Immunologic Tolerance to Organ Transplants

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The field of transplantation has witnessed an enormous growth over the past three decades. Much of this growth has been due to the advent of excellent drugs to control acute rejection, making transplantation possible. However, there are currently three major limitations to further progress in this field. The first is chronic rejection, which is the process that begins after the danger of acute rejection is past and inexorably continues to cause loss of organs over the ensuing years at the approximate rate of 7%. Second are treatment-related complications, mainly the side effects of the drugs required to prevent rejection, which include infections and malignancies. Awareness of these complications is what frequently keeps patients and their doctors from seeking a transplant until the original disease has progressed rather far. Third is availability of donor organs, which has become a problem, paradoxically, because of the success of transplantation as a mode of therapy. Thus there are now much greater numbers of patients waiting for an organ than there are cadaver organs available, and the disparity is continuously increasing. In addition, this limitation of availability has caused transplant physicians to impose stringent criteria to determine which recipients will receive the limited organs available. Therefore the number of potential transplant recipients for whom organ transplantation might be lifesaving is actually much larger than the waiting lists would indicate.

This laboratory has been involved in studies on the induction of tolerance to transplanted organs, which, if successful, could provide at least part of the solution to all three of these limitations. Thus tolerance to allografts would eliminate the need for chronic immunosuppressive medications, one major cause of complications in transplant recipients, and may also prevent chronic rejection.[1] Xenotransplantation may provide a solution to the problem of limited availability of organs, and because the immune response to such transplants is so strong, tolerance induction, at least to some of the most potent determinants, may be required to make the procedure feasible. One of the most promising approaches we have investigated involved the induction of tolerance through the establishment of "mixed chimerism," and our progress to date in applying this approach to both allografts and xenografts is the subject of this brief review.

BONE MARROW TRANSPLANTATION AND TOLERANCE

Transplantation tolerance for allografts following bone marrow transplantation has already been demonstrated both in experimental animal models and in the clinic. Thus it has been clear for some time that successful allogeneic bone marrow transplantation carries with it the induction of tolerance to other tissues or organs derived from the same donor. This phenomenon has been demonstrated repeatedly in large-animal systems[2-4] and, in addition, several clinical examples of such tolerance have also been observed.[5-7] The latter have involved cases in which bone marrow transplantation was performed from a human leukocyte antigen–matched sibling for treatment of hematologic malignancy. The recipients subsequently developed renal failure requiring kidney

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Supported by grants NIH1RO1 AI37692 and 1PO1 AI39755 from the National Institutes of Health.
Presented at the Thirty-Ninth Annual Meeting of The Society for Surgery of the Alimentary Tract, New Orleans, La., May 17-20, 1998.
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transplantation. These recipients received kidney transplants from the same sibling donor who donated the bone marrow. In all of these cases the transplant was accepted with little or no exogenous immunosuppression.

ADVANTAGES OF MIXED CHIMERISM

When bone marrow transplants are performed to induce tolerance rather than to treat a hematologic malignancy, it is neither necessary nor desirable to ablate the host's bone marrow completely and establish a fully allogeneic chimera. This is especially true when a major histocompatibility complex (MHC) barrier is crossed. In this case there are two major problems: (1) If mature T cells are not removed from the allogeneic bone marrow, then severe graft-versus-host disease results; and (2) if mature T cells are removed from the allogeneic bone marrow inoculum, then the chimeras that result are relatively immunoincompetent.

The probable reason for the immunoincompetence of fully allogeneic complete chimeras lies in the fact that T cells derive their specificity from education in the thymus. There they develop specificity for self MHC plus peptides of foreign antigens (so-called Self + X). New T cells that arise following ablative radiation and reconstitution with allogeneic bone marrow are of the allogeneic donor MHC type, but are educated in a thymus of host MHC type. These new T cells therefore acquire restriction specificities for host MHC + X. However, the antigen-presenting cells, which are responsible for presenting environmental antigens to mature T cells in the periphery, are also replaced by the bone marrow transplant and are therefore of donor MHC type. The mature T cells thus encounter a different MHC + X on antigen-presenting cells from that to which they were educated in the thymus. Some sharing of specificities is undoubtedly responsible for the weak immune responses that occur in such fully allogeneic chimeras, but the majority of MHC-restricted responses are disabled, leading to relative immunoincompetence.[8-10]

Herein lies the reason why mixed chimeras have a great advantage over fully allogeneic chimeras in terms of immunocompetence. Mixed chimeras possess bone marrow precursor cells of both host and donor origin.[11,12] Both types of mature T-cell populations develop in such animals and are restricted through positive selection in the thymus to the recognition of host MHC + X. However, now antigen-presenting cells are present in the periphery of the host type, and immunocompetent interactions can occur. In addition, donor antigen-presenting cells develop in such animals and among these are dendritic cells, which localize in the corticomedullary junction of the thymus and are thus capable of the same kind of negative selection that occurs for self during T-cell development.[13] Thus mixed chimeras are both immunocompetent and tolerant to both self and donor MHC. The relevant interactions occurring in the thymus of mixed chimeras are illustrated in Fig. 1, in which host MHC is indicated as "A" and donor MHC as "B."

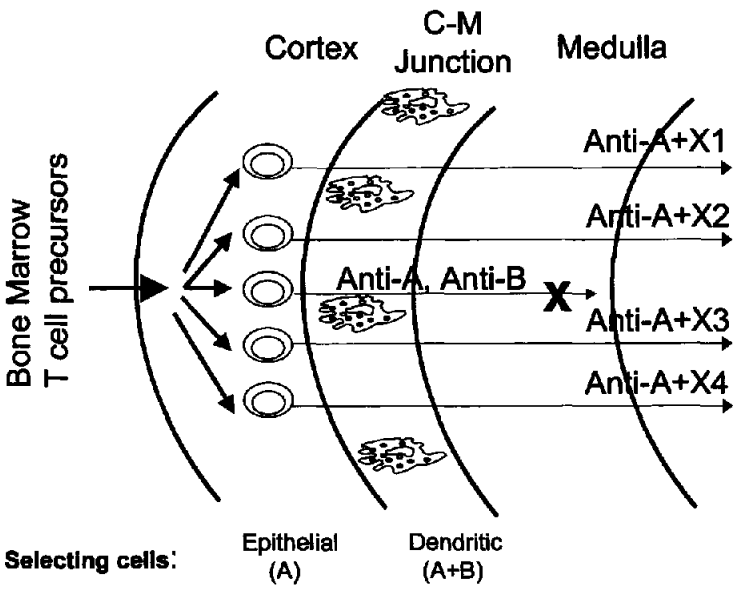

Fig. 1. T-cell development in the thymus of a mixed chimera. Positive selection occurs in the cortex on thymic epithelial cells of the host *(A)*; negative selection occurs at the corticomedullary junction on dendritic cells of both host *(A)* and donor *(B)*.

Our early studies of mixed chimerism involved lethal irradiation of recipient mice followed by reconstitution with mixtures of syngeneic and fully allogeneic bone marrow, both of which were depleted of mature T cells prior to reconstitution.[4,11,14] Such mixed chimeras were found to be specifically tolerant to skin grafts from the allogeneic donor strain, which were retained permanently, while they were capable of rejecting third-party skin grafts just as promptly as did normal animals. Thus these studies proved the principle that induction of mixed chimerism resulted in animals that were both immunocompetent and specifically tolerant.

However, the preparative regimen for those early mixed chimeras included lethal irradiation, much too toxic a procedure for clinical applications, except in the case of malignancy. Therefore we have subsequently developed methods for producing mixed chimerism without the need for lethal irradiation. One such regimen that we have studied intensively involves the use of monoclonal antibodies (anti-CD4 and anti-CD8) to remove the mature T cells from the host, which are the main impediment to allogeneic engraftment.[15] It was also found in studies of this preparative regimen that the mechanism by which antibody treatment leads to depletion of mature T cells does not function efficiently within the thymus. A boost of irradiation to the thymus was therefore added to the preparative regimen. Thus utilizing a protocol consisting of 300 R whole-body irradiation, 700 R thymic irradiation, and treatment with monoclonal antibodies to CD4 and CD8, mixed allogeneic chimerism could be produced in most recipient animals.[15] The pattern of reconstitution of animals prepared by this nonmyeloablative regimen was indistinguishable by fluorescence-activate cell sorting analysis from that of animals prepared by lethal irradiation and reconstitution with T cell–depleted host plus donor bone marrow. Similar to their counterparts prepared by the lethal preparative regimen, these mixed chimeras showed specific tolerance to subsequent skin grafts. However, these animals were far more healthy than those produced by the lethal preparative regimen and showed none of the toxic effects of lethal irradiation. Because this regimen was so much less toxic than the lethal preparative regimen, we have developed it further for clinical applications.

MIXED CHIMERISM IN CYNOMOLGUS MONKEYS

Using a protocol based on that developed in mice, we have extended the nonmyeloablative regimen to the induction of tolerance to renal transplants in cynomolgus monkeys as a preclinical large-animal model.[16,17] Pairs of animals were selected following serologic and mixed lymphocyte reaction testing to ensure that they were fully MHC mismatched. Recipients received 3.0 Gy of whole-body irradiation, 7.0 Gy of thymic irradiation, and horse antihuman antithymocyte globulin preoperatively. Bilateral nephrectomy, splenectomy, orthotopic kidney transplantation, and donor bone marrow administration were all performed on day 0. To supplement suppression of mature T cells by antithymocyte globulin, treatment with cyclosporine intramuscularly was begun on day 1 and continued for 4 weeks. No further immunosuppression was administered after this time.

Monkeys treated by this regimen became pancytopenic on day 8 but recovered thereafter, becoming hematologically normal by day 30. Sequential flow cytometry analyses showing clear evidence for chimerism in all three leukocyte subpopulations was generally detected first on about day 8, and persisted until day 30. Thereafter the levels of detectable chimerism decreased progressively, becoming difficult to distinguish from background staining by 2 months in all animals. Nevertheless, in eight of nine animals treated by this protocol, transplantation tolerance was induced, as assessed by mixed leukocyte reaction assays, by monitoring of kidney transplant function, and in one case by acceptance of a full-thickness skin graft from the kidney donor.[16] Because the chimerism was transient in these animals, it is likely that although bone marrow chimerism was essential for the induction of tolerance, the kidney itself may have contributed to the maintenance of tolerance in these animals, possibly by a peripheral mechanism. In none of these animals was any additional exogenous immunosuppression administered after day 30. We therefore concluded that specific transplantation tolerance can be induced in primates by this procedure.

EXTENSION TO XENOTRANSPLANTS

Shortly after determining that mixed bone marrow reconstitution of lethally irradiated recipients could produce long-term mixed chimerism and transplantation tolerance in an allogeneic model, we demonstrated its effectiveness in a concordant xenogeneic rat-to-mouse species combination.[4,18] We have subsequently extended our nonmyeloablative protocol to concordant xenogeneic transplants as well.[19] In this case it was found necessary to deplete not only mature T cells but also natural killer (NK) cells from the recipients to achieve mixed chimerism and prolonged skin graft survival.

We have also recently demonstrated that this procedure is effective in the concordant primate species combination baboon to cynomolgus monkey.[20,21]

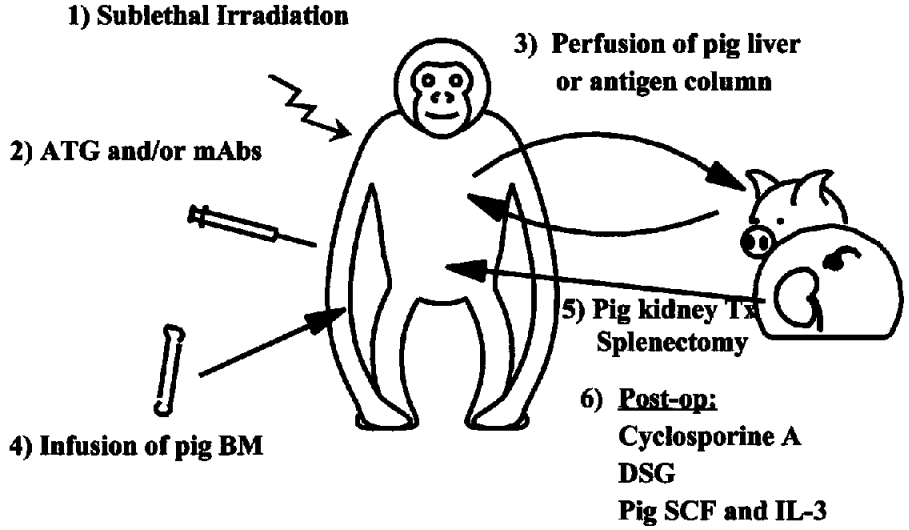

Fig. 2. Schematic representation of the nonmyeloablative protocol being attempted in this laboratory for induction of tolerance by mixed chimerism across the discordant species barrier pig→baboon. BM = bone marrow; ATG = antithymocyte globulin; mAbs = monoclonal antibodies; DSG = deoxyspergualin; SCF = stem cell factor; IL-3 = interleukin-3.

However, although nonhuman primates would provide the closest potential donors for humans phylogenetically, there are a variety of reasons, including availability and ethical considerations, that mitigate against wide-scale use of primates as xenograft donors. We and others have therefore turned our attention to more discordant species as a potential xenograft donor source. The miniature swine that we have inbred over the past 20 years as a large-animal model for transplantation[22,23] have a variety of advantages over other potential xenograft donors. These include size, availability, and especially breeding characteristics, which make it possible to manipulate the genetics of these animals within a relatively short time. We currently maintain three herds of MHC homozygous pigs as well as several intra-MHC recombinant strains.[23] The size of these animals is very similar to that of human beings, with maximal weights of approximately 250 pounds. Thus one might choose an appropriate donor for any potential human recipient.[24]

We have attempted to extend our nonmyeloablative regimen for production of mixed chimerism to the discordant pig→primate combination.[25-28] The one main difference from previous protocols involves the need to avoid the effects of natural antibodies in the recipient's circulation in order to avoid hyperacute rejection. This has been accomplished in our model by preoperative plasmapheresis and perfusion of the monkey's plasma through specific antigen-bearing columns immediately prior to bone marrow and renal transplantation.[29,30] The solid matrix columns bear α-1,3-galactose sugar linkages based on reports demonstrating that the majority of primate antiswine natural antibodies are directed to this epitope,[31,32] a finding that has been confirmed in our laboratory.[29] Our results with such columns have demonstrated that they are highly effective in removing natural antibodies sufficiently to avoid hyperacute rejection.[30]

The preparative regimen we have used is very similar to that used in our monkey allotransplantation model, as described previously. Sublethal irradiation consisting of three fractions of whole-body irradiation, 1.0 Gy each, were administered on days −6 and −5, and 7.0 Gy of thymic irradiation was administered on day −1. Splenectomy was performed on day 0 because of its apparent usefulness in avoiding antibody responses.[33,34] Antithymocyte globulin was administered on days −2, −1, and 0 (50 mg/kg intravenously), as in the allogeneic primate model described earlier,[16] followed by cyclosporine (15 mg/kg per day intravenously), to further suppress T-cell function in the immediate post-transplant period. We have tested a variety of immunosuppressive drugs postoperatively in an attempt to prevent the return of natural antibodies, but none has been fully effective in this regard.[35] As shown in Fig. 2, one such reagent is 15-deoxyspergualin, which has been reported to suppress antibody responses.[36] On day 0, recipients receive a pig kidney and bone marrow (5×10^8/kg), both harvested from the same donor.

Removal of natural antibodies has been successful in avoiding hyperacute rejection in more than 20 pig-to-primate xenotransplants we have performed to date.[37] However, the natural antibodies have invari-

ably returned after a period of 7 to 15 days, coincident with loss of the xenograft organs to an acute vascular form of rejection. Thus we have not yet observed the occurrence of "accommodation," which, if it occurred, might have been expected to protect the kidney from such rejection.[38] It is nevertheless encouraging that during the 1- to 2-week periods of xenograft organ survival, the pig kidneys have been capable of achieving normal blood urea nitrogen and creatinine levels and maintaining normal electrolytes. This finding suggests that if we can overcome the immunologic barriers to this form of xenograft, it should be able to support the life of a primate indefinitely.

Our studies in allogeneic systems have demonstrated that chimerism is a requirement for achieving lasting transplantation tolerance.[16,39] However, survival of pig bone marrow–derived cells in a primate appears to require the presence of some species-specific growth factors.[40,41] We have recently demonstrated that administration of pig recombinant cytokines (stem cell factor and interleukin-3) to primates (cynomolgus monkeys and baboons) for 2 weeks after the preparative regimen enables the pig bone marrow to survive for well over 6 months.[42]

Finally, we are also testing in baboons the use of fetal pig thymic tissue, which has recently been shown in a mouse model to be capable of inducing discordant xenograft tolerance.[43] It is our hope that by combining approaches directed toward the humoral xenograft response, which are being pursued in other laboratories, with our own approaches toward inducing tolerance at the cellular level, long-term acceptance of discordant xenografts in the pig-to-primate combination will eventually become a clinical reality.

I wish to thank Drs. John Iacomini and Joren Madsen for their helpful review of the manuscript and Ms. Cinde L. Clatterbuck for her expert secretarial assistance.

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Staging Laparoscopy for Pancreatic Cancer Should Be Used to Select the Best Means of Palliation and Not Only to Maximize the Resectability Rate

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Staging laparoscopy, based on the assumption that endobiliary stenting is the best palliation, allegedly saves an "unnecessary" laparotomy for incurable pancreatic cancer. Our aim was to determine survival of patients with *clinically resectable* pancreatic cancer that is found to be unresectable intraoperatively and thereby infer appropriate utilization of staging laparoscopy. A retrospective analysis was undertaken of 148 patients with ductal adenocarcinoma (1985 to 1992) with a clinically resectable lesion based on current imaging techniques. All were considered candidates for resection but were deemed unresectable at operation because of metastases to the liver (group I; 29 patients), the peritoneum (group II; 22 patients), or distant lymph nodes (group III; 44 patients) or because of vascular invasion (group IV; 53 patients). Overall median survival was 9 months (range 1 to 53 months), but by group was as follows: group I, 6 months; group II, 7 months; group III, 11 months; and group IV, 11 months. Individual comparisons showed shorter survival for patients with distant nodal, liver, or peritoneal metastases than with nodal or vascular involvement ($P < 0.03$). Staging laparoscopy should be performed to identify patients with liver or peritoneal metastases who have an expected survival of approximately 6 months, in whom short-term endoscopic palliation is satisfactory. Extended laparoscopy to identify lymph node or vascular involvement is contingent upon which palliation (operative vs. endoscopic) is considered most appropriate. Because we believe operative bypass provides better, more durable palliation in this latter group, we have not adopted extended laparoscopy. (J GASTROINTEST SURG 1999;3:111-118.)

KEY WORDS: Pancreatic cancer, staging laparoscopy, palliation, survival

Laparoscopic staging of patients with periampullary malignancies has been suggested as a minimal access technique to increase the rate of curative resection,[1-3] and thereby decrease the number of "unnecessary" noncurative, nonresective operations. By recognizing the presence of peritoneal and/or liver metastases,[2,4] or with more advanced laparoscopic techniques also determining distant nodal or vascular involvement,[3] the rate of resectability has been increased to 75% to 90%,[2-4] thereby *allegedly* saving many patients a nonresectional and thus unnecessary celiotomy.

Despite its ability to recognize disease that is unresectable for cure, we believe that staging laparoscopy should be considered not primarily to increase resectability rates but rather to select the best means for palliation of unresectable disease. Our hypothesis in this study is that patients with pancreatic cancer deemed "resectable" by currently accepted imaging techniques, but proven to be unresectable for cure only at the time of exploration for potential resection, have survival times that vary according to the reason for unresectability (i.e., liver vs. peritoneal vs. nodal metastases vs. vascular involvement), and thus the best means of palliation for these patients (endobiliary stent vs. operative bypass) may vary accordingly. Our aim in this group of selected, good-risk patients was to determine survival separately for those with either liver, peritoneal, or distant nodal disease and those with locally advanced vascular involvement, and thereby to infer the optimal means of palliation based on expected survival. Our bias is that for pa-

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Presented at the Thirty-Ninth Annual Meeting of The Society for Surgery of the Alimentary Tract, New Orleans, La., May 17-20, 1998.
An abstract of this work was published in *Gastroenterology* 114:A1407, 1998.
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tients with pancreatic cancer expected to live only about 6 months, endoscopically placed endobiliary stents are the best palliation,[5-7] but for those patients expected to live longer (~12 months), operative palliation with biliary and duodenal bypass combined with operative chemical splanchnicectomy offers better palliation.[8,9] Thus staging laparoscopy would provide an excellent means of choosing the appropriate palliative method for individual patients with incurable pancreatic cancer.

MATERIAL AND METHODS

We retrospectively reviewed the medical records of all patients at the Mayo Clinic between September 1985 and December 1992 who had either a mass in the head of the pancreas or a presumed pancreatic head cancer classified as *clinically resectable* after a thorough evaluation using state-of-the-art clinical and imaging modalities; all were considered good-risk surgical candidates to undergo operative exploration for potential pancreatic resection. The imaging studies consisted of at least dynamic contrast-enhanced computed tomography (CT). All CT scans were reviewed by a radiologist and the surgeon. Tumors were considered to be resectable when there was no evidence of distant extrapancreatic disease or involvement of lymph nodes outside the classic margins of resection. Encasement or occlusion of the superior mesenteric artery or vein, celiac artery, or portal vein were used as criteria for unresectability. Neither obliteration of perivascular fat planes nor blurring of retropancreatic soft tissue planes not accompanied by other signs were considered criteria for unresectability. Abdominal ultrasonography, endoscopic retrograde cholangiopancreatography (ERCP), and angiography were used only in selected patients.

Our study group was comprised of patients who fulfilled the preceding criteria but were found *intraoperatively* to have neoplasms that were unresectable for cure because of distant disease and/or locoregional involvement. All patients had histologic confirmation of ductal adenocarcinoma of the pancreas, and specimens from those surviving longer than 6 months were re-reviewed by one of us (L.J.B.) and confirmed for this study. Because of known differences in clinical presentation and outcome, and in accordance with the purposes of our study, we included only those patients who had cancer in the head of the pancreas. We excluded patients in whom no studies were done or read at the Mayo Clinic, one in whom preoperative assessment was directed at a presumed benign process (chronic pancreatitis) and who was found to have an unresectable tumor at operation, and one in whom preoperative assessment (with ERCP and stent placement) was hampered by necrotizing pancreatitis.

We divided this group of patients into the following four subgroups according to the reason for unresectability found *at operation:* (I) liver metastases, (II) peritoneal metastases, (III) lymph node metastases (outside the classical resection margins, e.g, clinically evident and histologically confirmed nodes in the common hepatic or celiac artery or at the base of the small bowel mesentery), and (IV) adherence to the superior mesenteric vessels and/or portal vein precluding a short wedge or segmental resection. When more than one site was present in a single patient, he or she was included in the subgroup indicating more advanced disease (i.e., a patient with peritoneal or liver metastases and vascular involvement was placed in the "peritoneal metastasis" category).

Data Analysis

To compare survival within the four subgroups, we used a nonparametric analysis with the Kruskal-Wallis test; for purposes of graph construction, we used a Kaplan-Meier plot. For individual comparisons between subgroups, Mann-Whitney two-tailed U tests were performed. Results were corrected with Bonferroni's method for multiple comparisons; P values of <0.05 were considered significant.

RESULTS

Among 155 patients who fulfilled our entry criteria, five died postoperatively (3%), and of the remaining 150, follow-up was complete in 148 (99%). There were 99 men and 49 women whose mean age was 65 years (range 32 to 90 years). After diagnostic and staging preoperative evaluation, all patients underwent exploratory celiotomy for potential resection; no one underwent preoperative staging laparoscopy.

Intraoperatively 29 patients (20%) were found to have liver metastases, 22 (15%) had peritoneal dissemination, 44 (30%) had metastatic lymph nodes outside the classic resection margins, and 53 (35%) had locally advanced disease with vascular involvement; these findings precluded the planned resection for cure.

Median and range of survival for each subgroup, as well as for the group as a whole, are shown in Table I. Survival differed among the four subgroups (Kruskal-Wallis, $P<0.001$). In contrast to patients with nodal and vascular involvement who survived for close to 1 year after operation, survival for patients with liver or

peritoneal metastases was dismal and quite similar to that known for *clinically unresectable* neoplasms (~6 months). Survival curves (Kaplan-Meier) are depicted in Fig. 1. Individual comparisons between subgroups further confirmed the differences; we found a longer survival in patients with lymph node metastases compared to those with liver ($P < 0.02$) or peritoneal ($P < 0.03$) metastases as well as a longer survival for patients with vascular involvement compared to those with liver ($P < 0.03$) or peritoneal ($P < 0.03$) metastases. Small differences in survival between patients with liver vs. peritoneal metastases, or patients with distant nodal disease vs. vascular involvement, were not statistically significant. Summarizing these results, good-risk patients with clinically resectable ductal adenocarcinoma of the head of the pancreas lived significantly longer if the reason for unresectability (found *only* at operation) was distant nodal or vascular involvement compared to those with liver or peritoneal metastases.

In an effort to determine whether characteristics other than the reason for unresectability may have had an influence on survival, we reviewed several features from these patients and compared them among the four subgroups. Age, duration and type of symptoms, performance status, tumor size and grade, biochemical laboratory abnormalities, and postoperative length of stay (mean 10 to 12 days) were similarly distributed among the four subgroups ($P > 0.5$).

Table I. Survival in patients with clinically resectable pancreatic cancer according to reason for unresectability (found at operation)

Site of metastases	No. of patients	Survival*	
		Months	Range
Liver	29	6	1-34
Peritoneal	22	7	2-36
Nodal	44	11	1-53
Vascular	53	11	3-30
OVERALL GROUP	148	9	1-53

*Median, $P < 0.001$ (ANOVA).

DISCUSSION

Because approximately 90% of patients with pancreatic cancer have unresectable neoplasms at diagnosis or at operative exploration, precise, timely, and cost-effective preoperative staging becomes a priority in order to identify the few resectable patients and provide optimal palliation for those patients in whom exploration would be of no benefit. The rationale for performing laparoscopy as part of the preoperative staging of these patients is based on its ability to identify small (<1 cm) peritoneal and liver metastases (common features of unresectability) that would have been missed by other (even the best) staging modalities, which have false negative rates of 20% to 40%.[10-12] In such cases, resection of the primary neoplasm offers no apparent survival benefit and exposes the patient to the potential morbidity and mortality of a major operation.

Staging laparoscopy for abdominal neoplasms, first entertained in 1911,[13] was revived by Cuschieri et al.[1]

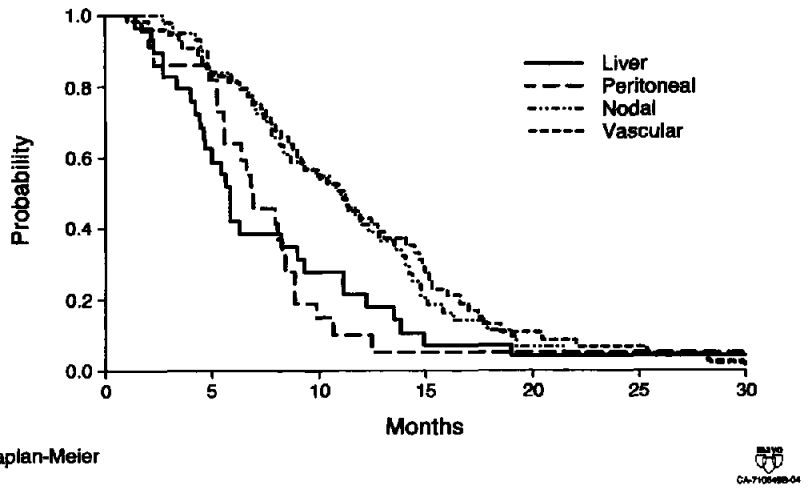

Fig. 1. Kaplan-Meier survival curves for patients with liver, peritoneal, and nodal metastases and vascular invasion.

in 1978. Recently several authors have claimed that laparoscopy will identify peritoneal, omental, or hepatic metastases in 35% to 63% of patients thought to have resectable disease.[14-16] Conlon et al.[3] further developed this concept using "extended" laparoscopy to assess also the lesser sac, porta hepatis, duodenum, transverse mesocolon, and celiac and portal vessels, thus "reproducing" the open exploration to determine resectability for cure. They reported positive and negative predictive indices of 100% and 91%, respectively; with this approach the resectability rate increased from 35% to an impressive 76%.

The central argument around which staging laparoscopy revolves is that it "increases resectability rates" (which undoubtedly it does) and thus "decreases the number of unnecessary operations." In this context an unnecessary operation is defined by proponents of staging laparoscopy as an operation not involving a curative resection of the neoplasm. However, the following issues should be raised: (1) Is this the main reason for performing staging laparoscopy? (2) Do results obtained by laparoscopy necessarily and always change our approach in the management of these patients? (3) Are all of the so-called unnecessary operations really unnecessary and not indicated?

Proponents of staging laparoscopy have shown that metastases can be recognized by laparoscopic examination, thereby preventing a noncurative abdominal operation. However, an inherent assumption is that such patients are better palliated by an endobiliary stent than by operative biliary bypass (with or without gastroenterostomy). This contention is based primarily on studies composed of patients with preoperatively recognized unresectable pancreatic cancer with known limited survival and conducted at times when the mortality (15% to 30%) and morbidity of operative palliation in such patients were unacceptably high or even prohibitive.[5-7,17,18]

To define the role of routine staging laparoscopy, the following questions should be answered: (1) What is the best method of palliation for the patient with unresectable cancer of the pancreas? (2) Does optimal palliation depend on the expected length of survival? (3) Is expected survival different in patients in whom the reason for unresectability was encountered preoperatively compared to those in whom it was found at operation undertaken with the intent for resection? (4) Is length of survival in these latter patients different according to the reason for unresectability? (5) Should an operation be "indicated" only if it entails curative resection or also when it provides a better means of palliation for patients with unresectable disease? (6) What is the current morbidity and mortality of operative palliation in such patients?

We hypothesized that patients with pancreatic cancer who are deemed resectable after a thorough preoperative staging workup and then undergo operative exploration and are found to be unresectable at operation have a longer survival than patients with known metastatic or locally advanced disease recognized preoperatively.[19] Thus we selected our study group very carefully on the basis of the following criteria:

1. *Histopathology.* We included only patients with ductal adenocarcinoma of the pancreas.
2. *Location of neoplasm.* Patients with cancers of the body or tail of the pancreas generally do not need operative palliation, and the only reason they should be operated on is for a curative resection. Indeed staging laparoscopy has demonstrated a higher yield for metastatic disease in these patients (44%),[2] and we believe its role in this context is established.
3. *Performance status.* We included only patients deemed able to undergo pancreaticoduodenectomy; in patients considered poor operative candidates regardless of tumor resectability, endobiliary stents are an ideal method for palliation.
4. *Preoperative staging workup.* All of our patients underwent a complete staging workup that included state-of-the-art imaging with abdominal CT, and in selected cases ultrasound, ERCP, and/or angiography.
5. *Status of resectability.* In no patient was there definite evidence of unresectability preoperatively. This point is important because the randomized studies in the literature comparing endobiliary stenting with operative palliation included patients known to have unresectable disease who have a short survival expectancy of 6 months or less.[5-7,18]

Our study shows that survival varied according to the reason for unresectability. Patients with hepatic or peritoneal metastases had a median survival similar to patients with clinically unresectable tumors (~6 mo).[5,7,8] Endoscopic biliary stenting provides optimal palliation in patients with extrahepatic biliary obstruction and a short expected survival. We believe that the operative mortality and morbidity of surgical palliation and the obligate postoperative convalescence outweighs the known morbidity of biliary stents and the expected 10% to 15% incidence of duodenal obstruction[5,6,8,20] as the neoplasm grows.

When nodal metastases or vascular involvement were the reasons for unresectability for cure at operation, patients had a longer survival (~1 year). The benefits of endoscopically placed biliary stents for

longer periods of time are offset because of the increased risk of stent occlusion, which usually requires readmissions, antibiotic treatment, and stent changes with their implicit morbidity.[21] Gastric outlet obstruction becomes more of a possibility as well. For these reasons we believe that operative palliation with a bilioenteric bypass, gastroenterostomy (either palliative or prophylactic), and celiac plexus block serves this select population better than endobiliary stents.

When laparoscopic exploration is negative for hepatic or peritoneal metastases, we do not pursue and do not recommend "extended" staging laparoscopy directed at detecting nodal and vascular involvement.[3] The procedure is cumbersome, not widely available for the "general" laparoscopist, and adds related costs. More important, the findings would not change the management, because according to our results (and our philosophy) these patients are better managed with operative palliation.

The initial enthusiasm for the role of staging laparoscopy has been moderated by other recent studies as well. In a retrospective review, although the sensitivity of staging laparoscopy for metastatic pancreatic and periampullary cancer was 93%, CT accurately staged 87% of patients missing only one with liver and peritoneal metastases amenable to laparoscopic detection.[22] Similarly, in another study, staging laparoscopy would have modified the operative strategy in only 13% of patients with cancer of the pancreatic head.[23] Moreover, Rumstadt et al.[24] believe that truly accurate assessment of local unresectability is possible only by open operative exploration; in a cohort of 398 patients, only 29 (7%) with peritoneal carcinomatosis or hepatic metastases would have avoided an unnecessary celiotomy and benefited from a laparoscopic examination.

We acknowledge the dynamic nature of management algorithms in pancreatic cancer and recognize that the principles we have outlined above may (will) be subjected to modification when new staging and therapeutic modalities are introduced. In fact, the experience of some groups has shown that the yield of detecting unresectable periampullary neoplasms via the use of laparoscopy has decreased from 40% in the past decade to 24% in the present decade[2,14]; this trend could be the result of better preoperative imaging techniques or due to the fact that patients are now referred earlier in their disease. Self-expanding metallic stents have decreased the incidence of stent occlusion[25,26]; although expensive, they may increase the long-term success rate of endoprostheses and may alter recommendations concerning endoscopic vs. operative palliation. Laparoscopic biliary-enteric bypass has been accomplished primarily with the use of the gallbladder as the biliary conduit[27]; this approach has inherent limitations because of cystic duct obstruction and a greater incidence of "earlier" occlusion by the tumor.[28] In the future, advanced laparoscopy may not only allow the performance of extended staging procedures but also safe and effective palliation of biliary and gastric outlet obstruction, as well as pain. Experienced centers have reported the development and use of laparoscopic hepaticojejunostomies, gastroenterostomies, and celiac plexus block.[29,30] New immunocytologic and cytomorphologic techniques have increased the yield of malignant cells in peritoneal cytology and may in the near future provide "metastaging" capabilities to the test, which could offset these current preoperative recommendations and even lead to a reclassification of pancreatic cancer.[31] Advances in technology and expertise in minimally invasive procedures will undoubtedly have an impact on algorithms of management and may allow patients with unresectable disease to be managed nonoperatively or with minimally invasive methods, provided they truly *maximize* palliation. Until then we believe staging laparoscopy should direct the surgeon to the best method of palliation (endoscopic vs. operative). Simple staging laparoscopy aimed at detecting liver or peritoneal metastases can be performed by the "general" laparoscopist who, on the basis of results obtained and according to our proposed algorithm, can continue managing the patient or refer to a tertiary care facility for exploration for resection.

Another role for laparoscopy is diagnosis and staging for neoadjuvant chemo/radiotherapy protocols.[32,33] Patients to be considered for this treatment modality require tissue diagnosis and assurance of the absence of distant disease. Laparoscopy is the ideal procedure for this novel approach.

In summary, in the algorithm of management of pancreatic cancer (Fig. 2), staging laparoscopy should optimize palliation rather than just increase the resectability rate. We currently recommend staging laparoscopy for clinically resectable periampullary malignancies. When liver or peritoneal disease is encountered, endoscopic palliation is usually offered. If these sites are clear of metastases, we do not recommend extended laparoscopy.

Maybe the definition of a "necessary" operation in patients with pancreatic cancer should be expanded to include not only curative resection but also the optimal operative palliation of those with unresectable disease. Aiming at continuously higher resectability rates, per se, may not necessarily or always be in all patients' best interests.

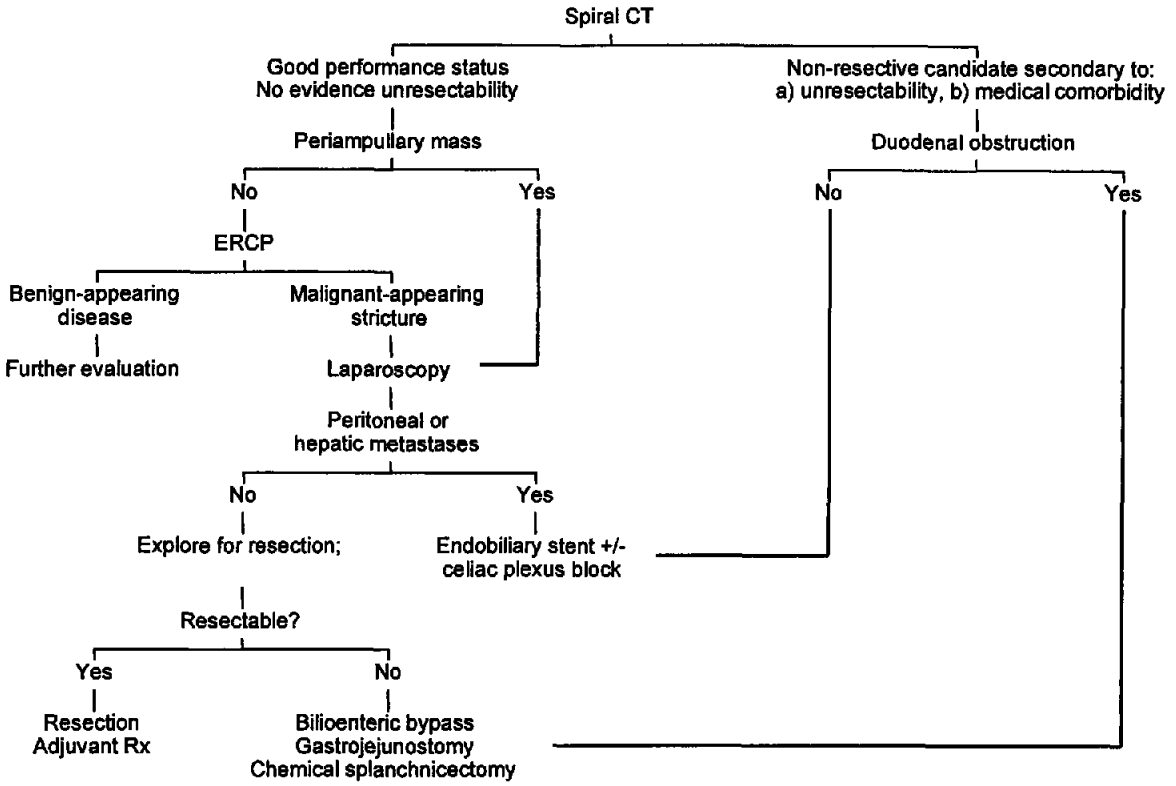

Fig. 2. Algorithm for management of suspected periampullary malignancy.

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Discussion

Dr. C. Fernandez-del Castillo (Boston, Mass.). I agree that we are seeing more patients with nonresected pancreatic cancer who survive for long periods of time and that the issue of adequate palliation for these patients probably needs to be revisited constantly. It is interesting that without doing a single laparoscopic procedure, you arrive at the conclusion that laparoscopy would have helped 51 of your patients. We and others have shown that anywhere between 22% and 70% of patients with apparently localized pancreatic cancer by CT criteria have evidence of distant metastasis by laparoscopy. I also agree that the purported advantages of extended laparoscopy remain to be proved and that by keeping laparoscopy simple, it becomes an effective triage tool in the hands of any general surgeon and can potentially help more patients.

Over a 7-year period, 150 patients you reviewed underwent operative exploration and were found to be unresectable. Can you tell us how many patients in that same time period were found to be resectable so that we may know the denominator and therefore the true incidence of findings with laparoscopy? Second, I have a question related to patients with unresectable disease as shown by CT who do not undergo operative exploration. You state that their survival is only 6 months. The Mayo Clinic many years ago published a paper stating that radiation and 5-fluorouracil can actually double the survival of patients with localized pancreatic cancer that is unresectable. So how can you identify these patients or how do you currently treat patients with unresectable cancer by CT? Wouldn't laparoscopy also be useful in this setting to identify those patients who have distant metastasis and those who do not? Finally, it seems somewhat artificial to separate liver and peritoneal metastasis. The liver lesions that are seen by laparoscopy are truly superficial implants and are peritoneal metastases as opposed to the deep-seated liver metastases that probably come through a different route.

Dr. E. Luque-de León (Rochester, Minn.). Over a 7-year period, approximately 150 patients were resected, so the yield would be around 50%. We agree that another role for laparoscopy is in diagnosis and staging of patients for neoadjuvant treatment. Indeed the patients considered for this treatment modality require tissue diagnosis and assurance of the absence of distant disease, so laparoscopy would be the ideal procedure in this setting.

Dr. N. Soper (St. Louis, Mo.). How have the advances in CT imaging since 1992 changed the diagnostic algorithm? Also, it should be noted that there is palliative therapy that might be rendered laparoscopically, such as gastrojejunostomy or biliary bypass. Do you have any data regarding the mode of exodus in these patients that would guide you?

Dr. Luque-de León. There is a dynamic evolution in the management of pancreatic cancer. Once we can be assured that laparoscopic palliation is satisfactory, this algorithm would change dramatically. Also, I agree that preoperative imaging techniques have improved and this would also change the algorithm.

Dr. J. Roslyn (Philadelphia, Pa.). Reviewing the records of patients with this disease from 1985 to 1992 may not be relevant to 1998 when we not only have improved CT imaging but also magnetic resonance pancreatography and angiography, as well as endoscopic ultrasound. In this day and age, shouldn't we be able to identify most patients preoperatively who have vascular involvement or nodal disease? In that context, how many patients are you seeing and evaluating currently and deciding preoperatively that they are unresectable? You have told us how many of your patients underwent operative exploration and how many were resectable and unresectable, but what is the real denominator?

Dr. Luque-de León. Again, I agree with the substantial advantage of the more recent preoperative diagnostic and staging modalities. In fact, the yield for staging laparoscopy has decreased over the years from 40% to approximately 25%, and I believe this trend of a decreased yield will continue. I cannot tell you the exact denominators for 1998, only the ones I have already discussed.

Pancreaticoduodenectomy for Metastatic Tumors to the Periampullary Region

Heriberto Medina-Franco, M.D., Norman B. Halpern, M.D., Joaquin S. Aldrete, M.D.

Although operative resection of metastatic lesions to the liver, lung, and brain has proved to be useful, only recently have there been a few reports of pancreaticoduodenectomies in selected cases of metastases to the periampullary region. In this report we present four cases of proven metastatic disease to the periampullary region in which the lesions were treated by pancreaticoduodenectomy. Metastatic tumors corresponded to a melanoma of unknown primary site, choriocarcinoma, high-grade liposarcoma of the leg, and a small cell cancer of the lung. All four patients survived the operation and had no major complications. Two patients died of recurrence of their tumors, 6 and 63 months, respectively, after operation; the other two patients are alive 21 and 12 months, respectively, after operation. It can be inferred from this small but documented experience, as well as a review of the literature, that pancreaticoduodenectomy for metastatic disease can be considered in selected patients, as long as this operation is performed by experienced surgeons who have achieved minimal or no morbidity and mortality with it. (J GASTROINTEST SURG 1999;3:119-122.)

KEY WORDS: Pancreaticoduodenectomy, periampullary, metastatic, tumors, pancreas

Metastatic lesions to the head of the pancreas and the periampullary region are uncommon, and the reported incidence of these types of lesions in autopsy studies ranges from 4% to 11%.[1,2] Operative resection of metastatic lesions to the liver, lung, and brain has proved to be useful in order to improve the quality of life or the survival of patients with metastatic lesions to these sites.[3-5] Only recently has the usefulness of pancreaticoduodenectomy in selected cases of metastasis to the periampullary region been reported.[6]

In recent years an improvement in hospital perioperative morbidity and mortality rates for pancreaticoduodenectomy has been documented,[7,8] therefore extending the indications for this operation.[9] However, the treatment of metastatic disease to the pancreas has not been clearly defined. In this report we present the personal experience of the two senior authors (N.B.H. and J.S.A.) with four patients undergoing pancreaticoduodenectomy for proven metastatic disease to the periampullary region. A review of the literature is also included.

CASE REPORTS

Case 1

A 60-year-old white man presented with upper abdominal pain; he had jaundice but no other signs were noted. His total bilirubin was 4.5 mg/dl, alkaline phosphatase 740 IU/L, aspartate aminotransferase 293 IU/L, and alanine aminotransferase 414 IU/L. CT scan, percutaneous transhepatic cholangiogram, and ultrasound suggested the presence of a pancreatic tumor. There was no evidence of distant or regional metastatic disease. In May 1983 he underwent celiotomy for a mass involving the duodenum and extending into the head of the pancreas. Because the mass was resectable and appeared to be malignant, an intraoperative biopsy was not done; therefore a pancreaticoduodenectomy with pylorus preservation was performed. His recovery was uneventful. The resected specimen showed an 8 × 5 cm mass involving 80% of the circumference of the duodenum. Light and electron microscopic examinations showed metastatic malignant melanoma with involvement of the common bile duct, peripancreatic lymph nodes, and the head of the pancreas, but the surgical margins were reportedly free of tumor. The site of the primary melanoma was not found. After leaving the hospital, the patient received no further treatment. In October 1983 he presented with disseminated disease and died in November 1983, 6 months after his operation.

Case 2

A 36-year-old white woman presented with upper abdominal pain. Two years previously she had had a hysterectomy for choriocarcinoma. Postoperatively she was treated with chemotherapy. It was noted that her mother had died

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of pancreatic cancer at age 47. In the hospital she was evaluated for a possible diagnosis of pancreatitis vs. pancreatic carcinoma. All laboratory test results were normal. A CT scan showed a small mass in the head of the pancreas suggestive of a focal inflammatory process. She remained asymptomatic and was discharged. A follow-up CT scan 8 months later showed that the pancreatic mass had doubled in size and appeared cystic. Surgery was advised, which was performed in December 1989. A localized lesion in the head of the pancreas was found and removed. A needle biopsy did not show tumor; however, the enlarging mass was present and was amenable to pancreaticoduodenectomy. The surgical specimen showed a central cystic lesion measuring 2.3 × 0.8 cm in the head of the pancreas. Surgical margins were free of tumor without lymph node invasion. Epithelial membrane antigens were strongly positive for keratin. Retrospectively the slides of her previous endometrial tumor were reviewed and found to be histologically similar to the resected pancreatic lesion. Sections of the two specimens were stained immunohistochemically for human chorionic gonadotropin and placental alkaline phosphatase. The uterine tumor had equal staining characteristics showing that the pancreatic tumor was a metastasis to the pancreas that had originated in the trophoblastic neoplasm. After the pancreaticoduodenectomy, the patient received chemotherapy and she was then followed up with serial CT scans. In February 1993 a CT scan showed a recurrent mass near the superior mesenteric vein. She underwent a second celiotomy that showed a recurrent tumor seated at the confluence of the splenic and mesenteric veins. Biopsies showed the same tumor. Because of its location and fixation to the surrounding tissues, it was deemed unresectable. The patient was given cytotoxic chemotherapy and localized radiation therapy. She showed a partial response and was well until December 1994 when she was found to have disseminated tumor in her abdomen. She died in March 1995, 63 months after her pancreaticoduodenectomy.

Case 3

A 40-year-old white man presented with a high-grade pleomorphic liposarcoma in his right calf. It was resected in November 1995, and postoperatively he received chemotherapy. In October 1996 he was found to be anemic, and esophagogastroduodenoscopy showed a large friable lesion in the second portion of the duodenum. Biopsy results showed a sarcoma similar to the original one that had been resected. Results of physical examination and laboratory studies were unremarkable. CT scans of the abdomen and thorax did not show any other organs to be involved with metastases. In October 1996 the patient underwent a celiotomy. A well-localized tumor was found in the periampullary region, which was removed by means of a pylorus-preserving pancreaticoduodenectomy. Recovery was uneventful. The tumor measured 5 cm in diameter, and histologically it corresponded to a high-grade liposarcoma identical to the previously resected calf lesion on the right leg. Immunoperoxidase stain for vimentin was strongly positive. These staining characteristics supported the diagnosis of metastatic liposarcoma. Subsequently two CT scans of the abdomen were performed and interpreted as free of recurrent tumor. Twenty-one months after the pancreaticoduodenectomy, the patient is alive and well; however, he did require a thoracotomy in March 1998 to remove a similar metastasis from his right lung.

