Toward a More Perfect Society

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It is traditional for the President to read the addresses of the previous distinguished presidents of the Society. I have done so, not only out of tradition, but also because these addresses give historical perspective to the development and future directions of the Society. I will review the highlights of these addresses and then discuss some issues that I believe lie before the Society. In attempting to summarize the key point of each prior address, total justice cannot be done to the messages given by our past presidents. I have organized these addresses into the following four arbitrary categories: Clinical-Historical, Political-Philosophical, Research and Education, and SSAT Specific (Tables I to IV). It has been a humbling experience to read these eloquent addresses because I have come to realize that what I have to say is not terribly new, although I may be more direct about certain topics. Dr. Claude Welch made three marvelous observations in his Presidential Address given 34 years ago: (1) ". . . wise presidents confine their addresses to historical summaries or scientific subjects. . . . (2) "Even wiser men say nothing at all. . . ." In fact, the first three presidents of the SSAT, Drs. Cole, Waugh, and Maddock, did not give addresses. (3) "Only the foolish will stoop to philosophy or exhortation."[20] Welch then went on to admonish surgeons about the necessity for continuing education.

I am deeply honored to be standing here and so far I have joined the ranks of the "wise presidents." But now, I am about to join the ranks of my "foolish" predecessors. I must also tell you that I am quite nervous because what I am going to say may not be politically correct. However, my intent is not to offend. If I do so, it is because of my sincere belief that there are issues that we, the members of the SSAT, need to consider and bring to conclusion if we are to remain successful as an organization in the future.

Competitive swimmers know that the harder you swim, the resistance of water increases as the square of the increase in power. Doubling the power increases the resistance fourfold. We cannot defy the laws of physics, but we as members of a competitive organization know that despite resistance, we must push harder in order to be a winner. I believe that this will require our addressing at least seven issues: Digestive Disease Week, SSAT membership, our annual meeting, standards, research, the JOURNAL OF GASTROINTESTINAL SURGERY, and training in surgical gastroenterology.

DIGESTIVE DISEASE WEEK

We are indeed a unique surgical society in the United States because we are devoted to both the science and application of such science exclusively to the physiology and surgical diseases of the entire digestive tract. What also sets The Society for Surgery of the Alimentary Tract apart from other surgical organizations is our privilege of being a full participant in Digestive Disease Week (DDW), where we integrate our thoughts and expertise with our colleagues belonging to the American Gastroenterological Association, the American Society of Gastrointestinal Endoscopy, and the American Association for the Study of Liver Disease. This partnership has been of enormous mutual intellectual and economic benefit to the participating organizations. These benefits have been in existence for nearly a quarter of a century for the

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Table I. Clinical-historical topics of prior presidential addresses

Rhoads[1]	1968	Reviewed history of parenteral nutrition and the beginnings of the modern era of total parenteral nutrition
Dunphy[2]	1969	Reviewed some historical aspects of gastrointestinal injuries and research in gastrointestinal healing
Hardy[3]	1970	Highlighted controversies involving malignant cachexia, genetics, mechanism of peptic ulcer, metabolism, angiology, and transplantation
Scott[4]	1971	Reviewed current treatment of hyperlipidemia and atherosclerosis
Hoerr[5]	1972	Presented the results of his 20-year experience with surgery for duodenal ulcer
Warren[6]	1973	Discussed the need for controlled surgical clinical trials and special problems facing surgeons involved in human experimentation
Nyhus[7]	1975	Discussed current status of vagotomy and his current research interests
Cohen[8]	1977	Made a plea for educating patients and physicians in the earlier diagnosis of gastrointestinal malignancies
Jordan G[9]	1979	Gave historical perspective to development of alimentary tract surgery
Polk[10]	1986	Outlined contributions of alimentary tract surgery to the modern understanding of infection control in surgical patients
Jordan P[11]	1984	Traced the history of description and surgical treatment of peptic ulcer disease
Jones[12]	1985	Honored his former teachers and mentors
Jaffe[13]	1985	Presented arguments for the involvement of gastrointestinal surgeons in visceral transplantation

Table II. Political-philosophical topics of prior presidential addresses

Altmeier[14]	1974	Discussed some of the social and economic changes that affect the training and practice of surgeons
Longmire[15]	1976	Made a plea that medical training be supervised not by the government but rather by a concerned profession
Zeppa[16]	1978	Urged that SSAT join Digestive Disease Council to make our thoughts known in Washington
Carey[17]	1981	Spoke about surgeon participation in changing costs of health care
Nahrwold[18]	1990	Discussed the forces that have changed the medical profession
Warshaw[19]	1998	Emphasized the necessity of collaboration among physicians, surgeons, and scientists

Table III. Research-education topics of prior presidential addresses

Welch[20]	1966	Stressed need for continued education
Rosoff[21]	1980	Discussed development of academic surgeon and private practice surgeon
Moody[22]	1982	Gave arguments in support of developing a fellowship in surgical gastroenterology
Thompson[23]	1984	Advocated vigorous pursuit of research
Hermann[24]	1989	Emphasized importance of role models in the education of surgeons
Cameron[25]	1992	Advocated alimentary tract fellowship programs with the SSAT overseeing such fellowships
Way[26]	1995	Pointed out complexity of new technology and advocated fellowship training to deal with the most complex procedures

Table IV. SSAT topics of prior presidential addresses

Zollinger[27]	1963	SSAT is only society devoted to entire gastrointestinal tract and many unsolved problems regarding treatment of gastrointestinal diseases
Wilson[28]	1965	Reviewed events leading to formation of SSAT
Turell[29]	1967	Reviewed origins of the SSAT and proposed establishment of a research fund
Tompkins[30]	1987	Argued for development of specially trained gastrointestinal surgeons and SSAT playing a more active role in improving gastrointestinal surgical training and research
Silen[31]	1991	Membership should be made available to a wider group of surgeons
Kelly[32]	1993	Urged public recognition of surgical gastroenterology by broadening membership, defining practice guidelines, developing research and educational programs, and promoting national and international cooperation in gastrointestinal surgery
Langer[33]	1994	Favored specialty training; journal to play important role in education; public education; identify research problems; consensus conferences; standards of care; become more involved in public policy issues
DeMeester[34]	1997	Historical review of how the SSAT has evolved; emphasized integration of surgery into other disciplines of medicine

SSAT. In fact, we as an organization participate in what is the most prestigious and most informative gastrointestinal meeting that draws participants from around the world. However, we must be mindful of forces that could change the very fabric of DDW. We, as members of the SSAT, must take an increasingly active role in perpetuating the cooperative spirit that led not only to the formation of DDW but also to its many successes.

SSAT MEMBERSHIP

As an organization, the SSAT has evolved from an exclusive society consisting mostly of members from the academic community with special interests in the gastrointestinal tract to an open society serving all surgeons with some interest in the gastrointestinal tract. On the one hand, this change has upset some members who feel that an open society no longer recognizes their expertise or accomplishments. On the other hand, we must consider that most of general surgical practice involves the digestive tract. Thus an extraordinary clinical expertise also exists in the private practice community. In my view, a new and important strength of the SSAT is that it has brought an increasing number of academic and private practice surgeons together to discuss mutual interests. Our annual meeting is a special opportunity for surgical scientists to learn about issues concerning the private practitioner. Similarly our annual meeting is a wonderful opportunity for the private practitioner to hear up-to-date basic and clinical research presentations. In many instances a strict distinction between scientist and clinician does not exist because most surgical scientists are also clinicians and most clinicians want to know the future directions of gastrointestinal surgery. The SSAT should provide the best information for all, should they be a clinician, a scientist, or both, with any degree of interest in surgical problems of the digestive tract.

I believe we need to expand our membership even more and include members of other surgical organizations with special interests in the digestive tract. Such membership should also extend to the international surgical community. Participation from other surgical organizations already occurs to some degree. Since 1992, the International Society for Digestive Surgery (ISDS) (formerly known as the Collegium Internationale Chirurgiae Digestivae) has supported the State-of-the-Art Lecture at the annual SSAT meeting. More recently the Society of American Gastrointestinal Endoscopic Surgeons has participated with the SSAT in joint symposia during DDW.

Four mechanisms are already in place to attract new members. First, DDW is a unique meeting. Second, the cost of SSAT membership is among the lowest of national surgical societies in the United States. Furthermore, among the many advantages of membership is that one does not pay a registration fee in order to attend DDW. The third mechanism involves the International Council of Surgical Gastroenterology, which was formed in 1998. The SSAT is one of six organizations from around the world that comprise the Council. Among the missions of the Council is "To unite and strengthen . . . established . . . surgical gastroenterology organizations of international dimensions in order to speak with a single voice on issues related to surgery of the alimentary tract." Among the objectives of the Council is "To expand the involvement of surgical gastroenterology in ma-

jor international events such as DDW. . . ." The fourth mechanism is related to the fact that our meeting improves each year.

The importance of involving members from other organizations lies not only with greater scientific and clinical expertise or potentially increased wealth of the Society. There is a fragmentation of surgical organizations in the United States and abroad interested in the whole or parts of the alimentary tract. There is no single voice to express the parochial interests and concerns of gastrointestinal surgery to the managers, be they public or private, of our systems of health care or our systems of surgical education. Other organizations have been successful in influencing legislation, reimbursement, and standards of care for nonsurgical problems of the alimentary tract. Should we, the SSAT, not learn from this example and further our interests in doing what is best for our patients at reasonable cost and at the same time achieve a more uniform standard of surgical treatment of disease of the alimentary tract? Should we not educate concerned parties about areas of controversy and that there are surgical alternatives to expensive long-term medical therapies?

ANNUAL MEETING

As a surgical society, the SSAT is fairly traditional in its format. However, we need to be more innovative and more responsive to the interests of our members (old and new). We attend the annual meeting for many reasons, not the least of which is our need to learn and to share what we have learned. Given the expenses of travel, hotels, and especially time, we need to maximize our efforts. We should not be content to be like most members of an arctic dog sled team—if we do not become the lead dog, the view never changes! However, if one of our goals is indeed to attract more members and maintain the interest of our current members, then we must be innovative in addition to continuing to do what we do well. Specifically, our meetings are well organized; we participate with other societies in many outstanding clinical symposia; our consensus conferences are unique; and the quality of presentations progressively increases. However, I am sure most, if not all, of us have attended a 10-minute research presentation thinking we knew something about the subject. Two and one-half minutes after the speaker begins, we realize we do not know as much as we thought we did. Two and three-quarter minutes later we realize we know very little and by 7 minutes we are lost in thoughts about how our patients are doing at home. By 8 ± 1 minutes into the talk, we are in a different world (Fromm D, unpublished observations). These minutes turn into nanoseconds if the research presentation involves a field we know little or nothing about, let alone understand the terminology. If we are going to continue to have a mixture of research and clinical presentations given at a single session, would not our attention span be increased and our understanding enhanced if each research presentation began with a clear statement as to the importance of the work relative to the overall context of the field and if presenters geared their talks to an audience of diverse backgrounds? This would enhance our understanding without detracting from the scientific validity of the presentation and would increase audience participation. Should we consider state of the art research conferences specifically directed to those of us with primary clinical interests or primary expertise in other areas of investigation?

It is always easy to suggest that our Society be more innovative but the execution of innovation is not always easy. The many thoughtful hours that members give to Society committees, while insufficiently recognized, is an important source of innovative ideas. However, greater input is required from the membership about the content and format of our meetings. We need to consider new venues. Should we have technical sessions relating to how we perform certain complicated procedures or how we handle operative indiscretions? Should we have hands-on sessions using a variety of models? There is an incredible amount of surgical adeptness in this room, but we need more innovative forums that tap this expertise. Many of you have thought about and contributed to the success of the Society. Yet participation in the annual business meeting is sparse. Perhaps this is because of the necessary, and sometimes mundane, presentation of information that is in part dictated by our bylaws. We might consider a change and set aside time at the business meeting for a critique and free exchange of ideas about our meetings and future. This would be the time to bring up issues that may not be apparent to the administrative structure of the Society. Our Board of Trustees has become increasingly concerned about the thoughts of the members. We need to define more clearly what our goals are and to this end, our Board will analyze in depth the results of the recently completed survey of the membership.

STANDARDS

As surgeons, we publish in diverse areas that include pathophysiology or basic research (some of which has and some which does not have apparent clinical application), technical aspects, issues relating to training, administration, politics, and clinical studies. However, the SSAT, like virtually all other surgi-

cal societies, continues to accept presentations of case series that are encumbered by all of the imperfections of retrospective studies. We try to get around this by saying the case series is prospective because the patients were all followed after their treatment, but this does little to diminish the array of confounding variables that, even unavoidably, lead to the drawing of erroneous conclusions. All of us know that prospective randomized studies are the "gold standard," but we engage in too few such studies. So we play on words by saying that we have done a retrospective analysis of prospectively collected data! I am not implying at all that retrospectively or prospectively followed case series are without value, but we must be wary of the conclusions. Our critics constantly point this out, but our selective auditory disorder kicks in. If we do not start to listen more intently, we run the risk of drowning in our own conceit. Dr. Richard Horton, the editor of *Lancet*, pointed out in a scathing editorial in 1996: "To retain their academic reputation surgeons must find imaginative ways to collaborate with epidemiologists to improve the design of the case series and to plan randomized trials. In addition to safety and efficacy studies, more pragmatic trials are needed to determine acceptability, effectiveness and efficiency by comparing new interventions with currently preferred treatments."[35] Because the SSAT is the only surgical organization in this country exclusively devoted to all aspects of surgery of the gastrointestinal tract and because the SSAT is the only surgical organization in this country that publishes the only journal devoted exclusively to surgery of the gastrointestinal tract, we de facto set a standard. However, we must ask ourselves how lofty a standard we wish to achieve.

I suspect that few, if any, of us had the foresight to predict the technological revolution leading to laparoscopic approaches to the digestive tract. We are a technologically oriented society and we thrive on technology both as providers and consumers of medical care. Laparoscopic images and approaches are dazzling as are the surgical tours de force that have been accomplished with this new technology. However, there is, I believe, an important issue that we have not dealt with directly. Are we, as devotees of alimentary surgery, abusing this technology for personal gain and prestige? As a result of the widespread belief that laparoscopic cholecystectomy is associated with less morbidity than an open procedure, the number of cholecystectomies has increased, but has there been a concomitant increase in the incidence of symptomatic gallstone disease? Should not we, as members of the SSAT, insist on more extensive evaluation of this technology and more rigorously compare its outcomes to the results of our time-honored open surgical techniques? Many may sneer at this suggestion because in their opinion there is no question laparoscopic techniques are superior. However, we sometimes forget that many of us treated our patients with standard incisions in an unintentionally biased fashion postoperatively. For example, a recent report indicates that following open sigmoid resection performed under combined epidural-spinal anesthesia, 87.5% of patients had an oral intake of at least 2 liters and 93.8% had resumed defecation by 48 hours![36] We are presently caught up in laparoscopic approaches, but the data are not uniform that a laparoscopic or quasi (i.e., assisted)–laparoscopic approach for certain surgical problems is better than a classic open approach.[37] My point is not to debate the merits of laparoscopic surgery but rather to indicate that it took a technological revolution to make us reexamine our postoperative care of patients who undergo open operations. As surgeons we have an interest in developing and evaluating new technology, but we must ask ourselves whether we are posing the right questions when we do comparative studies. We, as members of the SSAT, should have been more actively engaged in this process years before laparoscopic surgery became widely practiced. We have not been the lead dogs!

RESEARCH

The advances in research are staggering and are difficult to keep up with. However, as clinicians we do a disservice by not keeping abreast of research developments. We do an even greater disservice if we do not insist on the development of new generations of surgeons engaged in basic research problems devoted to the gastrointestinal tract. Otherwise, we run the risk of becoming subservient physicians in the care of our patients. Dr. Jim Thompson, in his 1983 presidential address to the SSAT, said it very succinctly: "If we fail today to vigorously pursue research, the medicine of tomorrow will be the medicine of today."[23] Dr. Leon Rosenberg put it another way: "Some might ask if, at a time when there are increasing numbers of well-trained Ph.D.'s, it matters if the physician-scientist disappears. The answer is that in the absence of physician-scientists, the bridge between bench and bedside will weaken."[38]

Patient care is becoming increasingly complex and this will escalate as receptor modification and gene therapy become more applicable. As senior surgeons, we need talented younger surgical scientists to teach us the new methodology and serve as the link between basic research developments and patient care. As younger surgical scientists, we need senior surgeons for mentoring and helping us to maintain our clinical

credibility. As pure clinicians, as pure scientists, and as a mixture of both, we need to ensure the fertility of the bed-bench breeding ground. This approach allows both the academic clinician and the surgical scientist to become inscribed in academic immortality.

Every academic surgeon is aware of the increasing pressures of clinical practice, which unavoidably takes time from work in the laboratory. To be both a successful researcher and clinician implies an extraordinary time commitment. This has two rate-limiting steps: the number of hours in a day that one can function effectively and the dollars available for research. This being the case and with increasing demands on time spent taking care of patients, we are in jeopardy of shrinking the surgical gastroenterologic research force and thus weakening the surgical perspective applied to basic problems. If we, the SSAT, are going to continue to be a major force in surgical gastroenterology, we must place even greater emphasis on the training and success of young creditable researchers.

The SSAT has been proactive in two areas relating to research. One is the Residents Research Conference, which is a first-class and unique experience for residents who present papers at our annual meeting. The second is the Career Development Program, which has been most successful in helping individuals with basic research backgrounds begin their careers as independent investigators.[34] Industry, especially Ethicon, has been generous in its support of these awards during a time when it has been increasingly difficult for the Society to monetarily support such fellowships without significantly increasing dues. However, industrial support is precarious as philosophies and financial pressures change. For example, the Resident Research Conference, which has been very successful because of the enthusiasm and largesse of Ross Laboratories, has become a financial burden to its sponsor. The SSAT Board of Trustees felt so strongly about this conference and its ability to interest surgical residents to include alimentary research as part of their future careers that the Society will, beginning next year, assume approximately half the expense of the conference. We must not only continue to seek other joint ventures with the industry that alimentary surgery supports but also establish a Research Foundation under exclusive control of the Society.

Most clinicians would agree that the fundamental task of the biomedical researcher is to establish new information that improves the diagnosis and treatment of disease. However, I believe that both biomedical researchers and many clinicians push aside what should be our obligation as members of the SSAT. Why do we often exclude participation of clinicians and why do clinicians frequently decline to participate in well-planned clinical studies? Would it not be a grand contribution if we, as members of the SSAT, irrespective of our biomedical research or clinical backgrounds, participate in SSAT-sponsored prospective randomized studies? This is a simple concept in theory but is difficult, yet not impossible, to implement. Think how exciting it would be if many of us in this room participated in studies that not only answer important questions but are also published in the *Journal of Surgical Gastroenterology.*

JOURNAL

The SSAT has done very well in establishing a new journal devoted exclusively to surgery of the alimentary tract. Even though a survey of the membership indicated that the majority believed a new journal was unnecessary, our Board of Trustees forged ahead. I must admit I was among the skeptics, but wow was I wrong! The JOURNAL OF GASTROINTESTINAL SURGERY is a quality publication that has received accolades. Take, for example, the review by Eubanks and Albright[39] that appeared last October in *JAMA.* "This journal has rapidly established itself as one of the leading surgical journals and will likely enjoy a long and highly successful future." In less than 2 years, the JOURNAL became listed in *Index Medicus.* The JOURNAL has also been a modest financial success due to the generous support of United States Surgical Corporation.

If the SSAT is to become the vehicle for dissemination of the best information relating to surgical gastroenterology, we must engage in more active strategic planning for the continuing success of the JOURNAL on both a scientific and financial basis. I am virtually certain that all of you feel the same as I do about the increasing number of journals—it is overwhelming! However, which journals do we read first and to which journals do we send our best work? If the JOURNAL OF GASTROINTESTINAL SURGERY is to reach first-tier stature, we must insist on progressively increasing stringent editorial policies so that the JOURNAL indeed becomes the international repository of the best information relating to research and clinical aspects of surgery of the alimentary tract. The editors of our journal have done a magnificent job in the hatching, but they must now carefully guide the JOURNAL into its future role with the help of members of the Society.

TRAINING

Surgery of the alimentary tract is not officially recognized as a specialty. However, few would argue that there are a number of general surgeons with special

interest and expertise in the surgery of the gastrointestinal tract. This is the basis of our Society. On the other hand, those who believe that gastrointestinal surgery is indeed a specialty are strong advocates for the SSAT supporting and even certifying clinical fellowships in gastrointestinal surgery and establishing criteria for gastrointestinal surgical training. The motivations for these proposals vary, and substantive data supporting the necessity for such fellowships are difficult to obtain. I have great concerns about clinical fellowships in surgical gastroenterology. A bevy of alimentary tract fellowships will not only fragment surgery further but also has the potential for franchising that serves narrow interests. An even more worrisome adverse effect involves the diversion of energy from the training of general surgeons (Ritchie WP Jr: Personal communication, 1995). Given the emergence of surgical societies with more-or-less narrow interests in various aspects of the gastrointestinal tract, do we really want to continue to segregate alimentary surgery into organ-specific surgeons, laparoscopic and open surgeons, or benign and malignant surgeons? Can we really afford to support clinical fellowships? Can the majority of surgical services in this country afford to be organ or technique specific? Should we really be involved in certification? Should we, as a society of general surgeons with a special interest in the gastrointestinal tract, be more concerned with establishing reasonable standards of practice based on data? If we did the latter, I submit that training standards would in effect be set.

Many of the advocates for specialty training feel that additional training is necessary for surgeons to be involved in complex gastrointestinal procedures. I believe that, in fact, such training already exists in the form of quasi-clinical fellowships in which we mentor younger faculty and younger members of our private practice groups. Pragmatically, it is our referral patterns that lead to "specialization." In other words, I strongly suspect it is the practice environment that determines what procedures we do far more than our specific training. Let us also not forget, as Silen[40] has pointed out, that one to two years of fellowship do not result in true virtuosity and that many of the gastrointestinal surgical giants did not develop their expertise as a result of fellowships. I would also submit that we already have in place mechanisms for dealing with complex gastrointestinal surgical problems in the form of tertiary referral centers, both academic and private.

Some have even suggested that the SSAT be involved in establishing standards for general surgical residency training involving the digestive tract. My bias is that we should leave this issue to other organizations such as the American Board of Surgery and the Residency Review Committee. However, if we as a society establish standards of practice, as we are starting to do with our published patient care guidelines and the publication of the JOURNAL OF GASTROINTESTINAL SURGERY, then surely these standards will work their way into residency training.

CONCLUSION

I have touched on the following points: perpetuation of the spirit of DDW, membership expansion, innovation in our annual meeting, greater membership input, fertilization of the bed-bench breeding ground, support from industry, establishment of a research foundation, strategic planning for our journal, and the setting of standards. I have also asked some questions: Do we want more uniform standards of practice? Do we insist on a more rigorous evaluation of technology? How lofty a standard do we wish to achieve? Are we asking the right clinical questions?

We must actively participate in and contribute to the future of surgery of the alimentary tract so that we are indeed among the best. All of us are proud to be members of the SSAT. Now and in the future we can state with progressively greater pride that we are members of the best surgical organization devoted to the alimentary tract because we swim harder and we are becoming the lead dogs!

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Remarks on Accepting the SSAT Founders Medal

Wallace P. Ritchie, Jr., M.D., Ph.D.

I am deeply grateful and truly honored to receive the Founders Medal from The Society for Surgery of the Alimentary Tract (SSAT). It was totally unexpected but I can assure you that it is totally appreciated.

I would also like to thank Dr. Warshaw for his very gracious introductory remarks (I now fully understand the definition of hyperbole). As I was listening to him catalogue the reasons for this award, I could not help but reflect on how very lucky I have been to be able to participate at one time or another and in one way or another in so many facets of the academic surgical life.

And if that participation has resulted in some small measure of good for the discipline of surgery, in general, and for gastrointestinal surgery, in particular, that is gratifying and all to the good. For me personally, however, the experience was most memorable because it was so very enjoyable. I enjoyed being a clinically active gastrointestinal surgeon; I enjoyed my time in the laboratory; I even enjoyed being a chairperson (at least 50% of the time—the 50% I was out of town); I enjoyed my service on the Study Section and on the Residency Review Committee for Surgery; and I truly enjoyed and continue to enjoy my work at the American Board of Surgery, despite occasional vicissitudes.

And I have certainly enjoyed my long association with the SSAT, which has been ongoing now for some 25 years. This Society has been very good to me; it allowed me to present my research work at an early stage of my career, even when it represented more of a work in progress than a polished finished product—and it extracted no penalties for that. It also indulged me in several offices, including that of Recorder where, I confess, my track record was something less than stellar. I realized that when one of the principal officers of the Society took me aside and told me in no uncertain terms that I was the worst recorder he had ever seen: I couldn't see, I couldn't hear, and I couldn't spell (all of which remains true today). Most important, however, the SSAT provided me the opportunity to become acquainted with and to learn from some of the ablest, brightest, and most talented clinical and research gastrointestinal surgeons in the world. For all of that I am extremely grateful.

As Dr. Fromm pointed out in his fine Presidential Address, this is a viable and engaged organization, very much on the move. When I first joined the SSAT one quarter of a century ago, it was mostly a "gentlemen's club"; it was quite satisfied to be so. It was somewhat parochial and, probably because of the placid nature of the times, it was largely uninvolved in any issues beyond its narrow focus on clinical and, to a lesser extent, experimental gastrointestinal surgery. Obviously all that has changed, and particularly so in the last decade thanks, I believe, to a remarkably able leadership that has been impressively resourceful, imaginative, energetic, forward-thinking and, most important, not afraid to take risks (witness the JOURNAL OF GASTROINTESTINAL SURGERY; it was a huge risk but the payoff has been equally huge and will only increase).

Because of that leadership, the SSAT has achieved the following:

- It has expanded its membership dramatically and now embraces all elements of gastrointestinal surgery in its most catholic sense, including the practicing general surgeon.
- It has greatly improved the quality of its programs to reflect cutting-edge clinical and basic science. The best example of this is the outstanding consensus conferences of the past several years, which have been both unique and impressive.
- It has, as a consequence, achieved intellectual parity with its partners in Digestive Disease Week and, in so doing, has earned their attention and respect.
- It has become aggressively international in its approach so that it is now universally considered to be

an unquestioned world leader among societies devoted to gastrointestinal surgery and worthy of global imitation.
- It has, as already noted, created, and nutured *the* premiere gastrointestinal surgical journal, without equal anywhere.
- It has not been afraid to engage itself fully in addressing the larger issues including the most vexing of all, the appropriate way to train gastrointestinal surgeons.

As to this last point, I firmly believe that the SSAT approach has been mature and responsible. While it understands that specialization is the natural offshoot of technological progress and new knowledge, it has also been cognizant of the fact that alimentary tract surgery is so much the core of general surgery training and practice that to institutionalize and codify specialized training in this area has the very real potential of creating unhappy and serious consequences, even if unintended. These include fragmentation and franchising, which can only result in "balkanization" of the basic discipline—the creation of surgical "sushi." As Dr. Fromm clearly indicated, the leadership of the SSAT understands this fundamental problem and is committed to protecting the corpus of general surgery, regardless of whatever else may transpire. For this I am extremely grateful because it benefits the public, even though the public has no knowledge or understanding of the problem.

Although much remains for the SSAT to accomplish, it is clear that it is a vibrant organization with a very bright future.

I am proud to be a member.

Thank you once again for this wonderful honor.

Short-Term Outcomes in Open vs. Laparoscopic Herniorrhaphy: Confounding Impact of Worker's Compensation on Convalescence

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Over a 28-month period, 123 patients with a unilateral inguinal hernia were recruited into a randomized controlled trial comparing open herniorrhaphy (OH) to laparoscopic inguinal herniorrhaphy (LH). The primary end point was duration of convalescence. Sixty-five patients underwent OH and 58 underwent LH. Both groups were well matched for all baseline parameters, although LH patients anticipated a shorter convalescence than OH patients (14.3 ± 9.4 days vs. 18.5 ± 10.8 days; $P = 0.021$). The median duration of hospital stay was one day in both groups. No difference was observed in the duration of convalescence (LH 9.8 ± 7.4 days; OH 11.6 ± 7.7 days) across groups. However, when the data were analyzed after removing patients receiving disability ("worker's") compensation (21 patients), patients undergoing LH recovered on average 3 days faster (LH 7.8 ± 5.6 days; OH 10.9 ± 7.5 days; $P = 0.02$). Patients not receiving worker's compensation appear to have a shorter convalescence after LH compared to OH. Disability compensation is a major confounding variable in determining convalescence and needs to be controlled for in any future trial design. (J GASTROINTEST SURG 1999;3:575-582.)

KEY WORDS: Outcomes, laparoscopy, herniorrhaphy, randomized trial

The benefits of laparoscopic techniques of inguinal hernia repair over open approaches remain controversial. Several published randomized trials have suggested that the laparoscopic approach may result in decreased postoperative pain and discomfort[1-4] as well as an earlier return to normal daily activities or work.[3,5-7] Other trials, however, have failed to show a difference in convalescence.[2,8] We undertook a prospective randomized controlled trial to objectively assess the comparative effectiveness of the most popular types of open herniorrhaphy (OH) and laparoscopic herniorrhaphy (LH) approaches. The primary end point was duration of convalescence, with secondary end points being postoperative pain and quality of life. The study was not designed to detect differences in hernia recurrence rates because of limitations in sample sizes.

In a previous preliminary report we suggested that although LH patients experienced less pain postoperatively, there was no significant difference in the duration of convalescence.[9] For the purposes of this final report, we specifically examined whether worker's compensation could be a confounding variable in our comparison of convalescence after LH and OH.

MATERIALS AND METHODS

Between March 1993 and July 1995, a total of 123 patients were recruited into the study at two McGill University hospitals. All patients, aged 18 to 82 years, referred to participating surgeons for elective hernia repair were eligible for entry into the study. Excluded from the study were patients deemed unfit for general anesthesia, pregnant women, and those who refused

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random group allocation. The study protocol and consent sheets had been approved by the institutional review boards. Eligible and consenting patients were stratified according to age (<50 years vs. >50 years) and type of hernia based on clinical assessment (direct or indirect). Randomization to either the OH or LH group was carried out using computer-generated random numbers. Each of the four participating surgeons had performed at least 30 laparoscopic hernia repairs and had had experience with multiple open techniques before operating on his first study patient. A separate randomization box was given to each surgeon to ensure an equal proportion of LH and OH patients among individual surgeons.

Research assistants not participating in the care of the patients collected preoperative and postoperative data. All data were collected prospectively through direct patient interviews. Anesthesia and operative data were collected from the surgeon immediately after each operation and confirmed by the study assistants using operative records.

Surgical Technique

Laparoscopic Technique. General anesthesia was used in all patients. A transabdominal preperitoneal (TAPP) technique was the most common procedure used as previously reported[10,11] because not enough experience had been accumulated with a totally extraperitoneal repair. The peritoneum was incised above the internal ring, dissected, and reflected inferiorly as a flap to directly expose the entire inguinal floor and its bony and ligamentous boundaries after the sac and preperitoneal lipomatous tissue had been reduced. Prolene mesh was used to reinforce the entire myopectineal orifice. Using an endoscopic staple tacker (EMS Hernia Stapler, Ethicon Endosurgery, Cincinnati, Ohio), the mesh was secured to the pubic tubercle medially, along Cooper's ligament inferiorly, to the ileopubic tract, and superiorly along the transverse abdominal arch. Careful attention was taken to avoid damaging cord structures, the inferior epigastric vessels, and the nerves inferior to the ileopubic tract laterally. Occasionally a slit was made in the mesh through which the cord could pass. Before the abdominal incisions were closed, the peritoneum was tacked closed over the mesh. Early in the study, in the presence of a very well-defined defect and a strong inguinal floor, an intraperitoneal onlay technique was used, thus avoiding the preperitoneal dissection.[12] The sac was either left unreduced or divided at the internal ring. The mesh was fixed intraperitoneally using the same peripheral boundaries as those used with the TAPP technique. All LH procedures were performed under general anesthesia.

Open Technique. The technique of open repair was left to each surgeon's preference, which was usually based on the operative findings, type of hernia, and strength of the floor. In performing a "tension" repair, we most commonly employed a modified Shouldice technique using a two-layer monofilament approximation of the transversalis fascia. Occasionally a McVay repair approximating Cooper's ligament to the reflected edge of the inguinal ligament was used. "Tension-free" repairs were performed using a plug and patch[13] or Lichtenstein[14] technique. Type of anesthesia at OH was decided jointly by the patient and the anesthetist.

Patient Assessment and Outcome Evaluation

Patient Assessment. Baseline data included patient demographics, body morphology, occupation, type and duration of hernia, and symptoms. The American Society of Anesthesiologists scoring system[15] was used to categorize the severity of comorbid conditions. Physical activity both at and outside of the workplace was documented, as were the expectations of the patients regarding their anticipated time to complete convalescence.

Operative information included the members of the operating team, length of time in the operating room and duration of anesthesia (for costing purposes), type of anesthesia, type of repair, the presence of a conversion, and surgeon satisfaction at the end of the procedure.

Outcome Evaluation. The principal outcome measure of the study was the duration of convalescence following surgery. Convalescence was defined as complete when a patient could perform all usual home activities if unemployed or usual duties at the workplace if employed. The duration of convalescence was measured and cross-validated by the study assistants according to four end points including the percentage of resolving disability over time (at 1 week and 1 month following the operation), and specific elements of the Nottingham Health Profile Questionnaire (NHPQ).[16]

Both the surgeon and the study assistants gave all patients standardized perioperative instructions; immediately after discharge from the hospital, patients were allowed to perform any activity, provided there was no undue discomfort. Return to activities was thus evaluated according to patient response rather than any preset standard. Secondary outcome evaluations included measures of postoperative pain, duration of hospital stay, quality of life, and morbidity.

Postoperative pain was assessed by administration of the McGill Pain Questionnaire[17] within the first 24 hours following surgery, and by measuring the

amount of narcotics (as morphine dosage equivalents in milligrams) used both during the hospital stay and at home. The presence of ongoing discomfort at the time of 1-week follow-up was also noted.

Quality-of-life measurements were made preoperatively, 1 week, 1 month, and 3 months following surgery. Two different instruments were used: the NHPQ and a Visual Analogue Scale (VAS).

All complications were rigorously noted at each postoperative visit utilizing a hernia repair follow-up sheet devised and previously validated by Fitzgibbons et al.[18] The suspicion of a hernia recurrence was confirmed through direct examination by another surgeon, or by herniography.

All results were analyzed according to the "intention to treat" principle.

Statistical Methods

Continuous descriptive variables are expressed as mean ± standard deviation. For the duration of hospital stay, which has a skewed distribution, the median and ranges are given. Between-group differences for continuous variables were assessed for statistical significance by the use of Student's *t* test and the nonparametric Wilcoxon's rank-sum test where applicable. The chi-square statistic or Fisher's exact test was used for comparison of categorical variables. Changes from baseline values for the quality-of-life measures were evaluated using paired *t* tests, whereas between-group differences with respect to these changes were assessed by nonpaired procedures.

For the time to full convalescence, the Kaplan-Meier method was used to construct life tables and the nonparametric log-rank test was chosen to evaluate the statistical significance of between-group differences. A post hoc multivariate analysis was performed using stepwise regression as well as modeling using Schwarz' criterion, which describes the probability that a given variable is significant for modeling in our data set.

Based on anticipated convalescence in each group, a sample size calculation was performed prior to the start of the study and a target accrual of 75 patients in each group was sought. All calculations were performed using SAS 6.12 for Windows software (SAS Institute Inc., Cary, N.C.).

RESULTS

Sixty-five patients were randomized to the OH group and 58 to the LH group. Recruitment was stopped short of the goal of 75 of 75 (150 patients total) because of difficulties in patient accrual. Two surgeons (J.S.B. and M.J.W.) operated on 76% of the patients. Two patients, randomized to the OH group, elected on the day of operation to undergo LH. One patient undergoing LH required conversion to OH because of bleeding. Table I shows that there was no significant difference between groups in any of the main baseline variables except for the "anticipated time to return to full activities." Prior to surgery and after they knew which group they had been randomized to, OH patients thought that they would take on average 18.5 ± 10.8 days to recover, in contrast to 14.2 ± 8.3 days for LH patients ($P = 0.021$). The 95% confidence interval (CI) on the difference between the two groups was 0.64 to 7.9 days.

The vast majority of LH operations were done using the TAPP approach (82.8%) rather than intraperitoneal onlay mesh (17.2%), as shown in Table II. OH techniques were equally distributed between tension (modified Shouldice, McVay) and tension-free (Lichtenstein, Rutkow-Robbins) repairs. Operating room time was longer in the LH group (88.8 ± 34.0 minutes vs. 73.9 ± 26.1 minutes; $P = 0.008$; 95% CI 4.0 to 25.8 minutes). Most of the OH repairs were done under local anesthesia (60%), whereas all of the

Table I. Baseline variables

	Open (n = 65 patients)	Laparoscopic (n = 58 patients)	
Mean age (yr)	51.1 ± 16.7	49.6 ± 13.4	
Occupation			
Desk	18	19	
Manual	17	19	
Retired	25	25	
Unemployed executive	2	3	
Worker's compensation	10	11	
Anticipated return to full activities (days)	18.5 ± 10.8	14.2 ± 9.4	($P = 0.021$) 95% CI 0.64 – 7.9

P values are two tailed.
95% confidence interval (CI) is the 95% confidence limits of the difference in duration.

Table II. Operative results

	Open (n = 65 patients)	Laparoscopic (n = 58 patients)	
Technique			
IPOM	NA	17.2%	
TAPP	NA	82.8%	
Tension-free	50.8%	NA	
Modified Shouldice, McVay	49.2%	NA	
Operating room time (min)	73.9 ± 26.1	88.8 ± 34	$P = 0.008$ (95% CI 4.0 − 25.8)
Anesthesia time (min)	106.9 ± 34	126.4 ± 37.7	$P = 0.004$ (95% CI 6.4 − 35.5)
Type of anesthesia			
General	40%	100%	
Regional	60%	0%	
Surgeon satisfaction			
Average	4.6%	1.7%	NS
Good	39%	53.4%	NS
Very good	56.3%	44.8%	NS

IPOM = intraperitoneal onlay mesh; TAPP = transabdominal preperitoneal; NS = not significant.
P values are two tailed.
95% CI is the 95% confidence limits of the difference in quantity or duration.

Table III. Postoperative outcome

	Open (n = 65)	Laparoscopic (n = 58)	
Still on narcotics (day 7)	15.6%	10.5%	NS
Narcotics consumption after 7 days (mg equivalent of morphine)	25.2 ± 22.8	15.5 ± 25.8	$P = 0.03$ (95% CI 1.0 − 18.4)
Total narcotics (mg equivalent of morphine)	41.9 ± 33.7	27.8 ± 34.8	$P = 0.02$ (95% CI 1.9 − 26.3)
Convalescence (days)			
All patients (123)	11.6 ± 7.7	9.8 ± 7.4	NS
Patients not on worker's compensation (102)	10.9 ± 7.5	7.8 ± 5.6	$P = 0.02$ (95% CI 0.5 − 5.6)
Recurrence (all patients)	2	1	NS
Quality-of-life			
NHPQ			
Baseline score	5.3 ± 6.9	5.3 ± 6.8	
30 days postoperative	2.3 ± 3.4	1.6 ± 3.9	NS
95% CI on difference	1.3 − 4.1	2.0 − 5.4	
P value	= 0.001	<0.0001	
VAS			
Baseline score	7.9 ± 2.0	7.9 ± 2.1	
30 days postoperative	8.9 ± 1.6	9.1 ± 1.3	NS
95% CI on difference	0.4 − 1.72	0.6 − 1.7	
P value	= 0.002	<0.0001	
Patient satisfaction			
Somewhat or not satisfied	11.7%	7.1%	
Neutral	18.3%	7.1%	
Strong*	51.7%	28.6%	
Very strong	18.3%	57.1%	

NHPQ = Nottingham Health Profile Questionnaire; VAS = Visual Analogue Scale; NS = not significant.
*Likelihood ratio = 2.21 (95% CI 1.4 to 3.4 [$P = 0.001$]). *P* values are two tailed; 95% CI is the 95% confidence limits of the difference in quantity or duration.

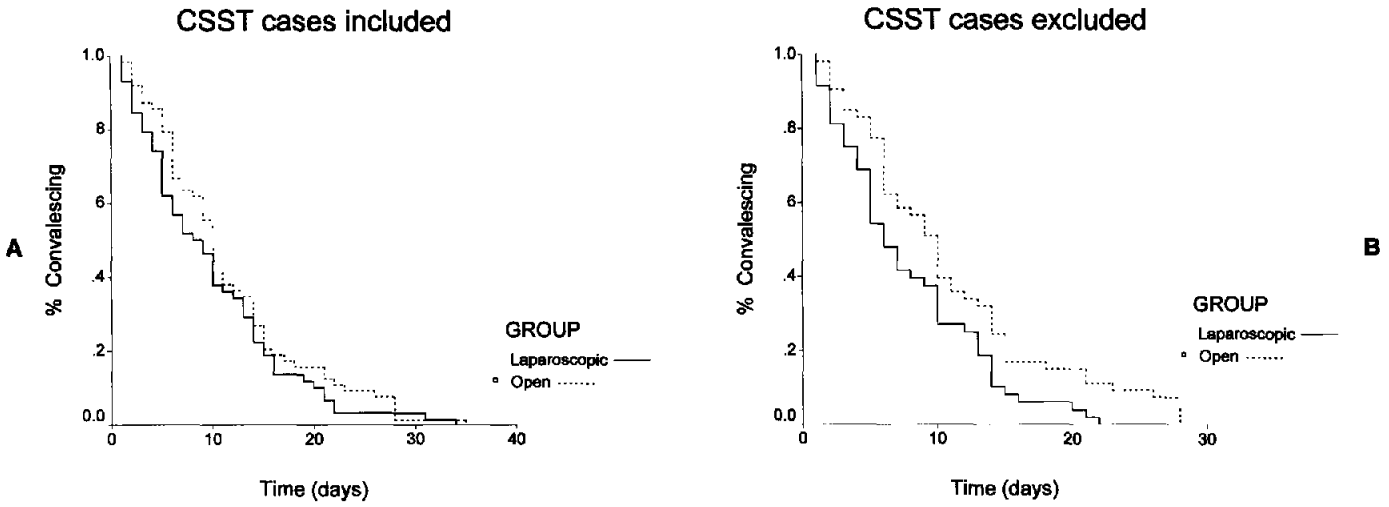

Fig. 1. A, Kaplan-Meier life-table analysis demonstrating the rate of convalescence after surgery across groups. $P = 0.2$ for the slopes of both curves by nonparametric log-rank test statistic. **B,** Kaplan-Meier life-table analysis demonstrating the rate of convalescence after surgery across groups after removal of patients on worker's compensation. $P = 0.02$ for the slopes of both curves by nonparametric log-rank test statistic.

LH repairs were performed under general anesthesia. Surgeon satisfaction with the repair immediately postoperatively was similar across the two groups (see Table II).

Table III summarizes postoperative outcomes. Significantly greater quantities of postoperative analgesics were required by the OH group both over the first 7 postoperative days (25.9 ± 23.5 mg equivalent of morphine vs. 15.9 ± 27.2; $P < 0.005$) and in the total quantity used (41.9 ± 33.7 mg equivalent of morphine vs. 27.8 ± 34.8; $P < 0.02$). At first glance the duration of convalescence did not differ significantly across the two groups (OH 11.6 ± 7.7 days; LH 9.8 ± 7.4 days). When convalescence was analyzed after exclusion of patients receiving worker's compensation (21 patients), a significant difference was found favoring the LH group (OH 10.9 ± 7.5 days; LH 7.8 ± 5.6 days; $P = 0.02$; 95% CI 0.46 to 5.6 days). Over a median follow-up of 4½ years, two patients in the OH group had recurrence vs. one in the LH group.

Fig. 1 shows the Kaplan-Meier survival curves illustrating convalescence time for both OH and LH patients before ($P = 0.2$) and after exclusion of patients receiving worker's compensation ($P = 0.023$). Both groups showed similar improvements in their quality of life as measured by the NHPQ and the VAS at 30 days after operation when compared with baseline values. Nevertheless, a greater percentage of LH patients would be more willing to have the same procedure repeated on the other side (LH 28.6% strongly and 57.2% very strongly; OH 51.7% strongly and 18.3% very strongly; likelihood ratio = 2.21; $P = 0.001$; 95% CI 1.4 to 3.4).

DISCUSSION

Several prospective comparative and randomized controlled trials have been conducted contrasting LH and OH techniques.[1-3,5,6-9,19-22] In most cases the focus has been on outcomes such as pain, return to work, and return to normal activities.[1-9,23] Although the most important question might well be the issue of hernia recurrence, only the study by Liem et al.[5] had the power and length of follow-up to address this issue; the results favored LH over OH. In almost all short-term trials, the LH repairs appear to be associated with patient benefits, particularly with respect to convalescence. Payne et al.[6] objectively measured such improvements in convalescence in a trial where exercise tolerance was found to improve earlier postoperatively following LH repairs. Some of these results appear to be at odds with those of the current report, which updates the final analysis of the McGill herniorrhaphy trial. The following highlights the ways in which it resembles or differs from currently available literature.

Unlike other groups, we were able to detect a significant difference in patient groups with respect to "anticipated return to full activities." This measure, although arbitrary, is important because it highlights a preoperative bias, which may taint any results seeming to favor the LH patients. To minimize this effect,

the surgeons and study nurses purposely reiterated similar instructions to both groups with respect to expected postoperative behavior and "medical" expectations. In spite of this, it is difficult to ascertain the extent to which differential preoperative patient expectations might have determined subjective perioperative outcomes. In the future, better indicators of possible preoperative bias will be needed to validate this finding and quantify it, because it is a recurrent theme in any unblinded study comparing two operative techniques.

As did others, we found that analgesic consumption was significantly increased in the open group, keeping in mind that the OH patients underwent a mix of tension and tension-free repairs. Whereas others have shown a benefit favoring tension-free[8,23] or laparoscopic[1-4] repairs over conventional tension repairs, it is not possible in this trial to measure the effect of each subtype of open repair. This is both because the study did not randomize patients within the OH group itself, and in view of the small numbers of patients undergoing each subtype of OH repair. It should be noted that over the course of this trial, tension-free techniques took over as the type of open repair favored by the participating surgeons.

The primary outcome measure in the trial was the duration of postoperative convalescence. In keeping with our preliminary report,[9] we found no statistical difference in the rates of convalescence between LH and OH groups of patients. In an effort to determine why our results differed from those of others, we hypothesized that certain variables other than the type of surgery may be paramount in determining the duration of convalescence. It has been previously reported that worker's disability compensation status may prolong the duration of convalescence after open inguinal hernia repairs.[24,25] We therefore reanalyzed our data not according to the initial randomization group, but rather according to the disability insurance status of the patient at the time of entry into the trial. There were a total of 21 patients receiving benefits from the state-run worker's compensation program, equally distributed between the two groups (11 OH and 10 LH). On regression analysis, this was found to be a significant predictor of convalescence in 100% (probability = 1.0) of models, exerting a delay in postoperative return to work by 8.2 days ($P < 0.001$) on average. This finding establishes that worker's compensation represents a true confounding variable in this data set, as we did not control for it at the time of randomization. It was, in fact, the strongest variable in any of the models studied, and we found that its ability to predict convalescence was actually much greater than the type of surgery performed. Once patients receiving worker's compensation were removed from the analysis, in order to eliminate this strong confounding variable, the LH group was found to exhibit a significantly shorter convalescence than the OH group ($P = 0.02$; 95% CI 1.9 to 26.3).

