 5% of all bleeding patients, it typically is associated with an operative mortality rate of less than 10%.[5,7,9-11] Gastrointestinal hemorrhage in critically ill patients is notable for rebleeding and a mortality rate approaching 50%.[12] Angiographic embolization is uncommonly considered or employed in the management of patients with acute nonvariceal upper gastrointestinal bleeding. The angiographic and endoscopic findings in acute bleeding correlate in 72% to 83%, and embolization is similar to surgery in its directed mechanical interruption of the bleeding vessel, making angiography a potentially accurate and definitive treatment.[13,14] The aim of this study was to determine whether angiography with embolization

From the Departments of General Surgery and Radiology (M.G.), The Cleveland Clinic Foundation, Cleveland, Ohio.
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Reprint requests: R. Matthew Walsh, M.D., Department of General Surgery, The Cleveland Clinic Foundation, 9500 Euclid Ave., Desk A80, Cleveland, OH 44195.

can obviate the need for surgical control of nonvariceal, nonmalignant acute gastrointestinal bleeding in poor-risk patients and to identify factors that could predict angiographic failure so as not to delay an eventual operation.

METHODS

A retrospective review was undertaken of all patients who underwent visceral angiography for massive gastric or duodenal bleeding at The Cleveland Clinic Foundation over a 7-year period ending in March 1998. Patients were identified by a computer-generated list of diagnostic codes in the Department of Radiology. Enrollment required an endoscopic diagnosis and failed treatment of an upper gastrointestinal bleeding source within the reach of a 100 cm gastroscope. Additionally, patients had to require at least four units of packed red blood cells (PRBCs) for a discrete bleeding episode that would otherwise require surgical therapy. All patients were empirically judged to be at high operative risk. Data referable to surgical risk were retrospectively collected and included APACHE III (acute physiology, age, chronic health evaluation) score,[15] admitting diagnosis, presence and number of comorbid diseases including chronic renal insufficiency, history of congestive heart failure, arrhythmias, or myocardial infarction, diabetes mellitus, malignancy, and immunosuppression resulting from chemotherapeutic agents of human immunodeficiency virus. The presence of concurrent renal, cardiac, pulmonary, or hepatic organ failure at the time of gastrointestinal bleeding was determined as previously defined by Deitch.[16] Coagulopathy was defined as the presence of thrombocytopenia (platelet count of $<50{,}000/mm^3$), international normalized ratio greater than 1.5, or therapeutic heparinization.[12,17] Transfusion requirements were determined for PRBCs, fresh-frozen plasma, and platelet pack units in relation to the performance of the angiography. Admission to a medical intensive care unit at our institution routinely includes initiation of stress ulcer prophylaxis with a histamine H_2-receptor antagonist. The specific medication and duration of prophylaxis were not collected.

All endoscopies were emergently performed in the intensive care unit with sedation, antecedent intubation, and hemodynamic monitoring as guided by the patient's condition. A portable, video endoscopic cart was used and procedures were performed with a therapeutic Olympus GIF2T100 gastroscope (Olympus America, Inc., Lake Success, N.Y.). Endoscopic data included site of bleeding (gastric vs. duodenal), therapy attempted, and number of procedures. The retrospective nature of this study did not allow for a reliable assessment of endoscopic stigmata of ulcer bleeding such as the presence of a "visible vessel." All patients with variceal and malignant sources of bleeding were excluded.

Diagnostic angiography and subsequent catheter interventions were performed from an inguinal approach. After sterile preparation and instillation of local anesthesia, either the right or left common femoral artery was cannulated with a 6 or 7 Fr sheath. Via this sheath, selective or subselective angiography was then performed typically with a 5 Fr catheter, although 6.5 Fr catheters were used for some of the earlier studies. Once the site of bleeding was ascertained, an attempt was made to advance a 5 or 6.5 Fr catheter into the appropriate branch vessel. If this was not possible, a 3 Fr catheter was advanced coaxially through the larger catheter and into the target vessel. All embolizations were performed with particles, coils, or a combination of particles and coils. The particles were either Ivalon (polyvinyl alcohol) or Gelfoam (cellulose sponge). The coils were Gianturco (Cook, Inc., Bloomington, Ind.) stainless steel coils (if a 5 or 6.5 Fr catheter was used) or platinum microcoils (if a coaxial 3 Fr catheter was used). The end point was angiographic evidence of occlusion of the target vessel or cessation of extravasation of contrast material. The time from the onset of bleeding to the first angiography was determined. In addition, the number of procedures, presence and location of active bleeding, and procedure-related complications were determined.

Outcome was primarily assessed by the ability to stop an acute bleeding episode as defined by avoidance of surgery or a postangiography transfusion requirement of less than 4 units of PRBCs. Statistical methods were performed by the Department of Biostatistics and Epidemiology. The predictors of angiographic failure were tested using univariate and multivariable Cox proportional hazards models. Certain associations were tested using two-sided chi-square and Fisher exact tests. Significance levels were defined to be 0.05.

RESULTS

A total of 50 consecutive patients underwent angiography for acute major upper gastrointestinal bleeding. There were 35 men (70%) and 15 women (30%) whose mean age was 64 years (range 24 to 84 years). The principal diagnosis at admission to the hospital was gastrointestinal bleeding in 17 patients (34%). Most patients were in fact admitted for another medical or surgical disease that was subse-

quently complicated by gastrointestinal bleeding. These admission diagnoses included complications of coronary artery disease in 13 patients, nongastrointestinal malignancy in five, sepsis in three, and organ transplant rejection in two. All 50 patients required admission to an intensive care unit for their primary medical disease or resuscitation at the time of intestinal bleeding, with a mean length of stay in the intensive care unit of 15 days (range 1 to 78 days). The number of major comorbid diseases averaged 1.75 per patient (range 0 to 5). At the time of the diagnosis of gastrointestinal bleeding, 32 patients (64%) had evidence of at least one organ in failure, averaging 2.3 failed organs per patient, 1.5 per patient for the entire study group. Complete information was available for APACHE III scoring in 37 patients (74%), with a mean score of 79 and a predicted mortality rate of 29%. Ten patients (20%) who underwent angiography had previously failed surgical treatment for gastrointestinal bleeding; four of them had had operations performed at another hospital.

All patients underwent emergency endoscopy with a mean of 2.1 endoscopies per patient (range 1 to 5). The source of bleeding was determined to be primarily gastric or duodenal. Duodenal ulceration was responsible for bleeding in 37 (74%), and 13 (26%) had a gastric course. Thirty-eight patients (76%) were evaluated by surgery after the endoscopy and prior to angiography.

Numerous transfusions were required and these were categorized by amounts given prior to and following angiography, and the total amount, which included amounts given with any subsequent surgery. Patients received a mean of 13.8 units of PRBCs prior to angiography (range 4 to 56 units), 8.2 units after angiography (range 0 to 41 units), and 24.3 units total (range 5 to 75 units). Thirty-six patients (72%) received units of fresh-frozen plasma, a mean of 4.0 units prior to angiography (range 0 to 24 units), 3.2 units following angiography (range 0 to 33 units), and 10.2 units total (range 0 to 120 units). Twenty-six patients (52%) received a transfusion of platelets, a mean total of 3.5 packs per patients. Bleeding associated with coagulopathy was present in 22 patients (44%) including three who were given heparin, which is appropriate for deep venous thrombosis and/or pulmonary embolism.

Angiography was performed at a mean of 4.2 days following the diagnosis of bleeding. An average of 1.3 arteriograms were obtained per patient (range 1 to 3). Active bleeding at angiography was documented in 25 patients (50%), which included 22 (59%) of 37 duodenal and 2 (23%) of 13 gastric sources of bleeding that were endoscopically determined. In patients with a bleeding duodenal ulcer, the bleeding vessel was found to be the gastroduodenal artery in 15 patients, an inferior pancreaticoduodenal artery in two, both in three, a branch of the splenic artery in one, and the right gastric artery in one. Patients with an actively bleeding gastric source had bleeding from the left gastric artery. Embolization was performed in a total of 46 patients (92%) including the offending bleeding vessel and feeding branches in the 25 patients with active bleeding and empiric embolization of the presumed offending vessel in 21 patients. Thirty-four patients underwent embolization of one major artery and 12 had embolization of more than one artery. No patients were given intra-arterial vasopressin or antidiuretic hormone (ADH) infusion for control of bleeding.

A total of 24 patients (48%) demonstrated persistent bleeding that resulted in death or required surgery and were considered angiography failures. The remainder (52%) were treated definitively with angiography and embolization including three patients with persistent bleeding documented at repeat angiography who responded to repeat embolization and three patients who underwent repeat angiography without radiographically documented rebleeding. Successfully treated patients were compared with those in whom treatment failed to determine which factors may predict outcome (Tables I and II). Patients unsuccessfully treated with angiographic embolization had a significantly longer time to angiography (5.8 vs. 2.5 days, $P < 0.017$, risk ratio 1.10) and a greater number of total red blood cells transfused (34 vs. 14.6 units, $P < 0.003$, risk ratio 1.05) both by univariate and multivariate analyses. An additional significant predictor of failure of embolization included prior surgery for bleeding ($P < 0.024$, risk ratio 2.8). Fewer units of PRBCs transfused prior to angiography tended to predict successful treatment (11.2 vs. 17.1 units, $P < 0.08$). As expected, the mortality rate was found to be significantly higher in those who failed angiographic therapy (62.5% vs. 17%, $P < 0.0015$, risk ratio 7.8). There was no predictive value for failure based on age (64.5 vs. 64.7 years,

Table I. Predictors of angiographic outcome

Variable	Success	Failure	*P* value
Days to angiography	2.5	5.8	0.017
Total PRBCs	14.6	34.0	0.003
Prior PRBCs	11.2	17.1	0.084
Prior surgery	2 (8%)	8 (33%)	0.024
No. of deaths	5 (17%)	16 (62.5%)	0.0012

PRBCs = packed red blood cells.

Table II. Nonpredictors of angiographic outcome

Variable	Success	Failure	*P* value
Coagulopathy	9 (35%)	13 (54%)	0.12
FFP prior	2.4	5.4	0.39
FFP total	3.4	16.9	0.70
Duodenal source	19 (73%)	18 (75%)	0.15
EGD	1.26	2.8	0.15
Bleeding at angiography	15 (58%)	10 (42%)	0.19
Bleeding on admission	13 (50%)	4 (16%)	0.31
Age (yr)	65	64	0.43
No. of comorbid diseases (mean)	1.5	2	0.52
No. of failed organs (mean)	1.3	1.62	0.36
APACHE III score	75	84	0.96

FFP = fresh-frozen plasma; EGD = esophagogastroduodenoscopy.

$P = 0.43$), admission for gastrointestinal bleeding ($P = 0.31$), number of comorbid diseases ($P = 0.76$), number of organs in failure ($P = 0.59$), APACHE III score (83.5 vs. 75.5, $P = 0.96$), length of stay in the intensive care unit or hospital ($P = 0.39$ and 0.095, respectively), units of fresh-frozen plasma prior to angiography or total transfused ($P = 0.39$ and 0.70, respectively), number of esophagogastroduodenoscopies ($P = 0.15$), bleeding at angiography (42% vs. 58%, $P = 0.19$), gastric or duodenal source of bleeding ($P = 0.15$), or presence of coagulopathy ($P = 0.12$). All patients successfully treated with angiography underwent embolization. Three patients who ultimately failed angiographic intervention did not have radiographic evidence of bleeding and did not undergo empiric embolization for technical reasons.

Ultimately surgery was attempted to control bleeding in 17 (71%) of the 24 patients who had failed embolization. Twelve of these operated patients had persistent bleeding from a duodenal ulcer, six of whom died. Seven underwent a truncal vagotomy and pyloroplasty or antrectomy, and five had oversewing of the bleeding ulcer alone. Five of the operated patients had a gastric ulcer, three of whom died. All of the patients who died had oversewing of the bleeding site and the two who survived had a gastric resection. The total surgical mortality rate was 52% and included four patients with persistent postoperative gastrointestinal bleeding. Only one surgical patient had an anastomotic leak, and multisystem organ failure was responsible for most surgical deaths.

Overall 20 patients (40%) died including two (4%) from angiographic complications; one patient died 12 hours after angiography of cardiopulmonary arrest and the other after surgery for a perforated duodenal ulcer 5 days after embolization. Those who were treated successfully with angiography ultimately died of their underlying diseases.

DISCUSSION

The study group selected in this retrospective review clearly represents patients at high risk for death from acute gastointestinal hemorrhage. These patients had profuse bleeding, frequently with coagulopathy, and two thirds were initially hospitalized for other medical diseases. Although complicating the care of only 1.5% of critically ill medical intensive care unit patients, gastrointestinal bleeding requiring transfusion has been shown prospectively to result in a 48.5% mortality rate.[12] Our overall 40% mortality rate underscores the challenge faced in the care of critically ill patients with major gastrointestinal bleeding. The predicted 29% mortality rate by APACHE III scoring was perhaps less reliable in estimating the actual outcome because the bleeding that was responsible for death in half the group was not present in most of the patients at the time of their admission to the intensive care unit.

Angiographic embolization was found to be successful in half of these patients with gastrointestinal bleeding. Success tended to occur in patients with fewer transfusions and a shorter time to angiographic intervention. This success was achieved despite the fact that these patients were similar with regard to the number of comorbid diseases, degree of organ failure, age, and APACHE III score. The ability to achieve success in treating bleeding in critically ill patients appears to be dependent on early intervention rather than the severity of the underlying medical disease. Although 17% of these successfully treated patients ultimately died, their deaths were a consequence of their underlying disease, and not the result of ongoing bleeding. Angiography and embolization were accomplished with low procedural morbidity and mortality. Intestinal ischemia following embolization[18] was initially reported 4 years after the first embolization and likely contributed to the postembolization perfo-

ration of a duodenal ulcer. The concern for ischemic injury has led some to attempt arterial vasoconstrictive infusions with ADH and vasopressin for gastric bleeding sites, unfortunately with rebleeding rates of 25%.[19] Vasoconstrictive infusions are ineffective for duodenal hemorrhage because of the dual blood supply from the pancreaticoduodenal arcade.[20] The combination of these factors has influenced our selection of embolization as the primary angiographic treatment for gastric and duodenal bleeding.

The failure of angiographic embolization to control bleeding portends a poor prognosis. Fifteen (62.5%) of 24 patients who demonstrated ongoing bleeding ultimately died, which is similar to findings in other smaller angiographic series.[21,22] Surgery was able to salvage only half of those who failed angiographic treatment. Of the 10 patients who had already failed a prior attempt at surgical treatment, angiography also failed in eight, making reoperation the recommended treatment. Active bleeding was present at angiography in half of the entire group and did not predict the outcome of embolic treatment. Empiric embolization was performed in nearly all others and has been advised for gastric bleeding when the endoscopic site is in the distribution of the left gastric artery, and the patient is already critically ill.[23] Indeed, empiric embolization was used slightly less frequently in patients who failed angiographic intervention. Our results also do not predict whether an endoscopically established gastric or duodenal source was more likely to respond to angiographic treatment. Gastric lesions have been reported to have a higher success rate with embolization, presumably because it targets treatment to the left gastric artery, which supplies 85% of the bleeding sites.[14,21,24] The preponderance of duodenal bleeding may have adversely affected our outcome.

In summary, angiography with embolization is successful in controlling acute massive gastroduodenal bleeding in half of the critically ill patients studied. If angiography is entertained in the management of bleeding, it should be performed expeditiously, early in the course of bleeding, with surgical salvage soon thereafter should there be evidence of continued bleeding. Aggressive treatment may still be associated with a poor outcome.

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Discussion

Dr. J. Fischer (Cincinnati, Ohio). In your group that underwent angiography, you had patients who received 11 and 17 units of blood prior to your intervention. To those who take care of these patients, I suspect that seems excessive. Is there an explanation?

Dr. R. Walsh. I think it correlates with the degree of bleeding. You will remember that in many of these patients angiography was performed within 2 days, so that is still a great deal of bleeding in a short period of time. Since half of the patients were in the intensive care unit, and had significant comorbid diseases, they were poor risks and were more likely to undergo repeat endoscopy and receive additional transfusions before other types of therapy such as angiography or surgery were considered.

Dr. L. Rikkers (Madison, Wis.). I realize that these were patients who were very ill and difficult to treat. However, there seemed to be an excessive amount of blood transfused before definitive action was taken, whether it was angiography or surgery. I believe that the most desperately ill patients require definitive action sooner rather than later. This is especially true of patients with lesions that are easily remedied by surgery such as bleeding duodenal ulcers. There is morbidity associated with these techniques. I recently saw a 40-year-old woman who underwent embolization of a bleeding posterior duodenal ulcer. She developed severe ischemic pancreatitis, a duodenal stricture, and a distal biliary stricture. She required 3 or 4 months of hospitalization. I believe that embolization is particularly useful in patients who are extremely poor surgical risks and patients with bleeding that is difficult to control surgically, for example, proximal gastric bleeding. On the other hand, I believe surgery still plays a major role and should be used early when endoscopic therapy has failed.

Dr. Walsh. I would not disagree with anything you have said but I would add that only 70% of the patients who had angiography had a surgeon involved before the decision was made to perform the angiography.

Dr. C. Filipi (Omaha, Neb.). We have been interested in this problem and have worked on developing endo-organ techniques for control of duodenal ulcer bleeding, but we have experienced some difficulty, particularly because access to the ulcer is limited by the intraluminal approach. We examined patients by means of intraoperative ultrasound who have undergone ligation of bleeding duodenal ulcers and found that the sutures do not occlude the gastroduodenal artery. In your experience what is the anatomy of the bleeding vessel in a posterior duodenal ulcer?

Dr. Walsh. In the patients who had a bleeding duodenal ulcer, the artery that was embolized almost universally was the gastroduodenal artery. As in the example I presented, embolization of feeding branches from that vessel may be required.

Dr. M. Callery (Worcester, Mass.). What is the incidence of ischemic pancreatitis with this technique?

Dr. Walsh. I do not know the reported incidence. We did not have any patients who had that complication.

Appendectomy in the Pre- and Postlaparoscopic Eras

Davis B. Nguyen, William Silen, M.D., Richard A. Hodin, M.D.