Case 4

A 64-year-old man was admitted in April 1997 because of jaundice. Previously, in November 1995, he was found to have a lesion of the right lung and he underwent a right upper lobectomy for removal of a 3.7 × 4 × 5 cm pulmonary mass with free margins of resection but with a metastatic peribronchial lymph node. Histologic examination showed a poorly differentiated large cell squamous carcinoma with lymphatic and bronchial cartilage invasion. The patient recovered uneventfully. In May 1996 he was found to have a mass on the left side of his neck. A CT scan showed a 3 cm lymph node in his left internal jugular chain. The biopsy proved it to be a squamous cell tumor. The patient was treated with radiation therapy and neck dissection in March 1997. There was no evidence of recurrent tumor in the surgical specimen.

At the time of his admission for jaundice, his total bilirubin was 18.4 and alkaline phosphatase was 430 IU/L. An abdominal CT scan showed a 4 × 5 × 4.5 cm mass in the head of the pancreas with dilation of the biliary system. Chest x-ray examination showed no evidence of recurrent tumor.

Except for the lesion in the pancreas, there was no evidence of any other metastatic disease from the primary cancer of his lung; he therefore underwent surgery in April 1997. A localized mass was found in the head of the pancreas, which was treated by pancreaticoduodenectomy. The patient recovered uneventfully and left the hospital a few days later.

Histologic examination of the resected tumor showed sheets of tumor cells with abundant eosinophilic cytoplasm and large pleomorphic nuclei that were remarkably similar to the cancerous tumor that had been removed from the lung.

This patient was seen by his local physician 12 months after his pancreaticoduodenectomy and was doing well. Physical chest x-ray examinations showed no evidence of recurrence of his neck or pancreatic tumor.

DISCUSSION

Metastatic lesions of the pancreas are not common. Autopsy studies have reported an incidence of solitary metastases to the head of the pancreas between 4% and 11%.[1,2] Of 129 cases of pancreatic malignant lesions reported by Sloan and Wharton[10] in 1954, only

15 of them were metastatic. Another analysis from the Mayo Clinic reported an incidence of metastatic pancreatic tumors of only 2%.[8] This study comprises the personal experience of the two senior authors with 193 pancreaticoduodenectomies from 1970 to 1997 showing only the four cases (2.07%) that are the subject of this report.

The most common primary tumors that have been found to metastasize to the periampullary region are colon,[11] lung,[12] kidney[13] melanoma,[14] and sarcomas.[15] In a retrospective review of 27 patients with metastatic lesions of the pancreas, Roland and VanHeerden[16] found a mean survival of 8.7 months.

In the past 15 years, the morbidity and mortality observed in patients undergoing pancreaticoduodenectomy has greatly decreased.[7,8,17,18] However, the role of these operations in the management of patients with metastatic tumors to the pancreas has not been clearly defined. Karakousis et al.[19,20] described a patient with a metastatic melanoma from an unknown primary site that was found in the periampullary region and could be removed by pancreaticoduodenectomy. This patient was reported to be free of recurrent disease 4 years after pancreaticoduodenectomy.

Nakeeb et al.[6] reported using pancreaticoduodenectomy to treat six patients with locally recurrent or metastatic carcinoma, but only two of the latter had true metastasis to the pancreas. One patient survived 60 months after his primary tumor, a clear cell carcinoma of the right kidney, was resected; the other patient survived 12 months after pancreaticoduodenectomy for a metastatic nodule from a previously resected primary carcinoma of the breast.

The prominent clinical features of the four cases presented in this study are summarized in Table I. The clinical presentation of these cases was similar to that of any other patients with primary periampullary malignancies. It has been reported that neither ultrasound, nor CT scan, nor endoscopic retrograde cholangiopancreatography can differentiate primary from metastatic pancreatic lesions.[21,22] The presumptive diagnosis of metastatic liposarcoma and carcinoma of the lung was recognized preoperatively in only two of our patients. In the other two, the presence of metastatic disease of the pancreas was established only by histopathologic analysis (Table II).

In summary, we infer that pancreaticoduodenectomy should be considered for the management of certain metastatic malignancies of the periampullary region. There are several reasons for this. First, it is difficult to differentiate between primary and metastatic lesions to this region; second, some patients presenting with periampullary tumors have a history of previous primary cancers; and third, these metastatic lesions from the pancreas can be resectable. Some previous reports[6] and our own experience showed that sometimes these patients can remain alive with good quality of life for prolonged periods of time, if their lesions are localized and pancreaticoduodenectomy can be done with minimal morbidity.

Table I. Demographics, site of primary tumor, symptoms, signs, diagnostic tests, and lesion recognized prior to pancreaticoduodenectomy in four patients undergoing pancreaticoduodenectomy for metastases to the periampullary region

Case	Sex	Age (yr)	Primary tumor	Symptoms	Signs	Diagnostic studies prior to PDMY	Metastatic lesion of pancreas recognized prior to PDMY
1	M	60	Melanoma, unknown site	Yellow sclera	Jaundice	Ultrasound Abdominal CT PTC	No
2	F	36	Choriocarcinoma	Abdominal pain	Abdominal tenderness Pancreatitis (no jaundice)	Repeated abdominal CTs showing enlarging cystic mass of the pancreas	No
3	M	40	Liposarcoma, right calf	Upper gastrointestinal bleeding	Tumor seen at endoscopy Biopsy proven (no jaundice)	EGD Abdominal CT Thoracic CT Bone scan	Yes (diagnosis confirmed by EGD and biopsy)
4	M	64	Carcinoma of the lung	Yellow sclera	Jaundice	Abdominal CT Thoracic CT	Suspected

PDMY = pancreaticoduodenectomy; CT = computerized tomography; PTC = percutaneous transhepatic cholangiogram; EGD = esophagogastroduodenoscopy.

Table II. Site of primary tumor, time elapsed to discovery of metastasis to the periampullary region, treatment used after removal of primary tumor, coadjuvant therapy, time and site of recurrence, and outcome in four patients undergoing pancreaticoduodenectomy for metastasis to the periampullary region

Case	Primary tumor removed (time elapsed between removal of primary tumor and PDMY)	Treatment after primary tumor removal	Treatment after PDMY	Time to recurrence of tumor after PDMY and site of recurrence	Outcome after PDMY and length of survival
1	Melanoma, unknown primary site (?)	None	Chemotherapy	4 mo Abdomen	Death—6 mo
2	Choriocarcinoma (24 mo)	Chemotherapy	Chemotheray Radiation	9 mo Abdomen	Death—63 mo
3*	Liposarcoma of the right lower leg (11 mo)	Chemotherapy	Chemotherapy	0 mo Chest metastasis Thoracotomy	Alive—21 mo
4	Squamous cell carcinoma of the lung (17 mo)	Chemotherapy Radiation	None	0 mo	Alive with no recurrence of tumor—14 mo

PDMY = pancreaticoduodenectomy.
*Patient underwent thoracotomy 16 months after PDMY for removal of another metastasis (primary liposarcoma of leg) from his right lung.

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Fungal Pseudotumor Masquerading As Pancreatic Cancer—A Sequela of New Technology?

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Fungal infection resulting in chronic pancreatitis is rare. We report a case of chronic pancreatitis due to fungal infection causing common bile duct obstruction and abdominal pain mimicking pancreatic cancer. Treatment included resection to cure the pain and rule out malignancy. Long-term effects of fungal infection may be seen more frequently as total parenteral nutrition, antibiotics, and foreign bodies (e.g., stents, drains, central venous catheters) are more often being used in the treatment of many diseases. (J GASTROINTEST SURG 1999;3:123-126.)

KEY WORDS: Pancreatitis, fungal, stent, endoprosthesis, Whipple

A sudden attack of acute pancreatitis may develop into chronic "smoldering" pancreatitis. The incidence in an operative series for chronic pancreatitis was 17%.[1] In North America most cases of chronic pancreatitis are due to alcohol abuse; however, in up to 20% of cases the etiology is "idiopathic." In addition, as many as 15% of operations for chronic pancreatitis in the pancreatic head are performed with a high index of suspicion for malignancy.[1] We report a case of acute pancreatitis progressing to chronic pancreatitis that mimicked pancreatic cancer because of a tight common bile duct stricture due to fungal microabscesses. The stricture was associated with a pancreatic head mass as shown by computed tomography (CT). Although fungal infection is common with acute pancreatitis, the association with chronic pancreatitis is rare. Only one other similar case has been reported,[2] and with advances in technology (i.e., stents, total parenteral nutrition, antibiotics) more cases may be seen. We believe that the primary risk factors for the development of chronic pancreatitis due to fungal infection in this case were a combination of total parenteral nutrition (TPN) and the prolonged use of a preoperative endoscopic pancreatic duct stent. A brief discussion of the literature and our practice pattern for the use of preoperative pancreatic or biliary stents is included in the discussion.

CASE REPORT

A 43-year-old man, who was previously in good health and taking no medications, was admitted to another hospital after 3 weeks of abdominal pain, nausea, and vomiting. He was found to have an elevated amylase of 145 U/L (normal 0 to 88 U/L) and alkaline phosphatase of 279 U/L (normal 39 to 117 U/L). The clinical and chemical findings were consistent with acute pancreatitis. He had no history of pancreatitis, and although he had a history of serious alcohol abuse 15 years earlier, he reported only minimal intermittent use since then. Initial imaging studies included abdominal ultrasound, which showed a normal gallbladder without stones, and a CT scan, which demonstrated a pancreatic phlegmon and a pancreatic tail pseudocyst. Results of an upper endoscopy were unremarkable. A peripherally inserted central venous catheter was placed on admission and TPN was initiated. On the third hospital day the patient became febrile with temperatures up to 39° C, and despite negative cultures he was given a 6-day course of intravenous Unasyn (Pfizer/Roerig, New York, N.Y.). A repeat CT scan on the fourth hospital day showed mild improvement of the pancreatic inflammation. He was discharged on the thirteenth hospital day on home TPN and oral pain medication.

He continued to have unrelenting abdominal pain and was unable to eat. TPN support was used for 1 month after he was discharged, at which time he was able to make the transition to a liquid diet. A CT scan 18 and 36 days after presentation showed improvement in the pancreatic edema and pseudocyst.

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Moderate symptoms persisted during the 4 months after presentation prompting endoscopic retrograde cholangiopancreatography (ERCP), which demonstrated a normal common bile duct (CBD) and a mildly irregular main pancreatic duct consistent with chronic pancreatitis. It was the endoscopist's impression that the main pancreatic duct was draining poorly so a 7 Fr, 3 cm long polyethylene stent was placed. The pancreatic stent was removed 6 weeks later as there had been no clinical improvement. Over the next 2 months the patient continued to have symptoms of smoldering pancreatitis. He was again hospitalized (now 7 months after original presentation) for 2 days and placed back on TPN via a new peripherally inserted central venous catheter. A CT scan showed resolution of the pancreatic tail pseudocyst, but an enlarged, noncalcified pancreatic head and uncinate process were observed with an irregular, dilated pancreatic duct in the body/tail. A second ERCP (now 8 months after initial presentation) demonstrated a double duct sign. The intrapancreatic CBD contained a tight smooth stricture (3 cm long) with proximal dilatation to 11 mm. There was also a main pancreatic duct stricture in the pancreatic head (2.5 cm long) with distortion of the secondary radicals, and a dilated main pancreatic duct in the body/tail to 8 mm (Fig. 1). Endoscopic brushings of the CBD stricture were negative for malignancy.

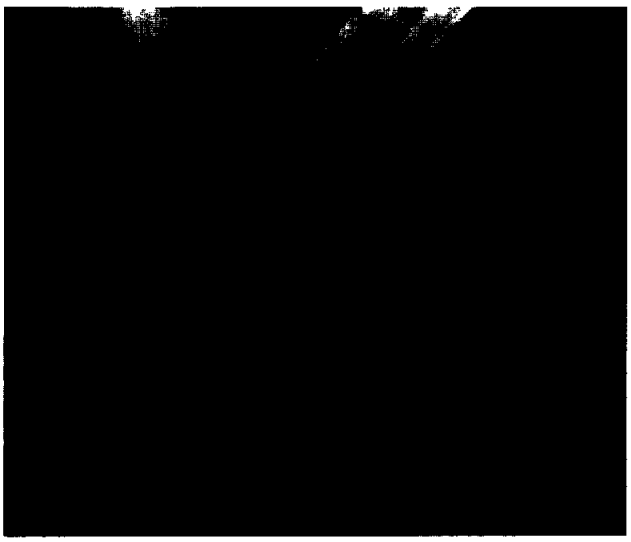

Fig. 1. ERCP 8 months after patient presentation demonstrating a double duct sign. There was a progressing long CBD stricture and a stricture of the main pancreatic duct in the pancreatic head worrisome for malignancy.

Nine months after initial presentation, surgical consultation was obtained. At this time the patient remained on TPN (second course—now at 1 month duration) and required a fentanyl patch (Janssen Pharmaceutical, Inc., Titusville, N.J.) in addition to oral narcotics for continuous epigastric pain, which he described as "9 to 10 over 10." Physical examination showed moderate epigastric and right upper quadrant tenderness without mass. Laboratory values included a normal hematocrit, white blood cell count and differential, aspartate aminotransferase, alanine aminotransferase, total bilirubin, and CA19-9 (Corning, Nichols Institute, San Juan Capistrano, Calif.). The serum amylase level was mildly elevated at 90 (normal 0 to 88), as was the akaline phosphatase level at 167 (normal 41 to 128). A helical CT scan demonstrated an enlarged pancreatic head, which measured 4 × 3 cm and abutted the superior mesenteric vein and artery with CBD dilatation proximally (Fig. 2). A visceral angiogram demonstrated a narrowed gastroduodenal artery in the pancreatic head with a surrounding soft tissue blush suggestive of an inflammatory process such as pancreatitis. The clinical impression was chronic smoldering pancreatitis but pancreatic cancer was also thought

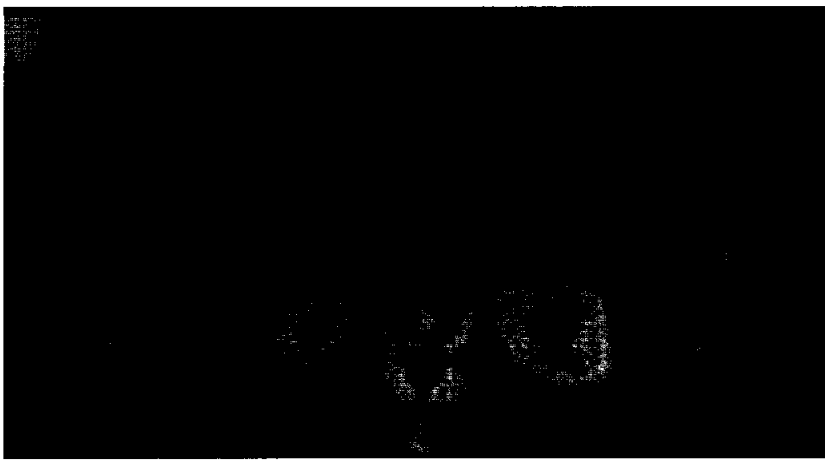

Fig. 2. Helical CT scan of the abdomen performed just prior to operation. Between the duodenum (arrow) and the superior mesenteric vein, an enlarged pancreatic head is observed. The enlarged head contained a dilated CBD. In addition, a loss of the fat plane between the pancreatic head and the mesenteric vessels was noted.

to be a distinct possibility. Resection of the pancreatic head was recommended for poorly controlled abdominal pain associated with ductal obstruction and to rule out malignancy.

Intraoperatively a discrete firm mass was palpated in the head of the pancreas without obvious adenopathy or metastasis. A cholangiogram confirmed an extremely tight CBD stricture (Fig. 3), and a pylorus-preserving Whipple procedure was performed. There were dense adhesions of the pancreatic head to the superior mesenteric and portal vein somewhat mimicking malignancy; however, gross and frozen section examinations of the specimen were consistent with chronic pancreatitis. Later microscopic examination of the permanent slides showed extensive granulomatous inflammation and microabscess formation throughout the pancreatic head parenchyma but not in the bile or pancreatic duct walls. The granulomas and microabscesses were found to be comprised of budding fungal elements and septate fungal hyphae (Fig. 4) that could not be further identified. There was no evidence of malignancy.

Fig. 3. Intraoperative transcystic cholangiogram confirming a tight, suspicious-appearing CBD stricture.

Fig. 4. Within the pancreatic parenchyma were many microabscesses, one of which is depicted in this paraffin section. Budding fungal elements (dark oval shapes) and septate hyphae (dark fibers) are shown. (Gomori's methenamine-silver stain.)

The patient was discharged on the eleventh postoperative day off of TPN and eating a regular diet. He was started on itraconazole (Janssen Pharmaceutical), 400 mg by mouth each day, for a total course of 3 months. An evaluation for an immunocompromised state or fungal source was initiated and included the following: HIV test, serum protein electrophoresis, serum quantitative immunoglobulins, and transesophageal echocardiography, all of which were unremarkable. At 11 months' follow-up the patient is without abdominal pain and eating a regular diet.

DISCUSSION

There has been a rapid increase in the incidence of serious fungal infections worldwide. In fact, *Candida* has become the fourth most common isolate from blood cultures in the United States.[3] Historically, fungal infection of the pancreas is uncommon and usually manifests as pancreatic abscess complicating acute pancreatitis.[4] Fungal infection of the pancreas leading to a chronic "smoldering" or "relapsing" pancreatitis is rare.[2,5] Attendant complications of this chronic inflammatory process are the same as other forms of chronic pancreatitis—recalcitrant abdominal pain, CBD stricture, pancreatic duct stricture, or an inflammatory pancreatic mass (pseudotumor). The anatomy imaged may be indistinguishable from pancreatic cancer. A review of the literature found only one similar case of a 43-year-old alcoholic South African man with a 2-year history of anorexia and weight loss who presented with obstructive jaundice and a pancreatic head mass causing CBD obstruction worrisome for malignancy.[2] A Kausch-Whipple (pancreatoduodenectomy with hemigastrectomy) resection was performed, and final pathologic examination showed an inflammatory pancreatic head mass containing *Candida albicans* microabscesses without malignancy. No known risk factors for fungal infection were cited. He was discharged without pain and eating.

It may be that this entity is not uncommon and could be more frequently observed as resections are more commonly performed for the severe forms of this disease. Current practice in the management of patients with pancreatitis often includes multiple potential risk factors for invasive fungal infection. These factors include the administration of broad-spectrum antibiotics, prolonged hospitalization, prolonged TPN, invasive procedures (e.g., endoscopy, ERCP, percutaneous drainage of fluid collections or pseudocysts), and placement of chronic indwelling foreign bodies (e.g., endoscopically placed stents, percutaneously placed drains or stents, central venous catheters). There may be additional patient risk factors such as advanced age, chronic corticosteroid use, diabetes, cancer, or cancer treatment with chemotherapeutic agents.[3] Therefore it may be reasonable to

minimize antibiotic use, frequently change central lines, closely monitor the quality of TPN preparations, and draw fungal as well as bacterial cultures if fever arises or if there is failure to improve clinically, and *obtain a fungal culture of the specimen if a resection is performed.*

Our patient was young and immunocompetent; however, many other known risk factors were present including the prolonged presence of a central venous catheter (two separate catheters each present for 1 month), use of a broad-spectrum antibiotic (one 6-day course), prolonged TPN (two separate 1-month courses), multiple invasive procedures (i.e., two ERCPs, endoscopy, two central venous catheters), and prolonged use of an indwelling main pancreatic duct stent for 6 weeks.

Another possible etiologic factor is the development of invasive fungal infection in damaged pancreatic duct/peripancreatic tissue resulting from acute pancreatitis.[5] In those patients who after an episode of acute pancreatitis develop a chronic "smoldering" course, an infectious etiology should be considered.

It is known that pancreatic duct stenting alone may lead to pancreatic ductal changes of chronic pancreatitis (i.e., stasis from side branch occlusion), which we believe could allow a "portal of entry" for invasive fungal infection in addition to the presence of a chronic foreign body.[6] Our current practice is to not use endoprostheses as long-term options for the treatment of main pancreatic duct stenoses. Although a distal CBD stricture was present, this patient did not become overtly jaundiced and did not have a biliary stent placed. In those patients who develop *distal* biliary obstruction, we selectively use short-term endoscopically placed stents in patients with deep jaundice and poor nutritional status. The use of stents in patients with *proximal* biliary obstruction (hilar) is associated with a high rate of procedural complications, a low success rate, and a high rate of subsequent infectious complications.[7,8] Preoperative biliary drainage was supported by several retrospective studies, which suggested increased morbidity and mortality in the jaundiced patient. However, more recent prospective randomized trials have not confirmed these findings and have instead noted either no benefit or increased risk of preoperative cholangitis, postoperative wound and intra-abdominal sepsis events along with the attendant morbidity associated with the additional procedure.[7,9-12] This appears to hold true whether the biliary drainage is internalized with an endoscopic stent or externalized with a percutaneous drain.[7] Thus indiscriminate preoperative endoscopic stenting of the biliary or pancreatic duct is unwarranted and unnecessary, and that technology should be used only in selected cases as a short-term option.

In summary, this uncommon case suggests that the combination of long-term TPN, antibiotics, and lengthy main pancreatic duct stenting may increase the chance that an opportunistic colonizer such as fungal species may become invasive and cause serious complications. The presence of fungal infection should be considered in those patients with idiopathic pancreatitis or in those with an unusual clinical history. Finally, resection for severe complications of chronic pancreatitis resolves the problem of chronic abdominal pain and inability to eat plus it rules out the possibility of malignancy.[1] We cannot comment on the long-term sequellae of residual fungal disease or the use of oral fungal suppressants in this case without long-term follow-up.

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G207, Modified Herpes Simplex Virus Type 1, Kills Human Pancreatic Cancer Cells In Vitro

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Pancreatic cancer is often fatal, and further effective therapeutic options are needed. This study was designed to assess whether the replication-restricted herpes simplex virus, G207, was effective in killing human pancreatic cancer cells in vitro. G207, a multimutated strain of herpes simplex virus type 1 carrying *lacZ* reporter gene, is capable of efficient cytolytic growth in many dividing cells, including certain tumor cells, but not in nondividing cells. Three human pancreatic cell lines, AsPC-1, MIA PaCa-2, and BxPC-3, were infected with G207 at different multiplicities of infection. After 24 hours, expression of the *lacZ* reporter gene was tested using a histochemical X-gal assay. In addition, cell lines were infected with G207 for 24 to 48 hours; then the virus obtained from cell pellets and media supernatant was used to infect Vero cells to obtain G207 titers by plaque assay. To assess whether increasing viral immediate early gene expression would improve cytolysis and virus production, similar experiments were performed with the addition of 0.5 mmol/L of hexamethylene bisacetamide (HMBA) 1 hour after viral infection. Finally, MTS cell viability assays were performed to measure viable cells at 24 to 96 hours post infection. The X-gal assay data revealed a viral dose-dependent β-galactosidase expression, indicating G207 infectivity and expression of the *lacZ* reporter gene. Plaque assays demonstrated a viral dose-dependent increase in plaque formation, indicating viral production from all three cell lines. In addition, HMBA data indicated a modest increase in viral production. The MTS assay data indicated a dose-dependent cytotoxicity for G207 in the cell lines tested. G207 infects, replicates in, and is cytotoxic to the above-listed human pancreatic cell lines in vitro and warrants therapeutic evaluation in models of pancreatic cancer. (J GASTROINTEST SURG 1999;3:127-133.)

KEY WORDS: Pancreatic cancer, gene therapy, herpes simplex virus

Pancreatic cancer is the fourth leading cause of cancer death in the United States after lung, colorectal, and breast cancer. To date, its etiology remains mostly unclear, early diagnosis difficult, and prognosis poor. The overall mortality rate is approximately 95% throughout the course of the disease. The following are several of the factors responsible for this poor prognosis: (1) lack of specific symptoms in the early stages of disease leading to delays in diagnosis; (2) the aggressive nature of the disease, as evidenced by the high rate of local spread and/or distant metastasis at the time of diagnosis; and (3) the lack of a significant response to currently existing chemotherapeutic and radiotherapeutic options. Surgery offers a possibility of cure but only in a small, select group of patients. The 5-year survival rate after surgery is only approximately 15% to 21%, mainly because of tumor recurrence. Because the response to existing therapies is so poor, formulation of additional management strategies is actively being sought.

Recent attempts to treat neoplastic disease include gene therapies that use cell transduction with retroviral or adenoviral vectors with the herpes simplex-thymidine kinase (HS-tk) gene inserted into their genomes, followed by ganciclovir administration.[1-5] Although these approaches remain promising, one or more of the following problems remain: (1) the need for in vitro transduction/transfection of cancer cells,

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Supported by the Wilmot Cancer Research Fellowship Program funded by the James P. Wilmot Foundation.
Presented at the Thirty-Ninth Annual Meeting of The Society for Surgery of the Alimentary Tract, New Orleans, La., May 17-20, 1998.
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which are then injected into the animals for further study[2,4,5]; (2) the low transduction rate, for both in vivo and in vitro transduction, requiring multiple and prolonged exposure to high doses of transducing vectors[2,3]; (3) induction of a severe host immune response caused by high titers of adenoviral vectors[1]; or (4) antitumoral efficacy limited to metastatic lesion without much shrinkage of the primary tumor.[1]

Replication-restricted viruses, such as G207, may offer an alternative to retrovirus- or adenovirus-based transduction vectors. Replication restriction denotes a viral gene vector that is capable of replication only within a desired cell type, such as cancer cells. G207 is an attenuated and multimutated form of herpes simplex virus type 1 (HSV-1), with deletion of both copies of $_{\gamma 1}$34.5 gene and insertion of *E. coli. lacZ* gene at the ICP-6 coding region.[6,7] $_{\gamma 1}$34.5 Gene product contains two domains: (1) a domain responsible for neurovirulence, which is responsible for encephalitis, and (2) a domain responsible for viral escape of cell apoptosis as a response to viral infection.[8-13] ICP-6 region codes for the larger subunit of ribonucleotide reductase, which is required for efficient viral growth in nondividing cells.[6,7] This potential for preferential viral replication in the target cells makes it an ideal vector for cancer therapy; unlike other vectors previously used, it provides high target cell replication specificity and eliminates the need for multiple cell transduction with high doses of transducing vectors. G207 was specifically engineered to be used in the central nervous system. Previous studies by Mineta et al.[6] and Yazaki et al.[7] indicate that G207 is cytotoxic to malignant human meningioma and glioma cells. In addition, direct intracerebral injection of G207 into nude mice and owl monkeys caused no deaths or neuropathic changes.[7] Furthermore, G207 features higher temperature sensitivity and retained ganciclovir sensitivity.[6,7] Other studies on different mutant forms of HSV-1 demonstrate that HSV-1 mutants are capable of infecting and expressing its genes in a wide variety of cells including hepatocytes.[14]

The objective of this study was to determine the efficacy of G207 in killing human pancreatic cancer cells in vitro. Several issues were examined as follows: (1) viral infectivity, (2) viral productivity, (3) the effect of an early gene expression enhancing agent, hexamethylene bisacetamide (HMBA),[15] on viral production, and (4) viral cytotoxicity. Our results indicate that G207 infects, replicates in, and is cytotoxic to tested human pancreatic cell lines AsPC-1, MIA PaCa-2, and BxPC-3 in vitro. These data are the first to show that replication-restricted viruses, particularly G207, are potentially important new agents for the study of pancreatic cancer, and perhaps its treatment.

MATERIAL AND METHODS

Cell Lines and Virus

Three human adenocarcinoma cell lines of pancreatic origin were used in our experiments: AsPC-1, MIA PaCa-2, and BxPC-3,[16-18] all of which were obtained from American Type Culture Collection (Rockville, Md.). AsPC-1 was maintained in 80% RPMI 1640 with 20% non–heat-inactivated fetal bovine serum (FBS), 1× penicillin/streptomycin (P/S), and 1 mmol/L sodium pyruvate. MIA PaCa-2 was maintained in 87.5% Dulbecco's modified Eagle medium (DMEM) with low glucose, 10% non–heat-inactivated FBS, 2.5% donor horse serum (DHS), and 1× P/S. BxPC-3 was maintained in 90% RPMI 1640 with 10% non–heat-inactivated FBS and 1× P/S. The Vero cell line (ATCC) was used to grow G207 (a gift from Dr. S. Rabkin) and also was used in the titering plaque assay. Vero was maintained in 95% DMEM with high glucose, 5% non–heat-inactivated FBS, and 1× P/S. All cell lines were incubated at 37° C with 5% CO_2.

G207 was grown in Vero cell lines. In a 100 mm tissue culture plate containing approximately 2×10^6 Vero cells, G207 was added at a multiplicity of infection (MOI) of 0.02. After a 1-hour incubation period at 37° C with 5% CO_2, the virus was allowed to grow at 34° C with 5% CO_2 for 72 hours. The media supernatant and cells were collected with a scraper and centrifuged at 1100 rpm for 10 minutes at 4° C. The supernatant was divided into aliquots and stored at −70° C. The cell pellet was resuspended in 1 ml of cell media, then underwent three cycles of quick freezing and thawing in a mixture of ethanol and dry ice followed by three cycles of 30 seconds' sonication at 4° C. The suspension was again centrifuged at 1100 rpm for 10 minutes at 4° C. The supernatant from the sonicated cells was divided into aliquots and stored at −70° C.

Viral Infectivity

In 24-well plates, all cell lines were plated at a density of 2×10^5 cells per well and were then allowed to adhere overnight in 1 ml of culture medium. G207 was added to each well at a serial dilution ranging from MOIs of 0.5 to 5×10^{-5}. After 1 hour of incubation at 37° C with 5% CO_2, the medium was aspirated and 1 ml of fresh medium was added. After 24 hrs of incubation at 34° C, the plates were removed

and medium aspirated. The wells were washed with cold 1% glutaraldehyde (Sigma, St. Louis, Mo.) for 10 minutes, followed by three washes with cold phosphate-buffered saline (PBS) solution. X-gal histochemical assay was performed and blue-forming units were measured.

X-gal Histochemical Assay

A solution containing 20 mmol/L potassium ferricyanide, 20 mmol/L potassium ferrocyanide, and 2 mmol/L magnesium chloride (all from Sigma) in PBS was freshly prepared. A 1/40 volume of 20 mg/ml solution of X-gal (5-bromo-4 chloro-3-indolyl-β-D-galactopyranoside; Gibco, Grand Island, N.Y.) in dimethylforamide (Sigma) was added to the previous solution and kept in the absence of light. A total of 250 μl of the final solution was added to each well in a 24-well plate; 24 hours later the blue-forming units and plaques were examined under a reverse-phase contrast light microscope.

Viral Production

Estimates of viral production from each of the cancer cell lines were performed by plaque assay. In six-well plates, each cell line was plated at 1×10^6 cells per well in 3 ml of their respective media; they were then allowed to adhere overnight at 37° C with 5% CO_2. The cells were infected with G207 at a MOI of 0.05 and incubated for 1 hour at 37° C with 5% CO_2. The cells were washed with PBS, and 3 ml of fresh culture medium was then added to each well. Twenty-four and 48 hours later, the supernatants and cell pellets were harvested, divided into aliquots, and stored as described previously.

For plaque assay, in 24-well plates, Vero cells were placed at a density of 2×10^5 cells per well and then allowed to adhere overnight in 1 ml of culture media. Previously aliquoted viruses were thawed in ice and added to Vero cells in serial dilution. After 1 hour of incubation at 37° C, medium was aspirated; then 0.5 ml of 1% low-melting agarose (Sigma) and DMEM with 5% FBS was added to each well at 40° C. The plates were incubated for 72 hours at 37° C with 5% CO_2, and X-gal histochemical assay was then performed. The plaque-forming units were measured by counting each plaque under the microscope.

Similar experiments were carried out with the addition of 0.5 mmol/L HMBA (Sigma) to each well 1 hour after infection with G207 and exposing it for 4 hours.

Cytotoxicity

Qualitative assessment of G207 cytotoxicity was first evaluated by plating 2×10^5 cells per well in 24-well plates. Cells were allowed to adhere overnight at 37° C with 5% CO_2, and G207 was then added to each well in serial dilutions ranging from MOIs of 0.001 to 10. Plaque assay, as described earlier, was performed 72 hours later, and the number of plaques was noted.

For quantitative measurement, in 96-well plates, AsPC-1, MIA PaCa-2, and BxPC-3 cells were plated at a density of 5000 cells per well in 0.1 ml of their respective cell media and allowed to adhere overnight at 37° C with 5% CO_2. G207 was added to each well in serial dilutions ranging from MOIs of 0.001 to 10. 3-(4,5-dimethylthiazole-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay (Promega Corp., Madison, Wis.), as described by the manufacturer, was performed for 24 to 96 hours in 24-hour intervals by measuring absorbance at 490 nm in a 96-well enzyme-linked immunosorbent assay plate reader.

RESULTS

G207 Infectivity

To determine G207 infectivity to pancreatic cancer cell lines, G207 was added to culture plates containing the cells and their growth media. After 24 hours of incubation, X-gal histochemical assay was performed. As shown in Fig. 1, all three cell lines are susceptible to G207 infection. As mentioned earlier,

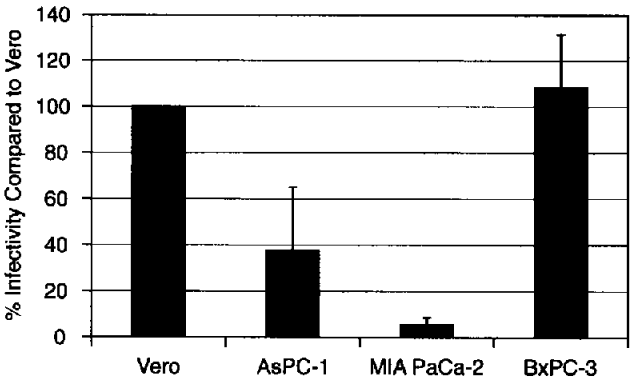

Fig. 1. G207 infectivity for AsPC-1, MIA PaCa-2, and BxPC-3. Each cell line was infected with G207 at MOI of 0.0005. G207 infectivity to the Vero cell lines was used to compare G207 infectivity to the above-mentioned pancreatic cell lines. AsPC-1 showed 38%, MIA PaCa-2 showed 6%, and BxPC-3 showed 109% infection rate compared to Vero cells.

E. coli. lacZ gene is inserted within the ICP-6 coding region, which is a β-gene; its expression demonstrates that these cell lines support viral immediate early gene expression. In addition, there is a viral MOI-dependent increase in the rate of infection. Fig. 1 reveals a differential quantitative measurement of the infection rate among the three cell lines; at MOI of 0.0005 AsPC-1 showed an average infection rate of 38%, MIA PaCa-2 showed a rate of 6%, and BxPC-3, with an infection rate of 109%, was comparable to that of Vero.

Viral Production

Virus growth was evaluated in the different cancer cell lines. An elapsed time of 48 hours represents sufficient time for inactivation of unabsorbed virus particles from the initial inoculum and also represents slightly more than one replication cycle for G207. Table I demonstrates that there is a time-dependent increase in viral production for all cancer cells tested. During the time interval 24 to 48 hours, there is an approximate 10-fold and 100-fold increase in viral production for BxPC-3 and AsPC-1, respectively. In addition, as shown in Table II, a trend toward a modest increase in viral production is observed when cells were exposed to 0.5 mmol/L HMBA for 4 hours. HMBA is an agent that increases viral production and titer by increasing immediate early gene expression in certain herpes simplex virus mutants.[15] it was noted that higher concentrations of HMBA were toxic to all of the cell lines tested. Results shown are those of a representative experiment; consistent results were found in repeated experiments.

Cytotoxicity

The cytopathic effect of G207 on human pancreatic cancer cells was qualitatively determined by performing plaque assays at 72 hours post infection. A plaque results when a single infectious particle creates a zone of cell death and lysis in a cell monolayer grown in tissue culture. Whereas Vero and BxPC-3 cell lines grew as monolayers, AsPC-1 and MIA PaCa-2 cell lines did not. Consequently, whereas G207 formed plaques on the monolayers of Vero and BxPC-3, only blue clumps were observed on AsPC-1 and MIA PaCa-2. Fig. 2 shows a representative plaque on BxPC-3. As shown, there is a central clearing, which represents cell death, surrounded by a rim of blue cells. Quantitative measurements of G207 cytotoxicity were performed by MTS cell viability assays. Absorbance at 490 nm correlates with the number of viable cells.[19,20] Fig. 3 displays the results of MTS assays. For MOIs of 1 and 10, viral cytotoxicity was observed as early as 24 hours post infection. For all three cell lines, at MOI of 10, almost complete cancer cell killing was observed by 96 hours post infection. This observation was also confirmed by direct microscopic evaluation of each well. Of the three cell lines, BxPC-3 was the most sensitive to G207; cell killing was demonstrated at the lower MOIs of 0.1 and 0.01.

Table I. Viral production for each of the cell lines after G207 infection

	Viral titer (pfu)	
Cancer cell line	**24 hr post infection**	**48 hr post infection**
AsPC-1		
Supernatant	400	400,000
Pellet	7000	600,000
TOTAL	7400	1,000,000
MIA PaCa-2		
Supernatant	60	140
Pellet	500	1000
TOTAL	560	1140
BxPC-3		
Supernatant	0	10,000
Pellet	2000	20,000
TOTAL	2000	30,000

Each cancer cell line was plated at 1×10^6 cells per well and infected with G207 at MOI of 0.05 (total viral particles = 5×10^4) for 24 to 48 hours. The viral particles are represented in plaque-forming units (pfu). As time increases, a definite increase in viral production is noted for all three of the cell lines tested.

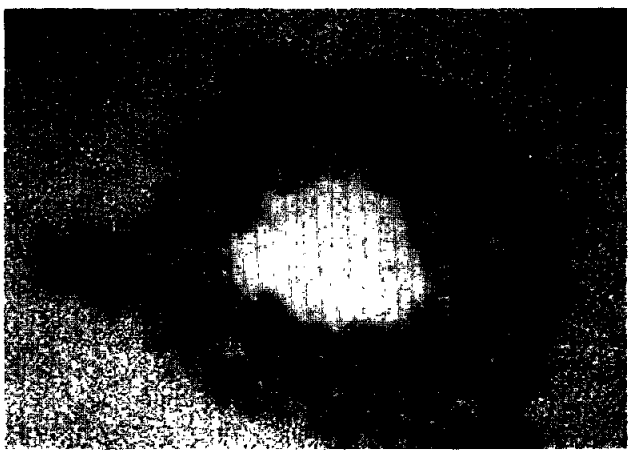

Fig. 2. A G207 plaque on BxPC-3. Qualitative measurement of G207 cytotoxicity to BxPC-3 is demonstrated. The BxPC-3 monolayer is infected with G207; 72 hours later X-gal histochemical assay is performed. Note the central plaque with a rim of blue cells, which represents G207-infected BxPC-3 cells.

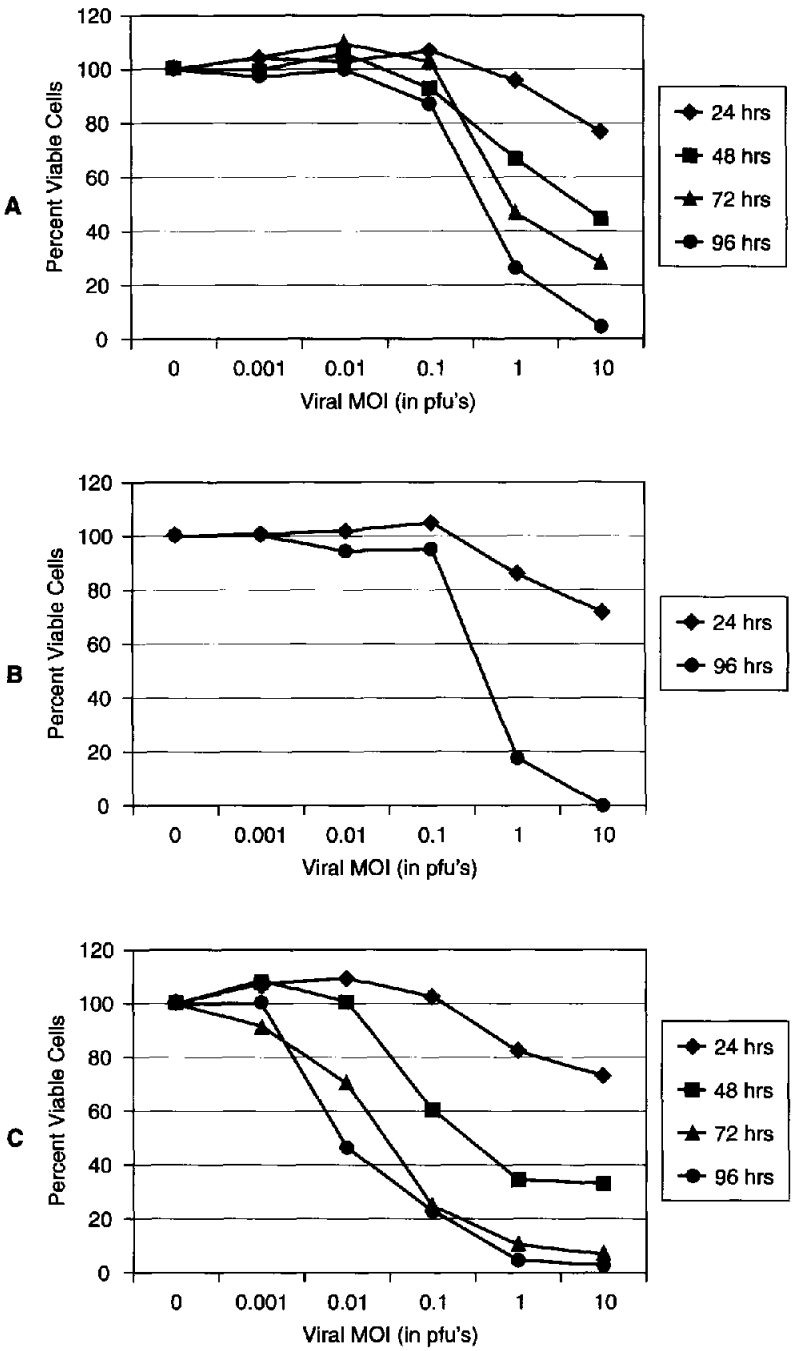

Fig. 3. A, MTS cell viability assay for AsPC-1. Percentage of viable cells, as measured by absorbance at 490 nm, is plotted against viral MOI (pfu's = plaque-forming units). For MOIs of 1 and 10, clear cytotoxicity is noted even as early as 24 hours post infection. Note the time-dependent increase in cell death, with almost complete cell killing by 96 hours for MOI of 10. **B,** MTS cell viability assay for MIA PaCa-2. Percentage of viable cells plotted against viral MOI. Again, at MOIs of 1 and 10, viral cytotoxicity is noted as early as 24 hours, with almost complete cell killing by 96 hours for MOIs of 1 and 10. **C,** MTS cell viability assay for BxPC-3. Dose- and time-dependent cell killing, even at lower MOIs (0.1 and 0.01), is noted. By 72 hours post infection, virtually complete cytotoxicity is observed for MOIs of 1 and 10.

DISCUSSION

The dismal prognosis associated with pancreatic cancer has been linked to difficult early detection and lack of effective therapeutic options. Recently some investigators have turned to gene therapy via liposome-mediated gene transfer[21] or by incorporating the HS-tk "suicide" gene into the retroviral or adenoviral vectors that were consequently used to transduce cancer cells in vitro and in vivo.[1-5] Although these studies offer new alternatives and are potentially promising, they nevertheless face many of the obstacles that are inherent in their respective systems. These systems often require in vitro transduction of cancer cells, which are then injected into the animals for in vivo studies. Furthermore, the transduction rate, despite multiple and prolonged exposure, remains low. Some studies do demonstrate success with in vivo transduction, but the problems of low cell specificity and a low transduction rate remain. In addition, adenoviral vectors, often requiring MOIs ranging from 100 to 1000, have the added complication of mounting profound immune responses in the host, possibly preventing further in vivo applications.[1,3]

Replication-restricted viruses such as G207 may offer a novel solution to these problems. G207 was initially developed for the treatment of central nervous system cancers. This modified HSV-1 contains multiple mutations that make reversion to wild type rather unlikely.[6] Previous studies by Mineta et al.[6] and Yazaki et al.[7] showed a marked decrease in neurovirulence in nonhuman primates compared to the wild-type viruses. In addition, by inactivating the gene for the larger subunit of ribonucleotide reductase, efficient viral production is limited to actively growing cells such as cancer cells. Their in vitro studies on rat cortical astrocytes and cerebellar neurons showed no cytopathic effect, and furthermore, their in vivo studies on nude mice and nonhuman primates demonstrate the feasibility of using G207 to treat solid tumors with no appreciable adverse effects on the host. In addition, herpes virus is known to infect a wide variety of cell types. Other studies indicate the possibility of using non–replication-restricted herpes simplex virus mutants in transducing gastrointestinal cells such as hepatocytes[14] or pancreatic β cells.[22]

Our data, as shown in Fig. 1, indicate that G207 infects selected human pancreatic cancer cells in vitro and, in addition, expression of its early viral genes is supported. This opens up the possibility of introducing different genes into cells by inserting such genes into the nonessential region of the herpes simplex virus early genome, which will present opportunities for more specific gene therapies. Furthermore, viral growth is supported by each of the cell lines tested (see Table I). Although highly unlikely, it is possible that viral particles present at 24 hours post infection may represent initial viral inoculum. Even so, by 48 hours post infection there are dramatic increases in viral titer for AsPC-1 and BxPC-3. MIA PaCa-2, on the other hand, does show a much lower viral yield; however, from 24 to 48 hours, approximately a twofold increase in viral titer is noted. In addition, Fig. 3, *B* demonstrates a time-dependent increase in cytotoxicity, offering indirect support for continued viral production in MIA PaCa-2. Although a higher resolution time course is needed to precisely determine the replication time and burst size of G207 in these cell lines, our data indicate that viral growth is supported in each of the cell lines tested. As for *in vivo* application, support for viral production eliminates the need for repeated cell transduction by retrovirus vectors carrying the HS-tk gene. Because of active production of G207 by infected cells, it is possible that *in vivo* application may result in ongoing virus production and progressive cytolysis of neighboring cancer cells. Moreover, the obvious advantage of retained ganciclovir sensitivity provides another level of therapeutic intervention.

Closer analysis of our data in Fig. 1 and Table I reveals that MIA PaCa-2 cells have a lower rate of infectivity and viral production compared to Vero cells and the other pancreatic cancer cells tested. As these data represent only a preliminary characterization of G207 in its ability to kill pancreatic cancer cells in vitro, we can only speculate on the reasons for such differences. A lower burst size (viral particle produced/blue-forming unit ratio) for MIA PaCa-2 compared to other cell lines suggests that the lower yield in MIA PaCa-2 cells is not simply a result of lower viral infectivity. Attempts were made to increase viral production by adding 0.5 mmol/L HMBA to the medium 1 hour post infection. It has been shown that transient exposure of infected cells to HMBA increases immediate early gene expression in the herpes simplex virus mutant *in*1814.[15] Although the existing data indicate that HMBA was optimal at a concentration of 5 mmol/L, our results showed 5 mmol/L HMBA to be toxic (data not shown). Our data showed a modest increase in viral production after 4 hours of exposure to 0.5 mmol/L HMBA for all three cell lines tested (see Table II). The effect was least pronounced in the MIA PaCa-2 cell line, which also suggests the following for MIA PaCa-2: (1) immediate early gene expression did not limit viral production and (2) further interference with viral production exists at a later phase of viral production.

At this point the host range of replication restriction for G207 has not been defined in humans. Al-

Table II. Effect of HMBA on viral production

Cell type	G207	G207 + HMBA	Increase
AsPC-1	600,000	1,000,000	1.66-fold
MIA PaCa-2	1000	1500	1.5-fold
BxPC-3	20,000	700,000	35-fold

Each cancer cell line was plated at 1×10^6 cells per well and infected with G207 at MOI of 0.05 (total viral particles = 5×10^4) for 48 hours; 0.5 mmol/L HMBA was added 1 hour post infection and was present for 4 hours. The cell pellet was harvested, and the viral particles in the cell pellet were measured using plaque assay. The viral particles are represented in plaque-forming units per milliliter. Representative data are shown above. As shown, a trend toward a modest increase in viral production is noted. A rather noticeable increase in viral production was seen for BxPC-3.

though Mineta et al.[6] demonstrated both its nonvirulence in nontransformed neural cells in vitro and its decreased neurovirulence in vivo, the specificity of infection, of replication, or of cytotoxicity is not yet established. Our own preliminary data (unpublished observations) indicate that G207 is not demonstrably pathogenic in nude mice. The data presented herein clearly demonstrate that G207 infects, replicates in, and is cytotoxic to selected human pancreatic cancer cells and that G207 is a potentially useful therapeutic agent that warrants further investigation.

