Management of Barrett's Esophagus

*The SSAT, AGA, ASGE Consensus Panel**

Questions Addressed by the Consensus Panel

1. What is the pathologic classification of Barrett's esophagus and what are the prognostic implications of this categorization?
2. What is the natural clinical and pathologic history of untreated Barrett's esophagus?
3. What is the appropriate surveillance of patients with Barrett's esophagus?
4. What is the effect of treatment of Barrett's esophagus, both in terms of reversing existing Barrett's esophagus and preventing the development of esophageal carcinoma?
   - Antiacid and antireflux medical therapy
   - Antireflux surgery
   - Nonsurgical ablative treatment
     - Laser/electrocoagulation ablation
     - Photodynamic therapy
   - Surgical resection
5. What new areas of clinical investigation should be emphasized in the future?

General Summary

1. Barrett's esophagus is defined by the presence of endoscopically visible columnar epithelium within the esophagus, which on biopsy has metaplastic columnar epithelium, as defined by the presence of acid mucin–containing goblet cells.
2. Barrett's esophagus is initiated by chronic gastroesophageal reflux, which leads to esophagitis. Some patients with reflux-induced esophagitis develop metaplastic columnar epithelium within the esophagus, and a small subset of these patients develops dysplasia, a precursor of adenocarcinoma. The overall risk of developing adenocarcinoma is low in patients without dysplasia.
3. A standardized endoscopic surveillance protocol, to include periodic examinations with biopsies of columnar epithelium in patients with Barrett's esophagus, allows the physician to diagnose cancer at an early, and thus a potentially more curable, stage while averting an esophagectomy in most patients. The frequency of endoscopic surveillance is determined by the severity of dysplasia. Data are insufficient for a consensus regarding the precise protocol or frequency of endoscopic surveillance.
4. Proton pump inhibitors and antireflux surgery are highly effective in controlling the symptoms of gastroesophageal reflux disease (GERD) and healing the esophagitis in patients with Barrett's esophagus, but very few published data suggest the efficacy of any treatment in inducing the regression of the established metaplastic epithelium or preventing the progression from metaplasia to malignancy.
5. When surveillance identifies cancer, the patient should be offered an esophagectomy. Those with no dysplasia should continue periodic surveillance. Patients with low-grade dysplasia should have more frequent surveillance. Those who have high-grade dysplasia are at substantial risk for harboring an invasive carcinoma and should be considered for esophagectomy. At present, data are insufficient to provide a consensus as to the magnitude of the risk of carcinoma associated with high-grade dysplasia.
6. The role of endoscopic ablative therapies in patients with Barrett's esophagus and the impact of these therapies on reversal of metaplasia and the subsequent development of adenocarcinoma is investigational and should be employed exclusively as part of a formal clinical trial.

Diagnosis and Natural History

In Barrett's esophagus, the stratified squamous epithelium that normally lines the distal esophagus is replaced by a metaplastic intestinal-type (columnar) epithelium, as defined microscopically by the presence of acid mucin–containing goblet cells. Biopsy specimens taken to search for dysplasia can be graded as negative or indefinite for dysplasia, or as containing low-grade or high-grade dysplasia. The detection of dysplasia in biopsy specimens identifies patients at risk

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for developing adenocarcinoma of the esophagus and who therefore need either more intensive endoscopic biopsy surveillance and/or some form of therapeutic intervention.

Barrett's esophagus develops when the reflux of noxious gastric material injures esophageal squamous cells and stimulates epithelial repair associated with replacement by columnar metaplasia. Although it is clear that modern antireflux therapies are highly effective for controlling GERD symptoms in patients with Barrett's esophagus, data are insufficient to establish the efficacy of any form of GERD treatment, including antireflux surgery, in preventing the development of GERD complications such as esophageal stricture, preventing the developing of more metaplastic epithelium, inducing the regression of the metaplastic epithelium already present, or preventing the progression from metaplasia to malignancy.