The role of laparoscopic appendectomy remains controversial since many authors have suggested that overall morbidity is primarily a function of the degree of appendicitis rather than the operative approach. We have reviewed our appendectomy experience to determine the advantages and/or disadvantages of the laparoscopic technique in cases of acute appendicitis, and furthermore to ascertain whether the extent of disease should affect the surgical approach used. Data were accumulated for all 1158 patients who underwent appendectomy at a single institution during the following three time periods that span the pre- and postlaparoscopic eras: period I (1987 to 1990), period II (1991 to 1993), and period III (1994 to 1997). Cases were categorized with regard to pathologic findings and operative approach (i.e., open or laparoscopic appendectomy). The percentage of appendectomies performed laparoscopically increased with time (0%, 27%, and 79% for periods I, II, and III, respectively). Overall, the total operating room time was slightly shorter for laparoscopic compared to open appendectomy (99 vs. 102 minutes; $P < 0.05$). Operating room times for open appendectomy remained unchanged, but the times for laparoscopic appendectomy decreased from period II to period III (119 to 94 minutes; $P < 0.001$). In cases of gangrenous/perforated appendicitis, the times for laparoscopic appendectomy were significantly shorter than those for open appendectomy (98/115 vs. 120/125 minutes; $P < 0.001$ for both). Overall, the hospital stay was shorter for patients undergoing laparoscopic appendectomy (1.63 vs. 4.21 days; $P < 0.001$), and the difference was maintained in all three time periods. The differences in length of hospital stay for laparoscopic vs. open appendectomy were most dramatic in gangrenous/perforated cases (1.8/3.0 vs. 4.0/9.0 days; $P < 0.001$), whereas there was only a slight difference in cases of simple appendicitis, for example, 1.6 vs. 2.1 days (laparoscopic vs. open appendectomy, period III). There was a significant decrease in the percentage of perforated cases in which surgical treatment had been delayed (>8 hours) (21%, 5%, and 5%) over the three time periods, but the rate of "negative" appendectomies was similar (10%, 8%, and 8%). The complication rates following laparoscopic and open appendectomies during period II were 5.4% and 7.5%, respectively ($P > 0.05$). Laparoscopic appendectomy results in a marked decrease in the length of hospital stay and similar postoperative morbidity compared to open appendectomy. In cases of gangrenous or perforated appendicitis, laparoscopic appendectomy appears to be especially worthwhile in regard to both operating room time and hospital stay. (J GASTROINTEST SURG 1999;3:67-73.)

KEY WORDS: Appendectomy, laparoscopy, surgery

Appendicitis is the most common cause of abdominal pain requiring surgery. The first appendectomy in the United States was performed in 1885.[1] Since the landmark description of the diagnosis and treatment of appendicitis by Fitz[2] in 1886, appendectomies have classically been performed through a right lower quadrant oblique or transverse incision, with little change in this method over the past 100 years. Recently, operative laparoscopy has proved to be useful in both the diagnosis and treatment of a variety of intra-abdominal conditions. Semm[3] introduced the laparoscopic technique for appendectomies in 1983, and since then a number of surgeons have published favorable results with laparoscopic appendectomy. Both retrospective and prospective studies have shown that despite increased operating room time, other parameters such as length of hospital stay, complications, and postoperative discomfort are reduced after laparoscopic appendectomy in comparison with the open operation.[4-28] Laparoscopic appendectomy has not been universally accepted, however, and a few reports have suggested that the laparoscopic method

From the Department of Surgery, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Mass.
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Reprint requests: Richard A. Hodin, M.D., Beth Israel Deaconess Medical Center, 330 Brookline Ave., Boston, MA 02215. E-mail: rhodin@bidmc.harvard.edu

is contraindicated in cases of complicated appendicitis, for example, gangrene or perforation, because of increased operating room time, hospital stay, and the risk of postoperative complications.[5,7,12,20,25] As such, the role of laparoscopic appendectomy remains controversial, since many authors have suggested that the overall morbidity is primarily a function of the severity of appendicitis rather than the operative approach. We have reviewed our experience over the past decade (1987 to 1997) at Beth Israel Hospital to determine the advantages and/or disadvantages of the laparoscopic technique in cases of acute appendicitis, and furthermore to ascertain whether the extent of disease should affect the surgical approach used.

PATIENTS AND METHODS

We analyzed the medical records and pathology reports of all patients who underwent appendectomy at Beth Israel Hospital (Boston, Mass.) from January 1987 through June 1997 (1158 cases). Incidental appendectomies and cases of nonappendicitis, such as appendiceal tumor and Crohn's disease of the appendix, were excluded from the study. Patients who underwent appendectomy for chronic right lower quadrant pain or interval appendectomy were also excluded. Patients were stratified into one of the three following categroies based on both the operative and pathologic findings: (1) acute, (2) gangrenous, or (3) perforated. When the surgeon's diagnosis and the pathology report did not correlate, the patient was assigned to the more complicated category (e.g., perforated vs. acute). Gangrenous appendicitis was defined as transmural necrosis of the appendix without rupture. Perforated appendicitis was defined as free appendiceal rupture with intra-abdominal purulence.

The operative approach, laparoscopic (LA) or open appendectomy (OA), was noted and conversions from LA to OA were considered separately. Patient characteristics, operating room time, and length of hospital stay were determined from the medical record. The operating room time represents the time from the patient's entry into the operating room to the exit from the operating room. Postoperative complications were determined from review of morbidity and mortality reports.

Statistical Analysis

Data are presented as mean ± standard error, with $P < 0.05$ considered statistically significant. Complication rates are compared using Z approximation, with $P < 0.05$ considered statistically significant.

RESULTS

From January 1987 through June 1997, a total of 1158 appendectomies (490 LAs, 639 OAs, and 29 conversions) were performed at Beth Israel Hospital. The conversion rate was 5.6%. The age and sex distributions were similar among the three groups. In cases of acute and gangrenous appendicitis, there was a similar distribution for LA vs. OA. However, in cases of perforation, there was a greater proportion of OA than LA ($P < 0.05$; Fig. 1). Overall the total operating room time was slightly shorter and the hospital stay was dramatically shorter in LA patients compared to OA patients (Table I).

The evolution of both LA and OA is evident when the three pivotal time periods are compared: period I (1987 to 1990), only OA performed; period II (1991 to 1993), transition period; and period III (1994 to 1997), majority LA (Fig. 2). During period II LA took longer to perform than OA (119 vs. 106 minutes; $P < 0.05$), but during period III operating room time for LA was significantly shorter than that for OA (94 vs. 104 minutes; $P < 0.001$). Length of hospital stay was shorter for LA during period II (2.57 vs. 3.85 days; $P < 0.001$), and during period III the difference became more pronounced (1.41 vs. 3.37 days; $P < 0.001$). The operating room time for OA patients has remained relatively stable as a function of time, whereas it has shown a marked decrement in LA patients (Fig. 3, *A*). The mean hospital stay steadily de-

Table I. Appendectomies 1987 to 1997

	LA (N = 490)	OA (N = 639)	Conversion* (N = 29)
Age (yr)	33	38	41
Sex ratio (M:F)	219:271	331:308	18:11
Operating room time (min)†	99 ± 1	102 ± 1	165 ± 11
Length of hospital stay (days)‡	1.6 ± 0.1	4.2 ± 0.1	3.8 ± 0.4

LA = laparoscopic appendectomy; OA = open appendectomy.
*Conversion group not included in either LA or OA.
†$P < 0.05$ (LA vs. OA).
‡$P < 0.001$ (LA vs. OA).

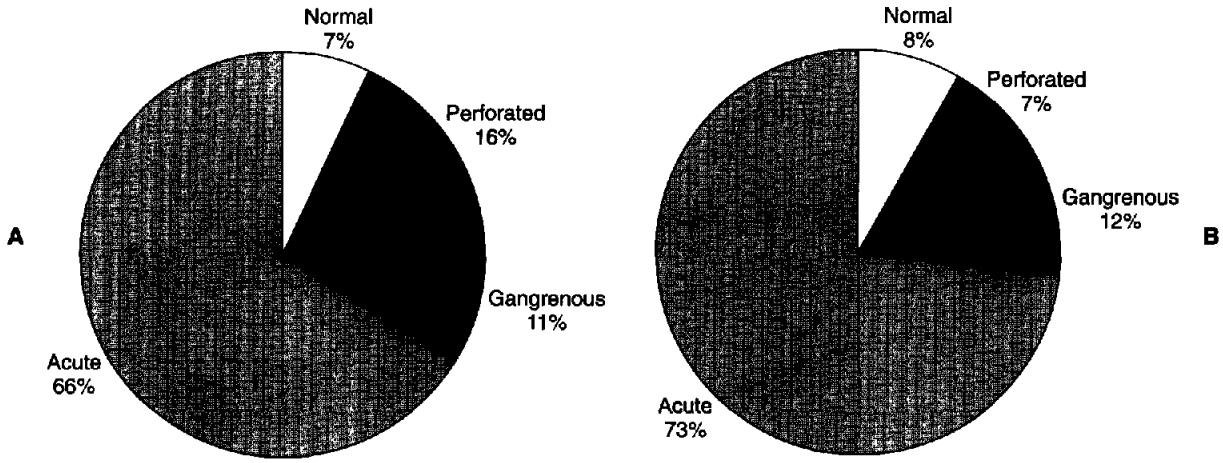

Fig. 1. Distribution of pathologic and/or operative findings, 1987 to 1997, following open appendectomy **(A)** and laparoscopic appendectomy **(B)**.

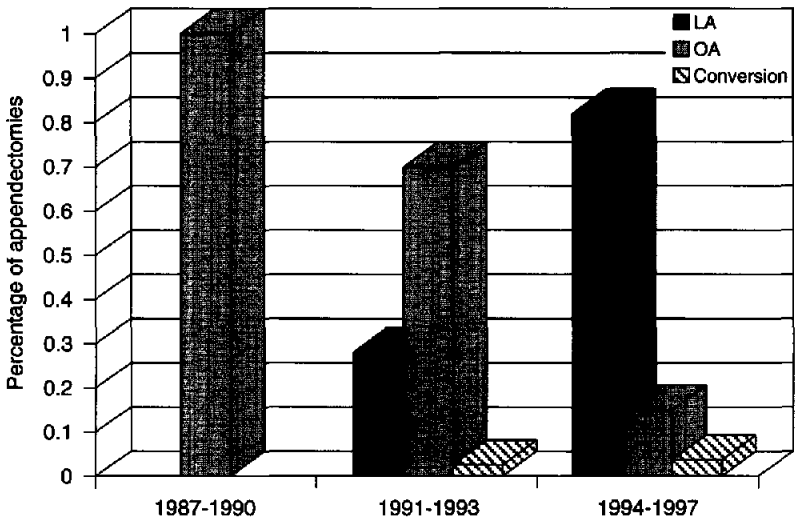

Fig. 2. Evolution of appendectomies performed during three pivotal time periods. All appendectomies were performed using the standard open technique during 1987 to 1990. A transition period occurred during 1991 to 1993, and the majority of appendectomies during 1994 to 1997 were performed laparoscopically.

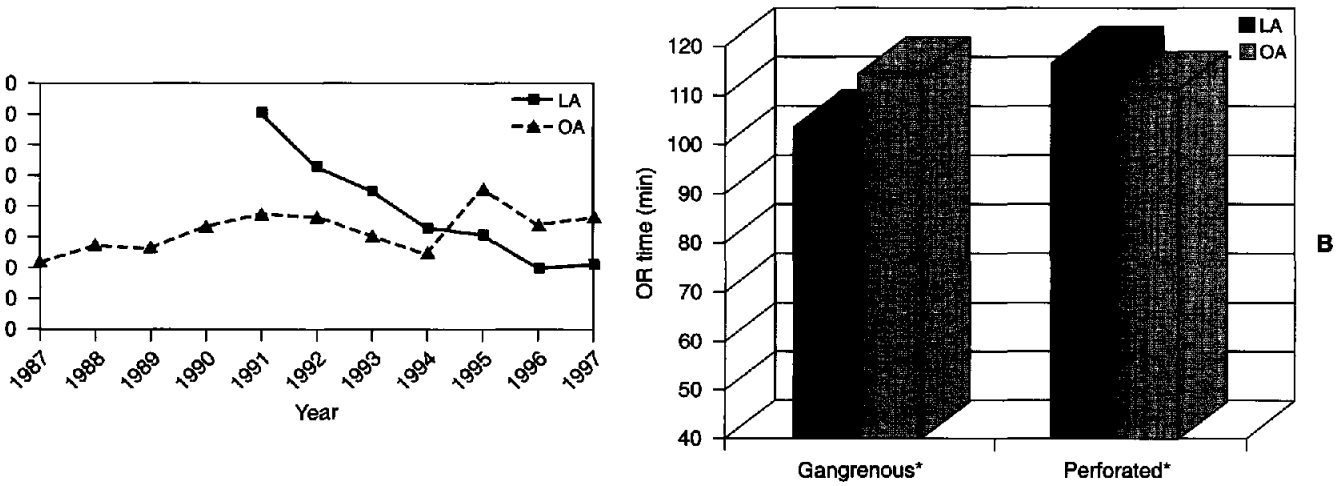

Fig. 3. Comparison of operating room *(OR)* times for laparoscopic appendectomy *(LA)* vs. open appendectomy *(OA)* as a function of time **(A)** and according to extent of disease **(B)** (* = $P < 0.05$). NOTE: OR time represents time from patient's entry into the operating room to exit from the operating room.

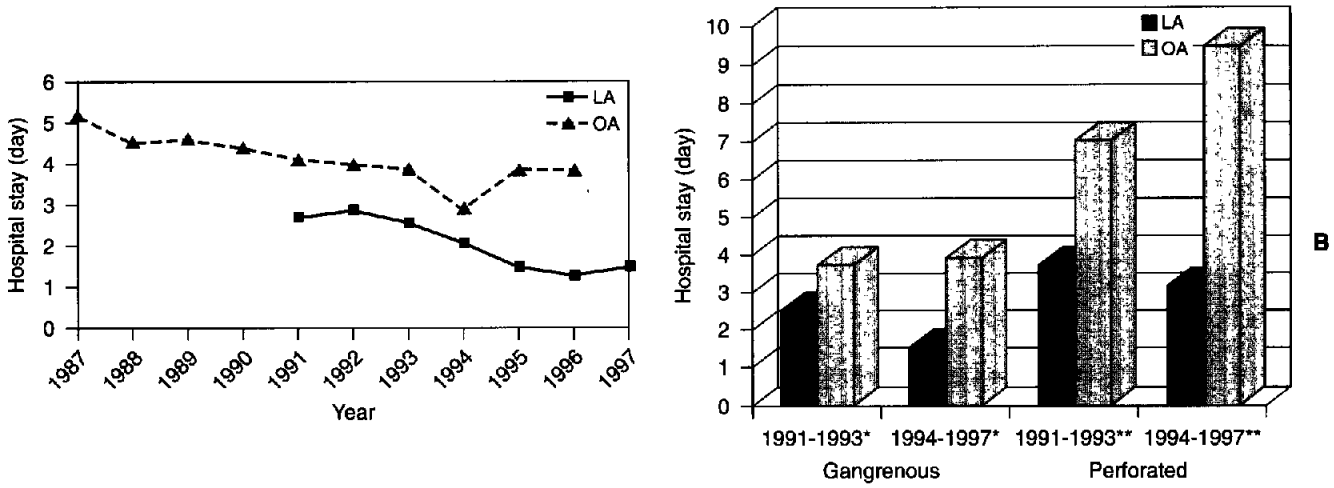

Fig. 4. Comparison of hospital stays for laparoscopic appendectomy *(LA)* vs. open appendectomy *(OA)* as a function of time **(A)** and according to time frame and extent of disease **(B)** (* = $P < 0.05$; ** = $P < 0.001$).

creased for both LA and OA patients, although it was always shorter in the LA group (Fig. 4, *A*).

Stratification of Patients

Acute Appendicitis. There were 731 cases of simple acute appendicitis over the 10½-year period. The percentages of these cases that were initially observed (for 8 hours or longer) for the three time periods were 15%, 16%, and 7%, respectively. The operating room time was longer for LA than OA in period II (120 vs. 106 minutes; $P < 0.05$), but during period III there was no difference in the operating room time between LA and OA patients (92 vs. 98 minutes). The LA group had a slightly shorter hospital stay compared to the OA group during both periods II and III (2.77 vs. 3.27 days, $P < 0.05$, period II and 1.39 vs. 2.36 days, $P < 0.001$, period III).

Gangrenous Appendicitis. There were 119 cases of gangrenous appendicitis. The percentages of these cases that were initially observed (for 8 hours or longer) for the three time periods were 17%, 0%, and 9%, respectively. When both periods II and III are considered collectively, the operating room time in gangrenous cases was similar to the times for LA and OA (Fig. 3, *B*). However, when period III was isolated, there was a dramatic difference in operating room times between LA and OA (100 vs. 124 minutes; $P < 0.001$). The hospital stay was also significantly shorter for LA compared to OA during both periods II and III (Fig. 4, *B*).

Perforated Appendicitis. There were 138 cases of perforated appendicitis. The percentages of these cases that were initially observed (for 8 hours or longer) for the three time periods were 21%, 5%, and 5%, respectively. There was no statistical difference in operating room time between LA and OA, but the hospital stay was significantly shorter for LA compared to OA during both periods II and III (Figs. 3, *B* and 4, *B*).

Table II. Postoperative morbidity 1991 to 1993

	LA (N = 93)	OA (N = 228)
Urinary retention	0	9
Wound infection	0	2
Pulmonary embolus	0	2
Abscess	0	1
Hematoma	1	1
Fever	1	1
C.difficile colitis	1	1
Bleeding	1	0
Stump leak	1	0
TOTAL*	5 (5.4%)	17 (7.5%)

LA = laparoscopic appendectomy; OA = open appendectomy.
*Not statistically different (LA vs. OA).

Complications. Postoperative morbidity was compared between LA and OA during period II, when both procedures were performed. LA was performed in 93 cases and OA in 228 during this period. In cases of gangrenous or perforated appendicitis, 11 LAs and 58 OAs were performed. Complications following OA included urinary retention (9), pulmonary embolus (2), wound infection (2), hematoma (1), fever (1), abscess (1), and *Clostridium difficile* colitis (1). Complications following LA included hematoma (1), fever (1), bleeding (1), stump leak (1), and *C.difficile* colitis (1). OA had an overall complication rate of 7.5%, which was not statistically different from the rate in the LA

group (5.4%). One of the two wound infections following OA, as well as the hematoma and stump leak following LA, involved cases of perforated appendicitis (Table II). There were no deaths in either group.

DISCUSSION

Many studies have reported a longer duration of operation for LA compared to OA,[15-19,21,26] whereas a few have reported little or no difference between the two approaches.[4,20,21,27] An important feature of our study is that the operating room time for LA was actually shorter than that for OA. As seen in numerous studies on operative laparoscopy, we noted evidence of a learning curve, with a 25-minute decrease in operating room time for LA between periods II and III. It is important to observe that previous studies have suggested greater expense for the LA procedure based on longer operation times and higher technology costs.[15,22] Although equipment costs are clearly increased with LA, since we saw no marked difference in operating room time, and the hospital stay was shorter with LA, it is likely that costs are not greater for LA.

The potential for LA to improve patient outcomes and decrease health care spending by shortening the hospital stay has been suggested by many studies.* Although it is true that the hospital stay for OA has decreased in our study, the hospital stay for LA has always been shorter than that for OA, and during period III the difference became more pronounced. Nonetheless, some have argued that it is difficult to ensure the absence of bias in determining postoperative recovery, especially "fitness for discharge." If a surgeon believes that laparoscopic surgery is advantageous, his or her attitude toward patients and their management may be altered.[4,19] Some authors have suggested that differences in hospital stay between LA and OA might be attributed to a higher percentage of patients with perforation undergoing the open procedure.[15,24] However, our study stratified patients according to extent of disease, and the differences in hospital stay between LA and OA were maintained. In fact, the advantage of LA over OA in terms of hospital stay was even greater in cases of perforated appendicitis.