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Cholecystokinin-A Receptor Messenger RNA Expression in Human Pancreatic Cancer

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Cholecystokinin (CCK) is a gut peptide hormone known to stimulate postprandial gallbladder contraction and pancreatic enzyme secretion. It has also been shown to induce the growth of normal pancreas and of malignant and premalignant lesions in rodents. Although CCK has been shown to promote the growth of human adenocarcinoma cell lines, its role in the growth of human pancreatic adenocarcinomas in vivo is less clear. Localization of CCK receptors to neoplastic cells within resected human tissue specimens would be suggestive of its potential action as an in vivo promoter of human pancreatic cancer. Resected tissue specimens of pancreatic adenocarcinomas were therefore studied by both reverse transcriptase–polymerase chain reaction (RT-PCR) and in situ hybridization for the presence of CCK-A receptors. Ninety percent of studied tumors demonstrated CCK-A expression by RT-PCR, and this expression was localized to neoplastic cells by in situ hybridization. An increase in the expression of CCK receptors is a mechanism by which pancreatic malignancies may gain a significant growth stimulus. (J GASTROINTEST SURG 1999;3:134-140.)

KEY WORDS: Pancreatic neoplasms, in situ hybridization, cholecystokinin

Pancreatic adenocarcinoma is the fifth leading cause of cancer death in the United States.[1] Eighty-five percent of patients with this disease are incurable at the time of presentation, and only 20% of the remainder experience long-term survival following operative resection.[2] Given the dismal prognosis associated with the conventional treatment of pancreatic cancer, hormonal manipulation of this disease has gained increasing interest. The role of hormone antagonists in the successful treatment of breast and prostate cancer suggests that agents which regulate normal pancreatic growth and function may be appropriate targets for therapeutic blockade in the treatment of pancreatic malignancies.

The development of safe and specific cholecystokinin (CCK) receptor antagonists has confirmed the role of CCK in the stimulation of normal postprandial pancreatic exocrine secretion in humans.[3] CCK has also been shown to induce the growth of normal rat pancreas,[4] and to enhance tumor production and growth in animal models of pancreatic carcinogenesis.[5,6] Risk factors for pancreatic cancer such as prior gastrectomy and a high-fat diet are also associated with elevated serum levels of CCK.[7,8] Finally, CCK appears to promote the growth of some but not all human pancreatic cell lines in tissue culture or when xenografted into nude mice.[9-19]

Neither animal studies nor the behavior of immortalized cultured pancreatic cancer cell lines is likely to exactly predict the behavior of human cancers in vivo. The examination of resected pancreatic adenocarcinomas for receptors for CCK provides more direct evidence of the potential role for CCK in the growth of these malignancies. Because CCK can bind to gastrin (CCK-B) receptors in addition to cholecystokinin (CCK-A) receptors, simple ligand binding studies cannot characterize the exact CCK receptor composition with absolute specificity. To avoid the ambiguity associated with CCK ligand binding studies, we have examined resected specimens of pancreatic cancer for CCK-A receptor messenger RNA by reverse transcriptase–polymerase chain reaction (RT-PCR).

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Supported by a grant from the Medical Research Service of the Department of Veterans Affairs and by a grant from Astra Pharmaceuticals.
Presented at the Thirty-Ninth Annual Meeting of The Society for Surgery of the Alimentary Tract, New Orleans, La., May 17-20, 1998.
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In addition, complementary in situ hybridization studies were performed to identify the cell population responsible for CCK-A receptor expression in these often histologically heterogeneous tumors.

MATERIAL AND METHODS

Clinical Specimens

Resected human tissues were taken directly from the operating room, snap frozen in liquid nitrogen, and stored at −80° C until used. All specimens were obtained in accordance with protocols approved by the institutional review boards of the University of Cincinnati and the University of Washington. Additional frozen specimens of human malignancies were obtained from the Cooperative Human Tissue Network (Philadelphia, Pa.).

RNA Isolation

Specimens were rapidly homogenized and extracted in a single reagent combination of guanidinium thiocyanate/phenol/chloroform (RNAzol, Tel-test, Friendswood, Tex.). Total RNA was resuspended in diethyl pyrocarbonate–treated water (Sigma, St. Louis, Mo.) following isopropanol precipitation and a 75% ethanol wash. Purified RNA was quantitated by spectrophotometric absorbance at 260 nm, and only specimens demonstrating an $OD_{260/280}$ ratio greater than 1.8 and intact 18s and 28s bands by agarose-gel electrophoresis were used.

Reverse Transcriptase–Polymerase Chain Reaction

Following incubation of 1 μg of total RNA at 70° C for 10 minutes, reverse transcription was carried out with 20 U/μl of RNAse(−) reverse transcriptase (Superscript RT, Gibco Biological Research Laboratories, Grand Island, N.Y.) in 20 μl of a solution containing 20 mmol/L Tris-HCl (pH 8.4), 50 mmol/L KCl, 0.5 mmol/L dCTP, dATP, dTTP, dGTP, 10 mmol/L DTT, and 2.5 ng/μl random hexamer primers.

One microliter of resultant cDNA was PCR amplified in a solution containing 0.05 U/μl of Taq DNA polymerase (AmphTaq, Cetus, Norwalk, Conn.), 10 mmol/L Tris-HCl (pH 8.3), 50 mmol/L KCl, 2.5 mmol/L $MgCL_2$, and CCK-A receptor-specific primers (nucleotides 166-190 and 1073-1094) in a 25 μl reaction. These primers were originally based on the rat CCK-A receptor cDNA sequence but demonstrate greater than 90% homology with the human CCK-A cDNA sequence and do not share significant homology to related human sequences, including the CCK-B receptor, based on a National Cancer Institute BLAST database search.

Following a 2-minute incubation at 95° C, DNA Taq polymerase was added to complete the reaction components at 85° C, and amplification was performed for 40 cycles at 94° C for 1 minute, 60° C for 1 minute, and 72° C for 2 minutes (DNA Thermal Cycler, Perkin-Elmer Corp., Norwalk, Conn.). PCR product was analyzed by 1.5% agarose-gel electrophoresis and ethidium bromide staining. Each reaction was run in triplicate, and only specimens demonstrating a positive result in each reaction were considered positive.

Southern Blot Analysis

Resultant gels were denatured in 0.4 N NaOH and transferred to nylon membranes (HyBond, Amersham, Cardiff, U.K.) by capillary action. Membranes were baked at 80° C for 1.5 hours in a vacuum oven. Twenty-five nanograms of full-length CCK-A receptor cDNA (a gift from Stephen A. Wank, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, Md.) was labeled with ^{32}P-CTP by random primer extension to a specific activity of 5×10^8 cpm/μg. Hybridization was carried out at 65° C overnight in a solution containing 50 mmol/L PIPES, 100 mmol/L NaCl, 50 mmol/L sodium phosphate, and 1 mmol/L EDTA. Blots were washed under high-stringency conditions and exposed to emulsion-coated film (Fuji x-ray film, Fuji Photo Film Co., Ltd., Tokyo, Japan) with intensifying screens.

In Situ Hybridization

Tissue Preparation. Frozen tissue specimens were cryostat sectioned at −20° C, affixed to electrostatically charged slides (Superfrost/Plus, Fischer Scientific, Pittsburgh, Pa.), and stored at −80° C. After a 5-minute fixation at 4° C in 5% paraformaldehyde, slides were treated with acetic anhydride in triethanolamine to decrease background binding, delipidated in chloroform, and dehydrated through 75%, 95%, and 100% ethanol. Representative slides were stained with hematoxylin and eosin to confirm preserved histologic specimens. Separate representative slides were also digested for 30 minutes at 37° C in 20 μg/ml RNAse A (Boehringer-Mannheim, Indianapolis, Ind.), 10 mmol/L Tris-HCl, 500 mmol/L NaCl, 1 mmol/L EDTA, and rinsed in the same buffer without RNAse. All slides were prehybridized in a hybridization buffer containing 50% formamide, 10% dextran, 0.3 mol/L NaCl, 10 mmol/L Tris-HCl, 1 mmol/L EDTA, 1× Denhardt's solution, and 2 mg/ml yeast tRNA.

Probe Labeling and Hybridization. The full-length human CCK-A receptor cDNA was subcloned into the plasmid pGEM −32f(−) (Promega Corp., Madison, Wis.) with the 5′ end of the sense strand oriented toward the SP6 transcriptional promoter site. One microgram of RcaI (Boehringer-Mannheim) digested construct was incubated for 1 hour at 37° C in a solution containing 40 mmol/L Tris-HCl (pH 8.0), 6 mmol/L $MgCl_2$, 100 mmol/L DTT, 2 mmol/L spermidine, 500 μmol/L ATP, CTP, and GTP, 34 μmol/L UTP, 17 μmol/L ^{33}P-labeled UTP (Amersham Life Sciences), 2 U/μl RNAsin (Promega Corp.), and 2 U/μl SP6 or T7 bacterial RNA polymerase (Boehringer-Mannheim), to yield a sense probe identical to nucleotides 1 to 668 or an antisense probe complementary to nucleotides 1268 to 1500 of the CCK-A receptor mRNA, respectively. The antisense probe corresponded to the area of maximal nonhomology with the CCK-B receptor, and demonstrated no significant homology to any previously described human sequence according to a National Cancer Institute BLAST database search. Following a 15-minute incubation of the reaction with 2 U/μl RNAse-free DNAse (Boehringer-Mannheim), labeled probe was column purified (Nensorb 20, NEN Research Products, Boston, Mass.) and hybridized to slides in a humidified chamber overnight at 62° C in hybridization buffer. The expected size of each probe produced in a similar reaction but without radiolabeled UTP was verified by denaturing agarose-gel electrophoresis. Slides were subjected to high-stringency washes and incubated at 37° C with 20 μg/ml RNAse A to degrade single-stranded unhybridized probes, dehydrated in serial alcohols, and exposed in duplicate to emulsion-coated film or dipped in liquid emulsion at 42° C (NTB-2 Emulsion, Kodak, Rochester, N.Y.). Following a 2-week exposure and development, emulsion-coated slides were stained with hematoxylin and eosin.

RESULTS

CCK-A receptor mRNA expression was examined by RT-PCR in human gallbladder, brain, normal pancreas, and in 30 operative specimens of histologically confirmed adenocarcinoma of the pancreas (Fig. 1). The expected 928 base-pair amplicon was detected in human gallbladder and human pancreas, and in 27 of 30 cancer specimens, and was not detected in the expected negative control, the human fibrosarcoma cell line Hs 913T. Hybridization of the labeled full-length CCK-A probe to Southern blots of PCR product demonstrated specific hybridization to the expected band, suggesting that the amplified product was derived from the CCK-A receptor mRNA.

In situ hybridization was performed on human gallbladder, pancreas, gastric body, and 25 human pancreatic cancers. Specific signal was detected in gallbladder muscle and in the deep layer of the gastric mucosa (Figs. 2 and 3). No specific signal was detected in eight specimens of normal pancreas. Twenty-one pancreatic adenocarcinomas were examined by in situ hybridization, and in 10 of these specimens signal clearly localized to neoplastic cells (Fig. 4). In the remaining specimens, signal did not clearly localize to malignant cells or any other discrete cell population.

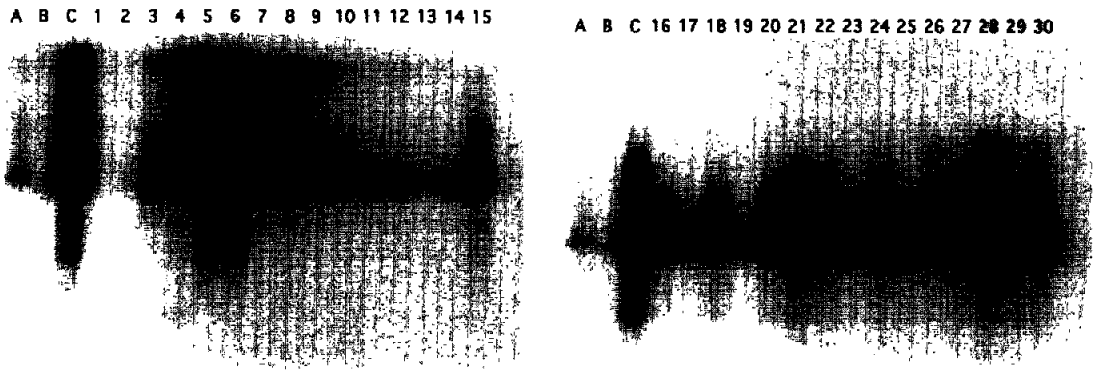

Fig. 1. Southern blot analysis of normal human pancreas *(A)*, human fibrosarcoma line Hs 913 T *(B)*, human gallbladder *(C)*, and 30 human pancreatic adenocarcinoma specimens hybridized to ^{32}P-CTP–labeled CCK-A receptor cDNA. The expected 928 base-pair amplicon is visible in all lanes except the expected negative control and carcinoma specimens 1 and 2.

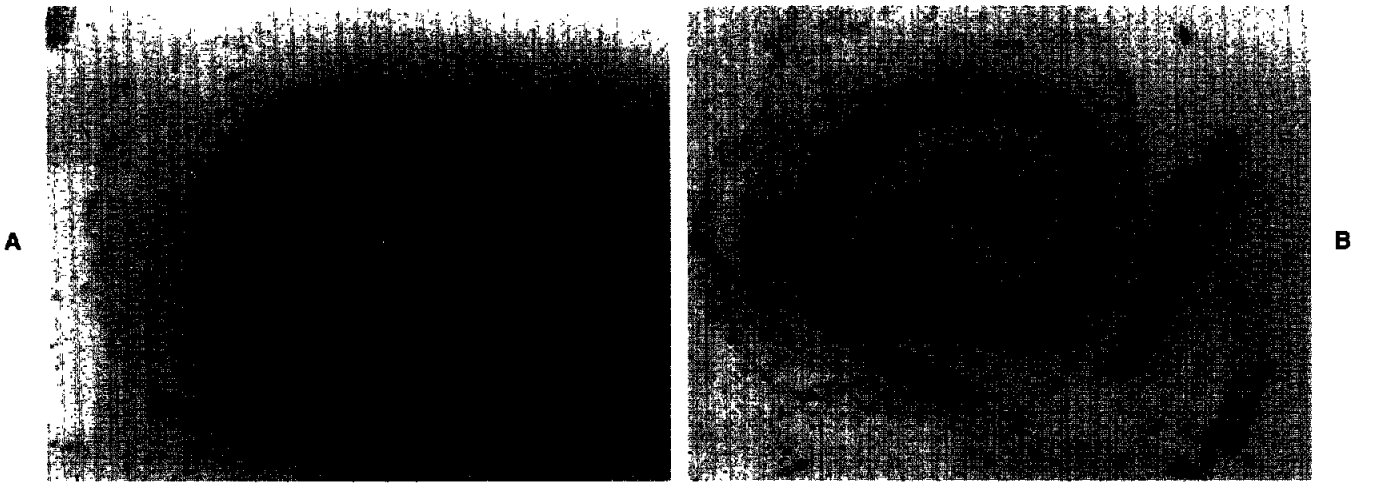

Fig. 2. A, In situ hybridization to the gallbladder shows CCK-A receptor mRNA localized to muscularis. **B,** The signal is lost following RNAse digestion.

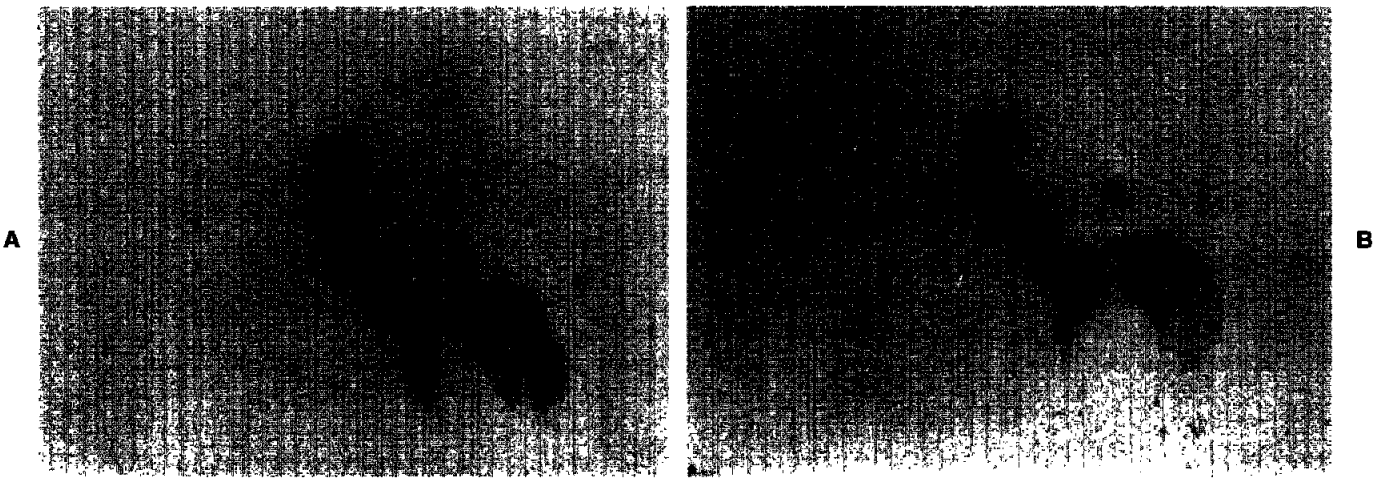

Fig. 3. A, In situ hybridization to the fundus of the stomach localizes CCK-A receptor mRNA expression to the deep layer of the mucosa. **B,** The signal is lost following RNAse digestion.

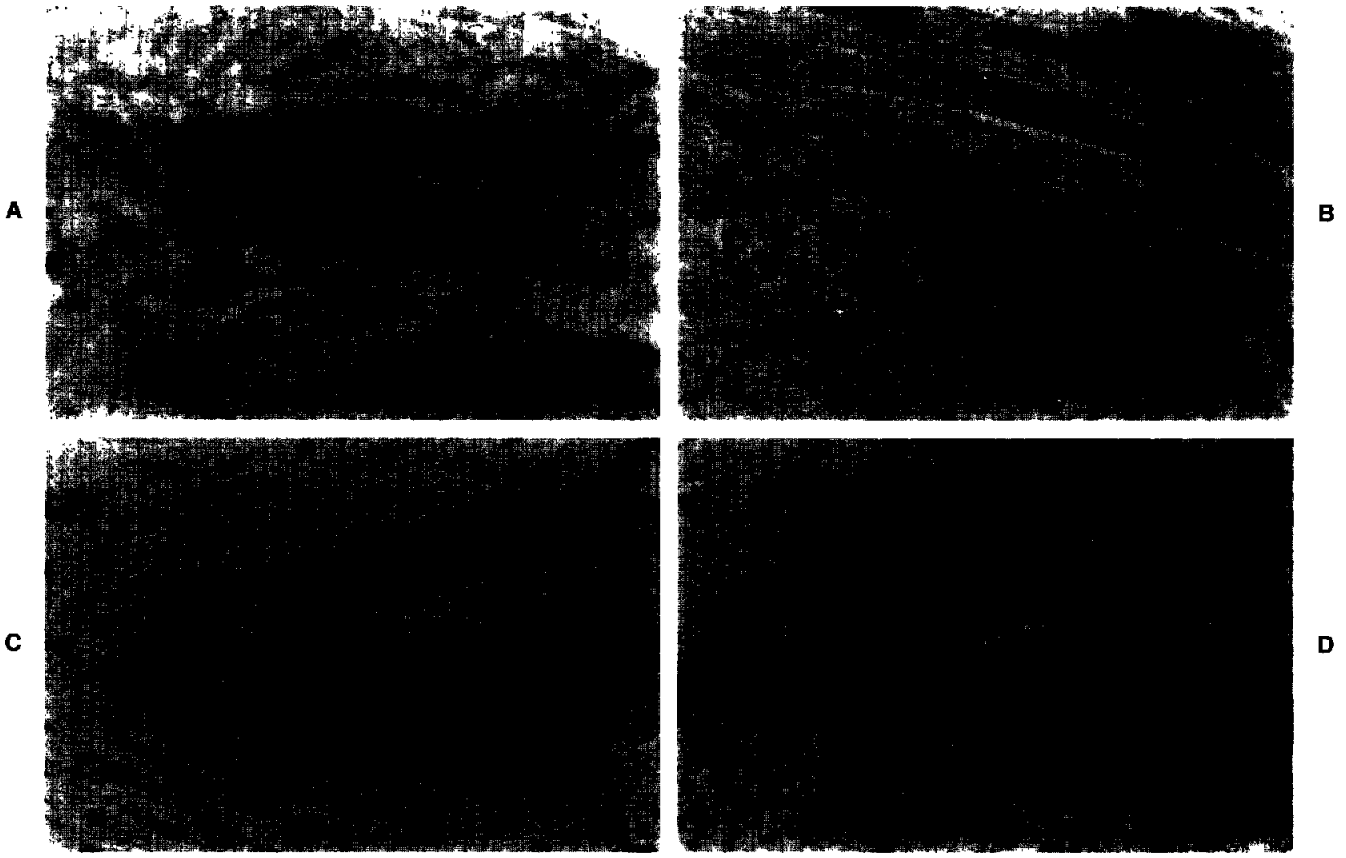

Fig. 4. In situ hybridization to four different specimens of adenocarcinoma of the pancreas: Moderately differentiated primary tumor **(A)**, tumor metastatic to the abdominal wall **(B)**, metastatic tumor **(C)**, and primary tumor **(D)**. CCK-A receptor mRNA expression is localized to neoplastic ductal adenocarcinoma cells. By comparison, hybridization of a "sense" riboprobe revealed no signal (not shown).

DISCUSSION

Only recently has the anatomic and histologic distribution of CCK-A and CCK-B receptors on normal human tissues been described. The presence of CCK-A receptors on the muscularis layer of the human gallbladder is well established and represents the most convenient specimen with which to validate techniques assessing CCK-A mRNA expression. CCK infusion leads to a significant increase in biliary secretion into the duodenum,[3] and meal-associated biliary secretion is significantly decreased by the concurrent administration of selective CCK-A receptor antagonists.[20] CCK-A receptor mRNA has been demonstrated in the gallbladder by Northern blot studies and RT-PCR.[21,22] The presence of functional receptor has been shown through ligand binding studies, in which the level of functional CCK receptors correlated with gallbladder contractility.[23] Most significantly, detection of CCK binding to the gallbladder muscularis by autoradiography, which is not substantially inhibited by gastrin, strongly suggests the presence of CCK-A receptors in this tissue[24] and is confirmed by both our RT-PCR and in situ hybridization results.

The presence of CCK-A receptors in the stomach is reasonably well established. CCK has been shown to have a significant impact on the rate of gastric emptying in humans, which is effectively blocked by selective CCK-A receptor antagonists.[25] The presence of CCK-A receptors in the deep mucosa of the human gastric body has been convincingly shown by direct CCK binding to tissue sections, and by the failure of such binding to be inhibited by gastrin.[26] We have confirmed these findings by in situ hybridization.

The presence of CCK-A receptors on normal human pancreas remains an area of controversy that we were unable to resolve. Meal-associated stimulation of pancreatic exocrine secretion is clearly one of the major physiologic actions of CCK, and such secretion is significantly suppressed by the preprandial administration of selective CCK-A receptor antagonists in vivo.[20] However, the ability of atropine to block the

effects of CCK on the human pancreas suggests that CCK may function via vagal afferent pathways and not directly on the pancreas,[27,28] a hypothesis substantiated by the inability of CCK to stimulate amylase secretion in preparations of isolated human pancreatic acinar cells except in doses substantially higher than those generally measured in vivo postprandially.[29] Direct CCK ligand binding to human pancreatic tissues has been consistently shown to be inhibited by gastrin and highly selective gastrin receptor antagonists, strongly suggesting that CCK binding in these experiments is mediated by gastrin/CCK-B receptors.[24,30] We were unable to demonstrate the presence of CCK-A receptor mRNA in normal pancreas by in situ hybridization. Although we and other investigators have found CCK-A receptors in human pancreas by RT-PCR,[31,32] the failure of other investigators to duplicate this finding[21,22] suggests that CCK-B receptors are the predominant species on human pancreas, with CCK-A receptors possibly confined in expression to a subset on pancreatic or peripancreatic tissues such as nerves, islets, or stroma.

The role of CCK in the promotion of the growth of pancreatic adenocarcinoma in humans is a subject of ongoing investigation. The detection of CCK-A receptors in tissue specimens by RT-PCR and their localization to tumor cells by in situ hybridization suggest pancreatic cancer cells may gain a growth advantage by increasing the expression of these receptors. The inconsistent ability of CCK to promote the growth of human pancreatic cancer cells in tissue culture or as xenografts in nude mice may be due to varying degrees of CCK-A receptor mRNA expression and varying degrees of success in translating this message into a membrane-bound protein. Although prior in situ studies have also shown CCK-A receptors in pancreatic adenocarcinoma specimens,[22] these results have not been confirmed by direct ligand binding.[30] These complementary modes of detecting CCK-A receptors have also been shown to have contradictory results in studies of the rat brain.[33] Such findings can be explained by a greater sensitivity of in situ hybridization over CCK ligand binding, failure of detected mRNA to be translated, or post-translational modification, which prevents the receptor from recognizing its ligand. A clearer understanding of these mechanisms is required to assess the clinical significance of CCK-A receptor mRNA expression in pancreatic adenocarcinoma.

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Discussion

Dr. J. Peters (Los Angeles, Calif.). Are CCK-A receptors and mRNA expressed in normal human pancreatic tissue?

Dr. R. Moonka. It is a point of controversy as to what is the major type of CCK receptor in human pancreas. Using RT-PCR, three groups out of four examining this question have found that CCK-A receptors are expressed in normal human pancreas. However, if you look at CCK binding to human pancreas, it appears that most of the binding is mediated by CCK-B (gastrin) receptors and not by CCK-A receptors. Normal human pancreas may express both A and B receptor mRNA, but the B receptors probably have more physiologic significance. If both normal pancreas and pancreatic cancer express CCK-A mRNA, it cannot be a marker for tumor. The PCR methodology would have to be quantitative to be useful in that setting.

Dr. D. Weinberg (Philadelphia, Pa.). We have reported our studies of CCK-A and CCK-B receptor expression in normal human pancreas and pancreatic adenocarcinomas. There is no expression of CCK-A mRNA by RT-PCR in normal human pancreas.

Dr. Moonka. As I said, most groups, including ours, who have looked at this issue do find some CCK-A receptor message in normal human pancreas. Yours was a well-done study, and it is difficult to square your results with ours and those of others. I do think that studies on CCK-A receptor PCR would have to be done quantitatively to use it diagnostically.

Dr. Weinberg. I might mention that we are about to publish data using quantitative PCR that confirm our previous report.

Discontinuous Appendiceal Involvement in Ulcerative Colitis: Pathology and Clinical Correlation

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Continuous mucosal involvement from the rectum proximally is one of the hallmarks of ulcerative colitis. However, recent pathologic series report appendiceal ulcerative colitis in the presence of a histologically normal cecum, representing a "skip" lesion. The clinical significance of this finding has not been established. Eighty patients, 54 males and 26 females, average age 37.9 years (range 14 to 82 years) who underwent proctocolectomy for ulcerative colitis from January 1990 to September 1995 were examined to determine the rate of discontinuous appendiceal involvement. Excluded were 12 patients with prior appendectomy and 11 with fibrotic obliteration of the appendiceal lumen. Of the remaining 57 patients, seven (12.3%) had clear appendiceal involvement in the presence of a histologically normal cecum. These seven patients clinically were indistinguishable from the 50 patients without skip involvement of the appendix in terms of age at surgery, pretreatment medications, type of surgery, interval from diagnosis to definitive procedure, complications, functional results, and clinical course. Discontinuous appendiceal involvement was found in 12.3% of patients undergoing proctocolectomy for ulcerative colitis, and clinically these patients behave as those without this feature. (J GASTROINTEST SURG 1999;3:141-144.)

KEY WORDS: Ulcerative colitis, appendiceal colitis, skip lesions, ulcerative appendicitis

Ulcerative colitis (UC) is a form of inflammatory bowel disease affecting the colorectal mucosa in what has been classically described as continuous involvement from the anorectal junction proximally without skip lesions. The appendix, which develops embryologically with the colon and has colonic-type epithelium, may be involved with the same mucosal inflammatory changes that are seen in the colon. Ulcerative appendicitis (UA) was described in early pathologic descriptions of UC,[1] but the condition of the adjacent cecum was not addressed. More recent reports differ on the presence of UA as a skip lesion; some describe up to 20% skip involvement,[2,3] whereas others maintain that UA only occurs in the presence of adjacent UC.[4,5] No known previous publication has addressed the clinical outcome of these patients. The authors reviewed their recent experience with UC, with particular emphasis on the pathology of the resected specimens and the clinical outcome of patients who were identified as having discontinuous appendiceal involvement with UC.

MATERIAL AND METHODS

Review of computerized hospital records from January 1990 to September 1995 identified 80 patients who underwent total or restorative proctocolectomy for UC at the Ochsner Foundation Hospital. Each case was analyzed for pathologic and clinical data.

The pathology reports for these cases were reviewed, with particular attention to the proximal extent of gross and microscopic involvement of the colonic mucosa with UC. Where available, this was correlated with preoperative endoscopic findings. The slides of cases in which the pathology of the appendix and cecum were not clearly recorded were reexamined. Strict criteria for normal cecal and appendiceal mucosa were used. Evidence of mucosal damage or

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repair (e.g., crypt abscesses, mucosal ulcerations, disordered glandular orientation, failure of the crypt bases to reach the muscularis mucosa, thickened mucosa, or submucosa) was considered evidence for UC or UA. This was differentiated from appendicitis in which the mucosa is normal and the appendiceal wall contains inflammatory cells.

Clinical data recorded for each case included age and sex, duration of symptoms, prior surgery, preoperative medical therapy, type of surgery performed, and complications. Postoperative follow-up data gleaned from the clinical record included duration, long-term problems, and functional results in patients who underwent restorative proctocolectomy.

RESULTS

Of the 80 patients initially identified, 54 were male and 26 were female; average age was 37 years (range 14 to 82 years). Twelve patients had undergone prior appendectomy, but UA was not identified at the time of surgery in any of them. Fibrotic obliteration of the appendiceal lumen was identified in 11 patients. This

Table I. Pathology (n = 57)

	Appendix (+) UC	Appendix (−) UC
Cecum (+) UC	23 (40%)	10 (18%)
Cecum (−) UC	7 (12%)	17 (30%)

UC = ulcerative colitis.

Table II. Perioperative data

	Appy (+) Cecum (−)	Appy (+) Cecum (+)	Appy (−) Cecum (+)	Appy (−) Cecum (−)
No. of patients	7	23	10	17
Age (yr)	34.7	33.8	35.0	39.2
Interval (mo)	72	63	88	79
Preoperative medications				
Steroids (%)	100	91	80	88
ASA preparations (%)	86	74	70	82
Azulfidine	3	8	4	9
Asacol	2	8	2	3
Dipentum	1	1	1	2
Azathioprine/6-mercaptopurine (%)	14	9	20	29

Appy = appendiceal involvement; ASA = acetylsalicylic acid.

Table III. Surgical procedures (n = 57)

	Appy (+) Cecum (−)	Appy (+) Cecum (+)	Appy (−) Cecum (+)	Appy (−) Cecum (−)
IPAA (%)	57	82	70	88
Ileostomy (%)	43	18	30	12

Appy = appendiceal involvement; IPAA = ileal pouch–anal anastomosis.

Table IV. Follow-up data

	Appy (+) Cecum (−)	Appy (+) Cecum (+)	Appy (−) Cecum (+)	Appy (−) Cecum (−)
No. of bowel movements/day	6.0	6.0	8.6	4.2
Obstruction (%)	0	13	10	17
Sepsis (%)	0	9	0	0
Pouchitis (%)	14	9	10	0
Stricture/sinus (%)	28	9	0	12
Late-diagnosis Crohn's disease	0	0	0	0

Appy = appendiceal involvement.

may occur as a normal consequence of aging and is not related to the presence of inflammatory bowel disease. The remaining 57 patients with evaluable appendices were included in this report.

Using the strict histologic criteria described, in seven patients (12.3%) discontinuous UA was identified in the presence of a normal cecum. In four of these patients, the ulcerated colon extended into the ascending colon, but in three of them involvement was only distal to the hepatic flexure. Twenty-three patients (40.4%) with UA had adjacent cecal UC. Normal appendices were identified in 27 cases; in 10 (17.5%) UC involved the cecum and in 17 (29.8%) it was normal (Table I). No cases of appendicitis were identified.

No differences were noted during clinical evaluation between patients with and without discontinuous appendiceal involvement. Age at surgery, duration of symptoms, and preoperative medications used were also similar (Table II).

Restorative proctocolectomy was the surgical treatment of choice for most of the patients in this series (Table III). According to the clinical records, doubts about the diagnosis of UC were not a contributing factor in the decision not to perform an ileal pouch procedure on patients who received a permanent ileostomy.

Follow-up time averaged 12.7 months (range 2 to 55 months); follow-up data are summarized in Table IV. Although there was a trend toward more stricture formation in the discontinuous group, no patients were later diagnosed with Crohn's disease. Functional results are given for those patients with an ileal pouch–anal anastomosis.

DISCUSSION

Since the appendix is derived embryologically from the colon, it is not surprising that it may be involved with the same pathologic processes that affect the colon. UC, a form of idiopathic inflammatory bowel disease that classically involves the colonic mucosa in a continuous fashion from the rectum proximally, is one such condition. This absence of skip lesions is one of the features that distinguishes UC from Crohn's colitis. Appendiceal mucosal ulceration consistent with UC has long been described in conjunction with colonic disease, having been found in approximately half of the cases.[1,6] However, the presence of UA as a true skip lesion has been debated in the literature since it was first reported in a single case in 1974 by Cohen et al.[7]

In a review of 65 cases of UC to assess appendiceal involvement, Jahadi and Shaw[4] documented its presence in 47%.[4] Adjacent cecal disease was present in all cases. Goldblum and Appelman[5] examined the appendix and cecum of 87 patients who underwent colectomy for UC. All patients had pancolitis. Colitic changes were present in 62% of the appendices and correlated well with the level of activity in the nearby cecum. These investigators concluded that if strict histologic criteria for involvement of the cecum are used, UC can be found in all cases where the appendix is diseased. However, none of their patients had left-sided colitis alone.[5]

Three recent pathologic studies refute the preceding findings. Of 62 patients reviewed by Davison and Dixon,[2] seven had active UA in the presence of a normal cecum. The authors carefully distinguished these patients from those with mild but recognizable colitis.[2] Groisman et al.[8] used strict histologic criteria similar to those used by Goldblum and Appelman[5] to compare disease activity in the cecum and appendix of 160 consecutive patients, separating patients into universal and nonuniversal colitis groups. Groisman et al.[8] found that discontinuous appendiceal involvement was as prevalent as continuous disease. Kroft et al.[3] noted discordant appendiceal colitis in half of their patients, with a histologically proved skip lesion present in 15%.

Our study confirms the presence of discontinuous appendiceal involvement as a histologically proved skip lesion in UC, occurring in 12.3% of our patients. Additionally, a normal appendix was found in patients with pancolitis in 17.5%, for a total discordance rate of nearly 30%, which is similar to the rates reported by Kroft et al.[3] In four of their patients the ascending colon marked the proximal extent of colonic involvement. To explain this finding it has been argued that the repair in quiescent colitis may approach normal histologic appearance.[5] However, using strict criteria for normal mucosa, this study and others have shown this not to be the case. It also fails to account for the presence of the appendiceal skip lesion in patients with more limited colitis, as in three of our cases.

None of the previous pathologic studies correlated their findings with the clinical course of these patients. The results of restorative proctocolectomy are dependent on correctly identifying patients with UC and avoiding those with Crohn's colitis; the ultimate test of whether this appendiceal skip lesion is truly UC lies in the clinical course of patients identified with this skip lesion. In our series no discernible difference was detected in patients with discontinuous appendiceal UC compared to patients without. Preoperative duration of symptoms and medication usage were the same, as were the rates of early and late complications. The types of acetylsalicylic acid preparations used by the patient groups were similar. Functional results of patients who underwent restorative

proctocolectomy were identical in patients with and without discontinuous appendiceal UC. Most important, none of the patients with discontinuous appendiceal involvement have been subsequently diagnosed as having Crohn's disease.

CONCLUSION

Discontinuous appendiceal involvement with UC exists as a histologically proved skip lesion, occurring at a rate of 12.3% in our population. The clinical course of these seven patients was identical to that of the 57 patients without such skip lesions. Restorative proctocolectomy can be offered to patients with skip lesions with the expectation of equivalent clinical outcomes.

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Clinical Subtypes of Crohn's Disease According to Surgical Outcome

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Patients with Crohn's disease are typically classified into perforator or nonperforator groups. The perforator group includes those who present with acute perforation, fistulas, or abscess formation. The nonperforator group presents with stricture, obstruction, or unresponsiveness to medical therapy. Our purpose was to investigate whether perianal disease constitutes a separate predictor of surgical outcome. The form of presentation was classified as perforator, nonperforator, or perianal disease in 91 patients undergoing 232 operations for Crohn's disease. Those with perforating complications presented with the highest Crohn's Disease Activity Index, followed by those with nonperforating complications, and then the perianal disease group. However, the perianal disease group appeared to have the most rapid rate of recurrence and subsequent surgery, followed next by the perforator, and then the nonperforator group. Recurrence rate and subsequent operation intervals for the perforator group appeared to lengthen when those patients were treated with steroids and/or immunosuppressants, as compared to nonsteroidal and/or antimicrobial agents. Recurrence rate and subsequent operation intervals appeared to lengthen for the nonperforator and perianal disease groups when they were treated with nonsteroidal and/or antimicrobial therapy, as compared to steroids and/or immunosuppressants. Our data indicate that perianal disease, as a form of presentation of Crohn's disease, has independent predictive value, although this is not accurately reflected by the Crohn's Disease Activity Index. (J GASTROINTEST SURG 1999;3:145-151.)

KEY WORDS: Crohn's disease, perianal disease, postoperative therapy, perforator, nonperforator

Crohn's disease (CD) is an inflammatory process that can affect any segment of the gastrointestinal tract. It has been suggested that patients who undergo surgery for complications of CD can be classified into one of two clinical subtypes, perforating or nonperforating, based on their operative findings.[1-8] The perforator group presents with complications such as acute perforation, fistulas, or abscess formation, whereas the nonperforator group presents with stricture, obstruction, or unresponsiveness to medical therapy.[1-4]

Approximately one third of patients with CD also present with perianal disease.[9-14] Manifestations of perianal disease include perirectal abscess, rectal mucosal ulceration, and perianal fistulas. These may occur either simultaneously with intestinal findings or independent of gastrointestinal symptoms.[12] Currently the stigmata of perianal disease are not considered to be a true subtype of CD but rather a complication of either the perforator or nonperforator CD subtypes. Because patients with perianal disease have a different clinical course from those with intestinal CD, and because this disease may occur independently, a variety of systems have been developed to classify and index the severity of perianal disease involvement.[12,14-16]

Perforating and nonperforating complications of CD have been shown to be influenced by the anatomic location and time course of the disease. Perforators are considered more aggressive and nonperforators more indolent.[1-5,7,17] Perforating complications have been shown to recur at a rate faster than nonperforating complications and to require subsequent operations sooner.[1,5-9,18] In addition, perforating complications appear to be more common anatomically as one moves distally along the digestive tract, peaking in the ileocolic region.[1,6,8-10,13,19,20] Nonperforating complications tend to occur with the greatest incidence more proximal in the small intestine.[1,6,8-11,17,19]

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Much about CD remains a mystery including its frequent unresponsiveness to pharmacologic therapy and its numerous recurrences. It is the goal of this study to provide new insight into these characteristics of CD and to ascertain whether patients who present with perianal disease constitute a separate group of CD with regard to surgical outcome.

MATERIAL AND METHODS

The study consisted of 91 consecutive patients, 49 women (54%) and 42 men (46%). These patients were referred for surgery while one of us (R.H.R.) was on staff at the Center for Health Sciences, University of California, Los Angeles. Data from previous operations performed at other institutions, as well as previous recurrences, were obtained for all patients and entered into the analysis, with follow-up by telephone. Data processed by statistical analysis include age, sex, tobacco use, family history, date of diagnosis, date of each operation, date of each recurrence, postoperative medical therapy, Crohn's Disease Activity Index (CDAI), subtype of CD, number of recurrences, and number of surgeries. Patients were placed into one of three clinical subtypes—perforator, nonperforator, or perianal disease—based on their operative findings. Inclusion criteria for the perforator group included the following operative findings: acute perforation, intestinal fistulas, or abscess. Inclusion criteria for the nonperforator group included the following operative findings: stricture, obstruction, or unresponsiveness to medical therapy. The perianal disease group included those patients presenting with perianal fistulas, anal mucosal ulceration, or perirectal abscess without concurrent intestinal manifestations. Those patients presenting with simultaneous perianal disease and intestinal findings were classified according to their intestinal complications. When combined or overlapping features of both the perforating and nonperforating complications existed simultaneously in a patient, the patient was allocated to the perforator category since it is considered the more severe complication.[1-5,7,17] The 91 patients underwent a total of 232 surgeries for complications arising from CD. After each operation, patients were reclassified into the proper category of CD based on their operative findings.

To examine the effect of the type of postoperative medication on rate of recurrence and interval to subsequent surgery, patients were classified into one of three groups: (1) those receiving no pharmacologic therapy (including total parenteral nutrition or nasogastric tube aspiration); (2) those receiving aminosalicylic acid (ASA) compounds and/or antimicrobial agents, and (3) those receiving steroids and/or immunosuppressants. In the event that a patient was receiving both total parenteral nutrition or nasogastric tube aspiration along with either ASA/antimicrobials or steroids/immunosuppressants, they were classified according to the pharmacologic therapy. This decision regarding classification was based on the fact that both ASA/antimicrobial therapy and steroids/immunosuppressants have been shown to be superior to treatment with placebo.[21-25] When a case of overlapping medical therapy arose, such as a patient receiving both steroids and ASA compounds, they were placed in the steroid/immunosuppressant category because of the stronger pharmacologic effect of steroids/immunosuppressants over that of first-line drugs such as ASA and/or antimicrobial agents.[10,21,23,24]

As a means of quantitating the severity of the patients' disease complications, the CDAI was recorded for each operation and recurrence of symptoms. For the purposes of this study, a recurrence was considered (1) a relapse of symptoms that required hospitalization or surgery or (2) a relapse of symptoms that was supported by diagnostic evidence such as sigmoidoscopy, radiography, or endoscopy. In cases where a patient had an ostomy, a numerical value of 21 was entered as an estimate of stools per week for calculation of the CDAI.

Intervals to the first postoperative recurrence and between subsequent operations were recorded to examine differences due to CD categories and postoperative therapy.

Data were analyzed using one-way analysis of variance with the Student–Newman-Keuls method, and Z-test.

RESULTS

The initial perforator group consisted of 33 patients (36%), the initial nonperforator group contained 39 patients (43%), and the perianal disease group consisted of 19 patients (21%).

Following reclassification of the original 91 patients based on operative findings from their subsequent 232 surgeries, the complete number of operations performed for perforating complications totaled 77 (33%), which included those presenting with a perforation, enterocolic fistula, enteroenteric fistula, enterovesical fistula, or abscess at the time of surgery. The number of operations performed for nonperforating complications was 98 (42%), which revealed an operative finding of obstruction, stricture, enteritis, terminal ileitis, or colitis. The complete number of operations performed for perianal disease manifestations totaled 57 (25%), which included patients who presented solely with perianal fistulas, mucosal ulceration, or perirectal abscess.

Following the initial operation on the 91 patients in the study, patients were treated postoperatively as follows: 25 patients (27%) received no pharmacologic

therapy (including total parenteral nutrition or nasogastric tube aspiration), 21 patients (23%) received ASA compounds and/or antimicrobial agents, and 45 patients (50%) received steroids and/or immunosuppressants.

No significant difference in mean age at diagnosis, onset of symptoms, sex, family history, tobacco use, total number of surgeries, total number of recurrences, or number of recurrences between subsequent operations was found among the three clinical subtypes.

The average CDAI value for the original classification of the 91 participating patients was 208.4 ± 94.6 for the perforator group, 164.4 ± 65.6 for the nonperforator group, and 156.1 ± 53.1 for the perianal disease group ($P < 0.02$). When compared pairwise, the perforator group was found to be significantly different from both the nonperforator and the perianal disease groups ($P < 0.05$); however, the perianal disease group and the nonperforator group were not significantly different (Table I).

The average time interval between the first surgery and the first postoperative recurrence was 3.6 ± 4.5 years for the perforator group, 6.1 ± 6.2 years for the nonperforator group, and 2.2 ± 2.2 years for the perianal disease group ($P < 0.02$). When crossed pairwise, the nonperforator group was found to be significantly different from both the perforator and the perianal disease groups ($P < 0.05$); however, the perforator and the perianal disease groups showed no significant difference (see Table I).

The average interval between the first surgery and the second surgery was 5.3 ± 7.4 years for the perforator group, 7.9 ± 8.5 years for the nonperforator group, and 3.1 ± 2.4 years for the perianal disease group ($P < 0.05$). When crossed pairwise, the nonperforator and perianal disease groups were significantly different ($P < 0.05$); however, the perforator group did not differ significantly when crossed with either the nonperforator or the perianal disease group (see Table I).

The average interval between the first surgery and the first recurrence varied for the three categories depending on the postoperative medication. The average interval for the perforator group was 5.8 ± 5.7 years when patients were treated postoperatively with steroids and/or immunosuppressants, as opposed to 1.7 ± 2.2 years for those treated with ASA and/or antimicrobial therapy, and 2.1 ± 2.8 years for those receiving no pharmacologic therapy (not significant [NS]). The average interval in the nonperforator group was 3.8 ± 4.4 years for those treated with steroids and/or immunosuppressants, 11.7 ± 6.4 years for treatment with ASA and/or antimicrobial therapy, and 7.2 ± 7.2 years with no medication ($P < 0.01$). When crossed pairwise, steroids/immunosuppressants and ASA/antimicrobial therapy for the nonperforator group were significantly different ($P < 0.05$); however, the no therapy group was not found to be significant when paired with those receiving either steroids/immunosuppressants or ASA/antimicrobial therapy. The average interval for the perianal disease group based on postoperative medication was 2.0 ± 1.8 years with steroids/immunosuppressants, 2.8 ± 2.7 years with ASA/antimicrobial therapy, and 1.7 ± 2.2 years with no medication (NS) (Table II).

The average interval between the first and the second surgery was also affected by postoperative medical therapy. The average interval for the perforator group was 6.4 ± 7.5 years when treated with steroids and/or immunosuppressants, 2.3 ± 2.2 years with ASA/antimicrobial therapy, and 5.3 ± 9.3 years with no therapy (NS). The interval between the first and the second surgery for the nonperforator group was 3.8 ± 4.0 years when they were treated with steroids/immunosuppressants, 12.9 ± 6.8 years with ASA/antimicrobial therapy, and 12 ± 11.9 years with no therapy ($P < 0.005$). When crossed pairwise, the steroid/immunosuppressant group was found to be significantly different from both the ASA/antimicrobial and the no therapy groups ($P < 0.05$); however, there was no significant difference between the ASA/antimicrobial and the no therapy groups. The interval between the first and the second surgery for the perianal disease group was 2.9 ± 1.7 years with steroids and/or immunosuppressants, 3.5 ± 3.3 years with ASA and/or antimicrobial therapy, and 2.7 ± 2.2 years with no medication (NS) (see Table II).

Patients were followed through their first three surgeries to see if they would remain in their original

Table I. Crohn's Disease Activity Index (CDAI), recurrence, and subsequent surgery intervals of disease clinical subtypes

	Perforator	Nonperforator	Perianal disease
Average presenting CDAI	208.4* ± 94.6	164.4 ± 65.6	156.1 ± 53.1
Average interval first surgery-first recurrence (yr)	3.6 ± 4.5	6.1* ± 6.2	2.2 ± 2.2
Average interval first surgery-second surgery (yr)	5.3 ± 7.4	7.9* ± 8.5	3.1 ± 2.4

Data are mean ± standard deviation.
*$P < 0.02$.