This study is one of the few to have measured quality-of-life parameters[26]; we found that both groups improved to a similar degree over the first 30 days after hernia repair, regardless of the operative technique, as measured by the NHPQ and VAS. This observation may appear to be at odds with the further observation that patients in the LH group were more satisfied with their operation (odds ratio 2.21; 95% CI 1.4 to 3.4). One possible explanation for this discrepancy is that short-term postoperative quality of life in hernia patients is related to but not totally determined by the aftereffects of the operation. Our group has already described this finding.[27]

CONCLUSION

In a randomized trial comparing laparoscopic repair with other types of open repair, patients in the LH group required less narcotics after surgery and were significantly more satisfied with their perioperative experience. Although there was no observed difference in convalescence between the two groups, disability insurance was a major confounding variable on post hoc analysis that may have masked a shorter convalescence in the laparoscopic group. Unless controlled for in trial design, this factor may introduce significant bias into outcome assessments of surgical procedures.

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Discussion

Dr. L.W. Traverso (Seattle, Wash.). Aren't you really comparing apples and oranges here? The tension-free laparoscopic repair is being compared to a tension repair in half of your patients undergoing open operations. Did you compare just the open tension-free cases with the tension-free laparoscopic cases for their narcotics use and satisfaction?

Dr. J. Barkun (Montreal, Quebec, Canada). We compared the group undergoing open tension-free repairs to the group undergoing a laparoscopic procedure only with respect to convalescence, although we are now looking at other variables. When this trial was begun in 1992, there were fewer surgeons performing tension-free repairs than there are now, so the design was thought to reflect what was going on at that time, given that it is not a single surgeon who performed all of the operations. In a subgroup analysis, we were not able to show an effect that could be attributed to the surgeon or the type of open repair. Because the sample size calculations were made on the whole trial group, I cannot tell you if there might be significant differences between tension repair and tension-free repair in the open group.

Dr. C. Filipi (Omaha, Neb.). The comprehensiveness of this study is impressive. Also, I would like to commend you for enrolling only patients with unilateral hernias. I am curious about several things. First, what was the incidence of bilaterality in patients with a TAPP repair that required exclusion from the study? Also, was it truly a tension-free repair in the group that had the Lichtenstein repair? That is, was a slight fold or buckle of the mesh present? Perhaps your study cannot address this, but I have a bias that laparoscopic hernia repair is better than open repair in young, very active individuals. I wonder about exercise tolerance, as Dr. Payne has shown with exercise testing that laparoscopic hernia repair is superior to open herniorrhaphy.

Dr. Barkun. There were six patients who ended up having bilateral inguinal hernias in the laparoscopic group. In regard to your second question, there were a few Lichtenstein repairs and those repairs were done in the way that has been reported in the *American Journal of Surgery.* We tend to employ mostly tension-free repairs using the plug and patch technique now. Finally, we do not have results of exercise tolerance tests. We had some interesting findings

that I cannot formally present here because they are still preliminary, but they do relate to convalescence, namely, that the presence of a large symptomatic hernia or a muscular patient build (independent of body mass index) affected recovery time.

Dr. S. Canale (New Orleans, La.). In building on the differences within your group of patients who underwent open repair, you had a group that had general anesthesia and a group that had local or regional anesthesia. Were you able to stratify these patients in terms of their quality of life, their level of pain control, and how much narcotic they needed? Presumably many of these patients may have been given some kind of preemptive analgesia.

Dr. Barkun. We have done that and we were not able to show significant differences. We left the choice of anesthesia type to the patient so as to reflect the reality of everyday practice. The sample size was calculated on a larger group, not for subgroup analyses. The power of the study decreases when you get to the subgroup analyses.

Dr. J. Kolbasnik (Hamilton, Ontario, Canada). What was your incidence of negative laparoscopy during this study? Sometimes, with my staff physicians, we operate on the patient and perform a laparoscopy only to find there is no defect.

Dr. Barkun. Like any surgeon's, our incidence was zero, obviously. In certain cases of inguinal hernia, the laparoscope may be inserted but a hernia may not be seen until the peritoneum is taken down. Then a defect may actually be seen, usually a direct defect. I have had that experience personally, although neither I nor Dr. Wexler, the other major contributor to this report, had that experience during this trial.

Laparoscopic Toupet Fundoplication Is an Inadequate Procedure for Patients With Severe Reflux Disease

Karen D. Horvath, M.D., Blair A. Jobe, M.D., Daniel M. Herron, M.D., Lee L. Swanstrom, M.D.

Recently we have shown that laparoscopic Toupet fundoplication is associated with a high degree of late failure when employed as a primary treatment for gastroesophageal reflux disease (GERD). This study defines preoperative risk factors that predispose patients to failure. Data from 48 patients with objective follow-up performed as part of a prospective long-term outcomes project (24-hour pH monitoring, manometry, and esophagogastroduodenoscopy [EGD] at 6 months, 3 years, and 6 years) was analyzed. Preoperative studies of patients with documented postoperative failure (n = 22), defined as an abnormal 24-hour pH study (DeMeester score >14.9), were compared to preoperative studies of patients with normal 24-hour pH studies (n = 26). Outcomes were assessed at a mean of 22 months (range 18 to 37 months) postoperatively. Of the 22 patients in the failure group, 16 (77%) were symptomatic and the majority (64%) had resumed proton pump inhibitor therapy. Preoperative indices of severe reflux were significantly more prevalent in the failure group including a very low or absent lower esophageal sphincter (LES) pressure on manometry, biopsy-proved Barrett's metaplasia, presence of a stricture, grade III or greater esophagitis, and a DeMeester score greater than 50 with ambulatory 24-hour pH testing. Comparison of pre- and postoperative manometric analysis of the LES revealed adequate augmentation of the LES in both groups and there were no wrap disruptions documented by postoperative EGD or manometry, indicating that reflux was most likely occurring through an intact wrap in the failure group. Esophageal dysmotility was present before surgery in four of the nonrefluxing patients and in three of the failures. Intact wraps were noted to have herniated in eight patients, all of whom had postoperative reflux. Laparoscopic Toupet fundoplication is associated with a high rate of failure both clinically and by objective testing. Surgery is more likely to fail in patients with severe GERD than in patients with uncomplicated or mild disease. A preoperative DeMeester score greater than 50 was 86% sensitive for predicting failure in our patient population. Laparoscopic Toupet fundoplication should not be used as a standard antireflux procedure particularly in patients with severe or complicated reflux disease. (J GASTROINTEST SURG 1999;3:583-591.)

KEY WORDS: Laparoscopic, fundoplication, Toupet, outcomes, partial fundoplication

The Toupet fundoplication and its variants have been advocated as a more physiologic alternative to the traditional Nissen repair for patients with gastroesophageal reflux disease (GERD) and as the treatment of choice for patients with primary esophageal motility disorders. The described benefits of partial fundoplication are mainly the creation of an effective antireflux barrier without the side effects of postoperative dysphagia and gas bloat often seen with a 360-degree fundoplication.[1-6] Interest in these techniques became widespread with the introduction of laparoscopic approaches in 1991. This desire for a low morbidity surgical treatment has led several groups to adopt the laparoscopic Toupet fundoplication for all patients with medically resistant GERD.

In 1997 we reported the results of a prospective series of 100 laparoscopic Toupet fundoplications performed for all patients with documented reflux.[7] Although the procedure was safe and well tolerated, the long-term results were disappointing, with symptomatic failure rates of 20% and abnormal DeMeester scores in 59% at a mean of 22 months. This compares

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unfavorably with a described 9% symptomatic recurrence rate over a 10-year period after open Nissen fundoplication as well as with our own intermediate-term failure rate of 4.5% with the laparoscopic Nissen procedure.[8] We recommended limiting laparoscopic Toupet procedures to patients with severe motility disorders.

In spite of the overall high failure rate of the Toupet fundoplication, there remain patients for whom it is indicated and who do well with it, at least in the short-term. For this study we analyzed data from 48 patients who had objective postoperative tests that confirmed the failure or success of their antireflux surgery. A comparative analysis was carried out to determine preoperative predictors of success or failure and to better elucidate the causes of failure.

MATERIAL AND METHODS

This is a descriptive post hoc analysis of data from a prospectively gathered database containing 598 patients undergoing laparoscopic antireflux procedures (431 Nissen, 156 Toupet, and 11 other) performed between October 1991 and May 1997. Forty-eight patients were identified who had completed a full battery of postoperative objective tests after a laparoscopic Toupet fundoplication and who had a minimum of 1 year follow-up. This represents 32% of Toupet patients in our database. An additional 41% of patients had completed only part of their objective follow-up (mostly 24-hour pH testing) and were not included in this analysis. Also excluded from this analysis were those with paraesophageal hernias and achalasia.

Patients in whom testing was completed were divided into two groups based on their results: those whose procedures failed (n = 22) and those who had a successful outcome after a Toupet fundoplication (n = 26). Surgical failure was defined as an abnormal postoperative DeMeester score (>14.9), with or without symptomatic recurrence. All patients underwent their initial surgery between August 1992 and May 1997 for documented GERD. Indications for using the Toupet fundoplication included the presence of a primary esophageal motility disorder or participation in a prospective trial in which partial fundoplications were performed in all patients with GERD.[7]

Evaluation

Pre- and postoperative data had been prospectively gathered from all patients as part of a long-term outcomes study. The data from all patients were entered into a database that included pre- and postoperative symptom assessment, 24-hour pH monitoring, esophageal manometry, and esophagogastroduodenoscopy (EGD). Symptom assessment forms were completed at the time of the first visit, at 2, 12, and 24 months postoperatively, and every 2 years subsequently. A detailed questionnaire was used that covered all aspects of functional and perceived gastrointestinal symptoms. In this study only the primary and secondary presenting symptoms were analyzed (Table I).

Esophageal motility testing was done preoperatively and at 6 to 12 months and 2 to 3 years postoperatively. A standard four-port, water-perfused system was used (Arndorfer Medical Specialties, Greendale, Wis.) and analyzed with a computer software package

Table I. Patient demographic data: Nonpredictors of failure after laparoscopic Toupet fundoplication

	Success (n = 26)	Failure (n = 22)	
Age (yr)	49 (range 30-72)	51 (range 35-73)	NS (Student's *t* test)
Sex	16 M 10 F	17 M 5 F	NS (Fisher's exact test)
Presenting symptoms (primary and secondary)			
Heartburn	22	22	
Dysphagia	5	8	
Pulmonary	7	3	NS (Fisher's exact test)
Water-Brash	11	7	
Odynophagia	1	0	
Nausea/emesis	1	1	
No. of symptomatic years	10.3 (range 0.5-30)	13.7 (range 1-45)	NS (Student's *t* test)
Presence of hiatal hernia on preoperative EGD	16 (62%)	14 (64%)	NS (Fisher's exact test)
Date of surgery (mean)	5/30/94	10/15/94	NS (Student's *t* test)
Motility disorder	4(7%)	3(7%)	NS (Fisher's exact test)

NS = not significant.

(Synectics-Medtronic, Minneapolis, Minn.). Esophageal body hypomotility was defined as contraction amplitudes less than 30 mm Hg at two or more levels. Hypomotility only at level 5 was presumed to be secondary to acid reflux and was not counted as a motility disorder. The presence of esophageal dysmotility was defined as the presence of greater than 60% tertiary or simultaneous contractions or greater than 60% dropped peristalses.

Twenty-four-hour ambulatory pH testing was done using a single port probe (Synectics-Medtronic) positioned 5 cm above the distal high-pressure zone. Testing was done preoperatively in all patients and postoperatively at 6 to 12 months and 2 to 3 years with the patient off all peptic medication for 5 days.

Upper endoscopy (EGD) was performed in all patients preoperatively. Esophagitis was graded based on the Savary-Miller system.[9] Barrett's esophagus was defined by any length of intestinal metaplastic columnar epithelium on biopsy. Postoperative EGD was performed only as clinically indicated and always included retroflexed views to assess the integrity of the fundoplication and to look for mediastinal herniation.

Radionuclide gastric emptying studies were performed selectively if there were indications that delayed gastric emptying was a contributing factor to the patient's reflux. Preoperative barium esophagrams were also performed selectively as clinically indicated. All postoperative studies were carried out in the esophageal physiology laboratory of the Department of Minimally Invasive Surgery.

Surgical Procedure

All Toupet procedures were based on Jonsell's modification of the original Toupet to create a 270-degree posterior fundoplication.[10-12] Critical elements of the technique include a thorough dissection of the esophageal hiatus, mobilization of the gastroesophageal junction so that at least 3 cm of distal esophagus is below the diaphragm without tension, and routine division of the short gastric vessels. The hiatal defect is closed loosely with one or two posterior crural sutures. Both sides of the fundoplication are sewn to the right and left crura. The wrap is subsequently sewn at the 10-o'clock and 2-o'clock positions of the esophagus around a 56 Fr bougie to create a 270-degree wrap between 3 and 4 cm in length (Fig. 1).

Data Assessment and Statistical Methods

We compared pre- and postoperative studies for the 22 failed Toupet fundoplications and the 26 successful ones to determine if there were preoperative predictors of failure. Univariate statistical analysis was performed using Student's *t* test and Fisher's exact test as appropriate. Multivariate analysis was performed using both linear regression and logistic regression techniques. Both multivariate analyses included the following preoperative variables: number of years of reflux symptoms, presence of Barrett's metaplasia, presence of a stricture, presence of abnormal distal esophageal motility, grade of esophagitis, lower esophageal sphincter (LES) resting pressure of less than 5 mm Hg, and preoperative DeMeester score. In the linear regression the outcome variable was postoperative DeMeester score. In the logistic regression the outcome variable was success, defined by a postoperative DeMeester score less than 14.9, or failure, defined by a postoperative DeMeester score equal to or greater than 14.9.

Presence or absence of the binary predictor variables were assigned values of 1 and 0, respectively. Because of the greater scatter among higher DeMeester scores, the natural log (ln) of the DeMeester score was used to obtain a more normal data distribution. A logistic regression was performed using the backward elimination method. The initial model included eight coefficients: seven coefficients corresponding to each of the variables described above and a constant term. Variables with the least statistical significance were then serially removed until all remaining variables had a T value less than 0.05. The final model included two variables: esophagitis grade and log of the DeMeester score, as well as a constant term. A likelihood ratio test was used to assess the cumulative importance of the excluded variables. This gave a chi-square statistic of 2.06 with five degrees of freedom, corresponding

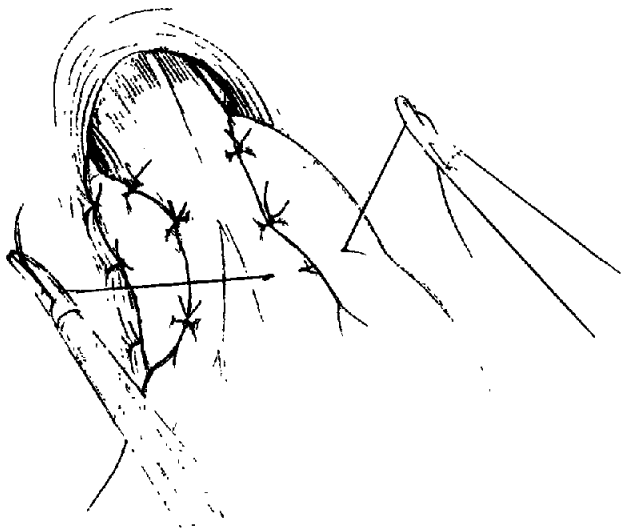

Fig. 1. Laparoscopic modified Toupet fundoplication.

to $P = 0.84$, confirming that these excluded variables were not significant in the logistic model.

In the linear regression model we performed a similar backward elimination. This analysis left three significant variables remaining: grade of esophagitis, LES pressure less than 5 mm Hg, and log of DeMeester score. Significance was defined by $P < 0.05$ for all analyses.

RESULTS

Univariate Analyses

Patients demographic data are summarized in Table I. Mean follow-up was 22 months with a range of 18 to 37 months. Univariate analysis suggested that failure was not associated with age, sex, primary or secondary presenting symptoms, or duration of symptoms prior to surgical evaluation. In addition, the presence of a hiatal hernia did not predict a higher failure rate.

Patients in the failure group had a significantly lower preoperative resting pressure in their LES than those in the success group (Table II). A preoperative LES pressure of less than 5 mm Hg was strongly predictive of failure after Toupet fundoplication; 15 of 22 patients with a severely hypotensive LES had abnormal postoperative 24-hour pH studies (Fig. 2).

Normal esophageal body motility was present in 75% of all patients preoperatively. Of the seven patients with a preoperative esophageal motility disorder, three were in the failure group and four were in the success group; this difference was not statistically significant. Of note, four patients in the failure group had evidence of aperistalsis confined to the distal esophagus, whereas none in the success group did; this difference, however, did not reach statistical significance ($P > 0.05$).

Preoperative endoscopy showed that 9 (41%) of 22 patients in the failure group had biopsy-proved Barrett's changes on preoperative EGD (two with low-grade dysplasia) as compared to only 4 (16%) of 25 in the success group (Fig. 3). Similarly, 45% of the patients who failed after a Toupet fundoplication had a preoperative stricture vs. only 12% of the successful cases (Fig. 4). All four patients who had both a stricture *and* Barrett's changes (Savary grade IV) failed Toupet fundoplication. Of note, more than 90% of patients with preoperative stricture underwent at least one dilatation preceding surgery.

The mean grade of preoperative esophagitis was significantly higher in the failure group. More than 70% of those in whom Toupet fundoplication failed had either grade III or IV esophagitis as compared to only 20% of the group in whom it was successful (Fig. 5).

Results of ambulatory 24-hour pH monitoring demonstrated that patients with higher amounts of esophageal acid exposure were at higher risk for failure after a Toupet fundoplication. As shown in Fig. 6, the preoperative DeMeester score was significantly higher in the patients who failed after a Toupet procedure.

Table II. Pre- and postoperative manometric data

	Success		Failure		
	Preoperative	Postoperative	Preoperative	Postoperative	
Mean LES resting pressure (mm Hg)	10.7[a,c]	13.6[b,c]	2.2[a,d]	11.9[b,d]	[a]$P = 0.006$ [b]NS [c]$P = 0.049$ [d]$P = 0.049$ (Student's t test)
Mean LES total length (cm)	1.3[e,g]	2.8[f,g]	0.3[e,h]	2.0[f,h]	[e]$P = 0.002$ [f]NS [g]NS [h]NS (Student's t test)
Esophageal body					
Normal	21	23	15	13	NS
Panhypomotile	2	1	1	3	NS
Dysmotile	2	1	2	1	NS
Distal amotile segment	0	0	4	4	NS
Not performed because of patient intolerance	1	1	0	1	

NS = not significant.

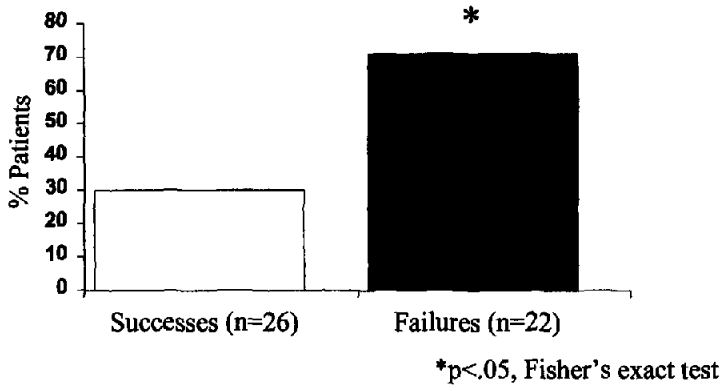

Fig. 2. Percentage of patients with LES pressure <5 mm Hg on preoperative manometry.

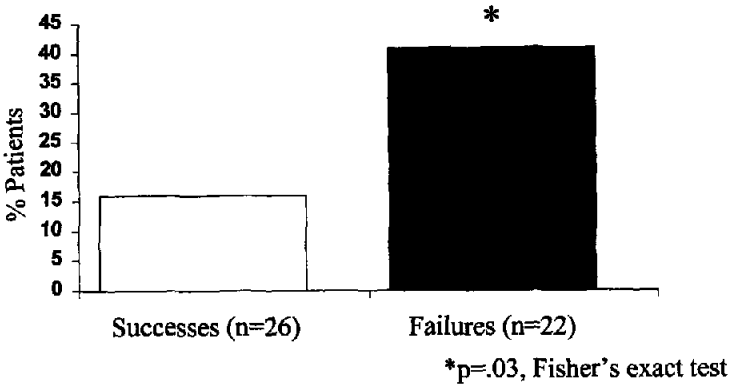

Fig. 3. Percentage of patients with biopsy-proved Barrett's signs on preoperative EGD.

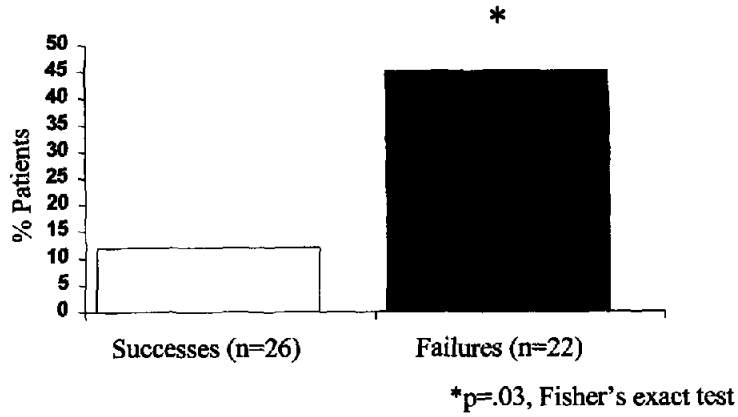

Fig. 4. Percentage of patients with stricture on preoperative EGD.

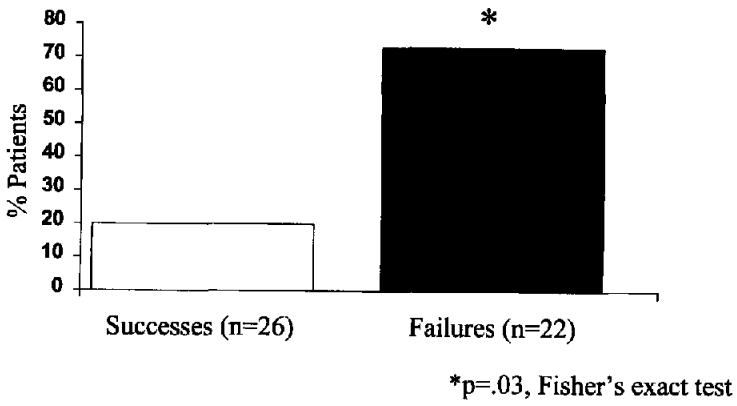

Fig. 5. Percentage of patients with grade III/IV esophagitis on preoperative EGD.

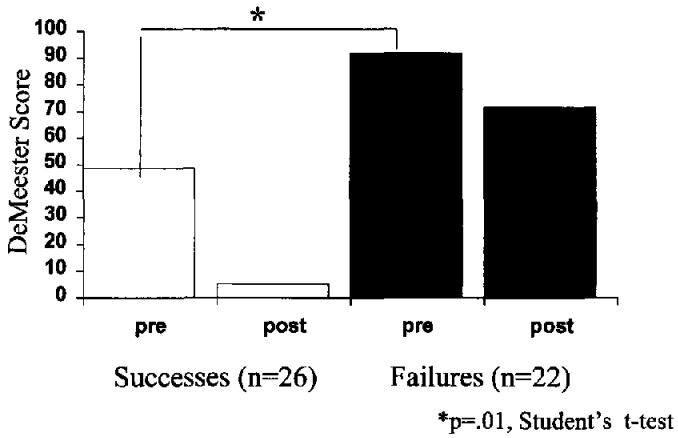

Fig. 6. Pre- and postoperative DeMeester scores.

Multivariate Analyses

In addition to the univariate analyses described earlier, we performed two multivariate analyses: a multivariate linear regression and a multivariate logistic regression. In both analyses we included the following preoperative variables: number of years of reflux symptoms, presence of Barrett's metaplasia, presence of a stricture, presence of abnormal distal esophageal motility, grade of esophagitis, LES resting pressure of less than 5 mm Hg, and preoperative DeMeester score. Of the 48 patients in the study, nine were excluded because of missing data points. In the linear regression the outcome variable was postoperative DeMeester score. In the logistic regression the outcome variable was success, defined by a postoperative DeMeester score of less than 14.9, or failure, defined by a postoperative DeMeester score greater than or equal to 14.9. Of the 39 subjects included in the final analyses, 21 (54%) had successful outcomes. The final model included two variables: esophagitis grade and log of the DeMeester score, as well as a constant term. A likelihood ratio test was used to assess the cumulative importance of the excluded variables. This gave a chi-square statistic of 2.06 with five degrees of freedom, corresponding to $P = 0.84$, confirming that these excluded variables were not significant in the logistic model.

In the linear regression model we performed a similar backward elimination. This analysis left three significant variables remaining: grade of esophagitis, LES pressure less than 5 mm Hg, and log of DeMeester score. The coefficients of these variables were 0.33, 0.84, and 0.39, respectively. Thus for a patient with grade III esophagitis, an LES pressure less than 5 mm Hg, and a preoperative DeMeester score of 20, we would predict the postoperative DeMeester score with the following equation:

$$\text{Natural log of DeMeester score} = 3 \times 0.33 + 1 \times 0.84 + \ln(20) \times 0.39 = 3$$

Table III. Other postoperative characteristics of the 22 patients who failed laparoscopic Toupet fundoplication

Symptomatic	17/22 (77%)
Back on chronic acid-reducing medication	17/22 (77%)
Cause of surgical failure	
Reflux through intact wrap	14/22 (64%)
Wrap herniation*	8/22 (36%)

*Three of these herniations were due to an unrecognized and untreated shortened esophagus at the first operation.

This gives a predicted DeMeester score of 20, suggesting probable failure.

Postoperative Characteristics

Table III lists the postoperative characteristics of the 22 patients in whom laparoscopic Toupet fundoplication failed. All patients had pathologic reflux as demonstrated by an abnormal postoperative DeMeester score; only 77% of them were symptomatic (16 had heartburn and one had dysphagia). The majority (64%) had resumed proton pump inhibitor therapy. Ninety-eight percent of patients had a LES that was appropriately augmented (pressure between 15 and 35 mm Hg; length between 2 and 4 cm) following surgery. Although the postoperative resting pressures and LES length were lower in the failure group, the differences were not statistically significant compared to values in the success group. Postoperative EGD showed an apparently intact wrap on retroflexion in 94% (16 of 17) of the patients examined, but a total of eight patients (17%) were determined to have varying degrees of mediastinal herniation postoperatively. All were documented with barium esophagrams, which showed four partial wrap herniations, two total mediastinal herniations, and two posterior herniations of the gastric fundus. There were no known wrap herniations in the success group.

Seven patients (32%) have undergone revision surgery. Two patients have had successful laparoscopic reoperations for reflux (one symptomatic and the other not) converting their Toupet to a 360-degree fundoplication. Both Toupet fundoplications were believed to be intact and adequate at the time of surgical revision. Another five patients had symptomatic reflux and wrap herniation. One patient with steroid-dependent chronic obstructive pulmonary disease and a long bout of postoperative emesis, had herniation of her wrap 2 weeks postoperatively and was returned to the operating room for a reinforced repair. A second patient underwent successful repeat fundoplication with a Nissen procedure for symptomatic reflux and a small paraesophageal gastric herniation. Finally, three patients had symptomatic herniation possibly as a result of failure to recognize and treat a shortened esophagus at the first operation. Two of these required a Collis gastroplasty along with the conversion to a Nissen fundoplication, and the third patient later had strangulation and perforation of the redone repair, requiring esophageal exclusion and subsequent replacement. Mean time to reoperation was 19 months (range 1 to 36 months).

DISCUSSION

The Nissen fundoplication is the current "gold standard" antireflux procedure. It has merited this distinction because of its proven effectiveness and durability after more than 10 years of follow-up.[8] It is generally accepted that partial fundoplications are indicated for patients with severe motility disorders. Advocates of the Toupet and other partial fundoplications have reported results comparable to those achieved with the Nissen procedure with fewer postoperative symptoms resulting from the hyperaugmentation of the LES, namely, dysphagia, gas bloat, hyperflatulence, and inability to vomit or belch.[1-6] This has led several groups to recommend the use of partial wraps in all patients with GERD. However, many of the outcome studies used to support this reported on only a small number of patients or relied on symptomatic assessment over short periods of time. In addition, at least one older study has shown a higher failure rate with the Toupet-type repair.[13] There is, and should be, concern about the long-term viability of this less competent repair when it is used as a routine antireflux technique for all patients with GERD. In fact, in a prospective series of 100 consecutive patients undergoing laparoscopic Toupet fundoplications, we reported an increasing failure in reflux control over time, with a symptomatic recurrence rate of 20% within 22 months of follow-up and objective evidence of recurrence approaching 60%.[7] With even longer follow-up there will undoubtedly be even more failures. These results prompted the current study, which is an effort to better understand the reasons for failure of Toupet fundoplication and to derive preoperative indices to predict postoperative results.

We carefully analyzed the subjective and objective outcomes data in two groups of Toupet patients. Failure (22 patients) and success (26 patients) was defined by postoperative 24-hour pH testing at a mean of 22 months. Table I shows that age, sex, primary and secondary presenting symptoms, presence of a hiatal hernia, and esophageal dysmotility were not predictive of failure following a Toupet procedure. Surprisingly,

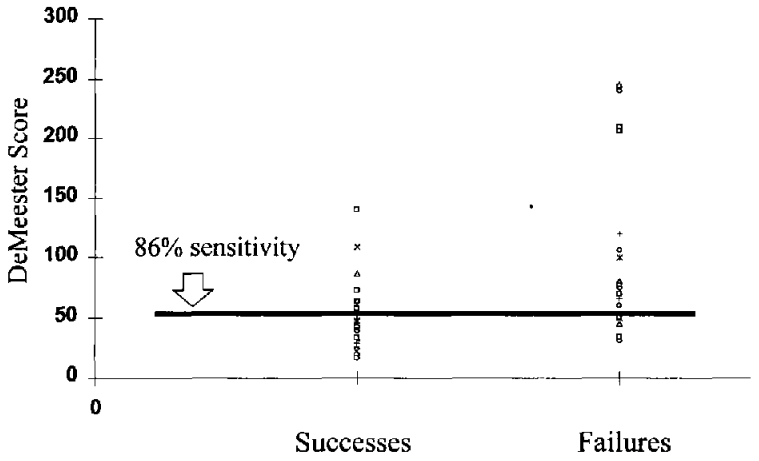

Fig. 7. Preoperative DeMeester scores for the Toupet success group and failure group. A DeMeester score greater than 50 is 86% sensitive for predicting failure after Toupet fundoplication.

the number of years of GERD symptoms before surgery also did not predict failure after Toupet fundoplication. These results confirm the known unreliability of symptoms as a correlate to all aspects of GERD including the presence of the disease, the severity of the pathologic condition, pathologic acid reflux on 24-hour pH testing, and the prediction of failure after fundoplication surgery.[7,14-16]

The most accurate predictors of failure were the measures of disease severity. It is generally accepted that patients with a severely hypotensive LES have more severe reflux disease. Our manometric analysis showed that patients with a LES pressure less than 5 mm Hg on preoperative manometry had a significantly higher risk of failure after a Toupet fundoplication than patients with a hypotensive, short, or normal LES (see Fig. 2). Severe reflux disease over a prolonged period of time is known to compromise distal esophageal body peristalsis.[16] The distal amotile segment can then lead to even greater esophageal acid exposure because of resulting poor esophageal clearance mechanisms. In fact, we found that all four patients with a distal amotile esophageal segment failed Toupet fundoplication (see Table III). Furthermore, all four of these patients had grade IV esophagitis with Barrett's metaplasia (1 with dysplasia) and two had concomitant strictures. In contradistinction, patients with primary esophageal dysmotility did not have more failures after Toupet fundoplication.

Results of preoperative EGD and 24-hour pH testing turned out to be the most important predictors of failure after laparoscopic Toupet fundoplication. As shown in Figs. 3, 4, and 5, presence of biopsy-proved Barrett's metaplasia (with or without dysplasia), strictures, and grade III esophagitis were each highly predictive of failure. High preoperative DeMeester scores were also highly predictive of failure (Fig. 6). As shown in Fig. 7, a preoperative DeMeester score greater than 50 can be used as a preoperative indicator of high risk for early failure after a laparoscopic Toupet fundoplication with a sensitivity of 86% (specificity 61%). With the inevitable increase in failure rates at longer follow-up, the DeMeester score cutoff for success may continue to fall. Close follow-up in patients with DeMeester scores nearing 50 is certainly important.

We also analyzed the impact of surgical technique. Since all procedures were performed by or under the direct supervision of the principal investigator, stratification by surgeon was not necessary. This surgeon had also performed more than 60 laparoscopic Toupet fundoplications before this group of patients and there was no difference in the dates of surgery in the failure group as compared to the success group, indicating that inexperience was not a factor in the failures (see Table I). Another indicator that the failures were not due to improper surgical technique is the demonstration of significant LES augmentation at follow-up motility testing. As shown in Table II, the LES resting pressure and total length were significantly greater after surgery in both groups when compared to their mean preoperative values and did not differ significantly between the two groups. These results compare favorably to others reported in the literature.[2,17] Finally, the presence of an intact wrap was confirmed on postoperative EGD in 16 of 17 in the failure group patients. These data suggest that in the majority of patients in the failure group, the reflux is occurring through an intact wrap.

We found that 32% of the 22 patients who failed after a Toupet fundoplication had herniation of their wraps into the mediastinum. It is our impression that

Table IV. Independent risk factors for surgical failure after laparoscopic Toupet fundoplication

- LES pressure <5 mm Hg on preoperative manometry
- Distal esophageal aperistaltic segment
- Biopsy-proved Barrett's metaplasia
- Presence of a stricture
- Grade III or IV esophagitis on endoscopy
- Preoperative DeMeester score >50

this represents a higher risk of mediastinal herniation than does the Nissen procedure, although this report did not examine a consecutive cohort of patients to establish the relative incidence of this finding. Wrap herniation, even if the wrap is intact, probably contributes to the failure of the fundoplication to prevent reflux. It is possible that the looser hiatal closure of the Toupet technique may predispose to cephalad migration of the wrap. Three patients in this group had a shortened esophagus, which was not recognized and treated at the first operation and which obviously predisposed them to subsequent herniation.

CONCLUSION

The laparoscopic Toupet fundoplication appears to provide a weaker antireflux barrier than the Nissen repair and is probably an insufficient procedure for patients with severe GERD. It may also predispose patients to postoperative mediastinal wrap herniation. Independent preoperative predictors of failure after Toupet fundoplication are summarized in Table IV. A highly sensitive predictor is a preoperative DeMeester score greater than 50. Although patients with less severe reflux appear to be effectively controlled with a Toupet fundoplication, it is still too early to evaluate follow-up data and definitive conclusions about the long-term fate of these patients cannot be made.

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Expression and Function of Inducible Nitric Oxide Synthase During Rat Colon Anastomotic Healing

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Nitric oxide plays a significant but incompletely understood role in fibroblast function and cutaneous wound collagen synthesis; however, the participation of inducible nitric oxide synthase (iNOS) in gastrointestinal anastomotic healing has not been studied. Male Sprague-Dawley rats underwent single-layer left colonic anastomosis. Animals were killed at 24-hour intervals postoperatively and the anastomosis was excised. Parallel uninjured colon tissue samples were also analyzed. Reverse transcriptase–polymerase chain reaction confirmed the absence of iNOS messenger RNA in control colon and expression of the gene in anastomotic tissue on all study days. Northern hybridization demonstrated maximal iNOS messenger RNA transcription on day 1 with decreased levels on days 3 and 5. iNOS enzyme activity, measured biochemically by the conversion of [^{3}H]-arginine to [^{3}H]-citrulline ex vivo, was also maximal on day 1 (7.35 ± 1.34 pmol/mg protein/min [± standard error of the mean], n = 10) and decreased on days 3 (4.37 ± 2.32 pmol/mg protein/min; n = 6) and 5 (2.80 ± 0.92 pmol/mg protein/min; n = 6). Immunohistochemical staining demonstrated that (1) iNOS expression is confined to a discrete cell population in the region of the anastomosis containing inflammatory cells; (2) those cells assume a highly conserved position on the luminal edge of the proliferating scar; and (3) the iNOS-expressing cells are present throughout the fibroplastic phase of healing. To functionally assess the role of iNOS in colonic healing, rats were treated with a continuous intravenous infusion of S-methylisothiourea (a selective inhibitor of iNOS) at a dosage of 200 mg/kg/day for 5 days after anastomosis. There was a significantly reduced anastomotic bursting pressure in rats treated with the inhibitor as compared to rats treated with intravenous normal saline solution (108.4 ± 13.2 mm Hg vs. 148.4 ± 10.3 mm Hg; $P < 0.05$). These results suggest that iNOS gene expression is induced during colonic anastomotic healing, that it is present through all phases of healing but is maximal through the inflammatory phase, and that iNOS activity is required for optimal anastomotic healing. (J GASTROINTEST SURG 1999; 3:592-601.)

KEY WORDS: Nitric oxide, colon anastomosis, wound healing

Colon anastomotic dehiscence continues to be a highly morbid and potentially lethal complication of bowel surgery. Historically there has been a great deal of study to delineate the most appropriate technique of colonic anastomosis. Debates over one- vs. two-layer techniques, stapled vs. hand-sewn anastomoses, and use of permanent vs. absorbable suture material continue. Fortunately, with adherence to good surgical principles, excellent results are often achieved regardless of the technique employed. However, very little is currently known about the cellular and subcellular interactions involved in anastomotic healing.

Recently there has been a growing body of evidence suggesting that the generation of nitric oxide (NO) from L-arginine may play a significant role in regulating fibroblast activity in the cutaneous wound. NO activity has been implicated as a regulator of collagen synthesis. Inhibition of the arginine-NO pathway significantly reduces cutaneous wound healing in mice.[1] Recently iNOS induction by the wound environment has been shown to influence collagen synthesis in both dermal and wound-derived fibroblasts.[2] Mice lacking the iNOS gene demonstrate delayed closure of open cutaneous wounds when compared to

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wild-type control mice; adenoviral transfer of the iNOS gene to the knockout animals restores healing time to control levels.[3] Transfer of the iNOS gene by injection of naked cDNA into subcutaneously implanted sponges enhances collagen deposition.[4] These studies suggest that iNOS expression and subsequent NO generation play an important role in healing wounds.

The aim of the present study was to investigate whether iNOS activity is induced following colonic injury and the level and site of its expression. We hypothesized that the temporal expression and activity of the iNOS gene in healing colonic tissue would parallel the period of maximal inflammation and cellular infiltration. We also sought to assess whether in vivo iNOS inhibition affects normal colonic anastomotic healing.

MATERIAL AND METHODS

Colonic Anastomosis

Male Sprague-Dawley rats (270 to 320 grams) were anesthetized by intraperitoneal injection of 50 mg/kg sodium pentobarbital, and their abdomens were shaved and painted with betadine. A small midline incision was made through all layers of the abdominal wall and the left colon was exposed. Approximately 1.5 cm proximal to the pelvic reflection, a small rent in the mesentery was made in the space between the marginal artery and the colon with care taken not to injure the marginal artery. The colon was then divided above the rent. If there were copious amounts of hard stool at that site, the stool was milked from both the proximal and distal loops, and delivered from the field. A single-layer inverted anastomosis was fashioned with interrupted 6-0 polypropylene sutures after which the colon was returned to its anatomic position. The abdominal wall incision was closed with a two-layer running 3-0 silk suture. Each animal undergoing colonic division and anastomosis was resuscitated with 25 ml/kg normal saline solution via subcutaneous injection.

At 24-hour intervals postoperatively, the rats were killed by intraperitoneal sodium pentobarbital overdose. The colonic anastomosis was cleared of overlying adherent tissue and excised with 0.5 cm of colonic tissue on either side of the anastomosis and opened longitudinally. The tissue was washed three times with ice cold saline solution, cut into longitudinal strips, and processed as described below.

Isolation of mRNA

Fifty to 100 mg strips were homogenized in approximately 1 ml of Trizol reagent (Sigma Chemical, St. Louis, Mo.). The samples were then stored in Trizol at −70° C until processing. All of the remaining steps were performed on ice at 4° C. The cell debris and insoluble material were removed by centrifugation of the sample at 12,000g for 10 minutes, and the RNA extracted from the supernate was washed twice in 75% ethanol. When ready for reverse transcriptase–polymerase chain reaction (RT-PCR) or Northern blot analysis, the pellets were dried by evaporation of the ethanol and resuspended in diethyl pyrocarbonate (DEPEC)–treated water, and RNA quantification, and purity was measured by spectrophotometry at A260 and A280.

RT-PCR for iNOS

RT-PCR was performed using the commercially available GeneAmpEZ rTth RNA PCR kit (Perkin-Elmer, Branchburg, N.J.). Briefly, 1 μg of mRNA was mixed with Bicine buffer, dNTPs, $Mn(oAc)_2$ solution, rTth DNA polymerase, and primers in a 50 μl volume (in manufacturer-recommended concentrations). The amplification was carried out with a GeneAmp PCR System 2400 (Perkin-Elmer) using preprogrammed sequences. The PCR products were electrophoresed on a 1.2% agarose gel containing ethidium bromide. The bands were documented on an ultraviolet lightbox and analyzed using Eagle Eye II software (Stratagene, La Jolla, Calif.).

The following primers were used:

iNOS 5'—TTG GGT CTT GTT AGC CTA GTC
iNOS 3'—TGT GCA GTC CCA GTG AGG AAC
GAPDH 5'—GTG GAG TCT ACT GGC GTC TTC
GAPDH 3'—CAT GCC AGT GAG CTT CCC GTT

Northern Blot Analysis of iNOS mRNA

Equal amounts of total RNA were electrophoresed on a 1.0% agarose gel (Ultra Pure, Gibco BRL, Gaithersburg, Md.) and transferred onto a nylon membrane (Hybond C, Amersham, Princeton, N.J.). The RNA was fixed by incubation at 90° C for 1.5 hours. The blots were prehybridized overnight in buffer containing formamide (50% volume/volume), 5% Denhardt's solution 5× SSPE buffer (Gibco BRL, 1% sodium dodecyl sulfate (SDS), and 100 μg/ml denatured salmon sperm DNA in water, followed by hybridization in identical buffer containing iNOS probe labeled with ^{32}P-d(CTP)(NEN Life Science Products, Boston, Mass.) (iNOS probe was a

generous gift of Dr. David Geller, University of Pittsburgh) instead of salmon sperm DNA. The blots were washed in 2% SDS/2% SSPE solution four times for 10 minutes each at 50° C, followed by six washes in 2% SSPE/0.2% SDS solution (four times at 50° C and twice at room temperature). Autoradiography was performed at −70° C.

^{3}H-Arginine to ^{3}H-Citrulline Conversion Ex Vivo—A Measure of iNOS Activity

[^{3}H]-arginine to [^{3}H]-citrulline conversion in anastomotic tissue was measured using a method adapted from Knowles et al.[5] Fresh colon samples were weighed accurately (wet weight) and homogenized in ice cold 40 mmol/L HEPES buffer containing 32 mmol/L sucrose, 1 mmol/L dithiothreitol (DTT), 2 μg/ml aprotinin, 10 μg/ml leupeptin, and 10 μg/ml soybean trypsin inhibitor. Samples were centrifuged at 100,000*g* for 30 minutes at 4° C and the supernates were saved. Sixty microliters of supernate was mixed with 150 μl of 50 mmol/L phosphate buffer (pH 7.4) containing 6 mmol/L L-valine, 100 μmol/L NADPH, 1 mmol/L MgC_{12}, 200 μmol/L CaC_{12}, 20 μmol/L L-arginine, and 2.5 μCi/ml L-[2,3-^{3}H]-arginine (NEN DuPont) and incubated at 37° C for 10 minutes. The reaction was terminated by adding 3 ml of ice cold phosphate-buffered saline containing 1% bovine serum albumin. [^{3}H]-citrulline was separated by passing the sample over a Dowex AG-50 column (Na^+ form, Bio-Rad Laboratories, Hercules, Calif.) and quantified in a beta counter (liquid scintillation analyzer 1600-TR, Packard Instrument Co., Downers Grove, Ill.). Parallel incubations in the presence of 1.0 mmol/L EGTA and 1.0 mmol/L L-N-G–monomethyl arginine (NMMA) were performed. All assays were performed in triplicate. Total protein in homogenate was measured using the Bio-Rad protein assay kit (Bio-Rad Laboratories).

The amount of newly synthesized citrulline was calculated from the percentage conversion of [^{3}H]-arginine to [^{3}H]-citrulline per sample. iNOS activity was calculated as the difference between the EGTA-inhibited activity and the NMMA-inhibited activity. Enzyme activity was reported as formation of picomoles of citrulline per milligram of protein (homogenate) per minute.

Immunohistochemical Staining

Immediately after washing, cut strips of anastomosis were placed in 10% formalin, refrigerated overnight, and subsequently embedded in paraffin. Paraffin-embedded blocks of tissue were sectioned at 4 μm, mounted on microscope slides, and deparaffinized by xylene and graded alcohol series. Antigen enhancement was achieved by boiling the slides for 30 minutes in sodium citrate buffer (10 mmol/L, pH 6 in water). Slides were then washed in distilled water, and endogenous peroxidase activity was quenched by incubating slides in methanol containing 0.3% hydrogen peroxide for 15 minutes at room temperature. Nonspecific binding was blocked by incubating sections with 2% horse serum in phosphate-buffered saline. Primary antibody against either iNOS (Transduction Laboratories, San Diego, Calif.; 1:400 dilution) or endothelial NOS (Transduction Laboratories; 1:3500 dilution) was diluted in blocking buffer, laid over the tissue sections, and incubated in a moist chamber at 4° C overnight. Slides were washed three times in phosphate-buffered saline and incubated with secondary antibody (biotinylated horse antimouse, Sigma Chemical; 1:400 dilution) for 1 hour at room temperature. DAB staining was achieved by the Vectastain Elite ABC kit (Vector Laboratories, Burlingame, Calif.). Negative control samples were obtained by incubating slides without primary antibody.

In Vivo Inhibition of iNOS

To assess the effect of iNOS inhibition on anastomotic healing, rats were subjected to continuous intravenous infusion of either normal saline alone (n = 11) or S-methylisothiourea (MITU) dissolved in normal saline solution at a dosage of 200 mg/kg/day (n = 9) via subcutaneously implanted miniosmotic pumps (Alzet, Palo Alto, Calif.), which were placed in subcutaneous pockets dorsally in the intrascapular region. A Silastic catheter (Dow Corning, Midland, Mich.) attached to the pump was tunneled over the right shoulder and placed in a central venous position via jugular venous access. Additionally, two polyvinyl alcohol sponges were placed subcutaneously in a more caudal dorsal position to obtain wound fluid. Animals subsequently underwent laparotomy, colonic division, and anastomosis (as above) during the same anesthesia. Postoperatively animals were allowed food and water ad libitum.

Anastomotic Bursting Pressure

On postoperative day 5, animals were killed by sodium pentobarbital overdose. The divided segment was isolated and ligatures of 3-0 silk were place 1 cm proximal and distal to the anastomosis. A 16-gauge intravenous catheter was inserted into the lumen of isolated bowel. The segments were then infused with normal saline solution using a Medfusion intravenous infusion pump (Medex Inc., Duluth, Ga.) at a rate of 3.5 ml/min; continuous pressure monitoring was accomplished by connection of the pump and anastomotic segment to a pressure transducer (American

Edwards Laboratories, Irvine, Calif.) via a three-way stopcock. Bursting pressure was measured as peak pressure attained prior to anastomotic disruption.

Plasma and Wound Fluid Total Nitrite and Nitrate Concentrations

The effect of iNOS inhibition by MITU was assessed by measuring stable end products of NO metabolism in plasma and wound fluid. At sacrifice, blood was drawn by intracardiac puncture and centrifuged to extract the plasma fraction. The subcutaneously inserted polyvinyl alcohol sponges were removed and squeezed into 2 ml tubes to collect accumulated wound fluid. The wound fluid was centrifuged to remove cellular debris. Plasma and wound fluid were stored at −70° C until processing. Plasma and wound fluid samples were filtered in 10,000 molecular weight (MW) cutoff Ultrafree-MC (Millipore Corp., Bedford, Mass.) filter units to remove protein and total nitrite and nitrate concentration. Stable end products of NO synthesis (NOx) of each sample were determined using a commercially available colorimetric assay kit based on a nitrate reductase reaction (Oxford Biomedical Research, Oxford, Miss.).