Surveillance

The rationale for surveillance in patients with Barrett's esophagus is that frequent endoscopic examinations with biopsies of columnar epithelium will allow the physician to diagnose cancer at an early and potentially curable stage in the small proportion of patients in whom it develops, while avoiding esophagectomy in most patients. The frequency of surveillance is influenced by the degree of dysplasia present. Detailed information about endoscopic findings should be recorded and the mucosal abnormalities correlated with the final pathologic diagnosis. Biopsies should be carried out in all four quadrants at specific longitudinal intervals (1 or 2 cm), as well as in all areas that are irregular or friable within the Barrett's epithelium. Several studies have emphasized the importance of appropriate and systematic mapping and the predictive value of an irregular mucosa, as well as the negative predictive value of a perfectly flat mucosa without any grossly visible abnormality. Brush cytology appears useful as an adjunctive diagnostic tool. Endoscopic examination during and after therapy allows for assessment of healing and possible regression of metaplasia. Data are insufficient to define the precise timing or biopsy protocol for surveillance.

Therapy

Treatment of severe GERD involves aggressive acid suppression through the administration of proton pump inhibitors, sometimes supplemented with H_2 receptor antagonists, as well as antireflux surgery. The evidence that elimination of acid reflux reduces the cancer risk in Barrett's esophagus is indirect and inconclusive.

Treatment should be based on a consideration of morbidity and mortality of esophagectomy versus the risk of ignoring a potentially curable esophageal cancer. A pathologist who is experienced with Barrett's esophagus, who reviews the biopsies, and correlates the histologic findings with endoscopic findings, should confirm the diagnosis of dysplasia. Patients with no dysplasia or low-grade dysplasia should continue with surveillance. Patients with a diagnosis of cancer should be offered an esophagectomy by an experienced surgeon who works in a center that performs a high volume of esophagectomies. Those patients who have high-grade dysplasia should be considered for esophagectomy after being engaged in a discussion that includes the significance of the endoscopic findings, their overall state of health, their age, and the risks associated with esophagectomy. The role of ablation therapy including photodynamic therapy, argon plasma coagulation therapy, and multipolar electrocoagulation therapy in patients with Barrett's esophagus and their impact on reversal of metaplasia and the subsequent development of adenocarcinoma requires further study, and their use should be limited to formal clinical trials.

Areas for Future Clinical Investigation

Future clinical trials should be planned with an intent-to-treat design. The following issues should be addressed: (1) What is the natural history of Barrett's esophagus, and (2) what is the long-term outcome of patients treated with medical antireflux therapy, antireflux surgery, and/or endoscopic ablative therapies? Finally, the establishment of specific biologic and clinical criteria for identifying patients with Barrett's esophagus at high risk for developing adenocarcinoma is urgently needed.

Members of the Consensus Panel were:

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Pathology of Barrett's Esophagus

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DEFINITION

The Practice Parameters Committee of the American College of Gastroenterology has recently defined Barrett's esophagus as the presence of endoscopically visible columnar epithelium within the esophagus, which on biopsy is confirmed to have intestinal metaplasia.[1] Intestinal metaplasia is defined by the presence of acid mucin–containing (Alcian blue–positive) goblet cells and will be referred to simply as "metaplasia" in the following discussion. The rationale for requiring metaplastic columnar epithelium for the diagnosis of Barrett's esophagus is that as many as half of all biopsies from the distal esophagus will contain gastric fundic or cardiac-type mucosa because of a squamocolumnar junction (Z-line) that is irregular, producing "tongues" of columnar epithelium extending into the esophagus, or which lies entirely within the distal esophagus.[2] In either case, the columnar epithelium does not normally extend more than 2 cm into the esophagus. Almost all columnar epithelium extending above the distal 2 cm of the esophagus is metaplastic. Occasionally only a few goblet cells may be present in metaplastic epithelium, making it appear not to be metaplastic unless an Alcian blue stain is used to identify the goblet cells. Only metaplastic columnar epithelium has been associated with an increased risk of esophageal adenocarcinoma; therefore the diagnosis of Barrett's esophagus should be restricted to patients in whom it is present.[3] Authorities on the diagnosis of Barrett's esophagus in children recommend applying the same criteria for the diagnosis in them as are used in adults.[4]

Not all cells with a goblet configuration are metaplastic, as defined by the presence of acid mucin. Gastric surface (foveolar) cells may become distended and assume a goblet configuration ("pseudogoblet" cells), but they do not contain acid mucin and are therefore Alcian blue negative.[3] Although pseudogoblet cells can usually be recognized on routine hematoxylin and eosin staining, they may occasionally be difficult to differentiate from true goblet cells. When there is doubt about the nature of goblet-shaped cells, an Acian blue stain should be applied.