Table II. Response of Crohn's disease (CD) clinical subtypes to postoperative therapy

CD subtype	Postoperative therapy	First surgery-first recurrence (yr)	First surgery-second surgery (yr)
Perforator	None	2.1 ± 2.8	5.3 ± 9.3
Perforator	ASA/antimicrobials	1.7 ± 2.2	2.3 ± 2.2
Perforator	Steroids/immunosuppressants	5.8 ± 5.7	6.4 ± 7.5
Nonperforator	None	7.2 ± 7.2	12.0 ± 11.9
Nonperforator	ASA/antimicrobials	11.7* ± 6.4	12.9* ± 6.8
Nonperforator	Steroids/immunosuppressants	3.8 ± 4.4	3.8 ± 4.0
Perianal disease	None	1.7 ± 2.2	2.7 ± 2.2
Perianal disease	ASA/antimicrobials	2.8 ± 2.7	3.5 ± 3.3
Perianal disease	Steroids/immunosuppressants	2.0 ± 1.8	2.9 ± 1.7

Data are mean ± standard deviation.
*P <0.01

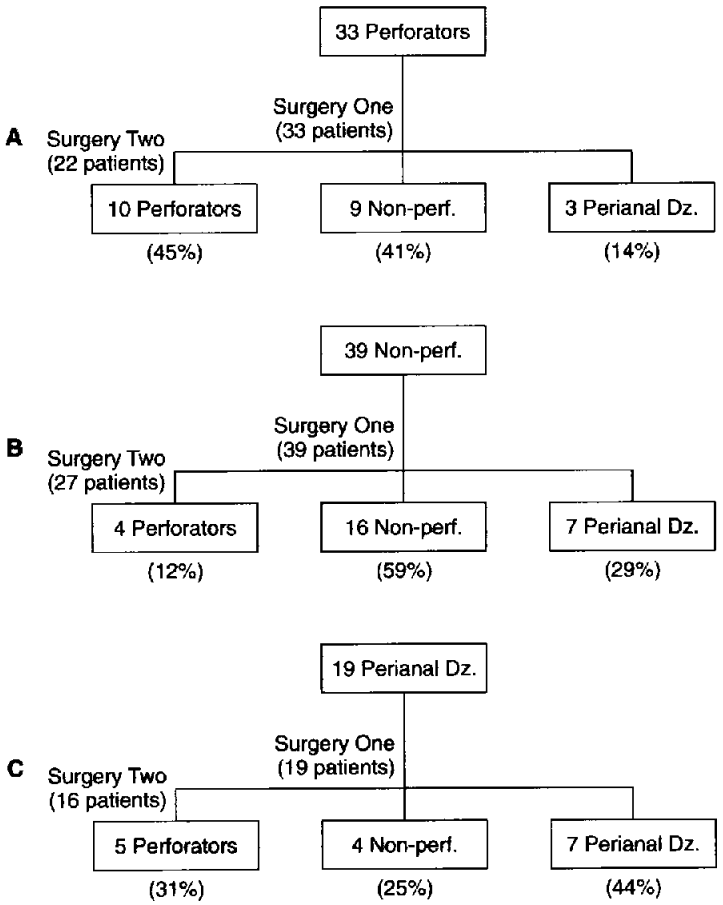

Fig. 1. A, Twenty-two of the original 33 patients in the perforator group had recurrences severe enough to warrant a second surgery at which time 45% remained in the perforator group, 41% converted to the nonperforator group, and 14% converted to the perianal disease group (NS). **B,** Twenty-seven of the original 39 patients in the nonperforator group underwent a second surgery at which time 59% remained in the nonperforator group, 12% converted to the perforator group, and 29% converted to the perianal disease group. When crossed pairwise with the perforator group, this was significant (P <0.001), but not significant when crossed with the perianal group. **C,** Sixteen of the original 19 patients in the perianal disease group required a second surgery, after which 44% remained in the perianal disease group, 31% converted to the perforator group, and 25% converted to the nonperforator group (NS).

subtype or if they would convert to another subtype. Following the first surgery for the original 33 patients in the perforator group, 22 experienced recurrences severe enough to warrant a second surgery, at which time 45% remained in the perforator group and 41% converted to the nonperforator group, and 14% converted to the perianal disease group (NS) (Fig. 1, *A*). Following the first surgery for the original 39 patients in the nonperforator group, 27 of them experienced recurrences severe enough to warrant a second surgery at which time operative findings showed 59% to remain in the nonperforator group as opposed to conversion of 12% to the perforator and 29% to the perianal disease group (Fig. 1, *B*). When nonperforators were crossed pairwise with perforators, this was significant ($P < 0.001$), but this was not significant for those crossed with the perianal disease group. Following the first surgery, 16 of the original 19 in the perianal disease group experienced recurrences severe enough to warrant a second surgery, at which time operative findings showed 44% to remain in the perianal disease group, along with conversion of 31% to the perforator and 25% to the nonperforator group (NS) (Fig. 1, *C*). Following the second surgery, the numbers were too small for statistical analysis.

DISCUSSION

Our study suggests that there are three subtypes of CD rather than the previously classified two. Our division of CD into three subtypes, perforators, nonperforators, and perianal disease, is based on operative findings. These subtypes appear to demonstrate differences in the following: severity of disease, rates of recurrence and intervals between subsequent surgeries, responses to postoperative medications, and recurrence tendencies within the same subtype following surgeries.

To evaluate the severity of each CD flareup in the patients, we used the CDAI, which was developed by the National Cooperative Crohn's Disease Study (NCCDS) to evaluate the clinical response of patients in a large trial. The CDAI takes into account many indices of the disease presentation and weighs them accordingly to evaluate each patient's clinical condition.[19,26-28]

To observe the effects of postoperative medication on recurrence rate and subsequent surgery interval, the patients were placed in one of three categories: no therapy, ASA and/or antimicrobial therapy, and steroids and/or immunosuppressants. Patients with overlapping medical therapy from both the ASA/antimicrobial and steroid/immunosuppressant categories were placed in the steroid/immunosuppressant group. This classification decision is supported by the results of the National Cooperative Crohn's Disease Study, which found the combination of ASA plus prednisone to be less effective than prednisone alone.[21,29] In addition, several investigators conducted studies that showed the combination of sulfasalizine plus 6-methylprednisolone offered no advantage over 6-methlyprednisolone alone.[21,29] This indicates that steroids/immunosuppressants are the major effectors in combination therapy.

Following the first surgery, all of the subtypes tended to recur within their original subtype, although this was only shown to be significant for the nonperforator group. This is consistent with recent studies regarding only the perforator and nonperforator subtypes.[1,5-7,17,18,30] Greenstein et al.[18] documented that 73% of their patients who presented with perforating complications at the first operation also presented with a perforating complication at the second operation. In contrast, only 29% of patients whose initial surgical indication was a nonperforating complication developed a perforating indication for the second operation. Greenstein et al.[18] observed these trends through the second and third operations. In our study, the sample size was too small to determine the role of postoperative medications in the conversion of one subtype to another.

In our study, perforating complications were the most serious, presenting with the highest CDAI values, which is consistent with current literature that considers perforating complications to be the more aggressive of the two recognized subtypes of CD.[1-5,7,17] The group with perforating complications appeared to exhibit a lower recurrence rate and a shorter interval to subsequent surgery than the perianal disease group, yet the values were greater than in the nonperforator group. This finding is in concordance with current literature, which has suggested that perforating complications recur faster than nonperforating complications.[7,9,18] This was demonstrated in a study by Aberhard et al.,[5] in which operations for perforating complications were followed by a subsequent operation twice as rapidly as operations for nonperforating complications. The perforator group appeared to exhibit the greatest postoperative recurrence interval, and first to second surgery interval when postoperative treatment consisted of steroids and/or immunosuppressants rather than ASA and/or antimicrobial therapy or no therapy, although this was not found to be significant. This trend was suggested by a study by Present et al.[31] in which the immunosuppressant 6-mercaptopurine was found to have signif-

icant effects on patients with intractable CD often complicated by fistulas.

The perianal disease group appeared to exhibit the fastest rate of recurrence and shortest interval to subsequent surgeries. Conversely, this group also presented with the lowest CDAI value, followed by the nonperforator, and then the perforator group. The rapid rate of recurrence seen in the perianal disease group is supported by a study which showed that CD has a higher likelihood of recurrence and tends to recur more quickly when there is perianal involvement.[17] In a study by Mackowiec et al.,[12] the rapid rate of perianal disease recurrence was noted to be 48% within 1 year. The finding that perianal disease appears to have a lower CDAI but a faster rate of recurrence is puzzling and may have two possible explanations. First is the fact that this subtype presents with external features that the patient can readily appreciate and identify, in contrast to the intestinal complications that may only be evident when accompanied by abdominal discomfort or other vague symptoms, which may be ignored until they become severe. This external expression could also be responsible for the higher rate of recurrence simply because this subtype is identified earlier. Second, the lower CDAI value could be explained by the fact that we used an index designed to measure severity of intestinal complications of CD to measure perianal disease severity. The CDAI has been shown to be inadequate in analyzing perianal disease associated with CD.[14,15] For this reason other indices such as the Perianal Disease Activity Index, which takes into account numerous factors associated with perianal disease, have been specifically designed to measure the severity of perianal involvement.[14,15] The perianal disease group appeared to exhibit the greatest interval to postoperative recurrence and the longest first to second surgery intervals when treated postoperatively with ASA/antimicrobial therapy rather than steroids/immunosuppressants or no therapy. However, this did not meet statistical significance. This trend is supported by a study that showed metronidazole to be effective in treating perianal CD.[32]

The nonperforator group expressed the lowest rate of recurrences and the longest interval to subsequent surgeries. Patients in this group had the second most severe form of CD based on the CDAI. The rate of recurrence and subsequent surgeries in the group with nonperforating complications fell substantially below the figures for the perforator group, which is consistent with current literature.[7,9,18] The CDAI value was intermediate falling below that of the perforator group and above that of the perianal disease group. This is consistent with studies suggesting that perforating complications are more aggressive than nonperforating complications.[1-5,7,17] The fact that the nonperforator group appeared to exhibit a higher CDAI than the perianal disease group in our study may suggest that nonperforating complications represent a more severe subtype than perianal disease, or the difference may be due to the inadequacies of the CDAI in evaluating perianal disease as discussed earlier. The nonperforating group exhibited the greatest postoperative recurrence interval and the longest first to second surgery interval when treated postoperatively with ASA and/or antimicrobial therapy rather than steroids and/or immunosuppressants or no therapy; this was found to be statistically significant.

Our study was hampered by a limited sample size, so in some cases the statistical power used to analyze the data was below the desired level. This increases the likelihood of a type II error in our analysis, and negative findings should be interpreted cautiously. In addition, because patients were not assigned in a randomized, prospective double-blinded manner to one of the three treatment groups, conclusions concerning efficacy of therapy must be guarded.

CONCLUSION

Although not all findings in our study were found to be statistically significant, the trends observed warrant further study. Our study suggests that there are actually three clinical subtypes of CD patients: perforators, nonperforators, and those suffering from perianal disease. These groups exhibited differences in severity, based on CDAI values, with the perforator group appearing to have the greatest severity followed by the nonperforator group, and then the perianal disease group. Differences in recurrence rate and subsequent surgery interval were observed with the perianal disease group showing the most rapid recurrence rate, followed by the perforator group, and then the nonperforator group. Recurrence rate and subsequent surgery intervals varied for the three categories based on postoperative therapy. Our data suggest CD patients suffering from perforating complications may be better treated with steroids and/or immunosuppressants, whereas patients with nonperforating and perianal disease complications may be better treated with ASA and/or antimicrobial therapy. The three CD clinical subtypes appeared to demonstrate the tendency to recur within the same subtype following surgery. It is our belief that following further study, recognition of these three different subtypes of CD and their appropriate therapies may prove beneficial in the management of CD in the future.

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Surgical Feeding Gastrostomy: Are We Overdoing It?

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Feeding gastrostomy is a commonly performed procedure in North America. Our aim was to study the outcome of patients undergoing feeding gastrostomy to better define patients who will benefit from the procedure as opposed to those in whom it may be futile. A cohort of the most recent 100 consecutive patients undergoing feeding gastrostomy in a community teaching hospital was retrospectively studied. The main indication for gastrostomy was neurologic disorder interfering with eating/swallowing (group A—54 patients), followed by debilitating systemic disease (group B—26 patients) and obstructive malignancy of the head and neck or esophagus (group C—20 patients). Forty-one patients died within 30 days of the procedure (41%). The overall 30-day survival rates in groups A, B, and C were 70%, 15%, and 85%, respectively. In four patients death was caused by intraperitoneal leak from the gastrostomy site; the remaining patients died of their underlying disease. Five patients required reoperation for gastric leakage around the gastrostomy within 30 days. Only nine patients could be traced who were alive and still using the gastrostomy a year after its placement: two in group A, none in group B, and seven in group C. APACHE II scores at tube insertion also predicted survival; 30-day survival rates in patients with scores of 10 and below, 11 to 15, 16 to 20, and over 20 were 96%, 71%, 48%, and 18%, respectively. No patient with an APACHE score above 15 belonging to group B (debilitating disease) survived more than 30 days. We conclude that to have a beneficial therapeutic effect feeding gastrostomy should be performed selectively. Severe debilitating systemic conditions that interfere with normal eating, when combined with a high APACHE II score, indicate the futility of gastrostomy. (J GASTROINTEST SURG 1999;3:152-155.)

KEY WORDS: Gastrostomy, feeding, surgery

The creation of a gastrostomy for the purpose of feeding only is a commonly performed procedure in North America. In other parts of the Western world, on the other hand, feeding gastrostomy is rarely performed. Published comparisons of the rate of feeding gastrostomy in the United States and in other countries are not available, but that such a difference exists is suggested by the paucity of publications on this topic in the non-American literature, by personal communications, and by a recent questionnaire conducted on SURGINET—an international surgical discussion forum on the Internet. There are two possible explanations for this marked discrepancy—that is, either surgeons in Europe, for example, do not perform this procedure often enough or it is overused in the United States.

Stuart et al.,[1] in a retrospective review of 125 patients undergoing open and percutaneous endoscopic gastrostomies in a community hospital between 1984 and 1986, reported a 30-day mortality rate of 28%. The authors suggested that methods of selecting patients for this procedure were flawed, and in retrospect certain patients should not have had a gastrostomy tube placed. Wilkinson and Pickleman,[2] in an earlier study of 67 patients undergoing open gastrostomy, disclosed a 30-day mortality rate of 30% with only 40% of patients surviving longer than 6 months.

Did surgeons take notice of such disquieting reports and alter their practices? Our aim was to study the outcome of patients undergoing feeding gastrostomy to define the current paradigm. In addition, we attempted to better define patients who would benefit from the procedure as opposed to those in whom gastrostomy would be futile.

MATERIAL AND METHODS

A cohort of the most recent 100 consecutive patients undergoing "open" feeding gastrostomy in a 550-bed university-affiliated, community teaching

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hospital was retrospectively studied. To retrieve this number of cases, procedures performed from 1995 onward had to be reviewed. Fifty-eight of the patients were women whose average age was 79 years (range 28 to 99 years). Sixty-eight gastrostomies were performed by means of the Stamm technique, whereas in 32 patients a Janeway-type gastrostomy was fashioned using a linear cutting stapler.

Patients were divided into three groups based on the indications for gastrostomy. Group A (n = 54) included those with neurologic disorders interfering with eating and swallowing (i.e., stroke, brain tumor). Group B (n = 26) included those with "debilitating" systemic diseases interfering with oral intake of food (i.e., metastatic cancer, end-stage cardiorespiratory disease). Group C (n = 20) included those with obstructive head and neck or pharyngoesophageal malignancies.

The severity of illness in all patients was retrospectively scored using the Acute Physiology and Chronic Health Evaluation (APACHE II),[3] which takes into consideration each patient's acute physiologic status, age, and underlying chronic health. APACHE II scores were determined based on the worst parameters recorded during the 24 hours prior to insertion of the gastrostomy tube. When an individual parameter was not available, it was scored as zero.[4]

In-hospital outcome was assessed from patients' charts. The outcome of patients who left the hospital alive was tracked by means of telephone calls to their families or nursing homes.

RESULTS

Early Results

Forty-one patients (41%) died within 30 days of the procedure. The 30-day survival rates in groups A, B, and C were 70%, 15%, and 85%, respectively. The mean APACHE II score was 18 in those who died and 9 in the survivors. In four patients the cause of death was an intraperitoneal leak from the gastrostomy site. The remaining patients died of cardiorespiratory complications and/or their underlying disease. Five patient required reoperation within 30 days to treat gastrostomy-related intra-abdominal infection.

Late Results

Only nine patients were traced who were alive and still using the gastrostomy a year after its placement: two in group A, none in group B, and seven in group C.

Table I shows the distribution of APACHE II scores in the three groups based on the indications for gastrostomy. It should be noted that all patients in group B scored above 10.

Table II correlates the survival rates with the indications for gastrostomy. The 6-month survival rates

Table I. Distribution of APACHE II scores based on indications for gastrostomy

Indication	No. of patients	APACHE II scores			
		0-10	11-15	16-20	>20
Neurologic (group A)	54	13	19	16	6
Debilitating disease (group B)	26	0	5	12	9
Obstructive malignancy (group C)	20	11	4	3	2
TOTAL	100	24	28	31	17

Table II. Survival according to indications for gastrostomy

Indication	No. of patients	Survival time		
		0-30 days	6 mo	1 yr
Neurologic (group A)	54	38 (70%)	14 (30%)	2 (4%)
Debilitating disease (group B)	26	4 (15%)	2 (8%)	0
Obstructive malignancy (group C)	20	17 (85%)	14 (70%)	7 (35%)
TOTAL	100	59	30	9

Table III. Survival according to APACHE II scores

APACHE II score	No. of patients	Survival time		
		0-30 days	6 mo	1 yr
0-10	24	23 (96%)	12 (50%)	6 (25%)
10-15	28	20 (71%)	13 (47%)	2 (7%)
16-20	31	15 (48%)	4 (13%)	1 (3%)
>20	17	3 (18%)	1 (6%)	—
TOTAL	100	59	30	9

in the three groups were 30%, 8%, and 70%, respectively. Only nine patients were traced who were alive and using the gastrostomy a year after insertion: two in group A, none in group B, and seven in group C.

Table III shows survival rates according various APACHE II scores. APACHE II scores at tube insertion were predictive of survival—that is, 30-day survival rates in patients with scores of 10 and below, 11 to 15, 16 to 20, and over 20 were 96%, 71%, 48%, and 18%, respectively. At 6 months these figures were 50%, 47%, 13%, and 6%, respectively. No patient with an APACHE II score above 15 belonging to group B survived for more than 30 days.

DISCUSSION

Open surgical, percutaneous endoscopic, and radiologic guided gastrostomies are all commonly performed in the United States. The choice of a specific route depends mostly on the speciality of the physician performing the procedure.[5,6] Irrespective of whether percutanous methods are safer, as suggested by the nonsurgical literature,[5] or the outcome of all methods is similar, as claimed by surgeons,[6] open surgical gastrostomy is still widely performed as shown in this study.

Our hypothesis was that this dismal experience in an "average," midsized, urban community teaching hospital reflects the trend nationwide. However, although single-center studies report a mortality rate of approximately 30%,[1,2] a recent study from the Mayo Clinic[6] relates a 1 month survival rate of 79% and a meta-analysis of 721 surgical gastrostomies yielded a 30-day mortality of 16.2%.[5] Unfortunately not many studies attempt to measure long-term results. Wilkinson and Pickleman[2] reported a 6-month survival rate of 40%, which is slightly better than our rate of 30%, but the Mayo Clinic survival rates for adult patients at 6 months and 1 year were 49% and 36%, respectively.[7]

There are several explanations for these conflicting results. First, a "study effect" implies that patients are better selected and managed when included in studies comparing, for example, surgical gastrostomies to percutaneous endoscopic gastrostomies. Second, a "publication bias" suggests that better results by "interested" clinicians from "more academic" centers tend to be published while poor practice is usually downplayed. Third, as in any field of surgery, patient selection and stratification has a major impact on prognosis and the analysis of results.

It is obvious that the outcome of gastrostomy depends on the specific indication for placement. Stuart et al.[1] reported a 30-day mortality rate in debilitated patients with "pulmonary cachexia" of 90%; the corresponding figures for patients with neurologic disorders and those with head and neck cancers were 28% and 12%, respectively. Wilkinson and Pickleman[2] indicated that the 30-day mortality in patients who were not in a coma at the time of gastrostomy was 37%, whereas it was 78% in comatose patients. The 6-month survival rate in comatose patients was 2%. Also, in our study the specific indication for gastrostomy strongly influenced the outcome; two thirds of the patients with obstructive malignancy and one third of those with neurologic disorders survived for more than 6 months. Group B "debilitated" patients, on the other hand, did not benefit from the gastrostomy at all, with 30-day and 6-month survival rates of 15% and 8%, respectively.

Scoring systems such as the APACHE II[3] measure the acute physiologic compromise of patients (including a coma scale), taking into consideration their premorbid health status and age. As its predictive value has been validated in myriad acute surgical conditions, it is disturbing that surgeons do not use it more often in clinical practice and/or research.[8] In this study APACHE II scores at tube insertion were predictive of survival—the 30-day survival rate in patients with scores of 10 and below was 96%. At 6 months and 1 year 30-day survival was 50% and 25%, respectively. Among patients with scores higher than 20, the 30-day survival rate was 18%; it was 6% and 0% after 6 months and 1 year, respectively. No patients with an APACHE II score greater than 15 belonging to group B survived more than 30 days.

As previously emphasized, gastrostomy insertions are not free from early major surgical complications.[2] In this series five patients underwent repeat laparotomy for intra-abdominal leakage from the gastrostomy site; four of them died. To minimize such complications it is crucial to carefully suture the gastrostomy site to the abdominal wall.[9]

It is difficult to justify any surgical procedure in a group of patients who are predicted to die within 30 days. If such ill-fated patients were to be identified prior to gastrostomy tube insertion, then the latter could be defined as an "unnecessary operation." We contend that most debilitated end-stage patients belonging to group B in our study underwent an "unnecessary" gastrostomy insertion. These patients did not have a well-defined swallowing disorder or mechanical pharyngoesophageal obstruction. Instead they either refused to eat or were too weak to eat or could not feed themselves. Differing medical, cultural, and financial attitudes in this country promote gastrostomy insertion under such circumstances. Elsewhere the solution would include dedicated spoon feeding by family members and/or a team of nurses. But for some it may appear more financially rewarding and less time consuming to insert a tube and simply drip down the feeding solution.

It has been suggested that the overall incidence of feeding gastrostomy in this country increased after the introduction of percutaneous endoscopic gastrostomy.[7] It may or may not be that percutaneous endoscopic or radiologic gastrostomies are associated with less early morbidity than open surgical insertions.[5,10] The 30-day mortality rates for all methods of insertion, however, remain the same,[5] the long-term attrition rate being determined by the indication for insertion and the patient's condition. Thus the availability of apparently less invasive methods of gastrostomy placement should not influence the indications nor should it justify "unnecessary" procedures in terminal patients.

Our experience supports previous assertions that a group of patients exists "who, on due consideration, should not have had gastrostomy tubes" and that in some patients "gastrostomy hastened death."[1] We agree with Wilkinson and Pickleman[2] that a demand for a gastrostomy tube from a referring physician is not an indication to proceed with the procedure. The short-term morbidity of the operation and the dismal prognosis in certain categories of patients should be explained by the surgeon to the patients and their families. A small-bore, soft nasogastric feeding tube is well tolerated and a reasonable alternative to gastrostomy in such "terminal" patients, particularly as it is known that gastrostomy is not associated with a lower incidence of pulmonary aspiration.[11]

We conclude that gastrostomy is often requested and performed in patients who are not expected to live more than a month; we suggest that such an unnecessary and futile procedure could be predicted and avoided. "One must ask whether it might be more humane to allow these individuals to die without a gastrostomy."[1]

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Venting Intraluminal Drains in Pancreaticoduodenectomy

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The utility of placing biliary, pancreatic, or enteric "venting"tubes (externally draining devices traversing the bowel or bile duct that have their distal tip located intraluminally near the biliary or pancreatic anastomosis) when performing a pancreaticoduodenectomy has received little attention to date. We hypothesize that these venting tubes do not decrease the morbidity or mortality associated with pancreaticoduodenectomy and may actually be a source of additional morbidity. To characterize our use of and the effect of these drains, we retrospectively analyzed 136 pancreaticoduodenectomies (127 partial, 9 total) performed over a 24-month period. Venting drain use, drain type and size, drain location, duration of intubation, hospital course, and postoperative complications were noted. Venting tubes were used in 80 patients (59%). The use of these drains had no significant relationship to postoperative length of stay, the development of major complications, overall morbidity, or mortality ($P > 0.05$). Such drains also did not significantly shorten the length of hospital stay ($P > 0.05$) or improve outcome when available to augment local control following luminal leak (n = 6) or regional abscess (n = 7). These drains were removed at a median interval of 29 days postoperatively (range 6 to 77 days). Seven patients had complications that were directly related to the venting drain; four of these patients had a documented intra-abdominal luminal leak from the site of drain removal, whereas the other three were hospitalized for presumed leakage secondary to immediate, severe abdominal pain following removal of the drain. These seven patients were elderly (mean age 70 years) and often harbored pancreatic ductal carcinoma (n = 6). Intraluminal drains afford no distinct advantage in terms of shortening the postoperative length of stay, decreasing operative morbidity and mortality, or improving local control with regional sepsis in pancreaticoduodenectomies. Furthermore, they may add an additional source of morbidity and we no longer employ them routinely. (J GASTROINTEST SURG 1999;3:156-161.)

KEY WORDS: Abdominal drains, pancreaticoduodenectomy, Whipple, surgical drains, venting drains

Likely the product of widely documented decreases in morbidity and mortality rates over the past two decades, pancreaticoduodenectomy is being performed with increased frequency in major medical centers.[1-9] Difficulties with the pancreaticoenterostomy, and less often the biliary-enteric anastomosis, continue to be the major sources of morbidity and mortality with this operation.[1,2,10,11] Among numerous operative techniques[5,8,10] and pharmacologic agents[12,13] employed to augment anastomotic integrity is the frequent use of intraluminal "venting" tubes. These externally draining tubes have their distal tip located within the bowel or bile duct in close association with the pancreatic or biliary anastomosis (Fig. 1). In theory, such tubes prevent overdistention of the afferent limb and divert potentially harmful secretions away from the anastomoses. Additionally, in instances of anastomotic leak or intra-abdominal infection, the presence of a venting tube for diversion may provide improved local control promoting a better outcome. The utility of and morbidity associated with such tubes has received little attention to date in the setting of pancreaticoduodenectomy. This retrospective analysis reviews our recent experience with these drains.

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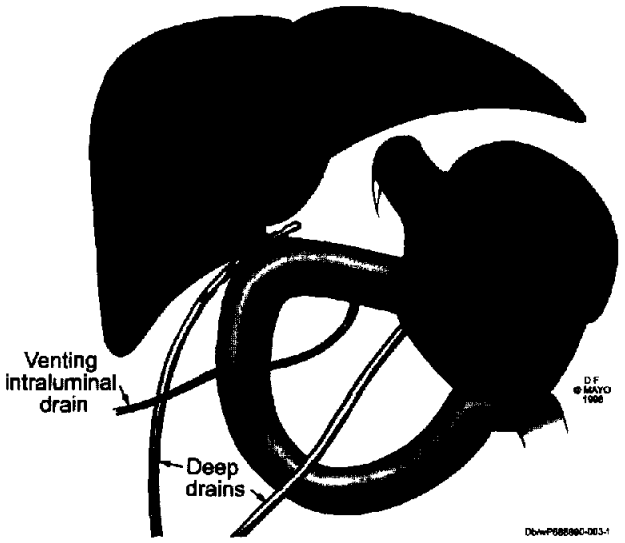

Fig. 1. Whipple reconstruction depicting a transjejunal venting intraluminal drain (dark colored) and two deep drains. (Reproduced with permission from the Mayo Foundation.)

METHODS

The medical records of 136 consecutive patients (July 1, 1993, through June 30, 1995) undergoing pancreaticoduodenectomy (127 partial, 9 total) were retrospectively reviewed. Pancreaticoduodenectomy was performed in the standard manner utilizing transabdominal drainage tubes placed extraluminally near the biliary-enteric and pancreatic-enteric anastomoses.[14] Venting tube usage was variable. In patients who had venting drains placed, the type and size of the drain, its location, and its duration of use were noted. All early and late postoperative complications were abstracted including those related to intraluminal drain removal. Outcome measures included postoperative length of stay, the development of major complications, overall morbidity, mortality, local control in the presence of luminal leak or regional abscess, and the outcome of venting tube removal.

The discrete response variables were analyzed by use of contingency tables. *P* values of 0.05 or less were considered statistically significant. Analysis for tables involving nonordered categories was carried out using the chi-square test or Fisher's exact test when the table contained cells with low expected values.[15] When the tables contained variables comprised of ordered categories, an exact Wilcoxon test for ordered categorical data was used.[16] When a significant association was found for a table containing more than two rows, a subsequent analysis was conducted comparing each row of the table with each of the other rows (a series of 2 × 2 tables). To compensate for the inflated type 1 error rate associated with this type of multiple-comparison testing, the resulting *P* values were adjusted using a Bonferroni correction.[17]

Continuous response variables were analyzed using two-sample *t* tests when the data approximately followed a gaussian distribution. When the data did not follow a sufficiently gaussian curve, Wilcoxon rank-sum tests[18] were used. *P* values of 0.05 or less were considered statistically significant.

This study had approximately 80% power to detect differences of a medium effect size between the group of patients who received venting drains and those who did not. As described by Cohen,[19] a medium effect size corresponds to a 0.5 standard deviation difference in means of a continuous variable, or a raw difference in proportions of 0.25.

Table I. Patient comorbidity

Comorbid conditions	No.	%
Hypertension	40	29
Other malignancy	27	20
Coronary artery disease	21	15
Diabetes mellitus	19	14
Chronic obstructive pulmonary disease	6	4
Chronic dysrhythmias	6	4
Peripheral vascular disease	5	4
Miscellaneous	34	25
Overall (one or more comorbid conditions)	93	68

RESULTS

Patient Population

The study group included 69 men and 67 women undergoing pancreaticoduodenectomy. Mean age at operation was 69.9 years (range 23 to 88 years, median 67 years). One or more comorbid conditions were present in 93 patients (68%) undergoing resection, whereas 43 individuals (32%) were entirely healthy (Table I). A total of 12 individuals underwent open exploration and biliary diversion (n = 10) or exploration and biopsy only (n = 2) at other institutions prior to referral. Additional preoperative interventions included endoscopic biliary tract stenting (n = 26) and percutaneous biliary diversion (n = 3).

Operative Management

Pancreaticoduodenectomies were performed by 10 different surgeons with most procedures (96%) concentrated among six individual surgeons. Resections were undertaken for a variety of malignant

(n = 114) and benign (n = 22) conditions (Table II). Advanced tumor burden among the 114 resections for cancer was evidenced by lymph node involvement (n = 53) and/or locoregional extension (n = 61) in 82 patients. Whipple-type procedures were undertaken in 127 patients and nearly equally divided between a standard approach (n = 64) and pylorus preservation (n = 63). Total pancreatectomy was performed in nine patients. The pancreaticoenterostomy was most commonly an end-to-side pancreaticojejunostomy (n = 109). End-to-end pancreaticojejunostomy (n = 11) and pancreaticogastrostomy (n = 5) were less prevalent. In one instance the pancreatic remnant was oversewn, and in an additional case the variety of pancreaticojejunostomy was not specified. Fibrin glue application to anastomotic sites (n = 11) and feeding needle catheter jejunostomy (n = 2) adjuncts were rarely employed. Postoperative hospitalization ranged from 6 to 47 days (median 11 days).

Morbidity and Mortality

One or more postoperative complications occurred in 55 patients for an overall morbidity of 40% (95% confidence interval [CI] = 32%-49%). Major complications (defined as hemorrhage requiring intervention or transfusion, intra-abdominal abscess, or anastomotic leak) developed in 24 patients. Six deaths occurred for an overall mortality rate of 4.4% (95% CI = 1.6%-9.4%). Pancreaticojejunal leak with resultant sepsis was notable in two of the patients who died. Hemorrhage (n = 3) and myocardial infarction (n = 1) were significant clinical factors in the other four patients who died. Malignant disease was present in four of the six who died. Nine patients underwent a total of 12 reoperations including completion pancreatectomy in three instances. No deaths or major complications occurred in the nine patients undergoing total pancreatectomy; however, minor complications (n = 4) developed in 44% (95% CI = 13.7%-78.8%).

Table II. Histopathologic findings

	No.	%
Malignant		
Pancreatic ductal carcinoma	58	43
Ampullary carcinoma	16	12
Mucinous carcinoma	12	9
Cholangiocarcinoma	8	6
Duodenal carcinoma	6	4
Other	14	10
	114	84
Benign		
Chronic pancreatitis	13	10
Other	9	6
	22	16

Intraluminal Drainage

Following resection, venting intraluminal tubes of various designs and sizes were used in 80 patients (59%) in a diverse manner (Table III). Most venting tubes were placed to lie near or traverse the biliary-enteric anastomosis, although in two instances they were used as stents for the pancreatic-enteric anastomosis. Internal anastomotic stents with no external component were placed intraoperatively in 65 patients (pancreatic in 63, biliary in 2), many of whom had typical venting drains as well (n = 56). Among the six patients who died perioperatively, venting tubes were used in four resections. Two of these four patients died with functioning intraluminal drains in place. In the remaining two patients the venting drain was removed prior to death allowing assessment of 78 patients with the potential for complications with removal of the venting drain. Data on intraluminal drain management following hospital charge were incomplete in 16 patients. The timing of extubation varied from 6 to 77 days (median 29 days) in the 62 patients for whom accurate documentation was available. Venting tubes were not placed in 56 patients (41%).

Table III. Venting tube location, tube type, and tube size in 80 patients

Point of entrance	No. (%)	Tube type	No. (%)	Tube size	No. (%)
Jejunum	30 (38)	Standard T tube	36 (45)	8.5 Fr	2 (3)
Cystic duct junction	29 (36)	Malecot	31 (39)	10 Fr	4 (5)
Common hepatic duct	14 (18)	Cope	9 (11)	12 Fr	17 (21)
Transhepatic	5 (6)	Pediatric feeding tube	3 (4)	14 Fr	29 (36)
Common bile duct	1 (1)	Not specified	1 (1)	16 Fr	11 (14)
Right hepatic duct	1 (1)			18 Fr	1 (1)
				Not specified	16 (20)

OUTCOMES

Length of Stay

Mean postoperative hospital stay was 13.8 days (range 6 to 47 days, median 11 days). Patients with venting drains were discharged after a mean of 14.2 days, whereas those without intraluminal devices were discharged after 13.2 days ($P = 0.10$).

Major Complications

One or more major complications developed in 24 patients (18%) (95% CI = 12%-25%). Eight patients required blood transfusions in the early postoperative period, in five instances prompting successful endoscopic (n = 2) or reoperative (n = 3) therapy. Two additional cases of disseminated intravascular coagulopathy developed, one following the pancreatic resection and the other following coronary artery bypass grafting for unstable angina. Intra-abdominal abscess was evident radiographically or at the time of reoperation in 11 cases. One such patient demonstrated contrast communication with the bowel lumen at the site of the enterotomy fashioned for venting drain placement. Anastomotic leaks developed in 11 patients, as evidenced radiographically or on reoperation (pancreaticojejunostomy in 7, pancreaticogastrostomy in 1, choledochojejunostomy in 1, and both the pancreaticojejunostomy and gastrojejunostomy in 1 each). In an additional patient with a leak apparent on reoperation, the precise location of the leak was indeterminate. These leaks led to exploratory operations in five patients. Venting intraluminal tubes were used in 54% (n = 13) of the patients in whom major complications occurred after resection. The remaining 11 patients with major complications did not have venting tubes placed.

Univariate statistical analysis showed no significant relationship between the presence of external venting tubes ($P = 0.61$), tube type ($P = 0.16$), tube size ($P = 0.80$), or tube location ($P = 0.68$) in lessening the development of major complications. Patient gender, operating surgeon, pathologic findings, preoperative biliary tract manipulation, type of pancreatic anastomosis, internal pancreatic duct stenting, and the presence of comorbid conditions likewise were of no statistical significance ($P > 0.5$) in delineating patients with major complications.

Overall Morbidity and Mortality

Excluding outcome of intraluminal drain extubation, 35 patients who received venting drains (44%; 95% CI = 33%-55%) incurred postoperative complications as opposed to 20 patients who did not (36%; 95% CI = 23%-50%). Use of external venting drains did not significantly lessen the likelihood of overall morbidity ($P = 0.35$). A statistical difference in mortality rates was not apparent in four vented (5.0%; 95% CI = 1.3%-12.3%) and two nonvented (3.6%; 95% CI = 0.4%-12.3%) persons who died postoperatively ($P = 1.0$).

Local Control

Venting drains were present in 10 (63%) of the 16 patients in whom an intra-abdominal abscess or luminal leak developed. The vented and nonvented cohorts with a luminal leak or abscess were of similar age (mean 65 and 66 years, respectively). Mean hospitalization was 27 days (median 24 days) in the vented group and 25 days (median 23 days) in the nonvented group ($P = 0.79$). Two patients with a luminal leak or abscess died postoperatively; venting drains had been placed in both.

Extubation Complications

Of the 78 patients with venting tubes surviving to tube removal, seven required readmission to our facility following extubation for a rehospitalization rate of 9% (95% CI = 4%-18%). Underlying factors and eventual outcome of these seven patients were varied (Table IV). Leakage was demonstrated radiographically (n = 3) or clinically (n = 1) in four of these pa-

Table IV. Rehospitalization on removal of venting tube

Age (yr)	Sex	Location	Tube type	Pathology	Outcome
55	F	Jejunum	T tube	Pancreatic ductal	Documented leak
81	M	Jejunum	T tube	Mucinous adenoma	Documented leak
69	M	Common duct	T tube	Pancreatic ductal	Documented leak
73	F	Common duct	T tube	Pancreatic ductal	Documented leak
62	M	Common duct	T tube	Pancreatic ductal	Pain, presumed leak
78	M	Common duct	T tube	Pancreatic ductal	Pain, presumed leak
71	M	Cystic duct stump	Malecot	Pancreatic ductal	Pain, presumed leak

tients. The remaining three patients had immediate and severe abdominal pain on drain removal. Although clinically stable, these three were admitted for presumed luminal leak and placed on empiric intravenous antibiotics. Both pancreatic ductal carcinoma and a standard T tube were found to be present in six of these seven patients ($P > 0.05$). Two additional patients (3%) experienced *difficulty* with drain removal. In one instance the external component was transected with no further attempts undertaken to remove the residual indwelling component. In the second individual, initial local attempts at drain removal were unsuccessful, but then an intact drain inadvertently dislodged prior to subsequent follow-up. Although never symptomatic, two additional patients (3%) had routine postoperative contrast studies performed through the venting tube, which identified free flow into the abdominal cavity secondary to drain migration prior to drain removal.

DISCUSSION

The use of transabdominal drains in which the tip is located intraluminally (venting drains) has received little attention in the setting of pancreaticoduodenectomy. Unique in design, their utility is largely theoretical and often reproducible via other modalities. The reported use of such venting tubes is varied. Trede et al.[20] describe the use of a Silastic Völker drain for biliary anastomotic stenting brought out through the jejunal wall via a Witzel-type canal. The drain is routinely removed after 3 weeks. In their experience, subsequent bile leaks occurred in 3% (n = 8) of 285 individuals undergoing pancreatic head resections.[21] Of these leaks, two were localized to the drain enterotomy and as such use of external venting tubes was advocated only when a narrow bile duct was encountered. Braasch[22] outlined routine placement of a venting drain via a jejunal approach to provide pancreatic anastomotic stenting and external diversion of exocrine pancreatic secretions as well as T-tube biliary-enteric drainage via choledochotomy. Others have described routine use of biliary anastomotic vents with or without pancreatic duct stents of both the internal and external variety.[5,11,23] Enteric venting tubes located between the biliary and pancreatic anastomoses that do not traverse the bile duct have been used by Keck et al.[10] via a separate Roux limb tacked to the anterior abdominal wall at the site of venting drain exit. In their reported experience involving 76 pancreatic head resections, only two pancreatic leaks and one bile leak developed, although in two instances this led to reoperation. Routine internal pancreatic duct stenting has also been advocated, sometimes in concert with biliary venting,[8] or without mention of biliary or enteric venting.[7,24,25] The latter report from the University of Texas M.D. Anderson Cancer Center involved 23 individuals undergoing attempted reoperative pancreaticoduodenectomy following the initial exploratory operation and referral from other institutions. The one individual in that series of difficult resections whose pancreatic duct was stented with an external venting tube, rather than the internal variety used in the remaining 22 individuals, developed an enterocutaneous fistula forcing an additional reoperation.[25] Avoidance of venting drainage or internal stents of any kind has also been reported with no late biliary anastomotic strictures noted.[9]

Venting drains require formation of an additional surgical defect, typically in the afferent jejunal limb or remaining bile duct. Venting drains thus differ from both external surgical bed drains or internal stents placed at operation or endoscopically, which do not violate luminal integrity. Usually secured to the viscera with an absorbable pursestring suture, venting drains should completely obturate the luminal defect during the convalescence period while provoking a fibrinous sheath formation to seal the defect on planned removal. Technical errors in placement, unintended drain traction, tissue failure, infection, and inadequate host fibrinous response prior to removal presumably contribute to morbidity associated with these drains. In our study this morbidity occurred in 15% of patients who received venting drains.

Theoretical advantages of intraluminal drains include the facilitation of creating a patent anastomosis with small ducts, diversion of exocrine pancreatic secretions from newly fashioned anastomoses, diversion of enteral and biliary fluid, and subsequently the diminishment of intraluminal pressure to which anastomoses are subjected. External venting tube diversion, in theory, may also provide improved local control of fluid collections and septic complications in the setting of abscess or luminal leak. Clinical data supporting these hypotheses and the benefit of such drainage tubes is, to our knowledge, nonexistent in this setting. To the contrary, use of venting tubes in previous studies did not significantly lower the likelihood of pancreaticoenterostomy leak[1] or decrease overall morbidity.[26] Practical advantages of these venting drains include their ability to stent either the pancreatic or biliary anastomoses, provision of access for either radiographic evaluation or further manipulation of the operative field, and in rare cases provision of an alternative route for enteral feeding. However, all of these practical advantages can be achieved through other means.

Our institution has previously reported variable use of venting drains (via biliary or jejunal access) in 78% of pancreatic resections[1] as compared to the 59%

prevalence in this series. A variety of drain strategies were employed in our current practice as dictated by individual surgeon preference and preoperative interventions to the operative field. However, drain type, drain size, drain location, or concurrent use with internal stents showed no significant relationship to the outcomes evaluated. Subgrouping of drain location to a biliary tree or jejunal approach similarly was not associated with the development of major complications ($P > 0.5$). Duration of intraluminal intubation also varied according to surgeon preference and clinical course as evidenced by the longer duration of intraluminal drainage in those patients with major complications (median 38 days) in comparison to those individuals without (median 27 days).

Our experience demonstrates no significant advantage in terms of shortening the length of hospital stay, decreasing the development of major complications, or lessening overall postoperative morbidity or mortality following pancreaticoduodenectomy with the use of venting drains. On the contrary, postoperative length of stay tended to be slightly longer and the total morbidity rate higher when venting tubes were employed. Patients with an abscess or luminal leak also showed no significant decrease in the length of stay or improvement in overall outcome when they had venting drains placed. Of equal concern, localizing studies implicated the venting drain enterotomy as the source of leakage in at least one of these individuals. Two other patients had inadvertent migration of their venting drains outside of the bowel lumen, although both remained asymptomatic. The additional morbidity incurred in seven patients was directly related to removal of the venting tube, although all of these individuals required only short-term rehospitalization. The overrepresentation of more elderly patients (with T-tube venting drains) and ductal pancreatic carcinoma suggests an identifiable risk group in which extreme caution should be exercised when contemplating external drainage. In our experience, routine use of venting intraluminal drains should be avoided.

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Characterization and Reduction of Ischemia/Reperfusion Injury After Experimental Pancreas Transplantation

Herbert Mayer, M.D., Jan Schmidt, M.D., Jochen Thies, M.D., Eduard Ryschich, M.D., Martha Maria Gebhard, M.D., Christian Herfarth, M.D., Ernst Klar, M.D.

Reperfusion injury after pancreas transplantation is a cause of early graft pancreatitis. The aim of this study was to quantify pancreatic microcirculation after pancreas transplantation in correlation with cold ischemia time. In a second step the effect of N-acetylcysteine on reperfusion damage was tested. Pancreas transplantation was performed in three different groups of male Lewis rats. Groups 1 and 2 received no special treatment. Cold ischemia time was 1.5 hours in group 1 and 16 hours in groups 2 and 3. In group 3 donor and recipient were both treated with N-acetylcysteine (300 mg/kg) 1.5 hours after reperfusion graft microcirculation was quantified by means of intravital microscopy. Rhodamine-labeled leukocytes, fluoroscein isothiocyanate–labeled erythrocytes, and fluoroscein isothiocyanate–albumin were used as fluorochromes. After a cold ischemia time of 16 hours, functional capillary density, erythrocyte velocity, and leukocyte-endothelium interaction were reduced significantly compared to a cold ischemia time of 1.5 hours ($P < 0.05$). After 16 hours of cold ischemia, treatment with N-acetylcysteine improved all of these parameters ($P \leq 0.05$). Ischemia/reperfusion injury after experimental pancreas transplantation is characterized by a disturbance of the pancreatic microcirculation exhibiting a correlation with the duration of cold ischemia. Treatment of donor and recipient with N-acetylcysteine resulted in prevention of cold ischemia–induced microcirculatory disturbance. (J GASTROINTEST SURG 1999; 3:162-166.)

KEY WORDS: Experimental pancreas transplantation, ischemia, reperfusion, therapy, intravital microscopy

Insulin-dependent diabetes mellitus leads to several late complications including small vessel disease, neuropathy, and renal disease. In up to 70% of patients, these complications can be prevented by an intensified insulin treatment[1] but it is not possible to avoid complications in all patients. For these patients, especially the patients developing end-stage renal disease, at present pancreas transplantation should be considered the therapy of choice.[2-6] Nevertheless, until now relatively few patients have undergone pancreas transplantation. Even among diabetic patients with end-stage renal disease, only about 10% to 20% of possible candidates undergo transplantation. Those who oppose transplantation cite reasons such as the need for life-long immunosuppressive therapy and the possibility of severe perioperative morbidity.[7,8] The most significant complication is graft pancreatitis, which occurs in up to 35% of transplant recipients and is mainly due to ischemia/reperfusion injury.[7,9]

The pathogenesis of ischemia/reperfusion injury is based on complex mechanisms. Oxygen radicals play a central role.[10] In earlier studies at our institution, it was shown that prophylactic treatment with the scavenger N-acetylcysteine was able to reduce the impairment of hepatic microcirculation after reperfusion in experimental liver transplantation.[11]

The aim of the present study was first to establish a model for quantifying pancreatic microcirculation by means of intravital microscopy after experimental pancreas transplantation. Second, we wished to investigate the influence of N-acetylcysteine on the microcirculatory disorder following reperfusion.

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Presented at the Thirty-Ninth Annual Meeting of The Society for Surgery of the Alimentary Tract, New Orleans, La., May 17-20, 1998.
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MATERIAL AND METHODS

All animal experiments were approved by the Committee on Animal Care (Regierungspräsidium Karlsruhe, Germany). The animals were randomized to three experimental groups before the study was begun (Table I). In each group six transplants were performed in male Lewis rats. Donors weighed 210 to 240 grams and recipients 240 to 260 grams. Twenty-four hours before the experiments, the animals had their food intake restricted but they were allowed free access to water. Anesthesia was induced with pentobarbital (10 mg/kg body weight intraperitoneally) and ketamine (40 mg/kg body weight intramuscularly). Heterotopic pancreaticoduodenal transplantation was performed using a modification of the technique described by Lee et al.[12] The pancreas of the donor was explanted after perfusion with University of Wisconsin (UW) solution and stored at 0 to 4° C.