To determine whether the decreased anastomotic bursting pressure was caused by decreased food intake exhibited by MITU-infused animals, a second experiment was performed. Fourteen rats underwent colonic division and anastomosis with implantation of miniosmotic pumps primed to infuse normal saline solution. One group of seven rats was offered food and water ad libitum over the course of the postoperative period. The other group of seven rats was pair matched and fed daily the amount of chow consumed by the MITU-infused rats in the previous experiment.

Statistical Analysis

Mean and standard error of the mean (SEM) were analyzed for the groups in the iNOS enzyme assay. Significant differences between groups were determined using Student's *t* test.

All animal experiments were performed in compliance with the guidelines set forth by the National Institutes of Health for the care and use of laboratory animals under protocols approved by the Sinai Hospital of Baltimore Institutional Animal Care and Use Committee.

Strict adherence to guidelines outlined by the National Institutes of Health and the Sinai Hospital of Baltimore Radiation Safety Committee was observed in the receipt, storage, handling, and disposal of radioactive material.

RESULTS

Quantification and Localization of iNOS in the Anastomosis

RT-PCR demonstrated the presence of iNOS in anastomotic tissue on all study days. There was no iNOS expression in uninjured colon (Fig. 1). Northern blot analysis of mRNA from control (unoperated) colon and anastomotic tissue harvested on postoperative days 1, 3, and 5 showed maximal iNOS expression on postoperative day 1 with continued although somewhat reduced iNOS expression on subsequent days (Fig. 2). iNOS enzyme activity as measured by the conversion of [^{3}H]-arginine to [^{3}H]-citrulline paralleled gene transcription demonstrating peak iNOS enzyme activity on postoperative day 1 (7.35 ± 1.34 pmol/mg protein/min (± SEM; n = 10) with activity falling sequentially on postoperative days 3 (4.37 ± 2.32 pmol/mg protein/min; n = 6) and 5 (2.80 ± 0.92 pmol/mg protein/min; n = 6) (Fig. 3).

Immunohistochemical staining revealed the presence of iNOS-expressing cells in anastomotic tissues on all studied days (Fig. 4). iNOS expression in the early anastomosis appeared to be confined to a discrete population of cells in the region of the anastomosis that also contains inflammatory cells. These cells assume a highly conserved position on the luminal edge of the proliferating scar and are present through postoperative day 10. iNOS-positive cells were identified in healing anastomotic tissue as late as 28 days (slides not shown). High-power microscopic examination of the iNOS-positive cells revealed them to be large, mononuclear cells. Endothelial NOS-positive cells were identified (in serial sections) in established vascular endothelium and in the neovasculature of the proliferating scar, but not at the same site as the iNOS-positive cells (see Fig. 4).

Effect of MITU Infusion on Anastomotic Healing

Twenty rats underwent colonic division and anastomosis with implantation of a miniosmotic pump and polyvinyl alcohol sponges. Nine rats received MITU infusion, whereas 11 were infused with normal saline solution. A total of three animals were excluded: one animal in each group had perianastomotic abscess formation and one other animal from the control group had a bowel obstruction at the anastomotic site secondary to surgical technique. Animals infused with MITU demonstrated a significantly greater postoperative weight loss as compared to the normal saline-infused control animals (Fig. 5). This weight loss was accompanied by decreased food intake (not shown) and a greater incidence of postoperative diarrhea (subjective observation).

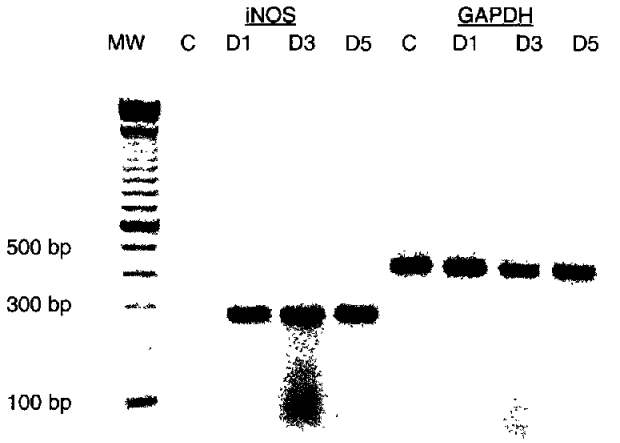

Fig. 1. RT-PCR of anastomotic mRNA using iNOS primers on postoperative days 1 *(D1)*, 3 *(D3)*, 5 *(D5)*, and unoperated colon tissue. GAPDH primers were used as controls.

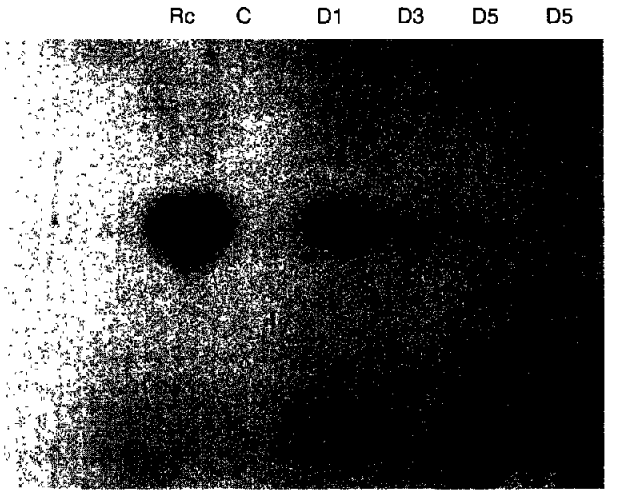

Fig. 2. Representative blot of Northern analysis of mRNA from unoperated colon *(C)* and anastomotic tissue on postoperative days 1 *(D1)*, 3 *(D3)*, and 5 *(D5)*; cultured mouse macrophages stimulated with lipopolysaccharide to produce iNOS was used as controls *(Rc)*.

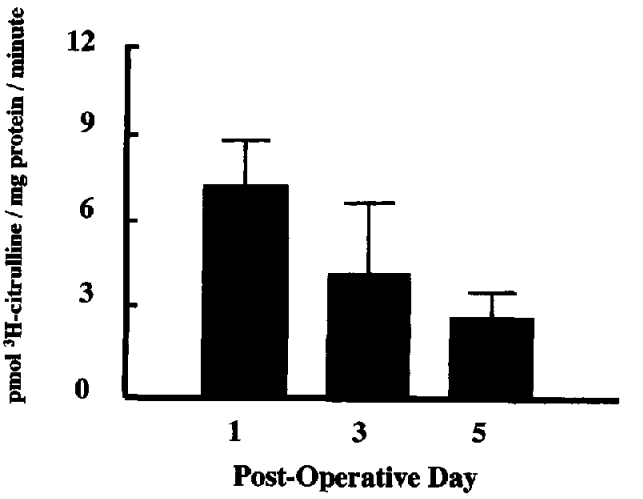

Fig. 3. Conversion of ^{3}H-arginine to ^{3}H-citrulline as a measure of iNOS protein activity. Results are expressed as pmol ^{3}H-citrulline/mg protein/min (represents mean ± SEM of 6 to 10 anastomoses per study day).

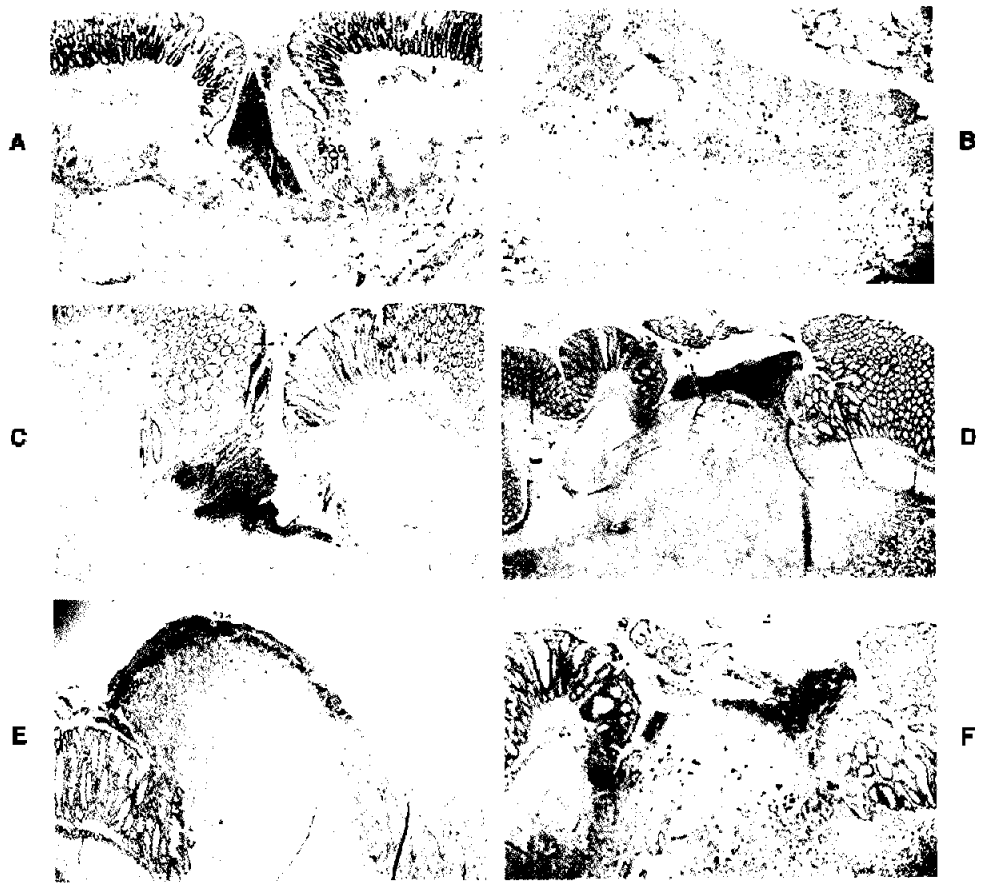

Fig. 4. A, Twenty-four-hour-old anastomosis (hematoxylin and eosin stain; ×40). Immunohistochemical staining against iNOS using a DAB counterstain. iNOS-positive cells (in brown) are initially scattered among the inflammatory cells of the anastomosis at 24 hours (B, ×100). iNOS-positive cells then align along the luminal surface of the proliferating scar by postoperative day 3 (C, ×40) and retain this position through postoperative days 5 (D, ×25) and 10 (E, ×40). F, Five-day-old anastomosis stained for eNOS *(white arrow)* (×40). All immunohistochemical sections were counterstained with hematoxylin.

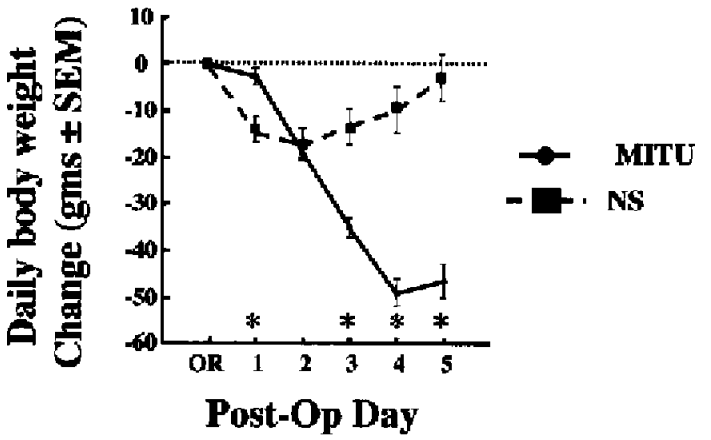

Fig. 5. Postoperative weight changes of MITU (n = 8)– and normal saline (*NS*; n = 9)–infused animals. * = $P < 0.05$; dotted line represents preoperative baseline weight.

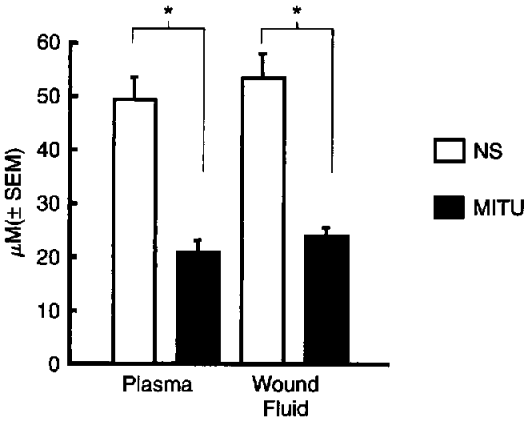

Fig. 6. Total plasma and wound fluid nitrite and nitrate concentrations in MITU (n = 8)– and normal saline (NS; n = 9)–infused animals. * = $P < 0.0001$.

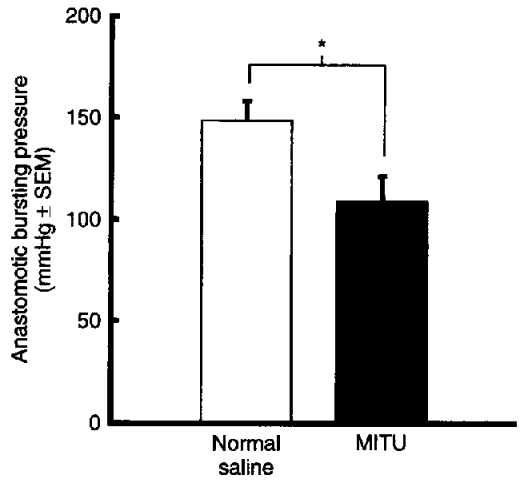

Fig. 7. Bursting pressure of 5-day-old anastomoses from MITU (n = 7)– and normal saline (NS; n = 9)–infused animals. * = $P < 0.05$.

MITU infusion resulted in a significant decrease in both plasma and wound fluid total nitrite and nitrate concentrations. There was a 58% reduction in plasma NOx (20.7 ± 2.2 μmol/L vs. 49.3 ± 4.2 μmol/L for normal saline controls; $P < 0.0001$) and a 55% reduction in wound fluid NOx (23.9 ± 1.6 μmol/L vs. 53.6 ± 4.4 μmol/L for controls; $P < 0.0001$) (Fig. 6). Additionally, MITU-infused animals demonstrated a 27% reduction in anastomotic bursting pressure (108.4 ± 13.2 mm Hg vs. 148.4 ± 10.3 mm Hg for controls; $P < 0.03$) (Fig. 7).

Pair-fed animals also demonstrated a significant increase in postoperative weight loss compared to control rats. (Fig. 8). There were no differences in either plasma (18.6 ± 2.3 μmol/L for pair-fed vs. 20.0 ± 1.9 μmol/L for control fed; $P = 0.63$) or wound fluid (17.6 ± 1.6 μmol/L for pair-fed vs. 18.2 ± 2.3 μmol/L for control fed; $P = 0.85$) NOx concentrations, although total concentrations measured in both groups were lower than those noted in the previous experiment. There were no differences between the bursting pressures of the two groups (146.5 ± 15.2 mm Hg for pair-fed (n = 4) vs. 153.0 ± 10.4 mm Hg for control fed (n = 7); $P = 0.72$ animals).

DISCUSSION

It is generally accepted that the phases of gastrointestinal anastomotic healing parallel those seen in cutaneous healing.[6] There is an inflammatory phase initiated by injury causing exposure of basement membrane ligands to platelets resulting in adhesion and degranulation. It is classically characterized by the gradual evolution of inflammatory cell populations in the wound environment from polymorphonuclear cells to lymphocytes and macrophages. This is then followed by a fibroplastic phase as seen by the influx

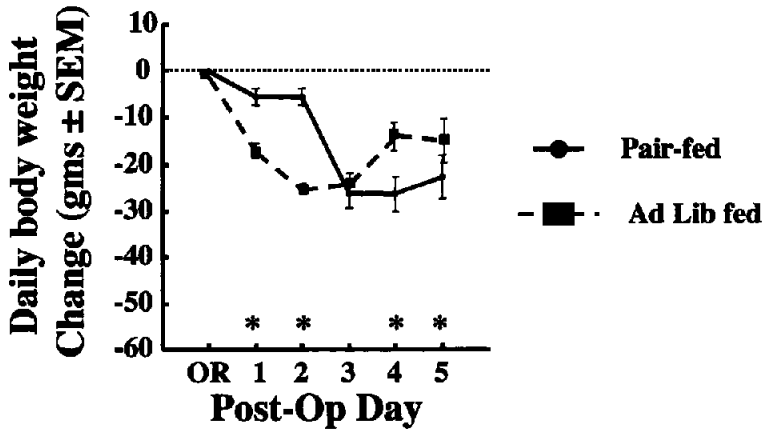

Fig. 8. Postoperative weight changes of normal saline-infused animals that were fed either ad libitum (n = 7) or in a pair-fed (n = 6) manner to match intake of previously MITU-infused group. * = $P < 0.05$; dotted line represents preoperative baseline weight.

and proliferation of fibroblasts and smooth muscle cells. It is at this point that collagen production accelerates and intrinsic bowel wall strength begins to be restored.

The alimentary canal is a hollow viscus whose function is to store, transport, and reabsorb water from succus or, more distally, feculent material. Unique to the healing of this type of wound is the need for immediate sealing after injury. This is accomplished at operative repair by serosal apposition (intraperitoneally) and postoperatively by extraluminal adherence of structures such as peritoneal fat, omenta, and other intra-abdominal structures to the anastomotic site. Gastrointestinal healing is further characterized by a marked increase in collagenolytic activity at the anastomosis. As a result of this activity, the early tensile strength of the anastomosis is entirely dependent on the sutures and the holding capacity of the bowel wall.[7] This phenomenon has been implicated as a factor contributing to anastomotic failure.[8]

In cutaneous healing, NO activity has been implicated as a regulator of collagen synthesis[1,2,4] The present experiments demonstrate that iNOS expression and activity may play a role in colonic healing. iNOS gene and protein activities are at their greatest during the initial inflammatory phase of anastomotic healing with peak activity for both occurring within the first 24 hours. Microscopic examination of the iNOS-expressing cells reveal them to be large mononuclear cells with morphology that is clearly distinct from that of the neutrophils seen in the earliest phases of healing or the lymphocytes noted later. That these cells may be macrophages is an attractive premise, as they are the principal producer of iNOS in response to varied inflammatory stimuli.[9] Virtually all of the cell types found in the healing anastomosis (fibroblasts,[2] neutrophils,[10] macrophages,[11,12] epithelial cells,[13] smooth muscle cells[14]) have been shown to produce iNOS under various stimuli and culture conditions. Our work, however, indicates that there is only a discrete cell population that produces iNOS in response to uncomplicated colonic injury.

The highly specific location of iNOS-producing cells within the anastomosis implies that they play an as yet unspecified role. Histologic examination of the anastomosis on serial postoperative days demonstrated that wounds heal unidirectionally from the serosa toward the mucosa, much like the secondary intention healing seen in cutaneous ulcers. Whether these iNOS-positive cells represent a "leading edge" stimulating growth of the proliferating scar (through regulation of collagen synthesis) or function as a protective barrier against the harsh luminal environment (via superoxide scavenging by NO) remains to be elucidated.

Disruption of iNOS activity by the infusion of MITU is accompanied by a significant decrease in anastomotic bursting pressure on postoperative day 5. This suggests that iNOS activity plays a role in the optimal healing of uncomplicated colon anastomoses. Unfortunately MITU infusion is also accompanied by a significantly greater postoperative weight loss as compared to normal saline-infused animals. This cause of weight loss is multifactorial and includes both decreased food intake and an increase in postoperative diarrhea, although the rats infused with MITU did not appear ill.

To discern whether the decrease in anastomotic bursting pressure was due to decreased nutrition, a second experiment was undertaken comparing normal saline-infused animals fed ad libitum with those pair fed to match the decreased food intake of the MITU arm of the previous experiment. Although the pair-fed animals demonstrated a significantly decreased weight loss compared to the ad libitum–fed control animals, the degree of weight loss was less than that seen in those animals treated with MITU (93% of preoperative body weight by postoperative day 5 vs. 85% for the MITU group). Despite this, decreased nutrient intake alone did not significantly reduce anastomotic bursting pressure.

Several studies have noted that immediate postoperative nutrition is critical to successful anastomotic healing; however, these results are from studies that examine healing in models of established malnutrition.[15-17] More recently, postoperative infusion of 5-fluorouracil in rats subjected to colon anastomosis demonstrated a similar weight loss as compared to control animals with no differences noted in anastomotic bursting pressure.[18] These animals were nutritionally replete at the start of the experiment.

Production of NO by iNOS depends not only on the iNOS gene and protein activity but also on substrate availability. The amino acid arginine is recognized as the sole precursor for NO production. Under normal nutritional conditions, arginine bioavailability is the result of both dietary intake as well as recycling of the arginine metabolites citrulline and ornithine through the urea cycle. However, recycling alone provides enough substrate to support normal cellular metabolic requirements.[19] Additionally, arginine has been characterized as a semiessential amino acid because of its nutritional requirement for the optimal growth and development of mammalian rodents but not for the maintenance of normal adult metabolic function.[20]

Arginine metabolism is intensified during the response to injury and in healing tissues. In the healing wound, at least two metabolic pathways are operative: nitric oxide synthase and arginase. iNOS appears to play a role in inflammatory cascades, whereas arginase

activity metabolizes arginine to ornithine, a known precursor to polyamine synthesis. The combined activity of these enzymes is so efficient that arginine levels in wound fluid are virtually undetectable in later phases of healing.[11]

Precisely how iNOS inhibition leads to decreased anastomotic healing is unclear because both increased and decreased NO production have been associated with poor healing. iNOS is an acute-phase protein that is markedly upregulated in several inflammatory conditions including rheumatoid arthritis,[21] glomerulonephritis,[22] and inflammatory bowel disease. In ulcerative colitis, strong iNOS activity has been identified in colonic epithelial tissue, and the resulting inflammation has been hypothesized to contribute to the colonic injury seen in this disease.[13,23,24] Lipopolysaccharide injection of animals substantially increases iNOS activity in anastomotic tissues, which is associated with impaired anastomotic collagen synthesis and decreased bursting pressure.[25] Conversely, disruption of iNOS activity impairs collagen synthesis and wound tensile strength.[1] Diabetes and steroid use are also associated with poor healing and decreased NO production in experimental models.[26-29] This suggests that optimal healing depends on the concentration of NO produced by iNOS in a fairly tightly regulated range. In our model, disruption of iNOS activity likely decreases the degree of inflammatory response seen early in the inflammatory phase of healing, which in turn may lead to decreased fibrosis.

CONCLUSION

The results of this study demonstrate that iNOS is induced during colonic injury and anastomosis. Peak gene and protein activity are noted on postoperative day 1 corresponding to the period of maximal inflammation, but there is continued expression and activity during later phases of healing. The results also suggest that iNOS activity is needed for optimal healing of uncomplicated colon anastomoses. Further study is required to elucidate the exact roles that iNOS and NO production play in anastomotic healing.

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Discussion

Dr. H. Sax (Rochester, N.Y.). How much arginine was in the feed that the rats received? What happens if you either deprive the animals of arginine or supplement them with arginine? In your methylisothiourea (MITU) group, could your observed differences merely be because the animals were not consuming as much food and what you are really seeing is the result of a lack of pair feeding?

Dr. Efron. The chow is normal laboratory chow. I do not know the exact amount of arginine. We have not yet supplemented or deprived animals of arginine in this model, although certainly the next step would be to flood the system with arginine when the MITU is given to see whether it is possible to recover that effect. To give a quick answer to your third question, we were very concerned with the postoperative weight loss, which is certainly multifactorial. Not only do these animals eat less, but they also have a significant amount of diarrhea compared to other animals. We did conduct a pair-fed study where we took all normal saline-infused animals divided into two groups; the first group was allowed access to chow ad libitum, and the second group was pair fed daily to match what the MITU animals of the previous experiment had taken. We found no difference in plasma or wound fluid nitrites and no difference in anastomotic bursting pressure. I think the ideal experiment would involve three groups: an MITU-infused group, a pair-fed group, and an ad libitum chow group at the same time. Having said that, I think that MITU is probably not the ideal inhibitor because it causes this effect and confounds the results to such a great extent. We are continuing to search for a better inhibitor, a specific inhibitor of iNOS.

Dr. F. Moody (Houston, Tex.). My question relates to the localization of the iNOS. As you know, iNOS is expressed constitutively in the ileum in the rat, but not in the colon, as you pointed out. Where in the colon did you do your sampling? Did you also obtain samples away from the anastomosis? Manipulation in the gut and/or any endotoxin that gets released really pushes iNOS levels sky high in the epithelial cells of the colon. Yet you saw none there, at least in terms of expression of the message.

Dr. Efron. We did not specifically obtain samples upstream or downstream, but we did not see iNOS expression in the anastomotic segment in the normal colon immediately proximal or distal to the anastomosis. What is also striking, I agree, is that all of the cell types in the anastomosis have, at some time or another, been shown to be able to produce iNOS if stimulated in some way, whether in vivo or in culture. But the anastomotic wound only has a specific cell type, which does express iNOS, and that begs the question of function. Is it nitric oxide itself or is there another function of the nitric oxide–stimulating cells being produced that is more important to anastomotic healing? That is a question we are going to continue to attempt to answer.

Effect of Photodynamic Therapy on Normal Fibroblasts and Colon Anastomotic Healing in Mice

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Photodynamic therapy as an adjuvant modality to surgical resection of colon cancer is feasible provided that it does not affect healing of the anastomosis. The aim of this study was to evaluate the effects of photodynamic therapy on the viability of normal fibroblasts and on the healing process of colonic anastomosis in mice. Both in vitro and in vivo methods were employed. For in vitro study, 2×10^5 human fibroblasts were incubated in triplicate with 5-aminolevulinic acid (2.5 μg/well) for 48 hours. Cells then underwent photoradiation at light doses of 50, 100, and 200 joules/cm^2 using a nonlaser light source. Viability was assessed by methylene blue dye exclusion. For in vivo studies, 60 mice were randomized into study and control groups and underwent laparotomy involving colonic anastomosis. The anastomosis underwent photodynamic therapy using 5-aminolevulinic acid (60 mg/kg) as a photosensitizer and a nonlaser light (40 joules/cm^2). On postoperative days 1, 4, 7, 14, and 21, six mice were killed and subjected to bursting pressure and histologic examinations. Results of in vitro study showed pretreatment cell viability to be 96% to 99% in both groups. Photodynamic therapy caused no significant change in fibroblast viability at all light doses. Results of in vivo studies showed that the mean bursting pressure of both groups dropped to a low peak on day 4. Subsequently there was a gradual increase in bursting pressure along the examined time points ($P < 0.001$). There was no difference in bursting pressure between the two groups for all time points examined. It was concluded that photodynamic therapy has no effect on viability of normal human fibroblasts and no adverse effects on healing of colonic anastomosis. (J GASTROINTEST SURG 1999;3:602-606.)

KEY WORDS: Anastomotic leak, adjuvant therapy, in vitro study, animal study, colorectal cancer, aminolevulinic acid

Colorectal cancer is one of the most common internal malignancies of Western society.[1] The primary treatment is surgical excision. Adjuvant modalities have been studied extensively in recent years because of the high incidence of recurrent disease inherent to the natural history of the disease.[2] One of the adjuvant modalities for colorectal cancer that has recently been proposed is photodynamic therapy (PDT).[3] Several studies have indicated adjuvant intraoperative PDT to be a promising approach for destroying tumor cells after surgical resection of the tumor.[3-5] Before including PDT in the management of colon cancer, it is essential to establish its safety and to ascertain that it does not interfere with the healing of anastomosis of colon.

Anastomotic breakdown or leakage is a severe and life-threatening complication of colonic surgery. The addition of PDT to the surgical resection of the tumor is feasible provided that it does not affect healing of the anastomosis in terms of weakening it and thereby increasing the risk of leakage. There are a few reports on PDT and healing of incisional wounds in rats,[6] but the direct effect of PDT on the healing of colonic anastomosis has not been adequately documented.

One of the methods for evaluating the strength of an anastomosis is to measure the resistance of the

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anastomosis to intraluminal pressure. The pressure at which leakage or rupture of the anastomosis occurs is defined as bursting strength or bursting pressure.[7] We studied the effects of PDT on fibroblasts and on the healing process of colonic anastomosis in mice using bursting pressure as an indicator of the anastomotic strength.

MATERIAL AND METHODS

Female Balb/c mice, 6 to 8 weeks old, were obtained from the animal colony of the Sackler School of Medicine, Tel Aviv University, and housed in the animal section of the Elias Sourasky Medical Center, Tel Aviv. This study was approved by the local committee for animal studies.

Normal human fibroblasts (kindly supplied by E. Fireman and A. Yellin) obtained from the healthy contralateral lung of a patient with lung cancer were prepared as a single cell suspension by a triple-enzyme technique.

5-Aminolevulinic acid (Medic Fintech Ltd., Technion City, Haifa, Israel) with a purity of 98% was dissolved in phosphate-buffered saline at a concentration of 80 mg/ml (pH 2.8) and used within 24 hours. In a series of preliminary studies, cells were incubated with various doses of 5-aminolevulinic acid (unpublished data). The maximum drug concentration that did not affect cell viability was 2.5 μg/ml and was the one used for further studies.

The Versa-Light (ESC Medical Systems Ltd., Yokneam, Israel) illuminator uses a xenon lamp as its source and fiberoptics as its light delivery system. The spectral output is limited to ranges of 580 to 720 nm and 1250 to 1600 nm. This system had been developed in accordance with the specific demands for PDT and was shown to be effective in both experimental studies and treatment of patients.[8]

Study Design

In Vitro Study. For in vitro study, 2×10^5 cells were incubated in triplicate with 5-aminolevulinic acid (2.5 μg/ml) for 48 hours. The cells were then washed twice with phosphate-buffered saline and subjected to photoradiation in 1 ml of phosphate-buffered saline. Photoradiation was performed with light doses of 50, 100, and 200 joules/cm^2 after which the cells were washed and their viability was assessed with methylene blue dye exclusion.

In Vivo Study. For in vivo studies, 60 mice were randomized into two equal groups (study and control). 5-Aminolevulinic acid, 60 mg/kg, was injected intraperitoneally into the mice in the study group. After 24 hours, these mice underwent laparotomy through a 2 cm midline incision under general anesthesia using Ketalar (Park Davis, Eastleigh, Hampshire, U.K.), 2.5 mg. The cecum was exposed and a circumferential cut of 320 degrees was made 1 cm from its tip, leaving the mesenteric wall intact. Anastomosis was then performed using Tri-Cron 6-0 continuous inverting sutures (Davis & Geck Ltd., Anyang, Korea). Indeed this experimental model does not fully mimic the clinical situation of colonic resection and 360-degree anastomosis. However, we believed that this would not affect the results from a scientific point of view. The anastomosis was subjected to photoradiation using a light dose of 40 joules/cm^2. The abdominal wall was then closed in two layers using silk 3-0 sutures (Teva Pharmaceutical Industries Ltd., Israel). The same technique was used in the control group but the photodynamic therapy was omitted. On each postoperative day (1, 4, 7, 14, and 21), three mice in each group were killed and autopsies were immediately performed. The right colon, 3 cm distal to the anastomosis and including a remnant of terminal ileum, was mobilized and excised. The ileal stump was ligated with silk 3-0 sutures. The resected segment was then subjected to a bursting pressure evaluation.

Bursting Pressure Evaluation

A cannula was inserted into the intestinal lumen through the distal end of the ascending colon to a distance of 1 cm and the intestinal wall was tied over it. The cannula was connected to two channels. The first was connected to the cecum and to a pressure transducer. The transducer was attached to an infusion pump using 0.9% saline solution at a rate of 2 ml/min. The second channel was connected via a pressure transducer to a pressure recorder. Bursting pressure was defined as the pressure at which saline first appeared to leak from the anastomosis.

Histologic Examination

On days 14 and 21, two mice from each group were killed and the colonic segment with the anastomosis underwent histologic evaluation. A trained pathologist who was blinded to animals' group assignments examined the specimens. The specimens were excised, fixed in 10% formalin, and embedded in paraffin. The blocks were cut at a thickness of 3 μm and stained with hematoxylin and eosin.

Statistics

The viability of the in vitro cells was evaluated using Student's *t* test and one-way analysis of variance (ANOVA). The increase in bursting pressure in each

group from day 1 to day 21 was evaluated by one-way ANOVA. The daily difference in bursting pressure between the study and control groups was evaluated by Student's *t* test.

Table I. Fibroblast viability before and after photodynamic therapy

5-Aminolevulinic acid (2.5 μg/ml)	Light (joules/cm²)	Before treatment (%)	After treatment (%)
None	None	99	98.7
Applied	None	98.7	99
Applied	40	99	99.7
Applied	60	98.7	98.7
Applied	100	98.7	95

RESULTS

In the in vitro study, pretreatment viability was 96% to 99%. PDT caused no significant change in the viability of fibroblasts at all light doses (Table I); thus fibroblasts were not affected by photodynamic therapy.

Regarding in vivo studies, there were no postoperative deaths among mice after the anastomoses were performed. On postoperative days 1, 4, 7, 14, and 21, three mice in each group were killed and autopsies were performed. Macroscopically all anastomoses were intact.

The mean bursting pressure of the control group dropped during the first experimental days and showed a low peak of 30.7 mm Hg on the postoperative day 4. This was followed by a gradual but significant increase in bursting pressure along the examined

Table II. Bursting pressure after colonic anastomosis in control (nontreated) and PDT-treated mice

Day	Control (mm Hg)	PDT (mm Hg)	*P* value (Student's *t* test)
1	36.5 ± 2.5	38.3 ± 2.1	NS
4	30.7 ± 9.6	25.0 ± 12.3	NS
7	85.5 ± 21.3	89.8 ± 17.5	NS
10	115.7 ± 4.7	95.8 ± 22.0	NS
14	117.5 ± 12.9	112.0 ± 36.2	NS
21	121.3 ± 24.1	119.5 ± 6.7	NS
One-way ANOVA	$P < 0.001$	$P < 0.001$	

Values are mean ± standard deviation; NS = not significant.

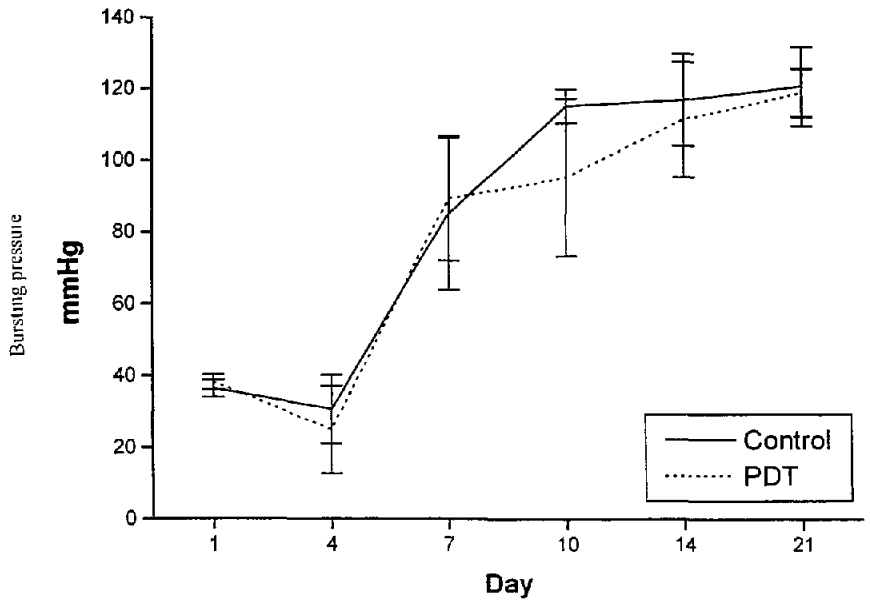

Fig. 1. Bursting pressure after colonic anastomosis (days 1 to 21) in PDT-treated mice and control mice (n = 3).

time points until a level of 121.3 mm Hg was attained on day 21 ($P < 0.001$). The change in bursting pressure of PDT-treated mice was similar to that of the control group. It dropped to 25 mm Hg on day 4 and increased gradually to 119.5 on the day 21 after the anastomosis ($P < 0.001$) (Fig. 1). There was no difference in bursting pressure between the two groups for all examined time points (Table II).

Histologic Findings

There was no difference between the groups in the degree and extent of inflammation at the anastomotic site on days 14 and 21 (Figs. 2 and 3). The anastomotic site showed transmural inflammation consisting of lymphocytes, granulocytes, and histiocytes.

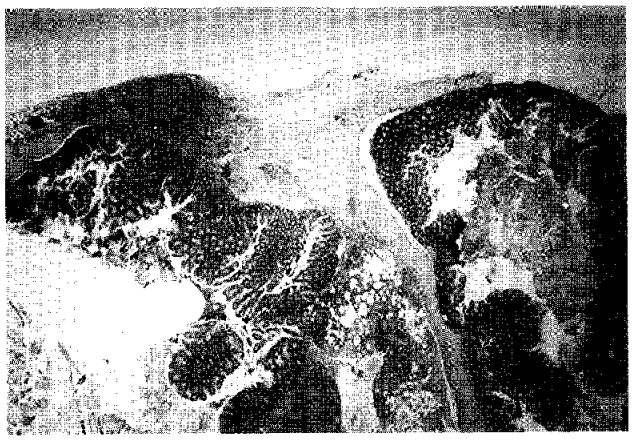

Fig. 2. Colonic wall with transmural inflammation in a PDT-treated mouse.

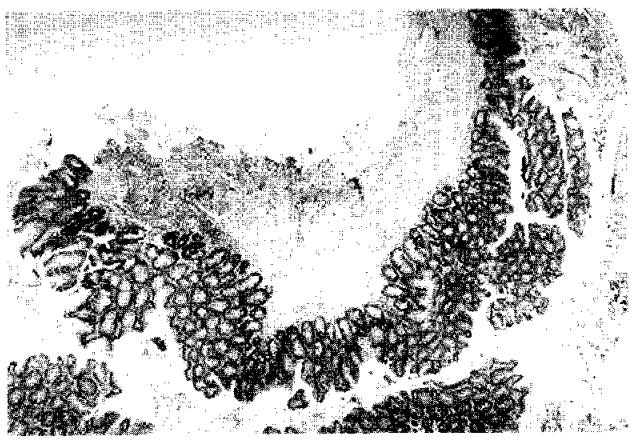

Fig. 3. Inflamed colonic wall in a control (nontreated) mouse. The histologic changes are similar to those seen in the PDT-treated mouse.

Granulation tissues with young fibroblasts and foreign body giant cells were also noted.

DISCUSSION

The earliest attempts to use photosensitizers to treat cancer were reported in 1903 by Tappenier and Jesionek.[9] In the past 15 years, following toxicology and preclinical studies, several human studies showed good results of PDT in tumors of various organs including colorectal cancer.[10-12] The aim of the study was to establish the safety of PDT as an adjuvant therapy for colorectal cancer in conjunction with colonic resection.

The mechanism of PDT is not completely understood. PDT-induced cytotoxicity is thought to occur through photooxidation reactions. Free radicals and peroxides, which are produced as a result of PDT photooxidation reactions, may damage a number of cellular structures or targets. Perhaps the most pronounced cellular response observed following PDT is damage to membranes, particularly the plasma membrane.[13]

The healing of colonic anastomosis is similar to wound healing elsewhere in the body. The early process is acute inflammation followed by proliferation of fibroblasts accompanied by collagen synthesis and ground substance.[7] Here we demonstrated that normal fibroblasts were not affected by photodynamic therapy. This confirmed the findings of other studies that used 5-aminolevulinic acid or other photosensitizers, which also demonstrated that in vitro PDT had no effect on human fibroblasts.[14,15]

The natural course of anastomotic healing is characterized by a significant reduction in its bursting strength, which reaches its lowest peak around postoperative day 4. Thereafter a progressive increase in the bursting strength occurs, reaching similar levels of preanastomotic integrity on day 21.[7] The effects of PDT on healing are not well established. Brouwer et al.[6] studied them in incisional wounds in rats and found that there was no difference in tensile strength after 21 days between PDT-treated rats and control rats. Kubler et al.[16] found the opposite effect, showing that PDT lowered the tensile strength of skin wounds in rats. In a more recent study, Loh et al.[17] measured the strength of the rat stomach in response to PDT and showed that PDT did not compromise the mechanical strength of the stomach wall. The results of the present study are in accordance with those claiming that PDT did not have a deleterious effect on the tensile strength of the skin—that is, PDT did not decrease the bursting strength of the anastomosis in our treated animals compared to the control mice.

Adjuvant and perioperative oncologic treatment, such as chemotherapy and radiation therapy, is in-

creasingly used in the treatment of malignant diseases. It appears that some chemotherapeutic agents given in the immediate perioperative period impair the early healing of colonic anastomoses, whereas the effects on late healing are less pronounced.[7] The increased risk of anastomotic dehiscence both in the early and late periods after radiation therapy is also well known. PDT is generally considered to be a treatment modality with relatively fewer side effects than chemotherapy or radiation therapy.[7] One of the weaknesses of PDT is its limitation in the tissue penetration of the light and hence in destroying bulky tumors. On the other hand, it is theoretically an excellent modality for treating residual and microscopic disease.

Intraoperative PDT was first applied clinically to patients undergoing resection of recurrent colorectal carcinoma and retroperitoneal sarcoma.[11,18] In a phase I study, Delaney et al.[4] demonstrated the feasibility of intraoperative PDT following debulking surgery. Allardice et al.[3] and Abulafi et al.[5] reported the results of intraoperative PDT of patients who underwent colonic resection with proven positive resection margins. Only one out of eight PDT-treated patients developed local recurrence compared with 12 out of 14 who underwent surgery alone.

In summary, we evaluated the effect of PDT on colonic anastomosis in mice using 5-aminolevulinic acid as a photosensitizer. We first demonstrated that in vitro PDT does not affect the viability of fibroblasts. We subsequently evaluated bursting pressure as an indicator of anastomotic strength and demonstrated that there was no difference in the bursting pressure between PDT-treated mice and control mice in the early or the late post-operative period (days 1 and 21). Our findings suggest that PDT does not cause a significant impairment in healing of colonic anastomoses. Therefore PDT may be used as an adjuvant modality for the treatment of colorectal cancer.

This study is dedicated to our colleague, the late Dr. A. Shnaper, a distinguished surgeon and researcher who tragically passed away shortly after the completion of this research.

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Malabsorptive Procedures for Severe Obesity: Comparison of Pancreaticobiliary Bypass and Very Very Long Limb Roux-en-Y Gastric Bypass

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The aim of this study was to determine the efficacy and safety of two malabsorptive procedures for severe obesity. Prospectively collected data from eight men and three women who underwent partial biliopancreatic bypass (PBB) and 19 men and seven women who underwent very very long limb Roux-en-Y gastric bypass (VVLGB) for superobesity (preoperative weight >225% above ideal body weight) were evaluated. Age (42 ± 3 years and 40 ± 2 years), body mass index (64 ± 4 kg/m^2 and 67 ± 3 kg/m^2), and percentage of excess body weight (183% ± 17% and 203% ± 12%) were similar (mean ± standard error of the mean). Median follow-up was 96 months (range 72 to 108 months) and 24 months (range 18 to 60 months) for the PBB and VVLGB groups, respectively. Weight loss expressed as percentage of excess body weight was 68% ± 4% 2 years and 71% ± 5% 4 years after PBB, and 53% ± 7% 2 years and 57% ± 5% 4 years after VVLGB. Current body mass indexes are 37 ± 2 kg/m^2 and 42 ± 2 kg/m^2 in the PBB and VVLGB groups, respectively. Hospital mortality was zero. Morbidity occurred in five patients after VVLGB (wound infection in four, wound seroma in one, and pulmonary embolus in one) and in two patients after PBB (abscess in two, anastomotic leak in one, and gastrointestinal bleeding in one). After PBB, one woman died of refractory liver failure 18 months postoperatively and two other patients developed metabolic bone disease. No such known complications have occurred to date after VVLGB. We conclude that VVLGB is safe and effective for clinically significant obesity, results in sustained weight loss, and improves quality of life. (J GASTROINTEST SURG 1999;3:607-612.)

KEY WORDS: Bariatric surgery, obesity, morbid obesity, gastric bypass, malabsorption

Medically complicated obesity, often referred to as morbid obesity, is a serious health problem that has repeatedly proved refractory to nonoperative therapy. The two commonly used operations to treat medically complicated obesity in North American centers, vertical banded gastroplasty and Roux-en-Y gastric bypass, have been condoned by a National Institutes of Health Consensus Conference on Bariatric Surgery.[1] Variations of both procedures have been advocated for use in superobese patients (>225% above ideal body weight and/or body mass index >50 kg/m^2) such as decreasing the diameter of the stoma to 4.5 cm in vertical banded gastroplasty[2] or increasing the length of the Roux limb in gastric bypass.[3,4] However, relative weight loss after such procedures has not been as satisfactory as in patients who weigh less than 200% of their ideal body weight. Scopinaro et al.[5] have demonstrated a dramatic weight loss and acceptable early morbidity and mortality after partial biliopancreatic bypass (PBB) and claimed few significant long-term problems. PBB has not been widely accepted in North America because of the preceding ill-fated jejunoileal bypass and, more important, because, in contrast to Scopinaro's findings, most other groups have noted unacceptable serious long-term morbidity related to PBB.[4,6]

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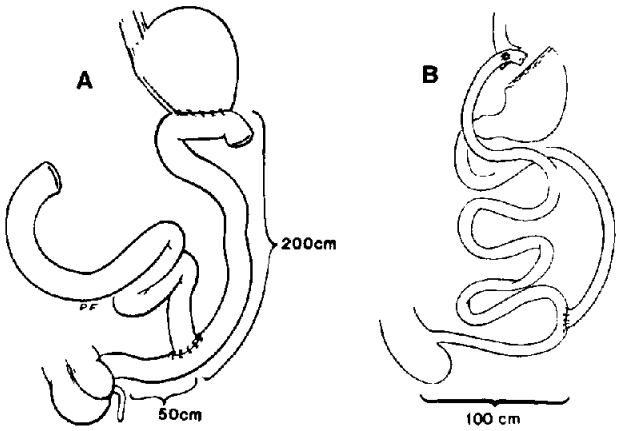

Fig. 1. A, Partial bilopancreatic bypass. **B,** Vertical disconnected very very long limb Roux-en-Y gastric bypass.

Based on the early reports by Scopinaro et al.,[5] we initially treated selected superobese patients with a modification of the PBB as originally described by this group (Fig. 1). We encountered an unacceptably high rate of unfavorable outcomes and subsequently began using a gastric bypass with a very very long Roux limb, the VVLGB. The purpose of this study was to report the operative mortality, short-term and long-term morbidity, and patterns of weight loss after VVLGB and PBB for clinically significant superobesity.

MATERIAL AND METHODS

Prospectively collected data from all patients who underwent bariatric surgery at Mayo Clinic from 1985 to the present were analyzed. The records of 37 consecutive patients were reviewed, including 11 who underwent PBB from 1989 to 1993 and 26 who underwent VVLGB from 1993 to 1997. Patient demographics, weight, perioperative mortality and morbidity, and subjective assessment of quality of life were analyzed. All patients were followed for at least 4 years after PBB and 25 of 26 to date after VVLGB; pertinent data points were collected prospectively by mailing a standard questionnaire at 3, 6, 12, 24, 36, and 48 months postoperatively. The questionnaire included detailed information on dietary and caloric intake, weight, level of activity, and a subjective assessment of quality of life. Patients were contacted by one of the authors to update follow-up at the time of completion of this study. Follow-up is complete to date for 35 of 37 patients; one PBB patient was lost to follow-up after 48 months and one VVLGB patient was lost to follow-up after 18 months, despite numerous attempts to contact him.