LA has previously been described as time consuming in cases of gangrenous appendicitis, with little difference seen in the length of hospital stay.[16,20] However, we have shown a significant advantage of LA over OA with regard to both operating room time and hospital stay in cases of gangrenous appendicitis. In cases of perforated appendicitis, Frazee and Bohannon[25] found the average hospital stay after LA to be 7 to 19 days and concluded that the prolonged hospitalization negates one of the primary advantages of the laparoscopic procedure. However, as in gangrenous appendicitis, our study of perforated appendicitis also demonstrated a significant advantage for LA over OA in terms of hospital stay. Complicated (gangrenous and perforated) appendicitis usually increases the morbidity and mortality rates.[2,29,30] It has been suggested that LA when performed for perforated appendicitis is associated with a greater risk of postoperative complications. Although we did find that the postoperative complication rate following LA for perforated appendicitis (18%) was higher than that following OA (2%) during period II, the total number of perforated cases was small, and we also expect complications to decrease with further laparoscopic experience. The decreased hospital stay following LA in perforated cases should also help to outweigh this potential disadvantage. A possible limitation of our study was that the difference in severity of peritonitis between the LA and OA patients was not examined (e.g., the exact amount of intraperitoneal pus was not measured).

It has also been suggested that the laparoscopic technique can result in a more complete and effective lavage of the peritoneal cavity.[6-8] This idea is supported by studies demonstrating fewer wound infections with the laparoscopic approach.[4,18,22] Although our study found the wound infection rate and the overall complication rate to be lower for LA compared to OA, this difference was not statistically significant. LA may also be advantageous in terms of optimizing exposure and thus decreasing the risk of retraction-associated tissue damage.[18,28]

It has become clear that laparoscopy is effective as a diagnostic tool in some patients with abdominal pain.[16,26] The number of perforated cases in our series has decreased with time (17% of total cases in period II and only 8% of total cases in period III). The explanation for this is unclear, but the advent of laparoscopy may have proved beneficial in this regard, since the number of perforated cases in which surgery had been delayed (>8 hours) was also decreased (Fig. 5). However, concern has been raised as to the possibility that surgeons will be "overly aggressive" in performing laparoscopy in such patients. Since it is the practice at our institution to remove the normal appendix if no other cause for the pain is found, we wondered whether there would be an increase in "negative" appendectomies. However, we found that the incidence of normal appendices has remained stable (8% to 10%) over the recent periods, and it is actually lower than that generally quoted in the literature (20% to 30%).[24,31,32] It does not appear, there-

*References 5, 13, 15, 17, 18, 20, 23, 26, 28.

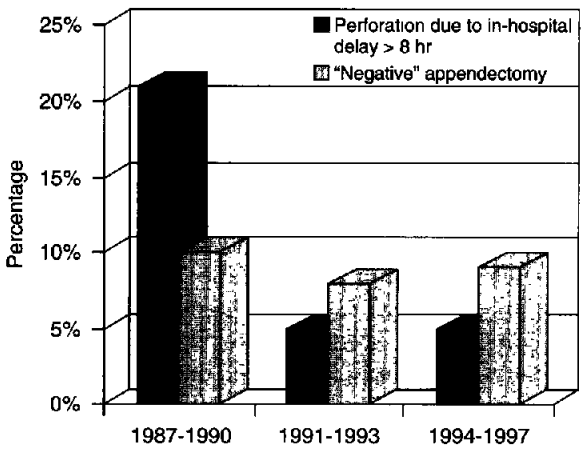

Fig. 5. Rate of perforated cases that were initially observed has decreased, whereas the "negative" appendectomy rate has remained stable as a function of time.

fore, that the surgeons at our institution have become too aggressive in regard to performing surgery in patients with suspected appendicitis.

In summary, LA results in a marked decrease in the length of hospital stay compared to OA, without a significant difference in operating room time or postoperative morbidity. In cases of gangrenous or perforated appendicitis, LA appears to be especially worthwhile with regard to both operating room time and length of hospital stay. The availability of LA for diagnostic purposes probably contributes to a reduced incidence of perforation associated with in-hospital delay. We conclude that LA is an effective surgical approach and may be superior to the traditional open method, especially in cases of gangrenous and perforated appendicitis.

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Discussion

Dr. R. Stephens (Phoenix, Ariz.). I think this is a classic presentation. Those of us who perform laparoscopic procedures have been fighting this battle for years. Those of us who treat acute appendicitis laparoscopically have always considered this to be a superior method, and for the first time you have proved it. I have two questions. Are you using the Endoloop (Ethicon, Inc., Somerville, N.J.) for tying off the stump or are you using a stapling device? Also, have you given consideration to making this totally an outpatient procedure? This is essentially what we are doing now.

Dr. D. Nguyen (Boston, Mass.). We are currently using the Endo-GIA stapling device (U.S. Surgical Corp., Norwalk, Conn.). We were using the Endoloop during the transition period when the laparoscopic technique first came into use, but we find that the stapler results in fewer complications. In regard to your second question, we are headed toward appendectomy for acute appendicitis becoming an outpatient procedure. The hospital stay is now about 1½ days. In interval appendectomies, where patients present with a mass, they are treated with antibiotics and come back about 10 weeks later for an elective appendectomy, which can be performed as an outpatient procedure.

Dr. E. Livingston (Los Angeles, Calif.). I am a believer in laparoscopic appendectomy, but I am not sure that your data support your conclusions. You stated that the hospital stay at your institution was roughly 4 days for the open technique and slightly less than 2 days for the laparoscopic technique. At our institution the hospital stay for an open appendectomy is usually about 2 days. Because this was a retrospective study, I suspect that the longer hospital stay at your institution for patients undergoing open laparoscopy reflects that this is a group of patients who are sicker and have more complicated disease. Second, you stated that your operating room time was shorter with the laparoscopic technique, 99 minutes vs. 102 minutes for the open technique. What was the standard error of the mean for that? I find it remarkable that you can find a statistically significant difference for operating room time, which is a parameter that tends to be all over the map?

Dr. Nguyen. We agree that the slight difference in operating room time is not clinically relevant. As far as the first question is concerned, we did stratify our patients according to extent of disease (gangrenous, perforated) so that we could determine whether there were sicker patients in the open group, or in the laparoscopic group. When we did that, the difference in the hospital stay was maintained. So, in the end, laparoscopic appendectomy resulted in a shorter hospital stay regardless of patient presentation.

Dr. Livingston. Let me just comment on your statistics. If those are your standard errors, then you could not have had a statistically significant difference.

Dr. P. Paik (Los Angeles, Calif.). Most of the reported deaths following appendectomy are the result of intra-abdominal abscess formation. How many of your patients had intra-abdominal abscesses following appendectomy? Are you comparing this incidence as well? Second, why did patients stay in the hospital longer after the open technique than after the laparoscopic procedure? This contradicts some of the previously reported data.

Dr. Nguyen. There was one intra-abdominal abscess in the open group and one in the laparoscopic group. As to why open appendectomy patients stay in the hospital longer than those undergoing laparoscopy, it appears that patients require less pain medication following laparoscopic appendectomy. There is a smaller scar and patients are able to recover more quickly.

Bak Expression and Cell Death Occur in Peritumorous Tissue But Not in Pancreatic Cancer Cells

Hans U. Graber, Ph.D., Helmut Friess, M.D., Arthur Zimmermann, M.D., Murray Korc, M.D., Guido Adler, M.D., Roland Schmid, M.D., Markus W. Büchler, M.D.

Bak is a pro-apoptotic member of the Bcl-2 family whose genes are involved in regulation of programmed cell death. Using in situ hybridization, immunohistochemistry, and Northern blot analysis, we studied the expression of Bak in specimens from 12 normal pancreata and 26 primary pancreatic cancers, and correlated the findings with the clinical and histopathologic data of the patients. By comparison with normal pancreas, Northern blot analysis demonstrated a 2.5-fold increase of Bak messenger RNA expression in the tumor samples ($P < 0.001$). Elevated levels were found in 15 of the 26 pancreatic cancer tissue specimens. In these samples Bak expression was increased 4.3 fold ($P < 0.001$). No association was detected between Bak expression and tumor stage. In situ hybridization and immunohistochemistry revealed that the tumor cells themselves and the stroma cells expressed only low levels of Bak. In contrast, in regions adjacent to the tumor, which showed chronic inflammation, there was always high expression in the acinar and inflammatory cells, explaining the increased Bak levels found in the tumor samples by means of Northern blot analysis. In the normal pancreas the expression of Bak was generally moderate in the acinar cells and low in the ductal and islet cells. In situ analysis using the terminal deoxynucleotidyl transferase method further showed that there was extensive cell death in the peritumorous areas with chronic inflammation. Taken together, these results suggest that in pancreatic cancer Bak expression and programmed cell death are present in cells that are localized in regions of chronic inflammation surrounding the pancreatic cancer cells but not in the tumor cells themselves, a situation that may facilitate tumor growth and spread. (J GASTROINTEST SURG 1999;3:74-81.)

KEY WORDS: Pancreatic cancer, carcinogenesis, apoptosis, Bak expression, in situ hybridization

Apoptosis as a form of programmed cell death is thought to play an important role in the prevention of malignant cell transformation by contributing to the elimination of transformed cells.[1] Several genes involved in the regulation of programmed cell death have been identified during the past few years including members of the Bcl-2 family, which are characterized by the presence of the two conserved domains, BH1 and BH2.[2] Proteins of this gene family either suppress or accelerate apoptosis. Apoptosis-inhibiting proteins include Bcl-2,[3] Bcl-x_L,[4] and several viral proteins, whereas Bak,[5,6] Bax,[7] and Bcl-x_S[4] are cell death–promoting members. In Bak, Bax, and in the non–Bcl-2 family–related Bik,[8] an additional conserved 9-amino acid domain (BH3) was detected, which is necessary and sufficient for cytotoxic activity and binding to Bcl-2 and Bcl-x_L.[8,9] Formation of heterodimers between apoptosis promoting and suppressing proteins may either accelerate[5] or inhibit[8] programmed cell death, suggesting that the ratio of these two protein groups in cells is important for induction or inhibition of apoptosis, and may therefore be critical for the regulation of cell growth and tissue homeostasis.

Pancreatic cancer has a poor prognosis and is presently the fourth or fifth leading cause of cancer-related deaths in Western industrialized countries.[10] According to Gudjonsson,[11] the overall survival rate

From the Department of Visceral and Transplantation Surgery (H.U.G., H.F., and M.W.B.) and the Institute of Pathology (A.Z.), University of Bern, Inselspital, Bern, Switzerland; the Department of Gastroenterology, University of Ulm (G.A. and R.S.), Ulm, Germany; and the Division of Endocrinology, Diabetes and Metabolism, University of California, Irvine (M.K.), Irvine, Calif.
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Reprint requests: Helmut Friess, M.D., Department of Visceral and Transplantation Surgery, University of Bern, Inselspital, CH-3010 Bern, Switzerland.

is 0.4% and the median survival time after diagnosis is approximately 5 months. Once pancreatic cancer is clinically evident, it progresses rapidly, and metastases have normally occurred at the time of diagnosis. Up to now, it has not been clear why pancreatic cancer grows in such an aggressive manner. Recent studies were able to demonstrate that the concomitant overexpression of epidermal growth factor receptor with its ligands epidermal growth factor and transforming growth factor-alpha[12] and/or amphiregulin[13] in pancreatic cancer is associated with shorter postoperative survival after tumor resection. In addition, overexpression of transforming growth factor-beta isoforms contributes to tumor aggressiveness and poorer prognosis.[14] However, the influence of apoptosis promoting or suppressing factors in pancreatic carcinogenesis has not been studied, and it is not known whether genes involved in the regulation of apoptosis are altered in pancreatic cancer cells. For that reason we used Northern blot analysis to quantify the messengerRNA (mRNA) expression of the pro-apoptotic gene Bak in primary pancreatic cancers and in normal pancreata, and we used in situ hybridization and immunohistochemistry to identify the cells expressing Bak. Furthermore, tissue samples of normal and cancerous pancreata were analyzed by the terminal deoxynucleotidyl transferase (TdT) method for nuclear DNA fragmentation, a hallmark of programmed cell death. We now report that Bak mRNA and protein are not expressed in pancreatic cancer cells but rather in the peritumorous tissue exhibiting morphologic changes similar to those of chronic pancreatitis. These areas of chronic inflammation surrounding the pancreatic cancer cells are further characterized by extensive cell death, indicating that cell death–promoting mechanisms are activated in areas adjacent to the tumor but not in the cancer cells themselves.

PATIENTS AND METHODS

Normal human pancreatic tissue samples were obtained from 12 previously healthy individuals (9 males and 3 females; median age 26 years [range 10 to 62 years]) through an organ donor program. Tissue samples of pancreatic adenocarcinomas were obtained from 18 male and 8 female patients undergoing pancreatic surgery at the University Hospital of Bern (Bern, Switzerland). The median age of the patients was 67 years (range 32 to 78 years). There were three patients with stage I disease, 12 with stage II disease, and 11 with stage III disease according to the classification of the International Union Against Cancer.[15] All tissues used in this study were taken fresh in the operating room directly after removal. Tissue samples destined for RNA extraction were immediately frozen in liquid nitrogen and stored at −80° C until further use. No signs of degradation were found when the samples were later analyzed using Northern blot analysis, in situ hybridization, or immunohistochemistry. Concomitantly, tissue samples were fixed in formalin and embedded in paraffin for analysis by in situ hybridization. All studies were approved by the Ethics Committee of the University of Bern, Switzerland.

cDNA Clones

A plasmid containing the complete 2.1 kb coding sequence of Bak was obtained from Dr. R. Brown (Glaxo Research and Development, Greenford, Middlesex, U.K.). A new clone (pBak clone) was generated by subcloning a 0.5 kb PstI DNA fragment of the coding sequence into the pGEM-3Zf(+) (Promega Corp., Zürich, Switzerland) plasmid vector, which contains promoters for the DNA-dependent SP6 and T7 RNA polymerases. The authenticity of the subcloned DNA fragment was confirmed by sequencing using the dye-terminator method (ABI 373A Perkin-Elmer, Rotkreuz, Switzerland).

A cDNA clone with a 0.19 kb insert coding for the mouse 7S cytoplasmic RNA that cross hybridizes with human 7S RNA was used as an internal control.[12]

Preparation of a Nonradioactive Bak Probe for In Situ Hybridization

Digoxigenin-labeled sense and antisense RNA probes for Bak were produced according to an earlier published protocol.[16] The pBak plasmid was used as a template along with the Ribomax System (Promega Corp.) resulting in transcription with strand-specific runoff probes that were digoxigenin labeled. The probes were shortened to a length of approximately 150 bp[17,18] and stored in diethyl pyrocarbonate–treated water at −80° C until further use.

Preparation of Radioactive Bak and 7S Probes for Northern Blot Analysis

Using a random primer labeling system (Pharmacia Biotech AG, Dübendorf, Switzerland), the cDNA probes specific for Bak (0.5 kb) and 7S cytoplasmic RNA (0.19 kb) were radiolabeled with [^{32}P]-deoxycytidine 5'-triphosphate (Du Pont International, Regensdorf, Switzerland) as previously reported.[12]

In Situ Hybridization

In situ hybridization was performed as previously described.[16,18] Briefly, the paraffin-embedded sections of normal and cancerous pancreata were immersed

three times in xylol, rehydrated in decreasing ethanol concentrations (100% to 0%), and incubated in 0.2 mol/L HCl for 20 minutes. After repeated washing with 2× sodium chloride/sodium citrate buffer (SSC), the samples were treated with proteinase K at a concentration of 25 μg/ml for 15 minutes at 37° C and postfixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) solution. Following repeated washing with 2× SSC and acetylation,[8] the sections were prehybridized at 50° C for at least 1 hour in 50% formamide (volume/volume), 4× SSC, 2× Denhardt's reagent, and 250 μg RNA/ml. We performed hybridization overnight at 50° C in 50% formamide (volume/volume), 4× SSC, 2× Denhardt's reagent, 500 μg RNA/ml, and 10% dextran sulfate (weight/volume). The final concentrations of the antisense and sense probes were approximately 2.25 μg/ml, respectively. Excess labeled RNA was removed by washing in 2× SSC and by RNase treatment with 200 U/ml RNase T1 (Boehringer Mannheim GmbH, Mannheim, Germany) and 0.2 μg/ml RNase DNase-free (Boehringer Mannheim GmbH) at 37° C for 30 minutes. Following washings in 2× SSC (55° C, 20 minutes) and 0.2× SSC (55° C, 20 minutes), the sections were incubated with an antidigoxigenin antibody conjugated with alkaline phosphatase (Boehringer Mannheim GmbH). For the color reaction, 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium (Sigma Chemie, Buchs, Switzerland) were used. The sections from all of the examined organ donors and patients with pancreatic cancer were processed simultaneously, and they were also examined by two independent observers. No signal was detectable when the sense probe was used for in situ hybridization in control experiments. The results of in situ hybridization were semiquantitatively analyzed and scored as follows: (−) no detectable signal; (+) weak detectable signal; (++) moderate signal; and (+++) strong signal. The in situ hybridization signals were evaluated by two independent pathologists blinded to patient status, followed by resolution of any differences by joint review and consultation with a third observer.

Northern Blot Analysis

Total RNA was isolated by the guanidine thiocyanate method, fractionated on 1.3% agarose/1.8 mol/L formaldehyde gels, and stained with ethidium bromide for verification of RNA integrity and loading equivalency.[19-21] Fractionated RNA was electrotransferred onto nylon membranes (GeneScreen, Du Pont International, Regensdorf, Switzerland), and cross linked with ultraviolet light. Prehybridization, hybridization with the radiolabeled cDNA probes specific for Bak (0.5 kb) and 7S cytoplasmic RNA (0.19 kb), and washing of the filters under high-stringency conditions were performed as described by Korc et al.[12] All of the blots were exposed at −80° C to x-ray films with intensifying screens. The intensity of the radiographic bands was quantified by densitometry (video densitometer model 620, Biorad Laboratories AG, Glattbrugg, Switzerland), as previously reported.[12] The obtained optical densities for Bak were divided by the corresponding optical densities for 7S cytoplasmic RNA to correct for nonequivalent RNA loading.[12]

Immunohistochemistry

Paraffin sections of normal and cancerous pancreatic tissues were deparaffinized, rehydrated, and treated for blocking endogenous peroxidase as previously reported.[12,14,22,23] After the slides were washed in Tris-buffered saline (TBS) (10 mmol/L Tris/HCl, 0.85% NaCl; pH 7.4) containing 0.1% bovine serum albumin (BSA), they were immersed for 30 minutes in 10% normal goat serum. The primary Bak-specific antibody used was a polyclonal one (Santa Cruz Biotechnology, Inc., Santa Cruz, Calif.) raised against a peptide corresponding to the amino acids 82 to 104 and showing no cross reactivity with other members of the Bcl-2 family. This antibody was diluted 1:400 in 10% normal goat serum and incubated for 1 hour at room temperature. After repeated washing in TBS/0.1% BSA, bound antibody molecules were detected using a biotinylated goat antirabbit IgG antibody and peroxidase-labeled streptavidin (Kirkegaard & Perry Laboratories, Gaithersburg, Md.), followed by exposure to diaminobenzidine tetrahydrochloride and H_2O_2.