After the designated cold ischemia time, the graft was rinsed with a defined cold solution (depending on the experimental group) and the implantation was performed using a microsurgical technique. The donor aortic cuff carrying the celiac and mesenteric arteries was anastomosed to the infrarenal aorta of the recipient. The portal vein of the donor was connected to the infrarenal vena cava.

One and one half hours after reperfusion, the recipient was placed on its right side on a specially designed microscopy stage. The head of the transplanted pancreas was exteriorized using very gentle traction at the duodenal loop and subjected to intravital fluorescence microscopy. The microscopic images were recorded on videotape for later off-line analysis of the following parameters: (1) capillary erythrocyte velocity; (2) rolling leukocytes in pancreatic postcapillary venules; (3) sticking leukocytes in pancreatic postcapillary venules; and (4) functional capillary density.

Analyses were carried out according to standard procedures as described previously[13] using fluoroscein isothiocyanate–marked erythrocytes, fluoroscein isothiocyanate–albumin, and rhodamin 6G. To quantify plasma amylase levels, heparinized blood was withdrawn shortly before reperfusion and at the end of the experiments. At the end of the experiments the animals were killed by an overdose of pentobarbital, and the grafts were removed and fixed in a 6% formalin solution for histologic examination. For statistical analysis between groups, the Mann-Whitney U test was applied.

RESULTS

The physiologic parameters, which included heart rate, blood pressure, and hematocrit, did not differ between groups during the experiments. Plasma amylase levels did not differ significantly between the groups either before reperfusion or at the end of the experiments.

Capillary Erythrocyte Velocity

After pancreas transplantation with a short cold ischemia time of 1.5 hours, the mean capillary erythrocyte velocity was 0.78 ± 0.17 mm/sec. After a long cold ischemia time of 16 hours, the capillary erythrocyte velocity was reduced significantly to 0.55 ± 0.13 mm/sec. As a result of treatment with N-acetylcysteine, the velocity was improved to the level of the short cold ischemia time (Fig. 1).

Functional Capillary Density

The functional capillary density after a short cold ischemia time was 155 ± 28 mm/mm^2. This parameter was reduced significantly in organs transplanted after a long cold ischemia time of 16 hours to 106 ± 45 mm/mm^2. Prophylactic treatment with N-acetylcysteine led to a significant improvement (Fig. 1).

Leukocyte-Endothelium Interaction

A long cold ischemia time led to a significant increase in sticking and rolling leukocytes as compared to a short cold ischemia time. Treatment with N-acetylcysteine resulted in a reduction in the number of rolling and sticking leukocytes (Fig. 2).

Table I. Experimental groups

Group	Cold ischemia time (hr)	Donor treatment	Rinsing solution	Recipient treatment
1	1.5	—	Saline solution	—
2	16	—	Saline solution	—
3	16	300 mg/kg N-acetylcysteine	Saline solution containing 3 mg/ml N-acetylcysteine	300 mg/kg N-acetylcysteine

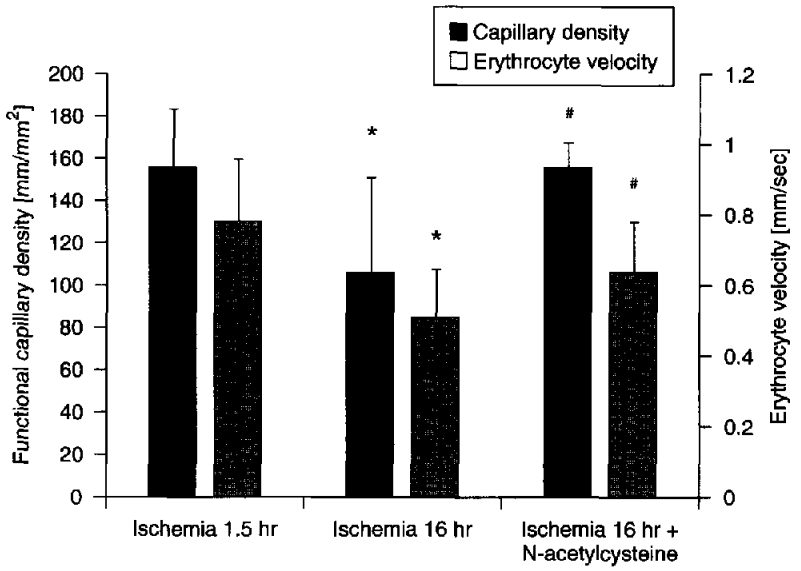

Fig. 1. Functional capillary density and capillary erythrocyte velocity after experimental pancreas transplantation. * = Significant differences 16 hours ischemia vs. 1.5 hours ischemia ($P \leq 0.55$; Mann-Whitney U test); # = significant differences 16 hours ischemia vs. 16 hours ischemia plus N-acetylcysteine ($P \leq 0.05$; Mann-Whitney U test).

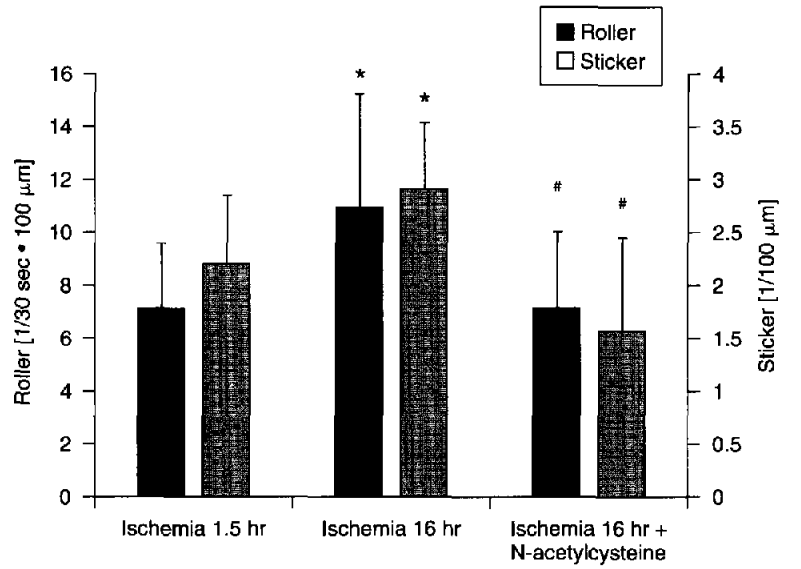

Fig. 2. Rolling and sticking leukocytes in postcapillary venules after experimental pancreas transplantation. * = Significant differences 16 hours ischemia vs. 1.5 hours ischemia ($P \leq 0.05$; Mann-Whitney U test); # = significant differences 16 hours ischemia vs. 16 hours ischemia plus N-acetylcysteine ($P \leq 0.05$; Mann-Whitney U test).

DISCUSSION

Ischemia/reperfusion injury represents the main problem in pancreas transplantation,[7,8] leading to graft dysfunction or even acute graft pancreatitis.[7,14] In several previous studies ischemia has been shown to play a central role in the development of pancreatitis.[15-17] Nevertheless, until now there has been no widely accepted strategy for such an acute graft pancreatitis. Therefore prophylaxis of ischemia/reperfusion injury seems to be important in preventing organ loss and systemic inflammatory response due to graft pancreatitis.

The principal finding in the current study is that ischemia/reperfusion injury can be significantly reduced by prophylactic treatment with N-acetylcysteine. N-acetylcysteine interferes with the production

of free oxygen radicals by inhibiting activated granulocytes. In addition, it promotes the inactivation of reactive oxygen species by acting as a classical scavenger and by inducing glutathione synthesis.[18]

The administration of scavengers to the graft during preservation, as is done with allopurinol as a constituent of UW solution,[19] is insufficient from a clinical standpoint. Our intention was to treat both the donor before explantation and the recipient before reperfusion with the scavenger N-acetylcysteine and to compare this approach with the standard pancreas transplantation procedure using UW solution alone.

For quantification of ischemia/reperfusion injury, intravital microscopy was chosen because it allows direct quantification of pancreatic microcirculation as previously validated.[20,21] For our study an extended cold ischemia time of 16 hours was chosen because it is known from previous experiments (unpublished) to induce a significant microcirculatory injury under standard UW preservation as compared to a cold ischemia time of only 1.5 hours. Prophylactic treatment with N-acetylcysteine led to a significant improvement in capillary erythrocyte velocity and functional capillary density as well as a reduction in leukocyte-endothelium interaction as a main determinant of microvessel perfusion.

Following a similar concept, N-acetylcysteine has previously been shown to limit ischemia/reperfusion injury in experimental liver transplantation.[11] These authors concluded that N-acetylcysteine was effective in that it specifically increased the liver glutathione level. In the present study we were able to demonstrate that N-acetylcysteine was equally effective in pancreas transplantation showing the potency of this drug to generally prevent ischemia/reperfusion injury in organ transplantation. Further experiments investigating survival rates and histologic morphology using this regimen are currently in progress.

CONCLUSIONS

Ischemia/reperfusion injury after experimental pancreas transplantation is characterized by a disturbance of pancreatic microcirculation correlating with cold ischemia time. Erythrocyte velocity and functional capillary density were significantly impaired after a cold ischemia time of 16 hours, in parallel with an induction of leukocyte-endothelium interaction. Treatment of donors and recipients with the scavenger N-acetylcysteine led to an improvement of the microcirculatory disorder after a prolonged cold ischemia time. These results are comparable to the effect of the drug in experimental liver transplantation. Our data will have to be further evaluated with respect to histologic findings and survival.

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Discussion

Dr. J. Peters (Los Angeles, Calif.). It seems to me that there have been studies of free radical scavengers in the clinical realm that have not borne fruit. Is there a reason to expect different results with N-acetylcysteine compared to other scavengers?

Dr. H. Mayer. N-acetylcysteine is not just a simple scavenger. It increases glutathione synthesis and interferes with granulocyte activation. We therefore think that it might be more potent in the treatment of ischemia/reperfusion injury than scavengers used previously.

Dr. E. Klar. To comment further on this question, I think that the main obstacle to showing a reduction in reperfusion injury in transplant patients is that the patients are not stratified properly. There are those who are at risk of developing complications of graft failure and those who will have an uneventful course. If you mix them all together, any treatment modality concerning reperfusion injury will fail to show a significant effect. To answer your question clinically, we have to look for better means to identify patients at risk for development of graft dysfunction.

Distal Splenorenal Shunts for the Treatment of Severe Thrombocytopenia From Portal Hypertension in Children

Joel Shilyansky, M.D., Eve A. Roberts, M.D., Riccardo A. Superina, M.D.

Profound thrombocytopenia resulting from portal hypertension may exacerbate gastrointestinal bleeding, precipitate spontaneous bleeding, preclude surgical intervention for associated disorders, and severely limit life-style because of the danger of splenic injury. Although splenectomy can reverse the thrombocytopenia, the procedure should be avoided in children. We reviewed our experience with distal splenorenal shunting (DSRS) in children, particularly when performed for the sole purpose of reversing severe thrombocytopenia resulting from portal hypertension. DSRS was performed in 11 children between the ages of 7 and 15 years: five for severe thrombocytopenia (group 1), four for advanced hypersplenism and congenital hepatic fibrosis prior to renal transplantation (group 2), and two for esophageal bleeding (group 3). One child in group 1 with severe heart disease and Child's class C cirrhosis due to hepatitis C died of progressive cardiac failure and was excluded from further analysis. Of the eight remaining patients in groups 1 and 2, four children had congenital hepatic fibrosis, two had portal vein thrombosis, one had hepatitis B, and one had Wilson's disease. After DSRS, the mean platelet count increased from 37,000 ± 18,000 to 137,600 ± 81,000 ($P = 0.01$). The platelet count improved significantly in all seven children with presinusoidal portal hypertension or stable cirrhosis but did not increase in the child with hepatitis B and Child's class B cirrhosis. The white blood cell count increased from an average of 3.3 ± 1.1 to 5.4 ± 2.6 ($P = 0.02$). There were no postoperative complications in this group. The improved platelet count allowed the four children with congenital hepatic fibrosis and renal failure to undergo renal transplantation with full posttransplant immunosuppression including azathioprine. Postoperative Doppler ultrasound examination demonstrated shunt patency at 6 months in all cases. Spleen size decreased appreciably in all children in groups 1 and 2. All children were able to resume full activity including contact sports. In summary, DSRS effectively controls profound thrombocytopenia resulting from presinusoidal portal hypertension or stable cirrhosis without sacrificing the spleen and should be the treatment of choice for this condition. (J GASTROINTEST SURG 1999;3:167-172.)

KEY WORDS: Thrombocytopenia, portal hypertension, distal splenorenal shunt

The treatment of bleeding esophageal varices has evolved over the past decade. Endoscopic sclerotherapy can control bleeding with low associated mortality and lower risk of encephalopathy and hepatic failure.[1,2] Portal decompression is reserved for patients in whom endoscopic sclerotherapy is not successful.[3-5] Portal hypertension in many children results from presinusoidal obstruction, and these children usually have well-preserved hepatic function. However, chronic portal hypertension may result in hypersplenism with accompanying thrombocytopenia and leukopenia. In some children the platelet count may become sufficiently low to cause spontaneous bleeding and exacerbate gastrointestinal hemorrhage. Children with hepatic fibrosis and renal insufficiency may benefit from renal transplantation. However, these children may be precluded from transplantation because leukopenia severely limits the choice of medications, particularly antiproliferative medications such as azathioprine that are required for immuno-

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suppression, and thrombocytopenia increases the hazards of surgery. The severe splenomegaly also prevents children from participating in sports because of the concern about injury. Splenectomy and its possible long-term effects, postsplenectomy sepsis, portal vein thrombosis, and variceal bleeding, should be avoided if alternatives exist.[6-8] Splenomegaly and thrombocytopenia appear to improve in most patients after portal decompression with DSRS.[9] The reported morbidity and mortality of DSRS in children is lower than in adults probably because of preserved hepatic function.[10] We treated children with portal hypertension and profound symptomatic thrombocytopenia with DSRS and reviewed their clinical records to assess the safety and efficacy of this novel application of this procedure in a unique population of children.

PATIENTS AND METHODS

Children with portal hypertension and thrombocytopenia were referred for surgery in the presence of spontaneous bleeding or when the platelet count dropped to less than 30,000. Patients with congenital hepatic fibrosis and polycystic kidney disease requiring renal transplantation were considered for prophylactic DSRS if the platelet count was less than 60,000 and the leukocyte count was less than 3500. A third group of children was referred for DSRS for the treatment of bleeding esophageal varices poorly controlled by endoscopic sclerotherapy or with complications of endoscopic sclerotherapy, such as esophageal stricture, regardless of platelet count.

Abdominal ultrasound, color-flow Doppler, liver biopsy, and esophagogastroduodenoscopy were used to assess the portal circulation and arrive at the cause of portal hypertension. Alternative causes of thrombocytopenia were excluded based on reticulocyte count and bone marrow biopsy. Prior to DSRS, angiography and portography were employed to define the arterial and portal venous anatomy. Following the DSRS procedure, shunt patency was evaluated with ultrasound and color-flow Doppler on postoperative day 2, at 1 month, and at 6 months. Following surgery, prophylactic heparin was used to prevent shunt thrombosis. As soon as the child was able to eat, acetylsalicylic acid and dipyridamole were started and continued for 6 months, and the heparin was stopped.

The surgical procedure was previously described.[11] In brief, the abdomen was entered through a bilateral subcostal incision. The lesser sac was approached via the gastrocolic omentum. The pancreatic body and tail were mobilized superiorly to expose the splenic vein. The vein was exposed and isolated from its junction with the mesenteric vein to the splenic hilum. The splenic vein was disconnected from the mesenteric vein at their junction to avoid a cul-de-sac that may promote thrombus formation in the portal vein. The distal splenic vein was gently angled inferiorly and anastomosed to the renal vein. An end-to-side running anastomosis measuring 1.5 times the diameter of the splenic vein was fashioned using a 6-0 monofilament suture. Adrenal and gonadal veins were not routinely ligated. When DSRS was performed for the treatment of hypersplenism and thrombocytopenia, the gastroepiploic and coronary veins were not ligated. Prior to completion of the procedure, flow in the shunt was assessed with a hand-held Doppler probe.

RESULTS

DSRS was performed in 11 children with portal hypertension. The diagnoses and indications for shunting are outlined in Table I. Nine children underwent surgery principally to treat hypersplenism and profound thrombocytopenia (groups 1 and 2). Four of these patients (group 2) had congenital hepatic fibrosis and renal failure, and had shunts placed prior to kidney transplantation. The five children in group 1 had portal hypertension and hypersplenism from a variety of disease processes and underwent shunting primarily for platelet counts that were considered dangerously low (<30,000) or that were associated with abnormal bleeding. Some of the children in group 1 also had symptoms of abdominal pain from splenic enlargement, and most had been advised to limit their physical activity for fear of splenic injury. Two children without thrombocytopenia (group 3) were treated for recurrent esophageal and gastric variceal bleeding not controlled with an aggressive endoscopic sclerotherapy regimen.

Three of the four patients awaiting renal transplantation also had leukopenia (white blood count <3.5) on the basis of hypersplenism. This would have precluded them from taking the antiproliferative immunosuppressive medication azathioprine, which would have compromised their posttransplant care. Nine children had well-preserved liver function and were Child's class A, whereas one was Child's class B and one was Child's class C.

There were no perioperative deaths in the series. However, one child in group 1 with class C liver disease, hepatitis C, and severe pulmonary hypertension resulting from congenital heart disease died of his cardiac condition 8 weeks after surgery. He was excluded from the analysis of results.

Early shunt patency was documented by Doppler ultrasound in all 11 children. Follow-up studies at 1 and 6 months after surgery revealed a patent shunt in

Table I. Summary of diagnoses, indications, and results of distal splenorenal shunting

Group	Patient	Age (yr)	Diagnosis		Indication for DSRS	Platelet count (×1000)		Leukocyte count (×1000)	
			Primary	Secondary		Preoperative	Postoperative	Preoperative	Postoperative
1	C.T.	14	Wilson's disease	Cirrhosis Hypersplenism	Thrombocytopenia	22	126	2.4	3.2
1	M.R.	14	Portal vein thrombosis	Hypersplenism Controlled varices	Thrombocytopenia Menorrhagia	22	150	5	5.4
1	H.K.	5	Portal vein thrombosis	Trisomy 21 AVSD Hypersplenism	Thrombocytopenia Bleeding gums Epistaxis	34	101	3.8	5.9
1	H.J.*	14	Absent portal vein Hepatitis C	TOF Right heart failure Hypersplenism	Thrombocytopenia	22	33	7	7.4
1	N.D.	15	Hepatitis B Cirrhosis	Epistaxis, bruising Hypersplenism	Thrombocytopenia Epistaxis, bruising	22	21	2.1	2.2
2	H.J.	13	Congenital hepatic fibrosis	CRF Hypersplenism	Thrombocytopenia Renal transplant	66	202	2.1	8.2
2	W.M.	8	Congenital hepatic fibrosis	CRF Hypersplenism	Thrombocytopenia Renal transplant	22	293	3.5	9.7
2	H.A.	5	Congenital hepatic fibrosis	Hypersplenism	Thrombocytopenia Renal transplant	54	108	3.2	3.2
2	P.A.	11	Congenital hepatic fibrosis	CRF Hypersplenism	Variceal bleeding Thrombocytopenia Renal transplant	54	100	4.4	5.9
3	H.N.	16	Congenital hepatic fibrosis	Varices, GI bleeding	Variceal bleeding	181	103	6.1	8.2
3	N.N.	6	Portal vein thrombosis	Varices, GI bleeding	Variceal bleeding	120	128	5.8	5.3

AVSD = atrioventricular septal defect; CRF = chronic renal failure; ES = endoscopic sclerotherapy; GI = gastrointestinal; TOF = tetralogy of Fallot.
*Patient died of associated disease.

all surviving children. None of the children showed clinical evidence of hepatic encephalopathy based on neurodevelopmental testing. Liver disease did not progress in any of the children after shunting based on follow-up liver function tests, albumin, and coagulation parameters.

Esophageal variceal bleeding did not recur in any of the children. Recession of splenomegaly was noted on physical examination and ultrasound study of the abdomen. In groups 1 and 2, mean platelet counts after shunting rose from 37,000 ± 18,000 to 137,600 ± 80,900 (P = 0.01; Fig. 1). The leukocyte count increased from an average of 3.3 ± 1.1 to 5.5 ± 2.6 (P = 0.02; Fig. 2). The patient with Child's class B hepatic disease and hepatitis B had no change in platelet and leukocyte counts, in contrast to those with persinusoidal liver disease or cirrhosis with well-preserved hepatic function who all improved to normal or near-normal counts after shunts were placed. The median hospital stay was 8 days (range 4 to 57 days), and there were no postoperative complications in the surviving children. All four children with renal

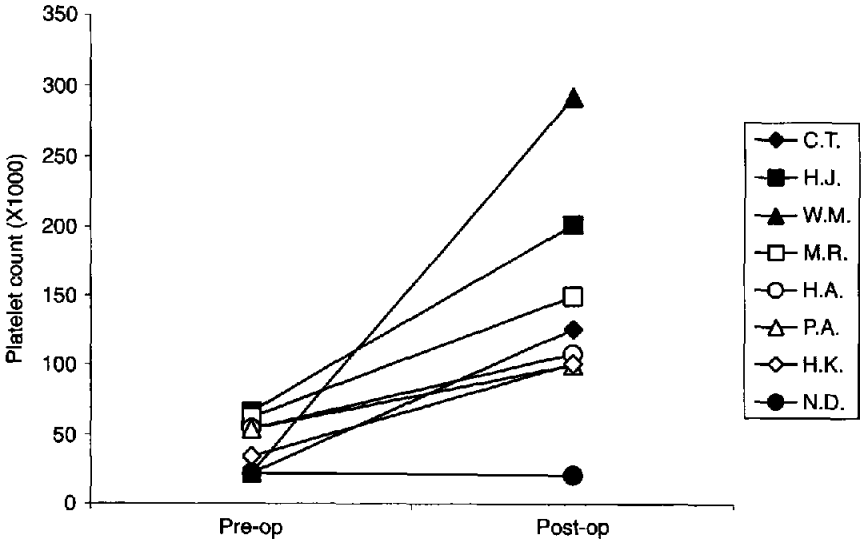

Fig. 1. Change in platelet count after DSRS in seven children with presinusoidal portal hypertension and hypersplenism. Mean preoperative platelet count was 37,000, whereas mean postoperative platelet count was 137,600 (P = 0.01).

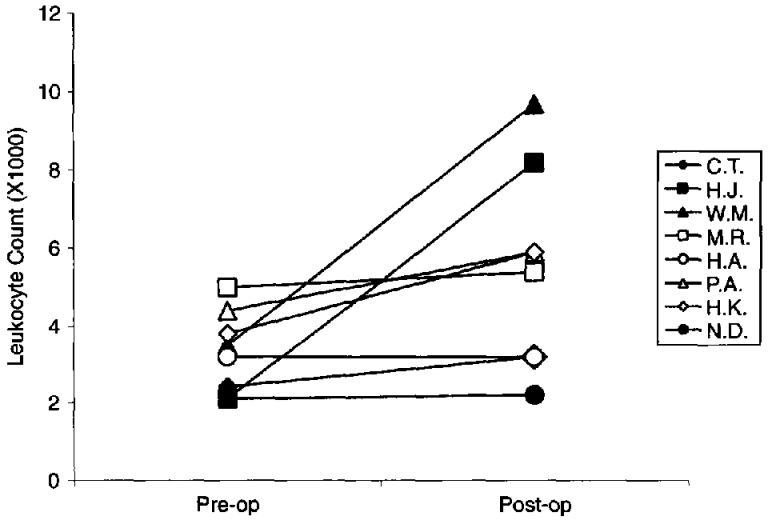

Fig. 2. Change in leukocyte count after DSRS in children with presinusoidal portal hypertension and hypersplenism. Mean preoperative leukocyte count was 3300, whereas mean postoperative leukocyte count was 5500 (P = 0.02).

failure underwent successful renal transplantation with full immune suppression an average of 3 months after DSRS. All children were able to resume full activity including sports after DSRS.

DISCUSSION

The DSRS procedure described by Warren et al.[11] was proposed for the treatment of variceal bleeding. During the procedure the gastrosplenic and hepatomesenteric venous systems are disassociated. Thus the varices are decompressed without diverting blood flow from the liver. By maintaining and possibly increasing the blood flow to the liver, hepatic function is preserved. The importance of preserving portal flow was underscored by the growing understanding of liver development and regeneration and the experience with auxiliary liver transplantation. It appears that high portal flow is a stimulus for initiating hepatic growth, whereas a drop in portal flow is a stimulus for hepatocyte apoptosis and a decrease in the size of the liver.[12] The incidence of hepatic encephalopathy is significantly lower after DSRS and has been rarely seen in children with presinusoidal portal hypertension.[13] Maksoud and Goncalves[4] and Maksoud and Mies[10] demonstrated that DSRS can be performed safely and with a high patency rate in children.

Koyanagi et al.[9] and Alvarez et al.[14] coincidentally noted recession of splenomegaly and hypersplenism after DSRS for portal hypertension. Hutson et al.[15] and McAllister et al.[16] suggested the use of portosystemic venous decompression for control of hypersplenism. The most consistent improvement was noted in patients with the most profound thrombocytopenia and leukopenia resulting from presinusoidal portal vein obstruction and associated with well-preserved hepatic function.[9,17] Improved platelet and leukocyte counts were observed by Koyanagi et al.[9] after DSRS only in patients with profound thrombocytopenia (platelet count <50,000) and associated leukopenia. Thus patient selection is critical for the successful outcome of this procedure.

Hypersplenism alone has not traditionally been considered an indication for DSRS. In the past it was rare to see patients with severe hypersplenism without recurrent bleeding from gastroesophageal varices. With the increasing success of variceal injection and banding as a way to control bleeding, isolated hypersplenism with its accompanying hematologic abnormalities is becoming a more frequently seen clinical entity. Untreated hypersplenism will eventually lead to massive splenic enlargement, increase the risk of splenic injury, cause abdominal pain from stretching of the splenic capsule, and lead to a severe decline in platelet and white cell counts, which in turn increases the risk of severe bleeding and serious infections.

We employed DSRS specifically for the control of hypersplenism in nine children. Seven had presinusoidal portal hypertension or cirrhosis with well-preserved liver function. Three children presented with spontaneous bleeding complications such as epistaxis, purpura, and menorrhagia. Because of marked splenomegaly and concern for splenic rupture, most of these children had also been restrained from full physical activity.

Shunting for hypersplenism alone was not done unless one of two conditions was met: (1) either the thrombocytopenia was associated with signs of abnormal bleeding such as epistaxis or menhorragia or (2) the platelet counts were so low that the patient was at risk for a life-threatening intracranial hemorrhage. In other disease states, such as idiopathic thrombocytopenia purpura, persistent platelet counts of 20,000 or less would be considered an indication for splenectomy.

Platelet and white blood counts increased in all patients with good liver function in groups 1 and 2. Renal transplantation in group 2 patients was carried out with adequate platelet counts avoiding bleeding during and after the transplant. In addition, ideal immunosuppression was implemented after transplantation in all four patients without the risk of further decreasing white cell counts. The spleen size diminished postoperatively and all children were able to resume full physical activity. No bleeding complications were observed during the follow-up period.

The two patients with advanced liver disease from hepatitis C and B did not have as gratifying a result. One died of complications of heart disease and had been given a shunt because he was considered a poor transplant candidate. The other has survived and may one day need a transplant for hepatitis B. It is thought that this latter patient may have developed an antiplatelet antibody and have thrombocytopenia on an immunologic basis.

In the past the high risk of shunt thrombosis discouraged surgeons from attempting DSRS. The experience in performing venous anastomosis in pediatric liver transplantation with portal vein patency rates of greater than 95% has led to increased confidence and proficiency in performing DSRS in small children. Patient selection and improved surgical technique resulted in 100% patency rates in the current series. These results correspond to the experience reported by Maksoud and Goncalves.[4]

Utilizing DSRS solely for hematologic considerations in children with portal hypertension is a novel approach. These children may be treated with splenectomy, but we are in agreement with El Khishen et al.[6]

who strongly discouraged that approach in patients with portal hypertension. Splenectomy may cause a predisposition to thrombocytosis, polycythemia, and a hypercoagulable state leading to portal vein thrombosis and worsening variceal bleeding. Overwhelming postsplenectomy infection is a particularly serious concern in children.[6,7,18-21] Infections with encapsulated organisms are more common in children than in adults and occur in approximately 10% of asplenic individuals.[8] In contrast, DSRS is not associated with overwhelming postsplenectomy infection or hypercoagulable states. The procedure may also decompress existing varices and may decrease the risk of future variceal bleeding.

We consider it important to preserve the coronary vein when performing DSRS for hypersplenism, particularly in cases of portal vein thrombosis. Tying off the coronary vein when the portal vein is obstructed is unlikely to improve mesenteric blood flow to the liver, and may precipitate ascites from increasing venous pressure in the mesenteric vascular bed. None of the patients in this series had recurrent or new onset of variceal bleeding when the shunt was performed for hypersplenism alone and the coronary vein was left intact.

CONCLUSION

DSRS is the treatment of choice in children with severe or symptomatic hypersplenism resulting from portal hypertension when liver function is still adequate. The procedure results in a significant increase in platelet and leukocyte counts in this group of children, carries no long-term morbidity, and results in the resolution of hypersplenism while preserving the spleen and its important immunologic functions. Patients with advanced liver disease did not do well in this series and probably should not be considered for this procedure, even if liver transplantation is not an option.

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Ogilvie's Syndrome in the Surgical Patient: A New Therapeutic Modality

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Acute colonic pseudo-obstruction, Ogilvie's syndrome, most often appears as a complication of other clinical conditions. It is characterized by massive colonic dilation in the absence of a mechanical cause. Therapy for this condition has traditionally been colonoscopic decompression via a flexible colonoscope. We performed a retrospective study to assess the efficacy of Cystografin enema for colonic decompression in Ogilvie's syndrome. We present a series of 18 patients who developed Ogilvie's syndrome while hospitalized for trauma (n = 10), burn (n = 1), gastrointestinal surgery (n = 4), and hip replacement (n = 3). The mean pre-enema cecal size was 13 cm (range 10 to 15 cm). The mean postenema cecal size was 8.5 cm (range 6 to 15 cm). Fifteen of the 18 patients underwent Cystografin enema as the primary mode of decompression. Three had undergone prior colonoscopy, which had failed. One of the 18 patients required repeat enema for inadequate decompression after the first enema and one underwent colonoscopy for recurrence. Two patients underwent operative intervention after the enema. There were no complications related to the enema. In all patients we were able to rule out a mechanical cause of large bowel obstruction. We believe the safety, efficacy, and ease of this procedure make Cystografin enema optimal first-line treatment for acute colonic pseudo-obstruction. (J GASTROINTEST SURG 1999; 3:173-177.)

KEY WORDS: Colonic pseudo-obstruction, therapeutic enema, Ogilvie's syndrome

Ogilvie's syndrome, also known as acute colonic pseudo-obstruction, is a nonobstructive colonic dilation.[1] The syndrome is thought to be caused by an imbalance of parasympathetic and sympathetic tone that results in loss of normal colonic peristalsis. Anticholinergics, narcotics,[2] and antidepressants may contribute to the syndrome. Patients most at risk for the syndrome appear to be those who are bedridden or nonambulatory, the elderly, and those with electrolyte disturbances, hypothyroidism, diabetes, and renal failure. Acute colonic pseudo-obstruction may follow a number of surgical procedures and is seen in the setting of severe trauma.

Ogilvie's syndrome is of concern as it may lead to spontaneous perforation of the colon. The cecum is the area most at risk for perforation.[3] The risk increases substantially once the cecal diameter reaches 12 cm. The condition is often diagnosed when the patient develops a distended, tympanitic abdomen. The abdomen is generally nontender and unlike a bowel obstruction, the patient generally does not have colicky abdominal pain.

Attempts to correct the underlying disorder causing the adynamic ileus should be undertaken. After resuscitation and correction of electrolyte disturbances, the physician must rule out mechanical obstruction of the large intestine. The standard of care in a patient with peritoneal signs or impending perforation is surgery.

Pharmacologic attempts to decompress the distended colon may be tried. The prokinetic agent cisapride[4] and the somatostatin analogue octreotide,[5] which promotes the migrating motor complex, have both been used in acute colonic pseudo-obstruction with variable results. The combination of guanethidine, to reduce sympathetic tone, and neostigmine, a parasympathomimetic, has also been used with moderate success.[6] A recent prospective randomized double-blind study of neostigmine showed excellent results of colon decompression but a significant risk of symptomatic bradycardia.[7]

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Presented at the Thirty-Ninth Annual Meeting of The Society for Surgery of the Alimentary Tract, New Orleans, La., May 17-20, 1998 (poster presentation).
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In patients with massive colonic dilation without perforation, the standard of care is colonoscopic decompression. Colonoscopy is currently considered the best means of decompressing the entire colon.[8,9] Colonoscopy often needs to be repeated[10] (11% to 40% of cases) for adequate decompression and carries a low but morbid 1% to 5% perforation rate.

The use of contrast enemas has been described in Ogilvie's syndrome to rule out a mechanical bowel obstruction.[11] We report here the use of the water-soluble diatrizoate meglumine (Cystografin, Squibb, New Brunswick, N.J.) contrast enema for diagnosis and decompression of Ogilvie's syndrome.

METHODS

The charts of surgical patients who had a Cystografin enema for Ogilvie's syndrome were retrospectively reviewed. Data obtained included age, admitting diagnosis, pre-enema colon diameter, postenema colon diameter, success rate, and need for further interventions. Pre-enema colon size was defined as that measured on the diagnostic anteroposterior radiograph leading to the intervention; postenema colon size was that measured on the anteroposterior radiograph the following morning.

Procedure For Colonoscopic Decompression

Dilute diatrizoate meglumine, Cystografin 18%, is a radiopaque water-soluble contrast agent. Each milliliter of solution contains 18 mg of diatrizoate meglumine, 0.4 mg of edetate sodium, and 85 mg of organically bound iodine. It is a moderately hyperosmolar solution of 349 mOsm/kg.[12]

The protocol is as follows:

1. A rectal tube with a balloon is placed in the rectum.
2. The colon is then filled with Cystografin by gravity drainage under fluoroscopic guidance. The bag of Cystografin is elevated approximately three feet above the patient to assist in flow. The patient is turned on the fluoroscopy table to facilitate cecal filling if necessary. Filling of the cecum generally requires 2 to 3 L of solution.
3. Once the cecum is filled, the Cystografin is left in place for 15 to 20 minutes. The bag is then placed on the ground and the colon empties under gravity drainage. If able, the patient may evacuate his or her own colon.
4. Repeat radiographs are obtained immediately after the procedure to rule out an acute perforation and then 8 to 12 hours later for follow-up assessment of colon diameter.

RESULTS

A total of 18 patients were evaluated. Patient age and admission diagnosis are given in Table I. Colon size before and after therapy is listed in Table II. Mean pre-enema cecal diameter was 13 cm. Mean postenema cecal diameter was 8.5 cm. Results are shown in Figs. 1 and 2. Fourteen (78%) of 18 patients

Table I. Patient age and admission diagnosis

Patient	Age (yr)	Admission diagnosis
1	49	Trauma
2	38	Trauma
3	27	Trauma
4	71	Trauma
5	72	Trauma
6	51	Hepatic abscess
7	18	Trauma
8	83	Cancer
9	68	Adhesiolysis
10	68	Total hip replacement
11	64	Trauma
12	64	Total hip replacement
13	29	Burns
14	71	Trauma
15	73	Total hip replacement
16	36	Trauma
17	76	Pneumonia
18	54	Trauma
	AVERAGE 56.2	

Table II. Colon diameter before and after enema

Patient	Pre-enema diameter (cm)	Postenema diameter (cm)	Change (cm)
1	11	Unknown	Unknown
2	13	6	7
3	12	6	6
4	14	7	7
5	14	7	7
6	13	7.5	5.5
7	12	8	4
8	12	8	4
9	10	8	2
10	14	8	6
11	13	8	5
12	14	8	6
13	12	9	3
14	13.5	9	4.5
15	15	10	5
16	13	10	5
17	13	10	3
18	15	15	0
	MEAN 13	8.5	4.6

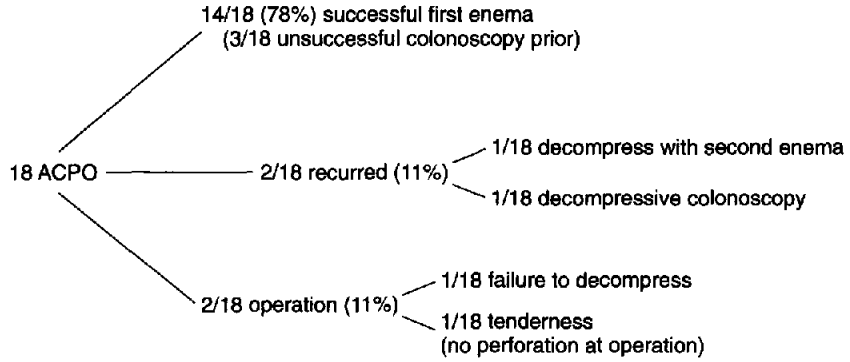

Fig. 1. Results of Cystografin enema in 18 patients with acute colonic pseudo-obstruction *(ACPO)*.

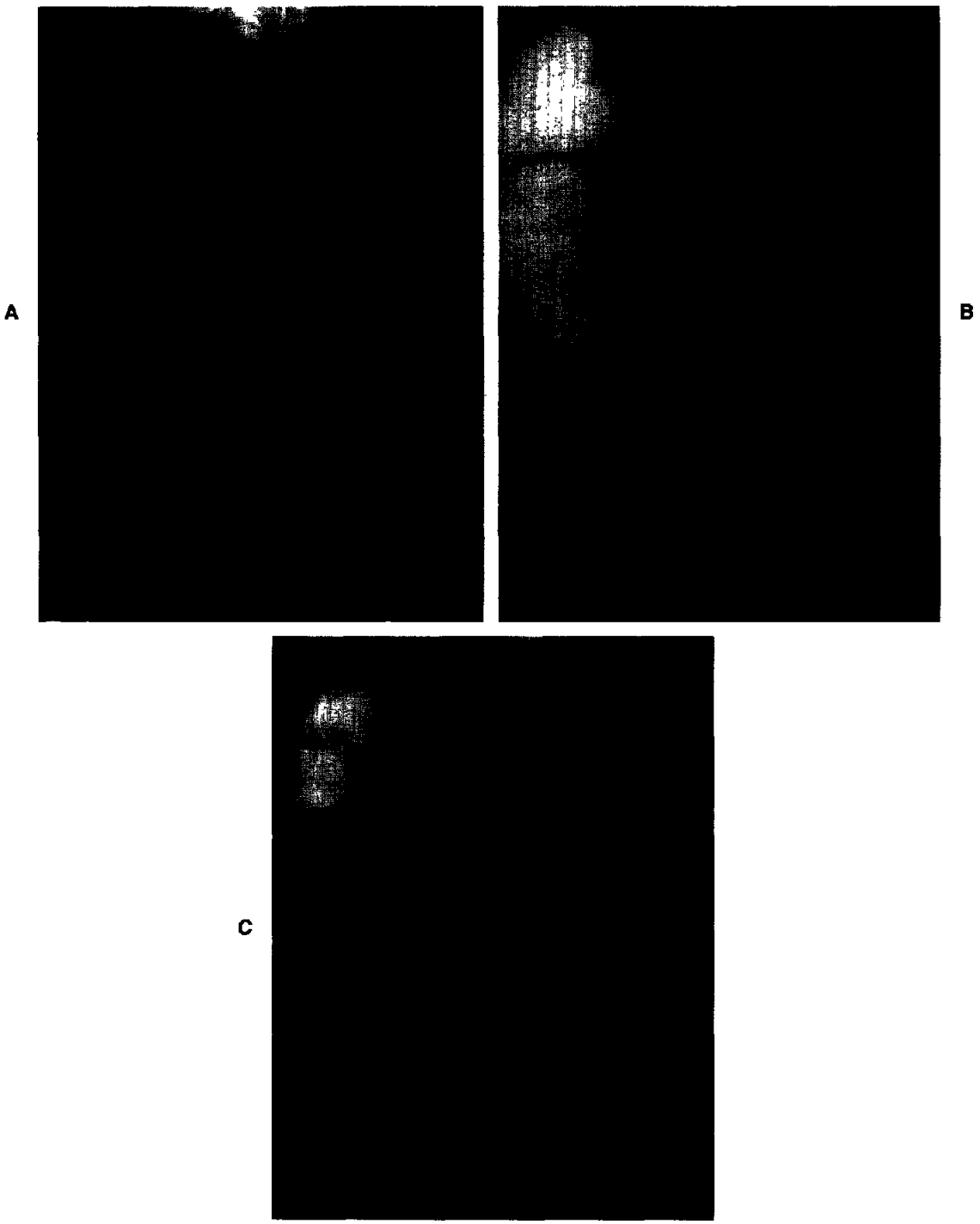

Fig. 2. Photographs of **(A)** pre-enema abdominal x-ray film, **(B)** immediate postenema x-ray film, and **(C)** 12-hour postenema x-ray film.

had successful decompression with one enema. Three of these patients had undergone colonoscopy as their primary mode of decompression, which had been unsuccessful. Two of 16 patients had recurrent or inadequate decompression with the first Cystografin enema. One patient (6%) had successful decompression with two enemas. One patient underwent colonoscopy for recurrence after initial enema decompression. Two patients (11%) underwent operative intervention, one for failure to achieve decompression, one for increasing tenderness. Neither had perforation at operation (Table III).

DISCUSSION

In this retrospective chart review, 78% of patients had successful resolution of acute colonic pseudo-obstruction when treated with Cystografin enema as the primary mode of decompression. A total of 84% had successful resolution with no procedure other than enema. In this small series there were no complications as a result of the therapy.

Table III. Failures of enema decompression

Patient	Age (yr)	Admission diagnosis	Pre-enema diameter (cm)	Reason for operation
15	73	Total hip replacement	15	Tenderness
18	54	Trauma	15	Failure to decompress

We suspect that Cystografin works to decompress the colon by increasing the water content and bulk volume of the stool because of its moderate hyperosmolality. The meglumine has a greater osmotic effect than the sodium salt of diatrizoate and is reportedly less cytotoxic.[13] The 18% Cystografin as opposed to 35% Gastrografin is less toxic to the mucosa. It also likely acts in a manner similar to a contact laxative with a net accumulation of fluid and electrolytes. The increase in volume then decreases intestinal transit time by promoting bulk flow. Rectal contraction is stimulated via hyperosmotic and irritant actions. There may also be reflex peristalsis after the sensory nerve stimulation of distending the colon with Cystografin.

The ease of therapy also makes this an attractive procedure as a first-line therapy. We recommend the algorithm in Fig. 3. Cystografin 18% comes in a large-volume bottle (500 ml) and does not need to be diluted. It can be used to determine whether or not mechanical obstruction is present, and if it fails it has acted as a cathartic to partially prepare the colon for subsequent colonoscopy. The total time the patient spends in the radiology department for the procedure is approximately 30 minutes. Published data for colonoscopic decompression range from 45 to 60 minutes. At our institution the charge for Cystografin

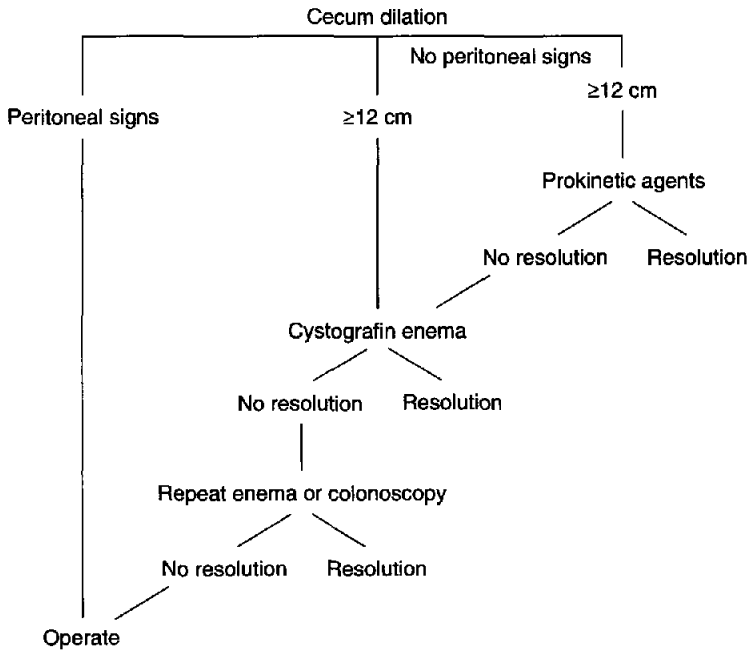

Fig. 3. Algorithm for treatment of acute colonic pseudo-obstruction.

enema for decompression is $248, which includes both physician and technical fees. The charges for the gastrointestinal laboratory technical fees alone for a colonoscopy are $578. We have also used this modality at the bedside for patients who are too sick to be moved to the radiology department. These patients were not included in this study, but we have anecdotal evidence of good success.

CONCLUSION

Cystografin enema is a safe first-line therapy for decompression of acute colonic pseudo-obstruction (Ogilvie's syndrome) and carries a 0% perforation rate in our series. Cystografin enema is less labor intensive and less expensive than colonoscopy for colonic decompression. Failure rates for Cystografin enema (11%) appear to be lower than those for colonoscopy (11% to 40%). Cystografin enema successfully decompresses the colon in 78% of cases when used as first-line therapy and in 84% of cases when used twice.

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Role of Transforming Growth Factor-Beta in Growth and Injury Response of the Pancreatic Duct Epithelium In Vitro

Carlos Alvarez, M.D., Barbara L. Bass, M.D.

Chronic pancreatitis is characterized by increased levels of expression of transforming growth factor-beta (TGF-β), particularly in and around the ducts. To examine the consequences of elevated exposure to TGF-β on the pancreatic duct epithelium, we cultured segments of the main bovine pancreatic duct in the presence of increasing doses of TGF-β. We also studied the effect of TGF-β on epithelial injury, produced in this model by exposure to a bile acid. The extent of proliferation, migration, and epithelial damage was measured morphometrically on sections stained with hematoxylin and eosin. Proliferation and apoptosis were qualitatively determined by means of immunohistochemical analysis. In this model of duct cell culture, TGF-β stimulated cell migration in areas of the explants where the native basement membrane of the duct epithelium was absent. In segments where the native basement membrane remained intact, proliferation was inhibited and apoptosis induced. When the explants were exposed to bile acid, extensive epithelial injury was observed. TGF-β exposure at high doses (1 nmol/L protected epithelial integrity, but cellular morphology was altered and the process of apoptosis appeared to be increased. Our results suggest that increased periductal levels ot TGF-β in the setting of pancreatic injury may be intended to promote repair of acute epithelial damage but may have detrimental long-term effects. (J GASTROINTEST SURG 1999; 3:178-184.)

KEY WORDS: Pancreatic ducts, pancreatitis, cell culture, growth factors, bile acids

The pathophysiology of chronic pancreatitis remains unexplained.[1] Increased expression of the peptide transforming growth factor-beta (TGF-β) has been noted in the pancreas of patients with clinical and histologic evidence of chronic pancreatitis.[2-4] Although it is hypothesized that this increase in expression is responsible for the extensive fibrosis that characterizes chronic pancreatitis, the exact role of TGF-β on pancreatic physiology is unknown.[5] TGF-β is known to inhibit proliferation and enhance restitution in epithelial systems, but its effects on pancreatic tissue have not been adequately studied.[6,7] Since the increase in growth factor expression occurs primarily in and around the pancreatic ducts, we were interested in determining the effects of TGF-β on isolated duct cells maintained in culture on their native basement membrane, using an explant culture method developed recently in our laboratory.

METHODS

Explant Harvest and Culture

All reagents were obtained from either Gibco (Grand Island, N.Y.) or Sigma (St. Louis, Mo.) unless otherwise noted. The method of explant culture has been previously described.[8] Briefly, the bovine main pancreatic duct and its major branches are dissected with sterile technique from freshly obtained pancreata. The ducts are divided into 10 to 20 mm × 5 to 10 mm segments (explants); between 5 and 12 explants can be harvested from a single organ. The explants are extensively washed in cold phosphate-buffered

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Supported by a grant from the Dean's Research Initiative Fund from the University of Maryland School of Medicine.
Presented at the Thirty-Ninth Annual Meeting of The Society for Surgery of the Alimentary Tract, New Orleans, La. May 17-20, 1998.
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saline containing a 2% antibiotic-antimycotic solution, then individually placed on gelatin (Gelfoam, Upjohn Company, Kalamazoo, Mich.) "rafts" in tissue culture plates. Waymouth's culture medium containing 5% fetal bovine serum, 2% antibiotic-antimycotic solution, and 0.1 mg/ml soybean trypsin inhibitor is added. Prior to experimental manipulations, the explants are allowed to recover from the harvest process in humidified 5% carbon dioxide/air at 37° C for 48 hours, with the medium exchanged after the first 24 hours.