All PBB procedures were performed similar in principle to the description of Scopinaro et al.[5] with a 200 to 500 ml proximal gastric pouch after ~80% distal gastrectomy, gastroileostomy, jejunoileostomy with a 200 cm Roux-en-Y ileal limb and a 50 cm common channel. The length of the biliopancreatic limb was thus approximately 300 to 400 cm. The last patient did not undergo the distal gastrectomy but simply had the proximal stomach partitioned with a double row of staples, leaving about a 100 ml proximal gastric pouch.

VVLGB involved a simple modification of the vertical disconnected Roux-en-Y gastric bypass operation as described previously.[7] The jejunal Roux limb is markedly lengthened to 300 to 400 cm by anastomosing the pancreacticobiliary limb to the Roux limb 100 cm proximal to the ileocecal junction. Initially we varied the length of the common channel in a manner inversely proportional to patient weight, but as our experience accumulated, we standardized the length of the common channel to 100 cm. This "common channel" of distal ileum permits the pancreatobiliary secretions from the "bypassed" small bowel to mix with ingested food emptying from the proximal gastric pouch into the very very long Roux-en-Y limb, thereby allowing digestion and absorption of ingested complex food stuffs to occur in the distal 100 cm of small bowel, thereby partially limiting absorption. A side-to-side anastomosis of the proximal jejunum to the "disconnected" small (15 ml) pouch of gastric cardia is performed with a No. 21 EEA stapler (U.S. Surgical Corp., Norwalk, Conn.) and reinforced with an outer layer of interrupted silk sutures. A cholecystectomy is routinely performed (if the patient has not had a previous cholecystectomy), as well as placement of a tube gastrostomy in the defunctionalized distal gastric remnant.

After both procedures, patients were started on sips of clear liquid diet on the second or third postoperative day. Diet was advanced to pureed food before patients were discharged from the hospital and was maintained for 6 weeks postoperatively; thereafter a more liberal diet of regular food was allowed over the ensuing 6 weeks.

The initial postoperative follow-up was at our facility and involved a visit with the surgeon, referring endocrinologist, and a dietitian. All patients had been strongly and repeatedly counseled in the hospital and were started on an oral multivitamin supplement plus minerals, monthly intramuscular injection of vitamin B_{12} (1000 μg), and oral calcium supplements. After PBB, patients were prescribed vitamin A and D supplements as well. Postoperative data were pooled from the responses to the periodic questionnaires, return visits to the Mayo Clinic, and phone interviews. Given the nature of our referral pattern, long-term follow-up in our facility was not always feasible for

some patients who lived a great distance away. These patients underwent periodic assessment at their local health care facilities after we corresponded with their home physician, counseling the latter in appropriate follow-up evaluation.

Analysis of Data

All data are reported as mean ± standard error of the mean unless otherwise specified. Student's *t* tests were employed, and $P < 0.05$ was considered significant.

RESULTS

Patient Demographics and Weight

Eight men and three women underwent PBB between 1989 and 1993, and 19 men and seven women underwent VVLGB between 1993 and 1997. All but two patients were superobese (i.e., >225% above ideal body weight). The two patients who did not fulfill the weight criteria for superobesity had both failed to maintain a weight loss after an anatomically intact vertical banded gastroplasty and had active weight-related severe morbidity; both were treated by PBB. Age and weight distributions were similar in the two groups (Table I). All patients had failed at least one supervised attempt at a nonoperative means of weight loss. All patients had one or more of the following comorbid conditions: hypertension, asthma, glucose intolerance, degenerative joint disease, or advanced venous stasis ulcers (Table II). In addition, sleep apnea (defined by multiple episodes of desaturation noted on a formal sleep study at our sleep disorders clinic) was documented in 5 (45%) of 11 patients undergoing PBB and 19 (73%) of 26 patients in the VVLGB group.

Table I. Patient characteristics*

	PBB (N = 11)	VVLGB (N = 26)
Age (yr)	42 ± 3	40 ± 2
Preoperative weight (kg)	187 ± 11	218 ± 11
Body mass index (kg/m^2)	64 ± 4	67 ± 3
Excess body weight (kg)	123 ± 11	146 ± 11
Excess body weight (%)	183 ± 17	203 ± 12

*Values are mean ± standard error of the mean.

Table II. Medication use for comorbid conditions before and after surgery*

	PBB		VVLGB	
	Before	After	Before	After
Asthma	4	0	2	2
Hypertension	3	2	10	4
Diabetes	1	2	2	1
Mechanical arthropathy	4	2	11	8

*Number of patients requiring treatment.

Table III. Surgical history and concomitant operations performed

	PBB	VVLGB
Previous bariatric surgery (No. of patients)	2	4
Common channel (cm)	50	108 ± 5 (range 60-150)
Cholecystectomy*	7/11 (63%)	21/26 (81%)
Umbilical/ventral hernia repair	6/11 (55%)	8/26 (31%)

*Includes all patients without a previous cholecystectomy.

Surgical History and Operative Procedures

Previous bariatric operations for weight loss had been performed in 4 of 11 patients in the VVLGB group, including jejunoileal bypass and vertical banded gastroplasty in one and three patients, respectively (Table III). Two patients in the PBB group had undergone a previous vertical banded gastroplasty but their weight loss had been insignificant despite intact anatomy of the operation. The last patient to have a PBB did not undergo a distal gastrectomy. The common channel in patients who underwent PBB was 50 cm. In the VVLGB group the common channel varied but had a mean of 108 ± 5 cm (range 60 to 150 cm). Concomitant procedures included cholecystectomy (7 of 11 and 21 of 26) if it had not been performed previously and repair of umbilical or ventral hernias (6 of 11 and 8 of 26) in the PBB and VVLGB groups, respectively.

Morbidity and Mortality

There were no operative deaths. After PBB, hospital morbidity (Table IV) included an anastomotic leak at the gastroileostomy that required operative repair. This patient also developed transient gastrointestinal bleeding, presumably from the gastroileostomy. One other patient developed a wound infection. After VVLGB, four patients (15%) developed wound infections and another had a wound seroma. An additional patient with a history of recurrent pulmonary embolism developed a pulmonary embolus on the tenth postoperative day, despite full postoper-

Table IV. Mortality and morbidity after PBB and VVLGB

Morbidity and mortality	PBB (N = 11)	VVLGB (N = 26)
Early morbidity		
Wound infection	1	4
Wound seroma	0	1
Anastomotic leak*	1	0
Gastrointestinal bleeding	1	0
Pulmonary embolus	0	1
Late morbidity		
Liver failure and death	1	0
Metabolic bone pain	2	0
Urolithiasis	1	2
Endoscopic dilatations	1	0
Stomal ulcer bleeding	1	0

*Requiring operative repair.

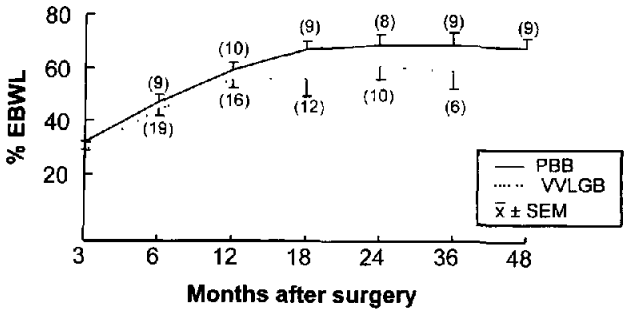

Fig. 2. Weight loss data expressed as percentage of excess body weight lost (*%EBWL*) PBB = partial biliopancreatic bypass; VVLGB = very very long limb Roux-en-Y gastric bypass.

ative anticoagulation beginning on the first day after operation. Median length of postoperative hospitalization was 11 days for the PBB group (range 6 to 54 days) and 8 days for the VVLGB group (range 5 to 33 days).

After PBB, long-term morbidity (mean follow-up 85 ± 9 months) involved complaints of diarrhea, steatorrhea, and foul-smelling stools in *all* patients; the latter was especially difficult to manage. One patient developed progressive liver failure associated with neuropathy 12 months postoperatively and died 6 months later despite aggressive vitamin and mineral supplementation. In speaking with her family, we learned that she had refused to take the prescribed mineral and vitamin supplements and never saw her physician despite numerous entreaties. Two other patients who were equally noncompliant developed metabolic bone disease with generalized bone pain, which has slowly responded to vitamin D and oral calcium supplements. In contrast, after VVLGB, no serious long-term nutritional complications have occurred (mean follow-up 32 ± 3 months) to date.

No patients have undergone reversal of either PBB or VVLGB. Similarly none have required lengthening of the common channel. One patient after PBB and two after VVLGB developed symptomatic urolithiasis; these stones were not of oxalate origin. After PBB, one patient required two episodes of endoscopic dilatation for (poorly documented) stenosis of the gastroileostomy, and another developed upper gastrointestinal bleeding, presumably from a stomal ulcer, during a prolonged course of nonsteroidal antiinflammatory drugs for arthritis.

Weight Loss

Mean weight loss, expressed as a percentage of excess body weight (% EBWL), appeared to plateau 18 to 24 months postoperatively in both the PBB and VVLGB groups, respectively (see Fig. 2). Weight loss was 68% ± 4% and 71% ± 5% at 2 and 4 years, respectively after PBB; after VVLGB, weight loss was 53% ± 7% at less than 24 months (n = 6) and 57% ± 5% at more than 24 months (n = 20). Current values for BMI are 37 ± 2 kg/m^2 (reduced from 64 ± 4 kg/m^2 preoperatively) and 42 ± 2 kg/m^2 (reduced from 67 ± 3 kg/m^2 preoperatively) after PBB and VVLGB, respectively. Two years after surgery, all patients with PBB and 62% of patients with VVLGB have lost 50% or more of their excess body weight. The difference in %EBWL at each time point is not statistically significant ($P > 0.05$).

Comorbid Conditions

The use of medications for asthma, hypertension and arthritis was reduced (see Table II) postoperatively. We cannot make any inferences concerning the effect of weight loss on glucose intolerance because of the somewhat surprisingly small number of patients in this cohort with clinically significant diabetes mellitus requiring medical treatment. Although many of these patients have discontinued their use of continuous airway pressure masks, we have no reliable objective data on the effect of weight loss on the presence or severity of sleep apnea.

Subjective Assessment of Quality of Life

All living patients in both groups were satisfied with the resultant weight loss after the operation and maintained that it markedly improved the quality of their lives. All patients indicated that they have recommended and will continue to recommend such op-

erative treatment to similar individuals with clinically significant obesity.

DISCUSSION

Operative treatment is the only modality that has reliably resulted in sustained weight loss in most patients with clinically significant obesity. The enthusiasm for pharmaceutical treatments has greatly faded after the incidental finding of an association between these widely used drugs and valvular heart damage.[8] Current acceptance of bariatric surgery as an option for the patient with clinically significant "morbid" obesity has been acknowledged by a National Institutes of Health Consensus Conference on gastrointestinal surgery for severe obesity.[1]

We have established a multidisciplinary team approach for the treatment of medically complicated obesity. Our operative treatment has evolved over the years reflecting our own experience based on critical analysis of our prospectively collected data. We have switched from nonbanded[9] to banded gastroplasty[10] and most recently to a disconnected Roux-en-Y gastric bypass as the primary modality of operative treatment for clinically significant obesity. Our initial operation of choice for the subgroup of superobese patients was pancreaticobiliary diversion based on the encouraging preliminary data from Scopinaro's group.[5] Although weight loss patterns after PBB were predictable and encouraging, we questioned the "radicality" of this procedure and specifically the need for distal gastrectomy. As our experience accumulated with this small cohort of patients, we noted untoward effects of the severe, generalized malabsorption induced by the short (50 cm) common alimentary limb. All patients had steatorrhea, diarrhea, and foul-smelling stools. One woman died of irreversible liver damage that could not be attributed to factors other than the PBB itself and the patient's noncompliance. Although all of the other patients who underwent PBB are alive and well, two other women developed severe bone pain requiring aggressive intervention. Although the weight loss was impressive after PBB, the need for supplemental vitamins and minerals and a very compliant patient for the remainder of his/her life makes this operation less attractive for most patients.

We postulated that we could achieve a similar degree of weight loss to reverse the weight-related morbidity without the untoward effects of the PBB operation by elongating the Roux limb (to 300 to 400 cm of a conventional disconnected Roux-en-Y gastric bypass) and controlling the length of the common channel. The VVLGB differs from the PBB in that we perform a disconnected Roux-en-Y gastric bypass using the proximal jejunum as the alimentary limb (Roux limb), and not the proximal ileum as in a PBB. Intestinal continuity of the alimentary and biliopancreatic limbs of the VVLGB is restored at the level of the distal ileum to form a 100 cm common limb. We fashion a very small pouch of gastric cardia, large enough to accommodate the anvil of a 21 mm circular stapler; we do not measure its volume but would estimate it at 15 ml or less.

Our data show that early weight loss patterns in the first 12 months are similar after the two operations. The trends after 1 year become parallel, and patients after PBB appear to attain greater weight loss at 24 and 36 months, albeit nonstatistically significant. These conclusions, however, are limited by the small number of patients who are at 3 and 4 years after a VVLGB. More important, with VVLGB the metabolic and nutritional consequences from PBB appear to have been avoided with a seemingly less radical operation. Similar results have been reported by Sugerman et al.[4] using an anatomic variant of gastric bypass; all five of their patients with a common limb of only 50 cm had to undergo revision because of development of protein-calorie malnutrition, and two patients died of hepatic failure. In the same report, weight loss after the common limb was elongated to 150 cm was 69% of excess body weight at 5 years; only 3 of 22 patients required elongation of this 150 cm common limb for metabolic complications.

It appears that the malabsorption after a VVLGB is less malignant than that after a PBB, presumably because the length of the common limb is longer (100 cm vs. 50 cm) and possibly because of a longer Roux-en-Y limb. The benefits of avoiding the metabolic consequences of a common limb of 50 cm as originally described by Scopinaro et al.,[5] in our opinion and that of others,[3,4,6] outweigh the modest increment of weight loss after PBB. The configuration of the VVLGB as we construct it may play a role in reducing the severity of malabsorptive syndromes. We employ the proximal jejunum as the Roux limb; the pancreaticobiliary limb is much shorter and consists of the duodenum and proximal 40 to 60 cm of jejunum as opposed to the entire jejunum with PBB. Therefore ingested nutrients are not excluded from the jejunum as in the PBB. We cannot justify distal gastrectomy because it considerably increases the operative time and has its potential complications. Although intestinal metaplasia of the bypassed stomach may develop,[11] long-term follow-up from centers that perform the gastric bypass has not shown an increased incidence of gastric remnant neoplasia or a need for later resection of the bypassed gastric remnant because of acute or chronic intragastric bleeding. Clearly, longer follow-up is needed to settle this issue.

It is worth noting that Scopinaro's group has reported a very low incidence of protein-calorie malnutrition and/or metabolic bone disease after PBB that paralleled a decrease in their referrals from southern Italy. Such regional, geographic, racial, and dietary variations may account for the high incidence of metabolic complications after PBB in North America where the diet of such obese individuals contains a high percentage of fat and lesser amounts of carbohydrates.[4] Nevertheless, the differences in our results and others after PBB are markedly different from those of Scopinaro et al.[5] and remain difficult to reconcile.

Weight loss in our patients after VVLGB is comparable to that reported from other centers employing malabsorptive procedures such as the distal gastric bypass (69% excess body weight at 5 years),[4] long limb gastric bypass (64% at 2 years)[3], and the PBB (72% at 8 years).[5] Vertical banded gastroplasty with a 5 cm collar resulted in an excess body weight loss of 54% and 50% at 2 and 5 years, respectively.[2] It is difficult, however, to judge the results of vertical banded gastroplasty because that report did not include the standard deviation or standard error of the mean. The preponderance of men in our patient population is worth noting and is at variance with other reports and with our own overall experience with bariatric surgery. It may be that men seek medical help when they reach a higher body mass index than women. The effect of this skewed sex distribution on weight loss remains to be evaluated.

Weaknesses of the Study

Meaningful conclusions regarding postoperative laboratory values to confirm the absence of subtle metabolic deficiencies have not been possible because our institution is a tertiary referral center. We acknowledge that relying on patients' reports of current weight and other conditions without on-site verification represents a pitfall of our study that we cannot correct because of the reasons mentioned earlier and our practice climate. Many of our patients live more than 100 miles away, and few have had long-term laboratory studies at our center. The relatively small number of patients in this study (n = 37) remains a potential weakness; nevertheless, this represents a series of malabsorptive procedures large enough to make some meaningful observations. Long-term follow-up of VVLGB may indeed unmask metabolic abnormalities that are not apparent at the present time. Currently we do not think it ethical to randomize patients to PBB vs. VVLGB after the untoward effects we have noted following PBB.

CONCLUSION

VVLGB is a safe and effective operation for the treatment of clinically significant obesity. VVLGB results in satisfactory weight loss, amelioration of comorbid conditions, and improvement in the quality of life. Maintaining a common channel of 100 cm of the distal ileum combined with a very small gastric pouch of cardia achieves adequate weight loss without late nutritional or metabolic consequences common to PBB. These results justify our abandonment of PBB and change in practice patterns. We currently recommend VVLGB for superobese patients, patients with severe comorbid conditions, and a body mass index greater than 60, and as a revisional operation for anatomically intact but failed previous bariatric surgery provided that the patient is compliant and well educated concerning the need for lifetime medical follow-up.

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Surgical Treatment of Roux Stasis Syndrome

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We wondered whether the slow gastric emptying of the Roux stasis syndrome could be improved by performing a corrective "uncut" Roux operation. Five dogs had a standard Roux gastrectomy and placement of serosal electrodes on the proximal jejunum and Roux limb. After recovery, baseline myoelectrical and gastric emptying data were collected. The animals then underwent a second operation: take down of the Roux limb, restoration of jejunal continuity, and construction of an "uncut" Roux limb. After the animals recovered, the tests were repeated. The slow frequency of pacesetter potentials (PPs) in the standard Roux limb (mean ± standard error of the mean 14 ± 0.4 cpm) was unchanged after the uncut Roux operation (14 ± 0.5 cpm, $P > 0.05$). However, a greater percentage of PPs propagated aborally in the uncut Roux limb (81% ± 4%) than in the standard Roux limb (53% ± 7%, $P < 0.05$). Nonetheless, gastric emptying of a 250 ml 10% dextrose liquid meal was not speeded by the uncut Roux operation (uncut Roux = 36% ± 5% emptied by 20 minutes vs. standard Roux = 35% ± 7%; $P > 0.05$). Bile acid concentrations in gastric aspirates were minimal after both operations (0.7 ± 0.2 μmol/L vs. 0.6 ± 0.1 μmol/L; $P > 0.05$). The conclusion was that more PPs propagated in the aborad direction in the uncut Roux limb than in the standard Roux limb, but gastric emptying was not speeded by the uncut Roux operation. Both operations were equally effective in preventing bile reflux into the gastric remnant. (J GASTROINTEST SURG 1999;3:613-617.)

KEY WORDS: Gastrectomy, gastric emptying, intestinal motility, small bowel transit, intestinal electrical activity

The Roux stasis syndrome, characterized by abdominal pain, nausea, vomiting, and postprandial bloating, appears in approximately 30% of patients at risk.[1] The syndrome results, in part, from the bowel transection required to create the Roux limb.[2,3] The transection disconnects the Roux limb from the duodenal pacemaker. Ectopic pacemakers then appear in the limb between 5 and 40 cm distal to its proximal end.[4] They beat at a slower frequency than the duodenal pacemaker. Also, they drive the pacesetter potentials (PPs), hence contractions, of the proximal portion of the Roux limb in an orad direction, toward the stomach. The slow PP frequency and the orad PP propagation delays gastric emptying and slows Roux limb transit.[2-5]

This study tested the hypothesis that the slow gastric emptying following standard Roux gastrectomy can be improved by taking down the Roux limb, restoring jejunal continuity, and performing an "uncut" Roux operation, with the new gastrojejunostomy being distal to the ectopic pacemakers in the old Roux limb. Uncut Roux operations have been used by others and ourselves in the past to avoid the Roux stasis syndrome and yet prevent bile reflux into the gastric remnant.[6-9]

MATERIAL AND METHODS

The experiments were performed according to the Guidelines of the National Institutes of Health and were approved by the Mayo Clinic's Institutional Animal Care and Use Committee on April 25, 1995.

Animal Preparation

After a 12-hour fast, five female mongrel dogs underwent resection of the distal 6 cm of the stomach, followed by reconstruction of the gastrointestinal

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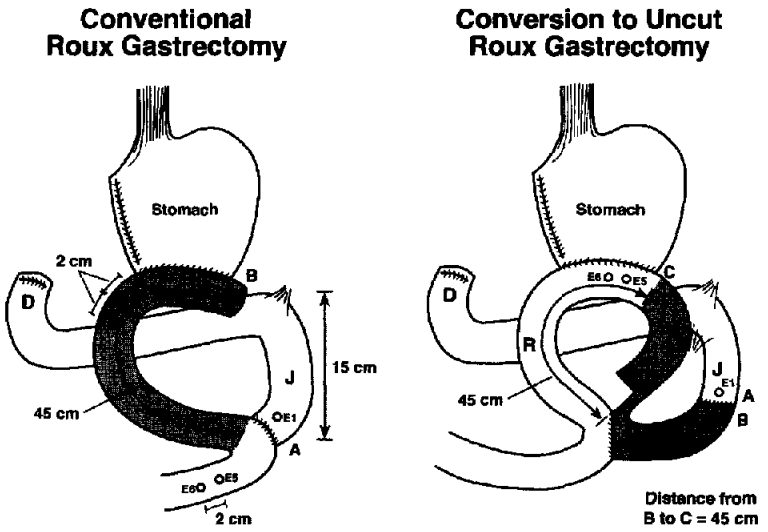

Fig. 1. Canine experimental preparations. **Left,** Distal gastrectomy, Roux-en-Y gastrojejunostomy, placement of electrodes *(E)*. D = duodenum; J = jejunum; shaded area = conventional Roux limb; A = distal end of proximal jejunum; B = proximal end of Roux limb. **Right,** Takedown of Roux gastrojejunostomy, restoration of jejunal continuity (*A* to *B*), uncut Roux limb construction, diverting side-to-side jejunojejunostomy. Distance from B to C is 45 cm. R = uncut Roux limb; shaded area = original conventional Roux limb.

tract with a standard Roux-en-Y gastrojejunostomy. The Roux limb was made of proximal jejunum and was 45 cm in length. Six monopolar silver-silver chloride electrodes were placed on the seromuscular layer of the proximal jejunum, the Roux limb, and the distal jejunum (Fig. 1, *left*). The electrodes were connected by insulated leads to a multipinned socket contained in a plastic cannula, the outer end of which was exteriorized and the inner end anchored to the abdominal wall in the left lower quadrant. In addition to the gastric operation, all dogs also underwent a lateral cervical esophagostomy to allow access to the stomach through the esophagus for gastric emptying studies. The cervical vagal nerves were carefully spared.

After acquisition of baseline myoelectrical and gastric emptying data, the animals underwent a second operation. The Roux limb was taken down, jejunal continuity was restored with an end-to-end jejunojejunostomy, and a side-to-side gastrojejunostomy was constructed using the jejunal segment 45 cm distal to the proximal end of the previous Roux limb. Five centimeters proximal to this anastomosis, a 1 cm circular band of jejunum was excised except for a 1 cm segment along the mesenteric wall. The mucosa of this remaining segment was stripped, thus creating a 1 cm in length neuromuscular bridge between the proximal jejunum and the newly formed Roux limb (Fig. 1, *right*). The bowel lumen distal to this bridge was closed in two layers using sutures. The lumen proximal to the neuromuscular bridge was incorporated into an end-to-side jejunojejunostomy at a site on the efferent jejunal limb 45 cm distal to the gastrojejunostomy. The neuromuscular bridge created by this operation maintains myoneural continuity between the new Roux limb and the more proximal bowel—hence the term, uncut Roux limb.

Myoelectrical Studies

Results of three fasting studies, performed on three different days, were obtained from each dog beginning 1 month after each operation. The studies, which lasted 3 hours each, were performed after the dogs had been fasted for approximately 16 hours overnight. During the study each fully conscious animal was placed in a Pavlov stand, with the electrodes connected to an electronic amplifier/computer acquisition system. The amplified analogue signals were converted to digital signals, sampled at 100 Hz, and displayed in real time on a VGA monitor while simultaneously stored on magnetic media for later analysis. The PP rhythm, configuration, frequency, and direction of propagation were determined at each site of electrode placement using computer analytic techniques previously described.[10] In particular, the direction of PP propagation was determined by which electrode had phase lead, that is, at which electrode the PP occurred first. We recognized that PP propagation cannot be proved without stimulation of an artificial PP and documentation of its pattern of detection by adjacent electrodes.

The shortest absolute difference between each PP event in channel E6 and that in its orad neighbor, channel E5, was determined. A minimum time difference of less than 1.5 seconds was considered to be a propagated PP. Propagated PPs that first occurred in the orad electrode were given a positive time designation and represented aborad propagation. Propagated PPs that first occurred in the aborad electrode

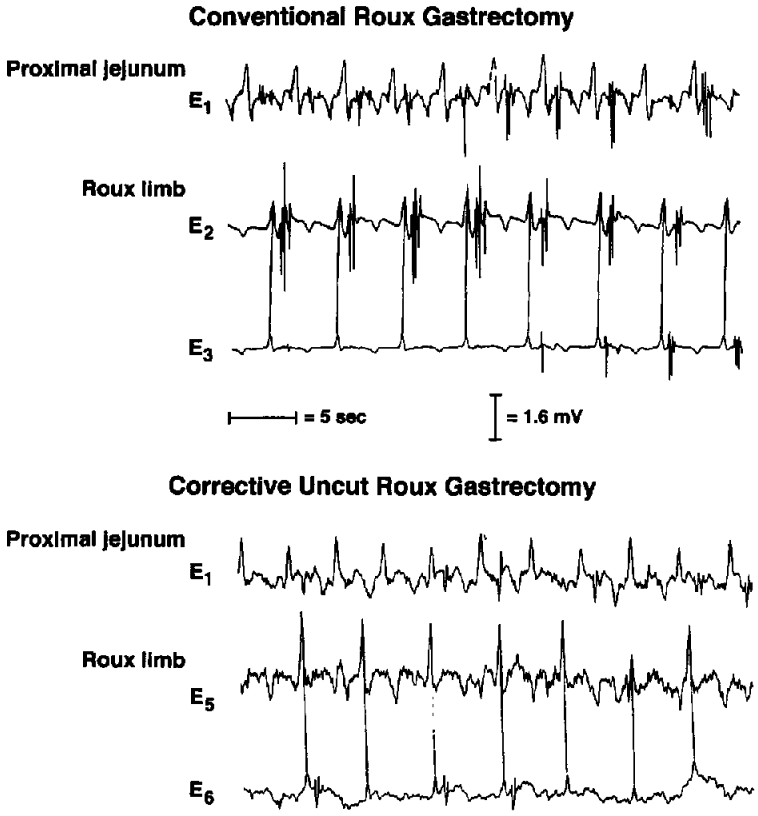

Fig. 2. Canine jejunal electrical tracings. **Upper,** Orad (backward) propagation of PPs in a conventional Roux limb from E_3 to E_2. **Lower,** Aborad propagation of PPs in an uncut Roux limb from E_5 to E_6. E = electrode; PPs = pacesetter potentials. For electrode positions, see Fig. 1.

were given a negative time designation and represented orad propagation. Simultaneous PP events in adjacent electrodes had a minimum time difference of 0, as expected. If no PP event was detected in the orad channel within 1.5 seconds of an event in the aborad channel, an arbitrary number, 3.5, was assigned. Thus each PP event in the aborad channel had an associated propagation time lag associated with it (3.5 for no propagation or a time lag between +1.50 and −1.50 seconds). The percentage of PPs that propagated orad and aborad was then calculated for the electrode pair during each experiment.

Gastric Emptying Studies and Bile Acid Concentration Measurements

After each operation, each dog underwent four studies to determine the rate of gastric emptying. We chose to study emptying of a liquid meal because it allowed us to assess the rate of gastric emptying and the presence or absence of enterogastric reflux using the same test. An orogastric tube was inserted through the esophagostomy and positioned in the stomach where a 250 ml solution of 10% dextrose tagged with 1% polyethylene glycol was instilled. In two studies the gastric content was aspirated 20 minutes later. In the other two studies, the stomach was aspirated 1 hour later. The stomach was then washed with 100 ml of distilled water and the wash collected.

The volume of gastric instillate emptied (VE) was determined (in milliliters) using the following formulas:

$$V_E = 250 - V_R \text{ and}$$

$$V_R = V_A (C_A/C_I) + V_W (C_W/C_I),$$

where V_E = volume emptied (ml), V_R = volume residual (ml), V_A = volume aspirated (ml), V_W = volume of wash (ml), C_A = concentration of polyethylene glycol in aspirated fluid (g/L), C_I = concentration of PEG in instillate (g/L), and C_W = concentration of polyethylene glycol in wash fluid (g/L). The samples of gastric aspirates were also used to determine the concentration of bile acids present in the gastric content.

RESULTS

Myoelectrical Patterns

The PPs in the Roux limb of the dogs with a conventional Roux gastrectomy had a configuration similar to that of the proximal jejunum (Fig. 2). The frequency of the PPs in the Roux limb, however, was

slower (14 ± 0.4 cpm) than that in the proximal jejunum (19 ± 0.3 cpm, $P < 0.05$). Moreover, approximately half of the PPs (53% ± 7%) in the proximal portion of the Roux limb were propagated in an oral direction toward the stomach (Fig. 2).

The frequency of the PPs in the original Roux limb remained slowed (14 ± 0.5 cpm) after the takedown of the limb with jejunal reconstruction. However, the frequency of the PPs in the uncut Roux limb connected to the original Roux limb by a neuromuscular bridge did not decrease further (13 ± 0.6 cpm, $P > 0.05$). Moreover, the percentage of PPs propagating aborally in the uncut Roux limb (81% ± 4%) was greater than in the conventional Roux limb before reconstruction (53% ± 7%; $P < 0.05$) (see Fig. 2).

Gastric Emptying

The dogs with the conventional Roux gastrectomy emptied 36% ± 10% of the gastric liquid meal from their stomachs in 20 minutes, whereas by 60 minutes they emptied 72% ± 13% of the meal ($P < 0.05$). Similarly, the volume emptied after the uncut Roux reconstruction at 20 minutes was greater than that emptied at 60 minutes (35% ± 15% vs. 71% ± 17%, $P < 0.05$). However, the uncut Roux operation did not speed either the 20-minute or the 60-minute rate of gastric emptying compared to that found after the conventional Roux operation.

Bile Acid Concentrations in the Stomach

Both operations were equally effective in diverting bile from the gastric remnant. The concentrations of bile acids in the gastric aspirates were low before and after the corrective operation (0.7 ± 0.2 μmol/L vs. 0.6 ± 0.1 μmol/L; $P > 0.05$).

DISCUSSION

These experiments confirm our previous findings that dividing the bowel during construction of a conventional Roux limb separates the limb from the proximal duodenal pacemaker. Ectopic pacemakers then appear in the limb at sites 5 to 40 cm distal to the proximal end of the limb. These pacemakers generate PPs at a slower frequency than the duodenal pacemaker. Moreover, the PPs they generate drive the portion of the limb proximal to them in a backward or orad direction, thus slowing transit through the limb and retarding gastric emptying.[3,5,11]

In the present tests, taking down the conventional Roux limb, restoring jejunal continuity with a hand-sewn jejunojejunostomy, and creating an uncut Roux limb distal to the old Roux limb did result in more aborally propagating PPs in the new Roux limb than in the old limb. By moving the gastrojejunostomy of the uncut Roux limb distal to the site of ectopic pacemakers in the old Roux limb, we were able to construct a Roux limb in which most PPs were propagating in the aborad direction, as is usually found in health. Because of this, we hypothesized that gastric emptying would be speeded. However, this did not prove to be the case; gastric emptying was not speeded after the reconstructive operation.

We believe gastric emptying was not speeded after the second operation because, although the uncut Roux operation did increase the percentage of PPs propagating aborally in the new uncut Roux limb, it did not increase the PP frequency. The continuing slow frequency of PPs in the new uncut Roux limb likely meant fewer propulsive contractions in the limb, slower Roux limb emptying, and slower gastric emptying. Another explanation might be that although the uncut Roux reconstruction did improve the percentage of PPs propagating aborally in the Roux limb, it did not restore the pattern to the 100% aboral propagation usually found in health. The 19% of PPs still propagating orally may have continued to slow gastric emptying. Clearly, in dogs when 100% aborad propagation is restored in Roux limbs by electrical pacing, Roux limb stasis is abolished and gastric emptying is restored to the control level.[5]

The neuromuscular bridge used to create the new uncut Roux limb did not seem to be the problem. The neuromuscular bridge apparently allowed PPs to pass from the more proximal bowel across the bridge into the new limb in these tests, because no drop in PP frequency across the neuromuscular bridge was detected after the corrective operation. However, such a drop might have been difficult to detect in these experiments because the bowel distal to the neuromuscular bridge would likely have been oscillating at about 14 cpm, even if propagation across the bridge were not occurring. Nonetheless, we have found in previous studies with uncut Roux limbs that the frequency of PPs in uncut Roux limbs is similar to that in the duodenum and proximal jejunum, no ectopic pacemakers appear in the limbs, and gastric emptying is not slowed.[12] Thus a neuromuscular bridge does preserve enteric myoneural continuity.

The persistent physiologic disturbance found with our present reconstructive operation was that the hand-sewn anastomosis we used to rejoin the conventional Roux limb to the proximal jejunum did not restore myoneural continuity sufficiently to allow PPs to propagate from the faster-beating proximal jejunum across the anastomosis into the old Roux limb—hence the continuing slow PP frequency in the

old Roux limb and therefore in the new uncut Roux limb. This interpretation is supported by past tests from our laboratory, which showed that restoring the frequency of PPs in the Roux limb to the faster frequency of healthy bowel using intestinal pacing restored gastric emptying to the control.[11]

Cheng et al.[13] have also studied gastric emptying after Roux gastrectomy. Their experiments suggest that preserving luminal continuity of the jejunum, rather than preserving neuromuscular continuity, is of greater importance in maintaining gastric emptying after gastrectomy. Two important differences exist between their tests and ours. All of their animals had gastric vagotomy with their gastrectomy, whereas none of ours did. Also, they studied gastric emptying of solids, while we studied emptying of liquids. These differences likely explain the differing results. Moreover, we found in past tests that disrupting myoneural continuity of the "unvagotomized" jejunum by transection and anastomosis, while maintaining luminal continuity, slows transit of liquid chyme through the intestine distal to the disruption.[4]

One important property of the conventional Roux-en-Y gastrojejunostomy is that it provides near-complete diversion of pancreaticobiliary secretions away from the gastric remnant. Fortunately the uncut Roux operation is as effective as the conventional Roux operation in this regard. The concentration of bile salts in the gastric lumen in the present tests was as small after the uncut Roux operation as it was after the conventional Roux operation. This was also true in previous tests we have done with an uncut Roux limb.[8]

In conclusion, taking down a conventional Roux limb after Roux gastrectomy, restoring jejunal continuity with a hand-sewn jejunojejunostomy, and constructing an uncut Roux limb with the gastrojejunostomy distal to the segment used for the original Roux limb increased the percentage of aborally propagating PPs in the new Roux limb. However, the operation did not completely restore aborad propagation of PPs in the new limb, nor did it speed the frequency of the PPs in the new Roux limb. Consequently it did not speed gastric emptying of a liquid meal. Thus, at this point, the operation described in this report cannot be recommended for patients with the Roux syndrome. Perhaps the use of a more precise microsurgical surgical technique to restore jejunal continuity would have allowed PPs to propagate across the jejunojejunostomy and into the uncut Roux limb. We have found recently that a microsurgical anastomosis can restore PP propagation, in part, across a jejunojejunostomy.[14]

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Targeting Molecular Pathways With Camptothecin as Novel Therapy for Gastric Cancer

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Novel chemotherapeutic agents are needed to treat gastric cancer for which the prognosis remains dismal. The antitumor alkaloid camptothecin (CPT) may be useful in the treatment of certain solid tumors; however, its effects on gastric cancer are largely undefined. The purpose of our study was to characterize the effects of CPT on human gastric tumors in vivo and to determine the cellular mechanisms involved in CPT-mediated inhibition. Two human gastric cancers, WIL and TOR, were transplanted subcutaneously into athymic nude mice. After tumors reached 50 to 100 mm^2, mice were randomized into three groups to receive injections of either low-dose CPT (5 mg/kg), high-dose CPT (10 mg/kg), or vehicle (control) intraperitoneally 3 days a week for 3 weeks. Tumors were measured and weighed, and protein levels of the cell cycle inhibitor, $p21^{Waf1/Cip1}$, and the antiapoptotic protein, Bcl-2, were assessed. Both dosages of CPT significantly inhibited growth of WIL and TOR gastric tumors. CPT (10 mg/kg) reduced tumor size compared to baseline, establishing this as a tumoricidal dosage. Treatment with CPT was associated with increased levels of $p21^{Waf1/Cip1}$ and decreased levels of Bcl-2. CPT effectively kills human gastric cancers associated with increased levels of $p21^{Waf1/Cip1}$ and decreased levels of Bcl-2. By activating cell cycle withdrawal and cell death through induction of $p21^{Waf1/Cip1}$ and downregulation of Bcl-2, CPT may be an effective agent for gastric cancer. (J GASTROINTEST SURG 1999;3:618-624.)

KEY WORDS: Gastric cancer, camptothecin, apoptosis, cell cycle, Bcl-2

Gastric cancer is the second leading cause of cancer-related deaths in the world and has reached epidemic proportions in countries such as Japan and Chile.[1] Although the incidence of this disease has decreased in the United States, 23,000 new cases are diagnosed annually and, furthermore, 15,000 people die of gastric cancer each year.[1] Moreover, because of the frequent occurrence of advanced disease, which precludes curative resection, overall survival has improved little in 50 years.[1,2] Unfortunately, to date, standard chemotherapeutic agents such as doxorubicin, 5-fluorouracil, and mitomycin C (alone or in combination) have produced only limited results.[1,2] Novel therapeutic agents, which target specific molecular pathways, are needed in the treatment of patients with gastric cancer.

Camptothecin (CPT), a plant antitumor alkaloid, inhibits topoisomerase I, an enzyme essential to DNA replication and repair. In addition to inducing programmed cell death (i.e., apoptosis) by introducing irreparable double-stranded breaks in cellular DNA,[3-6] CPT also has been shown to induce both expression of early response genes and cellular growth arrest in several types of cancer cells.[4-6] Furthermore, recent studies have demonstrated that novel semisynthetic derivatives of CPT may be effective against certain solid tumors, including gastric cancer.[3,7,8] Nevertheless, the effects of CPT on gastric cancer and the cellular mechanisms involved in CPT-mediated gastric cancer cell death have not been characterized completely.

Frequent cellular responses to chemotherapy include growth arrest and apoptosis. Cellular growth arrest is mediated, in part, by the complex interaction of cyclins, cyclin-dependent kinases, and the cyclin-dependent kinase inhibitors, which regulate transition through key checkpoints in the cell cycle.[9] An impor-

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tant and ubiquitous cyclin-dependent kinase inhibitor protein, $p21^{Waf1/Cip1}$ (p21), has been shown to play a significant role in mediating both cellular growth arrest and differentiation.[9,10] Furthermore, increased expression of p21 has been shown to be temporally associated with cellular differentiation and apoptosis in colon cancer and leukemic cells,[11,12] suggesting that p21 may be directly involved in the apoptotic pathway in certain cells. In addition to causing perturbations in the cell cycle, chemotherapeutic agents may mediate the differential regulation of members of the Bcl-2 family of apoptosis-related proteins (i.e., which consist of both promoters and inhibitors of apoptosis), thus inducing programmed cell death.[13] Cytotoxic agents may trigger cell death by either the inhibition of antiapoptotic proteins (e.g., Bcl-2 and Bcl-X_L) or the induction of proapoptotic proteins (e.g., Bax and Bcl-X_S). Furthermore, it has been suggested that the level of expression of these proteins in tumors may correlate with either tumor aggressiveness or response to treatment,[14-20] thus potentially providing a biologic marker that may better predict patient outcome and prognosis.

The purpose of our study was to characterize the effects of CPT on human gastric cancers xenotransplanted to athymic nude mice, and to determine the potential cellular mechanisms involved in CPT-mediated gastric cancer cell death.

MATERIAL AND METHODS

CPT (Sigma Chemical Co., St. Louis, Mo.) was dissolved in dimethyl sulfoxide (DMSO) (Sigma Chemical Co.) and diluted to the appropriate concentration with saline solution. The pH of the CPT solution was adjusted to 7.0 with sodium hydroxide and stored in plastic vials at 4° C in the dark. For control experiments, saline with 10% (volume/volume) DMSO was stored in plastic vials at 4° C in the dark.

Male athymic nude mice (Balb/c; 4 to 5 weeks old, 25 to 30 g) were housed under pathogen-free conditions with 12-hour light-dark cycles, in accordance with the recommendations of the National Research Council.[21] Mice received both standard rodent chow (Autoclavable Rodent Chow No. 5010; Ralston Purina, St. Louis, Mo.) and sterile water ad libitum.

Human Gastric Adenocarcinoma Xenografts and Experimental Protocol

The human gastric adenocarcinomas, WIL and TOR, were established in our laboratory as long-term tumor lines in athymic nude mice from patients with stage IV and stage II tumors, respectively. Both tumor lines were maintained in mice by the periodic retransplantation of small tumor pieces (3 mm^2) subcutaneously to the flanks of nude mice. The histology of these tumor lines was routinely checked to ensure that tumors maintained features of the original tumors. For experimentation, either WIL or TOR tumor pieces (9 to 16 mm^2) were transplanted subcutaneously into 24 nude mice. After tumors reached 50 to 100 mm^2, mice were randomized to three groups (n = 8 per group) to receive injections of either low-dose CPT (5 mg/kg), high-dose CPT (10 mg/kg), or vehicle (control) intraperitoneally 3 days a week for three consecutive weeks. Tumor size was measured biweekly. After 21 to 25 days, the mice were killed; tumors were measured and weighed and then immediately frozen at −70° C for protein extraction. Because of the significantly smaller size of tumors in the high-dose CPT groups, tumors in this group were pooled prior to protein extraction. In addition, a separate piece of each tumor was preserved in 10% buffered formalin for subsequent histologic examination.

Western Blot Analysis

After cellular protein was extracted from the tumors and concentrations were determined, cellular lysates (100 μg/lane for p21; 300 μg/lane for Bcl-2) were resolved by 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to immobilon-P membranes (Millipore Corp., Bedford, Mass.). Membranes were blocked in 5% nonfat milk overnight and then incubated with monoclonal mouse p21 or polyclonal rabbit Bcl-2 primary antibody (both from Oncogene Science, Inc., Cambridge, Mass.) for 3 hours. After washing, membranes were incubated with horseradish peroxidase–conjugated antimouse (p21) or antirabbit (Bcl-2) secondary antibody for 45 minutes and then developed using the enhanced chemiluminescence system (Amersham Corp., Arlington Heights, Ill.). To determine the relative expression of p21 and Bcl-2, densitometry was performed on all developed blots using the Eagle Eye II gel imaging system (Stratagene, La Jolla, Calif).

Statistical Analysis

All data were expressed as mean ± standard error of the mean (SEM). For comparison of densitometic findings for p21 and Bcl-2 for control and CPT-treated mice, Student's unpaired *t* test for independent means was used. For comparison of growth curves from control and CPT-treated mice, the Kruskal-Wallis test was performed. For comparison of body weight or tumor weight, analysis of variance with Fisher's least significant difference test for mul-

tiple comparisons was used. A P value <0.05 was considered significant.

RESULTS

CPT Effectively Inhibits the Growth of Human Gastric Cancers

There was one death in the WIL tumor–bearing group and one death in the TOR tumor–bearing group; each had been treated with high-dose CPT (10 mg/kg). There were no deaths in the low-dose CPT (5 mg/kg) group for either tumor. In addition, CPT treatment produced a significant weight loss in TOR tumor–bearing mice; there was a similar trend of weight loss in WIL tumor–bearing mice, but this did not reach statistical significance (data not shown).

CPT significantly inhibited the growth of the transplanted human gastric cancers, WIL (Fig. 1) and TOR (Fig. 2), in a dose-dependent fashion. Treatment with low-dose CPT (5 mg/kg) resulted in a 60% and a 50% decrease in WIL and TOR tumor sizes, respectively. Following treatment with high-dose CPT (10 mg/kg), there was a 79% and a 70% decrease in WIL and TOR tumor sizes, compared to the control group, respectively. Comparing the two dosages of CPT in WIL tumors, there was a significant (i.e., 51%) decrease in tumor size following treatment with high-dose CPT. In TOR tumors, there was a similar trend between the growth-inhibiting effects of the two dosages of CPT, but this did not achieve statistical significance. However, CPT (10 mg/kg) produced a significant (i.e., 54% and 30%) regression in tumor size from baseline in both WIL and TOR tumors, respectively, thus demonstrating that high-dose CPT is tumoricidal for these gastric cancers. CPT also significantly inhibited the weight of both gastric tumors. CPT (5 mg/kg) inhibited tumor weight by 64% and 70% in WIL and TOR tumors, compared to the control group, respectively. Furthermore, the tumoricidal dosage of CPT (10 mg/kg) produced a 87% decrease in WIL tumor weight and a 79% decrease in TOR tumor weight, compared to

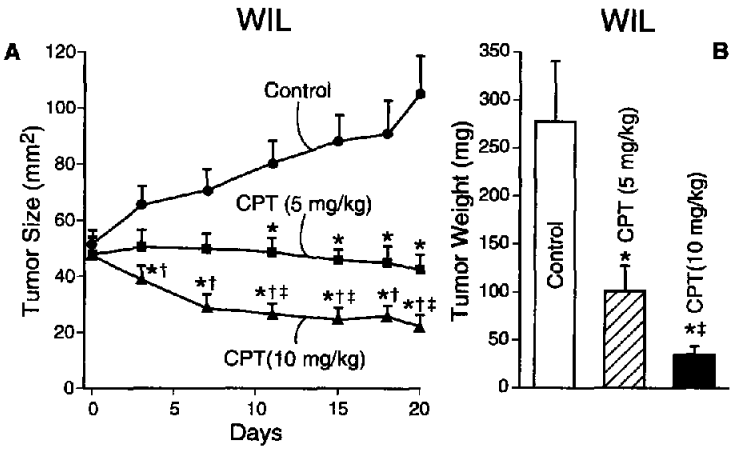

Fig. 1. Effects of CPT on tumor size **(A)** and weight **(B)** of WIL tumors treated with either control injection (white bar; n = 8), low-dose CPT (5 mg/kg; hatched bar; n = 8), or high-dose CPT (10 mg/kg; black bar; n = 8). Data represent mean ± SEM. * = P <0.05 vs. control; † = P <0.05 vs. baseline; ‡ = P <0.05 vs. low-dose CPT.

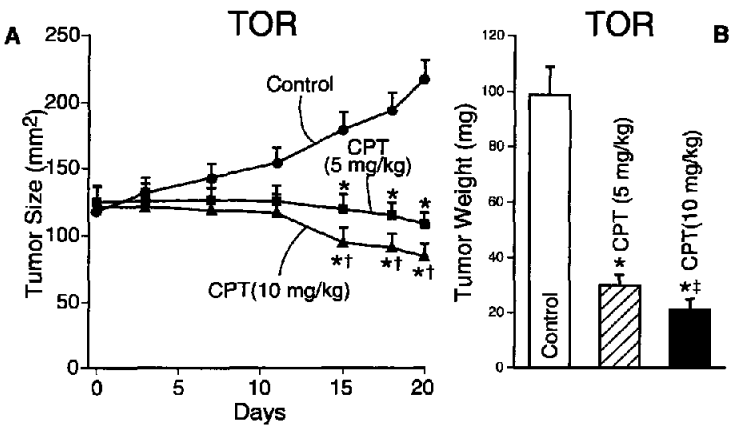

Fig. 2. Effects of CPT on tumor size **(A)** and weight **(B)** of TOR tumors treated with either control injection (white bar; n = 8), low-dose CPT (5 mg/kg; hatched bar; n = 8), or high-dose CPT (10 mg/kg; black bar; n = 8). Data represent mean ± SEM. * = P <0.05 vs. control; † = P <0.05 vs. baseline; ‡ = P <0.05 vs. low-dose CPT.

control values. Comparing the different dosages of CPT, there was a 65% decrease in tumor weight in WIL tumors and a 30% decrease in tumor weight in TOR tumors following treatment with high-dose CPT. Moreover, histologic examination of both WIL and TOR tumors demonstrated loss of overall cellular mass and replacement with fibrosis (Fig. 3).