Not all Alcian blue–positive cells are metaplastic. Alcian blue–positive mucin occurs normally in the gastric mucous neck cells, the stem cells of the gastric mucosa. With injury, as in reflux disease or *Helicobacter* infection, these stem cells proliferate and may move onto the surface of the mucosa. Although some investigators believe this represents "pre-Barrett's" esophagus,[2] it is such a common finding that it cannot be used to stratify patients into risk categories for the development of adenocarcinoma.

In patients in whom no endoscopically visible segment or "tongue" of columnar epithelium is visible in the esophagus, the significance of a few metaplastic glands on biopsy is unknown, and it therefore seems appropriate to avoid using term "Barrett's" esophagus for this finding.[6] The significance of this intestinal metaplasia at the gastroesophageal junction is currently an area of controversy and the subject of much research.[6,7] One school of thought suggests that it is due to gastroesophageal reflux and is related to Barrett's esophagus,[8] whereas another suggests that it is related to *Helicobacter pylori* infection of the stomach.[9]

NEOPLASTIC PROGRESSION IN BARRETT'S ESOPHAGUS

Neoplastic progression in Barrett's esophagus is initiated by chronic gastroesophageal reflux, which leads to esophagitis. A subset of patients with reflux-induced esophagitis develops metaplastic columnar epithelium within the esophagus, and a subset of these patients develops dysplasia, a precursor of adenocarcinoma.[5,10] Dysplasia is defined as neoplastic epithelium that is still confined within the basement membrane of the gland or epithelial surface within which it arose. Biopsy specimens taken to search for dysplasia can be graded as negative or indefinite for dysplasia, or as containing low-grade or high-grade dysplasia.[5] Molecular genetic studies of dysplastic epithelium reveal clonal abnormalities that are identical to those found in the cancers that may accompany it, confirming that the cancers arose from the dysplastic epithelium. The detection of dysplasia in biopsy specimens

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identifies patients at higher risk for developing adenocarcinoma of the esophagus, and who therefore need either intensive endoscopic biopsy surveillance or some form of therapeutic intervention. Not all patients with high-grade dysplasia in Barrett's esophagus will develop cancer.[11,12] Studies in which patients with high-grade dysplasia remain under close endoscopic surveillance indicate that fewer than half develop adenocarcinoma during follow-up of about 4 years, and in those who did develop cancer, an average of 5 years was required to do so.[12]

PROBLEMS WITH THE USE OF DYSPLASIA AS AN INDEX OF CANCER RISK IN BARRETT'S ESOPHAGUS

Sampling error is perhaps the most significant problem in the detection of dysplasia because it may be localized to small areas of mucosa when it first develops. Thus, in order to detect dysplasia if it is present, four-quadrant biopsies at intervals of 2 cm or less throughout the length of the Barrett's segment, and additional biopsies of any endoscopically visible abnormalities, are recommended.[13] In patients with high-grade dysplasia who are going to continue to undergo periodic endoscopic surveillance, the interval between the four-quadrant biopsies should be reduced to 1 cm to provide the best chance of detecting small foci of early cancer when they develop.[13]

Inflammation that may be present within the Barrett's epithelium can produce reactive changes that are difficult to distinguish from dysplasia.[5] For this reason it is appropriate for the patient to have received medical therapy, usually in the form of a proton pump inhibitor, in an attempt to reduce the inflammation prior to further endoscopic biopsy surveillance for dysplasia.