Experimental Conditions

Basal medium was replaced with a similar solution where the fetal bovine serum is reduced to 1%. To this "test" medium, TGF-β was added at varying doses (0, 0.01, 0.1, and 1.0 nmol/L, n = 5 to 7 per dose) (Calbiochem Corp., San Diego, Calif.). Although little data are available on quantitative tissue and serum levels of TGF-β, it has been shown that concentrations greater than 0.01 nmol/L inhibit pancreatic acinar cell growth in vitro.[9] A second set of explants (n = 5 to 7) was exposed to taurodeoxycholic acid (TDCA, 1 mmol/L), added at the same time as the various doses of TGF-β. We have previously shown that 1 mmol/L TDCA for 48 hours consistently produces damage to more than 50% of the explant epithelial surface.[8] The explants were returned to the incubator for 48 hours, after which they were then harvested. They were fixed in 4% paraformaldehyde overnight, embedded in paraffin, and cut into 7 μm sections. Sections were stained with hematoxylin and eosin for morphometric assessment of the epithelial injury or processed for immunohistochemical analysis.

Immunohistochemistry

Explant sections were deparaffinized, rehydrated, and washed with phosphate-buffered saline solution. Inherent peroxidase activity was quenched with dilute hydrogen peroxide in methanol for 5 minutes. The sections were then blocked with nonimmune serum for 1 hour at room temperature. This was followed by incubation with primary antibody raised against proliferative cell nuclear antigen (PCNA; 1:100, Neomarker, Freemont, Calif.).[10] Negative control sections were incubated with nonspecific immune serum. The sections were then washed and incubated with horseradish peroxidase–linked secondary antibody (1:200; Vector Labs, Burlingame, Calif.) for 1 hour, followed by exposure to horseradish peroxidase substrate (DAB reagent, Vector Labs), counterstaining with eosin, and examination by microscopy.

Apoptosis

Apoptosis was detected histologically in paraffin-embedded, 7 μm tissue sections using a kit (FragEl) according to the specifications of the manufacturer (Calbiochem).[11] Briefly, sections were deparaffinized and rehydrated from 100% to 70% ethanol, then permeabilized with proteinase K (0.02 mg/ml) for 20 minutes. The sections were rinsed and positive control sections were exposed to DNAse I (1 μg/μl) for 20 minutes. The sections were washed again and incubated in dilute (3%) H_2O_2 in methanol for 5 minutes. They were then placed in equilibration buffer for 30 minutes, rinsed, and labeled by incubating them in a humidified chamber at 37° C for 1.5 hours with excess biotin-labeled and unlabeled deoxynucleotides and terminal deoxynucleotydil transferase (TdT), which identifies and repairs damaged DNA. Negative control sections were generated by omitting TdT. The transferase reaction was stopped and blocking buffer was added, followed by streptavidin–horseradish peroxidase conjugate, which in the presence of horseradish peroxidase substrate and peroxide (DAB reagent) specifically stains apoptotic nuclei dark. The sections were then counterstained with methyl green to accentuate horseradish peroxidase–negative nuclei and with eosin to define cell margins.

Assessment of Damage and Growth

As described previously, an optical micrometer was employed to determine the length of the epithelial surface of each explant, measured between the cut edges of the duct.[8] Mucosal damage was defined as the aggregate length of sloughed (Fig. 1, *A*, *arrowhead*), blebbed (Fig. 1, *A*, *curved arrow*), or flattened (Fig. 1, *D*, *arrow*), epithelium. Measurements were made by two blinded observers on three separate sections from each explant and the average score was used. The degree of mucosal damage is reported as a percentage of the total explant length to justify for different explant sizes. As a measure of cellular proliferation or loss in the native epithelium, the number of epithelial cell nuclei per high-power (200×) field was counted. The totals from three separate areas of representative sections from each explant were averaged and reported as epithelial density in terms of nuclei per high-power field. We observed that under normal culture conditions the epithelium consistently extended from the explant edge (where the duct had been cut open) onto the bare connective tissue. The extent of this proliferation (growth from the edge) was measured with the optical micrometer. Statistical comparisons between treatment groups were made using the unpaired Student's *t* test, with statistical significance accepted at $P < 0.05$. Each treatment

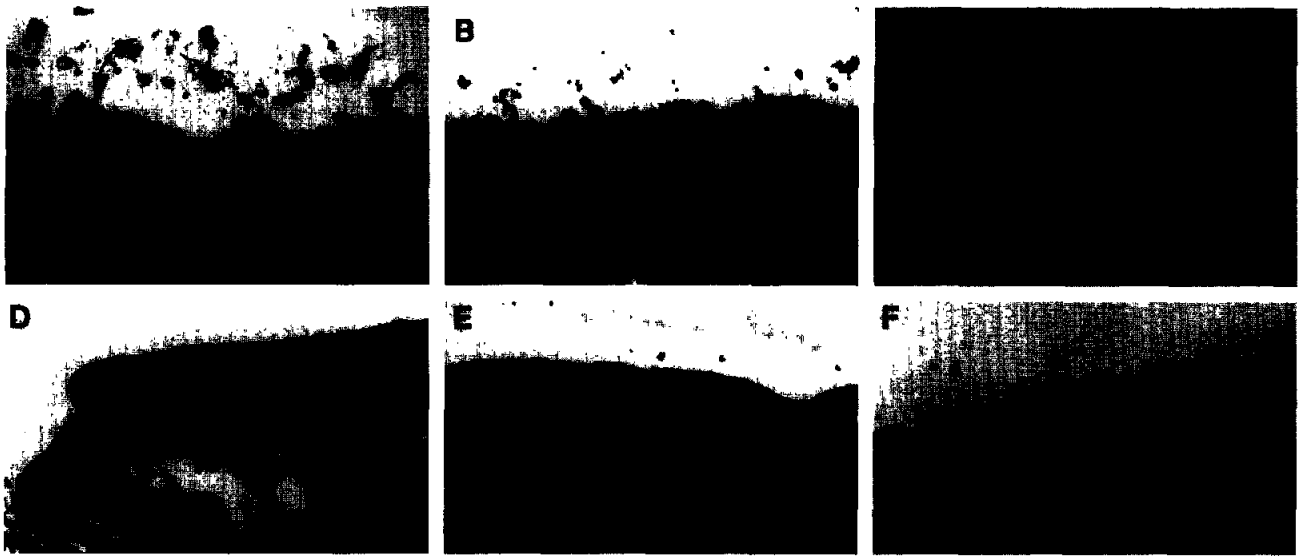

Fig. 1. Histologic effects of TGF-β on pancreatic duct explant damage induced by TDCA. **A, B,** and **C,** Explant treated for 48 hours with 1 mmol/L TDCA alone demonstrating extensive epithelial blebbing *(curved arrow)* and sloughing *(arrowhead)*. **C,** The surface epithelium has been extensively sloughed and the supporting cells stain positive for apoptosis. **D, E,** and **F,** Segment of explant treated with both 1 nmol/L TGF-β and 1 mmol/L TDCA for 48 hours showing flattening *(arrow)* of the epithelium and pyknotic nuclei. **E** and **F,** Neighboring nuclei stain positive for either PCNA or apoptosis. **A** and **D,** Hematoxylin and eosin stain; **B** and **E,** Immunostaining of explant epithelium anti-PCNA antibody; **C** and **F,** Cytochemical staining against apoptic nuclei. (× 80.)

group contained between five and seven individual explants.

RESULTS

Dose-Response Effect of TGF-β

During the process of harvesting, some cells are lost from the duct surface. After 48 hours in culture, the normal single-cell, cuboidal appearance of the pancreatic duct epithelium (PDE) returns, and this appearance persists throughout the 48 hours used for our experimental period and beyond (Fig. 2, *A* and *B*). With increasing doses of TGF-β, there appeared to be an increase in cell density in both the PDE and the underlying connective tissue (Fig. 2, *C* and 3). However, PCNA staining did not corroborate this suggestion of increased proliferation. Although a number of cells stained positive for PCNA in untreated explants (Fig. 2, *E*), with the higher doses of TGF-β (0.1 and 1.0 nmol/L), PCNA staining disappeared from the "native" epithelium (Fig. 2, *F*). It is probable that TGF-β treatment led to contracture of the explant, artificially increasing the cell density. PCNA expression persisted in the segment of PDE extending out from the cut edge of the explant even at the highest dose of TGF-β (area to the right of the arrowhead, Fig. 2, *F*). The untreated PDE contained only a sporadic cell that stained positive for apoptosis (Fig. 2, *H*). Starting with the lowest dose (0.01 nmol/L) of TGF-β, we noted an increase in apoptotic cells in the epithelium, so that at the highest dose (1.0 nmol/L) nearly all cells stained positive (Fig. 2, *I*). In PDE cells extending out from the cut edge of the explant, there was increased evidence of apoptosis, but the pattern of expression was not consistent.

Effect of TGF-β on Bile Acid–Induced Damage

When added alone to the culture medium, TDCA (1 mmol/L) induced damage to 59% ± 11% of the explant epithelium and reduced cellular extension from the edge of nearly 70% (Fig. 3) ($P < 0.05$). TGF-β had no effect on PDE density in the presence of TDCA. At intermediate doses (0.01 and 0.1 nmol/L of TGF-β, migration out from the cut edge was preserved, if not stimulated, in the presence of TDCA ($P < 0.05$), but at the 1.0 nmol/L dose, migration was severely impaired. As shown in Fig. 1, *D*, the epithelium in the majority of TDCA-treated explants exposed to

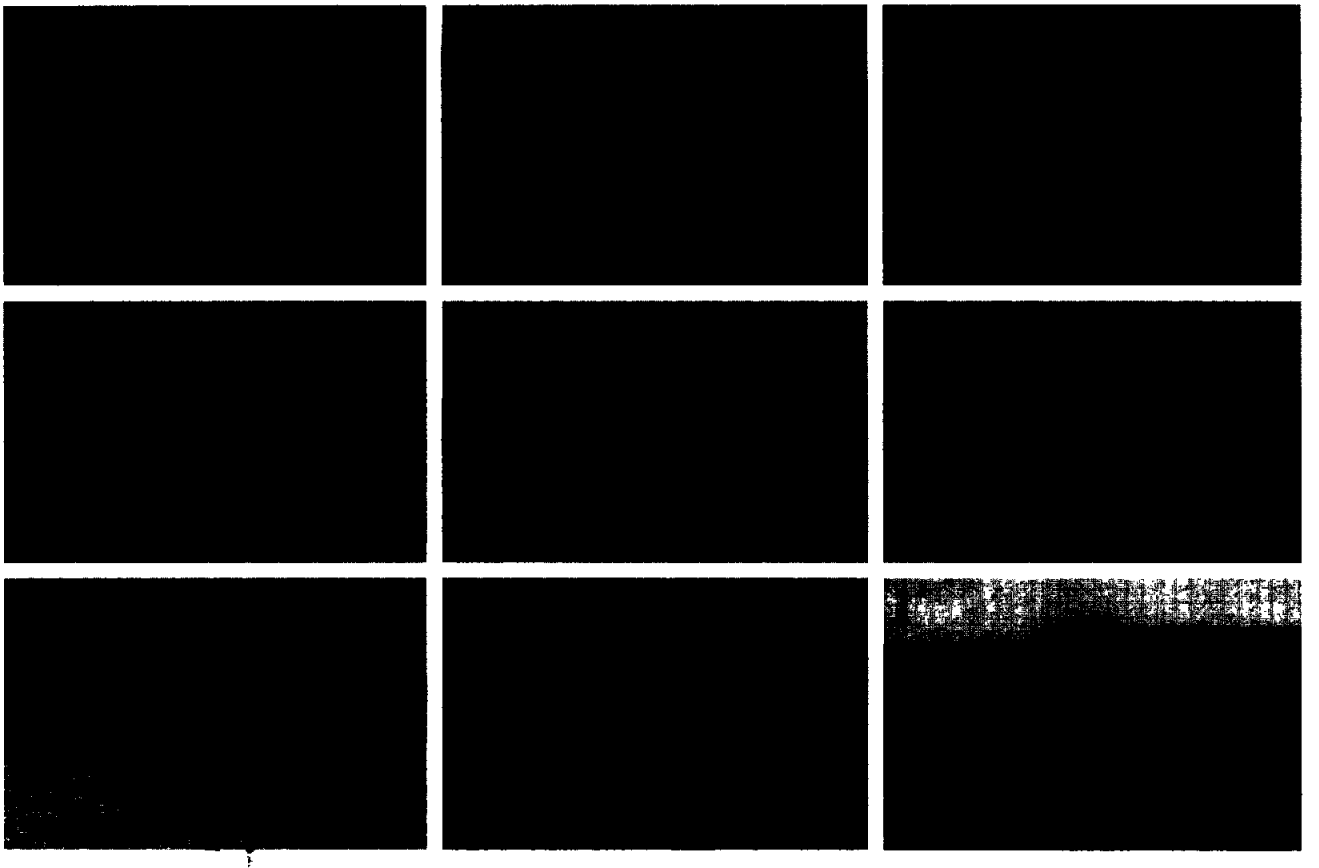

Fig. 2. Effects of TGF-β on pancreatic duct explant histologic appearance. **A,** Edge of explant demonstrating outgrowth of new epithelium to the right of the arrowhead. **B, E,** and **H,** Midsection of untreated explant; note normal epithelial appearance, occasional PCNA nuclear staining, and rare evidence of apoptosis. **C, F,** and **I,** Central ("native") segment of explant treated with 1 nmol/L TGF-β for 48 hours showing flattening of the cells, pyknotic nuclei, and apoptotic staining (**C** and **I**), and (**F**) edge of similarly treated explant, with reduced PCNA staining to the left of the arrowhead in the native explant and persistent PCNA staining in the new growth region to the right of the arrowhead. **A, B,** and **C,** Hematoxylin and eosin stain; **D, E,** and **F,** Immunostaining of explant epithelium with nonspecific immune serum (**D**) or anti-PCNA antibody (**E** and **F**) depicting positive staining in nuclei of proliferating cells; **G, H,** and **I,** Cytochemical staining against free-OH ends of fragmented DNA after treatment with DNAse as a positive control (**G**) or in the native state, demonstrating apoptotic nuclei. (×80.)

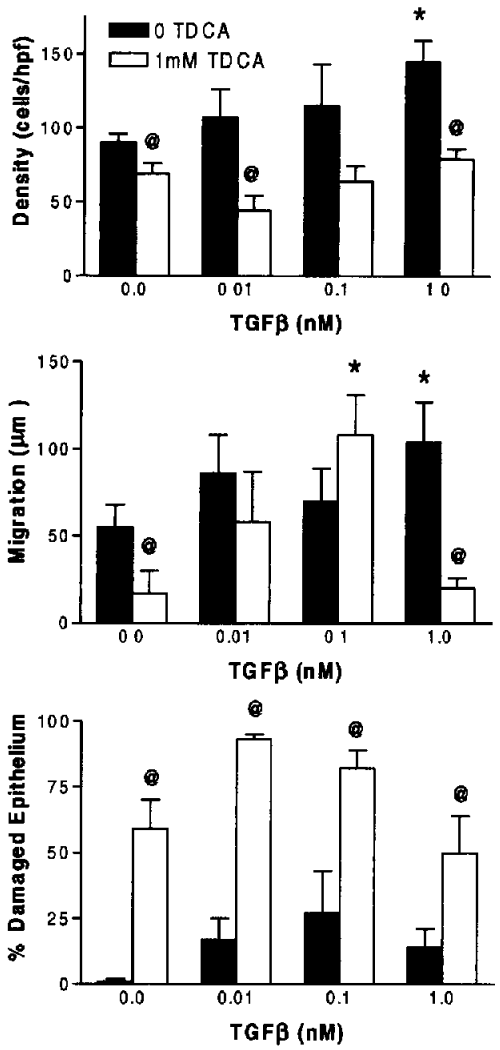

Fig. 3. Effects of varying doses of TGF-β in the presence or absence of 1 mmol/L TDCA on duct epithelial cell density, migration, and epithelial damage as measured morphometrically. * = $P < 0.05$ vs. 0 TGF-β; @ = $P < 0.05$ vs. 0 TDCA; n = 5 to 7 in all groups.

TGF-β was preserved, but cells appeared flattened and abnormal. Because in our grading system flattening of the epithelium was considered a sign of damage, there was no difference in the total percentage of damage with TGF-β treatment. The appearance of the TDCA- and TGF-β–treated PDE was suggestive of apoptosis, and indeed, evidence of apoptosis was detected throughout the epithelium of those explants (see Fig. 1, *F*).

DISCUSSION

Chronic pancreatitis most frequently results from chronic and severe alcohol consumption.[1] In the remaining group of patients, the disease develops either because of a genetic predisposition, because of metabolic abnormalities (hyperlipidemia, hypercalcemia, and protein malnutrition), or for indeterminate reasons (idopathic). Although most authors favor hypotheses that focus on acinar cell dysfunction, we clearly do not understand the mechanisms involved in the evolution of this disease.

The histologic appearance of established chronic pancreatitis is distinguished by extensive replacement of normal acinar tissue by fibrosis, as well as dilatations and strictures of the ductal system.[1] This fibrosis has been implicated in the development of the pain that characterizes this disease and is the most common indication for surgical intervention.[12] Why the pancreas affected by chronic pancreatitis has the propensity to become fibrotic remains unexplained. TGF-β has been implicated in this process because pancreatic tissue levels of TGF-β are increased.[6,7] One of the best-described effects of this peptide growth factor is its ability to stimulate fibroblast growth and extracellular matrix formation.[2-4] Indeed a number of other chronic fibrosing disorders, including glomerulonephritis, hepatic cirrhosis, and idiopathic pulmonary fibrosis, are also characterized by increased TGF-β expression. Pancreatic fibrosis can be induced by either transgenic overproduction of TGF-β in mice or by exogenous administration during repeated episodes of experimental pancreatitis, further supporting this concept.[5,13]

In addition to its effects on connective tissue, TGF-β is generally regarded as an inhibitor of epithelial cell proliferation, although its biologic effects are dependent on the cell type under scrutiny and the context of the study.[6,7] We were able to show here that TGF-β inhibits proliferation of PDE as determined by the level of expression of PCNA. Others have obtained similar results with isolated guinea pig PDE cells, as well as with other cells of pancreatic origin including acinar cells and pancreatic tumor cells.[9,14,15] This is the first evidence that this effect is preserved when duct cells remain attached to their native basement membrane and that the inhibition of growth is associated with apoptosis.[16]

The importance of the basement membrane in regulating epithelial growth is well established, but it is emphasized by our results.[17,18] Epithelial proliferation at the edge of the explant, where the basement membrane ends and the underlying connective tissue (collagen) lies exposed, persisted and appeared to be promoted by low doses of TGF-β together with injury-inducing concentrations of TDCA. This response is consistent with another important biologic function of TGF-β, enhancing repair and re-epithelialization.[6,19-21] In experimental models of pancreatitis, such a role is suggested by the transient increase in TGF-β tissue levels during the development and res-

olution of the inflammatory process.[22-24] The influence of TGF-β appears limited to repair processes. We did not observe a protective effect on the native explant epithelium in the face of TDCA-induced damage. This contrasts with the effect of epidermal growth factor in this setting, where a cytoprotective effect was evident.[8] The low level of TGF-β expression noted in the normal pancreas, particularly in and around the smaller most proximal ductules,[2,25] may serve both to regulate duct cell proliferation and stimulate repair of low-level epithelial breakdown.

Treatment with TGF-β did preserve epithelial integrity, but the increased level of apoptosis in the PDE suggests that preservation of the epithelium was achieved at a price. Necrotic cell death, demonstrated by extensive sloughing of the TDCA-treated epithelium, was converted to apoptotic cell death when TGF-β was added. Slowed loss of the epithelium and the barrier it forms to extravasation of luminal contents would have a teleologic short-term advantage. This may explain why TGF-β expression increases immediately after the induction of acute pancreatitis in animals.[23-25] Indeed the level of apoptosis has been shown to be inversely related to the severity of experimental pancreatitis.[26-28] A number of cytokines whose levels are increased during pancreatitis also regulate apoptosis, but TGF-β appears to be a potent factor in this process in pancreatic cells and probably has a dominant role in the setting of pancreatitis.[29] The short-term benefits of this conversion to apoptosis would be lost if the triggering insult were not removed and the epithelium were not allowed to regenerate, as would be the case with chronic alcohol consumption. At that point the fibrotic response to persistently high TGF-β levels may predominate over any protective effects on the epithelium, leading to the typical histologic changes observed in chronic pancreatitis.[2,3,5,6,13,30]

We thank Kathleen Lally and Richard Milanich for their expert technical assistance.

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Cholangiography During Laparoscopic Cholecystectomy—Cumulative Sum Analysis of an Institutional Learning Curve

Mark Molloy, M.D., Robert H. Bower, M.D., Per-Olof Hasselgren, M.D., Barbara J. Dalton, R.N.

The ability to perform intraoperative cholangiography during laparoscopic cholecystectomy is an essential skill for the laparoscopic biliary surgeon. The volume of experience required to be able to consistently obtain a cholangiogram during laparoscopic cholecystectomy has not been determined. Cumulative sum analysis is a statistical technique which generates a graphical display that identifies periods of performance that fall below a predetermined standard for a given task. The cumulative sum (S_n) for a series of observations is defined as: $S_n = \sum_{i=1}^{n} X_i - X_o$, where $X_i = 0$ for a success, $X_i = 1$ for a failure, and X_o is the acceptable failure rate for the process under study. This function is plotted against the number of observations to create a curve. When the curve has a positive slope, the acceptable failure rate is being exceeded. When it reaches a plateau, the observed failure rate is equal to the acceptable failure rate. When the curve has a negative slope, the observed failure rate is lower than the acceptable failure rate. We performed a cumulative sum analysis of the first 97 intraoperative cholangiograms attempted during laparoscopic cholecystectomy at our institution. The results demonstrated that 46 cases were required to reach a level of proficiency where a cholangiogram could be obtained in 95% of attempts. Success rates of 85% and 90% were achieved at 16 and 25 cases, respectively. This form of analysis is a useful tool for estimating the number of attempts required to achieve a desired success rate when learning new procedures. (J GASTROINTEST SURG 1999;3:185-188.)

KEY WORDS: Laparoscopy, cholecystectomy, cholangiography, learning, experiential

Performance of an intraoperative cholangiogram during laparoscopic cholecystectomy can identify unanticipated common bile duct stones and prevent, or at least facilitate the indentification of, bile duct injuries. It is critical that the laparoscopic biliary surgeon be able to obtain one in a high percentage of attempts. The volume of experience required to be able to consistently obtain a cholangiogram when needed has not been determined.

We performed a cumulative sum (CUSUM) analysis of the first 97 cholangiograms attempted at our facility to determine the number of attempts required to reach a level of proficiency with the technique where a cholangiogram could be obtained in 85%, 90%, and 95% of attempts. The CUSUM (S_n) for a series of observations is defined as: $S_n = \sum_{i=1}^{n} X_i - X_o$, where $X_i = 0$ for a success, $X_i = 1$ for a failure, and X_o = a predetermined acceptable failure rate.[1] For example, assume that a given process has a target success rate of 95%. The acceptable failure rate for the process is then 5%. The CUSUMs for each observation in a series of five trials consisting of a success followed by two failures, followed by two successes would be: −0.05, 0.90, 1.85, 1.80, and 1.75. These values are plotted against the number of observations to produce a curve. When the curve has a positive slope, the acceptable failure rate is being exceeded. When the curve is level, the observed failure rate is equal to the acceptable failure rate. When the curve has a negative slope, the observed failure rate is lower than the acceptable failure rate.

The effectiveness of the analysis can be enhanced by calculating a series of upper control limits (h) for the graph.[2] These limits are derived by establishing a

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Presented at the Thirty-Ninth Annual Meeting of The Society for Surgery of the Alimentary Tract, New Orleans, La., May 17-20, 1998 (poster presentation).

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rate of failure that is unacceptably high and are plotted as a series of sequential horizontal lines across the CUSUM graph at y = h, y = 2h, y = 3h, and so forth. Whenever the curve intersects one of these control limits from below, the unacceptable failure rate is being exceeded. After a control line has been crossed for the first time, the next one above it becomes the new upper control limit. The magnitude of the distance between these lines (h) is calculated as follows[1-4]:

$$h = \frac{a}{P + Q}, \text{ where } a = \ln \frac{1 - \beta}{\alpha}, P = \ln \frac{p_1}{p_0}, \text{ and } Q = \ln \frac{1 - p_0}{1 - p_1},$$

and α is the risk of a type 1 error, β is the risk of a type 2 error, p_1 is the acceptable failure rate, and p_0 is the unacceptable failure rate.

MATERIAL AND METHODS

Demographic and perioperative data points for all patients undergoing attempted laparoscopic cholecystectomy at the Veterans Affairs Medical Center (Cincinnati, Ohio) have been maintained in a prospective database since the procedure was introduced in the facility in early 1993. This registry was used to identify all patients who underwent attempted laparoscopic cholecystectomy between January 1, 1993, and November 15, 1997. The database indicates whether or not cholangiography was attempted and if attempted, whether or not it was successfully completed. Patients who required conversion to open cholecystectomy, or in whom no cholangiography was attempted, were excluded from consideration. The remaining cases were sequenced according to the order in which they were performed and assigned a case number.

CUSUM functions were then calculated as described above for target success rates of 85%, 90%, and 95% for cholangiography during laparoscopic cholecystectomy. These functions were plotted against case number to produce CUSUM graphs. When calculating the magnitude of the distance between control limits (h), the unacceptable failure rate (p_0) used was a level 5% above the acceptable failure rate. For example, when the target success rate was 95%, the acceptable failure rate (p_1) was 5% and the unacceptable failure rate (p_0) was 10%. The risks of both type 1 (α) and type 2 (β) error were set at 5%.

RESULTS

Laparoscopic cholecystectomy was undertaken in 139 patients between January 1, 1993, and November 15, 1997. Twenty-seven cases required conversion to open cholecystectomy, and no cholangiogram was attempted in 15 others. These cases were excluded from analysis, and the remaining 97 patients formed the basis of this study. Median patient age was 61 years (range 23 to 90 years). Eighty-six subjects (89%) were male. The indications for surgery were distributed as follows: biliary colic, 46 cases (47%); acute cholecystitis, 26 cases (27%); biliary pancreatitis, 15 cases (15%); and all others, 10 cases (10%).

A total of eight attending surgeons supervised the performance of the operations in this study. Thirteen of the procedures were done by five surgeons, each of whom supervised six or fewer operations. The remaining 84 operations were supervised by one of the three physician authors of this study. All of the surgeons involved used intraoperative cholangiography liberally if not routinely—a cholangiogram was attempted in 97 of the 112 cholecystectomies that were completed laparoscopically during the study period (87%).

Eighty-one (84%) of the cholangiograms that were attempted during the study were successfully completed. Seven failures occurred among the first 16 cases (44%). Six subsequent failures (cases 23, 25, 37, 46, 49, and 50) occurred between cases 16 and 50. Three failures (6%) were observed among the last 47 procedures.

These findings are graphically demonstrated by the CUSUM plot for an 85% success rate for attempted intraoperative cholangiography (Fig. 1). This curve reaches a plateau after case 16 and stays level until case 51, when it begins a gentle decline. The positive slope of the curve between cases 1 and 16 indicates that the target success rate of 85% was not being met during this period of time. Between cases 16 and 50 there were six additional failures, yielding an overall failure rate of 18% for these 34 cases. Since this failure rate is close to the acceptable failure rate (15% in this instance), the curve is flattened over these cases. The negative slope of the curve over the last 47 cases reflects the low overall failure rate (6%) during this portion of the experience.

Fig. 2 shows the CUSUM plot for a 90% target success rate for performing intraoperative cholangiography during laparoscopic cholecystectomy. The control limits depicted by the horizontal lines have been calculated assuming an unacceptable failure rate of 15%. This curve touches the first upper control limit at case 26, indicating less than a 5% chance that the failure rate up until this point exceeded 15% because of random "clustering" of failures alone. Thereafter the curve plateaus and remains fairly level for the rest of the series. The overall rate of failure during the last 71 cases was 10%—equal to the target rate for this curve. The next control line is therefore never approximated.

The CUSUM graph for a 95% target success rate for intraoperative cholangiography is shown in Fig. 3.

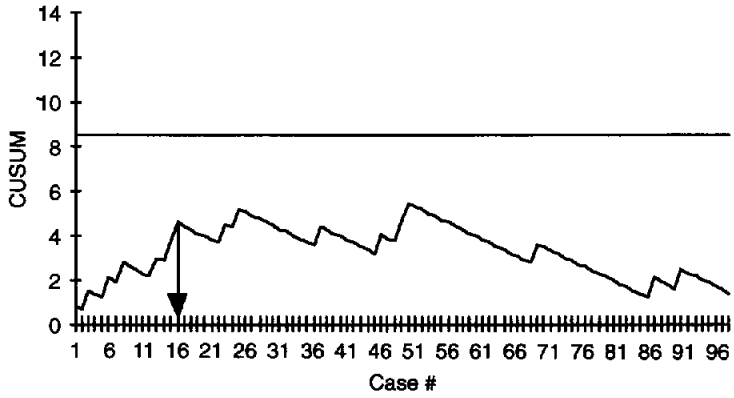

Fig. 1. CUSUM plot for 85% success rate for attempted cholangiography during laparoscopic cholecystectomy. The curve plateaus after case 16, indicating that the target failure rate of 15% is no longer being exceeded. The negative slope of the curve after case 51 indicates that the observed failure rate is lower than the target failure rate during the last half of the series. The first upper control limit is never approached.

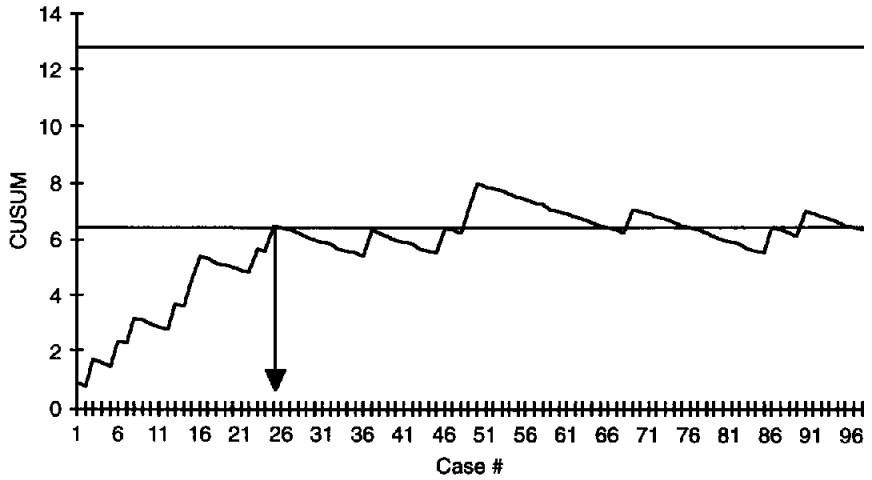

Fig. 2. CUSUM plot for a target success rate of 90% for cholangiography during laparoscopic cholecystectomy. The curve has a positive slope until case 26, then levels off. Intersection with the first control limit at that point indicates that the unacceptable failure rate of 15% has been exceeded up until that point. After case 26, performance remains acceptable for the duration of the series.

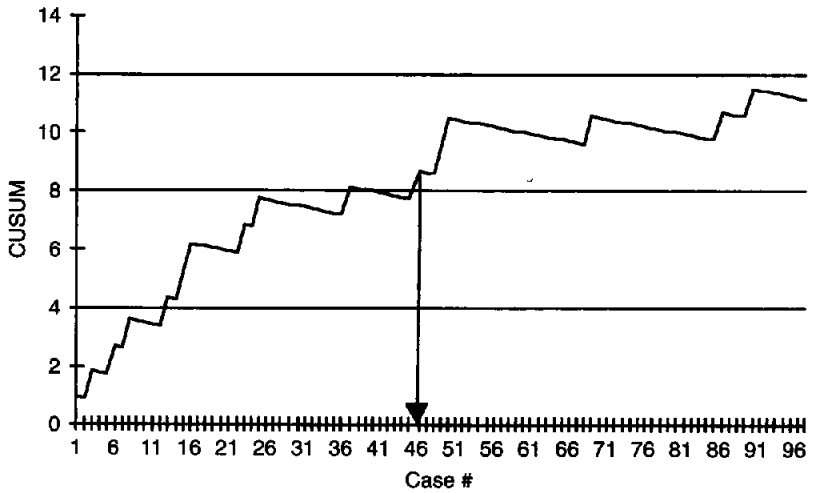

Fig. 3. CUSUM plot for a 95% target success rate for cholangiography during laparoscopic cholecystectomy. The curve increases through two control limits, passing the second one at case 46. Subsequent failures occur at cases 49 and 50, and then the curve plateaus. Although additional failures occur, the third control limit is never crossed, indicating that performance remains within the acceptable range for the duration of the series.

This graph maintains a steady upward slope through two control limits until case 46. Consecutive failures in cases 49 and 50 then cause another small upward surge in the function, which subsequently remains on a fairly stable plateau for the duration of the series. The third control limit is never exceeded, although it is approached after closely clustered failures in cases 86 and 90. This finding indicates that even after the "learning" portion of the experience ends at approximately 50 cases, continued performance at the 95% level remains a challenge to maintain.

DISCUSSION

The ability to perform cholangiography when needed is a critical element of the safe performance of laparoscopic cholecystectomy. It is essential that the laparoscopic biliary surgeon be able to successfully obtain a cholangiogram when needed. Occasionally, however, operative findings such as fibrous obliteration of the cystic duct preclude the performance of this study. Several large series suggest that typical success rates for attempted cholangiography during laparoscopic cholecystectomy range between 70% and 95%.[5-8] A standard of 100% success for attempted intraoperative cholangiography may therefore be unreasonably high and could even contribute to the incidence of biliary complications by encouraging persistent attempts to instrument the biliary tree in situations where this cannot be done safely. Establishing a target success rate for attempted intraoperative cholangiography and then monitoring for sustained achievement of that goal therefore seems to be a valid means of pursuing quality improvement in a laparoscopic biliary surgery program.

This study describes the "learning curve" of an institutional practice with intraoperative cholangiography during laparoscopic cholecystectomy. The findings indicate that approximately 50 cases are needed for a new program to reach a level of proficiency with this technique where a success rate of 95% can reasonably be expected to be maintained. Success rates of 85% and 90% were achieved after 16 and 25 cases, respectively. The fact that failed attempts are still occasionally observed near the end of the series reinforces the need to continue to try obtaining cholangiograms, especially in a relatively low-volume practice such as the one described herein.

The study population consisted of Veterans Administration beneficiaries and therefore had a high percentage of older male patients with acute cholecystitis who had "difficult" cholecystectomies. This effect may have been offset somewhat by the fact that all of the surgeons involved had 1 to 2 years of experience with laparoscopic cholecystectomy in other settings before the procedure was introduced into the Veterans Administration practice.

CUSUM analysis has the advantage of dealing with experience as a continuous variable when studying performance over time. If this series was randomly broken down into discrete blocks of cases for analysis, it would be difficult to account for the effects of random "clustering" of failures into some blocks and not others. In addition, the graphical output of the CUSUM plot provides a visual image of compliance with a given performance standard that is intuitive and easy to understand. It can be used both to evaluate the learning process as new skills are acquired and to monitor compliance with quality improvement goals after those skills have been mastered. The analysis can be performed using any standard spreadsheet program. It provides an objective measure of performance over time that can be an equally valuable tool for individual surgeons and surgical groups at all levels of experience.

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Interval Appendectomy in the Laparoscopic Era

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In the acute setting, patients with periappendiceal masses generally improve with broad-spectrum antibiotics with or without percutaneous catheter drainage, but whether or not to perform an interval appendectomy remains controversial. We have analyzed our experience over the past decade, comparing results from interval laparoscopic appendectomy (ILA) and interval open appendectomy (IOA). Medical records were reviewed for 56 patients who initially presented with the diagnosis of periappendiceal mass or abscess and who subsequently underwent interval appendectomy. Data were accumulated for both the initial hospitalization and interval appendectomy. Comparisons were made between period 1 (1987 to 1993) and period 2 (1994 to 1997). Follow-up data were obtained via telephone conversations with the patients. Patient characteristics with regard to age, sex, and comorbidities did not differ between the ILA and IOA groups. The number of patients undergoing CAT scan increased from 33% to 55%, whereas the initial hospital stay decreased from 7.42 to 4.61 days ($P < 0.001$). The percentage of interval appendectomies performed by the laparoscopic method increased from 30% to 85%. The total operating room time did not differ (95 vs. 103 minutes), but the hospital stay was much shorter in the ILA group (0.55 vs. 3.07 days, $P < 0.001$). There were no instances of intra-abdominal or wound infections in either group. In the later time period the mean hospital stay decreased to 0.38 days, with 59% of the operations performed on an outpatient basis. Following ILA, narcotic pain medication was used for an average of 1.3 days and the reported "time to return to full activities" was 2.5 days. ILA is a simple and safe procedure that can usually be performed on an outpatient basis. Given the minimal morbidity of the procedure, we believe that ILA should be considered for most patients who initially present with periappendiceal masses. (J GASTROINTEST SURG 1999;3:189-193.)

KEY WORDS: Appendectomy, interval, laparoscopy, appendicitis

In some cases of appendicitis, the inflammatory process becomes "walled off," resulting in a periappendiceal mass, either a phlegmon or an abscess. Compared to patients with uncomplicated acute appendicitis, these patients usually have a longer duration of their illness (>5 days), a higher fever, and a more elevated white blood cell count.[1] Controversy exists as to whether these patients should undergo immediate appendectomy or receive initial nonoperative treatment followed by elective interval appendectomy. The initial nonoperative approach has been advocated because surgery in the acute setting can lead to spread of the infection and damage to adjacent loops of bowel with subsequent fistula formation.[1] On the other hand, immediate appendectomy has been shown to decrease the total hospital stay by eliminating readmission for the interval procedure.

The initial treatment of a periappendiceal mass includes broad-spectrum antibiotics and in some cases radiologically guided drainage of an abscess to hasten clearance of the infection. Following successful treatment of a periappendiceal mass, the mean reported incidence of recurrent appendicitis is 13.7% (range 0% to 20%), with the greatest danger during the first year after the initial episode. Several authors have suggested that interval appendectomy is unnecessary because the recurrence rate is so low.[1,2]

Interval appendectomy is classically performed through a McBurney incision. Little has been written

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Supported by the Harvard Center for Minimally Invasive Surgery.
Presented at the Thirty-Ninth Annual Meeting of The Society for Surgery of the Alimentary Tract, New Orleans, La., May 17-20, 1998.
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Table I. Initial hospitalization for nonoperative treatment

	1987 to 1993 (N = 21)	1994 to 1997 (N = 35)
Length of illness prior to seeking attention (days)	9.35 ± 2.45	6.81 ± 0.88
Temperature on admission (°F)	101.4 ± 0.51	101.1 ± 0.32
Patients undergoing CAT scan or ultrasound (%)	33	55
Hospital stay for initial nonoperative treatment (days)*	7.42 ± 1.14	4.61 ± 0.45
Time until elective surgery (wk)	9.85 ± 1.61	8.79 ± 0.61

*$P < 0.05$.

Table II. Interval appendectomies 1987 to 1997

	ILA (N = 38)	IOA (N = 15)
Operating room time (min)	95 ± 5.7	103 ± 9.8
Hospital stay (days)*	0.55 ± 0.12	3.07 ± 0.36

ILA = interval laparoscopic appendectomy; IOA = interval open appendectomy.
*$P < 0.05$.

about the laparoscopic approach in this context. We have reviewed our experience over the past decade (1987 to 1997) at Beth Israel Hospital in Boston to determine whether there are advantages or disadvantages to interval appendectomy in regard to the laparoscopic and open techniques.

PATIENTS AND METHODS

We analyzed the medical records and pathology reports of all patients who initially presented with the diagnosis of periappendiceal mass or abscess and who subsequently underwent interval appendectomy at Beth Israel Hospital (Boston, Mass.) from January 1987 through June 1997 (56 cases). Data were accumulated for both the initial hospitalization and subsequent interval appendectomy. Comparisons were made between period 1 (1987 to 1993) and period 2 (1994 to 1997). The operative approach, interval laparoscopic (ILA) or interval open appendectomy (IOA), and conversions from ILA to IOA were noted. The operating room time represents the time from the patient's entry into the operating room to exit from the operating room. Follow-up data, including time to return to full activities and length of pain medication use, were obtained via telephone interviews with the patients. Because the study spanned a 10½-year period, many patients undergoing surgery before 1994 could not be contacted either because of relocation or death of the patient.

Statistical Analysis

Data are presented as mean ± standard error, with $P < 0.05$ considered statistically significant.

RESULTS

Initial Hospitalization

No significant difference in initial presentation (e.g., length of illness prior to seeking medical attention) was observed between periods 1 and 2. The number of patients undergoing CAT scan or ultrasound before administration of antibiotics increased from period 1 to period 2 (33% vs. 55%), whereas the initial hospital stay decreased (7.42 vs. 4.61 days, $P < 0.05$). Those patients not undergoing diagnostic imaging received empiric antibiotic treatment. A few patients presented with abscesses requiring percutaneous drainage (one during period 1 and three during period 2). There was no difference between periods 1 and 2 in terms of time until elective surgery (9.85 vs. 8.79 weeks) (Table I). Most interval appendectomies were open during period 1 and most were laparoscopic during period 2.

Interval Appendectomy

The percentage of interval appendectomies performed by the laparoscopic method increased from 30% to 85% between periods 1 and 2. From January 1987 through June 1997, 56 interval appendectomies (38 ILA, 15 IOA, and three conversions) were performed at Beth Israel Hospital. Patient characteristics with regard to age, sex, and comorbidity did not differ between the ILA and IOA groups. Overall the total operating room time did not differ (95 vs. 103 minutes), but the hospital stay was markedly shorter in ILA patients compared to IOA patients (0.55 vs. 3.07 days) (Table II).

Period 1 was subdivided into two distinct time frames: (1A) 1987 to 1990 (all interval appendec-

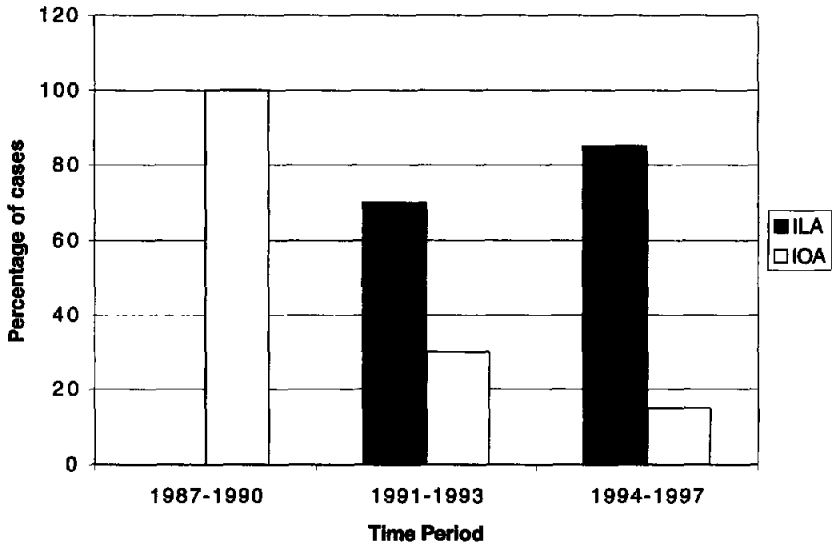

Fig. 1. Trend in performance of interval appendectomies. From 1987 to 1990 all interval appendectomies were open *(IOA)*, whereas from 1994 to 1997 most interval appendectomies were laparoscopic *(ILA)*. A transition period occurred from 1991 to 1993.

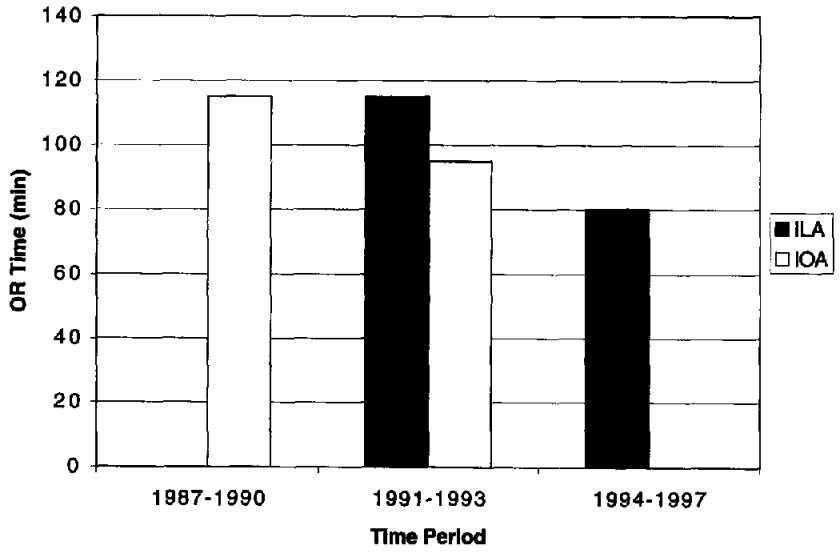

Fig. 2. Comparison of operating room *(OR)* time for interval laparoscopic appendectomy *(ILA)* vs. interval open appendectomy *(IOA)*.

tomies were open) and (1B) 1991 to 1993 (transition period). During period 2 (1994 to 1997) most interval appendectomies were laparoscopic (Fig. 1). The operating room time continued to be similar for ILA and IOA during each time period (Fig. 2). The hospital stay was always shorter for ILA compared to IOA, and during period 2 the hospital stay for ILA decreased to 0.38 days, with 59% of the operations performed on an outpatient basis (Fig. 3). There were no instances of intra-abdominal or wound infections in either group. Pathologic examination revealed that 34% of the appendices had acute inflammatory changes, 28% had chronic changes, 24% had fibrosis, 8% were normal, and 6% had both acute and chronic changes (Fig. 4).

Follow-up for the ILA patients showed an 89% response rate and demonstrated that narcotic pain medication was used for an average of 1.3 days, and the average time to return to full activities was 2.5 days. Follow-up for IOA patients showed only a 20% response rate because of patient relocation or patient death. Narcotic pain medication for IOA patients was used for 5.7 days, and the average time to return to full activities was 7.2 days (Table III).

Table III. Follow-up data

	ILA (N = 38)	IOA (N = 15)
No. of patients responding	34 (89%)	3 (20%)*
Length of pain medication use (days)	1.3	5.7
Time to return to full activities (days)	2.5	7.2

ILA = interval laparoscopic appendectomy; IOA = interval open appendectomy.
*NOTE: The poor response rate for IOA patients was related to the long time period since the operation.

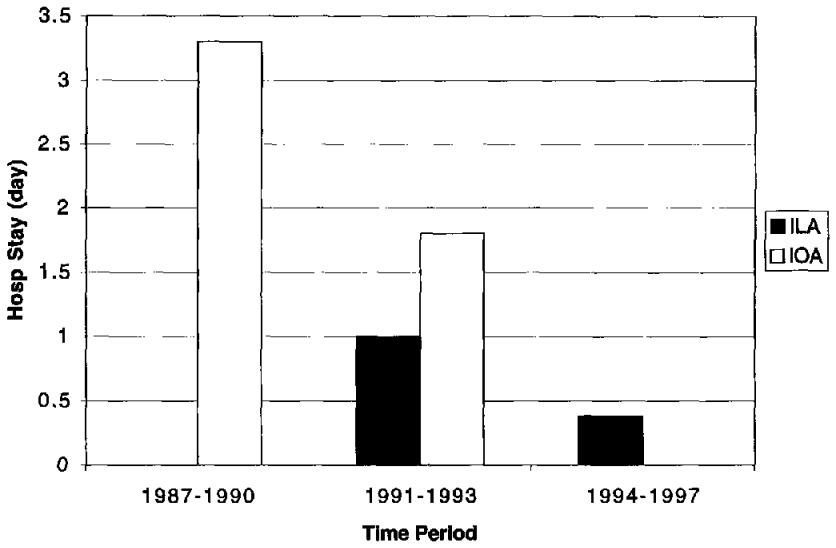

Fig. 3. Comparison of hospital stay for interval laparoscopic appendectomy *(ILA)* vs. interval open appendectomy *(IOA)*. From 1994 to 1997 59% of ILA cases were performed on an outpatient basis.