Tumor Regrowth Is Slowed or Completely Inhibited Following Treatment With CPT

To assess whether CPT produces long-term inhibition of tumor growth, WIL tumors, treated with CPT for 21 days, were observed for an additional 45 days after treatment (Fig. 4). The doubling time for CPT-treated tumors during this 45-day period of regrowth was 23 days, which was significantly longer than the doubling time of 19 days for control mice, as noted during the initial 21-day treatment period. In addition, two of the CPT-treated tumors did not regrow at all following cessation of treatment. Nevertheless, CPT-treated tumors, which did resume growth, eventually reached a maximal size comparable to that of untreated tumors. Collectively these results demonstrate that CPT effectively kills human gastric tumors and results in a significant slowing or complete cessation of tumor growth.

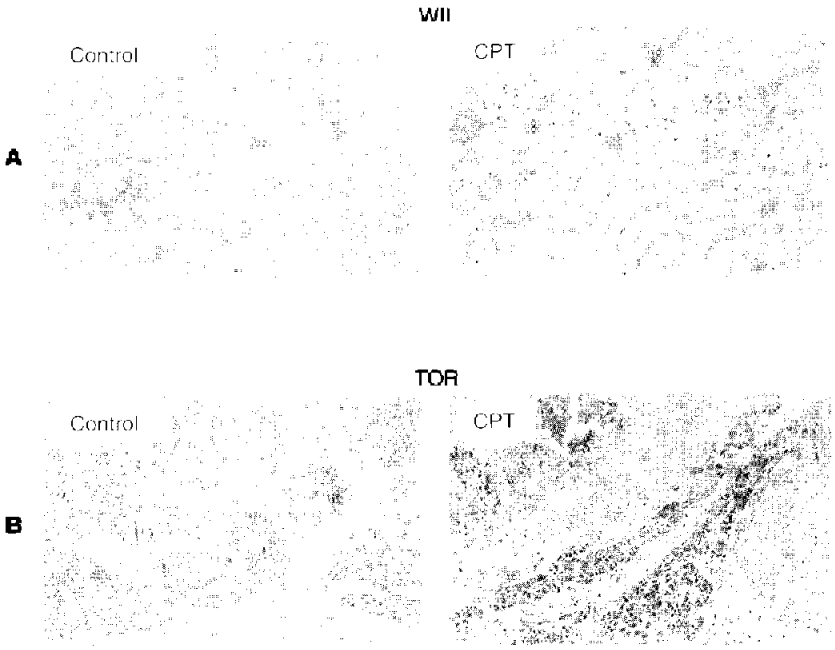

Fig. 3. Representative histologic sections of WIL (A) and TOR (B) tumors following treatment with either CPT (10 mg/kg) or control injection. CPT results in a significant loss of overall cellular mass and replacement by fibrosis.

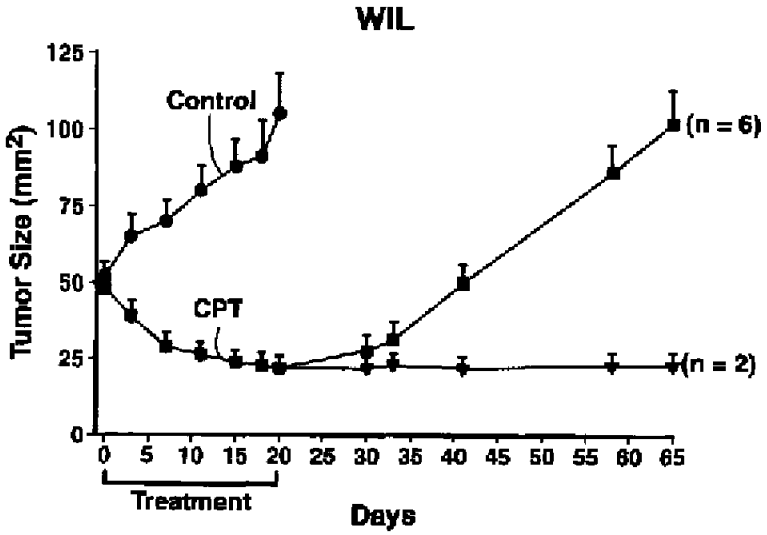

Fig. 4. Regrowth of WIL gastric tumors treated for 21 days with either CPT (10 mg/kg) or control injection and then monitored for an additional 45 days after treatment. The doubling time for CPT-treated tumors that resumed growth (n = 6) was significantly increased to 23 days compared to 19 days for the control group. Furthermore, two tumors did not resume growth following cessation of CPT treatment.

Treatment With CPT Increases p21 and Decreases Bcl-2 Protein Expression

To determine whether treatment with CPT alters expression of the cell cycle inhibitor protein p21, in association with growth inhibition, Western blot analyses were performed (Fig. 5, *A*). There was a 54% increase in the expression levels of p21 in WIL tumors treated with CPT (5 mg/kg) compared to control values. In addition, an increase in p21 expression was noted in the pooled gastric tumors from mice treated with CPT (10 mg/kg). These findings are consistent with our previous results for the human gastric cancer cell line, SIIA in which p21 messenger RNA and protein levels were increased following exposure to CPT.[22] Furthermore, recent studies have demonstrated a correlation between p21 tumor levels and responsiveness of patients with gastric cancer to chemotherapy,[14,18] suggesting that increased expression of p21 may be an important cellular mechanism contributing to gastric cancer growth inhibition in response to cytotoxic agents. Collectively these results suggest that CPT-mediated growth inhibition of gastric cancer may involve induction of p21.

To determine whether treatment with CPT alters expression of the apoptosis inhibitor Bcl-2, in association with growth inhibition of gastric tumors, Western blot analysis for Bcl-2 was performed (Fig. 5, *B*). Treatment with CPT significantly inhibited levels of Bcl-2 in WIL tumors, resulting in a 36% decrease in mean protein levels by densitometric analysis in low-dose CPT-treated mice compared to control mice. Furthermore, the level of Bcl-2 expressed in the tumors of high-dose CPT–treated mice approached undetectable levels. These findings of decreased Bcl-2 expression following treatment with CPT are consistent with our previous in vitro results using the SIIA gastric cancer cell line.[22]

DISCUSSION

In the present study we have identified both cytostatic and cytotoxic dosages of CPT for two transplantable human gastric cancers, WIL and TOR, with high-dose CPT causing regression of these tumors. In addition, we have shown that CPT, at a cytotoxic dosage, significantly or completely prevented the regrowth of residual tumors following cessation of treatment. Furthermore, we have determined that the growth inhibitory effects of CPT are associated with increased levels of the cell cycle inhibitor p21 and decreased levels of the apoptosis inhibitor Bcl-2 in the tumors.

Treatment of mice bearing human gastric tumors with CPT (5 and 10 mg/kg) resulted in increased protein levels of cell cycle inhibitor protein p21. Consistent with these findings, we noted previously the increased expression of p21 at both the gene and protein level in SIIA gastric cancer cells following exposure to CPT in vitro.[22] In addition, other studies have reported increased levels of p21 following treatment with CPT in breast[23] and brain[24] cancer cells, suggesting that induction of p21 may be a common cellular response to exposure to CPT. In addition to mediating cellular growth arrest, p21 may be closely linked to the apoptotic pathway[11,12] and has been suggested to be an important cellular mediator of tumor suppression.[16,17] Recent in vitro and in vivo studies[25,26] have suggested that the altered expression of cell cycle proteins, such as p21, may contribute to either gastric carcinogenesis or deregulated growth of established

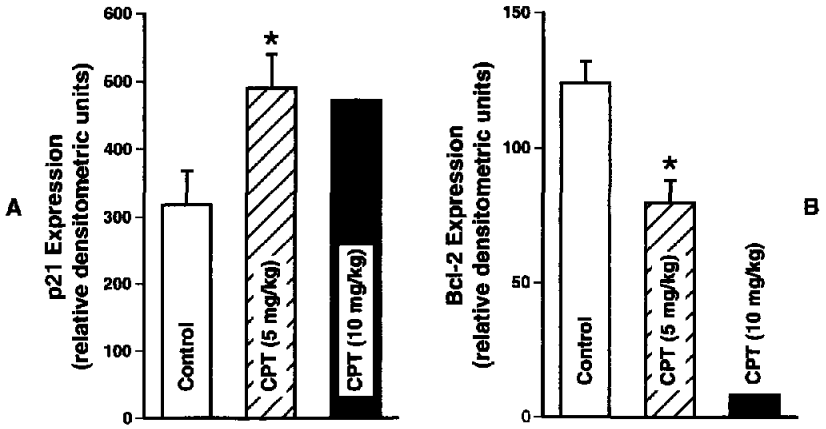

Fig. 5. Analysis of p21 **(A)** and Bcl-2 **(B)** protein expression by densitometry of Western blots in WIL tumor–bearing mice treated with either control injection (white bar; n = 6), low-dose (5 mg/kg) CPT (hatched bar; n = 4), or high-dose (10 mg/kg) CPT (black bar; pooled). Data represent mean densitometric values ± SEM. * = $P < 0.05$ vs. control.

cancers. Furthermore, the importance of p21 in the growth regulation of gastric cancer has been suggested by recent clinical studies,[14,18] which have shown that tumor levels of p21 correlate with the effectiveness of chemotherapy in patients with gastric cancer. Collectively, the results of our present study suggest that p21 may play a significant role in CPT-mediated growth inhibition and/or regression of gastric cancers.

Gastric tumor growth inhibition is associated with the downregulation of the antiapoptotic protein Bcl-2. Following treatment of tumor-bearing mice with CPT, we noted the decreased expression of Bcl-2, which reached nearly undetectable levels after treatment with a tumoricidal dosage of CPT (10 mg/kg). These data recapitulate our previous results, which demonstrated decreased Bcl-2 messenger RNA and protein levels in SIIA gastric cancer cells following treatment with CPT in vitro.[22] The downregulation of Bcl-2 has been associated with the induction of apoptosis in certain types of cancer cells following exposure to CPT, but this may be cell type specific.[24] Furthermore, the differential expression of Bcl-2 may be an important mediator in either chemotherapy-induced killing of gastric cancer (due to Bcl-2 inhibition) or, alternatively, gastric carcinogenesis (due to Bcl-2 upregulation). In support of this hypothesis, Bcl-2 levels have been shown to correlate with cisplatin- or 5-fluorouracil–induced apoptosis in either gastric cancer cell lines[27] or gastric tumors,[14,20,28] respectively. In addition, elevated levels of Bcl-2 in proximity to the gastric remnant following resection have been implicated in the development of gastric cancer subsequent to partial gastrectomy,[29] and the study by Cho and Kim[26] suggested that expression of Bcl-2 was associated with the early stages of gastric tumorigenesis. Collectively these results and the findings from our present study suggest that inhibition of Bcl-2 may be an important cellular mechanism contributing to CPT-mediated gastric cancer cell death and tumor growth inhibition.

Besides serving as potential targets for chemotherapeutic agents, a number of studies have suggested that either p21 or Bcl-2 may be a useful prognostic indicator for patients with gastric cancer.[14-17,19,20,30] Gomyo et al.[15] and Ogawa et al.[16] independently have shown that the 5-year survival rate in patients with p21-positive tumors is significantly higher than that for p21-negative patients and that loss of immunohistochemical expression of p21 in gastric cancers is associated with both increased stage (including metastatic disease) and risk of recurrence following resection, respectively. However, the role of Bcl-2 as a prognostic indicator may be less clear, as some recent studies have demonstrated a correlation between Bcl-2 expression and the efficacy of chemotherapy in patients with gastric cancer, whereas others have failed to show a similar correlation.[14,20,28,31]

CONCLUSION

We have demonstrated that CPT is an effective chemotherapeutic agent against human gastric cancers transplanted to athymic mice, capable of producing both tumor regression and long-term, if not permanent, growth inhibition. In addition, treatment of gastric tumors with CPT was associated with induction of apoptosis and the increased expression of the cell cycle inhibitor p21, and the decreased expression of the apoptosis inhibitor Bcl-2, suggesting that the differential expression of these proteins may contribute to CPT-mediated tumor suppression.

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Discussion

Dr. B. Bass (Baltimore, Md.). Have you had a chance to look at the actual cell cycle status of your tumors? Have you used flow cytometry, for example, to look for apoptosis or for specific patterns of cell cycle withdrawal?

Dr. Litvak. We have not looked at the cell cycle of the tumors. Previously we examined cells in vitro and noted that there was cell cycle withdrawal and growth arrest. We have done staining for apoptosis in these tumors as well, and there is evidence that this seems to be the method by which the cells undergo cell death induced by camptothecin.

Dr. R. Postier (Oklahoma City, Ok.). One of the major upregulators of p21 is p53. Could you tell us about the p53 mutation status of these tumors?

Dr. Litvak. Unfortunately it is not known. These tumors were collected in the operating room and have not been fully characterized. As I said earlier, we have looked at cells in culture and characterized them as far as the p53 expression in relation to the p21 expression that was induced by camptothecin. It appeared that there was a dependency on p53, although other investigators have not demonstrated in other cell lines that this is always the case with camptothecin.

The Continent Ileostomy: Long-Term Durability and Patient Satisfaction

Virginia R. Litle, M.D., Susan Barbour, R.N., Theodore R. Schrock, M.D., Mark L. Welton, M.D.

The long-term results of the continent ileostomy are controversial. Durability and patient satisfaction were evaluated by analyzing the outcome in 129 consecutive patients who had a continent ileostomy performed by one surgeon at the University of California, San Francisco, between 1975 and 1995. A quality-of-life questionnaire was sent to all patients for whom addresses were available (n = 121). Late outcome data could be obtained for 85 (66%) of the 129 patients. Three of the 85 patients died with their continent ileostomies but of unrelated causes. Fifty-one (60%) of 85 patients currently have the continent ileostomy (group A) (mean 15.1 years, range 2.7 to 21.7 years), whereas 31 (36%) of 85 have undergone conversion of continent ileostomy to conventional ileostomy (group B) (mean 5.4 years, range 0.2 to 20.4 years). Patients in group A underwent fewer major postoperative revisions (mean 0.7, range 0 to 4) than patients in group B (mean 1.3, range 0 to 8) (*t* test, $P = 0.088$). The indications for pouch removal included valve dysfunction (42%), refractory pouchitis (23%), multiple fistulas (26%), Crohn's disease (6%), and other (16%) (four patients had two indications). Eighty-seven percent of survey respondents in group A considered their present state of health to be better than before their continent ileostomies. Fifty-seven percent and 82% of respondents in group A were not limited at all in regard to vigorous or moderate activity, respectively. Although in approximately one third of patients the pouch had to be removed, 97% of the remaining two thirds have a good to excellent outcome. (J GASTROINTEST SURG 1999; 3:625-632.)

KEY WORDS: Ulcerative colitis, continent ileostomy

The continent ileostomy (CI) was first described by Kock,[1] in 1969, as an alternative to the conventional ileostomy. It became popular in the 1970s and early 1980s, but restorative proctocolectomy with ileoanal pouch has largely replaced it as the procedure of choice. CI has lost favor for three main reasons: (1) the operation is technically difficult; (2) complication and pouch resection rates are high; and (3) restorative proctocolectomy allows defecation per anus with no permanent stoma. Despite these issues, it was our impression that many patients with CI are satisfied with the operation. We reviewed our series to determine what factors, if any, might predict long-term pouch survival and patient satisfaction.

MATERIAL AND METHODS

Continent ileostomy was performed in 129 consecutive patients by one surgeon (T.R.S.) between December 1975 and June 1995. The patients included 64 women and 65 men. Ages ranged from 10 to 67 years (median 31 years). The primary disease was ulcerative colitis in 119 (92%) of 129 patients, familial polyposis in 7 (5%) of 129, Crohn's disease in 2 (2%) of 129, and multiple neoplasms in one (1%).

Patient satisfaction and pouch durability were evaluated in these 129 patients. A modified 36-item short form (SF-36) health survey was sent to all patients for whom addresses were available (n = 121). The survey included questions regarding general health status, function and maintenance of the CI, and overall long-term satisfaction with the procedure. Phone calls were made and one mail reminder was sent to patients asking them to complete the questionnaire. Chart review was performed to obtain additional information including number and complexity of revisions, status of the pouch, and indications for excision. Details of the CI procedure were

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obtained from operative reports and the personal records of the surgeon.

Major reoperations after the CI included transabdominal revisions and parastomal herniorrhaphies. Incision and drainage of parastomal abscesses, reduction of prolapse, and revisions under local anesthesia were classified as minor revisions.

Statistical methods applied included chi-square analysis and the unpaired *t* test.

RESULTS

Late outcome data could be obtained for 85 (66%) of the 129 patients. Addresses were not available for 8 of the 129 patients. Surveys were returned from 56 (46% response rate) of the 121 patients, and follow-up data were obtained by phone calls and review of medical records in an additional 29 (22%) of the 129 patients (Fig. 1). Follow-up ranged from 1 to 21 years (mean 11.4 years). Indications for operation included ulcerative colitis in 79 (93%) of 85, familial polyposis in 5 (6%) of 85, and multiple neoplasms in 1 (1%) of 85 patients. Thirty-three (39%) of the 85 patients had undergone an elective one-stage operation with total proctocolectomy and creation of a CI, whereas the remainder had undergone a variety of emergent and elective procedures prior to CI creation (Table I).

Table I. Initial procedure in patients who retained their continent ileostomy (group A) and those who underwent excision of their continent ileostomy (group B)

Initial procedure	Group A (n = 54)	Group B (n = 31)	Total (n = 85)
Proctocolectomy with continent ileostomy	26 (48%)	7 (23%)	33 (39%)
Emergent proctocolectomy with conventional ileostomy	3 (6%)	3 (10%)	6 (7%)
Elective proctocolectomy with conventional ileostomy	20 (37%)	16 (52%)	36 (42%)
Proctocolectomy with ileoanal anastomosis	3 (6%)	4 (13%)	7 (8%)
Right colectomy and abdominoperineal resection	1 (2%)	0 (0%)	1 (1%)
Left colectomy	1 (2%)	0 (0%)	1 (1%)
Total abdominal colectomy	0 (0%)	1 (3%)	1 (1%)

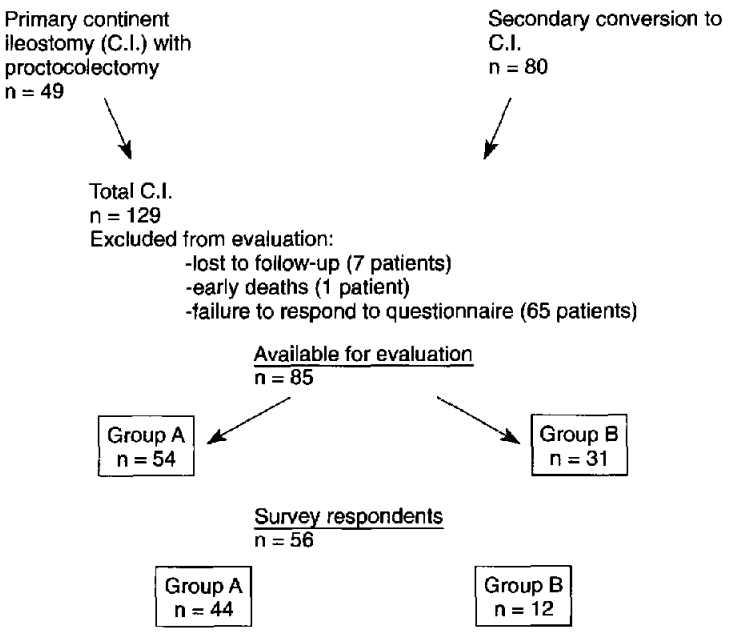

Fig. 1. Distribution of continent ileostomy *(CI)* patients and their availability for late outcome assessment obtained by questionnaire and chart review. Group A = CI retained; Group B = CI excised.

Seven of the 129 patients were known to have died of causes unrelated to the CI. Three patients had died with their CI intact, three had undergone pouch resection, and in one patient the CI status at the time of death was unknown. Of the 85 patients for whom follow-up data were available, 54 (64%) had a functional pouch (group A), whereas 31 patients (36%) had their CI excised (group B). The indications for pouch excision are presented in Table II. Indications classified as "other" included two patients with chronic bleeding from the CI, one patient with chronic difficulty intubating the CI, and a fourth patient with multiple revisions for prolapse. The fifth patient requiring pouch excision and classified as "other" had had familial polyposis and developed a desmoid tumor in the mesentery of the pouch.

In construction of the CI valve, polypropylene (Marlex, C.R. Bard, Murray Hill, N.J.) slings or pledgets were used to prevent slippage in 45 patients from September 1976 through November 1984. One patient had a Gore-Tex (W.L. Gore & Associates, Flagstaff, Ariz.) sling (1983) and another had a Teflon (C.R. Bard) sling (1987). Nineteen (40%) of 47 patients in whom foreign bodies (Marlex, Gore-Tex, or Teflon) were used to create the CI subsequently required excision, whereas 28 (60%) retained their pouch (Table III). Of the 38 patients who underwent major revisions of their CI, 21 (55%) had a foreign body present compared to 17 (45%) without a foreign body. The mean number of major revisions in patients with a foreign body and maintenance of the pouch (0.71 ± 1.18) was not significantly different from those with a foreign body and subsequent pouch excision (1.39 ± 2.03) (t test, $P = 0.161$). Marlex was used in 11 (73%) of the 15 patients with fistulas and Teflon in 1 (7%) of 15. No fistulas occurred in the patient in whom Gore-Tex was used. Forty-seven percent (9 of 19) of patients with a foreign body and subsequent pouch excision had fistulas, whereas 11% (3 of 28) of patients with a foreign body and pouch survival had fistulas.

Fifty-one (60%) of the 85 patients currently have their CI (group A) (mean duration 15.1 years, range 2.7 to 21.7 years), whereas 31 (36%) of 85 have undergone conversion of their CI to a conventional ileostomy (group B) (mean 5.4 years, range 0.2 to 20.4 years). Sixty-three percent of the patients with ulcerative colitis (50 of 79) retained their CI (group A), as well as 60% (3 of 5) of the patients with familial polyposis (Table IV). Sixty-eight percent (30 of 44) of women and 58% (24 of 41) of the men maintained their pouches over time (chi-square, $P = 0.485$). Pa-

Table II. Indications for excision of continent ileostomy (n = 31)

Indication	No. of patients (% of pouches excised)
Valve dysfunction	13 (42)
Refractory pouchitis	7 (23)
Multiple fistulas	8 (26)*
Crohn's disease	2 (6)
Other	5 (16)

Note: Four patients had two indications for excision.
*Six of eight patients had Marlex or Teflon used in valve construction.

Table III. Frequency of sling or pledget use (Marlex, Teflon, or Gore-Tex foreign bodies) in continent ileostomy patients and number of revisions and incidence of fistulas in patients with a foreign body for prevention of valve slippage

Continent ileostomy status*	No. of patients with sling or pledgets (n = 47)	Mean no. of major revisions (±SD)	Incidence of fistulas in patients with Marlex or Teflon
Group A (n = 54)	28 (60%)	0.71 (±1.18)†	3 (11%)
Group B (n = 31)	19 (40%)	1.39 (±2.03)†	9 (47%)

*Group A = continent ileostomy retained; group B = continent ileostomy excised.
†$P = 0.161$; t test.

Table IV. Indications for continent ileostomy in patients who retained their continent ileostomy (group A; n = 54) and those who underwent excision of their continent ileostomy (group B; n = 31) over the long-term period

Survey response	Ulcerative colitis (n = 79)	Familial polyposis (n = 5)	Multiple cancers (n = 1)
Respondents (n = 56)			
Group A	42	1	1
Group B	12	0	0
Nonrespondents (n = 29)			
Group A	8	2	0
Group B	17	2	0

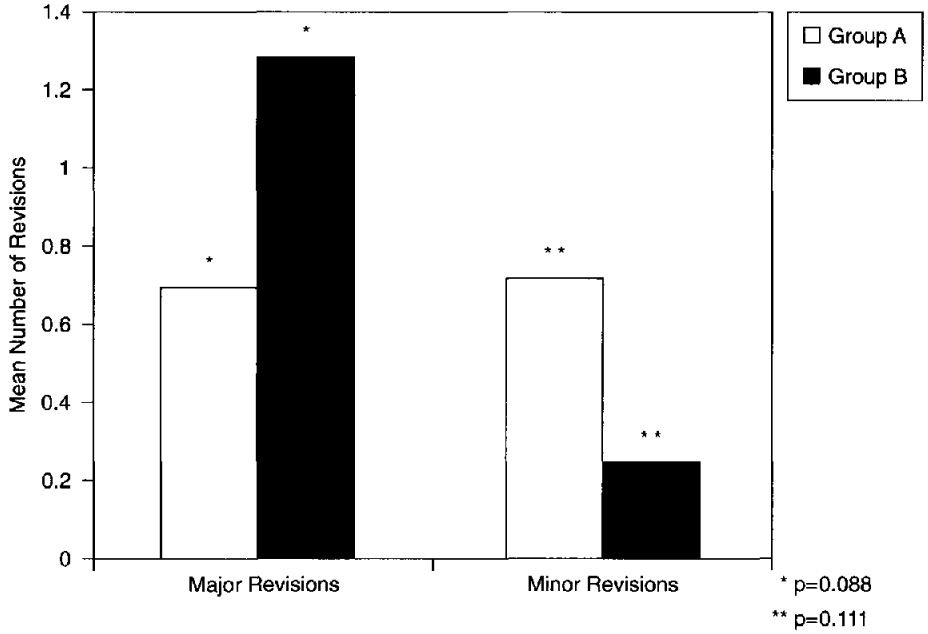

Fig. 2. Mean number of major and minor revisions in patients who retained their CI (group A; n = 54) and those who underwent CI excision (group B; n = 41) (*t* test).

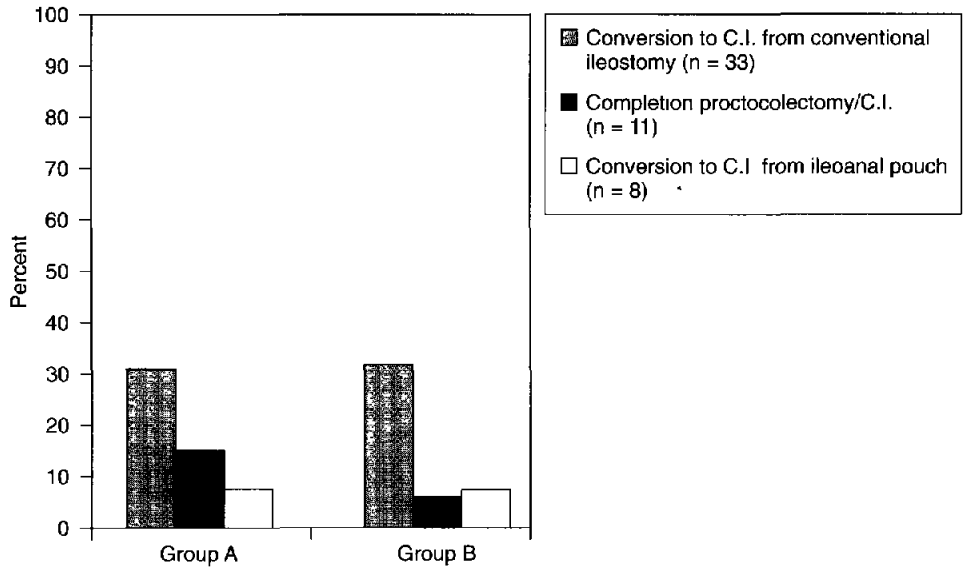

Fig. 3. Percentage of patients who underwent continent ileostomy *(CI)* as a secondary procedure and retained their CI (group A; n = 28) and those who underwent excision of their CI (group B; n = 24) (chi-square test, $P = 0.367$).

tients over 40 years of age did not have a significantly higher incidence of pouch removal (52% vs. 31%; chi-square, $P = 0.114$) or of major revisions (56% vs. 35%; chi-square, $P = 0.133$).

Major revisions occurred in 37% in group A and 61% in group B. Patients in group A underwent fewer major postoperative procedures (mean 0.73, range 0 to 4) than patients in group B (mean 1.29, range 0 to 8) (*t* test, $P = 0.088$); however, patients in group A underwent more minor postoperative procedures than those in group B (*t* test, $P = 0.111$) (Fig. 2). Seventy-eight percent of revisions involved the nipple valve. Eleven (41%) of 27 patients underwent valve reoperation within 6 months of creation of their CI, whereas 59% (16 of 27) occurred after 6 months (median interval 34.5 months, range 8 to 218 months). Valve revision within 6 months did not correlate with subsequent pouch failure (chi-square, $P = 0.873$).

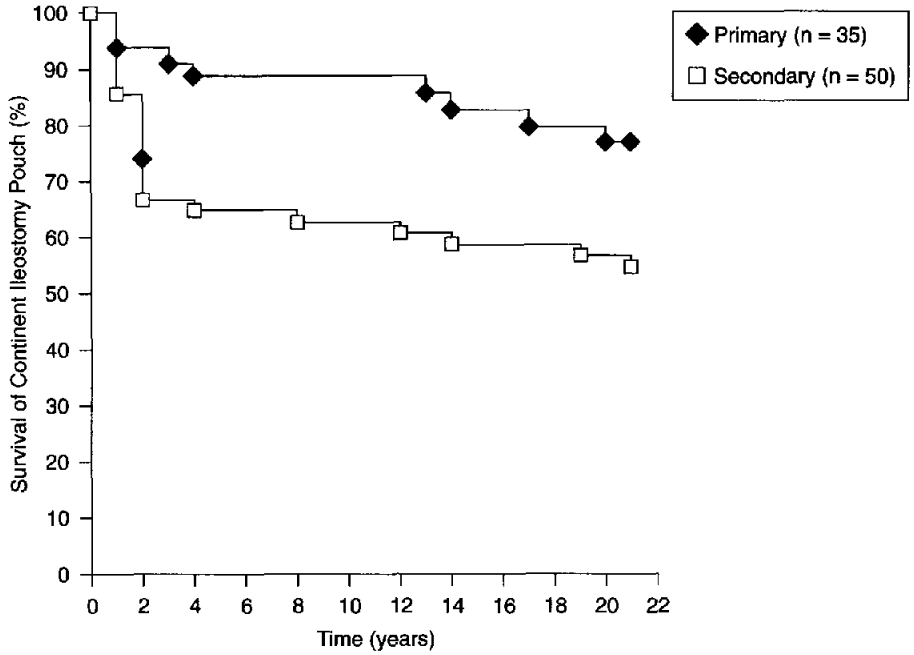

Fig. 4. Kaplan-Meier estimated survival curve for CI created primarily (one-stage total proctocolectomy) or secondarily (after previous conventional ileostomy or ileoanal pouch).

Pouch excision was indicated in approximately half (46%) of the 52 patients who underwent creation of a CI as a secondary procedure. Although the overall pouch failure rate was significantly higher for secondary vs. primary CI creation (46% vs. 23%; chi-square, $P = 0.05$), there was no statistically significant difference between the type of procedure done prior to the CI and subsequent pouch excision (chi-square, $P = 0.367$) (Fig. 3).

The probability of pouch survival was estimated in a Kaplan-Meier curve for the 35 patients who underwent creation of the CI as a primary procedure (with a total proctocolectomy) and for the 50 patients who underwent conversion to CI as a secondary procedure (from an ileoanal anastomosis or a conventional ileostomy with or without a completion proctocolectomy) (Fig. 4). At 21 years' follow-up CI pouch survival rates are 77% and 55% for primary and secondary creation, respectively.

Health Questionnaire

Forty-four (79%) of the 56 survey respondents still had their CI (group A) and 12 (21%) had undergone pouch excision (group B). Quality of life with the pouch for the two groups is compared in Table V. Fecal and flatus incontinence occurred in 25% (11 of 44) and 36% (16 of 44) of patients with the CI, respectively. Regardless of degree of continence, 89% of patients reported wearing a pad (sanitary napkin, gauze, paper towels) over their stoma daily to absorb mucus if nothing more. Three of the respondents (5%) had problems with fistulas (all in group A). In group A 24 respondents (54%) reported problems with the valve, six of whom (14%) had not had valve revisions. On average, 50% of patients in group A emptied their CI one to four times within 24 hours, whereas 18% emptied it seven or more times. Sixty-one percent of patients did not have to empty their CI at night, whereas 30% emptied it once or twice at night.

Forty-six percent of all respondents have experienced pouchitis (67% in group B; 41% in group A), but only three in group A (6%) were taking either metronidazole or ciprofloxacin chronically to prevent pouchitis. Sixty-one percent of survey respondents re-

Table V. Continent ileostomy function in survey respondents*

Continent ileostomy function	No. of patients in group A (%)	No. of patients in group B (%)
Pouchitis	18 (41)	8 (67)
Fecal continence	33 (75)	6 (50)
Flatus continence	28 (64)	3 (25)
Intubation difficulty	26 (59)	7 (58)
Bleeding	20 (45)	4 (33)
Fistulas	3 (7)	0 (0)
Skin irritation	27 (61)	5 (42)

Group A = continent ileostomy retained (n = 44); Group B = continent ileostomy excised (n = 12).

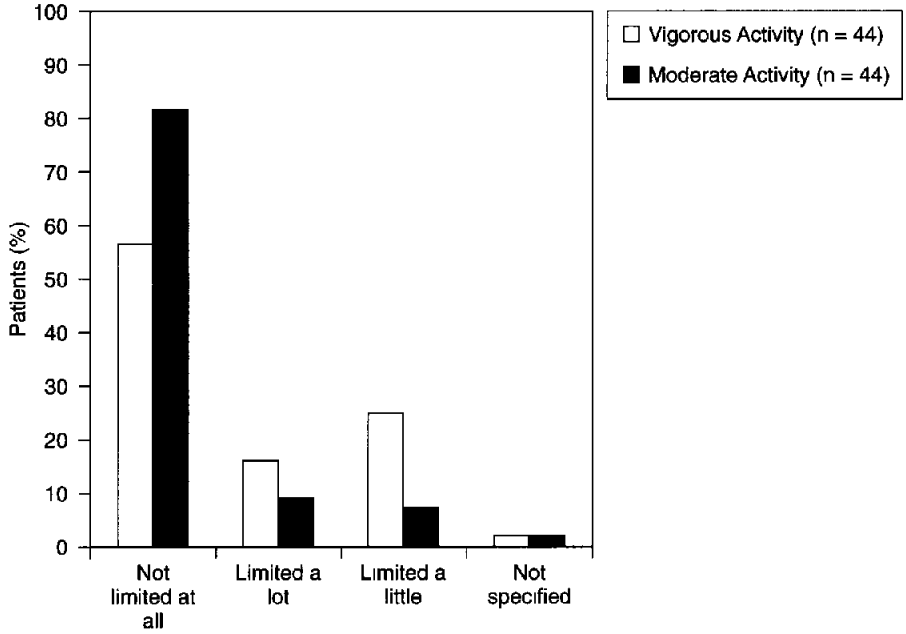

Fig. 5. Self-assessed limitations on moderate and vigorous activity in patients with a CI.

ported an average weight increase of 20 pounds over the years since their CI operation.

All six women in the survey respondent group who had given birth still had their CI. Three of the infants had been delivered vaginally and three had been delivered by cesarean section, and no women reported any complications during their pregnancies relating to the CI or any postpartum dysfunction of the CI.

In the quality-of-life questionnaire, vigorous activity was defined as running, lifting of heavy objects, and participation in strenuous sports, whereas moderate activity included bowling, housework, and playing golf. Fifty-seven percent and 82% of all respondents in group A were not limited at all in regard to vigorous or moderate activity, respectively (Fig. 5). After undergoing the CI procedure, 80% of the patients were able to resume the same type of preoperative employment. Two thirds of the patients did not feel restricted in any way by the operation. The overall results of the operation were considered good to excellent in 89% of respondents, and of those who retained their CI, 97% reported a good to excellent outcome.

DISCUSSION

Short-term results of patient satisfaction and durability of the pouch have been presented in several studies.[2,3] We have previously reported an improved quality of life in the short term (38 months) for patients with a CI.[4] We currently sought to determine the long-term durability and satisfaction with the pouch in a large group of patients after a 21-year follow-up by means of a quality-of-life questionnaire and review of medical records.

Excision of the CI was indicated in one third of our patients over a follow-up ranging from 1 to 21 years. Excision rates in the literature range from 3% to 28%,[2,5-8] with Kock's series of 36 patients having the longest follow-up of 20 years and an excision rate of 5%.[3] We found that patients who had undergone conversion to a CI from a conventional ileostomy or an ileoanal anastomosis had a shorter survival rate of their CI than the patients who had undergone a one-stage total proctocolectomy and CI creation. In addition, 80% of the continent ileostomies excised in this conversion group occurred within the first 2 years postoperatively. In contrast, the risk of CI excision in the total proctocolectomy group was stable over the long term. Surprisingly, two patients underwent pouch excision at 20 years.

End ileostomies require revision at rates ranging from 8% to 14%.[9,11] Revision rates of the CI in other series range from 6% to 59%.[2,8,12,13] In our study revision of the CI occurred at a frequency of 46%. Total number of major and minor revisions did not predict subsequent pouch excision. We found most revisions were necessary within the first postoperative year, which is consistent with other reports. Dozois et al.[14] reported that factors predictive of CI revision included male sex, age greater than 40 years, and conversion from conventional ileostomy versus a proctocolectomy with CI (42% vs. 24%). In our group of patients, women were equally as likely to need revi-

sion of the CI as men. Patients over 40 years of age at the time of CI creation underwent more major revisions than patients 40 years or younger, but the difference was not statistically significant. In addition, 48% of patients who had the CI created as a secondary procedure had one or more major revisions. Thus our findings are similar to those of Dozois et al. who found age and conversion from conventional ileostomy to be risk factors for pouch revision.

In 1972 the nipple valve was added to the procedure to improve continence of the ileal pouch.[15] Valve creation is still considered the most important, and perhaps the most difficult, part of the procedure. Reported valve revision rates range from 16.5% to 52%,[3,14,16] which are consistent with the 36% of the patients in our study needing valve revision. Overall 78% of our revisions involved the nipple valve. In our earlier series of patients, we reported that the rate of valve failure was higher in patients undergoing conversion from conventional ileostomy (46%) compared to those undergoing primary continent ileostomy creation (13%).[14] In long-term follow-up, valve failure was still higher in the conversion to CI group when compared to primary CI creation, but the difference was diminished (39% vs. 30%).

Technical modifications of the CI procedure were adopted by the surgeon (T.R.S.) after discussions with Dr. Kock in March 1979. Such changes included (1) the transition from hand-sewn to stapled valves to reduce the risk of valve failure, specifically prolapse; (2) the discontinuation of a mesenteric window, rotation sutures, and serosal scarification; and (3) the consistent use of a fascial or Marlex strip to minimize complete eversion of the valve due to gradual widening. Mesh was no longer used after 1984, to avoid the presence of the foreign body and to minimize the risk of fistulas. In our study the risk of pouch excision did not correlate with any of these major technical modifications (chi square, $P = 0.40$). We currently construct a 5 cm valve by applying the TA-55 stapler four times, and we no longer use mesh. The base of the valve is encircled with a nonabsorbable pursestring suture tightened around the indwelling catheter.

In addition to valve dysfunction with potential subsequent revision or excision, other complications of the CI include pouchitis, fistulas, and valve dysfunction not requiring revision. Forty-six percent of our survey respondents reported at least one episode of pouchitis, and three patients were placed on a regimen of long-term prophylactic antibiotics. Pouchitis rates in the literature range from 8% to 42%.[2,3,6,17]

The overall incidence of fistulas in our 85 patients was 15%. Multiple fistulas was the indication for resection in 26% of the 31 pouches excised. Halvorsen et al.[6] reported fistula complications in 22% of 32 patients, none of whom underwent pouch removal. In our study four patients had fistulas without pouch removal and three fourths of them had Marlex used in the construction of the valve of the CI. Fistulas have generally been attributed to Crohn's disease and to foreign bodies, primarily Marlex mesh, as previously reported by Kock et al.[18] However, in our series two patients had fistulas without a foreign body present and 70% (33 of 47) had Marlex or Gore-Tex without fistulas.

Two of the original 129 patients had a diagnosis of Crohn's disease prior to their CI, but no follow-up data were available for these two patients. Two other patients with a diagnosis of ulcerative colitis before creation of the CI underwent CI excision for a subsequent diagnosis of Crohn's disease.

None of our patients wears an ileostomy appliance, although 89% do wear a pad over the stoma daily. In Kock's series[2] of 259 patients with a nipple valve, 4% wore an ileostomy appliance at some time and in Failes' series[7] of 45 patients with 11-year follow-up, 7% of patients needed an external appliance. In a more recent report with a median follow-up of 4.6 years, Kohler et al.[13] reported that 93% of 313 patients with a CI had excellent fecal continence.

Conversion to CI from an ileoanal pouch has been reported previously in five patients with a follow-up of 1.3 to 3.7 years. The pouch was maintained in four patients.[19] The one patient who underwent excision had had slow-transit constipation prior to proctocolectomy. More than half (4 of 7) of the patients in our group who had undergone conversion from an ileoanal pouch required subsequent excision for valve dysfunction: three were incontinent and one patient had valve slippage.

Fazio et al.[5] note that most patients undergoing conversion from a conventional ileostomy to a CI are the most motivated and grateful patients. The patients in our survey who underwent conversion from a conventional ileostomy also reported a significant improvement in their quality of life with a return to normal life and alleviation of the chronic depression associated with the end ileostomy. Despite some incontinence of the CI, one patient still preferred the CI over conversion back to a conventional ileostomy.

Although our study includes the largest and longest follow-up of CI patients created by one surgeon reported in the literature, there are notable limitations to our results. Despite telephone calls and two mailings, we only were able to obtain late outcome data for 85 (66%) of the 129 patients. The mean response rate for published patient satisfaction studies from a multivariate analysis of 200 articles in 141 journals is 72.1%.[20] Our patient satisfaction results from the questionnaire may be limited by the 46% response rate; however, the 66% evaluation rate is approaching the mean of the multivariate analysis.

Overall, despite problems with leakage and fistulas, 97% of our survey respondents who retained their CI were very satisfied and considered their quality of life to be good to excellent with 82% enjoying moderately active lives. These results are similar to the 98% satisfaction rate in the report by Kohler et al.[13] with shorter follow-up (median 4.6 years).

The ileoanal pouch is the procedure of choice for patients undergoing proctocolectomy for ulcerative colitis or familial polyposis. Lower reoperation rates (11% to 26%),[9-11,13] a less restricted lifestyle,[13] and avoidance of a stoma are the major advantages of this procedure. The current indications for the CI may be limited but include patients needing a proctocolectomy for ulcerative colitis or familial polyposis and those with multiple cancers in whom an ileoanal pouch is contraindicated because of a hostile pelvis or weak anal sphincter. In addition, patients with a failing ileoanal pouch or with a conventional ileostomy may request conversion to a CI after discussing with their surgeon the real potential for pouch revision and excision.

CONCLUSION

Creation of a CI is a complex operation with a high rate of complications, particularly in the first two postoperative years. Primary disease, number of revisions, patient age, and sex are not predictive of pouch excision. Conversion from conventional ileostomy to CI predicts a greater likelihood of pouch removal than a one-stage total proctocolectomy with formation of a CI. Despite a 46% revision rate and a high pouch excision rate, long-term follow-up reveals excellent patient satisfaction. CI can be considered as an acceptable alternative to conventional ileostomy in selected patients who are willing to face the real possibility of pouch failure and subsequent excision.

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Ileal Pouch Salvage Following Failed Ileal Pouch–Anal Anastomosis

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Attempts have been made to salvage failed ileal pouch–anal anastomoses (IPAA) performed for ulcerative colitis or familial polyposis coli. These can be categorized as total reconstruction of the IPAA, partial transabdominal approach, and partial transperineal approach. The aims of our study were to determine the overall success of pouch salvage; to examine the demographics, indications, and outcomes for each approach; and to assess anorectal physiology and patient satisfaction in those with successful salvage operations. We reviewed data, including results of anorectal manometry, from 29 patients undergoing salvage procedures for failed IPAA. Seventeen salvage attempts were successful, 11 attempts failed, and one patient was lost to follow-up. Success rates were 100% in the total reconstruction group, 25% in the partial transabdominal group, and 55% in the transperineal group. In those undergoing total reconstruction of the IPAA (n = 9), functional outcome, as measured by incontinence, improved with 50% reporting incontinence preoperatively compared to 0% postoperatively ($P = 0.055$). Mean 24-hour stool frequency and nighttime stool frequency declined. All patients reported satisfaction with their outcomes. Sixty percent of patients who underwent ileal pouch salvage following IPAA have been successful in avoiding permanent ileostomy. These results suggest that a continued effort to salvage failed IPAA, including the use of total reconstruction, is a viable alternative to permanent ileostomy. (J GASTROINTEST SURG 1999;3:633-641.)

KEY WORDS: Ileal pouch–anal anastomosis, IPAA, ileal pouch salvage, ulcerative colitis, familial polyposis

Colectomy with ileal pouch–anal anastomosis (IPAA) has become a major surgical alternative for patients with ulcerative colitis and familial polyposis coli. This operation avoids a permanent ileostomy and, in most patients, provides excellent functional results and an improved quality of life. Nevertheless, IPAA is a complex procedure with an inherent risk of mechanical complications potentially resulting in pouch failure. When complications significantly impair the functional outcome, carefully selected and motivated patients may benefit from pouch salvage. Three revisional approaches are used to rectify functional pouch problems—total reconstruction of the IPAA or lesser procedures that are undertaken transabdominally or transperineally. The selection of patients for a particular approach requires consideration of a multitude of factors including surgical indication, functional status, number of failed prior operative attempts, age, and patient motivation.

We describe a series of 29 pouch salvage procedures in patients with failed IPAA. Our goal was to ascertain the overall success of pouch salvage while determining differences in demographics, surgical indications, and outcomes among the total reconstruction, partial transabdominal, and partial transperineal groups. Furthermore, in the total reconstruction group we were interested in functional outcome and preservation of anorectal physiology as reflected by incontinence, 24-hour stool frequency, nighttime stool frequency, leakage, anorectal manometry, and patient satisfaction.

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MATERIAL AND METHODS

Prospective data were obtained from the medical records of 29 patients referred to our institution for pouch salvage procedures following failed IPAA for ulcerative colitis or familial polyposis coli. Pouch salvage was defined as a procedure performed on the IPAA or the pouch itself. Demographic information, indications, and success rates were assessed for each of the three surgical approaches: total reconstruction of the IPAA, partial transabdominal procedures, and partial transperineal procedures. Total reconstruction of the IPAA involved either disconnection of the original anastomosis, resection and reconstruction of a new ileal J-pouch, and a neoileoanal anastomosis, or conversion of a Park's type S-pouch to a J-pouch. Partial transabdominal procedures included lysis of pouch-related adhesions, drainage of peripouch abscesses, fistula resections, and diverting ileostomies in combination with pouch revisions. Partial transperineal procedures included fistulotomy, Seton placement, repair of anal canal fissures, strictureplasty, mucosal flap advancement of the pouch, and incision and drainage of pouch-related abscesses.