To ensure specificity of the anti-Bak antibody, consecutive tissue sections were incubated either in the absence of the primary antibody or with nonimmunized rabbit IgG (Sigma Chemie). Under these circumstances, no immunoreactivity was observed.

Enzymatic In Situ Labeling of DNA Fragmentation

The labeling reaction was performed using the TUNEL (TdT-mediated dUTP nick-end labeling) technique. For that purpose, paraffin sections obtained from formalin-fixed normal and cancerous pancreatic tissues were dewaxed, rehydrated, and incubated in proteinase K solution (15 μg proteinase K/ml, 1.5 mmol/L $CaCl_2$, 10 mmol/L Tris/HCl; pH 7.5) for 15 minutes at 37° C. After blocking endogenous peroxidase with 0.3% H_2O_2 in methanol for 30 minutes and repeated washing with PBS, the slides were exposed to TUNEL mix (in situ cell death detection kit, POD; Boehringer Mannheim) for 30 min-

utes at 37° C, washed with PBS, and incubated for 30 minutes (37° C) with a ready-to-use antifluorescein antibody, conjugated with horseradish peroxidase (Boehringer Mannheim). To visualize the TdT reaction, the sections were immersed in DAB solution (0.2 mg diaminobenzidine tetrahydrochloride/ml, 0.0045% H_2O_2, 50 mmol/L Tris/HCl; pH 7.6) for 10 minutes and repeatedly washed with PBS to stop the color reaction.

As recommended by the manufacturer of the kit, labeling specificity was ensured by including tissue sections that were treated in the same manner as the regular ones but without using TdT during the labeling reaction. Under these conditions, labeling always turned out to be negative.

Statistical Analysis

For normal and cancerous pancreatic samples, the densitometric values obtained by Northern blot analysis were evaluated by stem-and-leaf display and box plots.[24] Results are expressed as median and range. The Mann-Whitney U test was used to compare differences in mRNA expression between normal and cancerous pancreata. The degree of association was evaluated by the Spearman rank correlation test. Significance was defined as $P < 0.05$.

RESULTS

Quantification of Bak mRNA

Northern blot analysis of total pancreatic RNA exhibited only low levels of Bak mRNA in all the normal pancreatic tissues (Fig. 1). In contrast, an increase in Bak mRNA levels was found in 15 of 26 pancreatic cancer tissue samples (Fig. 1). In two cancer samples the Bak mRNA was highly overexpressed (9.3-fold and 15.2-fold, respectively). Statistical evaluation of the densitometric data revealed a median and range of 1.22 and 1.16 for normal pancreas (n = 12), and 3.04 and 18.15 densitometric values for pancreatic cancer (n = 26), indicating a 2.5-fold increase in Bak mRNA levels in the cancer samples. The medians of the two groups differed in a highly significant way ($P < 0.001$). When only cancer samples with elevated Bak mRNA levels were compared to the control samples, the increase was 4.3-fold ($P < 0.001$). There was no correlation between the level of Bak mRNA expression in the cancer samples and the stage of the tumor ($r = 0.047$ [not significant]).

Localization of Bak mRNA By In Situ Hybridization

In normal pancreas the faint-to-moderate purple hybridization product for Bak mRNA was found in the cytoplasm of most acinar, ductal, and islet cells. In addition, there were strongly stained clusters of exocrine cells, which were typically scattered over the whole specimen. The staining intensity observed in the ductal and islet cells was usually weak.

In the pancreatic adenocarcinoma, the in situ hybridization signal of the cancer cells was either weak or absent and did not depend on the grade of differentiation or stage of the tumor (Fig. 2). In adjacent regions of the tumor with chronic inflammation surrounding the pancreatic cancer cells, there was strong Bak mRNA staining of acinar and inflammatory cells. However, only weak in situ hybridization signals were detected in cells of ductal origin undergoing metaplasia.

Localization of Bak Antigen By Immunohistochemistry

In the normal pancreas, weak-to-moderate immunoreactivity was observed in many acinar, ductal, and islet cells. Moreover, high levels of Bak antigen were found in randomly distributed clusters of acinar cells.

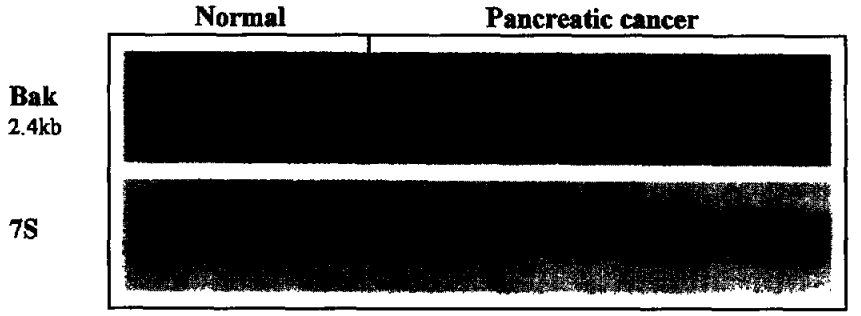

Fig. 1. Northern blot analysis of Bak mRNA expression using tissue samples from normal and cancerous pancreata. In the normal pancreas, only low levels of Bak mRNA were detectable (*Lanes* 1-5). In contrast, enhanced expression of Bak mRNA was present in some of the cancer samples (*Lanes* 6-15). The filters were rehybridized with a 7S cDNA probe to verify equivalent RNA loading.

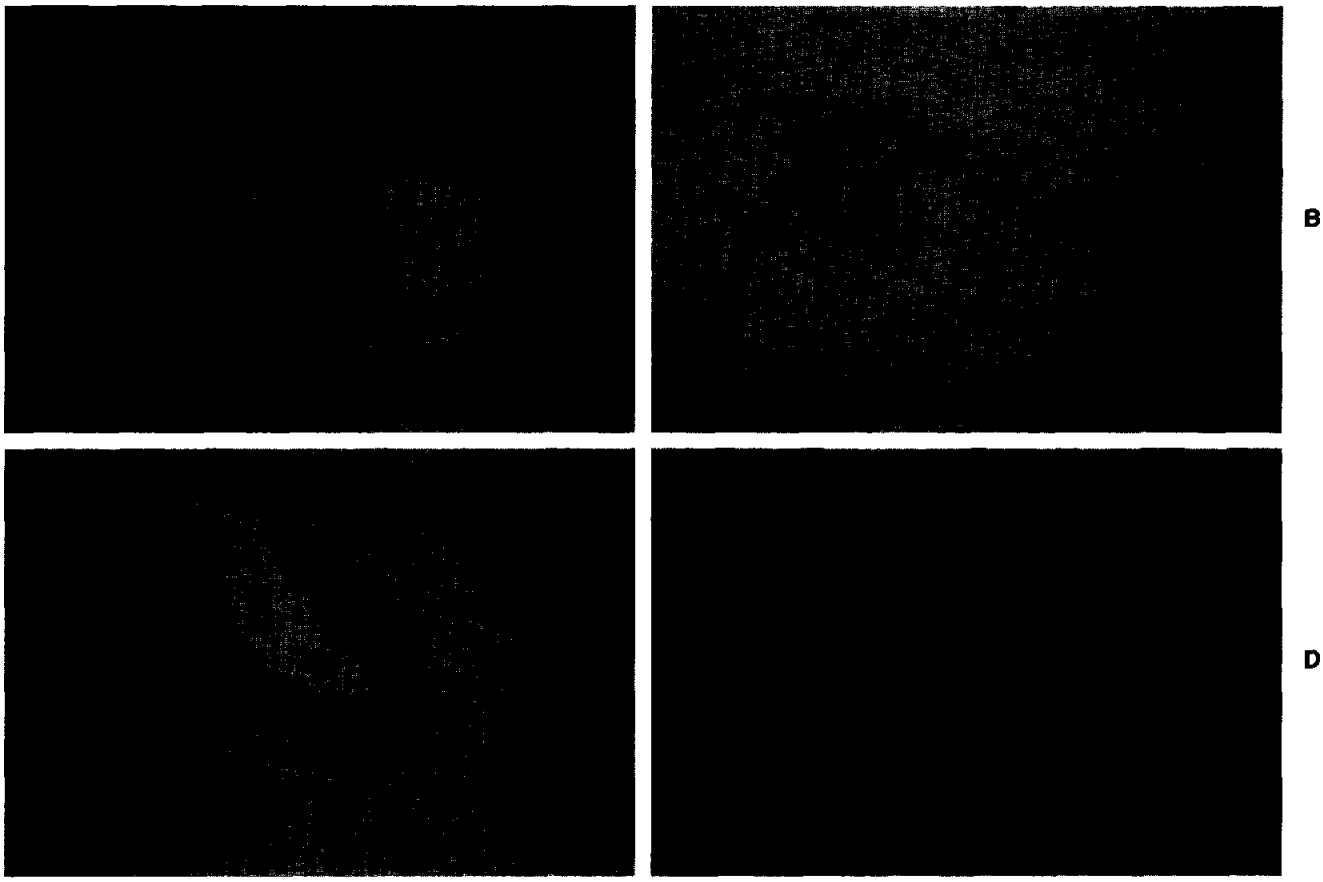

Fig. 2. Expression of Bak mRNA in pancreatic cancer tissue demonstrated by in situ hybridization. **A,** Ductal pancreatic adenocarcinoma in the vicinity of an area with chronic inflammation. The Bak mRNA expression is very low in the tumor cells themselves but increased in a few inflammatory cells (×200). **B,** Perineural invasion by pancreatic adenocarcinoma. Ductlike structures of cancer cells lacking Bak mRNA expression invade the perineurium of a small nerve *(center)* in the pancreas (×400). **C,** High expression of Bak mRNA in a cancer cell (arrow) leaving the tumorous cell clan. Expression levels of BAk mRNA are high in inflammatory cells and low in cancer cells (×400). **D,** Tissue adjacent to tumor with chronic inflammation. High levels of Bak mRNA are found in the acinar cells *(edge)*. In metaplastic ductal cells *(center)*, however, the in situ hybridization signal is very weak (×300).

In pancreatic cancer, the tumor cells were negative or only weakly positive for Bak immunoreactivity (Fig. 3, *A* and *B*). In addition, antigen expression was independent of the grade of differentiation and stage of the neoplasm. In areas adjacent to the tumor exhibiting chronic inflammation, there was normally moderate-to-strong Bak immunostaining of acinar and mononuclear cells. Immunoreactivity, however, was rarely detected in fibroblasts within these regions and within the stroma.

Localization of DNA Fragmentation

In all of the analyzed samples of normal pancreas, DNA fragmentation was detected in a few acinar, ductal, and islet cells. In addition, a usually small number of fibroblasts undergoing cell death were found in some specimens. The distribution of positive cells was apparently random and cells were detectable over the whole sample area.

In most of the pancreatic cancer samples, DNA fragmentation was not detectable in tumor cells (Fig. 3, *C*). In a few specimens, however, there were also areas in which cancer cells exhibited weak TUNEL positivity. In addition, labeled cells were observed within the lumen of some tumor tubuli. In contrast to the very limited occurrence of DNA fragmentation in cancer cells, extensive cell death was observed predominantly in mononuclear cells and fibroblasts, which were localized in tumor-adjacent

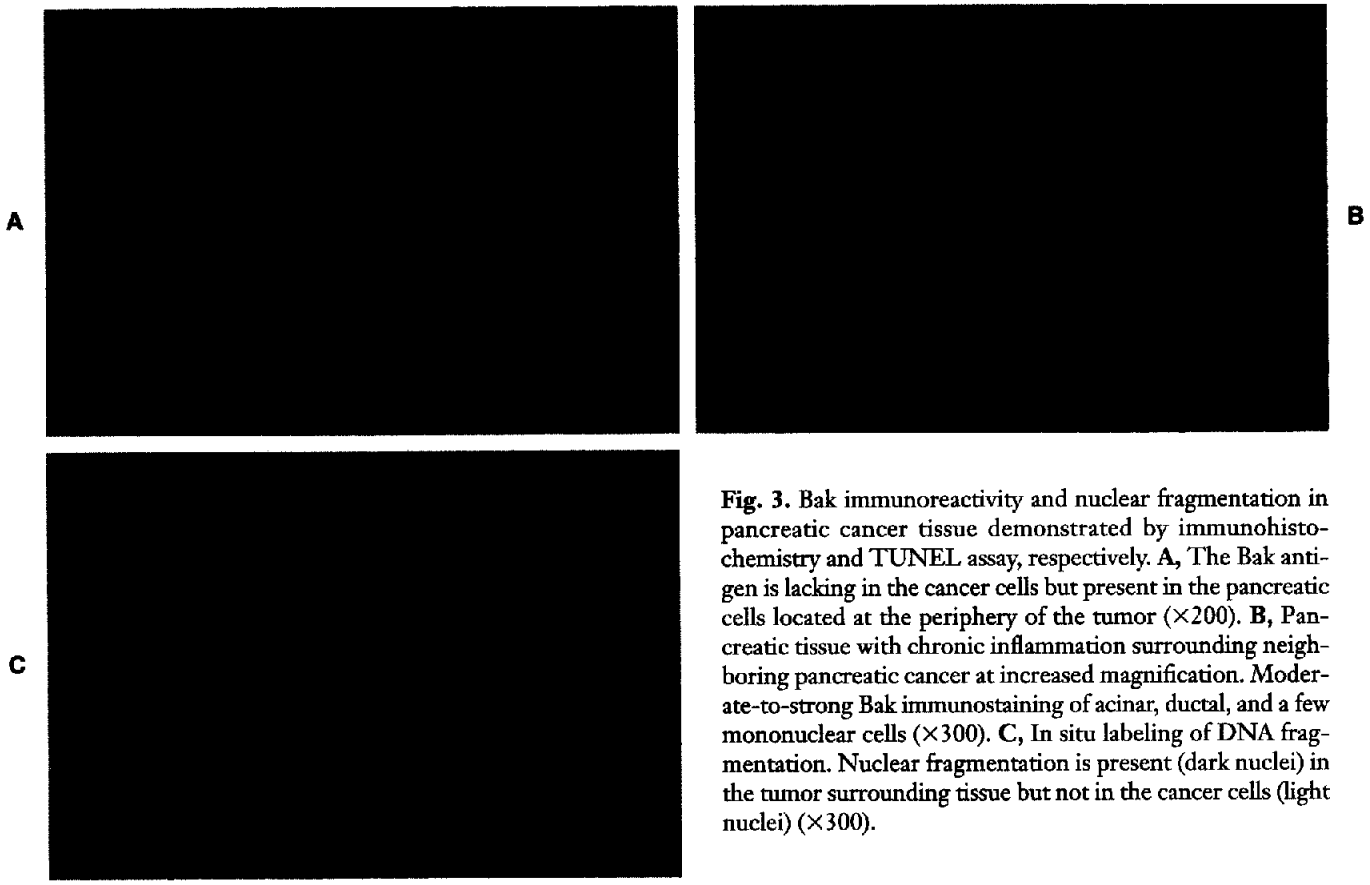

Fig. 3. Bak immunoreactivity and nuclear fragmentation in pancreatic cancer tissue demonstrated by immunohistochemistry and TUNEL assay, respectively. **A,** The Bak antigen is lacking in the cancer cells but present in the pancreatic cells located at the periphery of the tumor (×200). **B,** Pancreatic tissue with chronic inflammation surrounding neighboring pancreatic cancer at increased magnification. Moderate-to-strong Bak immunostaining of acinar, ductal, and a few mononuclear cells (×300). **C,** In situ labeling of DNA fragmentation. Nuclear fragmentation is present (dark nuclei) in the tumor surrounding tissue but not in the cancer cells (light nuclei) (×300).

regions showing chronic inflammatory changes (Fig. 3, *C*). Only a few positive cells were normally found in the stroma.

DISCUSSION

Using in situ hybridization and immunohistochemistry, we could clearly demonstrate that in pancreatic cancer the tumor cells themselves expressed low levels of Bak mRNA and protein, whereas there was strong expression in pancreas-specific and inflammatory cells within areas adjacent to the tumor. These results demonstrate that it is the variations in the extent of chronic inflammation surrounding the pancreatic cancer cells that are responsible for the increase and variation of Bak mRNA expression observed in the tumor group on Northern blots. Our results are consistent with those of Krajewska et al.,[25] who used immunologic methods for studying Bak expression in human primary colorectal adenocarcinomas. They found that immunoreactivity was reduced in the cancer cells in most of the carcinomas compared with normal mucosal epithelial cells. It seems therefore likely that downregulation of Bak is not specific for pancreatic cancer but may be a property of some malignant tumors. However, further studies using samples from different tumors will either confirm or reject this hypothesis.

As Bak is a pro-apoptotic member of the Bcl-2 family,[5,6] our results showing Bak overexpression in peritumorous areas of chronic inflammation suggest that there is extensive activation of programmed cell death in these areas. Indeed, this assumption is supported by the vast occurrence of nuclear DNA fragmentation, a hallmark of programmed cell death, which colocalizes largely with Bak expression. The reason for this is not clear. However, it is very probable that programmed cell death in peritumorous areas is a consequence of chronic inflammation and/or tumor growth. Krajewski et al.[26] demonstrated that Bak was hardly expressed in circulating lymphocytes and macrophages, suggesting that programmed cell death in areas of chronic inflammation surrounding pancreatic cancer cells is induced after having entered the perifocal tissue. Cells undergoing programmed cell death most probably fail to maintain their physiologic function so they are no longer able to stop the invasion of the tumor.

In contrast to peritumorous areas with chronic inflammation, there was no Bak expression and DNA

fragmentation in most of the cancer cells. These observations indicate that in tumor cells the pathways leading to death, including the one in which Bak is involved, are usually depressed. Inhibition of these mechanisms may be one way for transformed cells to protect themselves from exogenous, cell-killing factors and may therefore favor unlimited tumor progression, which is stimulated and supported by various growth factors such as epidermal growth factor,[12] transforming growth factor-alpha,[12] and isoforms of transforming growth factor-beta.[14]

Our data further demonstrate that Bak is not a unique apoptosis promotor, but that it can have cell-specific differences. In areas with chronic inflammation surrounding the pancreatic cancer cells, there were many fibroblasts that showed DNA fragmentation, but there were only a few with Bak expression. This indicates that in the mentioned cells, other members of the Bcl-2 family (e.g., Bax or Bcl-x_s) or unknown proteins may promote cell death.

In conclusion, our study demonstrates that Bak expression and cell death are both present within areas showing chronic inflammation surrounding pancreatic cancer cells but not in the cancer cells themselves. This discrepancy may contribute to improved proliferation and invasion of pancreatic cancer into neighboring tissue.

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Discussion

Dr. M. G. Sarr (Rochester, Minn.). You were looking for agents that might stimulate apoptosis in the cancer. If I were going to do this, I would look for things that would inhibit apoptosis in the cancer. Have you looked at Bcl-2 or any of those other factors in the primary neoplastic tissue?

Dr. Graber. Yes, we also looked for Bcl-2 and Bax in pancreatic cancer. Both factors were simultaneously studied in consecutive tissue sections, and we found enhanced Bax expression in many pancreatic cancer tissues; however, the frequency for Bcl-2 is much lower.