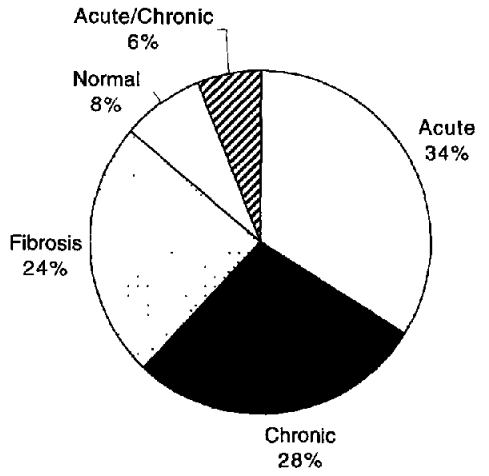

Fig. 4. Pathologic findings following interval appendectomies.

DISCUSSION

The issues surrounding interval appendectomy remain controversial. Many authors favor immediate appendectomy, suggesting that the hospital stay during the initial nonoperative treatment is long and costly.[2-4] Thomas[5] stated that the initial hospital stay for nonoperative treatment appears to be almost double that of patients having an early operation. Vargas et al.[3] also reported an initial hospital stay of 10 days. However, our study found a much shorter initial hospital stay of only 4.61 days during period 2. Other authors have pointed out that readmission for interval appendectomy adds further costs. However, Vargas et al.[3] wrote about their experience with ILA and reported a hospital stay of only 1 day, presumably reflecting the advantages of minimally invasive surgery. In our study the hospital stay for ILA was even shorter, 0.55 days. During the later time period the hospital stay was only 0.38 days, with 59% of ILAs performed on an outpatient basis. The short hospital stay for ILA is clearly advantageous when compared to IOA. The cumulative hospital stay for both the initial nonoperative treatment and for the stay following ILA was not very long (5.16 ± 0.57 days), in contrast to the median total stay of 21 days reported by Skoubo-Kristensen and Hvid.[4] Our study also differs from that of Ein and Schandling,[5] who reported that interval appendectomy patients spend an additional 3 to 13 days in the hospital. Barnes et al.[6] fur-

ther argued against interval appendectomy noting that a third of the appendices removed during the elective procedure showed no evidence of previous inflammation. However, our study found that only 8% showed no diagnostic abnormalities in pathology reports, with most other cases demonstrating inflammatory changes. Whether the pathologic findings at the time of interval appendectomy correspond to the probability of having recurrent appendicitis is not known.

Many studies have reported a longer duration of operation for laparoscopic appendectomy compared to open appendectomy.[7-13] In our study the operating room time for ILA was no different from that for IOA, thus negating one of the arguments against laparoscopic appendectomies. The operating room time for ILA decreased with time, reflecting the learning curve seen in other areas of laparoscopic surgery.[14]

The minimal morbidity of ILA is evidenced by the decreased hospital stay, lack of complications, short use of postoperative pain medication, and rapid return to full activities.

In summary, interval appendectomy following initial nonoperative treatment for an appendiceal mass may result in minimal or no increase in cumulative hospital stay. ILA results in a marked decrease in the hospital stay compared to IOA, without a significant difference in operating room time. ILA is a simple and safe procedure that usually can be performed on an outpatient basis. Given the minimal morbidity of the procedure, we believe that ILA should be considered for most patients who initially present with a periappendiceal mass.

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Endoscopic Laser Ablation of Nondysplastic Barrett's Epithelium: Is It Worthwhile?

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The clinical value of endoscopic ablation of nondysplastic Barrett's epithelium is controversial. It has been stated that ablation, combined with acid suppression or antireflux surgery, may reduce the risk of adenocarcinoma, thereby obviating the need for endoscopic surveillance in these patients. Eighteen symptomatic patients were enrolled in a prospective study of Nd:YAG laser ablation of Barrett's esophagus followed by treatment with proton pump inhibitors or antireflux surgery. All patients had intestinal metaplasia and no associated dysplasia or carcinoma. Laser treatment was performed with noncontact fibers and a power output of 60 watts. The mean number of treament sessions was three (range 1 to 5), and the mean energy delivered during each session was 2800 joules (range 600 to 4800 joules). All patients were given a standard dose of omeprazole (40 mg/day) throughout the study period. In two patients a mild distal esophageal stricture occurred and required a single dilatation. Macroscopic and histologic eradication of the specialized columnar epithelium was documented in 8 of 12 patients with tongues of Barrett's metaplasia, in one of four patients with circumferential Barrett's metaplasia, and in two of two patients with short-segment Barrett's esophagus. In five patients (28%) only a partial ablation could be achieved despite repeated laser treatment. Two patients (11%), one with tongues and the other with circumferential Barrett's metaplasia, were considered nonresponders. Adenocarcinoma undermining regenerated squamous epithelium was found, 6 months after eradication, in one patient who underwent esophagogastric resection. Twelve patients agreed to undergo antireflux surgery. Over a mean follow-up period of 14 months (range 4 to 32 months), two patients presented with recurrent Barrett's metaplasia: one at 8 months after successful Nissen fundoplication and the other after 1 year of continuous omeprazole treatment. Progression of Barrett's metaplasia was found in two other patients receiving pharmacologic therapy in whom a partial response to laser treatment had been obtained. In conclusion, Nd:YAG laser therapy of nondysplastic Barrett's esophagus, performed in conjunction with omeprazole treatment and followed by antireflux surgery, allows a partial regression of specialized columnar epithelium in most patients. However, this is a time-consuming procedure that produced only temporary eradication, did not prove effective in reducing cancer risk, and did not obviate the need for endoscopic surveillance. (J GASTROINTEST SURG 1999; 3:194-199.)

KEY WORDS: Barrett's esophagus, proton pump inhibitors, Nd:YAG laser, adenocarcinoma, Nissen fundoplication

Columnar-lined esophagus with specialized intestinal metaplasia, known as Barrett's esophagus, is a complication of gastroesophageal reflux disease and a premalignant condition. The incidence of adenocarcinoma of the esophagogastric junction in these patients is dramatically increasing. It has been estimated that the risk of developing cancer is 30 to 50 times greater in patients with Barrett's esophagus than in the general population; this corresponds to 500 cancers per year per 100,000 persons with Barrett's esophagus, a figure similar to the risk of lung cancer in white men older than 65 years.[1] At present, endo-

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Presented at the Thirty-Ninth Annual Meeting of The Society for Surgery of the Alimentary Tract, New Orleans, La., May 17-20, 1998.
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scopic surveillance is the only strategy that has the potential to improve long-term survival by allowing the detection of this lethal disease at an early stage.[2,3] Therefore a treatment that could effect the regression of columnar metaplasia in the esophagus might decrease the risk of adenocarcinoma and obviate the need for endoscopic surveillance.

Normalization of esophageal pH with high-dose proton pump inhibitor therapy[4] or with antireflux surgery[5-8] does not consistently result in regression of Barrett's esophagus; neither do these treatments halt the evolution to adenocarcinoma. Recently a number of studies on endoscopic ablation of Barrett's epithelium combined with medical or surgical therapy of gastroesophageal reflux have been published. However, patients with dysplasia or even with superficial carcinoma have been included by some investigators,[9-11] making it difficult to assess the impact of mucosal ablation on the incidence of cancer.

To assess the effectiveness of laser ablation combined with acid suppression therapy in the management of uncomplicated Barrett's esophagus, a prospective study was undertaken in a series of patients who were candidates for antireflux surgery.

PATIENTS AND METHODS

Between April 1994 and December 1997, 18 patients with biopsy-proved specialized columnar epithelium lining the distal esophagus were entered into the study. Patients over 70 years of age and those with evidence of dysplasia or adenocarcinoma were excluded from the study. All patients were concomitantly treated with proton pump inhibitors (omeprazole, 40 mg/day) and were given the option of antireflux surgery on completion of laser therapy. The protocol was approved by the Ethics Committee of the Ospedale Maggiore Policlinico of Milan. Informed consent was obtained from all patients.

There were 14 men and four women whose mean age was 55 years (range 32 to 70 years). All were symptomatic, and the duration of reflux complaints varied between 2 and 25 years.

Endoscopy with Lugol staining was performed with a standard video endoscope. All of the endoscopic procedures were videotaped. Multiple biopsies with jumbo forceps (Megabyte, Microinvasive/Boston Scientific Corp., Boston, Mass.) were performed at the four cardinal points every centimeter from the gastric cardia up to a level proximal to the squamocolumnar junction. An average of 19 biopsies were obtained from each patient (range 4 to 24). The length of Barrett's metaplasia, measured from the proximal margin of columnar-lined epithelium to the oral end of the gastric mucosal folds, averaged 4 cm (range 1 to 7 cm). The areas of Barrett's metaplasia were defined as circumferential, tongues, and short segments (less than 3 cm in length).

Esophageal manometry and 24-hour esophageal pH monitoring were performed in all patients. Fourteen of the 18 individuals had a manometrically defective lower esophageal sphincter, that is, pressure $\leq$6 mmHg, overall length $\leq$2 cm, or abdominal length $\leq$1 cm. All individuals had an abnormal esophageal acid exposure, that is, percentage of total time at pH $<$4 greater than 4.2%.[12]

Laser treatment was performed under local pharyngeal anesthesia and conscious sedation with diazepam. Intravenous Buscopan was administered whenever necessary to inhibit esophageal muscle contractions. After Lugol staining of the esophageal mucosa, photoablation of columnar epithelium was performed in an aboral direction using an Nd:YAG laser with a wavelength of 1064 nm (Sharpland 2100, Laser Industries, Tel Aviv, Israel). The energy was delivered continuously with a power output of 60 watts using noncontact fibers. The mean energy delivered for each session was 2800 joules (range 600 to 4800 joules). In patients with circumferential Barrett's esophagus, only half the circumference of the mucosa was treated during each session to avoid the risk of stricture. The most distal end point of laser ablation was the proximal border of the gastric mucosal folds.

After the first laser treatment, all patients underwent endoscopy on a regular basis every 4 weeks for up to 6 months. Whenever necessary, laser treatment was repeated to ablate residual areas of intestinal metaplasia.

Omeprazole at the standard dose of 40 mg daily was given to all patients continuously throughout the study period. On completion of laser therapy all patients were given the option to undergo antireflux surgery. In these subjects esophageal manometry and 24-hour pH monitoring were repeated within 6 months after the operation. The Mann-Whitney test was used to compare pre- and postoperative manometric and pH data. Statistical significance was established at the 0.05 level.

RESULTS

The mean number of endoscopic sessions per patient was three, ranging from a single session in patients with short-segment Barrett's esophagus to five sessions in patients with circumferential Barrett's metaplasia. Transient chest pain was recorded in all patients during or after laser therapy, but none of them required analgesic medications. In two patients with circumferential Barrett's metaplasia, a mild distal esophageal stricture developed and required a single

Table I. Outcome of endoscopic laser ablation in three subgroups of patients with Barrett's esophagus

	No. of patients	Mean length (cm)	Mean no. of sessions	Complete response	Partial response	No response
Tongues	12	4	3	8	3	1
Circumferential	4	6	3	1	2	1
Short segment	2	2.5	2	2	—	—

Table II. Results of esophageal manometry and 24-hour pH monitoring in 12 patients who underwent antireflux surgery

	Preoperative	Postoperative	P value
LES pressure (mm Hg)	9.8 ± 4.0	16.7 ± 5.1	<0.05
LES abdominal length (cm)	1.0 ± 0.6	2.2 ± 1.2	<0.05
Distal contraction amplitude (mm Hg)	41.3 ± 18	44 ± 21	NS
% Time pH <4 in 24 hr	37.8 ± 20	4.3 ± 7.2	<0.01

LES = lower esophageal sphincter. Values are expressed as mean ± standard deviation; NS = not significant.

dilatation for relief of dysphagia. Both patients underwent five sessions of laser therapy, with a total energy delivered of 18,000 and 24,000 joules, respectively.

An average of eight biopsies (range 4 to 20) were obtained from each patient after laser therapy. Macroscopic and histologic eradication of the specialized columnar epithelium was documented in 8 of the 12 patients with tongues of metaplasia, in one of the four with circumferential metaplasia, and in both patients with short-segment Barrett's esophagus. In five patients (28%) only a partial ablation could be achieved despite repeated laser treatment. Two patients (11%), one with tongues and the other with circumferential Barrett's metaplasia, were considered nonresponders. Table I shows the outcome of endoscopic treatment according to the type of Barrett's esophagus.

Adenocarcinoma undermining regenerated squamous epithelium was found, 6 months after complete Barrett's ablation, in one patient who had previously been subjected to 38 endoscopies and in whom dysplasia or cancer had never been documented. The tumor originated within the area of the previously treated tongue of metaplasia. Pathologic examination of the resected esophagus showed a pT1N0 adenocarcinoma without dysplastic areas at the border of the tumor (Fig. 1).

Twelve of the remaining 17 patients agreed to undergo antireflux surgery. Ten individuals had a laparoscopic Nissen fundoplication and one had a laparoscopic Toupet fundoplication. In one patient with a previously failed open fundoplication, an open Nissen fundoplication was performed. There was no morbidity. At a mean postoperative follow-up of 11 months (range 4 to 42 months), all patients were free of symptoms except the one who was operated on through an open approach. This individual complained of persistent heartburn and regurgitation. As a group, esophageal manometry showed a statistically significant increase in lower esophageal pressure ($P < 0.05$) and abdominal length ($P < 0.05$) compared to preoperative values. Contraction amplitude in the distal esophageal body remained unchanged. Esophageal acid exposure returned to normal in all patients except the one who was symptomatic. Table II shows the pre- and postoperative results of esophageal manometry and 24-hour pH monitoring.

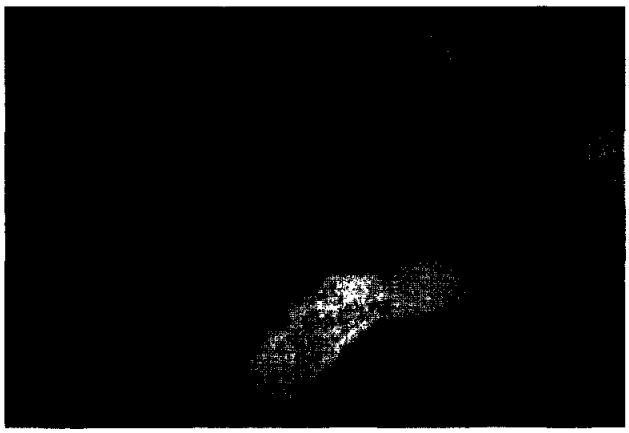

Fig. 1. Esophageal biopsy performed 6 months after successful laser ablation of Barrett's epithelium showing regenerated squamous epithelium overlying adenocarcinoma. (Hematoxylin and eosin stain. ×25.)

Over a mean follow-up period of 14 months (range 4 to 32 months), two of the patients with tongues of Barrett's metaplasia presented with recurrent metaplasia: one at 8 months after a successful Nissen fundoplication and the other after 1 year of continuous omeprazole treatment. Progression of metaplasia was

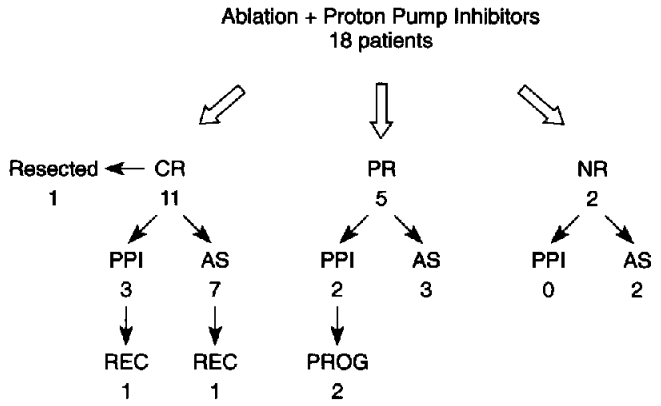

Fig. 2. Flow chart showing overall results of laser ablation therapy combined with medical and surgical antireflux therapy in 18 patients. CR = complete response; PR = partial response; NR = no response; PPI = proton pump inhibitors; AS = antireflux surgery.

found in two patients with circumferential Barrett's esophagus who were receiving medical treatment and in whom a partial response to laser treatment had been obtained (Fig. 2).

DISCUSSION

Several studies have indicated that limitation of esophageal acid exposure is not sufficient to promote reversion of columnar to squamous epithelium in patients with Barrett's esophagus. Therefore research efforts have been directed toward endoscopic ablation of the mucosa and concomitant suppression of gastroesophageal reflux to allow regeneration of squamous epithelium.[13,14]

In 1992 Brandt and Kauvar[15] treated one patient with short-segment Barrett's esophagus with Nd:YAG laser followed by omeprazole, 20 mg daily. Endoscopic follow-up 6 weeks after ablation reveled normal esophageal mucosa, but recurrence of columnar epithelium was documented 14 weeks after treatment. In 1993 Berenson et al.[16] reported on 10 patients treated with argon laser and acid suppression with omeprazole, 40 mg daily. Partial or complete regrowth of squamous epithelium occurred both by spread from contiguous squamous epithelium and from glandular tissue. Barham et al.[17] found squamous regeneration after KTP laser ablation in 16 patients with nondysplastic Barrett's esophagus treated with 40 mg omeprazole daily. However, in 11 patients, some glandular nests persisted beneath the regenerated squamous epithelium.

The problem with long-term medical therapy for Barrett's esophagus is that patients who are symptom free on proton pump inhibitors may still have abnormal gastroesophageal reflux.[18,19] Compared to omeprazole therapy, antireflux surgery has the potential to provide a totally reflux-free environment by preventing reflux of both gastric and duodenal contents. Salo et al.[20] treated 11 patients with Nd:YAG laser with a sapphire contact tip after successful antireflux surgery and found regeneration of squamous epithelium in all individuals followed for a mean of 26 months. However, two patients still had intestinal metaplasia in the gastric cardia.

The result of the present study confirm that squamous reepithelization occurs after laser ablation of Barrett's mucosa under chronic acid suppression therapy with omeprazole. Eleven (61.1%) of the 18 patients in our series had a complete macroscopic and histologic response. However, in one of these patients an occult adenocarcinoma was discovered 6 months later. To our knowledge the development of adenocarcinoma after endoscopic ablation in a patient with no previous evidence of dysplasia or cancer on endoscopic biopsies has never been reported. The fact that even in patients with apparently nondysplastic Barrett's mucosa the concealed glandular mucosa may carry a continuing risk of malignant progression is of great concern. This is the reason why partial regression of Barrett's esophagus is probably an inadequate end point, and surveillance for malignancy should continue in these patients.[21]

The results obtained with endoscopic laser ablation combined with medical or surgical antireflux therapy suggest that this multimodal approach has a major impact on the natural history of Barrett's esophagus. However, since the persistence of glandular tissue beneath the regenerated squamous epithelium may influence the risk of adenocarcinoma and does not obviate the need for endoscopic surveillance, laser ablation of Barrett's esophagus should still be considered investigational. In addition, it has been suggested that the increased proliferation of metaplastic cells induced by the laser thermal injury might even increase the risk of malignant transformation in patients with incomplete ablation of columnar mucosa.[22]

Recurrence of Barrett's mucosa after successful endoscopic eradication has previously been reported in the literature,[15,16] and it occurred in two patients in our series. Again, this finding suggests that laser photoablation does not eliminate the need for endoscopic surveillance; moreover, detection of malignancy is more difficult after laser ablation since reepithelization with squamous mucosa may harbor the adenocarcinoma. Therefore biopsy samples must be of sufficient size to permit detection of underlying glandular mucosa.

The small number of patients in our nonrandomized study does not allow us to determine whether antireflux surgery is superior to pharmacologic therapy. After complete macroscopic and histologic ablation,

there was one recurrence of Barrett's epithelium among the seven patients who underwent successful Nissen fundoplication and one recurrence among three patients undergoing medical therapy. Moreover, it should be noted that two patients with a partial response to ablation therapy had progression of metaplasia under medical therapy; conversely, in five patients who underwent fundoplication after partial or no response to ablation therapy, the extent of Barrett's epithelium remained unchanged. This may suggest that in patients with nondysplastic Barrett's epithelium, fundoplication is superior to continuous pharmacologic therapy.

CONCLUSION

Nd:YAG laser photoablation of nondysplastic Barrett's epithelium is a time-consuming treatment that can achieve eradication of metaplastic epithelium in approximately 50% of the patients. The procedure does not seem to decrease the risk of adenocarcinoma and does not obviate the need for regular endoscopic surveillance. Antireflux surgery is effective in restoring esophageal acid exposure to normal and appears to decrease the risk of glandular reepithelization.

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Discussion

Dr. C. Bremner (Los Angeles, Calif.). This is an important study because it highlights the difficulties and dangers that you have encountered with this procedure. First, the ablation is incomplete. Second, you have reepithelialization over columnarization, sometimes with underlying adenocarcinoma, and you have had progression and two mild strictures. So my questions are as follows: Is there a chance that this technique will ever be able to completely ablate columnarization? Should you perhaps be using vital staining to try and detect the remaining columnar epithelium? With ordinary endoscopy this is not being done, and this is not the only paper presented this week that has highlighted this problem of incomplete ablation. It is happening with all techniques. Finally, some good results have been re-

ported by Salo et al. (Ann Surg 1998;227:40-44) who performed a Nissen fundoplication first, followed by the ablation. Do you think there is room for that? Finally, because of the results reported generally at this meeting, do you think this technique should be abandoned completely in favor of other methods?

Dr. L. Bonavina. In answer to your first question, I believe that the end point of this multimodal approach should be eradication, that is, complete ablation of Barrett's metaplasia. We attempted to accomplish this by routinely staining the mucosa with Lugol's solution, but despite that, we were successful in only about 50% of the patients. In addition, we had a patient with adenocarcinoma undermining regenerated squamous epithelium, and this was of much concern to us. In answer to your last question, I am aware of the report by Dr. Salo and his group. We started this way because we thought it could be safer to do the ablation first followed by fundoplication. Salo et al. have reported excellent results and almost 100% eradication with a YAG laser. Probably there is a difference in technique. They used contact fibers. I do not know if this can affect the results of treatment. In line with the preliminary studies of your group, mechanical ultrasonic ablation may replace the laser technique in the future. The ultrasonic dissection combines the advantages of peeling of the mucosa and providing the ultimate cytologic examination.

Dr. J. Hunter (Atlanta, Ga.). We conducted a similar pilot study where we performed fundoplication first and then treated the patient with a laser. There have been many pilot studies and no long-term studies because it becomes very difficult. Attempting treatment inside the fundoplication with a laser is difficult because the valve is closed.

Dr. J. Maber (Iowa City, Iowa). We have been conducting a similar prospective pilot study using surgery beforehand and I have seen some fairly impressive results but, as Dr. Hunter stated, it is difficult to perform treatment inside the fundoplication. It also seems a bit difficult to tell when the Barrett's metaplasia is gone. Close to the esophagogastric junction, what you think is Barrett's metaplasia I might think is a normal esophagogastric junction. How do you make that distinction? How do we tell if the Barrett's metaplasia is really gone? In some cases there is no question that it has reverted to squamous mucosa, but it doesn't look like normal squamous mucosa; it looks very thin, and I wonder if we shouldn't be doing deeper biopsies for full evaluation, maybe something similar to the suction biopsies used by Drs. Polk and Richardson 10 to 15 years ago to look at esophagitis resolution after a Nissen fundoplication.

Dr. Bonavina. Regarding your first question, our end point was the eradication of Barrett's metaplasia, so we looked for the histologic disappearance of glandular epithelium. As far as your second question is concerned, I totally agree; in this study we routinely performed deep "jumbo" biopsies. I think it is the only way to find out whether residual intestinal metaplasia is still underneath.

Dr. Maber. How do you separate metaplasia of the cardia from metaplasia in the esophagus itself?

Dr. Bonavina. We rely mostly on Lugol staining, but you are probably correct—we may overlook some intestinal metaplasia around the squamocolumnar junction.

Caco-2 Cell Differentiation Is Associated With a Decrease in Stat Protein Levels and Binding

Shan Wang, M.D., Ph.D., B. Mark Evers, M.D.

Novel proteins of the Stat (signal transducers and activators of transcription) family have been associated with proliferation and differentiation of certain cells; the role of these transcription factors in gut differentiation has not been examined. The purpose of this study was to determine whether the cellular levels and actual binding of the Stat proteins are altered with intestinal differentiation using the Caco-2 cell line that spontaneously differentiates to a small bowel phenotype after confluency. We found that both Stat3 and Stat5 protein levels were increased in preconfluent and confluent Caco-2 cells; levels then decreased with postconfluency. Mobility shift assays demonstrated maximal binding of Stat3 and Stat5 at confluency and, similar to protein levels, binding activity decreased with postconfluency. The intestinal differentiation marker gene sucrase-isomaltase was increased by postconfluent day 1 with maximal levels by day 6. The progressive decrease of Stat3 and Stat5 protein levels and binding activity, occurring at a time associated with increased Caco-2 cell differentiation, suggests that a decrease in the cellular levels of these proteins may potentially play a role in subsequent intestinal cell differentiation. Delineating the cellular mechanisms responsible for intestinal differentiation is crucial to a better understanding of both normal gut development and aberrant gut growth. (J GASTROINTEST SURG 1999;3:200-207.)

KEY WORDS: Stat proteins, intestinal differentiation, signal transduction pathways

The mammalian intestine is lined by a complex and continuously renewing epithelium characterized by a highly regimented progression of cellular proliferation and differentiation.[1,2] Stem cells localized to the crypt give rise to four primary epithelial cell types: absorptive enterocytes, goblet cells, Paneth cells, and enteroendocrine cells.[3,4] When these four predominant epithelial cell types emerge from the proliferating crypt, they acquire differentiated characteristics and express a variety of terminally differentiated gene products that are maintained in a strict spatial- and temporal-specific pattern along the horizontal and vertical gut axes. The mechanisms responsible for cell growth arrest and establishment of differentiated cells occupying specific positions along the gut axes are largely unknown.

Stimulation of downstream transcription factors by various growth factors and cytokines is responsible for their ultimate effects on cellular growth and differentiation. An important advance in the understanding of the mechanisms by which these agents regulate cellular functions has been the identification and characterization of the Stat (signal transducers and activators of transcription) signal transduction pathway.[5-9] To date, six distinct but homologous members of the Stat family have been identified (designated Stat1 to Stat6).[10] Stat proteins 1, 3, and 5 are more ubiquitous, better characterized, and have been demonstrated to play a role in cellular proliferation, differentiation, and apoptosis.[11-18] In contrast, the remaining Stat proteins are relatively tissue specific and appear to participate more in immune function.[10] The Stat pro-

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Supported by grants R01 DK48498, R01 AG10885, and P01 DK35608 from the National Institutes of Health and the James E. Thompson Memorial Foundation.
Presented at the Thirty-Ninth Annual Meeting of The Society for Surgery of the Alimentary Tract, New Orleans, La., May 17-20, 1998.
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teins, located in the cytoplasm, are rapidly tyrosine phosphorylated in response to certain cytokines or growth factors and subsequently dimerize and translocate to the nucleus where they bind to variations of a consensus palindromic 9 base-pair recognition sequence and activate target genes.[10] Despite the fact that a number of studies have examined the mechanisms of Stat activation, the biologic function of the Stat proteins, particularly in the gut epithelium, has not been assessed.

Therefore the purpose of our study was to determine whether the cellular levels and actual binding of the Stat proteins are altered during the process of differentiation of the gut-derived Caco-2 cell line. Caco-2, a human colon cancer cell line, provides a unique and well-characterized model system for the evaluation of gut differentiation, since these cells undergo differentiation to a small bowel–like phenotype with microvilli, dome formation, and expression of sucrase-isomaltase (SI) noted after the cells have reached confluency.[19]

MATERIAL AND METHODS

Restriction, ligation, and other DNA-modifying enzymes were purchased from Promega Corp. (Madison, Wisc.) or Stratagene (La Jolla, Calif.). The protease inhibitors were from Sigma Chemical (St. Louis, Mo.). Poly(dI·dC) was purchased from Pharmacia LKB Biotechnology, Inc. (Piscataway, N.J.), and radioactive compounds were obtained from Du Pont–New England Nuclear (Boston, Mass.). Autoradiography film was purchased from Kodak (Rochester, N.Y.). Oligonucleotides containing a consensus *sis*-inducible enhancer (SIE) element, which binds both Stat1 and Stat3, and a consensus β-casein probe, which binds Stat5, were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, Calif.). In addition, antibodies to Stat1(sc-591), Stat3(sc-482), and goat antimouse immunoglobulin G (IgG) (sc-2005) were from Santa Cruz Biotechnology. Donkey antirabbit IgG (NA934) and the enhanced chemiluminescence system for Western immunoblot analysis were obtained from Amersham Corp. (Arlington Heights, Ill.). Monoclonal anti-Stat5 (s21520) was from Transduction Laboratories, Inc. (Lexington, Ky.). Tissue culture reagents were from Gibco Laboratories (Grand Island, N.Y.) and fetal calf serum was from Hyclone Laboratories (Logan, Utah). Immobilon-P nylon membranes for Western blots were purchased from Millipore Corp. (Bedford, Mass.). Nitrocellulose filters for Northern blots were from Sartorius (Göttingen, Germany). Antisense RNA probes were labeled using an in vitro transcription kit purchased from Promega. All other reagents were of molecular biology grade and were obtained from either Sigma or Amresco (Solon, Ohio).

Cell Culture

The human colon cancer cell line Caco-2, obtained from American Type Culture Collection (Rockville, Md.), was maintained in modified Eagle's medium supplemented with 15% (volume/volume) fetal calf serum. Cells were maintained in a humidified atmosphere of 95% air and 5% carbon dioxide at 37° C. Studies were performed on preconfluent (50% and 70% confluency), confluent, and postconfluent (1, 2, 3, and 6 days) Caco-2 cells.

RNA Extraction and Northern Blot Analysis

Cells were harvested, and RNA was obtained by the method of Schwab et al.[20] Polyadenylated [poly(A)$^+$] RNA was selected from all samples by oligo(dT) cellulose column chromatography, and the final RNA concentration was quantified spectrophotometrically by the absorbance at 260 nm. For Northern blot analysis, poly(A)$^+$ RNA (5 μg) was electrophoresed in a 1.2% agarose-formaldehyde gel, transferred to nitrocellulose, and hybridized with an [α-^{32}P] CTP-labeled cRNA probe (pHSI-1) containing 420 base pairs of the human SI gene subcloned into the *Sa/I/Eco*RI site of a Bluescript vector (kindly provided by Dr. Peter Traber, University of Pennsylvania).[21] Hybridization and washing conditions were described previously.[22]

Preparation of Nuclear Extracts and Western Immunoblot Analysis

Nuclear extracts were prepared from Caco-2 cells according to the method described by Schreiber et al.[23] over a time course. The extracts were quick-frozen and stored in aliquots at −80° C and used within 2 months of extraction. Western immunoblot analyses were carried out as described previously.[22] Briefly, nuclear protein samples (30 μg protein/lane) were resolved by sodium dodecyl sulfate–7.5% polyacrylamide gel electrophoresis (SDS-PAGE) and then electroblotted onto Immobilon-P nylon membranes. Filters were incubated overnight at room temperature in blocking solution (Tris-buffered saline containing 5% nonfat dry milk and 0.05% Tween 20), followed by a 4-hour incubation with the rabbit anti-Stat1 (1:500), anti-Stat3 (1:500), and mouse anti-Stat5 (1:1000). Filters were washed three times and incubated with a horseradish peroxidase–conjugated goat antirabbit or antimouse immunoglobulin as the secondary antibody (1:1000 dilution) for 1 hour. After

three final washes, the immune complexes were visualized using enhanced chemiluminescence detection.

Electrophoretic Mobility Shift Assay

Electrophoretic mobility shift assays (EMSAs) were performed as described previously.[24] Briefly, nuclear protein (10 μg) from Caco-2 cells was preincubated for 10 minutes at 4° C with 5× binding buffer and 1 μg of poly(dI·dC). The synthetic SIE or Stat5 double-stranded oligonucleotide probes were end-labeled on one strand with [γ-^{32}P]ATP and T4 polynucleotide kinase. EMSA mixtures containing 45,000 counts/min of ^{32}P–end-labeled oligonucleotide and 10 μg of nuclear protein in a final volume of 20 μl of 12.5 mmol/L HEPES (pH 7.9), 100 mmol/L KCl, 10% glycerol, 0.1 mmol/L EDTA, 0.75 mmol/L dithiothreitol, 0.2 mmol/L phenylmethylsulfonyl fluoride, and 1 μg of bovine serum albumin with 1 μg of poly(dI·dC) as nonspecific competitors and further incubated for 20 minutes at room temperature. Competition binding experiments were performed by first incubating the competitor fragment, in molar excess, with the nuclear protein extract and binding buffer for 10 minutes on ice. The labeled probe was then added, and incubation continued for 20 minutes at room temperature. The reaction mixtures were loaded onto 6% nondenaturing polyacrylamide gels in 0.5× Tris borate–EDTA buffer for 2 hours at 200 V. For antibody (supershift) studies, 3 μl of antiserum to either Stat1, Stat3, or Stat5 was added during the preincubation for 1 hour at room temperature before the addition of the labeled probe. To increase the resolution of the gel shift complexes, the electrophoresis time was increased to 3.5 hours, resulting in the elution of unbound probe from the bottom of the gel. The gels were subsequently dried and autoradiographed at −70° C with an intensifying screen.

RESULTS

Caco-2 Cell Differentiation Is Associated With a Decrease in the Levels of Stat3 and Stat5 Proteins

Caco-2 cells, which differentiate spontaneously to a small bowel phenotype when they reach a postconfluent state,[19] were harvested over a time course and analyzed by Northern blot to confirm the timing for induction of SI gene expression in our cell system. Consistent with the findings of other investigators,[25,26] SI mRNA levels increase after the cells become postconfluent with maximal levels achieved by postconfluent day 6 (Fig. 1).

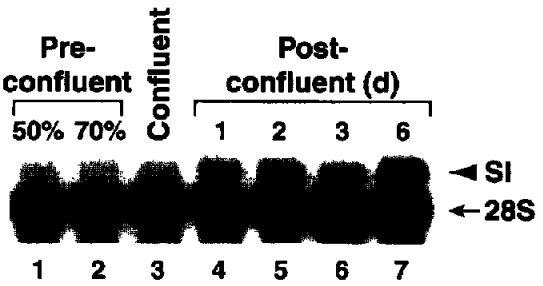

Fig. 1. Sucrase-isomaltase *(SI)* gene expression in Caco-2 cells. Northern blot analysis of RNA [5 μg poly(A)$^+$] from either preconfluent (50% and 70% confluency), confluent, or postconfluent (1, 2, 3, and 6 days) Caco-2 cells demonstrating that the intestinal differentiation marker gene SI (large arrowhead) was increased by postconfluent day 1 with maximal levels achieved by day 6. The 28S RNA is denoted.

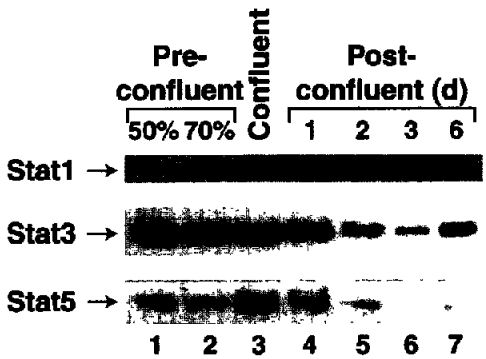

Fig. 2. Western blot analysis of Stat1, Stat3, and Stat5 proteins in nuclear extracts from preconfluent, confluent, postconfluent Caco-2 cells. Both Stat3 and Stat5 levels were increased in preconfluent and confluent Caco-2 cells; levels then decreased with postconfluency.

Using this time course, we next harvested Caco-2 cells for nuclear protein and determined alterations in the levels of Stat1, Stat3, and Stat5 by Western blot analysis (Fig. 2). The levels of Stat3 remained relatively constant until day 2 post confluency when nuclear protein levels were reduced by more than 50% compared with preconfluent Caco-2 cells. Stat5 nuclear protein levels, which were the highest at 100% confluency, progressively decreased after reaching postconfluency. Stat1 was expressed at low levels in preconfluent cells and then increased slightly on day 1 post confluency. The changes in Stat1, however, were not as dramatic as those noted for Stat3 and Stat5, suggesting that Stat1 may not play a role in the subsequent differentiation of Caco-2.

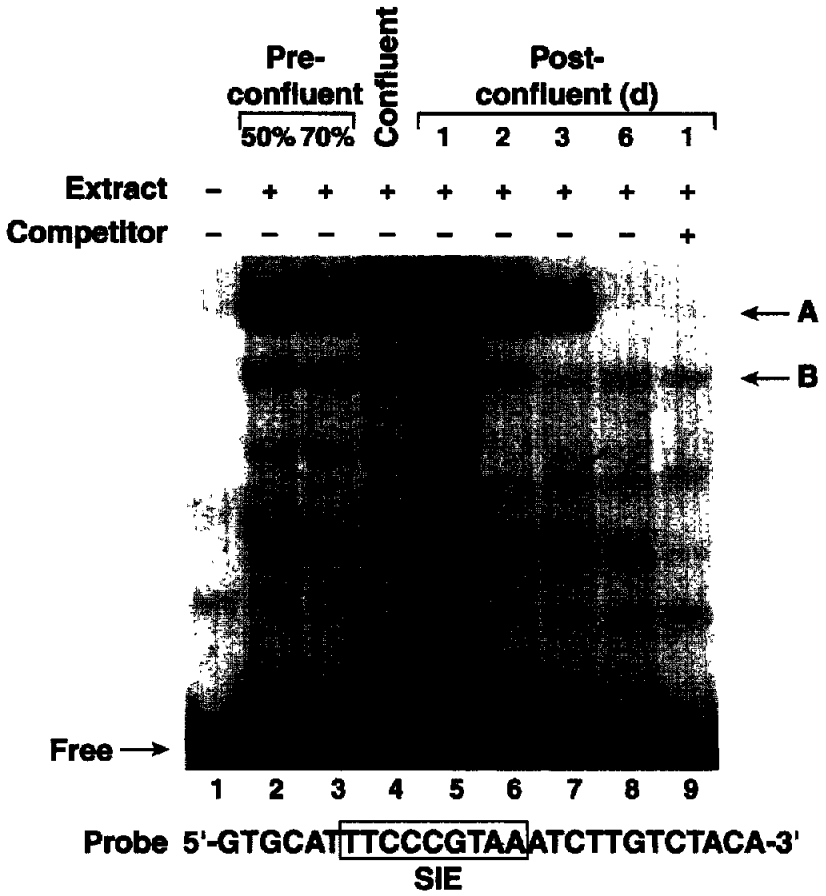

Fig. 3. Electrophoretic mobility shift assay of activated Stat3 and Stat1. Nuclear extracts (10 μg) from Caco-2 cells were analyzed for DNA binding activity using an oligonucleotide probe containing a consensus SIE binding site (5'-GTGCATTTCCCGTAAATCTTGTCTACA-3'). Two complexes (*A* and *B*) were noted. Maximal binding of activated Stat3 (complex A) was noted at confluency; binding activity decreased with postconfluency. Unlabeled SIE was used as competition DNA at a 200-fold molar excess (lane 9).

Caco-2 Differentiation Is Associated With a Decrease in Stat3 Binding Activity

To next assess whether the changes in steady-state levels of Stat3 and Stat5 proteins noted with Caco-2 differentiation were associated with concomitant changes in binding activity, we assessed the binding of the Stat proteins by EMSA. A labeled oligonucleotide containing a consensus SIE element from the c-*fos* promoter[27] was used to assess Stat1 and Stat3 binding, and an oligonucleotide with a consensus Stat5 binding site from the β-casein promoter was used to determine binding of Stat5.[28] Activated Stat1 and Stat3 proteins bind to the SIE element and can form three distinct gel shift complexes: an upper band (complex A) consisting of Stat3 homodimers, a middle band (complex B) consisting of Stat3 and Stat1 heterodimers, and a lower band (complex C) consisting of Stat1 homodimers.[29,30] Stat5 binds to the β-casein probe to form a single shifted complex.[31]

As shown in Fig. 3, an increase in protein binding to the labeled SIE probe (complexes A and B) was noted in Caco-2 cells at confluency and 1 day post confluency (lanes 4 and 5), and then, similar to the steady-state levels noted by the Western blot analyses, decreased after this point. A lower third complex was not noted in our study using the Caco-2 cell line. The specificity of the binding of the two complexes was confirmed by inhibition of complex formation using a 200-fold molar excess of the unlabeled SIE probe (lane 9). Therefore our findings demonstrate an increase of protein binding to the SIE probe in undifferentiated Caco-2 cells, which progressively decreases in differentiating cells. However, the exact nature of these complexes (i.e., pre-

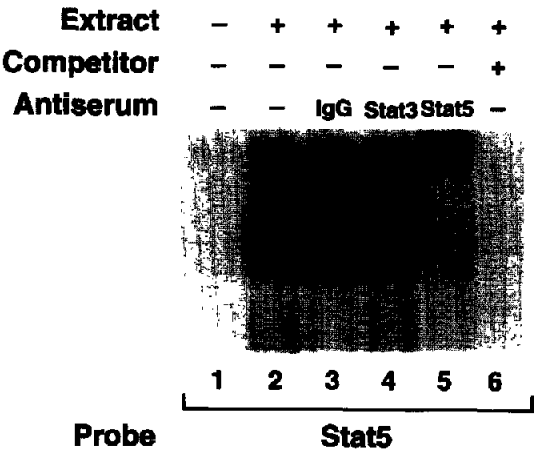

Fig. 4. Verification of activated Stat3 by supershift analysis. Nuclear extracts (10 μg/lane) from Caco-2 cells at confluency were preincubated with antisera to Stat1 or Stat3 proteins and analyzed by electrophoretic mobility shift assay. Complex A was almost entirely supershifted using anti-Stat3 antibody (lane 5); no supershift of the SIE complex was observed with either IgG or anti-Stat1 antibody (lanes 3 and 4, respectively). Unlabeled SIE was used as a competition DNA at a 200-fold molar excess (lane 6).

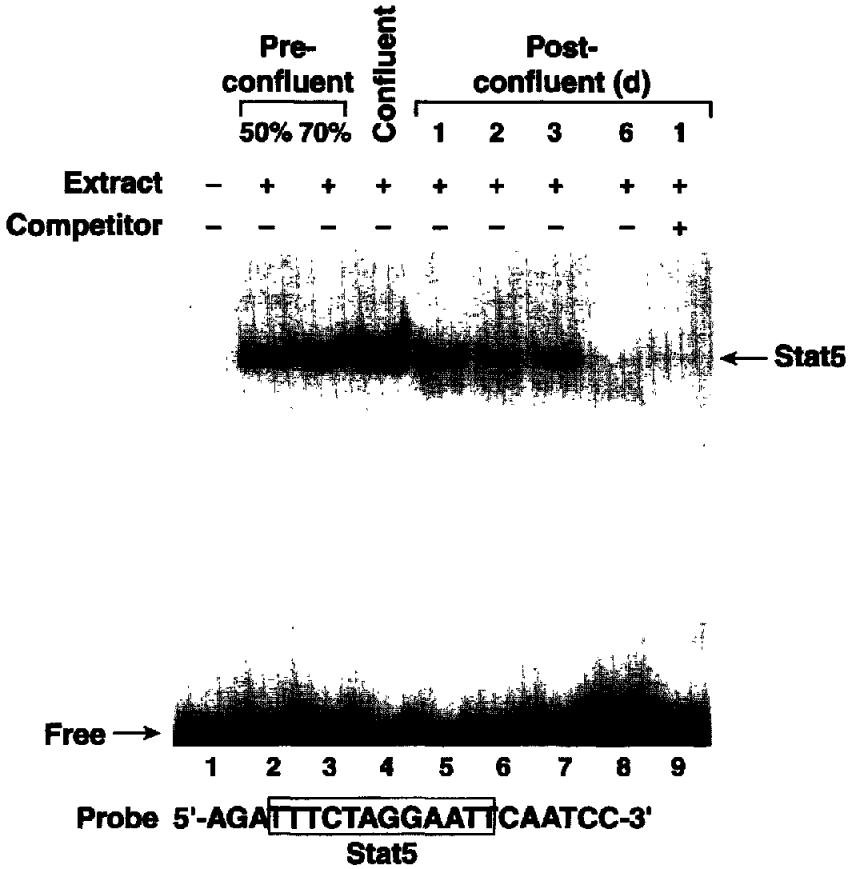

Fig. 5. Electrophoretic mobility shift assay of activated Stat5. Nuclear extracts from Caco-2 cells were analyzed using a labeled oligonucleotide containing the consensus Stat5 probe (5'-AGATTTCTAGGAATTCAATCC-3'). Maximal Stat5 binding activity was observed at confluency; Stat5 binding decreased with postconfluency. Unlabeled Stat5 was used as competition DNA at a 200-fold molar excess (lane 9).

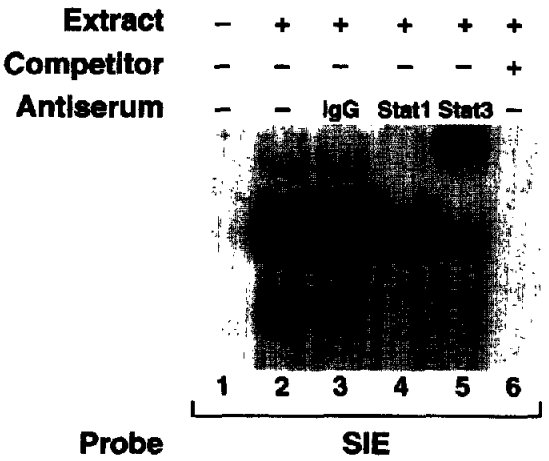

Fig. 6. Verification of activated Stat5 by supershift analysis. Nuclear extracts from Caco-2 cells at confluency were preincubated with antisera to Stat3 or Stat5 proteins. A supershift of the complex was noted with anti-Stat5 antibody (lane 5); no supershift of the complex was observed with either IgG or anti-Stat3 antibody (lanes 3 and 4, respectively). In addition, the binding activity was inhibited by a 200-fold molar excess of unlabeled Stat5 probe.

dominantly Stat1 or Stat3) could not be elucidated by this initial study.

To better determine which of the Stat proteins are changed during gut differentiation, an additional EMSA was performed using Caco-2 cell nuclear extracts at day 1 post confluency (Fig. 4). As previously demonstrated, the entire complex is readily competed with a molar excess of the SIE probe (lane 6). Addition of antibody to Stat3 (lane 5), but not IgG or Stat1 (lanes 3 and 4), produced a supershifted complex. Taken together these results demonstrate that the increase in protein binding to the SIE probe occurring in preconfluent and confluent cultures was predominantly the result of an increase in Stat3; Stat3 binding activity was then dramatically decreased in differentiating (i.e., postconfluent) Caco-2 cells.

To determine the binding activity of Stat5 during gut differentiation, we performed EMSAs using a labeled oligonucleotide containing the Stat5 consensus site (Fig. 5). Stat5 binding activity was increased in confluent Caco-2 cells; binding to the Stat5 oligonucleotide then progressively decreased with postconfluency (i.e., increased differentiation). Competition of the band using the unlabeled probe in molar excess confirmed specificity of binding (lane 9). To confirm that the protein binding to the labeled probe was indeed composed entirely of Stat5 protein, another EMSA was performed using nuclear extracts from confluent Caco-2 cells (Fig. 6). The entire complex was readily competed with a molar excess of unlabeled probe (lane 6). The addition of antibody to Stat5 (lane 5), but not IgG or Stat3 (lanes 3 and 4, respectively), produced a supershifted complex.

Collectively these findings confirm the differential induction pattern of the Stat proteins, as demonstrated by increases of both Stat3 and Stat5 protein levels and DNA binding, which occur in confluent cells; both the steady-state levels and binding activity of these proteins decreased with postconfluency. These decreases in Stat3 and Stat5, which occur at a time associated with Caco-2 differentiation (i.e., an increase in SI gene expression), suggest a potential role for these proteins in the process of Caco-2 cell differentiation.