Indications for reoperation were categorized as follows: (1) functional pouch problems (including inability to evacuate, incontinence, and severe pouchitis); (2) retained rectal mucosa; (3) anastomotic stricture; (4) intra-abdominal abscess/pelvic sepsis/anastomotic leak/fistula; and (5) perianal abscess/fissure/fistula/anastomotic sinus. Patients were classified by the primary indication for their pouch salvage. Overall success was measured as the percentage of patients whose satisfactory clinical outcome did not require conversion to a permanent Brooke ileostomy. Functional outcomes and patient satisfaction were recorded based on patient self-reports.

Anorectal manometry was performed where appropriate. A pneumohydraulic perfused catheter manometry system was used to obtain anorectal pressures, as described by Becker et al.[1] Resting pressure of the internal anal sphincter, basal and maximal squeeze pressures of the external anal sphincter, and estimates of ileal pouch capacity were assessed.[1]

Statistical comparisons were made with chi-square analysis, Fisher's exact test, analysis of variance, or paired *t* tests as appropriate. All statistical analyses were performed using SAS software (Statistical Analysis System, Carey, N.C.) licensed to Boston University.

RESULTS

Among the 29 patients referred for pouch salvage, 17 (59%) were females and 12 (41%) were males. The mean age at the time of pouch salvage was 36 years (range 14 to 63 years). Diagnoses included chronic ulcerative colitis in 24 patients (83%), familial polyposis coli in two patients (7%), and Crohn's disease originally diagnosed as chronic ulcerative colitis in three patients (10%). The original pouch types were as follows: 24 (83%) J-pouches, three (10%) S-pouches, one (3.5%) W-pouch, and one (3.5%) Y-pouch.

As depicted in Fig. 1, indications for pouch salvage surgery were as follows: three patients (10%) with functional pouch problems, five patients (17%) with retained rectal mucosa, six patients (21%) with anastomotic stricture, seven patients (24%) with intra-abdominal abscess/pelvic sepsis/anastomotic leak/fistula, and eight patients (28%) with perianal abscess/fissure/fistula/anastomotic sinus. Of the 29 patients requiring pouch salvage, nine underwent total reconstruction of the IPAA, nine had a partial transabdominal procedure, and 11 had a partial transperineal procedure.

There were no deaths associated with the 29 pouch salvage operations. Patients were followed for a mean of 2.5 years (range 0.02 to 11.4 years); one patient in the partial transabdominal group was lost to late follow-up. Over the follow-up period, 17 (61%) of 28 salvage attempts were successful and 11 (39%) of 28 salvage attempts failed. Successful surgical outcomes were as follows: 100% in the total reconstruction group, 25% in the partial transabdominal group, and 55% in the partial transperineal group.

Demographic information, surgical indications, and outcomes for each of the three groups are summarized in Table I. The partial transabdominal group (n = 9) included four females (44%) and five males (56%). The mean age at the time of reoperation was 42 years (range 22 to 63 years). Two of these patients were referred from other institutions for salvage procedures. All of these patients had prior local procedures. Chronic ulcerative colitis was the diagnosis in seven patients (78%), familial polyposis coli in one patient (11%), and Crohn's disease originally diagnosed as chronic ulcerative colitis in one patient (11%). The J-pouch was the original pouch type in all of these patients. Indications for pouch salvage consisted of one patient (11%) with retained rectal mucosa, two patients (22%) with anastomotic stricture, and six patients (67%) with intra-abdominal abscess/sepsis/anastomotic leak/fistula (see Fig. 1). Of the six patients in whom reoperation failed, four (67%) had intra-abdominal abscess/sepsis/leak/fistula including one patient diagnosed with Crohn's disease, one patient (17%) who had retained rectal mucosa with a chronic pelvic mucocele, and one patient (17%) who had an anastomotic stricture with secondary sepsis.

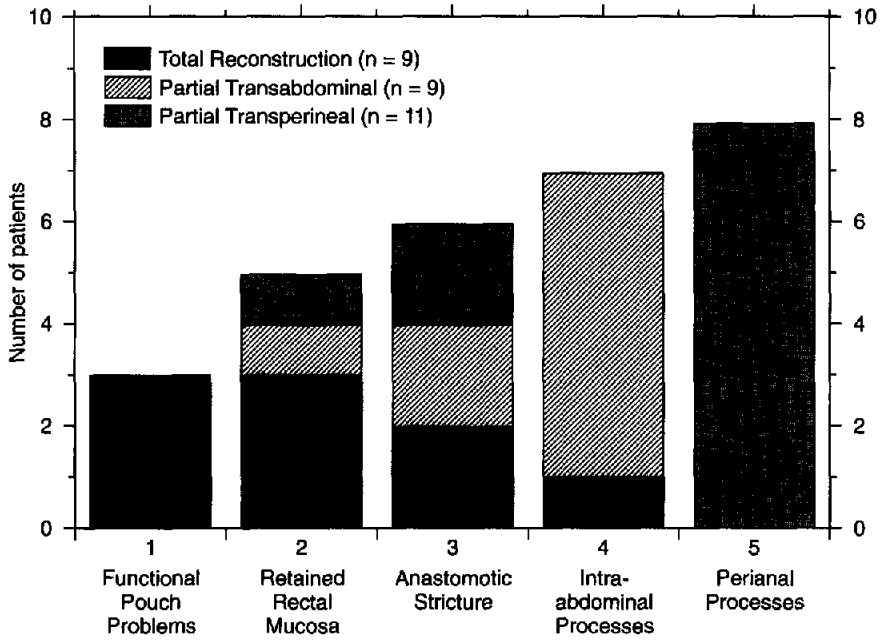

Fig. 1. Indications for pouch salvage by surgical approach. 1 = Functional pouch problems including inability to evacuate, incontinence, and severe pouchitis; 2 = retained rectal mucosa; 3 = anastomotic stricture; 4 = intra-abdominal abscess/pelvic sepsis/anastomotic leak/fistula; 5 = perianal abscess/fissure/fistula/anastomotic sinus.

Table I. Demographics, indications, and results of salvage by surgical approach

	Total reconstruction (n = 9)	Partial transabdominal (n = 9)	Partial transperineal (n = 11)
Age (yr)	28 ± 3.8	42 ± 4.1	36 ± 3.9
Sex (M:F)	4:5	5:4	3:8
Disease (CUC:FP:Crohn's)	8:1:0	7:1:1	9:0:2
Original pouch (J:S:Y:W)	6:2:0:1	9:0:0:0	9:1:1:0
Surgical indications			
Functional pouch problems	3 (33%)	0 (0%)	0 (0%)
Retained rectal mucosa	3 (33%)	1 (11%)	1 (9%)
Anastomotic stricture	2 (22%)	2 (22%)	2 (18%)
Intra-abdominal abscess/pelvic sepsis/ anastomotic leak/fistula	1 (11%)	6 (67%)	0 (0%)
Perianal abscess/fissure/fistula/ anastomotic sinus	0 (0%)	0 (0%)	8 (73%)
Successful pouch salvage	9 (100%)	2 (25%)*	6 (55%)

CUC = chronic ulcerative colitis; FP = familial polyposis.
All data are expressed as mean ± standard error of the mean.
*One patient was lost to follow-up.

Two patients (25%) had successful surgical outcomes, six patients (75%) had unsuccessful outcomes, and one patient was lost to follow-up.

The partial transperineal group (n = 11) included eight females (73%) and three males (27%) with a mean age of 36 years (range 13 to 62 years). Four of these patients were referred from other institutions for pouch salvage. Four patients had prior local salvage attempts, six patients had no prior salvage attempts, and one patient did not have these data available. Nine patients (82%) were diagnosed with chronic ulcerative colitis, and two patients (18%) had Crohn's disease originally diagnosed as chronic ulcerative colitis. Original pouch types included nine (82%) J-pouches, one (9%) S-pouch, and one (9%) Y-pouch (see Table I). Surgical indications were as

Table II. Indications, operations, and outcomes in patients undergoing total reconstruction of the IPAA

Sex	Diagnosis	Pouch type	Reason for salvage	Operative details	Pouch salvage	Postoperative incontinence	Patient satisfied	Follow-up (yr)
F	UC	S	Unable to evacuate	Resection ileal S-pouch to ileal J-pouch	Yes	No	Very	5.2
F	UC	S	Unable to evacuate	Resection ileal S-pouch to ileal J-pouch	Yes	No	Very	6.8
M	FP	W	Retained rectal mucosa, stenosis	Revision IPAA	Yes	No	Very	0.2
M	UC	J	Retained rectal mucosa, pouch perforation, stricture	Completion mucosal proctectomy, revision IPAA	Yes	No	Very	2.0
F	UC	J	Unable to evacuate, stricture	Revision IPAA	Yes	?	Yes	0.3
F	UC	J	Ileal pouch-vaginal fistula	Revision IPAA, resection ileal pouch-anal fistula	Yes	No	Moderately	0.3
F	UC	J	Ileal pouch-anal stricture	Revision IPAA	Yes	No	Very	0.4
M	UC	J	Unable to evacuate	Revision IPAA	Yes	No	Moderately	5.3
M	UC	J	Retained rectal mucosa, severe pouchitis, stricture	Revision IPAA	Yes	No	Very	1.3

IPAA = ileal pouch–anal anastomosis; UC = ulcerative colitis; FP = familial polyposis.

Table III. Anorectal physiology and functional outcomes in patients undergoing total reconstruction of the IPAA

	Preoperative	Postoperative
Anorectal function		
24-hour stool frequency	12.6 ± 2.9	7.8 ± 0.7
Nighttime stool frequency	4.8 ± 2.3	1.6 ± 0.5
Stool leakage (%)	86%	50%
Incontinence (%)*	50%	0%
Perianal pad usage (%)	33%	43%
Sexual dysfunction (%)	0%	0%
Anorectal physiology		
Pouch volume (ml)	75.4 ± 20.1	73.6 ± 7.9
Resting pressure (mm Hg)	67.6 ± 4.2	59.1 ± 9.6
Basal squeeze pressure (mm Hg)	36.0 ± 6.8	26.1 ± 5.9
Maximum squeeze pressure (mm Hg)	109.4 ± 17.1	90.9 ± 15.8

All data are expressed as mean ± standard error of the mean.
*$P = 0.055$.

follows: one patient (9%) with retained rectal mucosa, two patients (18%) with anastomotic stricture, and eight patients (73%) with perianal abscess/fissure/fistula/anastomotic sinus (see Fig. 1). Six patients (55%) had successful surgical outcomes, and five patients (45%) required pouch excision with conversion to a permanent Brooke ileostomy. Of the five patients in whom reoperation failed, three (60%) had perianal abscess/fissure/fistula/anastomotic sinus, one (20%) had retained rectal mucosa, and one (20%) had anastomotic stricture. Of the two patients diagnosed with Crohn's disease in this group, one required pouch excision with conversion to a permanent Brooke ileostomy. Of those in whom partial transperineal salvage failed, three patients (60%) had prior local salvage procedures. One patient (20%) had no prior local procedures and one patient (20%) did not have these data available.

Given the successful surgical outcome of total reconstruction of the IPAA (n = 9), functional outcome and patient satisfaction were further assessed in this group (Table II). Of these nine patients, five (56%) were females, and four (44%) were males. These patients were referred from other institutions with significant complications of the original IPAA and had been unresponsive to numerous local procedures. The mean age at the time of reoperation was 28 years (range 14 to 49 years). Chronic ulcerative colitis was the diagnosis in eight patients (89%), and one patient (11%) had familial polyposis coli. No patients were subsequently diagnosed with Crohn's disease. The original pouch types were as follows: six (67%) J-pouches, two (22%) S-pouches, and one (11%) W-pouch. Indications for salvage in these nine patients included: three patients (33%) with functional pouch problems, three patients (33%) with retained rectal mucosa, two patients (22%) with anastomotic stricture, and one patient (11%) with intra-abdominal abscess/sepsis/anastomotic leak/fistula (see Fig. 1).

Follow-up of these nine patients was a mean of 2.4 years (range 0.2 to 6.8 years). Several indicators of functional outcome were evaluated (Table III). Incontinence improved, with 50% reporting incontinence preoperatively compared to 0% postoperatively ($P = 0.055$). Mean 24-hour stool frequency declined nearly 40% and mean nighttime stool frequency was reduced by 66%. Stool leakage also declined from 86% preoperatively to 50% postoperatively; however, perianal pad usage was slightly increased from 33% preoperatively to 43% postoperatively. No patients reported sexual dysfunction pre- or postoperatively. All patients reported overall satisfaction with the functional results of the total reconstruction surgery, with six patients reporting that they were very satisfied, two patients reporting that they were moderately satisfied, and one patient reporting satisfaction. No patients reported dissatisfaction with their clinical outcome.

Pre- and postoperative manometric assessments of anorectal function in the total reconstruction group were performed (Table III). Pouch capacity remained essentially unchanged with a preoperative mean volume of 75.4 ml and a postoperative mean volume of 73.6 ml. Mean maximum resting pressure, basal squeeze pressure, and maximum squeeze pressure all demonstrated slight but nonsignificant reductions.

In this particular series, on average, approximately 30 cm (range 10 to 64 cm) of ileum was resected in those nine patients undergoing a total reconstruction. In two cases a significant portion of the pouch itself

was salvaged; however, in most cases all of the existing pouch was resected including some ileum proximal to the pouch. Despite the loss of healthy bowel during the reconstruction procedure, these patients reported a satisfactory functional outcome with no postoperative incontinence.

DISCUSSION

Despite the high success rate of the IPAA for patients with chronic ulcerative colitis and familial polyposis coli, complications still occur in as many as 30% to 50% of patients.[2,3] Significant complications can lead to pouch dysfunction and failure, which requires excision in some 5% to 20% of these patients.[4,7] Many patients with debilitating pouch dysfunction are responsive to local procedures performed through either a transabdominal or transperineal approach. However, there is a subgroup of patients with complex pouch problems that fail to respond to local procedures and require total reconstruction of the pouch.[2,4]

Pouch dysfunction and failure can occur for a variety of reasons. The most difficult is pelvic sepsis, which occurs in up to 25% of patients.[8-10] Pelvic sepsis leads to the development of pelvic fibrosis and the loss of ileal pouch elasticity. Studies have suggested that salvage in this subgroup yields a poor outcome resulting in subsequent pouch excision.[2,6] Crohn's disease, like pelvic sepsis, has also long been considered a contraindication to pouch salvage. Because of the difficulty in distinguishing the early stages of chronic ulcerative colitis and Crohn's disease, 5% to 7% of patients are originally diagnosed with chronic ulcerative colitis but are later found to have evidence of Crohn's disease.[11] Pouch excision has been reported to occur in approximately 25% to 30% of these patients.[11] Severe pouchitis can also lead to pouch dysfunction and failure. Although the cause of this inflammation of the ileal reservoir remains unclear, pouchitis has been reported to occur in 10% to 57% of patients depending on the diagnostic criteria.[7,12,13] Refractory pouchitis can require pouch excision in a small percentage of patients with an IPAA.

Pouch dysfunction resulting from surgical technique includes retained rectal mucosa, long efferent limbs, and anastomotic strictures. Avoiding damage to the anal sphincter during rectal mucosectomy led to the development of the double-stapled IPAA. However, this procedure has resulted in patients with retained rectal mucosa. These patients may experience recurrence of disease and/or dysplastic changes in this segment of tissue, necessitating a salvage procedure.[2,14] Revision is also often necessary in patients with Park's type S-pouches that have long efferent limbs. These limbs have been associated with an increased inability to completely evacuate the pouch. Patients must then choose between manual intubation, revision of the limb, or conversion to a J-pouch. Anastomotic strictures and ischemia can result from excessive tension on the IPAA in 8% to 17% of patients.[5,7,11] Most of these patients respond to a local procedure, such as dilatation, and rarely require complete revision of the IPAA.[11]

Local procedures are also quite successful in patients with fistulas, fissures, peripouch abscesses, and pouch-related adhesions. Fistulas can present as pouch-vaginal, pouch-perineal, entero-pouch enterocutaneous, rectovaginal, and perianal communications. Chronic fistulas may suggest an etiology of Crohn's disease. Fistulas or anastomotic leaks may lead to pelvic or presacral abscesses. These occur in 5% to 12% of patients and may require reoperation if they do not respond to percutaneous drainage.[7,8,11] Last, localized processes such as fissures and pouch-related adhesions may result in problems with the IPAA and are often managed with transabdominal and transperineal procedures.

Our experience with 29 patients with failed IPAA demonstrates an overall pouch salvage success rate of 61%, which is consistent with that of other series.[5,6,15] Pouch salvage, therefore, is a realistic option in carefully selected patients with a failed IPAA who wish to avoid a permanent ileostomy. The surgical approaches to pouch salvage can be categorized as total reconstruction of the pouch, partial transabdominal procedures, and partial transperineal procedures. Important differences exist in the mean age, surgical indications, prior salvage attempts, and outcomes among the three groups (see Table I). The mean age of the patients in the partial transabdominal and partial transperineal group was 42 years and 36 years, respectively, whereas the mean age of the total reconstruction group was 28 years. Younger patients may be more motivated to withstand further major surgery for cosmetic, social, and psychological reasons. Older patients are also more likely to carry additional surgical risks that would decrease the safety of total reconstruction of the IPAA.

The transabdominal and transperineal approaches are primarily undertaken for well-circumscribed problems (see Table I). In the transabdominal group, 67% (6 of 9) of patients were those with intra-abdominal abscess/sepsis/anastomotic leak/fistula, and in the transperineal group, 73% (8 of 11) of patients were those with perianal abscess/fissure/fistula/anastomotic sinus. In contrast, 89% (8 of 9) of the total reconstruction group consisted of those with complex technical problems such as functional pouch

problems, anastomotic stricture, and retained rectal mucosa.

As expected, there was a disparity in surgical outcomes among these three groups (see Table I). Success rates were 100% (9 of 9) in those with total reconstruction, 25% (2 of 8) in those with a partial transabdominal approach in which one patient was lost to follow-up, and 55% (6 of 11) in the partial transperineal group. The least successful approach appeared to be transabdominal with a success rate of only 25%. All of those patients had prior multiple local procedures. This may be related to pelvic sepsis and/or chronic pouch-related abscesses, which were present in six of nine patients. These have been shown to be poor prognostic factors because of the development of pelvic fibrosis and inelasticity of the pouch. As such, these patients would be considered less often as surgical candidates for a total reconstruction of the IPAA. Therefore older patients with pelvic sepsis as their primary pouch complication seem to have poorer outcomes than younger patients without pelvic sepsis as their primary pouch complication. Selection of better surgical candidates for a total reconstruction procedure may have influenced the higher success rate of this group. The transperineal group had an acceptable success rate of 55%. Seventy-three percent of these patients had a localized complication (i.e., fistula or fissure) with few to no prior salvage attempts. Patients with a diagnosis of Crohn's disease would also be considered less often as surgical candidates for a total reconstruction procedure. Of note, there were three patients diagnosed with Crohn's disease between the partial transabdominal and partial transperineal groups and none in the total reconstruction group. Salvage attempts failed in two of these patients (67%).

The total reconstruction group was the most successful, with 100% avoiding permanent ileostomy. These were carefully selected, highly motivated younger patients who had numerous failed attempts at salvage in the past. Our study demonstrated that this is a viable option for this subgroup of patients. Although a history of multiple local procedures may suggest failure of further local salvage attempts requiring pouch excision and conversion to permanent Brooke ileostomy, it does not appear to mitigate against successful reconstruction of the pouch and IPAA. Not only did they manage to avoid permanent ileostomy, but we found their functional status and anorectal physiology to be well preserved, Incontinence was nonexistent (see Table II) after reoperation and 24-hour stool frequency, mean nighttime stool frequency, and leakage all declined (see Table III). It is unclear as to why pad usage increased slightly. The manometry data indicate that integrity of the anal sphincter and capacity of the pouch were well preserved. It is important to note that all of these patients reported satisfaction with their functional outcome.

The goal of the surgeon undertaking a major pouch reconstruction is to "salvage" and reuse as much of the existing pouch ileum as possible. However, depending on case-by-case circumstances and the reasons for failure, significant portions of nondiseased ileum must be resected. In this particular series, on average, approximately 30 cm (range 10 to 64 cm) of ileum was resected in those nine patients undergoing a total reconstruction. In two cases a significant portion of the pouch itself was salvaged; however, in most cases all of the existing pouch was resected including some ileum proximal to the pouch. Despite the loss of healthy bowel during the reconstruction procedure, as mentioned, these patients continue to maintain normal anorectal function and all reported a satisfactory functional outcome with no postoperative incontinence (see Table II).

Although the success rate and functional outcome were greatest in the group of patients undergoing total reconstruction, lesser procedures using either a transabdominal or a transperineal approach are still appropriate in many cases. It is tempting to compare the outcomes among these three groups, but it is important to recognize that there is an inherent problem with selection bias in this type of study. For example, all of the patients in the total reconstruction group were referred to our institution and had failed prior salvage attempts. This was not the case for the transabdominal or transperineal groups. For each referral to our institution, there may be many patients who were treated successfully or who decided to no longer persist in their efforts to avoid permanent ileostomy. In addition, patients who underwent total reconstruction were younger, and they may have been more psychologically and physically appropriate for the rigors of a reconstruction procedure. Therefore the surgeon must consider on a case-by-case basis the surgical indications, functional status,[16] demographic information, and patient's level of motivation in determining which salvage approach, if any, is a feasible option.

CONCLUSION

Sixty-one percent of patients who underwent pouch salvage for failed IPAA were successful in avoiding permanent ileostomy. These results suggest that a continued effort to salvage failed IPAA, including the use of total reconstruction, is a viable alternative to permanent ileostomy.

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Discussion

Dr. R. Beart (Los Angeles, Calif.). You had three patients with Crohn's disease. Traditionally that has not been an indication for sphincter preservation. Did you attempt it in those patients; did they have a shot at preservation? Second, in order to minimize sphincter damage can you tell us how you approached the anus in your total reconstruction group or in the other transperineal group? Did you use any special techniques?

Dr. S. Saltzberg. In the patients with Crohn's disease, the disease had not been diagnosed until the pouch was removed and it was confirmed pathologically that they had Crohn's disease.

Dr. M. Dayton (Salt Lake City, Utah). I have two questions. How many of these patients had rectovaginal fistula repair? What was your success rate with that group? Second, did you restrip the mucosa, say the last 3 or 4 cm, in those patients in whom you did the complete repair? If so, how did you do it?

Dr. Saltzberg. In response to the first question, the majority of the fistulas that we found were pouch-perineal fistulas and not vaginal fistulas. Overall there were two patients; one was later found to have Crohn's disease and the other had a successful repair.

Dr. J. Becker (Boston, Mass.). In terms of mucosectomy, a number of these patients had a prior double-stapled ileal pouch–anal canal anastomosis and had persistent problems with the retained mucosa itself in terms of inflammatory changes or strictures in that area. In those cases we did do a completion mucosectomy, reconstructed the pouch, and performed an ileal pouch–anal anastomosis.

Dr. A. Sicular (New York, N.Y.). Are there any tricks to getting that extra 20 cm of vessels down when you reconstruct a pouch?

Dr. Becker. We have not found that to be a problem in any of our patients. By fully mobilizing the ileal mesentery, including sacrifice of the ileocolic artery (which is necessary in all cases), scoring the mesentery, and then flexing the operating table, we have been able to extend the pouch to the anus in all 500 patients including these nine patients who had a pouch reconstruction.

Dr. Z. Cohen (Toronto, Ontario, Canada). There have been a few articles recently on pouch salvage reporting approximately the same results, maybe not quite 100%. I agree with Dr. Becker as far as getting a pouch down—it is usually not very dificult. Even when you have to create a second pouch for these patients, it is not that difficult to bring the mesentery down. I am interested in knowing about the partial operations that were performed, either abdominally or perineally. In the total reconstruction group, all of your patients were referred and had undergone multiple local procedures before that. In your own group of patients, how many local procedures would you tolerate? If you have a failed local procedure, why then would you not go on to perform a total reconstruction in some of those patients whose procedures had failed and thereby increase your pouch salvage rate in the second and third groups?

Dr. Saltzberg. Again, the total reconstruction group consisted of carefully selected patients. In the partial transabdominal group, out of the six failures, there were five that had had pelvic sepsis and chronic abscesses. Several reports

in the literature have shown that this is a poor indicator for pouch salvage. They had also had multiple prior procedures in that group. So, I do not think that this is a group of patients in whom we would consider total reconstruction.

Dr. Cohen. I think the most recent papers from the Cleveland Clinic have shown that more than 80% can be salvaged just for septic complications alone. The report that we recently published showed that more than 75% of the pouches could be salvaged for septic complications alone.[4]

Dr. B. Harms (Madison, Wis.). There is a controversy related to the need for diversion. Do you know why these pouches failed in the first place? Didn't these patients undergo diversion during their primary procedures and then experience numerous problems? In the group that had a transabdominal reconstruction, do you have any data on why patients improved? Were you making the pouch larger or was it a matter of function? Do you have any compliance data or distensibility data you could examine to look at the pressure gradient between the pouch and the anal canal and why they improved?

Dr. Saltzberg. Much of the information from the institutions our patients were referred from was not complete, making it possible only to list a primary indication for reoperation. Three had functional pouch problems, three had retained rectal mucosa, two had anastomotic strictures, and one had a perianal complication. In response to your second question, we do not have complete data on the partial transabdominal or partial transperineal groups with respect to anorectal manometry.

T3N0 Rectal Cancer: Results Following Sharp Mesorectal Excision and No Adjuvant Therapy

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Adjuvant chemoradiation therapy following resection of T3N0 rectal cancer is recommended in order to reduce the incidence of local recurrence and improve survival. However, recent experience with rectal cancer resection utilizing sharp dissection and total mesorectal excision has resulted in a reduction in local recurrence rates to as low as 5% without adjuvant treatment. The purpose of this study was to determine if rectal cancer resection utilizing sharp mesorectal excision alone is adequate treatment for local control of T3N0 rectal cancer. Between July 1986 and December 1993, 95 patients with T3N0M0 rectal cancer underwent resection with sharp mesorectal excision and did not receive any adjuvant therapy. Various prognostic factors were analyzed for their association with local recurrence and survival. Seventy-nine patients had a low anterior resection, 10 of whom had a coloanal anastomosis, and 16 had an abdominoperineal resection. The median follow-up was 53.3 months. Six patients had local recurrence, 12 had distant recurrence, and three had local and distant recurrences. The overall local recurrence rate was 9% crude and 12% 5-year actuarial. The overall crude recurrence rate was 22%. The 5-year disease-specific survival rate was 86.6% with an overall survival of 75%. Postoperative complications occurred in 18 patients (19%). Five patients (6%) had a documented anastomotic leak. Perioperative mortality was 3%. No technical factors, including type of resection (low anterior vs. abdominoperineal), location of tumor, or extent of resection margin, were significant for determining local recurrence. The only histopathologic marker significant for determining local recurrence was lymphatic invasion ($P < 0.04$). Sharp mesorectal excision with low anterior resection or abdominoperineal resection for T3N0M0 rectal cancer results in a local recurrence rate of less than 10% without the use of adjuvant therapy. Therefore, in select patients with T3N0M0 rectal cancer, the standard use of adjuvant therapy for local control may not be justified. (J GASTROINTEST SURG 1999;3:642-647.)

KEY WORDS: Rectal cancer, mesorectal excision, radiation therapy, adjuvant therapy

Of the estimated 36,000 new cases of rectal cancer in 1999, 70% to 80% will present with disease beyond the rectal wall either by direct extension or lymphatic spread[1]; these patients are at increased risk for pelvic and distant recurrence. The pelvis is a frequent site of recurrence and a major cause of morbidity and mortality with salvage therapy often incomplete and providing only temporary palliation.[2,3]

Adjuvant chemoradiation therapy is recommended following curative resection of stage II and III (Astler-Coller stages B2 and C or TNM stage T3-4N0 or TanyN1) rectal cancer for improved local control and survival.[4] However, because of the significant side effects associated with pelvic radiation, as well as recent reports demonstrating that rectal cancer surgery incorporating a total mesorectal excision (TME) produces local recurrence rates of 5% to 7%,[5-8] in selected patients, the adequacy of "conventional" non-TME surgery has been brought into question. Also in question is the exact subset of rectal cancer patients who may benefit most from adjuvant chemoradiation therapy.

"Conventional" surgery for rectal cancer usually involves blunt pelvic dissection, which has an associated

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risk of mesorectal disruption and shedding of malignant cells into the pelvis. This may explain, in part, why local recurrence rates following conventional surgery for Astler-Coller stage B2 (T3N0M0) rectal cancer range from 15% to 35%.[2,9,10] The importance of surgical technique is further underscored by the broad range (10% to 50%) of local recurrence rates among individual surgeons.[11,12]

The purpose of this study was to determine if "optimal" rectal cancer resection incorporating a sharp mesorectal excision (SME) without the use of adjuvant therapy is adequate treatment for selected patients with transmural, node-negative (T3N0M0) rectal cancer.

PATIENTS AND METHODS

A review of the prospective colorectal cancer database at Memorial Sloan-Kettering Cancer Center between July 1986 and December 1993 identified 283 patients with T3N0M0 rectal cancer who underwent resection with either a low anterior resection or abdominoperineal resection. Of these, 95 patients were resected and received no other preoperative or postoperative therapy and formed the basis of this study. The decision for these patients to receive no other treatment was based on the preference of the operating surgeon and was generally related to lack of tumor bulk, adequate clearance of resection, and lack of adverse pathologic features. The remaining 188 patients received either preoperative or postoperative adjuvant chemoradiation therapy and were excluded from this analysis.

All patients were resected utilizing an SME technique. This involved sharp dissection under direct vision in an areolar plane along the visceral fascia that envelopes the rectum and its mesentery.[6] For mid to low rectal cancers, the entire rectal mesentery, including that distal to the tumor, was removed as an intact unit. For high rectal cancers, sharp mesorectal dissection was continued 5 to 6 cm distal to the tumor, and the mesorectum was excised at that level. Patients were followed prospectively, and the time to local and distant recurrence or death was recorded.

Histopathologic features analyzed in the resected tumors included the following: tumor grade (well, moderate, and poor differentiation); vascular, lymphatic, and/or perineural invasion; and the presence of mucin production, which was considered to be positive if more than 50% of the specimen contained mucin. Patients in whom a preoperative carcinoembryonic antigen (CEA) level was obtained were separated into those with CEA levels above and below 5 ng/ml.

The type of surgical resection was distinguished between abdominoperineal resection and low anterior resection, including patients who had a coloanal anastomosis. Tumor location, based on the distance between the lowermost edge of the tumor and the anal verge, was divided into three groups: between 0 and 5 cm (low), 5 to 10 cm (mid), and 10 to 15 cm (high).

The resection margin was measured from the pathologic specimen. Patients were then divided into those who had resection margins less than 2 cm or greater than or equal to 2 cm.

All perioperative complications were recorded. Perioperative mortality was defined as death occurring within 30 days of surgery.

Disease-free and overall survival rates were calculated by the Kaplan-Meier actuarial method, and results were compared by means of the log-rank test. Statistical significance was defined as $P < 0.05$.

RESULTS

Ninety-five patients were identified with T3N0M0 rectal cancer who were treated with surgery alone and received no other form of adjuvant therapy. There were 57 males (60%) and 38 females (40%). The average age of this group was 67 $\pm$ 12 years. The median follow-up time for this study was 53.3 months.

Recurrence Rates

Six patients (6%) had local recurrence only, 12 (13%) had distant recurrence only, and three (3%) had both local and distant recurrence. The overall crude local recurrence rate was 9%, with an overall recurrence rate of 22%.

Pathologic Features and Local Recurrence

Pathologic review of the specimens revealed four (4%) well-differentiated, 88 (93%) moderately differentiated, and three (3%) poorly differentiated lesions. Tumor grade was not a significant factor in determining local recurrence ($P = 0.20$) by log-rank analysis.

Analysis of the tumor revealed six (6%) with vascular invasion, three (3%) with lymphatic invasion, and four (4%) with perineural invasion. Mucin production of the tumor was seen in 12 cases (13%). Only lymphatic invasion was a significant factor in determining local recurrence ($P < 0.04$). Perineural invasion showed a trend toward determining local recurrence ($P < 0.07$) but did not achieve statistical significance.

Of the 95 patients in this study, 69 had a preoperative CEA level determined. In 51 (74%) the CEA level was less than 5 ng/ml and in 18 (26%) it was greater than 5 ng/ml. Preoperative CEA level did not influence local recurrence ($P = 0.82$).

Technical Factors and Local Recurrence

Seventy-nine patients underwent low anterior resection, 10 of whom had a coloanal anastomosis for reconstruction, and 16 underwent abdominoperineal resection. Seven patients (9%) undergoing low anterior resection and two (12%) undergoing abdominoperineal resection developed local recurrence ($P = 0.52$).

Twenty-three patients (24%) had tumors located 0 to 5 cm from the anal verge (low), 45 (47%) had tumors located 5 to 10 cm from the anal verge (mid), and 27 (28%) had tumors located 10 to 15 cm from the anal verge (high). Two (9%) of 23 patients with low rectal tumors, 6 (13%) of 39 with midrectal tumors, and 1 (4%) of 26 with high rectal tumors developed local recurrence. Location of the primary tumor did not influence local recurrence ($P = 0.39$).

In 76 cases (80%) the resection margins were ≥2 cm, whereas in 19 (20%) the margins were less than 2 cm. There were no patients with resection margins less than 2 cm who developed local recurrence, whereas 9 (12%) of 76 patients with resection margins ≥2 cm developed local recurrence ($P = 0.15$).

Table I. Perioperative complications

Complications	No.
Anastomotic leak	5
Wound infection	3
Myocardial infarction	2*
Pelvic abscess	2
Incidental splenectomy	1
Prolonged ileus	1
Colostomy slough	1
Fever of unknown origin	1
Deep venous thrombosis	1
Perineal wound infection	1
Respiratory failure	1*
Sepsis	1*

*Perioperative mortality (one patient with myocardial infarction).

Morbidity and Mortality

There were three deaths in the perioperative period, for an overall mortality rate of 3%. One patient died of a myocardial infarction on postoperative day 18, one died of respiratory failure on postoperative day 4, and one died of septic complications related to a small bowel fistula on postoperative day 30.

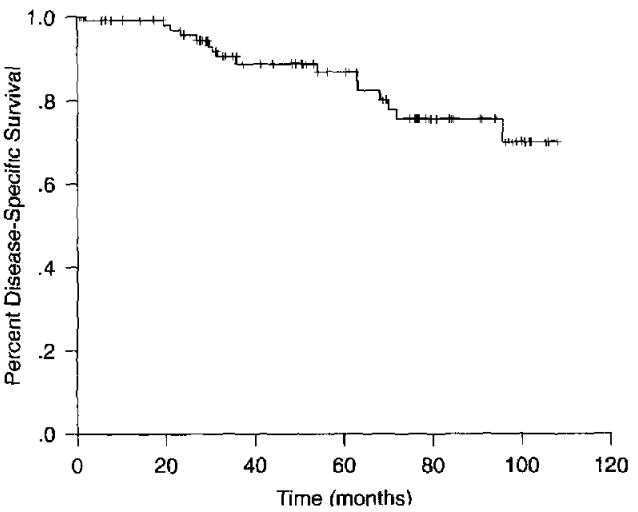

Fig. 2. Disease-specific survival was 86.6% at 5 years. Median follow-up time was 53.3 months (N = 95).

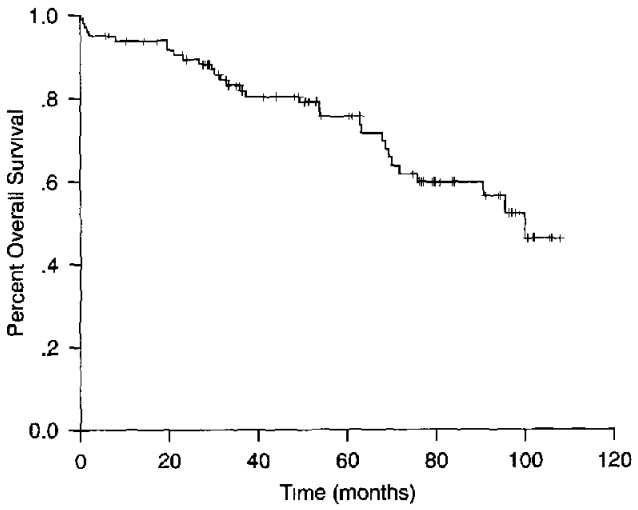

Fig. 1. Overall survival was 75.3% at 5 years, with a median survival of 100.3 months. Median follow-up time was 53.3 months (N = 95).

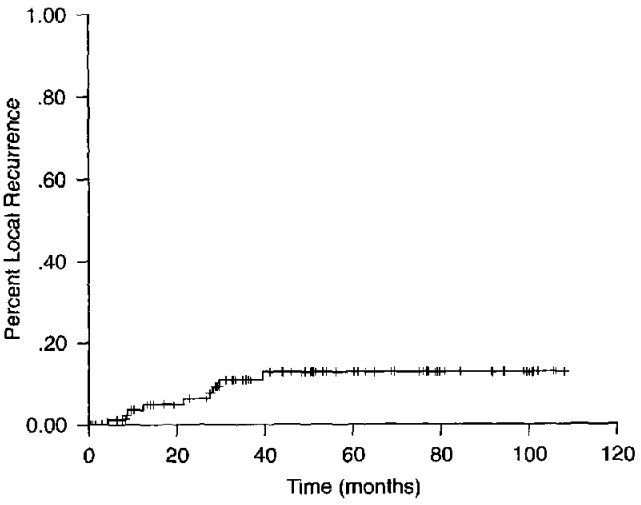

Fig. 3. Risk of local recurrence at 2 years was 6% and at 5 years was 12%. Median follow-up time was 53.3 months (N = 95).

Postoperative complications occurred in 18 patients, for an overall complication rate of 19% (Table I). However, only five patients (5%) had an anastomotic leak and two (2%) had a pelvic abscess unrelated to anastomotic leak. Postoperative complications, including anastomotic leaks, were not significant in determining local recurrence ($P < 0.15$).

Survival

The overall actuarial 5-year survival for this group of patients was 75.3% with a median survival of 100.3 months (Fig. 1). The actuarial 5-year disease-specific survival was 86.6%, with the median survival not yet reached (Fig. 2). The overall actuarial 5-year disease-free survival was 73.4%. The actuarial risk of developing a local recurrence in 2 years was 6% and at 5 years was 12% (Fig. 3).

DISCUSSION

Surgery for rectal cancer is a locoregional therapy and its oncologic efficacy is based on its rate of local control.[13] The major risk factors for local and distant relapse are the number of involved lymph nodes, extent of transmural penetration, and tumor grade.[14,15] However, inadequate surgical resection is also a predominant cause of pelvic relapse since a positive lateral margin correlates strongly with local recurrence.[7,16,17] "Conventional" surgical resection incorporating blunt pelvic dissection yields a positive lateral margin in 20% to 33% of cases. This is of clinical significance since 80% to 85% of patients with a positive lateral margin develop a local recurrence within 2 years, compared to only 10% of patients without circumferential involvement.[16,17]

Pelvic surgery incorporating SME has several advantages over conventional surgery, including improved lateral clearance resulting in a negative circumferential resection margin in 93% of cases.[7] Removal of the mesorectum as an intact unit facilitates enhanced removal of tumor deposits in the mesentery and decreases the risk of tumor spillage that can occur from a disrupted mesentery. In addition, dissection within the areolar plane surrounding the mesorectum allows for identification and preservation of autonomic nerves, thereby significantly decreasing postoperative urinary and sexual dysfunction rates.[18,19]

Several authors using this technique have reported local recurrence rates well below 10%[5-8] (Table II). However, these studies include a broad range of tumor stage as well as patients receiving adjuvant radiation therapy.[6] Our results show a crude local recurrence rate of less than 10% and a 5-year actuarial rate of 12% in a large group of patients with similarly staged transmural, node-negative (T3N0M0) rectal cancer treated by optimal surgery alone. These results compare favorably with the 11% actuarial incidence of local failure alone and the 23% actuarial incidence of local failure as a component of overall failure noted in the late 1980s.[20] In addition, in three series of patients with T3N0 rectal cancer resected before 1980, the incidence of crude local failure as the only site of failure ranged from 13% to 24%, whereas the crude local failure rate as a component of failure ranged from 27% and 35% following a curative resection.[21-23] The differences between these earlier results and ours may be explained, in part, by the fact that TME was not popularized until the late 1980s.

Despite its oncologic benefit, appropriate concern has been raised about the widespread use of TME for all rectal cancers.[24] The series by MacFarlane et al.[5] showed an anastomotic leakage rate of 17.4% for anastomosis created between 3 and 6 cm. This resulted in long-term failure and a permanent colostomy in 5% of patients.[5] A recent audit reveals an anastomotic leakage rate of 16% with the introduction of TME, compared to a leakage rate of only 8% prior to TME.[25] However, other series report anastomotic leakage rates ranging from 3% to 8%.[6,8] The anastomotic leakage rate in our series was 5% utilizing SME with transection of the mesorectum 5 cm below the lowermost edge of the tumor in the case of high rectal cancers and TME for mid to low rectal cancers. Although the number may be too small to draw any definitive conclusions, it did not appear to have an impact on local failure or survival.

Despite the results seen with SME, combined-

Table II. Results of total mesorectal excision for rectal cancer

Series	Stage	No. of patients	Radiation therapy	Local recurrence (%)	Survival (%)
Cawthorne et al.[7] (1990)	T123N123	122	n = 7	7	NS
MacFarlane et al.[5] (1993)	T3 or N123	135	None	5	78
Enker et al.[6] (1995)	T3 or N123	204	≈33%	6	74
Arbman et al.[8] (1996)	T123N123	128	n = 3	7	68

Adapted from Guillem JG, Paty PB, Cohen AC. Surgical treatment of colorectal cancer. CA Cancer J Clin 47:113-128, 1997.[19]

modality therapy has remained an integral part in the management of rectal cancer. In the United States, attempts to reduce the incidence of local recurrence and improve survival have emphasized postoperative adjuvant chemoradiation therapy since randomized trials have shown that adjuvant chemoradiation therapy improves local control and survival for patients with stage II and stage III rectal cancer.[26-29]

In addition to using conventional surgical techniques, these trials combined a heterogeneous population of patients (stage II and III). Therefore it is difficult to determine any stage-specific advantages to using adjuvant chemoradiation therapy.

There have been 11 randomized trials using low to moderate doses of intensive, short-course, preoperative radiation therapy for resectable rectal cancer.[30] Only six of these found a significant reduction in local recurrence rates. Other than the recently reported Swedish Rectal Cancer Trial,[31] no trial has shown a survival advantage. The Swedish Rectal Cancer Trial showed a significant decrease in the rate of local recurrence with 2500 cGy of preoperative radiation therapy compared to those treated with surgery alone (11% vs. 27%; $P < 0.001$). Local recurrence rates for Dukes' B tumors were also significantly improved in the preoperative radiation therapy group (10% vs. 23%; $P < 0.002$). Survival was also significantly improved in those who received preoperative radiation therapy compared to those undergoing surgery alone (58% vs. 48%; $P < 0.004$). There was no statistically significant difference in survival in patients with Dukes' B rectal cancers.

Several nonrandomized trials have shown enhanced sphincter preservation[32,33] and improved local failure and survival rates[34,35] with the use of preoperative combined-modality therapy. However, the true benefit of this therapy remains to be seen when compared to postoperative therapy or the use of SME alone. A phase III randomized intergroup trial (INT 0147) comparing preoperative versus postoperative combined-modality therapy for resectable rectal cancer was discontinued prior to completion because of a lack of accrual of patients. The NSABP R-03 trial evaluating the worth of preoperative multimodality therapy is ongoing, but accrual of patients has been slow.[36]

Although radiation therapy has benefits, the morbidity of radiation therapy is not inconsequential. Pelvic radiation therapy is associated with significant acute and long-term complications.[37-39] Patients who receive combined–modality therapy have been shown to have a significantly increased number of "cluster" bowel movements per day, nighttime bowel movements, difficulty with evacuation, and incontinence and wear pads more often than patients undergoing rectal cancer resection alone.[40,41]

Likewise, the addition of adjuvant chemotherapy to radiation therapy increases both the benefits as well as the morbidity. Grade 3+ toxicity in patients receiving radiation therapy alone is reported to be as low as 5% but increases to 25% to 50% in those receiving combined-modality therapy.[26,28] With an increasing emphasis on quality-of-life issues, the incremental but costly benefit of adjuvant therapy in patients undergoing optimal surgery with SME needs to be clearly defined. A two-arm randomized study of TME with or without preoperative radiation therapy for resectable rectal cancer has begun in The Netherlands.

It must be emphasized that the comparison of our results with those of the randomized trials of adjuvant therapy must be interpreted with caution, since selection bias and differences in clinicopathologic features may be responsible, in part, for these differences. Randomized trials are needed to determine these differences with certainty.

CONCLUSION

This study represents a large homogeneous population of patients with Astler-Coller B2, T3N0M0, rectal cancer who were treated with surgery using SME without the use of pre- or postoperative adjuvant therapy. The results of this study reveal an acceptably low local recurrence rate (9% crude and 12% 5-year actuarial). Based on these results and those of others,[5-8] the routine use adjuvant therapy for local control of T3N0M0 rectal cancers, particularly those without adverse pathologic factors, may not be justified.

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Duration of Antibiotic Prophylaxis in High-Risk Patients With Penetrating Abdominal Trauma: A Prospective Randomized Trial

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To evaluate the effect of varying durations of antibiotic prophylaxis in trauma patients with multiple risk factors for postoperative septic complications, a prospective randomized trial was undertaken at an urban level I trauma center. The inclusion criteria were full-thickness colon injury and one of the following: (1) Penetrating Abdominal Trauma Index ≥25, (2) transfusion of 6 units or more of packed red blood cells, or (3) more than 4 hours from injury to operation. Patients were randomly assigned to a short course (24 hours) or a long course (5 days) of antibiotic therapy. All patients received 2 g cefoxitin en route to the operating room and 2 g intravenously piggyback every 6 hours for a total of 1 day vs. 5 days. Sixty-three patients were equally divided into short-course (n = 31) and long-course (n = 32) therapy. This was a high-risk patient population, as assessed by the mean Penetrating Abdominal Trauma Index (33), number of patients with multiple blood transfusions (51 of 63; 81%), number of patients with an Injury Severity Score greater than 15 (37 of 63; 59%), number of patients with destructive colon wounds requiring resection (27 of 63; 43%), and number of patients requiring postoperative critical care (37 of 63; 59%). Differences in intra-abdominal (1-day, 19%; 5-days, 38%) and extra-abdominal (1-day, 45%; 5-days, 25%) infection rates did not achieve statistical significance. There continues to be no evidence that extending antibiotic prophylaxis beyond 24 hours is of benefit, even among the highest risk patients with penetrating abdominal trauma. A large, multi-institutional trial will be necessary to condemn this common practice with statistical validity. (J GASTROINTEST SURG 1999;3:648-653.)

KEY WORDS: Antibiotic prophylaxis, abdominal trauma, penetrating trauma

Short-duration antibiotic prophylaxis in penetrating abdominal trauma has evolved over a 20-year period ending in the early 1990s. None of the controlled studies addressing the issue has shown an advantage of long-term antibiotic therapy over a short-term course.[1-5] In fact, the literature would lead one to conclude that the number of organs injured, the specific organ(s) injured (liver, colon, pancreas), the Penetrating Abdominal Trauma Index (PATI), the number of units of blood transfused, but *not* the duration of antibiotic therapy, can be used to predict the likelihood of septic postoperative complications among patients with penetrating abdominal trauma.[2-12]

Despite these conclusions extracted from the surgical literature, anecdotal and published observations suggest that many patients receive longer "prophylactic" courses of antibiotics when they are thought to be at a particularly high risk for septic complications.[13-16] For whatever reason these practices occur, it is clear that controlled clinical trials studying the duration of antibiotic therapy in exclusively high-risk patients with abdominal trauma are necessary. Given the increased expense and the potential for side effects, toxicity, and antibiotic resistance associated with long courses of antibiotic therapy, the practice can only be justified if it results in a reduction in postoperative

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septic complications. Lacking such a study, clinical practice proceeds without scientific validity.