Dr. Sarr. Why do you think that the tissue surrounding the neoplastic tissue shows increased expression of proapoptotic agents? Is the cancer releasing something?

Dr. Graber. That is a very interesting question. However, I cannot give you a conclusive answer. If you investigate pancreatic cancer specimens, you often find chronic pancreatitis–like lesions adjacent to the cancer mass. This might be due to duct obstruction by the cancerous lesion or by factors released by the cancer cells. To further study these changes and the factors that are involved in this process will be one challenge in the future.

Dr. J. Norman (Tampa, Fla.). Can you quantitate that? Are the more aggressive cancers associated with a higher degree of apoptosis in the surrounding tissues?

Dr. Graber. We were looking at a subset of only 26 pancreatic cancer samples, and there was no correlation, for example, with survival of the patients after tumor resection or with histopathologic parameters such as differentiation of the tumor among others.

Loss of Matrix-Dependent Cytoskeletal Tyrosine Kinase Signals May Regulate Intestinal Epithelial Differentiation During Mucosal Healing

Yi-Wen Liu, Ph.D., Matthew A. Sanders, Ph.D., Marc D. Basson, M.D., Ph.D.

Intestinal epithelial restitution and the migratory phenotype appear regulated by the extracellular matrix. Since integrin-associated adhesion to matrix triggers tyrosine kinase activity, we hypothesized that matrix-specific tyrosine kinase signals might modulate the intestinal epithelial migratory phenotype, particularly via focal adhesion kinase. Caco-2 cells were seeded at two densities on collagen I, laminin, fibronectin, and tissue culture plastic. Four days later the first cells were confluent, whereas the second cells were not contact inhibited and expressed migratory lamellipodia. Cells were fractionated into membrane/cytoskeletal and cytosolic fractions. Cytoskeletal tyrosine kinase activity in static cells was matrix dependent and, unlike cytoscolic tyrosine kinase, correlated with adhesion, highest on collagen and lowest on plastic. Migrating cells exhibited matrix-dependent increases in cytosolic tyrosine kinase activity. Cytosolic changes in tyrosine kinase activity in motile cells exceeded membrane/cytoskeletal changes. However, matrix-dependent variations in increase in cytosolic tyrosine kinase activity correlated inversely with changes in cytoskeletal tyrosine kinase activity, suggesting cytoskeletal tyrosine kinase translocation to the cytosol during motility. Indeed cytoskeletal focal adhesion kinase activity decreased during migration on collagen. Tyrosine kinase inhibition by genistein both inhibited migration and stimulated expression of brush-border enzymes downregulated during motility. Although enterocyte-matrix interactions alter both cytosolic and cytoskeletal tyrosine kinase activity, matrix-dependent cytoskeletal events are likely to regulate adhesion and differentiation in static cells. Loss of matrix-dependent cytoskeletal tyrosine kinase signals such as focal adhesion kinase during restitution may trigger a phenotypic switch to the "dedifferentiated" migrating intestinal epithelial phenotype. (J GASTROINTEST SURG 1999;3:82-94.)

KEY WORDS: Caco-2, focal adhesion kinase, intestinal epithelial cell, migration, restitution, tyrosine kinase, extracellular matrix

Intestinal mucosal wound healing involves restitution from its earliest stage and cell proliferation if rapid restitution fails to resurface the defect. After injury, intestinal epithelial cells at the edge of a defect exhibit different characteristics from their conventional roles and as they migrate across the wounded area as a sheet.[1-5] Cell proliferation, which does not occur until 24 hours after the injury, serves primarily to replace cells lost during a major injury. Migrating cells lose their columnar morphology and assume a squamous morphology, downregulating the expression of brush-border digestive enzymes, which are common markers for intestinal epithelial differentiation, and exhibiting integrin and cytoskeletal reorganization adapted to cell motility.[3,6] However, the processes that regulate the adaptive phenotypic modifications which occur during intestinal epithelial sheet migration are poorly understood.

The extracellular matrix has been reported to be important for intestinal epithelial cell morphogenesis,[7] motility,[8-10] adhesion,[11-15] differentiation,[16-20] and both basal and growth factor–regulated proliferation.[21,22] The extracellular matrix substrate across which intestinal epithelial migration occurs not only determines the rate of cell motility but also modulates the degree of lamellipodial spreading in migrating cells in parallel with rates of cell motility.[8] In general, we and others have observed that migration and proliferation are more rapid on the type I collagen typical of the interstitial matrix, whereas conventional in-

From the Departments of Surgery, Yale University and Connecticut VA Health Care System, New Haven, Conn.
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Reprint requests: Marc D. Basson, M.D., Ph.D., Department of Surgery, Yale University School of Medicine, 310 Cedar St., P.O. Box 208062, New Haven, CT 06520-8062. E-mail: BASSON.MARC_D+@WEST-HAVEN.VA.GOV

testinal epithelial differentiation markers are most expressed on basement membrane proteins such as laminin.[8,16,18-21]

The effects of the matrix on intestinal epithelial cell differentiation and proliferation may be mediated at least in part by intracellular signals related to integrins, the best characterized cell receptors for matrix proteins.[6] In particular, integrin-mediated cell-matrix interactions have been demonstrated to initiate tyrosine kinase signaling events in both static and migrating cells, including some mediated via the membrane-associated tyrosine kinase called focal adhesion kinase (FAK).[23-26] Although integrin-associated cell adhesion to matrix is well described to initiate tyrosine kinase activity,[22-25] we previously observed in preliminary studies of cells migrating across tissue culture plastic that tyrosine kinase signaling increases during migration in both the cytosolic and membrane/cytoskeletal fractions of these cells, whereas tyrosine phosphorylation by FAK was enhanced in only the membrane/cytoskeletal fraction of migrating cells.[27] This suggests that intestinal epithelial migratory phenotype may be modulated by tyrosine kinase signaling including cytoskeletal FAK. Because cell motility has been demonstrated to vary with the matrix substrate across which migration occurs,[8-10] we therefore hypothesized that the intracellular signaling initiated by intestinal epithelial migration would also be likely to include matrix-specific tyrosine kinase signals.

We chose the human Caco-2_{BBE} cell line for these studies. Although derived from a well-differentiated colon carcinoma,[28,29] the Caco-2 cell is highly differentiated and exhibits many of the characteristics of nonmalignant intestinal epithelial cells.[28,29] The integrins expressed by Caco-2 cells are consistent with normal human intestinal mucosa.[30] They also exhibit characteristics of differentiated intestinal epithelial cells including polarized columnar morphology with an apical brush border, electrical and morphologic tight junctions, and transport properties similar to intestinal mucosa.[28,29] We have previously extensively used the Caco-2 cell line to model intestinal epithelial cell restitution.[8,10,31-33] We therefore sought to use this cell line to evaluate the hypothesis that the signaling of intestinal epithelial cell motility is matrix specific, modulated by tyrosine kinase signals in general and FAK activity in particular. We used a well-characterized "fence" migration model to quantitate the rate of Caco-2 cell motility with or without the effect of the tyrosine kinase inhibitor genistein.[8] In addition, we generated more homogeneous populations of static and migrating cells for biochemical studies of cell signaling using a model of differential initial density seeding previously described.[27]

METHODS

Cells

Caco-2_{BBE} cells[29] for these studies were maintained in cell culture at 37° C in 8% carbon dioxide. Caco-2 cell culture medium was prepared with Dulbecco's modified eagle medium (Gibco Laboratories, Grand Island, N.Y.), 10% fetal calf serum (Gibco), 10 mg/ml transferrin (Boehringer Mannheim Corp., Indianapolis, Ind.), 2 mmol/L glutamine, 1 mmol/L pyruvate, 10 mmol/L *N*-2 hydroxyethyl-piperazine-*N*'2-ethanesulphonic acid (HEPES), 100 units/ml penicillin G, and 0.1 mg/ml streptomycin. Cells in all experiments were within 10 passages.

Matrix Proteins

Laminin, fibronectin, and type I collagen were purchased from Sigma (St. Louis, Mo.), and the collagen I was repurified by phenol/chloroform extraction. Tissue culture plastic dishes (Falcon Labware, Becton Dickinson Labware, Franklin Lakes, N.J.) were coated with saturating concentrations of matrix substrates as previously described.[34]

Caco-2 cells were allowed to reach confluence prior to seeding for these studies. After rinsing with 10 ml of 37° C phosphate-buffered saline (PBS; 150 mmol/L NaCl, 2.8 mmol/L NaH_2PO_4, and 7.2 mmol/L Na_2HPO_4, pH 7.4), cells were detached from the flask using 5 ml of trypsin/EDTA solution (0.02% trypsin and 0.01% EDTA in PBS) at 37° C for 2 minutes. The trypsin was neutralized with 5 ml of 37° C Caco-2 media as stated earlier. Cells were then washed, resuspended in 2 ml of media, and seeded simultaneously at 26,000 cells/cm^2 and 6000 cells/cm^2 into 35 × 10 mm and 100 × 20 mm tissue culture plates without coating of matrix proteins, respectively. Meanwhile cells were seeded at 13,000 cells/cm^2 and 750 cells/cm^2 into 35 × 10 mm and 100 × 20 mm matrix protein–coated tissue culture plates, respectively. After 4 days, the first population of cells had reached confluence and become static, whereas the latter cell population was nonconfluent and freely motile (Fig. 1).

Cell Harvesting and Fractionation

Both the static and motile Caco-2 cells were washed twice with ice-cold PBS and harvested in 400 ml of lysis buffer containing 25 mmol/L Tris-HCl (pH 7.6), 5 mmol/L ethylene glycol-bis(b-aminoethyl ether) N,N,N',N'-tetraacetic acid (EGTA), 0.7 mmol/L $CaCl_2$, 1 mmol/L phenylmethyl sulfonyl fluoride (PMSF), 0.1 mmol/L TLCK Na-p-tosyl-L-lysine chloromethyl ketone (TLCK), 10 mmol/L leupeptin, 1 mmol/L sodium vanadate, and 1 mmol/L

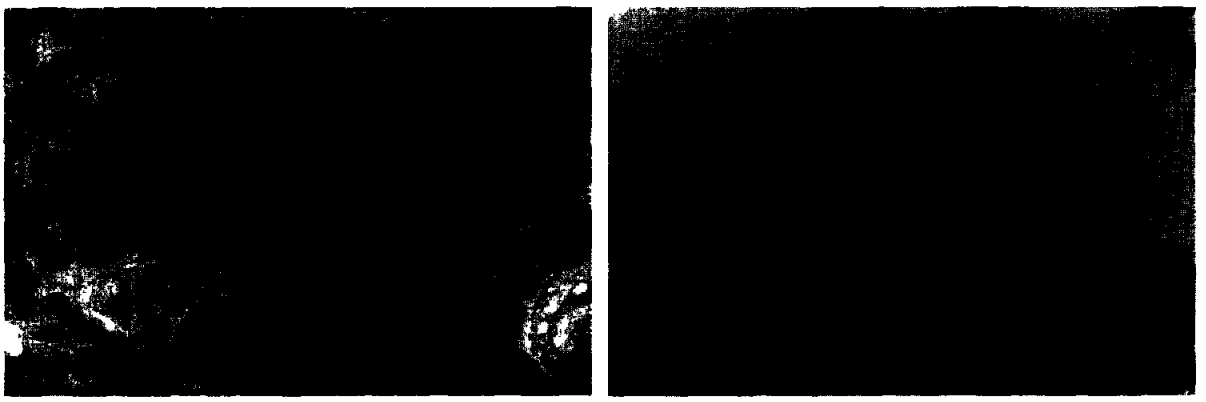

Fig. 1. Morphology of Caco-2 migrating and static cells cultured on type I collagen, photographed at 20× magnification. Cells were seeded simultaneously at 13,000 cells/cm² and 750 cells/cm². After 4 days, the first cell population was confluent (static), whereas cells in the second population were not contact inhibited (migrating). Cells were fixed in formalin, stained with hematoxylin, and counterstained with acidified 4% eosin stain. Static cells exhibited smaller cell areas and a polygonal cell shape without lamellipodia **(A)**, whereas migrating cells exhibited larger cell areas and thinned out lamellipodial edges in the direction of migration or spreading with nuclei frequently lagging behind the lamellipodial edges **(B)**.

dithiothreitol (DTT). The resulting cell lysates were then fractionated using differential Triton X-100 solubility to produce cytosolically enriched and membrane/cytoskeletal enriched fractions as previously described by Bissonnette et al.[35] After sonication using a Sonic Dismembrator 60 (Fisher Scientific Co., Fair Lawn, N.J.) at setting 10 for 10 seconds, the lysate was centrifuged at 10,000 × *g* for 10 minutes at 4° C. The supernatant, corresponding to a cytosolic fraction, was assayed directly for tyrosine kinase activity and FAK content and phosphorylation as described below. The pellet was resuspended in lysis buffer supplemented with 0.3% Triton X-100 and recentrifuged at 10,000 × *g* for 10 minutes at 4° C to produce a membrane/cytoskeletal enriched fraction.

Protein Assay

Protein concentrations in the cell lysate fractions were determined using bicinchoninic acid (BCA) reagent (purchased from Pierce Chemical Co., Rockford, Ill.) prior to subsequent study. Two milliliters of each cell lysate at 4° C was diluted with 98 ml of double-distilled deionized water in triplicate. A bovine serum albumin (BSA) stock (2 mg/ml) was serially diluted with water in triplicate from 0 to 200 mg/ml and used as standards with 100 ml total volume again in each tube. Two milliliters of BCA cocktail (BCA reagent: 4% $CuSO_4$ = 49:1 volume/volume) was added to each tube. All tubes were incubated in a 60° C water bath for 15 minutes; 200 ml of standards and samples were transferred to a 96-well flat-bottom assay plate and absorbances were measured using a spectrophotometer EIA 400 AT model (BioWhittaker Inc., Walkersville, Md.) at 562 nm wavelength. Protein concentrations were obtained by linear interpolation against the standard curve generated simultaneously with each experiment and back corrected for dilution in each assay. Triplicate values were averaged to yield a final result. All assays were conducted within the linear range of the assay.

Tyrosine Kinase Activity Assay

Tyrosine kinase activity was measured based on the phosphorylation of 1 μmol/L of synthetic tyrosine kinase substrate (biotin-K-V-E-K-I-G-E-G-T-Y-G-V-V-Y-K-amide) using an enzyme-linked immunosorbent assay–based technique (Boehringer Mannheim). Seventy-five to 100 μg of cytosolic or membrane fractions of the samples were incubated for 60 minutes at 37° C with assay buffer (50 mmol/L Tris-HCl [pH 7.5], 20 mmol/L magnesium acetate, 5 mmol/L NaF, 0.2 mmol/L Na-EDTA, 0.8 mmol/L EGTA, 1 mmol/DTT, 30 mmol/L sodium vanadate, 1 mmol/L adenosine triphosphate [ATP], and 10 mmol/L $MgCl_2$). The reactions were stopped by the addition of 120 mmol/L EDTA. After centrifugation at 10,000 *g* for 1 minute at 4° C, 50 μl of each supernatant was transferred to a streptavidin-coated 96-well plate in triplicate and incubated for 30 minutes at 37° C. After the plate had been washed three times with 10 mmol/L PBS, 75 μl of antiphosphotyrosine-peroxidase was added to each well and incubated for 1 hour at 37° C. The absorbance of each sample at 405 nm (reference 490 nm) was measured after 100 μl of 2,2'-azinobis-[3-ethylbenzothizoline-6-sulfonic acid] diammonium salt (ABTS) had been added to each well as a substrate

and the plate had been mixed using a Genie 2 Vortex (Fisher Scientific) at setting 3 for 2 minutes. A synthetic phosphopeptide was used to create a standard curve ranging from 0.1 to 3.1 pmol. Tyrosine kinase activity was obtained by interpolation against the standard curve generated simultaneously with each experiment and back corrected for dilution in each assay. Triplicate values were averaged to yield a final result and tyrosine kinase activity was expressed as pmol/mg/min. All assays were conducted within the range of the assay.

Immunoprecipitation of FAK

Immunoprecipitations contained 400 μg protein from either membrane/cytoskeletal or cytosolic fractions in 10 mmol/L Tris (pH 7.4), 150 mmol/L NaCl, 1 mmol/L EDTA, 1 mmol/L EGTA, 1% Triton X-100, 0.5% NP 40, 1 mmol/L Na_3VO_4, and 1 mmol/L PMSF. Samples were precleared for 1 hour at 4° C with 8 μl of rabbit antimouse IgG (Sigma) and 20 μl protein A sepharose CL4B (1:1 slurry in PBS; Pharmacia Biosystems, Piscataway, N.J.). After centrifugation (5000 rpm for 2 minutes in Biofuge 13, Heraeus Instruments Inc., South Plainfield, N.J.), supernatants were transferred to another tube incubated with 4 μg FAK monoclonal antibody (Transduction Laboratories, Lexington, Ky.) for 1 to 2 hours at 4° C. Eight microliters of rabbit antimouse IgG and 25 μl of protein A sepharose CL4B were then added, and immunoprecipitations were incubated for another hour at 4° C. Immunoprecipitations were continuously rotated throughout this procedure. Immunoprecipitations were then centrifuged at 5000 rpm for 2 minutes and rinsed three times with 0.5 ml immunoprecipitation buffer (with centrifugation between rinses). The samples were then resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

SDS-PAGE

After the final rinse and centrifugation in the immunoprecipitation procedure, 20 μl of 2× loading buffer was added to protein A sepharose pellets (1X = 50 mmol/L Tris[pH 6.8], 100 mmol/L DTT, 2% SDS, 0.1% bromophenol blue, 10% glycerol). After boiling for 5 minutes, samples were loaded on 3.5% stacking/7.5% resolving SDS-polyacrylamide gels. Gels were transferred overnight at 4° C, 0.1 Amp onto Hybond ECL nitrocellulose (Amersham Corp., Arlington Heights, Ill.) in gel-transferring buffer containing 39 mmol/L glycine, 48 mmol/L Tris, and 20% methanol.

Immunodetection of Phosphotyrosine and FAK Proteins

For immunodetection of phosphotyrosine, blots were blocked for 1 hour at room temperature in 5% nonfat dry milk in wash buffer (10 mmol/L Tris [pH 7.5], 100 mmol/L NaCl, 0.1% Tween-20). After five rinses with wash buffer, blots were incubated with a horseradish-peroxidase–conjugated phosphotyrosine antibody (RC20; Transduction Laboratories) in wash buffer at a 1:2500 dilution for 1 hour at room temperature. After five additional rinses in wash buffer, blots were detected using the ECL method (Amersham) and exposed to Hyperfilm ECL (Amersham). For immunodetection of FAK, blots immunodetected for phosphotyrosine were stripped in 100 mmol/L Tris hydrochloride, 0.0033% SDS, and 2 μmol/L β-mercaptoethanol at 50° C for 30 minutes. Blots were then rinsed 2 times with PBS and reblocked in 5% nonfat dry milk in wash buffer. FAK was immunodetected using an FAK monoclonal antibody (Transduction Laboratories) according to the manufacturer's protocol. In brief, blots were incubated with diluted antibody (1:1000 in blocking buffer) for 1 hour at room temperature. After five rinses in wash buffer, blots were then incubated for 30 minutes with horseradish-peroxidase–conjugated secondary antibody (sheep antimouse IgG; Amersham) at 1:2000 dilution in blocking buffer. After five further rinses in wash buffer, blots were visualized using the ECL method (Amersham).