DISCUSSION

To better elucidate the signaling mechanisms regulating the process of gut differentiation, we analyzed the changes of Stat protein level and DNA binding activity associated with intestinal cell differentiation using the gut-derived Caco-2 cell line, which spontaneously differentiates to a small bowel phenotype. We have shown that Stat3 and Stat5 levels and binding activities were increased in preconfluent and confluent Caco-2 cells; a reduction of steady-state Stat3 and Stat5 protein levels, as well as actual DNA binding, occurred with increasing differentiation (i.e., postconfluency). These findings provide evidence to suggest that the Stat pathway (particularly Stat3 and Stat5) may be involved in the differentiation process of the Caco-2 cell line.

Novel proteins of the Stat family transduce the signal of various cytokines and growth factors from the cell surface to the nucleus where they regulate gene transcription through the binding of conserved DNA sequences in target genes.[10] Members of the Stat family have been associated with proliferation, apoptosis, and differentiation in various cell systems. For example, Xu and Sonntag[18] found that growth hormone–induced activation of Stat3 is decreased with aging and contributes to the age-related decline in growth hormone receptor signal transduction and insulin-like growth factor I gene expression in the liver of B6D2 mice. Minami et al.[32] showed that myeloid differentiation and growth arrest in the murine leukemic cell line M1, induced by either interleukin-6 or leukemia inhibitory factor, were completely blocked by overexpression of a dominant negative Stat3 protein, indicating that Stat3 appears to be important for cytokine-mediated growth arrest and differentiation of this cell line. Stat3 is also activated by thrombopoietin, which supports the proliferation of megakaryocyte progenitors and induces their differentiation into large polyploid megakaryocytes.[33] In our present study we demonstrated changes of Stat3

steady-state protein levels and binding activity during Caco-2 differentiation. We found that the expression of Stat3 nuclear protein was increased in preconfluent and confluent Caco-2 cells; Stat3 levels and binding activity then decreased with postconfluency. These findings suggest that a decrease in Stat3 levels may be involved in the switch from proliferation to terminal differentiation in the Caco-2 cell line.

Stat5, or mammary gland factor, was discovered initially as a factor that binds to DNA sequences essential for a lactogenic hormone response.[28,34,35] Stat5 tyrosyl phosphorylation and/or DNA-binding activity is observed in response to many cytokines and growth factors in a number of cell types,[36-38] and activation of Stat5 is tightly linked to mammary gland differentiation.[39] Mutations in Stat5 binding sequence dramatically reduced the expression of milk protein genes in mammary cell lines[40] and in transgenic animals.[41,42] Chretien et al.[43] found that erythropoietin-induced differentiation in a human leukemia cell line, TF-1, correlates with impaired Stat5 activation, although direct evidence that Stat5 suppresses differentiation was not provided. Our results appear to be consistent with their report that Stat5 nuclear levels and binding activity were increased at confluency, but then progressively decreased in postconfluent Caco-2 cells. Thus both Stat3 and Stat5 patterns are similar in differentiating Caco-2 cells, suggesting that both of these proteins may be involved in Caco-2 cell differentiation. Although Stat1 has been shown to mediate cell growth arrest and induction of the cyclin-dependent kinase inhibitor $p21^{WAF1/CIP1}$ in fibrosarcoma cells,[11] Caco-2 cells expressed low levels of Stat1, which were only minimally altered with confluency and postconfluency. Therefore these results would tend to suggest that Stat1 does not play a significant role in the differentiation of Caco-2 cells.

CONCLUSION

We found that the nuclear levels and binding activity of Stat3 and Stat5, but not Stat1, were increased with confluency and then progressively decreased in postconfluent Caco-2 cells. This decrease in Stat levels was associated with increased SI gene expression. It is interesting to speculate that Stat3 and Stat5 may play a role in the switch from proliferation to differentiation in the Caco-2 cell line; however, future studies are required to determine whether these alterations of the Stat proteins are actually contributing to the process of intestinal cell differentiation or simply represent changes associated with cessation of proliferation. Identification of the cellular mechanisms responsible for intestinal differentiation is crucial to a better understanding of both normal intestinal development and aberrant gut growth.

We thank Eileen Figueroa and Karen Martin for manuscript preparation.

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These Guidelines have been written by the Patient Care Committee of The Society for Surgery of the Alimentary Tract (SSAT). Their goal is to guide physicians to the appropriate utilization of surgical procedures on the alimentary tract or related organs, and they are based on a critical review of the literature and expert opinion. Together these sources of information result in a consensus that is recorded in the form of these Guidelines. The consensus addresses the *range* of acceptable clinical practice and should not be construed as a standard of care. These Guidelines will require periodic revision to ensure that clinicians utilize procedures appropriately, but the reader must realize that clinical judgment may justify a course of action outside of the recommendations contained herein.

Surgical Treatment of Pancreatic Cancer

Pancreatic cancer, with 29,000 new cases diagnosed each year, is the second most common gastrointestinal malignancy. It is also the one with the worst prognosis, with only 17% of patients alive 1 year after diagnosis and 3% surviving 5 years. The only treatment with the potential for cure is an operative resection. Alternative treatments are reserved for more extensive disease and offer only possible palliation. Patients with a pancreatic mass or suspicion of pancreatic cancer should have surgical consultation for diagnosis and for therapy.

SYMPTOMS AND DIAGNOSIS

More than 90% of patients with pancreatic cancer present with pain, jaundice, and/or weight loss. Uncommonly, acute pancreatitis or recent onset of diabetes mellitus may be the initial presentation of pancreatic cancer. Vague upper abdominal symptoms may precede the onset of jaundice or overt pain by several months, and underscore the difficulty in establishing an early diagnosis in this disease.

Whenever pancreatic cancer is suspected, a CT scan of the upper abdomen should be obtained. If no mass is seen on CT scan, but clinical suspicion remains high, endoscopic retrograde cholangiopancreatography (ERCP) is indicated. A normal pancreatogram usually excludes the possibility of ductal adenocarcinoma. In selected cases endoscopic ultrasound may identify tumors that cannot be seen on CT scan or defined by ERCP.

STAGING

The purpose of preoperative staging in pancreatic cancer is to determine whether a patient (1) has a resectable tumor, (2) has a tumor that is still localized but is unresectable, or (3) has metastatic disease. Traditionally staging has been carried out with CT, angiography, and laparoscopy. The use of helical CT scanning with intravenous contrast with three-dimensional vessel image reconstruction is often preferred over angiography and yields an accurate assessment of metastatic disease, vascular invasion (that often precludes resection), and arterial anatomic variants. Endoscopic ultrasonography may be helpful in assessing vascular involvement, local nodal metastasis, and extrapancreatic tumor extension. Laparoscopy may be useful to identify patients with small metastatic hepatic and/or peritoneal implants in whom further surgery may be avoided.

TREATMENT

For the 15% of patients with resectable tumors in the head of the pancreas, the treatment is pancreaticoduodenectomy, with or without preservation of the pylorus. Preoperative or intraoperative histologic proof of malignancy is not required to carry out resection if the surgeon is experienced. A distal pancreatectomy with splenectomy is the procedure of choice for tumors of the body or tail of the pancreas but is possible in only 5% of patients. External beam radiation therapy coupled with 5-fluorouracil (5-FU) should be offered to all patients following resection. For the 85% of patients with unresectable tumors, treatment is directed primarily at palliation. If the patient has jaundice and gastric outlet obstruction, a biliary and gastric bypass are indicated. At the time of surgery, a celiac plexus block with 50% alcohol may be useful for the treatment or prevention of pain. For patients who have only jaundice, treatment is directed

by the availability of resources. An endoscopic stent is as effective as surgical bypass and has slightly less morbidity and lower cost. Patients who have no evidence of distant metastases may benefit from adjuvant therapy or some other more aggressive chemotherapy regimen. Patients with distant metastatic disease derive limited benefit from chemotherapy.

RISKS

The risk of dying from pancreaticoduodenectomy or distal pancreatectomy is currently very small (<3% in several large series). Five to 10% of patients experience significant complications following pancreatic resection. These include delayed gastric emptying, pancreatic fistula, and intra-abdominal abscess. Intra-abdominal or gastrointestinal bleeding, which was frequent in the past, is now uncommon. Reoperation for complications is rare. The complication and mortality rate in patients 70 years or older is no different from that in younger patients.

EXPECTED OUTCOMES

The median hospital stay following pancreaticoduodenectomy is usually less than 2 weeks and for distal pancreatectomy, less than 8 days. Following resection, patients have a 5-year survival of 5% to 20%. The median survival for patients with localized unresectable disease treated with chemoradiation therapy is 12 months. Patients with metastatic disease have a median survival of approximately 5 months.

QUALIFICATIONS

The qualifications of a surgeon to perform any operative procedure should be based on training (education), experience, and outcomes. At a minimum, operations for pancreatic cancer should be performed by surgeons who are certified or are qualified to be certified by the American Board of Surgery, the Royal College of Physicians and Surgeons of Canada, or their equivalent.

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Surgical Treatment of Cancer of the Colon or Rectum

Cancer of the colon and rectum (colorectal cancer) is the second most common form of cancer in the United States and is the second leading cause of cancer death; only lung cancer ranks higher in both categories.

Most colorectal cancers are sporadic, that is, there is no identifiable underlying cause. Perhaps as many as 20% of patients with colorectal cancer have an inherited predisposition to develop this disease. Familial adenomatous polyposis is one such syndrome and hereditary nonpolyposis colorectal cancer (HNPCC, Lynch syndromes) is another. Persons with HNPCC have an early onset of carcinoma, a tendency to develop cancers in the proximal colon, and synchronous and metachronous cancers. Some HNPCC syndomes include cancers of other organs, especially the endometrium. Other conditions associated with a predisposition to colorectal cancer include ulcerative colitis, Crohn's colitis, schistosomal colitis, exposure to radiation, and nonfamilial colorectal adenomatous polyps.

SYMPTOMS AND DIAGNOSIS

Screening measures used in asymptomatic persons to detect early cancers or premalignant polyps include digital rectal examination, fecal occult blood testing, and endoscopy.

Symptoms of colorectal cancer include rectal bleeding and change in bowel habits, and depend on the location and extent of the tumor. Constipation or diarrhea due to narrowing of the lumen, gross blood mixed with stool or mucus, and tenesmus (painful incomplete fecal evacuation) are common presenting symptoms. Systemic manifestations such as weight loss and fatigue due to chronic anemia suggest advanced disease. Obstruction, perforation, and acute bleeding may occur as complications of colon cancer.

Physical examination may reveal a palpable abdominal or rectal mass. Abdominal distention suggests high-grade rectal or colonic obstruction or, more rarely, the presence of malignant ascites.

The entire colon should be examined by colonoscopy or barium enema preoperatively in all patients with suspected cancer of the colon or rectum unless colonic obstruction or other circumstances preclude it. Colonoscopy is preferred because cancers can be seen and biopsied and synchronous neoplastic polyps can be removed if they are not contained within the segment of bowel to be resected.

The presence of metastases can be detected by chest x-ray examination, carcinoembryonic antigen (CEA) level, and liver function tests. CEA is not an accurate diagnostic test for colorectal cancer in a curable stage, but it may be helpful in detecting recurrence after curative resection. For this reason the preoperative serum CEA level is usually measured. Ultrasound, CT, or MRI scans of the abdomen are used primarily to search for hepatic metastases. Because of their cost and the need to operate regardless of the findings, some surgeons do not obtain these studies as a matter of routine. CT or MRI of the pelvis in patients with rectal cancer may be helpful in tumor staging by revealing spread beyond the limits of standard resection; such patients may be candidates for preoperative radiation therapy. Endorectal ultrasonography may be useful but only if local excision or preoperative or curative radiation therapy is under consideration.

If the primary lesion in the colon has the characteristics of a cancer, preoperative histologic confirmation is not required, but it should be done for primary lesions of the rectum. Preoperative histologic confirmation is also not routinely required for suspected liver metastasis since this can usually be obtained at the time of surgery.

SURGICAL TREAMENT

Surgical removal is the primary and preferred method of treatment of colorectal cancer. Surgical treatment is indicated in nearly all patients with a newly diagnosed tumor unless survival following the operation is unlikely or life expectancy is very short because of advanced cancer or other diseases. Even in the presence of metastases, palliative surgical resection of the primary tumor is advisable in most instances to prevent further bleeding and eventual obstruction of the lumen.

Surgical treatment consists of wide resection of the involved segment of the bowel and its regional lymphatic drainage system. Details vary with the location of the primary tumor. Primary anastomosis is possible in elective cases with a prepared bowel. In postmenopausal women, oophorectomy should be recommended because of the combined risks of micrometastases and primary ovarian cancer.

For rectal cancer, preservation of the sphincters and avoidance of colostomy is preferred if that goal is consistent with eradication of the cancer. All types of resections for cancer of the colon or rectum can be carried out by laparoscopic-assisted techniques, but they should be considered experimental at this time. Palliative treatments for unresectable rectal cancers include fulguration, laser photocoagulation, and radiation therapy.

Radiation therapy and chemotherapy are used in advanced disease and as adjuvants to surgical resection. Although radiation therapy has little or no role in management of cancer of the colon, it is an important treatment modality for *rectal* cancer. Bulky rectal cancers may be treated preoperatively to improve the potential for resection. Radiation therapy is a useful preoperative or postoperative adjuvant modality in rectal cancers that are stage II (invasion into the muscularis propria of the rectal wall) or stage III (metastases to regional lymph nodes). Chemotherapy in combination with radiation is an adjuvant modality for patients with stage II or III *rectal* cancer.

Patients with *colonic* cancers that extend through the wall with or without lymph node metastases should be considered for adjuvant chemotherapy.

RISKS

Postoperative complications of resection of colorectal cancer are mainly infectious and related to the bacterial flora of the large bowel. The most common postoperative complication is wound infection (2% to 4% in elective cases). This is minimized by mechanical and antibiotic bowel preparation and prophylactic intravenous antibiotics. Other risks include bleeding, pelvic abscess, damage to contiguous organs (such as the spleen or ureter), sexual and urinary dysfunction, and wound dehiscence.

EXPECTED OUTCOMES

Many patients return home on the fifth postoperative day or even earlier. Light physical activity begins within 2 to 3 weeks and full activity by 6 to 8 weeks. These estimates are lengthened considerably in elderly or debilitated individuals.

Bowel movements may be unchanged by the operation or may be looser and more frequent, depending on the portion and length of bowel removed. These changes seldom present problems, but disordered bowel habits after anterior resection with a very low anastomosis can be quite troublesome. Most patients with colostomies adjust well with the help of educational support groups and family. There are generally no dietary restrictions.

The clinicopathologic stage of the disease is the most important determinant of survival after surgical resection. Five-year survival rates vary from 90% for tumors confined to the mucosa and submucosa to less than 5% for those with distant metastases. Approximately 70% of patients can be operated for cure, 10% of lesions are not resectable at the time of operation, and another 20% of patients have distant metastases.

Follow-up programs after curative resection of colorectal cancer may involve measurement of serum CEA levels every 3 months for several years, annual fecal occult blood testing, colonoscopy every 3 years, chest x-ray examination every few years, ultrasonography, and either CT or MRI scanning at 1 year and every few years thereafter. The goals are early detection of metachronous colorectal cancer, prevention of metachronous cancer by removal of colorectal polyps, and diagnosis of recurrent or metastatic cancer. There is evidence that periodic colonoscopy to detect and remove adenomas reduces substantially the risk of subsequent cancer. The cost-benefit ratio of the diagnosis of recurrent or metastatic cancer is uncertain because so few asymptomatic recurrences detected during follow-up are amenable to cure.

Hepatic metastases detected during follow-up should be evaluated for possible resection. If one or a few lesions can be completely resected, survival is significantly prolonged.

QUALIFICATIONS FOR PERFORMING COLON OPERATIONS

Emergency as well as elective colon operations should be performed by surgeons who are certified or eligible for certification by the American Board of Surgery, the Royal College of Physicians and Surgeons of Canada, or their equivalent. These surgeons have successfully completed at least 5 years of surgical training after medical school graduation and are qualified to perform colon operations. The qualifications of the surgeon should be based on training (education), experience, and outcomes.

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Surgical Treatment of Diverticulitis

Colonic diverticulosis is among the most common diseases of western civilization and is the result of a low-fiber diet. In the United States approximately one third of the population develops diverticulosis by age 50 years and approximately two thirds by age 80. Diverticulitis describes the complication of acute bowel inflammation with the vast majority of cases being confined exclusively to the sigmoid and descending colon. Although most cases of acute diverticulitis can be successfully managed medically, recurrent attacks and the complications of diverticulitis require surgical intervention. The important complications of diverticulitis are diffuse peritonitis, a localized abscess, fistulas (such as a colovesical or colovaginal fistula), and obstruction.

SYMPTOMS AND DIAGNOSIS

Acute diverticulitis typically presents with left lower quadrant abdominal pain and local tenderness accompanied by fever. In most instances there is an inflammatory process confined to the colon and its mesentery and adjacent structures or peritoneal surfaces. If the patient has a macroscopic diverticular perforation, a pericolic or pelvic abscess may be present with associated high fever. Patients with perforation and diffuse peritonitis usually present with severe generalized abdominal pain and associated paralytic ileus. Diffuse peritonitis may lead to septic shock with prostration and cardiovascular collapse. High-grade colonic obstruction manifests as colicky abdominal pain, bloating, and constipation or obstipation.

The abdominal findings reflect the severity and localization of the septic process. The inflamed colon or pericolic abscess causes marked localized tenderness with or without a palpable mass. In cases of diffuse peritonitis, generalized tenderness, involuntary guarding, and decreased or absent bowel sounds are noted. The presence of pneumaturia or fecaluria signifies the presence of a colovesical fistula. Severe abdominal distention suggests bowel obstruction.

The diagnosis of acute diverticulitis is based on clinical findings and leukocytosis. Abdominal x-ray films may show some generalized ileus pattern, and uncommonly pneumoperitoneum in cases of diffuse peritonitis. Marked dilatation of the more proximal colon indicates sigmoid obstruction. For patients with localized disease, CT scanning has replaced a barium enema as the imaging procedure of choice. However, CT scanning is usually reserved for patients with suspected abscesses or perforations, those who fail to respond to medical therapy, or those in whom the diagnosis is not clear. Endoscopic evaluation is contraindicated in acute diverticulitis, as insufflation of air may cause free perforation and peritonitis. Following resolution of an acute attack, an endoscopy and/or barium enema are indicated to document the presence of colonic diverticula and to exclude colorectal carcinoma. In cases of a suspected colovesical fistula, the diagnosis is usually made on the basis of urinalysis, urine culture, and CT scan.

TREATMENT

Most patients with acute diverticulitis require hospitalization for intravenous hydration, broad-spectrum antibiotics, and bowel rest with or without nasogastric tube decompression. The initiation of medical therapy usually results in rapid clinical improvement with resolution of pain, fever, and ileus within 48 to 72 hours. Broad-spectrum antibiotics are continued for 7 to 10 days and oral feedings are gradually reintroduced as tolerated. Patients who recover from an attack of diverticulitis should be placed on a high-fiber diet to decrease the likelihood of repeated attacks.

Operation for diverticulitis and its complications may be required as either an elective or emergency procedure. Indications for elective operation include the following: (1) two or more acute attacks of diverticulitis successfully treated medically; (2) a single attack requiring hospitalization in a patient less than 40 years of age; (3) one attack with evidence of contained perforation, colonic obstruction, or inflammatory involvement of the urinary tract; and (4) inability to rule out a colonic carcinoma. Because the overwhelming majority of patients with acute diverticulitis have sigmoid colon involvement, resections of other portions of the colon are infrequent. Most patients deemed candidates for elective operation undergo a mechanical and antibiotic bowel preparation and are treated by sigmoid colectomy with primary anastomosis. If there is diverticulitis of the descending colon, a left hemicolectomy may be required. Isolated cecal or ascending colon diverticulitis is a rare condition, usually encountered during emergency operation for presumed acute appendicitis, and may require a resection.

Patients who present with diffuse peritonitis or pneumoperitoneum require prompt fluid resuscitation, intravenous antibiotics, and emergency surgical exploration. A resection of the perforated colonic segment (almost always the sigmoid) with descending end colostomy and closure of the rectal stump is usually required. The older three-stage approach of proximal colostomy and drainage followed by resection and subsequent colostomy closure is almost never employed as it does not consistently control the septic process.

Patients with acute diverticulitis without perforation who deteriorate or fail to improve after 48 to 72 hours of aggressive medical therapy should undergo prompt CT scanning of the abdomen. If no macroscopic abscess is identified, a laparotomy and colon resection is performed. If a large (5 cm or greater) abscess is identified, either surgical exploration with drainage of the abscess and colonic resection or CT-guided percutaneous catheter drainage may be performed. If the latter approach is chosen, a subsequent elective colon resection with primary anastomosis is performed after resolution of the abscess.

Patients whose acute diverticulitis is complicated by colovesical or other fistula rarely require emergent operation. Such patients are best treated medically with subsequent elective colon resection, fistula takedown, and primary anastomosis. When colonic obstruction attends diverticulitis, it is usually incomplete and allows a gentle mechanical and antibiotic bowel preparation and a nonemergent colon resection with primary anastomosis.

Recently, virtually all types of elective surgery for diverticular disease have been successfully performed using laparoscopic techniques. If significant adhesions, inflammation, bleeding, or other adversity is encountered during laparoscopic surgery, conversion to an open procedure may be indicated. Such conversion is not a complication and is appropriate to ensure safe completion of the operation.

RISKS

In the surgical treatment of diverticulitis, mortality rates for elective resection and primary anastomosis range from 0% to 2% compared to rates of 5% to 20% for emergency operations. Since the major morbidity and mortality attending emergency surgery derives from inadequately controlled sepsis, removal of the perforated colonic segment clearly has advantages over the older approach of proximal diverting colostomy and drainage of the perforation.

Technical complications that attend colonic surgery include bleeding, anastomotic leak with associated infection, and occasional inadvertent injury to adjacent organs, particularly the ureter. These risks are relatively low and in patients undergoing elective colectomy they total less than 5%. Although the risks of bleeding and adjacent organ injury are increased with emergency operations, the infrequency of primary anastomosis largely eliminates the problem of an anastomotic leak. As diverticulitis is often a geriatric disease, much of the risk of surgery, and most particularly emergency surgery, derives from comorbid conditions such as cardiac and pulmonary disease.

EXPECTED OUTCOMES

Among patients whose initial attack of diverticulitis is successfully treated medically, only 10% to 20% return with repeat attacks, but the need for surgical intervention in this latter group is very high. When an elective operation can be performed, a hospital stay of 5 to 7 days is typically required. Since an extensive colonic resection is rarely required for diverticulitis, postoperative bowel function is usually normal. Following successful resection and colonic anastomosis, the rate of recurrent diverticulitis is well below 5% but may be higher if the resection does not extend to the rectosigmoid junction.

Approximately 15% to 20% of patients presenting with an initial attack of acute diverticulitis require surgical intervention for peritonitis, abscess, or fistula. These patients incur greater perioperative morbidity and mortality, and often require a second operation for colostomy closure. However, following successful restoration of bowel continuity, these patients usually have normal bowel function.

QUALIFICATIONS FOR PERFORMING SURGERY FOR DIVERTICULITIS

The qualifications of a surgeon performing any operative procedure should be based on training (education), experience, and outcomes. At a minimum, emergency as well as elective colectomy should be performed by surgeons who are certified or eligible for certification by the American Board of Surgery, the Royal College of Physicians and Surgeons of Canada, or their equivalent. It is highly desirable that the surgeons performing laparoscopic colonic surgery have undergone specific advanced training in this area.

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Surgical Management of Hemorrhoids

Hemorrhoids are a common ailment. It should be recognized that every individual is born with hemorrhoids. It is only when they become enlarged and symptomatic that a patient is referred to as having "hemorrhoids." It is estimated that the prevalence of symptomatic hemorrhoids is 4.4% and that 50% of Americans over the age of 50 years will suffer with symptoms of hemorrhoids. Predisposing or associated conditions include hereditary factors, constipation, and increased intra-abdominal pressure (e.g., pregnancy, ascites, coughing, vomiting, strenuous work).

Hemorrhoids present with symptoms that are typical of most anal conditions. These symptoms include bleeding, pain, discharge, or presence of a mass. Hemorrhoids are generally categorized as internal (covered with mucosa) or external (covered with squamous epithelium). Internal hemorrhoids bleed and prolapse to give the effect of a mass. These hemorrhoids may protrude with bowel movements. Internal hemorrhoids are staged according to the degree of prolapse as follows:

Stage I = bleeding only, no prolapse
Stage II = prolapse, which reduces spontaneously, with or without bleeding
Stage III = prolapse that requires manual reduction, with or without bleeding
Stage IV = irreducible prolapsed hemorrhoidal tissue

External hemorrhoids generally do not bleed. They can thrombose and cause acute pain. Although external hemorrhoids can become necrotic and drain, most thrombosed hemorrhoids resolve spontaneously. Redundant skin "tags" may remain. These tags can cause pruritus in areas that are difficult to clean.

Acute complications occur with prolapse of internal hemorrhoids or thrombosis of external hemorrhoids. If the pain is acute in onset, it is usually constant and related to a visible mass. If the pain is more prevalent only after a bowel movement, it is rarely due to a hemorrhoid complication and is more likely due to a fissure or ulcerating anal mass. Chronic anal pain and pruritus are generally not symptoms of hemorrhoids but can be symptoms of other diseases including anal fissure, mucosal prolapse, anal mass, or anal fistula that can cause chronic pain or excessive moisture and associated pruritus.

SYMPTOMS AND DIAGNOSIS

The symptoms of hemorrhoids include local protrusion and swelling, pain related to the protruding or swollen masses, and bleeding that may be minor or exsanguinating. The symptoms are generally plebeian and the diagnosis should not be assumed. Other more serious conditions such as inflammatory bowel disease and cancer can mimic hemorrhoidal symptoms and should be ruled out.

The diagnosis is established with direct visualization by anoscopy or proctoscopy. All patients who have rectal bleeding should undergo examination of the colon to rule out proximal sources of bleeding regardless of the presence of enlarged hemorrhoids. Since most cases of bright red bleeding are within the reach of a flexible sigmoidoscope, most patients should undergo flexible sigmoidoscopy or colonoscopy, in addition to anoscopy, to rule out other causes of bleeding. Intermittent protrusion and/or intermittent bleeding do not require urgent consultation. However, patients with acute symptoms of bleeding, pain, or incarcerated protrusions should be seen promptly.

TREATMENT

Conservative therapy is suggested initially for chronic symptoms of hemorrhoidal disease. This includes stool bulking and local lubrication. Surgical treatment is reserved for patients with chronic symptoms that are irritating to the patient and acute symptoms such as pain, excessive blood loss, or suppuration. If the patient has evidence of anemia, more aggressive treatment is necessary. However, if conservative treatment fails to relieve the symptoms and the patient desires that the symptoms be resolved, then localized in-office therapy is appropriate.

If the patient has stage I, II, or III disease, then localized treatment is appropriate. This could be in the form of infrared coagulation, local injection, or rubber banding. Cryotherapy is to be avoided because of excessive post-treatment symptoms. Stage I and II disease is well treated by any of these modalities. Resolution of symptoms should occur in 90% or more of patients. Stage III disease is probably best treated by hemorrhoidal banding, which helps to remove redun-

dant tissue. Long-term resolution of stage III symptoms is likely in only 70% of patients treated by banding. Stage IV disease requires surgical intervention. Surgical treatment of hemorrhoids should be associated with long-term resolution of symptoms in 95% of patients.

There may also be symptoms from residual hemorrhoidal tissue after an episode of acute thrombosis of external hemorrhoids. These external anal tags cause difficulty with cleansing and may be locally excised if symptoms warrant.

RISKS

The risks of hemorrhoidal disease are continued symptoms, exsanguinating bleeding and, for hemorrhoids that undergo necrosis, sepsis.

Risks of treatment include bleeding and infection. The risk of bleeding after localized therapy is approximately 1%. The risk of infection after localized treatment is unknown, but it is much less than 1%. Localized pain is another symptom of localized treatment. Pain after banding and injection typically lasts 24 to 36 hours. If it continues longer than that, the patient should seek medical attention. If the pain is excessive after treatment, the patient may have difficulty urinating. Urinary retention is indicative of a localized problem and should be regarded as a sign of occult sepsis.

Bleeding, pain, and infection are greater risks after open hemorrhoidectomy. The risk is approximately 5% for each of these. Comorbid conditions such as diabetes, human immunodeficiency virus (HIV), and heart disease increase the risks of localized treatment but do not change the type of complications that occur. Anal incontinence is a rare complication of surgery for hemorrhoidal disease. There may be subtle changes in continence of gas, liquids, or solids following localized treatment or surgery, but they are rarely socially significant. Injury to the muscle is a recognized risk but is extremely rare when hemorrhoidectomy is performed by an experienced surgeon.

EXPECTED OUTCOMES

Following localized treatment, the symptoms of local protrusion and bleeding should be eradicated. The risk of recurrence of symptoms following localized treatment varies according to the extent of the localized disease. The risk of recurrent symptoms for stage I and II disease is 10%. The risk of recurrent symptoms for stage III disease is 30%. The risk of recurrent symptoms after hemorrhoidectomy is 5%.

Most hemorrhoidal disease can be treated in the office. Surgical hemorrhoidectomies can generally be carried out under local anesthesia with sedation. A brief stay in the hospital may be necessary for pain control if more than one quadrant of hemorrhoidal tissue is removed.

Symptoms following localized treatment are minimal after 24 to 48 hours. It should not require limited activity, although trauma to the perineum is to be avoided for 2 weeks. Patient activity need not be limited after localized treatment, although trauma to the perineum is to be avoided for 2 weeks. After surgical hemorrhoidectomy, the pain may persist for a number of weeks. Most moderate activity can be continued but 6 to 8 weeks may be required for the wounds to heal completely and for full activity to be resumed.

QUALIFICATIONS

Hemorrhoidectomy should be performed by surgeons who are certified or eligible for certification by the American Board of Surgery, the Royal College of Physicians and Surgeons of Canada, or their equivalent. These surgeons have undergone at least 5 years of surgical training after medical school graduation and in most instances are qualified to perform hemorrhoidectomy. In addition to the standard residency training, qualifications should be based on training, experience, and outcomes.

Adequate training in the management of anorectal disease is important to perform this procedure. Similarly, one should be able to deal with the complications (bleeding, infection, and urinary retention) should they occur. Therefore surgical training is necessary to manage hemorrhoidal disease.

Surgical Repair of Groin Hernias

Groin or inguinal hernias afflict mostly men but they can also occur in women of all ages. This guideline pertains only to adult patients. Repair of groin hernias is one of the most commonly performed outpatient surgical procedures. A groin hernia in an adult is really not a "rupture" but is best defined as a weakness in the abdominal wall that slowly develops as a result of weakening of the layers of the abdominal wall. A direct inguinal hernia develops when the posterior portion of the inguinal canal attenuates allowing the nearby contents of the abdominal cavity to protrude. An indirect inguinal hernia occurs along the spermatic cord or round ligament in the inguinal canal. A femoral hernia passes behind the area of a direct hernia and follows the femoral vessels. Femoral hernias are uncommon and usually occur in women.

SYMPTOMS AND DIAGNOSIS

Inguinal hernias can present in several ways. Typically they present as a vague ache in the groin and may or may not be able to be documented by physical examination. They can also be asymptomatic and discovered incidently during physical examination. Finally they can present as a bulge discovered by the patient. Since almost all hernias should be repaired, the patient should be referred to a surgeon for evaluation and operative treatment. Sophisticated tests to detect a groin hernia are not required because the diagnosis can usually be made on physical examination by a physician or the general surgeon. The best position for examining an inguinal hernia is with the patient standing and straining against a held breath. The use of ultrasound or a diagnostic x-ray examination is not usually necessary. The patient with groin pain but without a history of a groin bulge and without the physical finding of a hernia (by the primary physician and a surgeon) presents an uncommon diagnostic dilemma. These patients may not have a hernia but rather a groin muscle strain. In contrast, if the patient describes a groin bulge but on physical examination a hernia is not found, more than likely a hernia is present. A femoral hernia often presents with pain below the groin crease rather than a bulge. This can be a difficult diagnostic dilemma, particularly in the elderly or in obese females with sudden groin pain without the physical finding of a groin hernia of any type.

Most groin hernias are readily reducible, have no or minimal tenderness, and can be referred to a surgeon electively within a period of weeks. If the hernia is tender and not reducible, the patient should be referred immediately because of the risk of strangulation of bowel or other viscera. Aggressive attempts to reduce a groin hernia with sedation, ice packs, or sustained weight or pressure should *not* be pursued. Other symptoms such as nausea and vomiting suggest bowel obstruction and mandate immediate surgical referral.

TREATMENT

Patients with groin hernias are usually offered the opportunity for repair and therefore incarcerated (nonreducible) hernias are relatively uncommon. Urgent repair is required for the sudden presentation of a nonreducible hernia or any chronically incarcerated hernia that becomes acutely tender. This clinical history indicates impending strangulation and a surgeon should be consulted immediately. Severe morbidity and mortality can be avoided by prompt diagnosis; however, this clinical emergency results in the death of more than 2000 patients per year in North America.

Repair of almost all groin hernias is recommended. Inguinal hernias should ultimately be repaired because they enlarge over time making the repair more difficult with a higher risk of recurrence. Femoral hernias should always be repaired because of the high incidence of bowel strangulation. It is advisable that all patients with groin hernias undergo a surgical evaluation within a month after diagnosis. Urgent repair is required for all painful unreducible hernias. All asymptomatic hernias can be repaired electively. Elderly patients with minor comorbid conditions tolerate outpatient elective hernia repair very well. This obviates the emergency repair of chronically incarcerated hernias that occur mostly in elderly persons. The timing of repair is determined by the symptoms of the patient.

Every type of inguinal or femoral hernia operation attempts to repair the defect in the abdominal wall. There are three basic approaches—*open repair* (the standard repair, which uses the patient's own tissue); *open tension-free repair*, which uses mesh to cover the defect; and *laparoscopic* repair. The latter procedure also requires mesh and is a tension-free repair.

The open technique of hernia repair can be performed under local, regional, or general anesthesia. General anesthesia is required for laparoscopic hernia repair.

RISKS

Associated with all operations are risks of infection and hemorrhage. The risk of infection or a significant hematoma is approximately 1%. The incidence of hernia recurrence ranges from 5% to 10%. In the event of a recurrence, the hernia should again be repaired.

EXPECTED OUTCOME

Short-term outcome studies suggest a quick return to the normal activities of daily living following either open or laparoscopic hernia repair. The cost of a laparoscopic repair is currently greater than that of an open repair; therefore open repairs are more frequently performed. Out-of-the house activities can be resumed on the first or second day after the repair; a return to activities such as driving a car is governed by the patient's discomfort and can usually be resumed within 5 days or less after repair. Pain medication taken by mouth is needed for only a few days.

QUALIFICATIONS FOR PERFORMING INGUINAL AND FEMORAL HERNIA REPAIRS

Emergency as well as elective inguinal hernia repairs should be performed by surgeons who are certified or eligible for certification by the American Board of Surgery, the Royal College of Physicians and Surgeons of Canada, or their equivalent. These surgeons have successfully completed at least 5 years of surgical training after medical school graduation and are qualified to perform open inguinal hernia repair with and without the tension-free techniques. For laparoscopic groin hernia repair, advanced laparoscopic training is required. The qualifications of the surgeon should be based on training (education), experience, and outcomes.

Splenectomy

As our understanding of the role of the spleen in the host's immune surveillance system has evolved, the indications for splenectomy have changed as well. Newer methods of treatment of certain hematologic neoplasms and selected benign disorders, development of operative techniques for hemostasis and splenic salvage, and proliferation of intra-abdominal imaging techniques have markedly changed the way physicians and surgeons view splenectomy. Laparoscopic splenectomy is becoming increasingly common. It appears to be safe and is associated with less pain and a rapid convalescence.

INDICATIONS FOR SPLENECTOMY

Trauma

No longer is traumatic injury to the spleen considered an indication for immediate or mandatory operation or for splenectomy in either adults or children. CT scanning allows the diagnosis of splenic injury to be determined in the patient with blunt trauma to the abdomen or lower chest. Nonoperative supportive management with in-hospital observation for up to 5 days is indicated in both children and adults, provided that hemodynamic stability persists and no other intra-abdominal injuries that might require laparotomy are evident. In adults the significant accumulation of intraperitoneal blood (estimated at >1000 ml), the need for transfusion of more than 2 units of blood, a progressively decreasing hemoglobin concentration, or hemodynamic instability are currently accepted indications for operation. Even more aggressive nonoperative support can be justified in children under the age of 14 years.

When operative intervention is necessary, splenic preservation is warranted. If bleeding can be controlled quickly or there are no other life-threatening intra-abdominal injuries, splenic salvage is recommended. More aggressive attempts at intraoperative splenic salvage are probably justified in children under the age of 14 years. Splenic autotransplantation with a free graft is of unproved efficacy in maintenance of specific splenic immunity and should still be considered experimental.

Hematologic Diseases

Close cooperation with a hematologist is warranted when determining the indications for splenectomy for hematologic diseases. Common indications include hereditary spherocytosis, thalassemia major, and certain forms of idiopathic thrombocytopenic purpura unresponsive to medical management. Myeloproliferative disorders may lead to massive splenomegaly, which can cause symptoms that are best relieved by splenectomy. Splenectomy in these conditions is primarily for symptomatic relief. Splenectomy usually does not alter overall survival, and this information should be discussed clearly with the patient, usually in conjunction with a hematologist, prior to operation. The operative morbidity and mortality in these patients are greater because of their hematologic comorbidity. Thrombotic thrombocytopenic purpura and hairy cell leukemia unresponsive to other treatment strategies occasionally represent indications for splenectomy.

Hodgkin's Disease

Selected patients with clinical stage IA or IIA Hodgkin's disease may be candidates for a staging laparotomy. In the absence of obvious liver or intra-abdominal nodal disease, splenectomy becomes an integral part of the staging procedure to exclude splenic involvement, as this would alter the method of treatment.

Iatrogenic (Intraoperative) Splenic Injury

Uncommonly, the spleen may be injured inadvertently during intraperitoneal procedures, especially those involving the stomach, distal pancreas, or splenic flexure of the colon. These injuries may occur directly from operative retractors, but more often they are secondary to capsular adhesions that, when inadvertently avulsed, can lead to persistent bleeding. Attempts at hemostasis using suture plication, topical hemostatic agents, electrocautery, or argon beam coagulation are warranted to prevent the need for splenectomy. Hemorrhage severe enough to require blood transfusion if rapid hemostasis is not possible is better managed by formal splenectomy than by repeated attempts at splenic salvage. This is especially true in adults.

Other Indications For Splenectomy

Less common indications for splenectomy include splenic abscess, some splenic cysts, sinistral portal hy-

pertension secondary to isolated splenic vein thrombosis or obstruction, or splenic mass presumed to be a primary or undiagnosed neoplasm. Splenectomy is occasionally included as part of an en bloc resection for malignancy in an adjacent organ (stomach, colon, adrenal gland, or pancreas) that involves or is adherent to the spleen. Distal pancreatectomy for pancreatic disease usually includes splenectomy when preservation of the splenic artery and vein is either contraindicated (malignancy) or not technically possible.

MORBIDITY AND MORTALITY

Operative mortality for elective splenectomy should be less than 1%, except in patients with myeloproliferative disorders in whom postoperative problems with hemorrhage represent an increased risk. Trauma patients have a variable mortality rate depending on the extent of other injuries. Well-known early postoperative complications of splenectomy include hemorrhage, subphrenic abscess, pancreatic pseudocyst (secondary to inadvertent injury to the tail of the pancreas), and gastric fistula/perforation (secondary to injury/necrosis of the gastric wall during ligation of the short gastric vessels).

Late sequelae related to splenectomy are much more common in children, especially those younger than 6 years of age. Overwhelming postsplenectomy sepsis secondary to encapsulated organisms (pneumococcus, meningococcus, etc.), albeit unusual (<1%), is a well-recognized possibility in children before specific splenic immune function has become established outside the spleen. There also appears to be a slight increase in the susceptibility to similar infections in adults after splenectomy. Although the precise incidence is unknown, it is probably less than the incidence in children.

PROPHYLAXIS AGAINST POSTSPLENECTOMY SEPSIS

Most pediatricians believe that children who have had a splenectomy before the age of 5 years should be treated with a daily dose of penicillin until the age of 10 years. The use of prophylactic penicillin is less well defined in children over the age of 5 years and in adults. All patients who have undergone a nonelective splenectomy should be immunized with Pneumovax (a nonviable pneumococcal vaccine containing the more common virulent strains of the pneumococcus family). Patients for whom an elective splenectomy is planned should also be immunized with Pneumovax, preferably two or more weeks before operation. Children under the age of 10 years and all patients who are immunosuppressed or have an associated immunodeficiency should be vaccinated against pneumococcus, *Haemophilus influenza*, meningococcus, and hepatitis B.

QUALIFICATIONS FOR PERFORMING OPERATIONS ON THE SPLEEN

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

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Management of Colonic Polyps and Adenomas

Colonic polyps comprise a group of lesions that project above the surface of the colonic mucosa. Autopsy studies have shown that colonic polyps are very common, occurring in more than 30% of people over the age of 60 years. Not all polyps are neoplastic and not all polyps are benign. However, approximately 70% to 80% of resected polyps are adenomatous. The importance of adenomatous lesions is their very well-documented relationship to colorectal cancer. Therefore, because of the prevalence of polyps in the general population and the adenoma-carcinoma progression, these lesions represent a significant public health problem, because colorectal cancer is responsible for 10% of the cancer-specific deaths in the United States. Appropriate management of colonic polyps would greatly reduce the risk of death from colorectal cancer.

METHODS OF DIAGNOSIS

Several methods are available to detect colonic adenomas. These include flexible or rigid sigmoidoscopy, colonoscopy, and the combination of sigmoidoscopy with a double-contrast barium enema. It is now generally accepted that colonoscopy is the most accurate method to detect colonic polyps, and it also allows simultaneous removal of the vast majority of the lesions. Complete inspection of the colon by colonscopy is not possible in 10% of patients. The combination of double-contrast barium enema and sigmoidoscopy is better tolerated by some patients and is less expensive and safer than routine diagnostic colonoscopy. However, this method of diagnosis obligates many patients to a second procedure for therapeutic intervention. The incidence of significant bleeding and perforation is less than 1% for colonoscopy as compared to only 0.01% for the barium enema. Cost-efficacy and outcome studies for the different methods of diagnosis and treatment are inconclusive.

SYMPTOMS AND DIAGNOSIS

Most colonic polyps are asymptomatic, but they may present with occult or gross bleeding, iron deficiency anemia, or mucous discharge. Others may be detected by digital rectal examination. The remainder are discovered during the course of routine health screening examinations.

The yield of fecal occult blood tests for adenomas and carcinoma has been investigated extensively over the past three decades. In patients who have had one positive test for fecal occult blood, colonoscopy will detect an adenoma in approximately one third and a cancer in approximately 10% of cases. The yield for both adenomas and cancer increases with age in patients with positive fecal occult blood tests. Positive fecal occult blood tests performed during digital rectal examination are not as specific for adenomas as samples obtained after defecation.

An adenoma or carcinoma is found at the time of diagnostic colonoscopy in 22% and 11% of patients, respectively, who present with nonemergent rectal bleeding (blood mixed with the stool). In contrast, the incidence of an adenoma or carcinoma of the colon being detected is only 14% and 8%, respectively, in patients presenting with acute lower gastrointestinal hemorrhage. Colonoscopic screening of patients with a family history of colon cancer reveals an adenoma in 22% of first-degree relatives. Among symptomatic average-risk male patients over the age of 60 years, approximately 25% will have adenomas found at the time of colonoscopy. In general, the risk of harboring a colonic adenoma is greater with advancing age and in men.

TYPES OF POLYPS

The management of a colonic polyp is determined by histologic findings (hyperplastic, adenomatous, harmatomous), the size of the lesion, and morphology (sessile or pedunculated). Although hyperplastic polyps are the most common, they have no malignant potential. Only Peutz-Jeghers and adenomatous polyps have malignant potential. Although the risk of malignant transformation is low for an individual adenomatous polyp, the incidence of malignancy increases with the size of the adenoma and the age of the patient. Additionally, villous adenomas are more likely to progress to cancer than are the tubular adenomas.

MANAGEMENT OF COLONIC POLYPS

Patients undergoing colonoscopic treatment of colonic polyps require a mechanical bowel preparation. Most colonic polyps can be removed via the colonoscope using electrocautery techniques. Surgi-

cal removal is indicated only when an experienced endoscopist cannot remove the polyp safely or if the polyp contains an invasive malignancy. A total excision of the polyp is desirable; however, small polyps (0.5 cm or less) can be treated by biopsy and fulguration. The incidence of malignancy in these small polyps is less than 0.1%. Most pedunculated polyps are amenable to electrocautery snare polypectomy.

Sessile polyps larger than 2 cm usually contain villous features, have a high malignant potential, and tend to recur following colonscopic polypectomy. If complete or safe colonoscopic resection is not possible for technical reasons, the lesion should be biopsied and the patient referred for primary surgical therapy. In cases where the lesion can be removed via the colonoscope, follow-up endoscopy should be done within 3 to 6 months to determine whether the resection was complete. If residual adenomatous tissue is noted at follow-up colonoscopy, it should be removed and another confirmatory colonoscopy performed 3 months later. If any residual abnormal tissue is noted at the polypectomy site after two to three attempts at colonoscopic removal, surgical resection is recommended.

The resected polyp must be completely examined pathologically. Histologically the adenomatous polyps can show a benign adenoma (tubular, tubulovillous, or villous), carcinoma in situ, or invasive cancer. Colonoscopic removal is definitive therapy for patients with benign adenomatous polyps or polyps with carcinoma in situ. In cases of pedunculated polyps containing an invasive carcinoma, colonoscopic removal is considered adequate treatment when there are uniformly good prognostic indicators such as complete excision, no lymphovascular invasion, clear margins, and well-differentiated histologic findings. A follow-up examination is mandatory within 3 months to determine the presence or absence of residual or recurrent disease. All patients with lesions that do not meet these criteria should undergo an elective resection of the involved segment of the colon. Certainly if the risk of surgery because of comorbid conditions precludes operative therapy, then observation and repeat colonoscopy is appropriate.

POSTPOLYPECTOMY SURVEILLANCE

At the time of colonoscopic polypectomy, the entire colon must be examined to detect and remove all synchronous lesions. Approximately one half of patients will have a second polyp at the time of the initial colonoscopy. Metachronous polyps are found in 20% to 50% of patients within 5 years of the initial polypectomy. Once follow-up colonoscopy verifies that there are no residual polyps, colonoscopy should be repeated within 3 years. If the examination in 3 years is normal, follow-up colonoscopy every 5 years is sufficient. Patients with a solitary tubular adenoma smaller than 1 cm do not have an increased risk of cancer or metachronous polyps, and therefore no surveillance colonoscopy is indicated.

COMPLICATIONS OF COLONOSCOPIC MANAGEMENT OF COLONIC POLYPS

Colonoscopic polypectomy has an overall complication rate of 1% to 2%. The most common complication is bleeding. Other complications include free perforation of the bowel, microperforation, transmural electrocautery burn, pneumatosis cystoides intestinalis, splenic tear, and avulsion of a mesenteric blood vessel. Many of these complications can be treated expectantly, but peritonitis or unrelenting hemorrhage requires urgent laparotomy.

SURGICAL TREATMENT OF COLONIC POLYPS

The polyp that is deemed unresectable by the colonoscopic method requires surgical extirpation. Before operative resection, repeat colonoscopy is required so that the partially resected or flat lesion can be tattooed for localization at the time of surgery. It is important that the specimen be opened at the time of surgery to confirm resection of the suspicious lesion. Operative mortality for elective colectomy is less than 2% but may vary with associated comorbid conditions. Complications of colonic resection include wound infection, anastomotic leak, dehiscence, bleeding, and injury to other organs, most notably the ureter.

Laparoscopic colectomy for neoplasia should be considered investigational at the present time.

EXPECTED OUTCOMES

Long-term studies of sigmoidoscopy and polypectomy have shown a reduction of rectal cancer specific mortality to 15% of predicted levels. Similarly, long-term follow-up studies of colonic polypectomy suggest a two-thirds decrease in colon cancer specific mortality.

QUALIFICATIONS FOR TREATING COLONIC POLYPS

The qualifications of a surgeon performing any operative procedure including colectomy and colonoscopy should be based on training (education), experience, and outcomes. At a minimum, colectomy should be performed by surgeons who are certified or

eligible for certification by the American Board of Surgery or the Royal College of Physicians and Surgeons of Canada, or their equivalent.

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