Accordingly, we undertook a prospective randomized trial comparing short (1-day) and long (5-day) courses of antibiotic therapy exclusively among abdominal trauma patients considered to be at particularly high risk for septic complications.

PATIENTS AND METHODS

The protocol was approved by the Institutional Review Board of Los Angeles County–University of Southern California Medical Center. Of note, the study met National Institutes of Health criteria for a nonconsent study, facilitating plans for even the sickest patients to be enrolled. Consent was obtained from all patients (or, if not practical, from appropriate family members). All patients aged 16 years and over who were admitted to the trauma center were eligible for enrollment. The 30-month period of enrollment ended on December 31, 1997. This was a study consisting exclusively of patients deemed to be at highest risk for postoperative infections and included only patients with full-thickness injuries to the colon *and* one of the following criteria: (1) PATI ≥ 25, (2) transfusion of 6 units or more of packed red blood cells, or (3) more than 4 hours from injury to operation.

Exclusion criteria were as follows: (1) serum creatinine value greater than 2.5 mg/dl; (2) previous allergic or serious adverse reaction to cefoxitin or other cephalosporins; (3) being grossly underweight for height; (4) requirement for systemic antimicrobial regimen other than those in this study (e.g., intraperitoneal pus, complicated fractures, intra-abdominal prosthetic graft); and (5) exposure to any investigational agent within 30 days of entry into the study.

Patients were resuscitated and taken directly to the operating room under the supervision of a full-time in-house trauma attending surgeon. At the conclusion of the operation, the peritoneal cavity was irrigated with 6 liters of normal saline solution, and a sample of the remaining peritoneal fluid was cultured. Surgical management of colon injuries and abdominal wounds was at the discretion of individual attending surgeons; however, the prevailing institutional philosophy is liberal primary repair of colon injuries. Exceptions to this typically included those patients with low-lying rectal injuries where primary repair was thought to be precarious and required diversion. Skin wounds were left open and managed with delayed primary closure in the vast majority of patients.

All patients were given 2 g cefoxitin intravenously piggyback en route to the operating room, with a second dose at 4 hours in cases lasting that long. After surgical confirmation of eligibility, patients were then randomized to receive 24 hours vs. 5 days total length of cefoxitin therapy (2 g intravenously piggyback every 6 hours).

The randomization schedule was constructed as follows: 100 cards and envelopes were used, with 50 each designating either 1 day or 5 days of antibiotic therapy. Five cards from each designation (10 cards total) were placed together and shuffled, and then numbered consecutively. This process was repeated nine times to use all 100 cards. The cards were filed in a box in the trauma admitting area. As each patient was enrolled in the study, the topmost card was pulled by a coinvestigator who was not involved with the patient's clinical care, and the prescribed regimen was employed for that patient. Data elements captured for each patient included demographic data, preoperative clinical findings, time from injury to surgery, duration of operation, operative findings including calculation of PATI, Injury Severity Score (ISS), and number of units of blood. Surgical management including management of colon injury and mode of fascial closure was also documented. Postoperative course was prospectively analyzed, including number of days on the ventilator and in the intensive care unit, development of complications (septic or otherwise), length of stay, and survival.

Postoperative Complications

Complications were defined as any clinical occurrence requiring a therapeutic maneuver and prolongation of the hospital course. These included complications as varied as ileus, deep venous thrombosis, epididymitis, and adult respiratory distress syndrome. Septic complications were defined as any complication requiring antibiotic therapy and included (l) pneumonia (fever, leukocytosis, pulmonary infiltrate, and $>10^3$ bronchial alveolar lavage organisms), (2) urinary tract infection ($>10^5$ organisms in urine, fever, leukocytosis), (3) positive intravenous catheter culture (positive organisms, fever), and (4) infected pleural fluid (fever, positive organisms).

"Intra-abdominal infection" was designated to exist in any patient who was found by CT aspiration or reoperation to have an intra-abdominal infected fluid collection, a subfascial abscess, or any patient with any evidence of a wound infection characterized by infected fluid coming from the midline surgical wound. Patients were thus evaluated as to whether or not they developed a postoperative complication, and specifically septic intra-abdominal or extra-abdominal complications. In four instances, patients were started on empiric antibiotic therapy because of clinical deterio-

Table I. Comparison of risk factors for septic complications between short- and long-course antibiotic therapy groups

	1 day (n = 31)	5 days (n = 32)	*P* value
Mean age in years	27.9 (range 16-62)	29.7 (range 17-68)	NS
Mean PATI	34.4	32.4	NS
No. with PATI ≥25	30 (97%)	26 (82%)	NS
No. with destructive colon wounds requiring resection	14 (45%)	13 (41%)	NS
Mean Injury Severity Score	14.5	17.1	NS
No. with Injury Severity Scores >15	14 (45%)	23 (72%)	NS
No. with multiple blood transfusions	24 (78%)	23 (72%)	NS
No. with ≥6 units blood transfused	15 (49%)	10 (32%)	NS
No. with surgical intensive care unit stay	19 (61%)	18 (56%)	NS

Table II. Comparison of outcome parameters between short- and long-course antibiotic therapy groups

	1 day (n = 31)	5 days (n = 32)	*P* value
No. with intra-abdominal infection	6 (19%)	12 (38%)	NS
No. with extra-abdominal infection	14 (45%)	8 (25%)	NS
No. with both	2 (6%)	6 (19%)	NS
No. with any septic complication	18 (58%)	14 (44%)	NS
Average length of stay (days)	17.8	19.0	NS
Mortality	1 (3%)	5 (16%)	NS

ration without definitive diagnoses. In these cases the clinical course was evaluated and patients were designated by one of the "blinded" surgical investigators who were unaware of the antibiotic regimen that the patient had received.

Sample Size Determination and Statistical Analysis

We performed a power analysis considering figures derived from a logistic regression analysis by Nichols et al.[6,9] of risk factors for postoperative infections following penetrating abdominal trauma (high risk >70%; low risk ≤40%). Using a two-sided test with power of 0.80, a *P* value of 0.05, and an assumption that one mode of therapy would decrease the infection rate from 65% to 30%, 74 patients (37 in each arm) would be necessary to identify such a difference with statistical significance. Differences in morbidity and mortality were analyzed using the Yates corrected chi-square test. Assuming a 20% dropout after intention to treat, 93 patients would need to be enrolled initially.

RESULTS

Ninety-four patients had abdominal injuries of sufficient severity to be eligible for inclusion. Twenty patients were excluded on the basis of antibiotic regimens prescribed for associated injuries requiring antibiotic therapy (18 patients with open fractures, and 2 patients with prosthetic vascular grafts). Four patients met the criteria but died within the first 24 hours from associated head or chest injuries. Another seven patients met the criteria but were not enrolled because of violation of the protocol. The remaining 63 patients were enrolled and completely evaluated until the time of death or discharge from the hospital, and from the study group.

Overall there were 60 men (95%) and three women. There were 68 colon or rectal injuries in 63 patients. Fifty-five patients (87%) sustained gunshot wounds, seven had stab wounds, and one had a colonic perforation secondary to assault with a foreign body. Table I refers to the demographics and severity of injuries of the two groups. The groups were well matched in terms of average age, mean PATI, number of patients with a PATI of 25 or more, number of patients with an ISS greater than 15, number of patients requiring any blood, and number of patients requiring more than 6 units of blood.

Table II compares the outcomes of the two groups. The trend toward fewer intra-abdominal infections and more extra-abdominal infections in the group receiving short-term antibiotic therapy did not approach statistical significance. The one death that oc-

Table III. Characteristics of patients developing intra-abdominal infections

	1 day (n = 31)	5 days (n = 32)
No. of patients developing abdominal infections	6	12
No. of patients with positive peritoneal fluid cultures at first operation	3 (50%)	7 (58%)
Cefoxitin resistance among organisms at first operation	1/3	2/7
Mean No. of days after surgery with diagnosis of infection	5.7 (range 4-8)	7.3 (range 5-14)
Type of organisms		
Gram-negative	6	10
Gram-positive	2	6
Anaerobes	2	2
Fungi	2	0
Multiple	6	11
No. of patients with organisms resistant to cefoxitin	6 (100%)	12 (100%)

curred in the short-term antibiotic group resulted from brain injury in a patient with an associated gunshot wound to the head. In two of the five patients in the long-term antibiotic group who died, death was a direct result of intra-abdominal sepsis secondary to leakage of a colonic anastomosis.

Table III reveals the specifics of organisms causing intra-abdominal infections among the two groups. Approximately half of the patients who ultimately developed intra-abdominal infections had positive peritoneal fluid cultures at the original operation. Infections were diagnosed near the end of the first week in both groups.

DISCUSSION

One might wonder why another study of antibiotic therapy in trauma patients is needed. A 1991 review by Dellinger[2] of 18 papers examining various antibiotic prophylactic regimens and durations encompassed 2397 patients whose regimens included adequate aerobic and anaerobic coverage. One hesitates to draw conclusions from a heterogeneous group of studies except to point out the absence of a correlation between the duration of antibiotic therapy and the rate of postoperative infections (1-day treatment, 12% incidence of infection; 2 days and 7 days, 9% incidence; 3 days, 16% incidence; 5 days, 14% incidence). A large, well-controlled, prospective, randomized trial by Fabian et al.[5] would seem to put the issue to rest. This study of 515 patients with penetrating abdominal trauma in a double-blinded trial of 1 day vs. 5 days of cefoxitin or cefotetan found no overall benefit from 5 days of therapy in the entire group or in the subgroup of patients with hollow viscus injuries, colon injuries, or PATI >25. In discussing the results and reviewing the literature, the authors pointed out that patients at greatest risk for infections can be identified and should become the target population for future therapeutic trials. That is the purpose of this study.

Recommendations from the literature notwithstanding, there is ample evidence that many trauma patients receive more than 24 hours of antibiotic prophylaxis. A survey of the membership of the American Association for the Surgery of Trauma regarding the management of colon injuries was undertaken in 1997.[15] Eighty-eight percent of the 342 respondents held academic appointments, and 10% had published on the subject. When questioned about preferred duration of antibiotic administration for "a patient with an isolated colon injury" (who by definition would have a maximum PATI of 20 and therefore would not be severely injured enough to qualify for our study), only 36% stated they would prescribe antibiotics for less than 24 hours. Fifty-four percent said they would prescribe antibiotics for 1 to 3 days, and 9% would prescribe antibiotics for more than 4 days. Namias et al.[16] studied the prescribing practices in the surgical intensive care unit of a large university-based trauma center and found that 61% of patients receiving prophylactic antibiotics had the agents continued for more than 1 day. An ancillary study of a multicenter prospective randomized trial of recombinant interferon gamma therapy for severely injured patients observed the antibiotic prescribing practices among the 212 enrolled severely injured patients (ISS ≥20) with contaminated wounds.[13,17] That study, which involved 62% penetrating trauma victims, identified a common practice of prescribing multiple antibiotics for a prolonged period. An average of 15 antibiotic days (number of antibiotics × number of days prescribed) were prescribed for those 115 patients who never developed an infection during their hospital course.

The development of cefoxitin resistance is a troubling finding in this study. Table III demonstrates that 3 of the 18 patients in this study who developed postoperative intra-abdominal infections had cefoxitin-re-

Table IV. Combined intra-abdominal infection rates in two prospective randomized trials of 1 day vs. 5 days of antibiotic therapy (only highest risk patients)

Institution	No. of abdominal infections		
	1 day	5 days	*P* value
University of Southern California	6/31 (19%)	12/32 (38%)	
University of Tennessee[18]	5/27 (19%)	8/29 (28%)	
TOTAL	11/58 (19%)	20/61 (33%)	0.13

sistant organisms *(Pseudomonas, Enterobacter, Citrobacter)* recovered at the time of their first operation (cultures taken following a 6-liter peritoneal irrigation). By the time their postoperative intra-abdominal infections were diagnosed, all 18 of these patients had at least one cefoxitin-resistant organism, including six patients who received the drug for only 24 hours. Each institution must make individual antibiotic selection decisions based on the microbiologic data available locally.

The postoperative infection rate, although significant, is lower than the assumptions employed in the statistical power analysis. Therefore the trends toward more intra-abdominal infections in the 5-day group, and more extra-abdominal infections in the 1-day group, did not achieve statistical significance. The study by Fabian et al.[5] is the largest prospective randomized trial on duration of antibiotic therapy for penetrating abdominal trauma and has very carefully described subgroup analysis. The study included 515 patients: 235 with hollow viscus injuries, 111 with colon injuries, 74 with hollow viscus injuries, and a PATI >25. Their subset of 56 patients who had colon injuries and a PATI >25 bears the greatest resemblance to the patient population described here (Fabian T, personal communication). Because of very similar study designs, and in order to analyze a larger sample size, we combined postoperative abdominal infections in their highest risk subset with those seen in the present study (Table IV). A mini–meta-analysis of these two studies of exclusively the highest risk trauma patients only managed to increase our statistical power from 0.27 to 0.32. Given the incidence of intra-abdominal infections (1 day, 19%; 5 days, 33%), the sample size required to detect a 14% difference in incidence with an 80% power, and to avoid a type II statistical error (conclusion of no difference based on insufficient sample size), would be 344 patients (172 in each group). This is three times the number of patients with these highest risk criteria enrolled at two busy trauma centers over time periods of 2½ to 3 years. Therefore it should be clear that in order to definitively determine whether extending antibiotic therapy in even the highest risk trauma patients would decrease the postoperative complication rate, a multi-institutional trial is necessary.

CONCLUSION

Our study confirms that postoperative septic complication rates can be expected to be high in a group of patients with predominantly gunshot injuries to the colon, who commonly had a high PATI, major blood loss, and the need for postoperative intensive care. There is no evidence to date that extending antibiotic therapy decreases that high risk. A multi-institutional trial is necessary to confirm with statistical validity that all patients, regardless of risk, should receive only a short course of antibiotics following penetrating abdominal trauma. Until such a trial is completed, our practice will be to prescribe a short course (24 hours or less) of a broad-spectrum agent in all patients, and vigorous postoperative surveillance for developing infections, with appropriate antibiotic therapy given at that time.

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Kupffer Cell–Mediated Inhibition of Liver Regeneration After Combined Hepatectomy and Pancreatectomy

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Recently, simultaneous hepatectomy and pancreatoduodenectomy has been performed for the treatment of some biliary tract cancers in Japan. Postoperative hepatic failure is a common and potentially fatal complication. The aim of this study was to examine the reduction in the rate of liver regeneration after 70% hepatectomy (Hx) alone or in combination with 70% pancreatectomy (HPx). Male Sprague-Dawley rats underwent hepatectomy or simultaneous hepatectomy and pancreatectomy. The ratio of liver weight to body weight, the labeling index of hepatocytes in vivo, and DNA synthesis of the hepatocytes and/or Kupffer cells in primary culture were analyzed. The ratio of liver weight to body weight and the labeling index in HPx rat were found to be significantly lower than those values in Hx rats. There were no significant differences in plasma alanine aminotransferase levels between the two groups. The inhibitory effect on DNA synthesis was observed with coculture of hepatocytes and Kupffer cells when the portal plasma obtained 1 hour after operation was added. We further observed that the conditioned medium of Kupffer cells stimulated by the addition of the portal plasma that was obtained 1 hour after HPx inhibited DNA synthesis of hepatocytes. This effect was abolished after incubation at 56° C for 30 minutes. These results strongly suggest the existence of a growth inhibitory factor in portal plasma after HPx. This heat-labile growth inhibitory factor was released from Kupffer cells and would appear to act on hepatocytes in a paracrine manner. (J GASTROINTEST SURG 1999;3:654-661.)

KEY WORDS: Liver regeneration, hepatocyte culture, hepatectomy, pancreatectomy, growth inhibitory factor

Hepatectomy is at present a standard treatment for primary and secondary malignancies in the liver. Recently, simultaneous hepatectomy and pancreatoduodenectomy has been performed for the treatment of bile duct cancer in Japan.[1] The function of the remnant liver is a limiting factor for hepatic resections, and liver failure accounts for most of the postoperative deaths that occur after simultaneous hepatectomy and pancreatectomy.[2] Thus knowledge of the mechanisms that inhibit and stimulate liver regeneration is important.

In the rat, regeneration after a 70% hepatectomy is completed within approximately 10 days and the peak of DNA synthesis is seen after 24 hours,[3] preceded by the increased expression of proto-oncogenes within hours after the operation.[4-6] However, the factors that influence these regeneration mechanisms after simultaneous hepatectomy and pancreatectomy are not well known.

In the present study, a model for the combined resection of both the liver and pancreas in the rat was constructed. This model was used to investigate the liver regeneration and endocrine and paracrine mechanisms that stimulate and inhibit the primary cultured hepatocytes.

MATERIAL AND METHODS

Animals

The study was approved by the Animal Care and Use Committee of Tohoku University. Male Sprague-Dawley rats with an initial weight of 250 to 300 g (Funabashi Farm, Miyagi, Japan) were used for the

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operations. The rats were fed a diet of standard laboratory chow (Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water except on the morning before the operation. For isolation of the hepatocytes and Kupffer cells, a male Sprague-Dawley rat weighing approximately 150 g was used.

Operative Procedures

Operations were performed under light diethyl ether anesthesia. In one group of rats, a 70% hepatectomy (Hx) was performed as described by Higgins and Anderson.[7] In another group of rats, the spleen and splenic vessels were preserved at 70% pancreatic resection that included the splenic, gastric, and duodenal segments, according to the technique of Richards et al.[8] The pancreatic resection was combined with hepatectomy (HPx). A 70% pancreatectomy was performed first and immediately thereafter, a 70% hepatectomy was performed. The HPx procedure took approximately 15 minutes to complete. Sham operations consisting of laparotomy and peeling off the transverse mesocolon from the pancreas and manipulation of the liver and pancreas were also performed in the Hx group. Three to six rats were used in each group.

Sampling of Blood and Liver Tissue

The operated rats were killed by intraperitoneal pentobarbital injection at 1, 3, 6, 12, 24, and 48 hours after operation. At the time of death, portal blood was aspirated from each animal. To avoid the influence of platelets, the blood was first centrifuged at 2000*g* for 30 minutes using a special tube for measuring platelet factor 4 (SRS Co., Tokyo, Japan). The separated plasma was dialyzed with phosphate-buffered saline twice for 24 hours, filtrated by means of a 0.2 mm pore filter (Minisart NML, Sartorius AG, Goettingen, Germany), and stored at −20° C.

Immediately after the blood sampling, the liver was excised and, after the wet weight was determined, specimens were frozen in liquid nitrogen and preserved at −80° C.

Regenerated Liver Weight to Body Weight Ratio

The regenerated liver weight to body weight ratio (expressed as a percentage) was calculated from the body weight and the excised liver weight at sacrifice.

Labeling Index

As an index of liver regeneration, a proliferation kit (Amersham Pharmacia Biotech, Uppsala, Sweden) was used to calculate the DNA synthesis. One hour before the rats were killed, 5-bromo-2′-deoxyuridine (BrdU) was injected into the penile vein of each rat at a dose of 30 mg BrdU/kg body weight. Microscopic sections were then prepared for immunohistochemical staining of BrdU, and the cell number at the sham stage was determined microscopically. The BrdU index in 1000 hepatocytes is the so-called labeling index.

Blood Glucose, Alanine Aminotransferase, and Endotoxin in Portal Vein Blood

Blood glucose levels were determined by means of the glucose oxidase method, alananine aminotransferase levels were assayed by automated analysis, and endotoxin levels were measured using the limulus colorimetric test (Toxicolor Test, Seikagaku Co., Ltd., Tokyo, Japan).

Culture of Hepatocytes and Measurement of DNA Synthesis In Vitro

Isolation of rat hepatocytes was performed according to the method of Seglen[9] and Nakamura et al.[10] Briefly, the liver was perfused in situ through the portal vein with Ca^{2+}, Mg^{2+}-free Hank's balanced salt solution (HBSS) (Wako Chemicals Ltd., Osaka, Japan) at 37° C, followed by perfusion with 0.05% collagenase containing HBSS for 10 minutes. The liver was excised, cut into pieces in HBSS, filtrated with gauze, and centrifuged at 50*g* for 1 minute. Viability and cell number were determined by trypan blue exclusion test. Only samples with more than 90% viability were used in the following experiments. The concentration of hepatocytes was adjusted to 2.5×10^5/ml in Williams E (WE) medium (Gibco Laboratories, Grand Island, N.Y.) supplemented with 10% fetal calf serum, insulin (10^{-8} mol/L), dexamethasone (10^{-8} mol/L), penicillin (0.1 million unit/L), and streptomycin (100 mg/L), and then 1 ml of solution each was divided into 12-well type I collagen-coated microplates (Iwaki Co., Ltd., Chiba, Japan). After 3 hours' incubation in 5% carbon dioxide at 37° C, the medium was changed to a new serum-free WE medium supplemented with aprotinin (5 IU/ml), insulin (10^{-8} mol/L), penicillin (0.1 million unit/L), and streptomycin (100 mg/L), and the sample was added. After 22 hours' incubation, the medium was changed to a new WE medium supplemented with 10 ng/ml epidermal growth factor. After 12 hours' incubation, 1.25 μCi [^{3}H]-thymidine (0.3 Ci/mmol) was added for 24 hours. To estimate the amount of [^{3}H]-thymidine incorporated into the newly synthesized DNA, the DNA was precipitated by the addition of 7% ice-cold trichloroacetic acid and trapped by filtration on a glass filter disc (Whatman GF/C). The radioactivity

was counted after the addition of scintillation fluid. The protein concentration was measured by Bio-Rad protein assay kit (Bio-Rad Laboratories, Hercules, Calif.) and [^{3}H] radioactivity/mg protein was calculated.

Coculture of Hepatocytes and Kupffer Cells and Measurement of DNA Synthesis In Vitro

The Kupffer cells were separated according to the method of Munthe-Kaas et al.[11] Briefly, the liver was perfused with Ca^{2+}, Mg^{2+}-free HBSS solution, and 0.005% collagenase and was then cut into fine slices and made into a rough cell solution. To remove dead cells and hepatocytes, the cells were centrifuged several times for 1 minute at 50*g*. The supernatants were then further centrifuged for 5 minutes repeatedly at 500*g*. The cell pellets were used as nonparenchymal cells. The hepatocytes, separated as previously described, were put into 12-well 22 mm diameter dishes. Three hours later, the dead cells were removed and the nonparenchymal cells were added to the dishes. Approximately 2 hours later, noncontact cells were removed. The coculture of hepatocytes (2.5×10^5) and Kupffer cells (1×10^6) was then prepared. The cells were stimulated with 5% portal plasma collected 1, 3, 6, 12, 24, and 48 hours after the Hx and HPx operations, and DNA synthesis was measured as previously described.

Preparation of Conditioned Kupffer Cells Medium and Measurement of DNA Synthesis In Vitro

The separation and culture of Kupffer cells were performed as previously described. After 1 hour of incubation for recovery, the unattached cells were removed and the remaining Kupffer cells were transferred to a culture medium supplemented with 5% portal plasma obtained from 1 or 3 hours after the Hx or HPx operations. The Kupffer cells were cultured for another 20 hours and then transferred to the serum-free medium. After a further 24-hour cultivation, the conditioned medium was aspirated and the cells were removed by centrifugation, dialysis of phosphate-buffered saline twice, and filtration. To investigate the inhibitory effects on the hepatocytes caused by a factor that was produced by the Kupffer cells, DNA synthesis of the hepatocytes was measured as previously described. In addition, the influence of heating (at 56° C for 30 minutes) the conditioned Kupffer cells medium was studied.

Statistical Measurements

Mean, mean ± standard deviation (SD), and mean ± standard error of the mean (SEM) were used to express values for the various groups. After testing by means of analysis of variance, the protected least significant difference test difference was used only when $P < 0.05$.

RESULTS

Regenerated Liver Weight/Body Weight

The Hx and HPx groups did not differ during the first 6 hours after operation, but the liver weight/body weight ratios in the HPx group were significantly lower than those in the Hx group 12 hours after operation (Fig. 1).

Labeling Index In Vivo

In the Hx group, a peak in the labeling index curve appeared at 24 hours after operation. However, the peak in the HPx group appeared at 48 hours after operation and with a significantly lower value (24% vs.

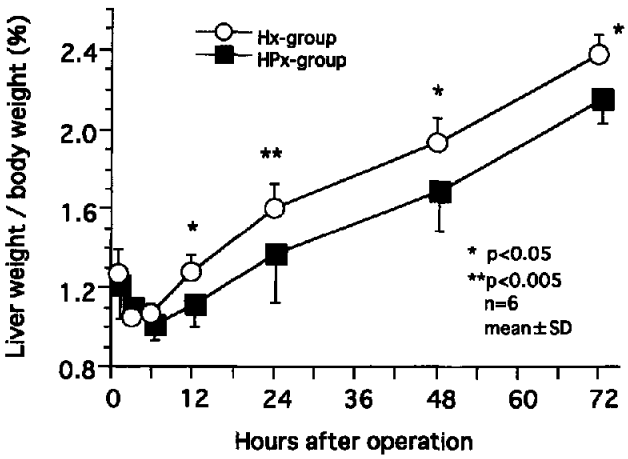

Fig. 1. Time course of regenerated liver weight/body weight after operation. Liver weight/body weight of 70% hepatectomy combined with 70% pancreatectomy is significantly lower than that of 70% hepatectomy alone after 12 hours. Each value represents the mean of the measurements (±SD) with six rats in each condition.

15%), thus indicating an apparent decrease of liver regeneration in the HPx group (Fig. 2).

Blood Glucose, Alanine Aminotransferase, and Endotoxin in Portal Blood

The blood glucose values reached their maximum in both the Hx and HPx groups 1 hour after operation and thereafter gradually decreased, but with no difference between the two groups except at 3 hours after operation (Table I). Alanine aminotransferase in the portal blood reached the peak value in both the Hx and HPx groups 12 hours after operation with no difference between the two groups (Table I). The endotoxin levels of the HPx group 3 hours after operation were slightly higher than those of the Hx group, but no significant differences were observed at 1, 6, and 12 hours (Table I).

DNA Synthesis of Coculture of Hepatocytes and Kupffer Cells With Portal Plasma

In coculture of Kupffer cells and hepatocytes stimulated by portal plasma taken 1 hour after operation, the value in the HPx group (34.7% ± 10.4%) was significantly lower compared to values in the control (serum-free) and Hx groups (90.5% ± 13.7%) (Fig. 3), whereas there were no differences between the groups in serum taken from 3 to 24 hours after operation. Serum taken at 48 hours induced lower DNA synthesis in the Hx group compared to the HPx and control groups. There were no differences between the control values of serum-free medium and plasma from unoperated rats (0 hours after operation). No difference was found in the alanine aminotransferase levels of the culture medium (data not shown).

DNA Synthesis of Hepatocytes With Conditioned Kupffer Cell Medium

When the hepatocytes were cultured with the conditioned Kupffer cell medium after stimulation by portal plasma obtained 1 hour after operation, the Hx group had a nonsignificant increase in DNA synthesis compared to the fetal calf serum control group (10.7 ± 1.6 vs. 7.3 ± 2.4 × 10^4 dpm/mg protein). On the other hand, the HPx group exhibited a significant decrease in DNA synthesis with plasma obtained 1 hour after operation compared to the Hx group (2.9 ± 1.0 vs. 10.7 ± 1.6 × 10^4 dpm/mg protein) (Fig. 4). With portal plasma obtained 3 hours after operation, a similar tendency was found, but the difference between the two groups was not significant ($P = 0.33$). When the conditioned Kupffer cell medium was heated at 56° C for 30 minutes, the inhibition of DNA synthe-

Table I. Plasma levels of glucose, alanine aminotransferase, and endotoxin after operation (n = 3 ~ 6)

	Time after operation									
	0 hour	1 hour		3 hours		6 hours		12 hours	
		Hx	HPx	Hx	HPx	Hx	HPx	Hx	HPx
Blood glucose (mg/dl)	112.1 ± 5.7	216.3 ± 11.1	239.3 ± 28.1	134.6 ± 13.5	197.0 ± 13.1*	90.3 ± 12.3	114.7 ± 27.8	133.0 ± 8.6	112.3 ± 11.3
ALT (IU/ml)	30.0 ± 3.2	229.9 ± 25.7	207.8 ± 16.4	240.6 ± 16.6	158.3 ± 22.0	396.9 ± 25.3	362.5 ± 50.9	453.9 ± 17.8	474.4 ± 91.8
Endotoxin (pg/ml)	4.3 ± 1.4	19.4 ± 4.3	23.1 ± 5.6	33.2 ± 6.1	61.0 ± 7.6*	28.4 ± 8.3	38.4 ± 5.6	35.6 ± 0.9	55.2 ± 12.9

Hx = hepatectomy alone; HPx = hepatectomy combined with pancreatectomy; ALT = alanine aminotransaminase.
Values are mean ± standard error of the mean.
*$P < 0.05$ compared to Hx.

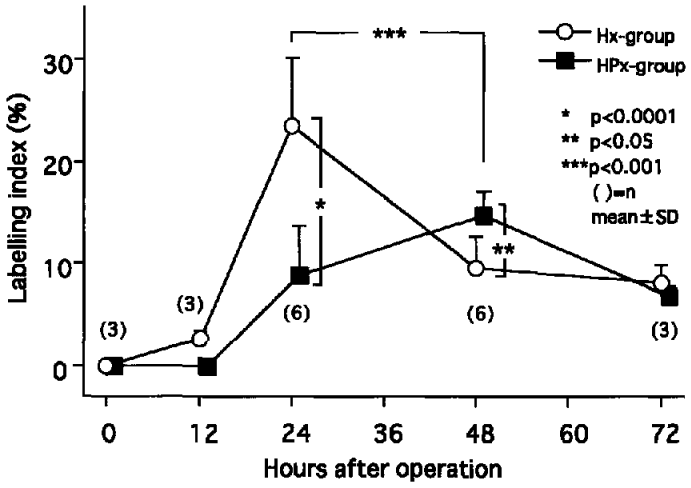

Fig. 2. Time course of the labeling index of the regenerated liver after operation. The peak time of the labeling index in the 70% hepatectomy group was 24 hours after operation, and that in the 70% hepatectomy plus 70% pancreatectomy group was 48 hours after operation. The peak level of the HPx group is significantly lower than that of the Hx group. Each value represents the mean of the measurements (±SD) with three or six rats in each condition.

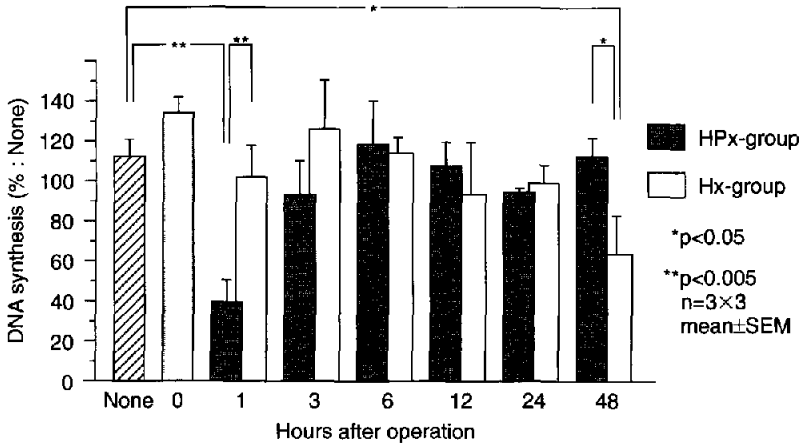

Fig. 3. Time course of DNA synthesis of rat hepatocytes and Kupffer cells in coculture by the addition of dialyzed portal plasma obtained from rats after operation. To each sample 5% portal plasma was added at 2 hours after the plating of Kupffer cells. The value in the HPx group 1 hour after operation is lower compared to values in the control (serum-free) and Hx groups. Each value represents the mean of triplicate measurements (±SEM) and the percentage for measurement of DNA synthesis in coculture without plasma.

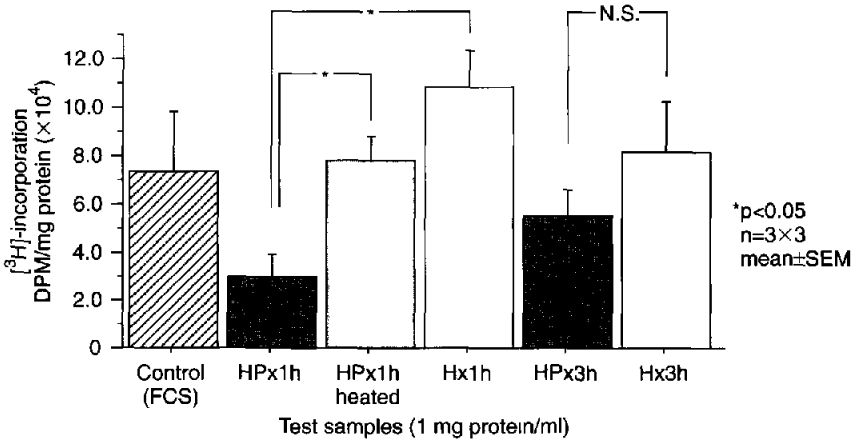

Fig. 4. [^{3}H]-thymidine incorporation of rat hepatocytes in primary culture with conditioned media from primary culture of Kupffer cells after stimulation by portal plasma collected after operation. To each sample was added 1 mg protein/ml at 3 hours after plating of hepatocytes. The HPx group exhibited a significant decrease in DNA synthesis compared to the Hx group with serum taken 1 hour after the operation. However, this inhibition of DNA synthesis was significantly improved with treatment at 56° C for 30 minutes. Each value represents the mean of triplicate measurements (±SEM). FCS = fetal calf serum.

sis was abolished but was still lower than that of the Hx group (7.9 ± 1.0 vs. 10.7 ± 1.6 × 10^4 dpm/mg protein).

DISCUSSION

As a result of advances in imaging techniques for the diagnosis of biliary tract cancer, the number of cases found in Japan has been increasing rapidly.[2] Complete resection of the tumor with removal of the lymph nodes is the approved operation. However, cancer in the extrahepatic biliary tract is usually situated at the boundary of the liver, pancreas, and duodenum, and may therefore involve the surrounding organs through direct invasion and then further spread into the adjacent portal vein and hepatic artery.[12-15] Connective tissues such as Glisson' s sheath in the hepatoduodenal ligament show discontinuous involvement by perineural and vascular spreading.[16,17] Thus simultaneous resection of the liver and pancreas is believed to be the operation of choice for bile duct carcinoma.[1,18,19] Because of the operative stress of simultaneous hepatectomy and pancreatectomy, severe complications may appear even in cases where the remnant liver seems to be sufficient. In one report, among 478 patients undergoing simultaneous hepatectomy and pancreatectomy, 70% had complications resulting from the operation, 92% of which were hepatic failure. Of those who died within 2 months after operation, 92% of the deaths were due to hepatic failure.[2] Therefore preventing hepatic failure will be an important factor in improving the operative outcome after simultaneous hepatectomy and pancreatoduodenectomy.

Because of the short history of simultaneous hepatectomy and pancreatectomy, studies on the pathophysiology of this operation have only just begun. To date, only one report concerning the endocrine and exocrine systems of the pancreas has been found.[20] To avoid impairment of liver function after operation, an understanding of postoperative liver regeneration would seem to be important. However, except for two studies on pancreatic hormones[21] and hepatic stimulator substance (HSS),[22] there are no reports concerning factors that stimulate and inhibit liver regeneration based on cultured hepatocytes in vitro.

Considering that in most instances simultaneous liver and pancreas resection is used to treat biliary tract carcinoma and that liver resection over the two segments results in incomplete liver function in most patients,[1,2] the model of Higgins and Anderson[7] with 70% resection of the liver seemed to be an appropriate rat model. In addition, in order to perform a pancreatic resection in the rat that resembles the pancreatoduodenectomy, the splenic and gastric segments comprising 70% of the pancreas were excised while maintaining the parabiliary segment and half of the duodenal segment together with the spleen and the splenic artery. Excision of the duodenal segment with 90% of the pancreas carries a high mortality rate because of the resulting diabetes mellitus. In our experience, all of the animals in our model survived at least 72 hours after operation. As shown in Table I, the blood glucose and alanine aminotransferase values revealed that there was no obvious difference in the extent of hepatic damage and the pancreatic endocrine system after 70% hepatectomy alone or in combination with 70% pancreatectomy. In addition, the ratio of regenerated liver weight to body weight and the labeling index indicate that liver regeneration was significantly decreased and delayed after simultaneous hepatectomy and pancreatectomy, suggesting the usefulness of this model for elucidating the stimulation and inhibition of liver regeneration after these operations.

To investigate the mechanism of stimulation and inhibition of liver regeneration, we used a coculture system of Kupffer cells and hepatocytes in which liver regeneration is inhibited through a paracrine mechanism by the Kupffer cells (Fig. 3). Stimulation of hepatic DNA synthesis by the Kupffer cells[23] and regulation of the liver regeneration by a balance of TGF transforming growth factor (TGF)–α and TGF-β secreted by the Kupffer cells[24] have been reported. In the present study, the hepatocyte proliferation after the addition of plasma taken from HPx rats 1 hour after operation was inhibited compared to that in control rats, and this tendency remained with plasma taken 3 hours after operation. This effect was not caused by the levels of insulin because excess insulin was added to the culture medium. Based on these results, there seems to be not only a stimulatory factor for hepatocyte proliferation in the portal plasma of rats in the HPx group, but also another factor that stimulates the Kupffer cells to secrete an inhibitory factor. Endotoxin was the candidate for this factor, but there was no difference between the Hx and HPx plasma levels taken at 1 hour after operation (see Table I). We hypothesize that the stimulated Kupffer cells secrete an inhibitory factor, the influence of which is stronger than that of the stimulatory factor in portal serum, thereby inhibiting the DNA synthesis of the hepatocytes.

Next we examined the effect of the conditioned Kupffer cell medium. As shown in Fig. 4, the portal plasma taken 1 hour after the HPx operation stimulated the Kupffer cells, and the conditioned medium had an inhibitory effect on hepatocyte proliferation similar to that of the coculture. The inhibitory effect of the conditioned medium was partially abolished after incubation at 56° C for 30 minutes, suggesting that the Kupffer cells release an inhibitory factor that is

heat unstable and therefore might be a protein. Interestingly, this inhibitory factor disappears from serum only 3 hours after operation. In addition, compared to nontreated medium, Kupffer cells also release growth-stimulating factors such as hepatocyte growth factor and/or epidermal growth factor, both of which are degenerated by heating.

Another possibility is that there is preexisting suppression factor from Kupffer cells that inhibits hepatocyte DNA synthesis and maintains hepatocytes in a normal state of quiescence. Following hepatectomy alone, production of this inhibitory substance is decreased, thereby releasing the hepatocyte to reenter the cell cycle. Alternatively, when pancreatectomy is combined with hepatectomy, there is no corresponding decrease in this suppression factor, or the decrease is diminished, resulting in maintenance of any higher percentage of cells in G0. These possibilities cannot be disregarded.

Future studies will reveal whether the inhibitory factor is novel or one of the known cytokines such as TGF-β,[25,26] activin A,[27] or interleukin-1β.[28] More detailed studies to purify and characterize the factor will surely follow shortly. Thus studies of the inhibitory effects of liver regeneration will provide more insight into the prevention of liver failure after simultaneous hepatectomy and pancreatectomy.

CONCLUSION

A model for simultaneous liver and pancreas resection in the rat is presented, and the effects on liver regeneration were studied. The regenerated liver weight and labeling index in the HPx group were significantly lower than values in the Hx group. The results suggest the presence of a factor that inhibits liver regeneration after combined resection of liver and pancreas. In the HPx group, a factor in the portal blood stimulates the Kupffer cells, which release another heat-labile factor that inhibits hepatocyte proliferation in a paracrine manner. More detailed studies to purify and characterize the factor secreted from Kupffer cells will surely follow shortly.

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Difluoromethylornithine Inhibits Crypt Fission

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Crypt fission is a physiologic mechanism of crypt reproduction. It increases in pathophysiologic situations where intestinal regeneration is required (e.g., radiation injury). Polyamine metabolism is important in the regulation of intestinal growth and recovery from injury in response to a variety of stimuli. Our aim was to determine whether inhibition of polyamine synthesis by difluoromethylornithine (DFMO) influenced crypt fission. Forty-eight rabbits underwent patch enteroplasty in the terminal ileum. One group served as a control group and the other took 2% DFMO orally. Animals (n = 6) from each group were killed at 7, 14, 21, and 28 days. Normal ileum adjacent to the enteroplasty was studied. Crypt dissection was performed 2 hours after vincristine was administered intravenously to determine crypt cell production rate, crypt depth, and proportion of bifurcating crypts (fission). DFMO administration decreased crypt fission (4 ± 2% vs. 11 ± 2% and 13 ± 1% vs. 34 ± 4% at 7 and 14 days) compared to control animals. There was a corresponding increase in crypt depth at 14 and 21 days. Crypt cell production rate was similar in both groups and did not change with time. Mucosal ornithine decarboxylase activity (11.9 ± 2.2 vs. 1.2 ± 0.3 specific activity at 21 days; $P < 0.05$) and polyamine content (323 ± 32 vs. 17 ± 8 and 382 ± 89 vs. 160 ± 47 pmol/mg at 14 and 21 days, control vs. DFMO; $P < 0.05$) were significantly lower in the DFMO group. The following conclusions were drawn: (1) DFMO administration inhibits crypt fission in stimulated intestinal epithelium; (2) this effect correlates temporally with reduced polyamine production; and (3) reduced crypt fission is another potential mechanism of inhibition of intestinal growth by altered polyamine metabolism. (J GASTROINTEST SURG 1999;3:662-667.)

KEY WORDS: Polyamines, intestinal crypts, intestinal growth

Homeostasis is maintained in the intestinal mucosa when the cellular output from the crypts matches the loss of enterocytes from the villus associated with those crypts. Total cell production by the crypts is influenced not only by proliferation rates in individual crypts but also by the number of crypts feeding the villus.[1] The crypt to villus ratio increases with age, is influenced by fasting and feeding, and decreases along the length of the gastrointestinal tract.[2,3] There is a low rate of ongoing crypt replication in steady state epithelium.[1] There is some evidence to suggest that the crypt size is regulated and when a certain crypt size is attained, bifurcation occurs.[1] However, crypt fission might also be induced as an independent process for increasing cellular output from the crypt, especially at times of stress-induced enterocyte demand.

Polyamine metabolism is an important regulator of cellular growth in the small intestine.[4-7] The polyamines stimulate growth by enhancing enterocyte proliferation, differentiation, and migration.[4,5] Ornithine decarboxylase, the rate-limiting enzyme of polyamine biosynthesis, and diamine oxidase, an enzyme that degrades polyamines, are the primary regulators of polyamine content. Both stimulation of ornithine decarboxylase activity and suppression of diamine oxidase activity increase polyamine production and enhance cellular growth.[4,6-12] Difluoromethylornithine (DFMO), an irreversible inhibitor of ornithine decarboxyline, reduces polyamine synthesis and inhibits cellular growth.[8-13] In a previous study, the effects of DFMO on intestinal regeneration were evaluated and no significant effects were noted despite decreased polyamine synthesis, villus height, and crypt cell production.[14] Consequently it was concluded that the lack of an effect on regeneration was due to unaltered cell migration, which is important in intestinal regeneration. However, effects, if any, of

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DFMO on intestinal growth due to alterations in crypt fission were not addressed in previous studies. Because crypt fission represents another mechanism for intestinal growth, the aim of the present study was to determine whether inhibition of polyamine synthesis by DFMO would influence the rate of crypt fission.

MATERIAL AND METHODS

Forty-eight male New Zealand white rabbits (2 to 4 kg) were included in the study. Two 2 × 5 cm ileal defects were patched with the adjacent colon serosal surface in each animal. One half of the animals were given water and rabbit chow ad libitum. The other half were given 2% DFMO (kindly supplied by Merrell Dow Research Institute) in drinking water and rabbit chow ad libitum. These animals drank an average of 190 ml water per day with an average dose of DFMO of 1426 mg/kg/day. Six animals in each group were killed 7, 14, 21, and 28 days after operation. Tissue ornithine decarboxylase activity, diamine oxidase activity, and polyamine levels were measured to assess polyamine metabolism. Crypt dissection was performed to determine crypt depth, crypt cell production rates, and proportion of bifurcating crypts.

Operations were performed after an overnight fast using sterile technique. Anesthesia was achieved with intramuscular ketamine (35 mg/kg) and xylazine (7 mg/kg). Intestinal patching was performed by identifying the distal ileum and making two 5 cm incisions on the antimesenteric border, the first beginning 20 cm proximal to the ileocecal junction and the second beginning 10 cm proximal to the ileocecal junction. The defects were closed with a 2 × 5 cm patch of adjacent colon serosal surface with a continuous inverting 4-0 silk suture. The abdomen was irrigated with 10 ml of sterile water containing 10 mg ampicillin, and the incision was closed. The animals received 100 mg of ampicillin intramuscularly preoperatively and twice daily for 3 days after operation. One hundred milliliters of 5% dextrose water was administered subcutaneously following the operation. The animals took nothing by mouth the first postoperative day, received fluids the second day, and took full alimentation after the third postoperative day.

Crypt cell production rate was determined using a metaphase arrest technique.[15] Vincristine sulfate (1 mg) was administered by intraperitoneal injection 2 hours before the animals were killed. The mucosal sample was fixed in Carnoy's fixative and then hydrolyzed at 60° C for 5 minutes with Schiff's reagent. Crypts and villi were separated from the underlying connective tissue and the crypts were dissected free using a dissecting microscope. Samples of crypts were transferred to a glass slide in 15% glacial acetic acid and squashed for determination of the number of metaphases per crypt in a minimum of 10 crypts. The mean of the metaphase counts of the crypts was taken as the reading for each sample. Crypt cell production was calculated assuming a linear accumulation for 2 hours. The proportion of bifurcated crypts was also determined (Fig. 1).

Polyamine levels were assayed in a mucosal homogenate containing 100 mg of mucosa in 50 mmol/L potassium phosphate. The polyamines (putrescine and spermidine) were separated by reverse-phase, ion-pair, high-performance liquid chromatography using a C18 column and quantitated by fluorescent detection after postcolumn derivatization with ophthalaldehyde.[7] The mobile phases used in this separation consisted of two buffers: buffer A contained 85% 0.1 mol/L acetic acid, 15% methyl alcohol, and 10 mmol/L heptanesulfonic acid; buffer B contained 75% 0.25 mol/L acetic acid and 25% methyl alcohol. The program was 2 minutes of 100% buffer B, which pumped for an additional 20 minutes. The height of the peak at the appropriate retention time was determined and concentrations were calculated based on a

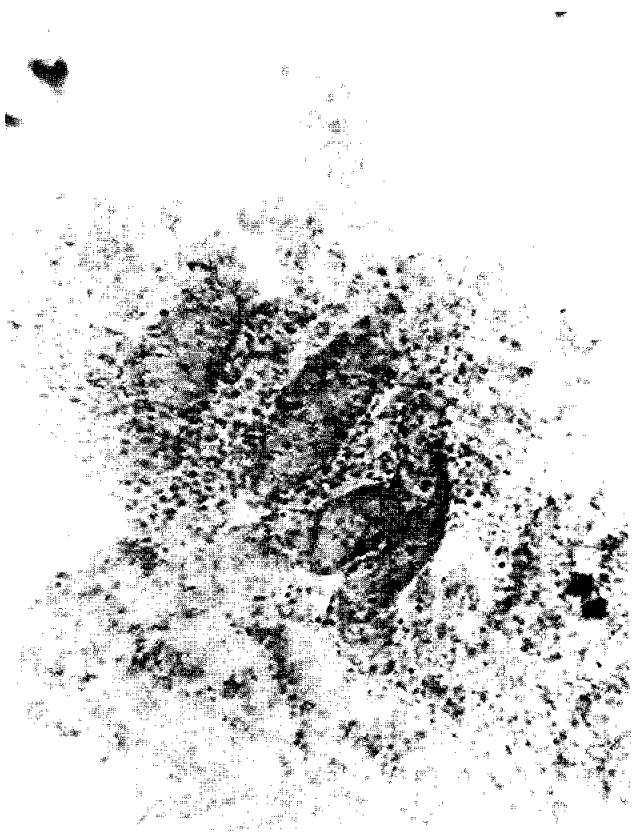

Fig. 1. Bifurcated crypts determined by crypt dissection. A crypt bifurcated for a third of its length is shown to the right of two nonbifurcating crypts.

standard curve. Polyamine levels were expressed as picomoles per milligram mucosa.