Densitometry

All resulting autoradiographs were scanned using a Microtek IIXE flat bed scanner, and densitometric analysis was performed using an IBM-compatible 486-based computer and SigmaScan/Image software (Jandel Scientific, Anaheim, Calif.). All bands scanned exhibited densities within the linear range of the scanner.

"Fence" Migration Model

Caco-2 cell sheet migration was quantitated using the "fence" migration assay previously described.[8,9] Briefly, cells were plated to confluence within the central well of a stainless steel fence, which was then removed, permitting outward radial migration across a collagen substrate. Immediately after removal of the fence, the cells were pretreated with 20 μg/ml mitomycin C for 2 hours to inhibit proliferation,[8] washed three times with PBS, and then cultured for 6 days during which migration occurred. Genistein (50 μg/ml) was added throughout the 6 days into the culture medium, which was changed every 2 days, and

control cells were treated with dimethylsulfoxide as a vehicle control. After 6 days, each monolayer was formalin fixed and stained with hematoxylin. Images of the stained monolayers were scanned into an IBM-compatible computer using a Microtek IIXE scanner, and the monolayer areas were measured using image analysis software (Sigma Scan/Image, Jandel Scientific). The migrated area was then determined by subtracting the area of the original central well of the fence from the total monolayer area.

Brush-Border Enzyme Assays

The brush-border enzymes alkaline phosphatase and dipeptidyl dipeptidase were assayed in protein-matched aliquots of cell lysates by synthetic substrate digestion, and the resulting reaction products were quantitated colorimetrically as previously described.[36]

Photography

For illustrative purposes, cells were rinsed with 37° C PBS and fixed with 10% formalin (Sigma) at 37° C for 1 hour. After rinsing with 37° C PBS three times, cells were incubated with 2 ml or 5 ml of filtered hematoxylin (Fisher Scientific) at 37° C for 1 hour. Cells were then rinsed with PBS three times and counterstained with 2 ml or 5 ml of acidified 4% eosin (with 2% HCl) stain for 3 minutes at room temperature. After three rinses with PBS, cells were air dried overnight and photographed using an Olympus C-35 AD-2 camera and an Olympus BH-2 phase-contrast microscope at 20× magnification and Kodak Tmax black and white film.

Statistical Analysis

Data obtained from each individual experiment with migrating cells were normalized to the mean value of the same parameter measured in static cells from the same passage seeded and studied simultaneously. Results were expressed as mean ± standard error of the mean (SEM). Differences between migrating and static cells were analyzed using an unpaired *t* test, whereas data of cells on various matrix substrates were analyzed using one-way analysis of variance with $P < 0.05$ being set a priori as the level of confidence sought for a positive result.

RESULTS

Morphology

Migrating Caco-2 cells cultured on collagen I (Fig. 1, *A*) exhibited larger cell areas and thinned out lamellipodial edges in the direction of migration or spreading, whereas static cells (Fig. 1, *B*) exhibited a rounded cell shape without lamellipodia. Nuclei were more readily visible in the migrating cells because of the decreased cytosolic cell height that accompanies cell spreading and motility.

Tyrosine Kinase Activity

Basal membrane/cytoskeletal tyrosine kinase activity in static cells appeared to be matrix dependent (Fig. 2, open bars). Caco-2 cells on collagen I exhibited significantly greater tyrosine kinase activity (0.74 ± 0.06 pmol/mg/min, n = 15) than cells on fibronectin (0.60 ± 0.03 pmol/mg/min, n = 8, $P < 0.05$) and plastic (0.49 ± 0.07 pmol/mg/min, n = 11, $P < 0.01$). The matrix-specific differences in membrane/cytoskeletal tyrosine kinase activity observed in static Caco-2 cells paralleled the matrix-specific differences in Caco-2 cell adhesion that we have previously described.[8] Membrane/cytoskeletal tyrosine kinase activity in migrating cells (Fig. 2, shaded bars), however, became relatively uniform (0.60 to 0.67 pmol/mg/min, $P > 0.05$, n ≥6). Only cells on collagen I exhibited significant differences between static and migrating cells ($P < 0.05$).

Basal cytosolic tyrosine kinase activity in static Caco-2 cells also varied with matrix substrate but did not correlate with cytoskeletal tyrosine kinase activity (Fig. 3, open bars). Cells on collagen I (0.39 ± 0.03 pmol/mg/min, n = 15) and plastic (0.32 ± 0.04 pmol/mg/min, n = 11) had significantly greater tyrosine kinase activity than cells on laminin (0.21 ± 0.03 pmol/mg/min, n = 6, $P < 0.001$ with collagen I, $P < 0.05$ with plastic) and on fibronectin (0.22 ± 0.01 pmol/mg/min, n = 6, $P < 0.001$ with collagen I, $P < 0.01$ with plastic). Migrating cells on collagen I exhibited greater cytosolic tyrosine kinase activity (0.70 ± 0.03, n = 15) than cells on laminin (0.49 ± 0.07, n = 6, $P < 0.01$), fibronectin (0.30 ± 0.05, n = 8, $P < 0.001$), and plastic (0.38 ± 0.06, n = 11, $P < 0.001$) with fibronectin exhibiting the lowest cytosolic tyrosine kinase activity during migration. Cells on all matrix proteins exhibited increased tyrosine kinase activity during migration (Fig. 3, shaded bars) as compared to static nonmotile cells ($P < 0.05$), with collagen I exhibiting the greatest increase in tyrosine kinase activity during migration. Cells on tissue culture plastic did not differ significantly in this regard.

Matrix-specific changes in both membrane/cytoskeletal and cytosolic tyrosine kinase activity were observed. In general, migrating cells exhibited increased cytosolic tyrosine kinase activity as compared to static cells (Fig. 3, shaded bars), but the magnitude of this increase varied with the matrix substrate. Matrix-dependent changes in membrane/cytoskeletal tyrosine kinase activity were also observed in motile

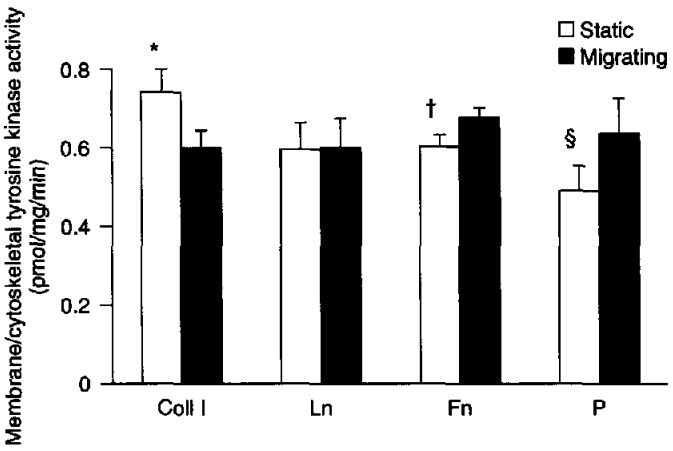

Fig. 2. Membrane/cytoskeletal tyrosine kinase activity of migrating *(shaded bars)* vs. static *(open bars)* Caco-2 cells cultured on collagen I (*Coll I*, n = 15), laminin (*Ln*, n = 6), and fibronectin (*Fn*, n = 8) extracellular matrix proteins and tissue culture plastic (*P*, n = 11). Cells were harvested in 400 μl of lysis buffer and fractionated using differential Triton X-100 solubility to produce cytosolically enriched and membrane/cytoskeletal enriched fractions. Tyrosine kinase activity was measured based on the phosphorylation of 1 μmol/L of synthetic tyrosine kinase substrate (biotin-K-V-E-K-I-G-E-G-T-Y-G-V-V-Y-K-amide) using an ELISA-based technique as detailed under "Methods." Tyrosine kinase activity is expressed as pmol/mg/min and is depicted as the mean ± SEM. Static Caco-2 cells on collagen I exhibited significantly greater tyrosine kinase activity than cells on fibronectin († = $P < 0.05$) and plastic (§ = $P < 0.01$). Membrane/cytoskeletal tyrosine kinase activity in migrating cells *(shaded bars)*, however, became relatively uniform (0.60 to 0.67 pmol/mg/min, $P > 0.05$, n ≥6). Only cells on collagen I displayed a significant difference between static and migrating cells (* = $P < 0.05$).

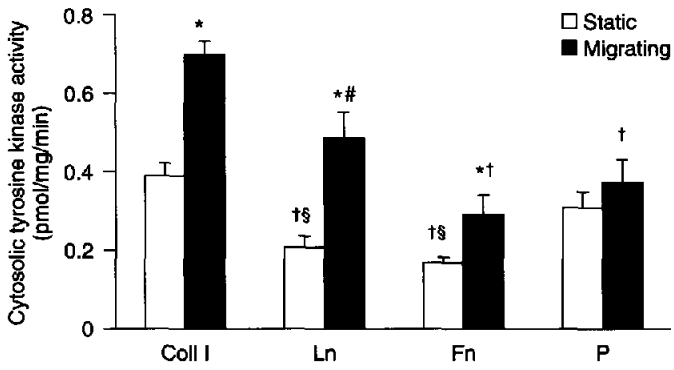

Fig. 3. Cytosolic tyrosine kinase activity of migrating *(shaded bars)* vs. static *(open bars)* Caco-2 cells cultured on collagen I (*Coll I*, n = 15), laminin (*Ln*, n = 6), and fibronectin (*Fn*, n = 8) and tissue culture plastic (*P*, n = 11). Cells were harvested in 400 μl of lysis buffer and fractionated using differential Triton X-100 solubility to produce cytosolically enriched and membrane/cytoskeletal enriched fractions. Tyrosine kinase activity was measured based on the phosphorylation of 1 μmol/L of synthetic tyrosine kinase substrate (biotin-K-V-E-K-I-G-E-G-T-Y-G-V-V-Y-K-amide) using an ELISA-based technique as detailed under "Methods." Tyrosine kinase activity is expressed as pmol/mg/min and is depicted as the mean ± SEM. Cells on collagen I and plastic had significantly greater tyrosine kinase activity than cells on laminin († = $P < 0.001$ with collagen I; § = $P < 0.05$ with plastic) and on fibronectin († = $P < 0.001$ with collagen I; § = $P < 0.05$ with plastic). Migrating cells on collagen I exhibited greater cytosolic tyrosine kinase activity than cells on laminin (# = $P < 0.01$), fibronectin († = $P < 0.001$), and plastic († = $P < 0.001$), with fibronectin exhibiting the lowest cytosolic tyrosine kinase activity during migration. Cells on all matrix proteins had increased tyrosine kinase activity during migration *(shaded bars)* as compared to static nonmotile cells (* = $P < 0.05$), with collagen I exhibiting the greatest increase in tyrosine kinase activity during migration. Cells on tissue culture plastic did not differ significantly.

Table I. Differences (pmol/mg/min) in tyrosine kinase activity between migrating and static Caco-2 cells on various matrix proteins (means ± SEM)

	Collagen I (n = 15)	Laminin (n = 6)	Fibronectin (n = 8)	Plastic (n = 11)
Membrane/cytoskeleton	−0.140 ± 0.034	0.001 ± 0.043*	0.050 ± 0.039†	0.143 ± 0.028†
Cytosol	0.304 ± 0.031	0.276 ± 0.046	0.110 ± 0.060†‡	0.064 ± 0.019†‡

*P <0.05 as compared to collagen I.
†P <0.001 as compared to collagen I.
‡P <0.01 as compared to laminin.

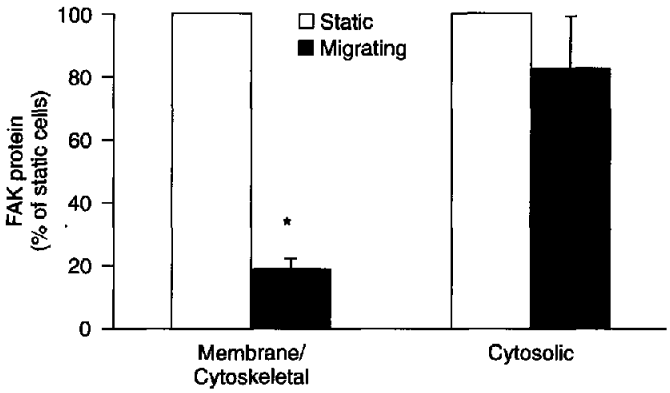

Fig. 4. FAK protein in the membrane/cytoskeletal and cytosolic fractions of migrating vs. static Caco-2 cells on type I collagen. Immunoprecipitates were resolved by 7.5% SDS-PAGE, and proteins were transferred to nitrocellulose membranes. FAK protein levels were detected using FAK monoclonal antibody and a horseradish-peroxidase–conjugated secondary sheep antimouse IgG antibody as described under "Methods." Blots were detected using the ECL method and densitometrically analyzed. A substantial decrease of 81.5% ± 3.6% (n = 3; * = P <0.01) in FAK protein in the membrane/cytoskeletal fraction of migrating cells was observed as compared with static cells. FAK protein also tended to decrease in the cytosolic fraction of migrating cells compared with static nonmigrating cells, but this did not achieve statistical significance (17.5% ± 16.3%, n = 4).

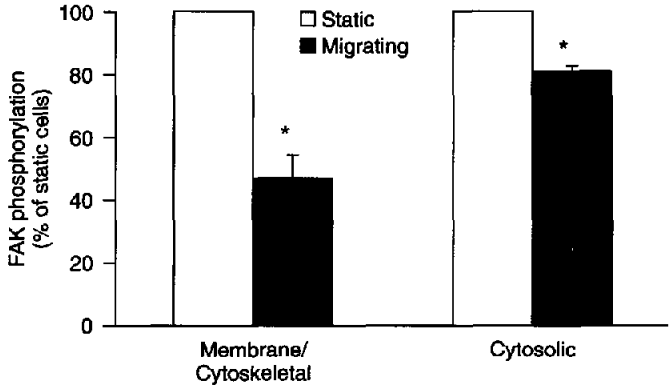

Fig. 5. Phosphorylated FAK in the membrane/cytoskeletal and cytosolic fractions of Caco-2 migrating vs. static cells cultured on type I collagen. Immunoprecipitates were resolved by 7.5% SDS-PAGE, and proteins were transferred to nitrocellulose membranes. Tyrosine phosphorylation of FAK was detected using horseradish-peroxidase–conjugated phosphotyrosine antibody (RC20) as described under "Methods." Blots were detected using the ECL method and densitometrically analyzed. FAK phosphorylation is expressed as a percentage of the static cells and is expressed as the mean ± SEM. Tyrosine phosphorylation of FAK blots demonstrated a decrease of 53.1% ± 7.4% (n = 4; * = P <0.05) in the membrane/cytoskeletal fraction of migrating cells as compared with static cells. The tyrosine phosphorylation of FAK was also decreased, to a lesser degree, in the cytosolic fraction of migrating cells by 19.3% ± 1.6% (n = 4; * = P <0.05) compared with static nonmigrating cells.

cells (Fig. 2, shaded bars), although the magnitude of these changes tended to be smaller than the magnitude of motility-associated changes in cytosolic activity. Furthermore, variations in the magnitude of the motility-associated increases in cytosolic tyrosine kinase activity (Fig. 3, shaded bars vs. open bars) correlated inversely with changes in cytoskeletal tyrosine kinase activity with motility (Fig. 2, shaded bars vs. open bars) on various substrates, suggesting the possibility of tyrosine kinase translocation during cell motility from the cytoskeletal to the cytosolic compartment. Table I summarizes these differences in membrane/cytoskeletal and cytosolic tyrosine kinase activity on different matrix substrates. Changes in membrane/cytoskeletal tyrosine kinase activity of cells on collagen I differed significantly from changes observed on laminin ($P < 0.05$), fibronectin ($P < 0.001$), or plastic ($P < 0.001$). No difference was found between fibronectin and plastic. Cells on collagen I and laminin had greater changes of cytosolic tyrosine kinase activity from static to migrating compared to fibronectin and plastic ($P < 0.001$ with collagen I, $P < 0.01$ with laminin).

Focal Adhesion Kinase Protein

Densitometric analysis of all the FAK protein blots demonstrated a substantial (81.5% ± 3.6%, n = 3, $P < 0.01$) decrease in membrane/cytoskeletal FAK protein in cells migrating across type I collagen as compared with static cells on type I collagen. FAK protein also tended to decrease in the cytosolic fraction of migrating cells, but this did not achieve statistical significance in this series of experiments (17.5% ± 16.3%, n = 4, not significant compared with static nonmigrating cells; Fig. 4).

Phosphorylated Focal Adhesion Kinase

Densitometric analysis of all the tyrosine phosphorylation of FAK blots demonstrated a decrease of 53.1% ± 7.4% (n = 4, $P < 0.05$) in tyrosine phosphorylated FAK in the membrane/cytoskeletal fraction of migrating cells as compared with static cells. Tyrosine-phosphorylated FAK was also decreased, to a lesser degree, in the cytosolic fraction of migrating cells by 19.3% ± 1.6% (n = 4, $P < 0.05$) compared with cytosolic tyrosine–phosphorylated FAK in static nonmigrating cells (Fig. 5).

FAK activity may be expressed as the ratio of phosphorylated (active) FAK to total FAK protein.[27] After normalizing to the static cytosolic FAK ratio for each experiment, Caco-2 cells migrating on type I collagen exhibited a greater ratio (2.95 ± 0.26) of FAK tyrosine phosphorylation to FAK protein in the membrane/cytoskeletal fraction (Fig. 6) (n = 3, $P < 0.05$).

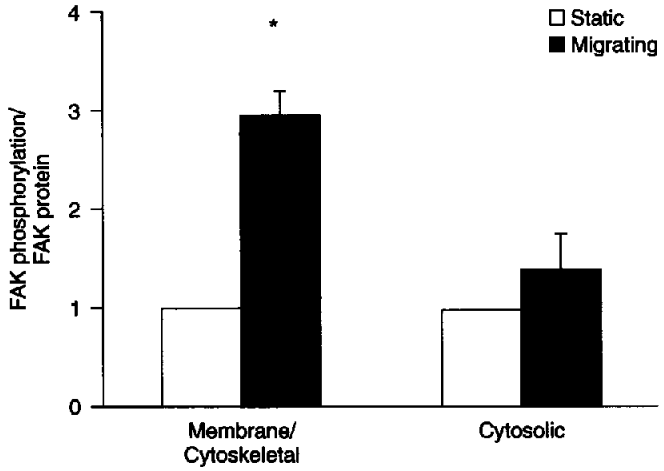

Fig. 6. Ratio of FAK phosphorylation/FAK protein in the membrane/cytoskeletal and cytosolic fractions of migrating vs. static Caco-2 cells cultured on collagen I. Immunoprecipitates were resolved by 7.5% SDS-PAGE, and proteins were transferred to nitrocellulose membranes. Tyrosine phosphorylation of FAK was detected using horseradish-peroxidase–conjugated phosphotyrosine antibody (RC20) as described under "Methods." Blots were detected using the ECL method and densitometrically analyzed. FAK protein levels were detected using FAK monoclonal antibody and horseradish-peroxidase–conjugated secondary sheep antimouse IgG antibody as described under "Methods." Migrating Caco-2 cells had a greater ratio (2.95 ± 0.26, n = 3; * = $P < 0.05$) of FAK tyrosine phosphorylation to FAK protein in the membrane/cytoskeletal fraction. The ratio of FAK tyrosine phosphorylation to FAK protein in the cytosolic fraction did not differ significantly between migrating and static cells.