The histologic diagnosis of dysplasia in Barrett's esophagus is a subjective interpretation and there is, therefore, some variation in the diagnosis and grading of dysplasia among different pathologists.[14] For this reason the diagnosis of high-grade dysplasia should be confirmed by a second pathologist experienced in its diagnosis before definitive therapeutic measures are implemented.

The natural history of dysplasia in Barrett's esophagus has not been well established in long-term prospective follow-up studies. However, as indicated earlier, it appears that up to 50% of patients with high-grade dysplasia who are continued on endoscopic surveillance do not develop invasive carcinoma after several years of follow-up.[11,12] In a few patients high-grade dysplasia may disappear. In most of these the disappearance is only apparent because of sampling error, and the dysplasia will be identified on subsequent biopsies. A small percentage of patients, however, have apparent complete regression of high-grade dysplasia with control of reflux.[11,12]

Brush cytology could theoretically play a role in the management of patients with Barrett's esophagus; however, one study found that it was not as sensitive or specific as histologic examination in detecting Barrett's metaplastic epithelium.[15] Although brush cytology was thought to be useful in detecting dysplasia in one study,[15] another found that its addition to histologic examination increased the cost but not the diagnostic yield for dysplasia or cancer.[16]

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Medical Treatment of Barrett's Esophagus

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In Barrett's esophagus, the stratified squamous epithelium that normally lines the distal esophagus is replaced by a metaplastic intestinal-type epithelium. This condition is judged to develop when the reflux of noxious gastric material injures esophageal squamous cells while simultaneously stimulating epithelial repair through columnar metaplasia.[1] The metaplastic columnar cells have a malignant predisposition, and Barrett's esophagus with specialized intestinal metaplasia is a major risk factor for esophageal adenocarcinoma.[2] The precise factors that induce the transition from metaplasia to malignancy are not known, but circumstantial evidence suggests that gastroesophageal reflux disease (GERD) may play a role in this process as well. Consequently, aggressive treatment of GERD has been prescribed for patients with Barrett's esophagus with the following goals: (1) to control GERD symptoms, (2) to prevent the development of GERD complications such as esophageal stricture; (3) to prevent the development of more metaplastic epithelium, (4) to induce the regression of the metaplastic epithelium already present, and (5) to prevent the progression from metaplasia to malignancy. Although it is clear that modern antireflux therapies are highly effective in controlling the symptoms of GERD in patients with Barrett's esophagus,[3] very few published data support the efficacy of any GERD treatment in accomplishing the latter four goals.

The mainstay of modern medical therapy for severe GERD is aggressive suppression of gastric acid through the administration of proton pump inhibitors (PPIs).[4] The profound acid suppression that can be achieved with the use of these agents has raised theoretical concerns regarding their long-term safety.[5] Protracted acid suppression can elevate the serum level of gastrin, a hormone that has trophic effects on the stomach and colon, and might result in bacterial overgrowth in the stomach and proximal small intestine. These effects conceivably could contribute to carcinogenesis. Furthermore, some data suggest that sustained acid suppression with PPIs might hasten the development of gastric atrophy in patients who are infected with *Helicobacter pylori*,[6] and that chronic PPI therapy might interfere with vitamin B_{12} absorption.[7] Despite these theoretical concerns, there are no reports of tumors or nutritional deficiencies clearly attributable to the use of PPIs after more than a decade of extensive clinical experience with these agents.[4] Some clinicians prescribe the combination of a PPI and a prokinetic agent (e.g., cisapride) for patients with severe GERD, but few data either strongly support or clearly refute this practice. In a large study that compared a number of maintenance therapies for patients with reflux esophagitis, 80% of those treated with omeprazole alone (20 mg every day) remained in remission at 1 year compared to 89% of patients treated with the combination of omeprazole (20 mg every day) and cisapride (10 mg three times a day).[8] However, the apparent difference in remission rates between these two groups was not statistically significant. Some clinicians add prokinetic agents to PPIs only for patients who have prominent symptoms that suggest dysmotility such as regurgitation, nausea, and bloating. Even in these patients, few published data establish that this combination therapy is superior to PPI therapy alone. It remains unclear whether the benefits of prokinetic agents outweigh their added expense, inconvenience, and risk for patients who are treated with PPIs.