Ornithine decarboxylase activity was determined by a radiometric technique in which the amount of {l4C} ornithine was estimated.[7] Ornithine decarboxylase activity was expressed in picomoles of carbon dioxide per milligram mucosa per 30 minutes. Diamine oxidase activity was determined using [14C] putrescine as the substrate and quantitating the radiolabeled pyrroline produced.[7] One unit of diamine oxidase activity equals 1×10^{-10} mole putrescine metabolized per hour at 37EC and pH 7.2.

Data are expressed as the mean ± standard error of the mean. Analysis of variance with the Bonferonni correction as appropriate was used for comparisons. Correlations were evaluated by linear regression analysis. Statistical significance was ascribed to P values <0.05.

RESULTS

Animals in both groups exhibited normal behavior. Control animals maintained body weight during the study. Animals receiving DFMO lost 10% to 15% body weight ($P < 0.05$) during the 14 days following operation, and body weight remained consistently below preoperative levels. Bowel movements were of normal consistency in all animals.

Patch enteroplasty resulted in a peak in crypt fission, crypt cell production rate, and crypt depth at 14 days (Fig. 2). DFMO administration decreased crypt fission at 7 and 14 days after operation, but the proportion of bifurcated crypts was similar to that in the control group at 21 and 28 days. Crypt depth increased significantly in DFMO-treated animals at 14 days and remained greater compared to control values at 21 days before returning to more normal levels. Crypt cell production rate was similar in both groups throughout the 28-day study period.

There was a significant positive correlation between crypt fission and crypt depth in the control group (r = 0.451, $P = 0.043$) (Fig. 3). There was not a significant correlation between these parameters in the DFMO-treated animals ($r = -0.159$; P = 0.508).

DFMO administration reduced polyamine content and enzyme activity associated with polyamine metabolism (Table I). Mucosal ornithine decarboxylase activity was lower in DFMO-treated animals at all time points, significantly so at 21 and 28 days. Mucosal diamine oxidase activity increased significantly at 14, 21, and 28 days in the control group. This increase was inhibited by DFMO with reduced diamine oxidase activity. Mucosal polyamine content increased significantly in the control group at 14, 21, and 28 days compared to 7 days. In DFMO-treated animals,

Table I. Assessment of polyamine metabolism

	Control				Difluoromethylornithine			
	7 days	14 days	21 days	28 days	7 days	14 days	21 days	28 days
Mucosal weight (mg/cm²)	29 ± 4	50 ± 5*	36 ± 3	30 ± 3	33 ± 4	36 ± 4	35 ± 3	40 ± 7
Ornithine decarboxylase activity (specific activity/mg mucosa)	7.2 ± 2.4	8.2 ± 1.7	11.9 ± 2.2	4.2 ± 0.4	4.7 ± 0.6	6.5 ± 1.1	1.2 ± 0.3*†	1.3 ± 0.3*†
Diamine oxidase activity (units/mg mucosa)	0.4 ± 0.1	2.0 ± 0.2*	2.3 ± 0.5*	1.7 ± 0.4*	0.4 ± 0.1	0.7 ± 0.2†	0.2 ± 0.1†	0.2 ± 0.1†
Polyamine content (pmol/mg)	65 ± 44	323 ± 32*	382 ± 89*	300 ± 24*	39 ± 24	17 ± 8†	160 ± 47*†	172 ± 46*†

*$P < 0.05$ vs. 7 days.
†$P < 0.05$ vs. control.

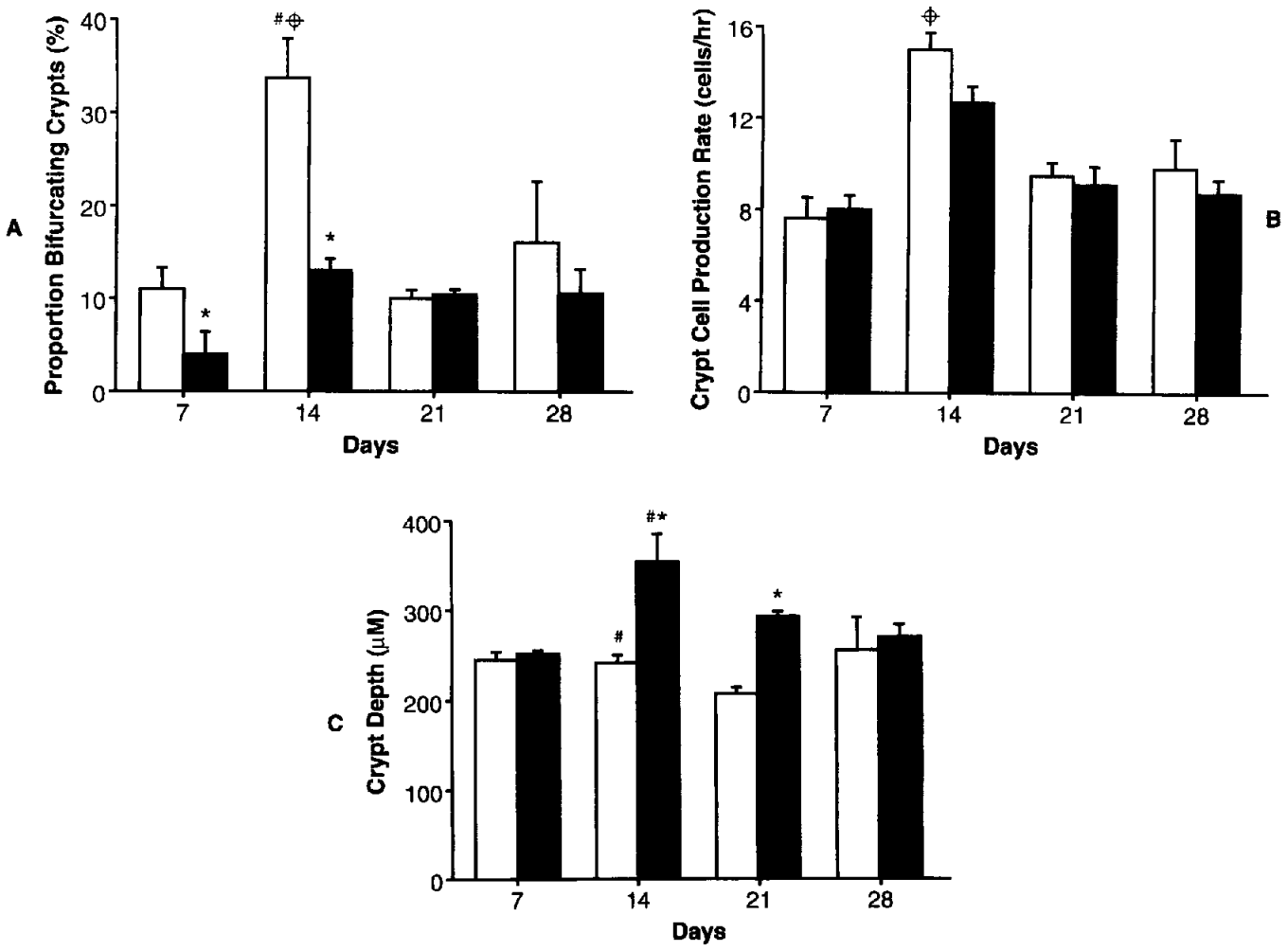

Fig. 2. Comparison of **(A)** crypt fission, **(B)** crypt cell production rate, and **(C)** crypt depth in the two groups at weekly intervals. * = $P < 0.05$ vs. control; # = $P < 0.05$ vs. 21 days; ⊕ = $P < 0.05$ vs. 7 days.

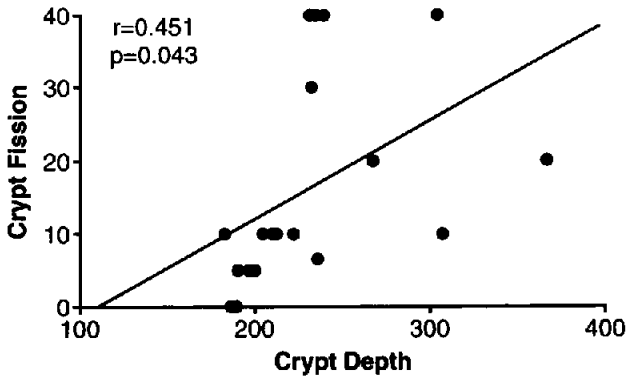

Fig. 3. Correlation between crypt fission and crypt depth in control animals.

polyamine content decreased significantly at 14 days but then increased above 7-day values at 21 and 28 days. However, all values were significantly lower than values in the control group.

DISCUSSION

Increased crypt cell production rate is usually the primary mechanism for increasing cellular output to the villus but in some situations of increased cellular demand, bifurcation of existing crypts occurs.[1,16,17] Patch enteroplasty transiently increased crypt fission and crypt cell production rate in the normal mucosa adjacent to the serosal patch in the present study. Increased crypt fission has been documented in several other circumstances as well, including the regenerative response to irradiation injury and after intestinal resection.[1,16,17] However, Hanson et al.[18] did not find increased crypt production in the intestinal remnant after resection but did note increased bifurcation adjacent to the anastomosis. Whether trophic stimuli such as polyamines or growth factors stimulate crypt fission is not clear. Although hypophysectomy decreases the ratio of crypts to villus, administration of growth hormone does not stimulate crypt fission.[19,20] We found previously that epidermal growth factor increased crypt fission in stimulated epithelium.[21] Crypt fission may play a role in abnormal tissue growth as well. Germline mutations of the APC gene result in an increased number of normally dividing crypts.[22] In this situation, crypt branching occurs on the lateral side of the crypt rather than the base, where bifurcation usually commences.

We sought to determine whether diminished polyamine production would inhibit crypt fission. In the present study, DFMO administration at a dose that reduced ornithine decarboxylase activity and mucosal polyamine levels inhibited rates of crypt fission in intestinal epithelium stimulated by patch enteroplasty. This effect was transient, however. This finding is consistent with previous reports and suggests that adaptation occurs despite the reduction in ornithine decarboxylase–ornithine decarboxylase mediated polyamine production.[10,14] Thus reduced crypt fission is another potential mechanism of inhibition of intestinal growth by altered polyamine metabolism. The present study does not clarify whether this is a specific effect of DFMO or is related to the reduced polyamine production. However, it emphasizes that this component of the adaptive mechanism must be considered in future studies of intestinal regeneration and adaptation.

Crypt fission did not increase until 14 days after patch enteroplasty. Similarly, Cheng et al.[16] found that crypt fission was increased at 3 weeks but not 1 week after 30% intestinal resection in mice. It has been suggested that crypt fission occurs in response to increased crypt depth, maintaining the size of crypts within some normal range.[1] Thus changes in rates of crypt bifurcation might not change until crypts elongate. There was a significant positive correlation between crypt depth and proportion of branching crypts in the present study. Crypt depth in DFMO-treated animals increased in parallel with reduced rates of crypt fission. This crypt elongation after DFMO administration has been reported by others.[8,23] This finding suggests that DFMO might block a normal signal to crypt progenitor cells or the crypt fission process itself. There was not a significant correlation between crypt depth and crypt fission in DFMO-treated animals.

An alternative explanation for the delayed response in crypt fission is that DFMO did not have a significant impact on ornithine decarboxylase activity and polyamine concentrations until 14 days. The reason for the delayed effect is not clear. Other investigators have reported a fairly prompt reduction of ornithine decarboxylase activity and polyamine synthesis within the first few days of administratiion.[9-12] Impaired absorption of DFMO in the early postoperative period is one possibility. The effects of DFMO are dose dependent.[9]

In summary, DFMO administration inhibits crypt fission in stimulated intestinal epithelium. This effect correlates temporally with reduced polyamine production. Reduced crypt fission is another potential mechanism of inhibition of intestinal growth by altered polyamine metabolism.

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Paraplegia Following Intraoperative Celiac Plexus Injection

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The technique for percutaneous and open neurolytic celiac plexus injection, using ethanol or phenol, for relief of intractable pancreatic cancer pain has been well described. Prospective randomized studies, demonstrating safety and efficacy with few complications, have led to widespread acceptance and use of this palliative procedure. The complications of neurolytic celiac plexus injection are rare, and are usually minor. However, transient or permanent paraplegia has been reported previously in 10 cases. The case described herein represents the third reported case of permanent paraplegia following open intraoperative neurolytic celiac plexus injection using 50% ethanol. The literature surveying the indications for this procedure, routes of administration, known complications, and their pathophysiology are reviewed. (J GASTROINTEST SURG 1999; 3:668-671.)

KEY WORDS: Celiac plexus injection, chemical splanchnicectomy, pancreatic cancer, cancer pain, paraplegia

Neurolytic celiac plexus injection (NCPI) for palliation of pain in patients with unresectable pancreatic cancer was first described by Copping et al.[1] Open and percutaneous approaches to NCPI were developed and gained increasing support as this technique was perfected for the treatment of patients with intractable pain resulting from pancreatic cancer.[2] A number of studies,[3-5] including a prospective randomized trial,[6] established the safety and efficacy of the procedure and its ability to decrease patients' requirements for opiate analgesics and their attendant adverse side effects. The elegant study by Lillemoe et al.[6] clearly established the ability of this procedure to improve actuarial survival for patients with unresectable pancreatic cancer.

Few complications have been reported following this procedure when it is performed in combination with exploratory laparotomy and biliary bypass, gastric bypass, or tumor biopsy. Major complications are extremely rare and usually involve transient or permanent paraplegia. The world literature reports only 10 cases of paraplegia following NCPI, eight of which involved permanent loss of motor function. Of these ten reports, only two have occurred following NCPI when performed as an open operative procedure. Table I presents a review of the world literature of reported temporary or permanent paraplegia following percutaneous or open NCPI. To date, the existence and frequency of these complications have not been reported in the surgical literature. This article describes the third reported case of permanent paraplegia following open NCPI, in order to increase awareness of this devastating potential complication, explore its pathophysiology, and reaffirm the role of this technique of palliation in the management of patients with unresectable pancreatic cancer.

CASE REPORT

A 42-year-old woman presented to her primary care physician with painless jaundice; CT scan showed a mass in the head of the pancreas. She subsequently underwent exploratory laparotomy, and at the time of operation was judged to have an unresectable pancreatic carcinoma compressing the distal common bile duct. A cholecystojejunostomy was performed to bypass the biliary obstruction, and biopsy specimens of the pancreatic mass showed adenocarcinoma of the pancreas. Following operation, she had a 4-month symptom-free interval and was thereafter referred to our practice with progressive symptoms of gastric outlet obstruction and intractable abdominal pain radiating to the

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back and left flank. The abdominal pain was not adequately controlled with increasing dosages of oral narcotic analgesics, and these further worsened the nausea and vomiting associated with the gastric outlet obstruction. CT scans were obtained, which showed a large mass involving the head and proximal body of the pancreas, with no evidence of obvious direct or nodal metastases. It was our recommendation that she proceed with exploratory laparotomy for an attempted Whipple procedure versus a gastric bypass procedure combined with a palliative NCPI. Informed consent was obtained for the above-mentioned procedures, following a detailed discussion of the relative risks of complications usually associated with the recommended operative procedures.

The patient subsequently underwent exploratory laparotomy, which revealed a sizable pancreatic mass that was firmly fixed to the retroperitoneum and encased the superior mesenteric vein. The tumor mass partially compressed the proximal duodenum, and a gastrojejunostomy was created to bypass the gastric outlet obstruction. NCPI was performed using the technique described by Lillemoe et al,[6] which employed a total volume of 50 ml of 50% ethanol divided equally between the left and right para-aortic regions adjacent to the takeoff of the celiac artery. The gastric bypass, NCPI, and remainder of the operation proceeded without difficulty, and following extubation the patient was transferred to the recovery room in stable condition.

Approximately 6 hours postoperatively the patient was alert and conversant, stating that her preoperative back and flank pain was entirely relieved. However, her bilateral lower extremities were noted to be flaccid and areflexic. Careful neurologic examination revealed loss of movement, pinprick, and temperature sensation below the T10 level, while light touch and proprioception were preserved. Emergent MRI was obtained and revealed images consistent with anterior spinal cord ischemia and infarction at the T9-10 level (Fig. 1). The anterior spinal artery was noted to be intact (Fig. 2) at levels superior to the T9-10 lesion

Table 1. Previous reported cases of temporary or permanent paralysis following celiac plexus neurolytic injection

Reference	Date	Tumor type	Technique	Fluoroscopy	Neurolytic agent	Duration
Jabbal and Hunton[14]	1992	Pancreatic	Percutaneous	Yes	50% ethanol 0.25% bupivicaine	Temporary
Wong and Brown[20]	1995	Pancreatic	Percutaneous	No	50% ethanol 0.25% bupivicaine	Temporary
Galizia and Lahiri[13]	1974	Pancreatic	Percutaneous	No	5% phenol	Permanent
Cherry and Lamberty[16]	1984	Pancreatic	Percutaneous	Yes	100% ethanol	Permanent
Woodham and Hanna[15]	1989	Not reported	Percutaneous	Yes	90% ethanol	Permanent
van Dongen and Crul[11]	1991	Colon	Percutaneous	Yes	96% ethanol 1% lidocaine	Permanent
DeConno et al.[18]	1993	Pancreatic	Percutaneous	Yes	50% and 95% ethanol	Permanent
Takeda et al.[19]	1996	Gastric	Percutaneous	Yes	Ethanol	Permanent
Kinoshita et al.[21]	1996	Pancreatic	Intraoperative	N/A	Ethanol	Permanent
Hayakawa et al.[17]	1997	Gastric	Intraoperative	N/A	99.5% ethanol	Permanent
Present case	1998	Pancreatic	Intraoperative	N/A	50% ethanol	Permanent

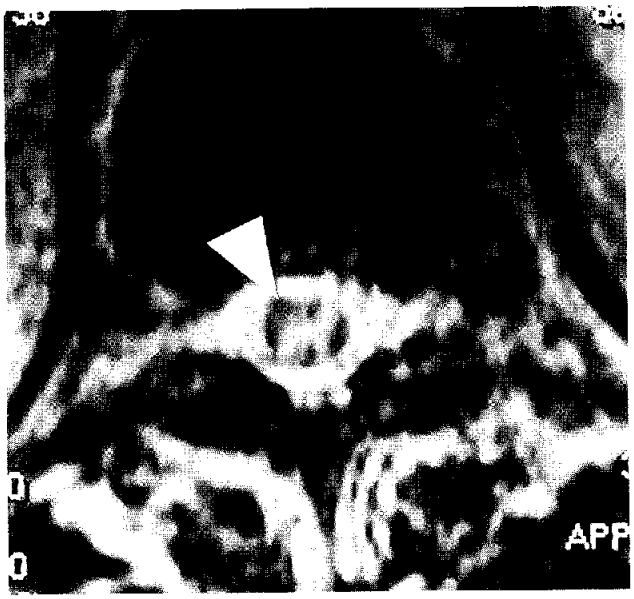

Fig. 1. T_2-weighted MRI of the thoracic spine at the T9-10 level. This image shows anterior spinal artery ischemia with infarction seen as characteristic "H" pattern (arrow).

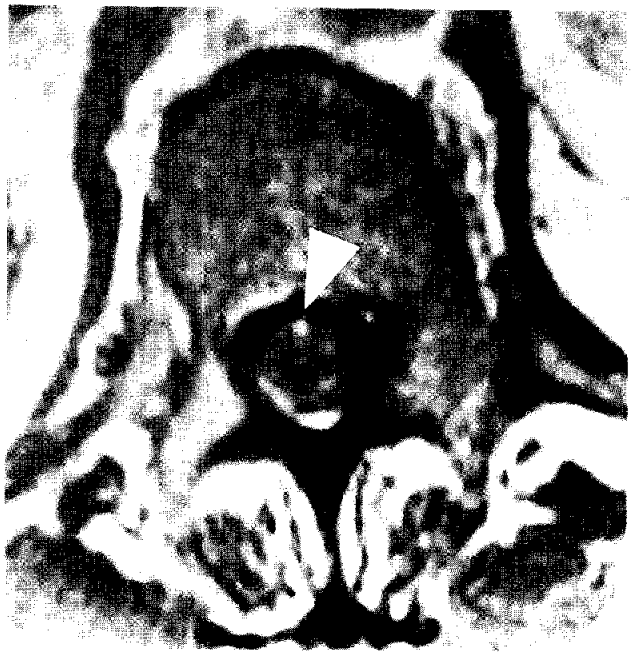

Fig. 2. T_2-weighted MRI of the upper thoracic spine in the same patient with anterior spinal artery ischemia and infarction. Arrow indicates flow through the anterior spinal artery of Adamkiewicz.

seen on these studies. Neurosurgical consultation was obtained, and the presumptive diagnosis was anterior spinal cord infarction secondary to spasm of the spinal artery of Adamkiewicz. Despite aggressive steroid pulse therapy during the ensuing 72 hours, the patient experienced no improvement in neurologic function following the operation. She remains pain free, and the gastric outlet obstruction symptoms have entirely resolved.

DISCUSSION

Serious complications following NCPI are rare.[4,7] Observed complications include paresis, temporary paralysis, seizures, and temporary unconsciousness following injection with phenol, ethanol, or various local anesthetic agents.[8] The world literature contains reports of 10 previous cases of paraplegia following NBPI, in which eight patients experienced permanent paraplegia. Only two of these eight cases occurred following NCPI performed as an open procedure combined with an abdominal operation, and the case presented in this article represents the third such case.

The etiology of this rare and devastating complication of NCPI remains uncertain. Patients in whom these symptoms develop demonstrate neurologic symptoms consistent with anterior spinal cord syndrome. This syndrome is characterized by loss of pinprick and temperature sensation, intact light touch sensation, complete loss of motor function, and radiographic findings consistent with ischemic injury to the anterior columns of the spinal cord. The ischemic injury to the cord is believed to follow transient or permanent reduction in flow from the anterior spinal artery of Adamkiewicz. The artery of Adamkiewicz typically originates from the aorta at the vertebral levels T9 to T11, although its origin has been variably described between T7 and L4.[9]

NCPI is performed at the level of the celiac artery, which originates from the aorta between the T12 and L1 vertebrae. The neurolytic agents preferred for NCPI—ethanol and phenol—are known to cause concentration-dependent contraction of vascular smooth muscle.[10]

Direct arterial injection or mechanical injury is of concern during NCPI, and most authors advocate aspiration prior to injection to ensure the absence of blood. When performing NCPI using the percutaneous approach, injecting a small amount of contrast material before the ethanol or phenol verifies that the needle tip is extravascular, but this has not eliminated the complication of paraplegia.[11]

During injection of the celiac plexus, Hardy and Wells[12] demonstrated that injected fluid typically spreads superiorly as high as the midthoracic and cervical levels. Thus the etiology proposed for development of paraplegia is spasm or thrombosis of the anterior spinal artery of Adamkiewicz, resulting from superior spread of injected ethanol or phenol, and induced vascular smooth muscle spasm.[11,13-19]

Direct spinal cord injection, injection into the cerebrospinal fluid or epidural space, and hematoma have been raised as possible causes of paraplegia following NCPI. However, the selective neurologic deficits resulting from anterior spinal column injury could not be created by these potential injuries.

The risk of paralysis following NCPI is impossible to determine precisely, since the total number of procedures performed annually remains unknown. This certainly is an extremely rare complication when NCPI is performed as an open procedure by surgeons, as this report represents only the third reported case in the world literature. The existence of paraplegia following NCPI should be recognized as a potential complication by surgeons who perform this procedure. Since risk of paraplegia following this procedure is so low, there may be institutional biases as to whether to discuss this risk when obtaining informed consent. In our practice we now include discussion of the potential risk of temporary or permanent paralysis with patients who are likely to require this procedure. During this discussion we stress the extremely

low risk of this complication and emphasize the significant benefits in terms of quality of life and survival for patients who receive this treatment.

The risk of temporary or permanent paralysis following NCPI represents a catastrophic potential complication. However, it is extremely important for surgeons not to lose sight of the clear scientific data that demonstrate excellent palliation of pain, improved quality of life, and increased survival[3,5,6,20] for patients undergoing this procedure. These benefits, and not the extremely small risk of complications, should support surgical practice to continue offering this procedure to all patients with unresectable pancreatic cancer.

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Effects of Intestinal Resection on Enterocyte Apoptosis

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Intestinal resection results in increased numbers of villus and crypt enterocytes. This adaptive response occurs secondary to increased crypt cell proliferation early after resection. Apoptosis also increases in both crypt and villus compartments early after resection. Our aim was to evaluate change in proliferation and apoptosis later during the adaptive period. Eighteen rabbits undergoing 50% proximal intestinal resection were killed 7, 14, or 21 days after resection. Intestine at the resection margin was evaluated before and after resection for morphometry, crypt cell production rate (CCPR), and in situ end labeling of DNA fragmentation. Apoptotic cells were identified by morphologic evaluation. Villus height (382 ± 51 μm vs. 505 ± 131 μm) and crypt depth (121 ± 10 μm vs. 163 ± 21 μm; $P < 0.05$) were significantly increased at 21 days. CCPR was also increased compared to preoperative values (5.8 ± 1.2 cells/hr vs. 8.6 ± 0.3, 7.9 ± 0.3, and 8.3 ± 1.6 cells/hr at 7, 14, and 21 days; $P < 0.05$). Apoptotic index (apoptotic cells per 100 cells) was significantly increased in cells at the tip of the villus at 21 days (32 ± 7% vs. 19 ± 7%; $P < 0.05$) but not in the lateral villus or total villus. Total cells per villus (83 ± 6 vs. 65 ± 3) and apoptotic cells per villus (9.2 ± 1.6 vs. 4.5 ± 2.5; $P < 0.05$) were also greater at 21 days. Mean DNA fragmentation scores were similar before and after resection. The crypt apoptotic index was increased only in the lateral crypt at 14 days. Total cells per crypt increased after resection, and the number of apoptotic cells per crypt was increased at 7 and 14 days (2.5 ± 1.7 and 2.3 ± 1.1 vs. 0.6 ± 0.6 days; $P < 0.05$). Thus the following conclusions were reached: (1) Apoptosis of villus tip cells remains elevated late in postresection adaptation; (2) crypt cell apoptosis returns to preoperative levels during the time interval when crypt cell proliferation is still stimulated; and (3) apoptosis in the crypt and villus compartments appears to be regulated independently. (J GASTROINTEST SURG 1999;3:672-677.)

KEY WORDS: Apoptosis, intestinal adaptation

Intestinal resection results in increased numbers of villus and crypt enterocytes.[1,2] This adaptive response occurs primarily because of increased crypt cell production after resection. Both an increased number of crypts per villus and stimulated crypt cell production in the individual crypts contribute to this enhanced cell production.[1-4] However, because enterocyte mass results from the balance between cell production and survival, alterations in rates of programmed cell death, or apoptosis, might also influence this adaptive response to resection.

A recent study in mice demonstrated that the number of apoptotic cells per crypt and villus increases early after resection.[5] However, the size of both crypts and villi increases after resection.[1,2] Thus it is not clear if the increased number of apoptotic cells simply reflects the expanded number of cells in these compartments or represents an actual increased rate of apoptosis. Apoptosis might increase in response to the stimulated proliferation in an attempt to maintain tissue homeostasis. Alternatively, changes in apoptosis may represent an independent process regulating adaptation. Furthermore, these observations in the earlier study were made during the early postoperative period. The aim of the present study was to evaluate changes in proliferation, apoptosis, and mucosal adaptation later during the adaptive period following intestinal resection.

MATERIAL AND METHODS

Eighteen male New Zealand white rabbits (3 to 4 kg) were included in the study. Animals (n = 6) were killed at 7, 14, and 21 days and the ileum was studied.

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Six preoperative specimens were used as control samples. Intestinal structure was assessed by morphometric measurements on histologic sections. Proliferative activity of the mucosa was evaluated by measuring mucosal crypt cell production rate (CCPR). Apoptosis was estimated by in situ end labeling of DNA fragmentation and morphologic assessment. The protocol was approved by the Omaha Veterans Administration Medical Center animal research committee.

Operative Procedure

Operations were performed after an overnight fast using sterile technique. Anesthesia was achieved with intramuscular ketamine (35 mg/kg) and xylazine (7 mg/kg) and maintained with halothane by inhalation. Through a midline incision, 50% of the small intestine was resected ending 20 cm proximal to the ileocolonic junction. Continuity was restored with a one-layer end-to-end anastomosis. The animals received perioperative ampicillin and supplemental subcutaneous fluid until they resumed oral intake on postoperative day 2. The rabbits were active, ate normally, and maintained body weight during the study.

Morphologic and Biochemical Measurements

Ileal segments were excised at sacrifice. Samples were processed histologically, and transverse sections were stained with hematoxylin and eosin. Villus height and crypt depth were measured at 10 sites around the circumference with the aid of an eyepiece micrometer.

CCPR was determined using the metaphase arrest technique with vincristine sulfate.[6] One milligram of vincristine sulfate was injected intraperitoneally 2 hours before sacrifice. The mucosal samples were fixed in Carnoy's fixative, hydrolyzed in acid at 60° C for 5 minutes, and stained with Schiff's reagent. The crypts were dissected free using a dissecting microscope. Samples of crypts were transferred to a glass slide in 15% glacial acetic acid and squashed for determination of the number of metaphases per crypt in a minimum of 10 crypts. CCPR was calculated assuming a linear accumulation for 2 hours.

In Situ End Labeling

In situ end labeling was performed using Klenow polymerase with detection of biotinylated nucleotides using a streptavidin-horseradish peroxidase conjugate (Frag EL, Oncogene Research Products, Cambridge, Mass.).[7] Paraffin sections (3 μm) were dewaxed with xylene and taken through alcohol (100% to 70%). The sections were rendered permeable with 40 μg/ml proteinase K in 10 mmol/L Tris, pH 8, for 60 minutes at room temperature and rinsed with Tris-buffered saline. Endogenous peroxidases were inactivated by incubation with 3% H_2O_2 for 5 minutes. Labeling was carried out after a 30-minute incubation in diluted Klenow equilibration buffer (0.5 mol/L Tris [pH 8], 0.5 mol/L NaCl, and 0.1 mol/L $MgCl_2$). Sixty microliters of labeling reaction mix (58.4 μl labeling reaction mix with 1.6 μl Klenow enzyme) was applied to each specimen, and the slides were incubated in a humidified chamber for 1.5 hours at 37° C. The reaction was terminated by incubation with 0.5 mol/L ethylenediaminetetracetic acid (pH 8) for 5 minutes. Sections were covered with 4% bovine serum albumin in phosphate-buffered saline for 10 minutes. Peroxidase streptavidin conjugate was applied for visualization of the avidin-biotin complex, and the section was incubated at room temperature for 30 minutes. Slides were rinsed and developed in 3.3 diaminobenzidine and lightly counterstained with methyl green.

Quantitation of Apoptosis

Apoptosis was evaluated by a blinded observer grading DNA fragmentation on the in situ end-labeled sections as well as quantitating cells with morphologic characteristics of apoptosis. Fragmentation was graded on a scale of 1 to 3 as follows: 1 = minimal staining and two or fewer densely stained nuclei/villus; 2 = diffuse light staining and five or fewer densely stained nuclei/villus; and 3 = diffuse staining with more than five densely stained nuclei/villus as described previously.[8] Twenty consecutive villi were graded on each section. Under high-power microscopy, cell morphology was studied to determine the presence of chromatin condensation in the nucleus, separation of the cell from adjacent enterocytes, and the presence of apoptotic bodies. Intraepithelial lymphocytes were excluded based on location and size. Enterocytes were counted in 10 consecutive axially oriented villi and apoptotic cells expressed as the number per 100 apical, lateral, and total villus cells. Enterocytes were similarly evaluated in 10 consecutive axially oriented crypts, and apoptotic cells expressed as the number per 100 basal, lateral, and total crypt cells. Apical villus cells and basal crypt cells included the five cells in that position on either side of the crypt or villus. Ten crypt cross sections were also examined to determine the number of apoptotic cells.

Statistical Analysis

Data are expressed as the mean ± standard deviation. Analysis of variance with the Bonferonni correction and the Mann-Whitney rank test were used for

comparisons. Statistical significance was ascribed to P values <0.05.

RESULTS

Villus height and crypt depth were significantly increased after resection. Villus height was significantly increased in the resected animals 14 and 21 days after resection compared to preoperative values (Table I and Fig. 1). Crypt depth was significantly increased at all time points after resection. Villus height increased approximately 30% and crypt depth approximately 35% compared to preoperative values at 21 days. Crypt cell production rate was also significantly increased approximately 40% above preoperative values at 7, 14, and 21 days.

Table I. Comparison of intestinal mucosa

	Preoperative	7 days	14 days	21 days
Villus height (μm)	382 ± 51	438 + 169	496 ± 104*	505 ± 131*
Crypt depth (μm)	121 ± 10	187 ± 32*	159 ± 23*	163 + 21*
CCPR (cells/hr)	5.8 ± 1.2	8.6 ± 0.3*	7.9 ± 0.3*	8.3 ± 1.6*

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

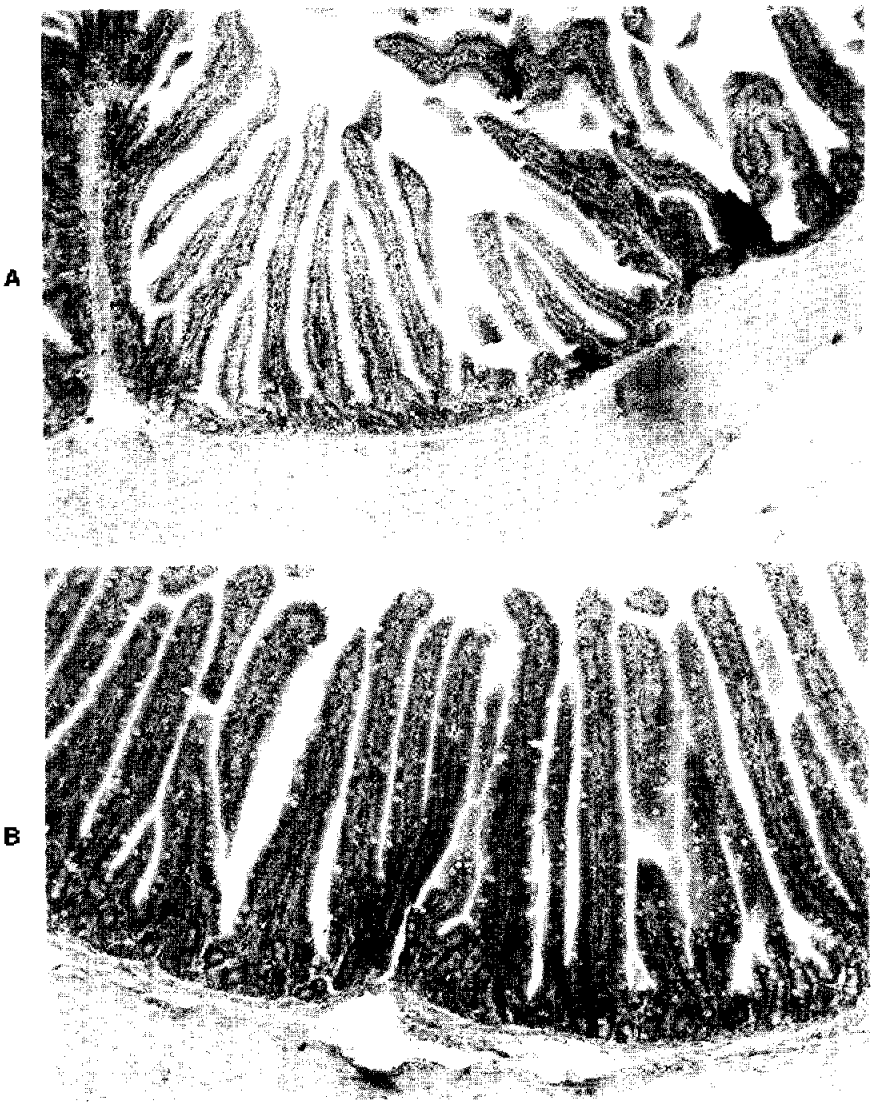

Fig. 1. Compared to preoperative mucosa **(A)**, villus height was significantly increased 21 days after resection **(B)**. (Hematoxylin and eosin stain, ×70.)

Parameters of apoptosis in villus enterocytes are shown in Table II. The number of apoptotic cells per 100 cells (apoptotic index) did not increase in villus enterocytes overall. Villus tip cells, however, did have an increased apoptotic index at 21 days. The apoptotic index was significantly increased at the villus tip compared to the lateral villus (Fig. 2). The number of cells along the longitudinal axis of the villi increased after resection. The number of apoptotic cells per villus increased significantly at 21 days. Mean DNA fragmentation scores of the villi were similar before and after resection (Fig. 3).

Parameters of apoptosis in crypt enterocytes are shown in Table III. The apoptotic index at the base

Table II. Comparison of apoptosis in villus enterocytes

Apoptotic cells/100 cells	Preoperative	7 days	14 days	21 days
Villus tip	19 ± 7*	20 ± 12*	25 ± 7*	32 ± 7†
Lateral villus	2 ± 1	2 ± 1	4 ± 2	3 ± 1
Total villus	6 ± 3	5 ± 3	7 ± 3	9 ± 2
Total cells/villus	65 ± 3	73 ± 7†	71 ± 10	83 ± 6†
Apoptotic cells/villus	4.5 ± 2.5	6.0 ± 3.0	7.0 ± 3.1	9.2 ± 1.6†

*P <0.05 vs. lateral villus.
†P <0.05 vs. preoperative.

Table III. Comparison of apoptosis in crypt enterocytes

Apoptotic cells/100 cells	Preoperative	7 days	14 days	21 days
Crypt base	1.0 ± 1.0	1.1 ± 0.4	0.8 ± 0.2	2.0 ± 1.2
Lateral crypt	0.1 ± 0.1	1.0 ± 0.9	1.0 ± 0.6*	0.6 ± 0.6
Total crypt	1.1 ± 1.0	2.5 ± 1.7	2.3 ± 1.2	2.5 ± 1.3
Total cells/crypt	16.5 ± 0.6	20.0 ± 0.7*	19.5 ± 1.2*	20.2 ± 0.3*
Apoptotic cells/crypt	0.6 ± 0.6	2.5 ± 1.7*	2.3 ± 1.1*	1.8 ± 1.6

*P <0.05 vs. preoperative.

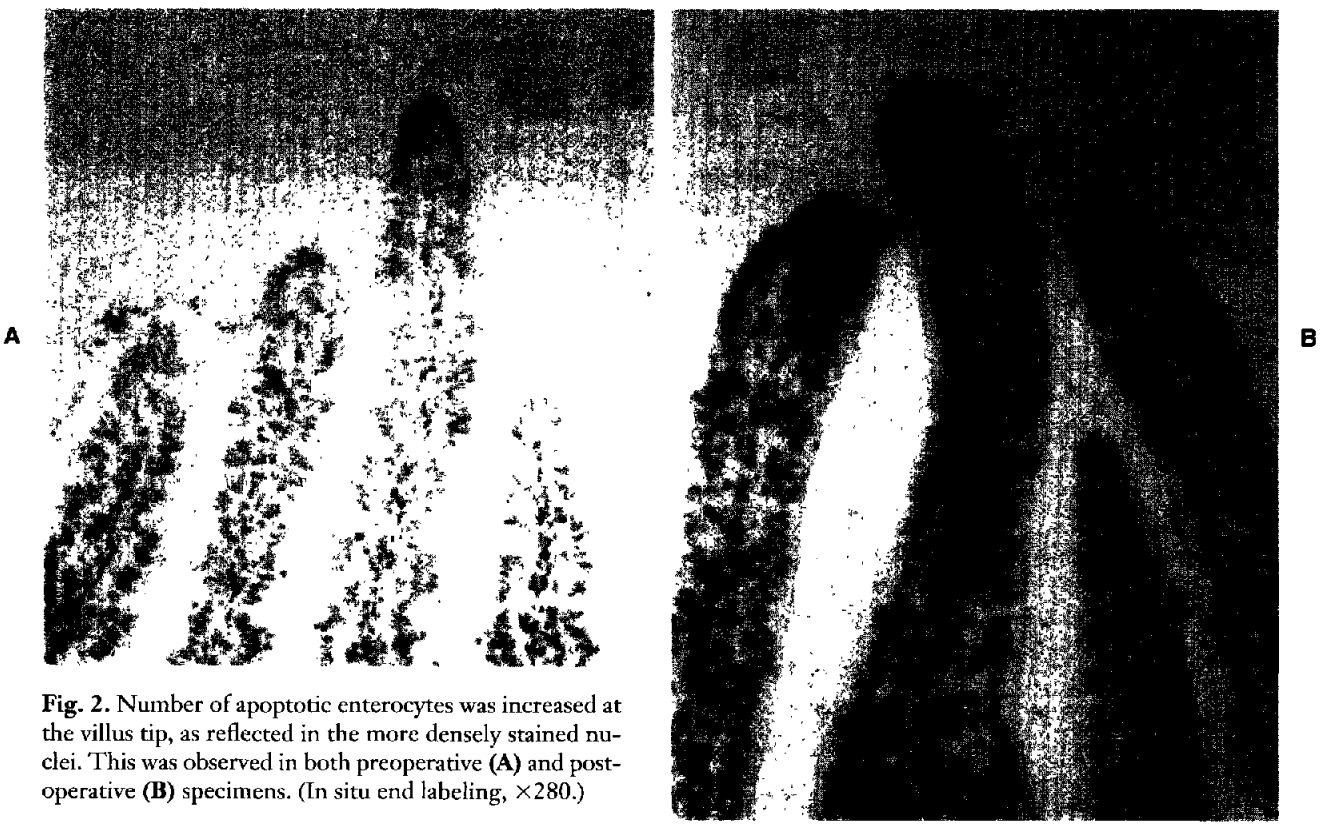

Fig. 2. Number of apoptotic enterocytes was increased at the villus tip, as reflected in the more densely stained nuclei. This was observed in both preoperative **(A)** and postoperative **(B)** specimens. (In situ end labeling, ×280.)

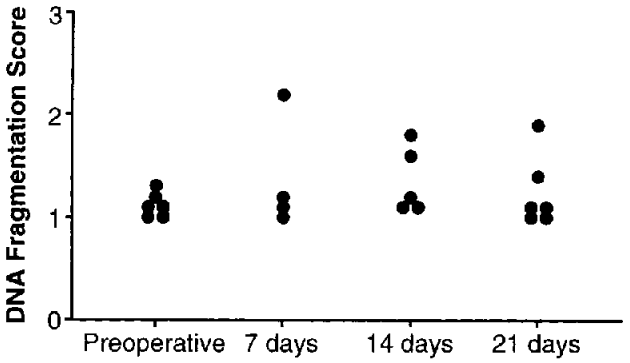

Fig. 3. DNA fragmentation scores were similar before and after resection.

of the crypt tended to be greater ($P = 0.07$) than on the lateral crypt in the preoperative ileum but not following resection. The apoptotic index was elevated only in the lateral crypt at 14 days. The number of cells along the longitudinal axis of the crypt increased significantly in all three resected groups. The number of apoptotic cells per crypt on these sections was significantly increased at 7 and 14 days. On crypt cross sections the apoptotic index was significantly increased only at 14 days (18 ± 7% vs. 10 ± 4%, 13 ± 9%, and 11 ± 5% at 14 days vs. preoperative, 7 days, and 21 days, respectively, $P < 0.05$).

DISCUSSION

Apoptosis (programmed cell death) is a highly regulated, genetically controlled process of cell deletion without any signs of inflammation or disruption of tissue architecture.[9] Apoptosis may be an important mechanism of normal cell loss from the intestinal villus.[10-13] There is a persistent low rate of spontaneous apoptosis in the intestine that is triggered by normal physiologic stimuli.[13] In the rat, apoptosis has a circadian rhythm and is increased by fasting.[14] Changes in apoptosis can be induced by a variety of other stimuli.[9,13] Growth factor deprivation is an important initiating factor that causes downregulation of survival genes and new gene expression leading to cell death.[15] Conversely, administration of various growth factors (e.g., epidermal growth factor and glucagon-like peptide-2) inhibits apoptosis.[16,17] Several types of injury, for example, irradiation and ischemia-reperfusion, also trigger this process.[18,19] Thus it is reasonable to speculate that apoptosis might play a role in the adaptive response to intestinal resection.

We confirmed the observation made by Helmrath et al.[5] in mice that both enterocyte proliferation and the number of apoptotic cells per crypt and villus were increased following 50% proximal resection. In their study proliferation was 40% greater than after sham operation at 7 days, which was similar to the present study. However, they found that apoptosis in the crypt progressively increased compared to normal mucosa from a twofold increase at 12 hours to a sixfold increase at 7 days, and apoptosis in ileal villi was increased fivefold at 7 days. The changes in the present study were of similar magnitude in the crypts but not nearly as dramatic in the villi. However, although the number of apoptotic cells per crypt or villus increases after resection, so do the number of enterocytes per crypt and villus. These observations are consistent with the changes in villus height and crypt depth. Expressing apoptosis as an index (apoptotic cells per 100 cells) reveals that relative rates of apoptosis do not increase significantly in villus enterocytes at 7 or 14 days but are increased at 21 days in villus tip enterocytes. Apoptotic indices in the crypt, both on the longitudinal and transverse axis, are elevated at 14 days but return toward normal at 21 days. The apoptosis index measurement can be influenced by the duration of the cell cycle and the apoptotic process, and also the mechanism of elimination of apoptotic cells, so it must be interpreted with caution.[20]

In the present study we demonstrated that villus enterocyte apoptosis remains elevated in the late stages of intestinal adaptation when morphologic changes have occurred. Apoptotic cells were more frequent at the tip of the villus compared to the lateral surface in both preoperative and postoperative mucosa. This is consistent with our previous observations in growth factor–mediated apoptosis.[8,17] Hall et al.[10] also found that apoptotic cells were more frequent at the tip of the villus. In the present study the proportion of apical villus cells undergoing apoptosis was significantly elevated and correlated with villus height. These findings suggest that this higher rate of apoptosis merely reflects the increased number of enterocytes in the villus compartment with relatively more senescent villus tip cells. The relatively constant proportion of total villus cells undergoing apoptosis suggests that apoptosis is not an independent regulator of the adaptive response. The findings with DNA fragmentation were similar to those of cells with morphologic changes of apoptosis (i.e., chromatin condensation).

The apoptotic index in crypts did increase early after resection but returned to more normal levels at 21 days. This increased apoptosis occurred primarily in the cells localized to the lateral crypt rather than at the crypt base. This is similar to the observations of others.[13] This enhanced apoptosis followed the resection-stimulated proliferation and increased crypt depth. However, crypt cell proliferation remained elevated at 21 days, whereas apoptosis decreased to pre-

operative levels. Apoptosis in the proliferative compartment may have a more profound and lasting influence on the size of the enterocyte compartment than apoptosis of villus cells.

Taken together these findings suggest that apoptosis in the crypt and villus compartments are regulated independently. Apoptosis in the crypt enterocytes occurs in response to increased proliferation and enlargement of the crypt but returns to more normal levels later after resection allowing a new steady state to occur in the balance between proliferation and cell death in the crypts. Overall, rates of apoptosis do not increase in villus enterocytes. However, as villus height increases, the proportion of cells at the 10 apical positions that are programmed to die increases, perhaps reflecting the increased migration time along the lengthened villus. These observations may be related to the known differences in distribution of apoptosis-regulating gene products and adhesion factors along the crypt-villus axis.[21] A better understanding of these processes may lead to the identification of new strategies for enhancing the intestinal adaptive response.[22]

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