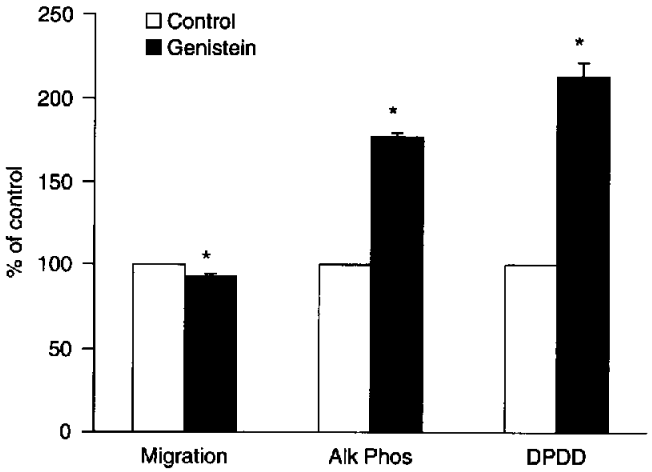

Fig. 7. Effects of the tyrosine kinase inhibitor genistein on Caco-2 motility and differentiation. Caco-2 cells were treated either with only a vehicle control (DMSO) or with genistein (50 μg/ml). Sheet migration was quantitated using the "fence" migration assay after pretreatment with mitomycin C to inhibit proliferation, and the specific activity of the brush-border enzymes alkaline phosphatase *(Alk Phos)* and dipetidyl dipeptidase *(DPDD)* was quantitated by the ability of protein-matched aliquots of cell lysates to digest specific synthetic substrates. Data were normalized to their respective vehicle controls. Inhibition of tyrosine kinase activity by genistein decreased cell migration by 6.7% ± 1.1% (n = 7; * = P <0.01). However, treatment with genistein increased activity of alkaline phosphatase by 76.3% ± 2.3% (n = 3; * = P <0.001) and dipeptidyl peptidase by 112.0% ± 8.4% (n = 5; * = P <0.001).

This calculated ratio in migrating cells was increased by a substantial decrease of FAK protein in migrating cells as compared with static nonmigrating cells, which exceeded the observed decrease in tyrosine-phosphorylated FAK. Thus membrane/cytoskeletal FAK appeared proportionally more activated in migrating cells on type I collagen, but total membrane/cytoskeletal FAK activity was actually decreased in these cells because of the decrease in membrane/cytoskeletal FAK protein. The ratio of FAK tyrosine phosphorylation to FAK protein in the cytosolic fraction did not differ significantly between migrating and static cells.

Tyrosine Kinase Inhibition by Genistein

Addition of genistein inhibited Caco-2 cell motility. Genistein is a potent global tyrosine kinase inhibitor that blocks tyrosine kinases by interfering with ATP binding by these enzymes.[37] Inhibition of tyrosine kinase activity by genistein decreased cell migration by 6.7% ± 1.1% (n = 7, P <0.01) (Fig. 7) and increased expression of differentiation markers, alkaline phosphatase by 76.3% ± 2.3% (n = 3, P <0.001) and dipeptidyl peptidase (DPDD) by 112.0% ± 8.4% (n = 5, P <0.001) (Fig. 7).

DISCUSSION

We and others have previously described modulation of diverse aspects of intestinal epithelial cell biology by matrix proteins, but the signaling pathways by which cell-matrix interactions trigger these phenotypic alterations is less well understood. In this study we have demonstrated not only that tyrosine kinase activity, a primary signaling modality for integrin-mediated signaling in other cell types,[38-40] is matrix dependent in human Caco-2 intestinal epithelial cells, but also that tyrosine kinase signaling during Caco-2 motility varies in a matrix-specific manner.

Cell migration has been studied using various models such as radial migration of cells from tissue explants[41,42] or the creation of wounds in cell monolayers[4,43] or intact tissues.[43,44] Wounding of the cell monolayer by scratching certain cells off the cell culture can cause disruption of the plasma membrane of cells remaining along the injured edge and may release previously intracellular bioactive agents such as growth factors.[43] This cytokine release may introduce a confounding variable into experimental models and render interpretation of cell motility data more complex. The "fence" migration model avoids cell injury and thus permits investigation of sheet migration under more controlled conditions.[8,9] Although suitable for quantitation of cell motility rates, this and similar cell culture models result in heterogeneous cell populations in which cells at the edge of the monolayer are migrating and cells behind the edges are completely surrounded and may not actively migrate. Although the "fence" model resembles the heterogeneous cell population characteristic of in vivo epithelium during restitution, it is difficult to isolate a ho-

mogeneous migrating cell population from this model, and we have previously demonstrated that such models therefore underestimate biochemical alterations associated with cell motility.[3] We therefore previously characterized differential density cell seeding as a mechanism for the generation of a more homogeneous motile cell population subcellular and molecular biology.[27] In previous studies using this model, we have observed that phenotypic alterations of motile Caco-2 cells in this model resemble the migratory front of the Caco-2 cells in the "fence" model and migrating gut epithelial cells in vivo based on morphology[8,27,45] and biochemical parameters[3] (Basson and Turowski, unpublished data).

We found that membrane/cytoskeletal tyrosine kinase activity in static cells was matrix dependent and correlated with our previous observations of rates of cell adhesion to matrix proteins,[8] highest on type I collagen and lowest on tissue culture plastic. Since intestinal epithelial adhesion to matrix depends largely on integrin engagement, these parallel observations may reflect differences in focal adhesion plaque formation on various matrix proteins, but this hypothesis awaits further study.

Tyrosine kinase inhibition by genistein slightly but statistically inhibited cell motility in these studies, consistent with our previous observations,[46] and suggesting a role for tyrosine kinase activity in regulating nonchemotatic intestinal epithelial cell motility. Furthermore, on all matrix substrates studied, migrating cells exhibited substantially increased tyrosine kinase activity in the cytosolic fraction as compared with static cells, suggesting the possibility that this cytosolic signaling may contribute to phenotypic alterations during cell motility. Since a large number of cytosolic tyrosine kinase signal proteins have been described,[47-50] which particular tyrosine kinases are actually involved in the increased cytosolic activity seen with cell motility is as yet unknown. Such signaling molecules as the mitogen-activated protein kinases and the Src family of kinases may well be involved.[47-50]

It is interesting to note that the relative magnitude of the increase in tyrosine kinase activity in the cytosolic fraction correlated inversely with changes in membrane/cytoskeletal tyrosine kinase activity during migration. Such opposing changes could be consistent with the possibility that some tyrosine kinase activity may translocate from the membrane/cytoskeleton to the cytosolic compartment during cell motility. We have previously observed similar translocation of cell signaling elements in a different model of cell-matrix interaction involving repetitive mechanical strain of the underlying matrix.[51] Alternatively, these opposing changes in tyrosine kinase activity may simply be an epiphenomenon representing the reverse effects on tyrosine kinase signaling in cytoskeleton/membrane and cytosol of matrix protein-specific signaling during cell motility.

Although cytosolic tyrosine kinase activity increased during cell motility, membrane/cytoskeletal tyrosine kinase activity tended to decrease, albeit to a lesser degree, and to lose much of its matrix-dependent variability. This is consistent with the model that such cells exhibit less strong adhesion and less focal contact formation in order to move across a substrate rather than adhere to it, as well as with the observation that intestinal epithelial cells in culture tend to lose their conventional differentiation characteristics to an equivalent degree when migrating on different matrix substrates (Basson and Turowski, unpublished observations). Membrane/cytoskeletal signaling during migration thus appears to involve a decrease in static levels of focal contact formation and associated signaling. Although we have yet to identify all of the molecules contributing to these membrane/cytoskeletal tyrosine kinase signals, FAK seems a likely candidate.

FAK is an important tyrosine kinase that has been implicated in integrin-mediated signal transduction during cell-matrix interactions in a variety of cell types.[23-25] It is a nonreceptor cytoplasmic protein tyrosine kinase involved in extracellular matrix-mediated integrin signaling and capable of phosphorylating cytoskeletal proteins such as paxillin, which are associated with focal adhesions where the actin cytoskeleton terminates at transmembrane integrin clusters.[47,52,53] FAK binds to β-integrin subunits directly via the FAK amino terminal domain, whereas the interactions of FAK with other tyrosine kinase families, such as Src protein tyrosine kinases, and focal adhesion–related proteins are associated with the FAK carboxyl-terminal domain.[52] Indeed we found here in further studies comparing static and migrating cells on type I collagen that membrane/cytoskeletal FAK protein and phosphorylation were decreased significantly in migrating cells compared to static cells. The substantial decrease of membrane/cytoskeletal FAK protein in migrating cells results in the greater ratio of FAK phosphorylation to FAK protein. Loss of membrane/cytoskeletal FAK protein in migrating Caco-2 cells on type I collagen could contribute to translocation of tyrosine kinase from membrane/cytoskeleton to cytosolic compartment.

We chose to characterize the effect of migration on FAK activity in cells on type I collagen because our tyrosine kinase assays had suggested that the greatest differences between confluent and migrating cells would be likely to be observed here, and would thus be most readily detected by this semiquantitative method. Based on these experiments, the extensive literature on FAK in other cell types, and our inability to demonstrate the FAK-homologous related adhe-

sion focal tyrosine kinase[54-57] in these cells (Sanders and Basson, unpublished data), it seems likely that alterations in FAK contribute to motility-associated alterations in membrane/cytoskeletal tyrosine kinase activity on other matrix substrates as well.

It is interesting to note that total FAK phosphorylation and FAK protein of Caco-2 cells on collagen I in the present studies contrast with results in cells migrating on tissue culture plastic in a previous preliminary study.[27] Total FAK phosphorylation and FAK protein were each decreased in both membrane/cytoskeletal and cytosolic fractions of cells migrating on collagen I but increased in cells migrating on tissue culture plastic in these previous observations.[27] These changes seem to correlate with differences of changes in membrane/cytosolic tyrosine kinase activity between collagen I and plastic, further confirming the matrix-dependent tyrosine kinase activity. However, cell motility increased the proportion of membrane/cytoskeletal FAK activation (ratio of membrane/cytoskeletal FAK phosphorylation to FAK protein) similarly in both cells cultured on collagen I and cells migrating on plastic, suggesting a complex regulatory pattern in which matrix may regulate the amount of membrane/cytoskeletal FAK available, whereas motility alters the activity of that FAK which is available.

It must also be acknowledged that although FAK is likely to be important here, it is unlikely to be the only tyrosine kinase involved in membrane/cytoskeletal signaling during intestinal epithelial cell motility. A variety of other tyrosine kinases have been implicated in downstream integrin-associated signaling.[47-50,52,53] Phosphorylation of FAK allows the binding of FAK to Src family protein tyrosine kinases, which then phosphorylate FAK and promote the binding of FAK to growth factor receptor–bound protein 2 (Grb2). Binding of Grb2 to FAK then leads to activation of the Ras signal transduction pathway, in which the mitogen-activated protein kinases are among the downstream kinases.[47-50,52,53] Thus, although the FAK-homologous related adhesion focal tyrosine kinase seems unlikely to play a significant role here, other less well-characterized tyrosine kinases may also be involved during cell motility.

In summary, although the cell-matrix interactions involved in intestinal epithelial cell adhesion and motility alter both cytosolic and membrane/cytoskeletal tyrosine kinase activity, the correlations between these data and our previous observations suggest that matrix-dependent cytoskeletal events seem likely to regulate adhesion and differentiation in static cells. Loss of matrix-dependent membrane/cytoskeletal tyrosine kinase signals such as FAK during cell motility may trigger a phenotypic switch to the "dedifferentiated" migrating intestinal epithelial phenotype.

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Paraesophageal Herniation as a Complication Following Laparoscopic Antireflux Surgery

Matthias H. Seelig, M.D., Ronald A. Hinder, M.D., Paul J. Klingler, M.D., Neil R. Floch, M.D., Susan A. Branton, M.D., Stephen L. Smith, M.D.

Paraesophageal herniation of the stomach is a rare complication following laparoscopic Nissen fundoplication. We retrospectively reviewed our experience with 720 patients undergoing laparoscopic Nissen fundoplications. Seven patients were found to have postoperative paraesophageal hernias requiring reoperation. The clinical presentation, diagnostic workup, operative treatment, and outcome were evaluated. There were no deaths or procedure-related complications. Clinical presentation was recurrent dysphagia in four, nonspecific abdominal symptoms in one, and acute abdomen in one. One additional patient was asymptomatic. Preoperatively the correct diagnosis was able to be confirmed in four of six patients by barium esophagogram. Four patients underwent successful laparoscopic repair. Two patients had a thoracotomy including one conversion from laparoscopy to thoracotomy. One patient had a laparotomy to reduce an intrathoracic gastric volvulus. At a mean follow-up of 2.5 months no patient had further complications. Paraesophageal herniation is a rare complication following laparoscopic Nissen fundoplication and a definitive diagnosis is often difficult to establish. Early dysphagia after surgery should alert the surgeon to this complication. Redo laparoscopic surgery is feasible but an open procedure may be necessary. (J GASTROINTEST SURG 1999;3:95-99.)

KEY WORDS: Hiatal hernia, fundoplication, paraesophageal hernia

As experience with laparoscopic antireflux procedures has increased, so the rate of complications has declined. The laparoscopic Nissen fundoplication is performed with very low morbidity and mortality.[1] Although good clinical results can be achieved in 80% to 90% of patients, most patients who have persistent symptoms or develop recurrent symptoms have a technical failure of the procedure. Besides various complications related to the wrap itself, paraesophageal herniation of the stomach is a serious and dangerous complication. This complication can be easily overlooked and may finally result in gastric volvulus and perforation.[2,3] Several reports have shown that paraesophageal herniation may be diagnosed in up to 6.3% of patients after the laparoscopic Nissen fundoplication.[4] We reviewed our experience with this complication after laparoscopic Nissen fundoplication and discuss the management of these patients and the pitfalls of diagnosis.

PATIENTS AND METHODS

The charts of all patients who underwent a laparoscopic Nissen fundoplication for severe gastroesophageal reflux disease, performed by the senior author (R.A.H.) at Creighton University, Omaha, Nebraska, or at the Mayo Clinic, Jacksonville, Florida, between 1991 and 1997 were reviewed. Patients who underwent reoperation for a paraesophageal hernia form the basis of this study. Data were available through a continuously updated computer database. Clinical follow-up was performed in each patient at least 1 month after the final operation.

RESULTS

Among 720 patients who underwent a laparoscopic fundoplication, six (0.8%) required reoperation for paraesophageal herniation of the stomach. Another patient had undergone a laparoscopic Nissen fundoplication elsewhere and is included in this study. An overview with relevant data on these patients is presented in Table I. There were four men and three women (mean age 45.3 years; range 14 to 66 years). The mean interval between the initial operation and the reintervention was 11 months (range 7 days to 30 months). The main clinical symptom that led to reevaluation was dysphagia in four of seven patients.

From the Department of Surgery, Mayo Clinic Jacksonville, Jacksonville, Fla.
Reprint requests: Ronald A. Hinder, M.D., Mayo Clinic Jacksonville, Department of Surgery, 4500 San Pablo Rd., Jacksonville, FL 32224. E-mail: hinder.ronald@mayo.edu

Table I. Overview of patients who underwent redo operation for paraesophageal hernia following laparoscopic Nissen fundoplication

Patient	Age (yr)	Sex	Closure of hiatus/ No. of stitches	Interval*	Radiologic diagnosis	Clinical symptoms	Intraoperative finding	Procedure
1	60	M	Yes/1	7 days	Elevated left hemidiaphragm	Dysphagia	Paraesophageal hernia of 50% of stomach	Toupet fundoplication
2	40	M	Yes/1	15 mo	Paraesophageal hernia	Tingling	Paraesophageal hernia, slipped fundoplication	Fundopexy at crura
3	66	M	Yes/1	18 days	Atelectasis, elevated hemidiaphragm	Acute abdominal pain	Intrathoracic gastric volvulus	Laparotomy, reduction of gastric volvulus
4	14	M	Not known	5.5 mo	Hourglass stomach	Dysphagia	Paraesophageal hernia	Thoracotomy, Belsey fundoplication
5	48	F	Yes/1	13 mo	Paraesophageal hernia	Dysphagia	Paraesophageal hernia	Thoracotomy, hiatal repair
6	46	F	Yes/1	30 mo	Paraesophageal hernia	Dysphagia	Paraesophageal hernia, failed Nissen fundoplication	Laparoscopic redo Nissen fundoplication
7	43	F	Yes/2	13 mo	Paraesophageal hernia	Asymptomatic	Paraesophageal hernia	Laparotomy, redo Nissen fundoplication, hiatal repair

*Time between the original operation and the complication.

One patient presented with an acute abdomen due to gastric volvulus, one patient reported a tingling feeling and a splashing noise arising from his stomach when he flexed his abdominal muscles, and one was asymptomatic. The patient who was found to have gastric volvulus at emergency laparotomy had a plain abdominal x-ray examination prior to operation (Fig. 1). The gastric volvulus was misinterpreted by the radiologist as atelectasis with an elevated hemidiaphragm. In the remaining six patients a barium esophagogram was performed. In four patients a paraesophageal hernia could be diagnosed by means of the barium esophagram prior to the operation (Fig. 2). In the first patient in whom the barium esophagram did not show the hernia and who could never swallow appropriately after a laparoscopic Nissen fundoplication, endoscopy revealed a tight stenosis at the presumed location of the wrap. This stenosis was repeatedly dilated with a balloon and a Savary dilator, but this failed to significantly improve the severe dysphagia. After the endoscope was passed into the stomach, no hernia could be detected. The barium esophagram disclosed a tight J-shaped stenosis at the gastroesophageal junction but did not show herniation of the stomach (Fig. 3). In the other patient the diagnosis of an hourglass stomach was made radiologically prior to the operation, but intraoperatively he was found to have paraesophageal herniation of the stomach. Reoperation was performed via emergency laparotomy in one case because of gastric volvulus. In two patients the operation was attempted laparoscopically but conversion to an open procedure became necessary. One of these was a 14-year-old boy who was found to have paraesophageal herniation of the stomach into the chest together with extensive adhesions. A left-sided thoracotomy was performed together with a Belsey fundoplication. In the other patient the pleura was entered during the dissection of the hiatus. As the pneumoperitoneum could not be maintained without respiratory embarrassment, conversion to an open procedure was required. Patient 5 (see Table I) developed a paraesophageal hernia about 13 months after a laparoscopic Nissen fundoplication. This was repaired laparoscopically. One year later she again presented with an endoscopically and radiologically proved paraesophageal hernia. At this time the

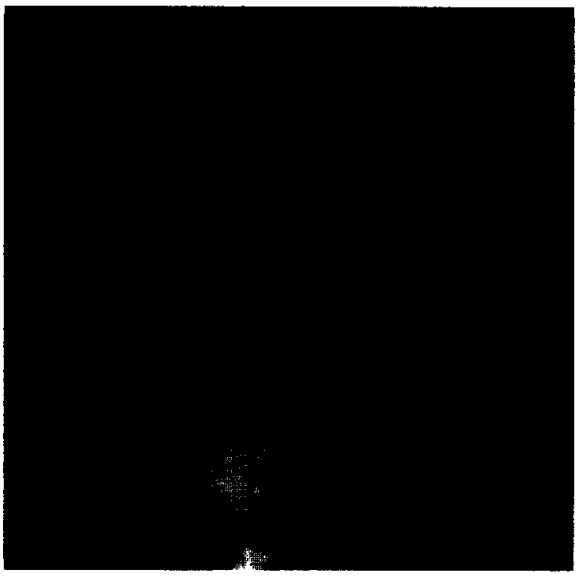

Fig. 1. Patient 3, who presented with intrathoracic gastric volvulus. Plain abdominal film shows an air bubble in the left hemithorax. This situation was misinterpreted as an elevated hemidiaphragm with atelectasis.

Fig. 2. View of the gastroesophageal junction during barium esophagram (patient 2). Paraesophageal hernia is clearly visualized.

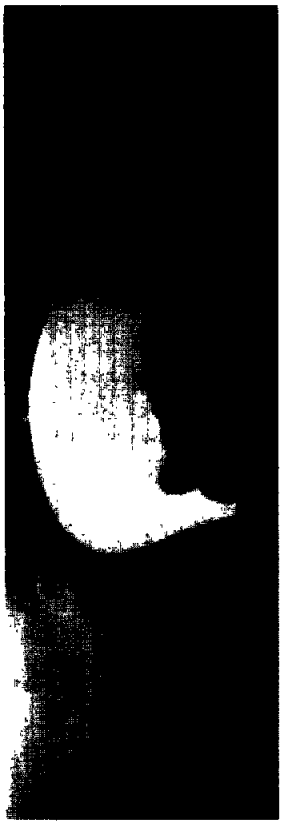

Fig. 3. View of the gastroesophageal junction during barium swallow (patient 1). J-shaped stenosis is demonstrated after two sessions of esophageal dilatation.

repair was performed via a left thoracotomy. At operation the wrap was intact but had herniated through a slightly open hiatus. The hernia was reduced and a hiatal repair was performed. The remaining three patients were operated on laparoscopically and had their hernias reduced followed by either a Toupet fundoplication with fixation of the fundoplication to both crura (patient 1), reduction and fixation of the fundoplication to the edges of the crura (patient 2), or redo of the disrupted Nissen fundoplication (patient 6). No deaths occurred and no major complications were observed following the redo operations. Two patients were lost to follow-up. After a mean follow-up of 2.5 months (range 1 to 8 months), all patients were without complaint.

DISCUSSION

The use of the laparoscopic Nissen fundoplication for gastroesophageal reflux disease has steadily increased since this procedure was introduced in 1991. Numerous series have been reported demonstrating the efficacy, safety, and superiority over long-term medical treatment. Despite the enthusiasm for this highly effective surgical procedure, it must be realized that potential complications may occur influencing the short- and long-term outcome. These are usually caused by failure of the wrap, which may break down, be too tight, or slip.[5] Severe complications such as paraesophageal herniation with intrathoracic volvulus have been reported.[3] In our experience with 720 laparoscopic antireflux procedures, the incidence of postoperative paraesophageal herniation is 0.8%. Other authors report an incidence of 0.8% to 6.7% of paraesophageal herniation as a complication following laparoscopic Nissen fundoplication.[4,6,7] Several reasons for the development of a postoperative paraesophageal hernia have been proposed. It is possible that reduced postoperative pain, which allows patients to return to their normal activities earlier than after open surgery, may be a factor. This results in increases in intra-abdominal pressure before sufficient scar tissue has developed at the operative site. This may force mobile organs in the abdominal cavity through the hiatus into the chest or may lead to rupture of the hiatal repair. Gastric distension or vomiting due to eating and drinking within 24 hours of surgery or postoperative bowel obstruction may have a similar effect. Extensive mobilization of the fundus to create the fundoplication may cause the "floppy" fundus to herniate through the hiatus. Following on extensive mobilization of the gastric fundus, this may be sucked through a small opening in the hiatus by the negative intrathoracic pressure. Secure closure of the esophageal hiatus by approximating the crura behind the esophagus may play an important role in preventing this sequence. We close the hiatus behind the esophagus using interrupted nonabsorbable sutures. This is required since we perform a wide dissection at the hiatus prior to fundoplication. After the fundoplication has been completed, no additional stitches are used to fix the wrap to the diaphragm or the crura. Watson et al.[4] originally left the esophageal hiatus open and reported the highest incidence of paraesophageal herniation. Subsequently they changed their operative approach and now close the hiatus behind the esophagus. Based on the observation of three episodes of wrap migration into the chest, Gotley et al.[8] also suture the crura snugly behind the esophagus. However, other authors never close the hiatus and report no increased risk for the development of paraesophageal herniation.[9] In the presence of a large hiatal defect, it has been proposed to reinforce the hiatal repair with mesh.[10] It has also been proposed to anchor the wrap of the Nissen fundoplication by additional sutures to one of the crura as is performed in the Toupet procedure.[11]

Complete herniation of the stomach may result in severe ischemia followed by necrosis and perforation

of the stomach.[2] In our series only one patient presented with complete paraesophageal herniation of the stomach into the chest but without ischemic changes and with the wrap remaining in the correct position.

One of our patients was unable to swallow properly after his primary surgery and spat up foamy saliva soon afterward ("foam sign"). This finding is suggestive of obstruction at the gastroesophageal junction. Obstruction may be enhanced by reduced esophageal body motility, since the esophagus rapidly looses its propulsive force when obstructed.

The diagnosis of acute paraesophageal herniation was difficult to establish and was misinterpreted by an experienced radiologist. In three of seven patients the diagnosis could not be confirmed preoperatively. In patients 1 and 3 the diagnosis of paraesophageal hernia was missed on plain abdominal films. Both were interpreted as elevated hemidiaphragm in combination with a suspected atelectasis. The barium esophagram in one revealed a J-shaped appearance of the distal esophagus ("J sign," see Fig. 3). This may be caused by the herniated fundus compressing the distal esophagus resulting in the J-shaped distal esophageal stenosis. The symptom of recurrent dysphagia after an antireflux procedure should suggest the possibility of an iatrogenic paraesophageal hernia. An additional clinical hint may be the "foam sign." A plain x-ray film will show what appears to be elevation of the left hemidiaphragm, and a barium esophagogram can confirm the diagnosis of paraesophageal herniation of the stomach, especially in the presence of the typical J sign.

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Achalasia

Achalasia of the esophagus is an uncommon disease characterized by defective esophageal emptying. Emptying of the esophagus is impaired by lack of adequate esophageal peristalsis, lack of relaxation of the lower esophageal sphincter (LES) with swallowing, and in some patients is worsened by high pressures in the LES. Defective esophageal emptying leads to progressive dilatation of the esophagus. Squamous cell carcinoma of the esophagus develops in patients with achalasia with greater frequency than in the normal population.

SYMPTOMS AND DIAGNOSIS

The two most common symptoms in patients with achalasia are dysphagia and regurgitation. Dysphagia, observed most commonly earlier in the evolution of the disease, is caused by the inability of the LES to relax. As the esophagus becomes progressively dilated and accumulates large amounts of solid food within it, overflow regurgitation occurs. Nighttime regurgitation, enhanced by recumbency, can lead to aspiration pneumonia and pulmonary abscesses. The disease is insidious and it usually takes months to years for patients to notice impairment of esophageal emptying. Inability to swallow adequately leads to weight loss in more than half of the patients.

Diagnosis requires the use of esophageal manometry, endoscopy, and radiography. Manometry is the diagnostic test of choice when achalasia is suspected. Typical findings include lack of esophageal peristalsis, inadequate relaxation (inability of the esophagus to relax to the zero baseline), and hypertensive LES. Endoscopy must be performed in all patients to rule out more common causes of dysphagia such as Schatzki's ring, benign strictures, or malignancy. The finding of a dilated esophagus with a tight LES is characteristic of achalasia. With a gentle push, the esophagoscope can easily be passed through the LES into the stomach. This is in contrast to patients with malignant strictures where the rigid tumor prevents the advancement of the endoscope into the stomach. Barium esophogram shows the typical findings of a "bird beak" appearance at the distal esophagus and a dilated proximal esophagus. An air-fluid level behind the heart can occasionally be seen on plain x-ray films of the chest. Endosonography of the esophagus is also helpful and usually shows a thickened hypertrophic inner layer of the muscularis propria of the esophagus.

TREATMENT

Treatment is directed at lowering the resistance to flow through the cardia. This can be achieved by medications, balloon dilatation, or surgery. Calcium channel blockers or nitrate-based compounds are ineffective in the majority of patients. Recent experience with endoscopic injection of botulinum toxin into the distal esophageal wall suggests that symptoms can be alleviated in approximately two thirds of patients, particularly elderly ones. Unfortunately the effect is transient, and even when the initial injection leads to a satisfactory resolution of dysphagia, the effect does not usually last longer than 3 to 9 months.

Balloon dilatation of the esophagus is effective in more than two thirds of patients but usually requires multiple sessions and can be complicated by esophageal perforation. Furthermore, when dysphagia is alleviated, more than half of the patients develop gastroesophageal reflux. Like botulinum toxin injection, the effects of balloon dilatation may be transient with recurrence of symptoms within 2 to 5 years.

Esophageal myotomy should be considered for good-risk patients. The procedure is performed using an open (laparotomy or thoracotomy) or minimally invasive (laparoscopic or thoracoscopic) approach. A 5 to 6 cm myotomy that crosses the cardioesophageal junction and divides the muscularis of the lower esophagus and upper stomach is effective in relieving dysphagia in 95% of patients. If the procedure is performed through the abdomen, a partial fundoplication can be added to prevent reflux. This procedure has the long-standing benefits of restoring the ability to swallow without the problems associated with reflux.

RISKS

In patients undergoing elective esophageal myotomy, the risk of death is less than 0.3%. The risk of recurrence of dysphagia in patients with classic manometric findings is approximately 5% to 10%. In patients with atypical manometric findings (e.g., vigorous achalasia), the risk of recurrence is higher. The main operative complication has been laceration of

the mucosa. This is usually easily recognized and can be repaired with sutures. The consequences of not performing any procedure in patients with achalasia are progressive malnutrition and aspiration pneumonia. Whether or not operation prevents the development of cancer is unknown. If a video endoscopic approach (laparoscopy or thoracoscopy) is chosen, conversion to an open procedure for difficulties in delineating the anatomy should not be regarded as a complication but as an appropriate decision to safely perform the operation.

EXPECTED OUTCOMES

Following esophageal myotomy, 90% of patients have long-term relief of symptoms. Reflux is uncommon if partial fundoplication is added. If not, reflux occurs in approximately 50%. In the majority of good-risk patients (ASA I and II), esophageal myotomy requires 1 to 3 days in the hospital. Older or higher risk patients (ASA III and IV) may require longer postoperative stays. After an uneventful esophageal myotomy, patients can begin taking liquids by mouth on the night of surgery or as soon as they have recovered from the effects of pneumoperitoneum (primarily nausea). A solid diet is usually tolerated within 1 to 2 days. Patients should be placed on a mechanical soft diet for 4 to 5 weeks. If symptoms of reflux develop postoperatively, an evaluation for reflux should be initiated.

QUALIFICATIONS

The qualifications of a surgeon performing any operative procedure should be based on training (education), experience, and outcomes. At a minimum, operations for achalasia should be performed by surgeons who are certified or eligible for certification by the American Board of Surgery, the Royal College of Physicians and Surgeons of Canada, or their equivalent. If the laparoscopic or thoracoscopic approach is used, it is highly desirable that the surgeon have advanced training in video endoscopic techniques.

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Surgical Treatment of Reflux Esophagitis

Gastroesophageal reflux is a condition that occurs when acidic gastric contents reflux into the esophagus. Approximately 10% of adult Americans have daily symptoms of heartburn and about 2% of them progress to severe esophagitis. Repeated episodes of acid reflux damage the esophageal epithelium causing esophagitis. A hiatal hernia may or may not coexist with gastroesophageal reflux and many patients with a demonstrated hiatal hernia have no evidence of gastroesophageal reflux. In most cases the underlying cause of reflux is a defective lower esophageal sphincter. This may be compounded by disorders of esophageal clearance of refluxed material. The symptoms of heartburn can usually be controlled by medical therapy directed at buffering or suppressing secretion of gastric acid.

Patients with documented reflux esophagitis who are dependent on continuous medical therapy, who cannot be controlled by medical therapy, or who have regurgitation and aspiration of gastric contents into the tracheobronchial tree can be effectively treated by a surgical procedure directed at creating an effective functional lower esophageal sphincter.

SYMPTOMS AND DIAGNOSIS

Gastroesophageal reflux results in a typical substernal burning discomfort or heartburn that characteristically is relieved by antacids. Some patients also experience esophageal spasm with a squeezing chest pain that is often mistaken for angina. Refluxed acid can be aspirated into the larynx causing hoarseness or into the tracheobronchial tree causing wheezing and coughing. In a minority of patients, dysphagia from an esophageal stricture occurs.

The diagnosis of reflux esophagitis can usually be made on the basis of a detailed history. To confirm the diagnosis, and to determine the extent of damage to the esophageal epithelium, several investigations are useful. The mainstay of diagnosis is flexible esophagoscopy. Endoscopic demonstration of mucosal erosion or ulceration is evidence of reflux damage. Endoscopy is also essential to diagnose Barrett's mucosa (replacement of the normal squamous epithelium of the lower esophagus by columnar cells). Barrett's mucosa is a consequence of acid reflux and is associated with an increased risk of adenocarcinoma of the esophagus. Barium esophagography should also be performed if an obstruction is suspected. Before planning surgery, it is important to utilize esophageal manometry to determine the function of the lower esophageal sphincter and to evaluate the peristaltic activity in the body of the esophagus. Absence of peristalsis, as is seen in scleroderma and achalasia, may require a modification of the usual surgical procedure. Since the aperistaltic esophagus may be unable to overcome the resistance of a surgically created normal lower esophageal sphincter, postoperative dysphagia can result. Other investigations such as 24-hour pH monitoring allow documentation of reflux episodes by indicating a drop in esophageal pH to very acid levels (<4.0). This test is useful in patients who have atypical symptoms or in those who have typical symptoms with normal endoscopic findings.

TREATMENT

Patients with a history of typical gastroesophageal reflux symptoms should initially be managed with lifestyle modifications. They should avoid foods and beverages that can weaken the lower esophageal sphincter. These include chocolate, peppermint, fatty foods, coffee, and alcoholic beverages. Foods and beverages that can irritate an inflamed esophageal mucosa, such as citrus fruits and juices, tomato products, and pepper, should also be avoided. Elevating the head while sleeping, not lying down immediately after eating, and not smoking are also helpful.

Medical therapy is directed at reducing the acidity of refluxed material by the use of antacids, H_2 receptor–blocking drugs, or proton pump inhibitors. The success of treatment seems to be related to achieving a high degree of acid inhibition. In theory, promotility drugs (including cisapride, metoclopramide, and domperidone) improve esophageal clearance, lower esophageal sphincter tone, and gastric emptying but in practice are of little benefit for patients with severe reflux symptoms.

Although medical therapy is highly effective in controlling the signs and symptoms of gastroesophageal reflux, approximately 80% of patients will relapse within 3 months if therapy is discontinued. However, complete control can often be accomplished by continuous therapy with omeprazole.

Surgical procedures are usually very effective in controlling severe gastroesophageal reflux disease.

Fundoplication was found to be more effective than ranitidine plus metoclopramide for 2 years in one study. Surgery for gastroesophageal reflux disease is indicated for patients who do not respond to medical therapy, who have complications of gastroesophageal reflux, who refuse to comply with medical therapy, and who are unable to discontinue medical treatment without developing a recurrence of their symptoms. The expense of long-term medical therapy and uncertainty regarding the sequelae of chronic proton pump inhibitor treatment also lead patients to consider surgery. The surgical approach to treatment can be carried out by means of the Belsey procedure or by the Nissen or Toupet fundoplication. The fundoplication operations can be performed using either open or laparoscopic approaches.

Surgical approaches are designed to correct gastroesophageal reflux by creating a new functional lower esophageal sphincter and to repair a hiatal hernia when present. The most popular approach is the Nissen fundoplication, or a modification of this technique. It involves mobilization and wrapping of the fundus of the stomach around the lower esophagus. As pressure increases in the stomach, it compresses the lower esophagus, preventing reflux. The procedure is performed after first placing a large dilator in the esophagus to avoid making the wrap too tight. Fundoplication performed by either a traditional open or laparoscopic technique should be identical, except access to the esophagus by laparoscopy is through a series of four or five punctures rather than by an upper abdominal incision. The advantages of the open technique include the ability to see structures in three dimensions and to palpate these structures. Laparoscopy provides a clear magnified view of the area of surgery and is associated with less pain and more rapid recovery postoperatively.

RISKS AND EXPECTED OUTCOMES

The most common risks include bleeding or damage to structures such as the spleen, esophagus, or stomach, and these total less than 5%. These uncommon but potentially serious complications may occur after either open or laparoscopic techniques. Respiratory complications such as atelectasis or pneumonia are less frequent after laparoscopic surgery than after open upper abdominal surgery.

Up to two thirds of patients will experience some difficulty in swallowing after surgery, especially with solid foods. This is usually temporary, and almost all patients are able to swallow normally and tolerate an unrestricted diet by 6 weeks after surgery. Another possible problem after fundoplication is the gas-bloat syndrome, which is a sensation of bloating associated with inability to belch. Many patients with reflux esophagitis swallow frequently in a subconscious effort to neutralize refluxed gastric acid with saliva. This may result in large volumes of swallowed air. If the wrap is tight, it is difficult to expel the gas by belching, an almost effortless event preoperatively. This usually resolves with time.

In the majority of good-risk patients, fundoplication requires a short hospital stay of approximately 3 days after laparoscopic surgery and about 5 days after open surgery. Hospitalization may be prolonged if the patient has comorbid conditions or encounters any postoperative complications.

Limited data suggest that long-term outcome is equivalent after either open or laparoscopic procedures. Recurrence of reflux symptoms is uncommon after fundoplication and implies that the wrap was too loose, has come undone (dehisced), or has slipped down around the stomach. In such cases further investigation is required to delineate the cause of the failure and to determine the appropriate remedial treatment, either medical or surgical.

QUALIFICATIONS FOR PERFORMING SURGERY FOR GASTROESOPHAGEAL REFLUX

The qualifications of a surgeon performing any operative procedure should be based on training (education), experience, and outcomes. At a minimum, laparoscopic or open fundoplication should be performed by surgeons who are certified or eligible for certification by the American Board of Surgery or the Royal College of Physicians and Surgeons of Canada, or their equivalent. When performing laparoscopic fundoplication, it is highly desirable that the surgeon have advanced laparoscopic skills.

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