As mentioned, one of the goals of medical therapy for GERD is to prevent the development of complications such as esophageal stricture. For patients with established peptic esophageal strictures, PPI therapy both improves dysphagia and decreases the need for subsequent esophageal dilations.[9,10] Few reports have documented the development of peptic strictures in patients known to have uncomplicated GERD, however, and no study has established that medical therapy prevents the formation of these strictures.[11]

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Severe GERD is a chronic and often unrelenting condition and, until recently, authorities assumed that Barrett's esophagus progressed in extent with time as more and more columnar mucosa replaced reflux-damaged squamous epithelium. A report by Cameron and Lomboy[12] seriously challenged this widely held assumption, however. A review of the records of 51,311 patients who had endoscopic examinations at the Mayo Clinic between 1976 and 1989 (before the advent of PPIs) revealed 377 cases of benign Barrett's esophagus. The prevalence of the disorder was found to increase from 0% in patients 0 to 9 years of age to a maximum of 0.9% in patients 80 to 89 years of age. Unlike the age-related rise in prevalence, however, the length of esophagus lined by Barrett's epithelium did not increase significantly with age. Furthermore, no progression in extent of Barrett's esophagus was found among 101 patients who had follow-up endoscopic examinations performed after a mean interval of 3.2 years. This report and others suggest that established esophageal metaplasia usually does not progress substantially with time, even in the absence of PPI therapy.[13] Thus there is little support for the notion that aggressive antireflux therapy is needed to prevent the progression in extent of Barrett's esophagus.

Metaplasia is a potentially reversible process if the responsible pathogenetic factors can be controlled.[1] Unfortunately, control of GERD (the factor responsible for metaplasia in Barrett's esophagus) rarely, if ever, results in the complete reversal of the metaplastic epithelium.[14] Partial regression of Barrett's esophagus (with the appearance of islands of squamous epithelium within the metaplastic columnar lining) is observed frequently in patients treated with PPIs, but the importance of this phenomenon is not known.[14] In a prospective study, Sharma et al.[15] obtained 39 biopsy specimens from squamous islands in 22 patients with Barrett's esophagus, most of whom had been treated with PPIs. Intestinal metaplasia underlying squamous epithelium was found in 15 (39%) of the 39 specimens, suggesting that the partial regression of metaplasia induced by PPI therapy might have little effect in decreasing the cancer risk.

The notion that control of acid reflux prevents the progression from metaplasia to malignancy in Barrett's esophagus is based on circumstantial evidence and conjecture. GERD appears to cause Barrett's esophagus, and GERD is clearly a strong risk factor for esophageal adenocarcinoma.[16] However, it is not clear whether GERD predisposes to malignancy by causing the initial metaplasia, by promoting the transition from metaplasia to neoplasia, or both. Chronic reflux esophagitis might be expected to predispose to cancer by increasing the turnover and proliferation of metaplastic epithelial cells, and few would argue that antireflux therapy is indicated for the treatment of reflux esophagitis. However, no study has established that the healing of reflux esophagitis with antireflux therapy reduces the risk of cancer in Barrett's esophagus.

Fitzgerald et al.[17] have demonstrated that biopsy specimens of Barrett's esophagus maintained in organ culture exhibit cellular hyperproliferation when exposed to short pulses (1 hour in duration) of acid, whereas continuous acid exposure blocks cellular proliferation. These findings suggest that the short pulses of esophageal acid exposure that occur frequently in patients with Barrett's esophagus might induce cellular hyperproliferation and thereby promote carcinogenesis. Conventional PPI therapy for GERD does not eliminate brief episodes of acid reflux in most patients. Indeed, Castell et al.[18] have shown that approximately 70% of individuals (normal subjects as well as patients with GERD and Barrett's esophagus) who are treated with a PPI twice a day experience nocturnal gastric acid breakthrough (defined as a gastric pH <4 for more than 1 hour at night), and that brief episodes of acid reflux occur frequently during these breakthrough periods.[19,20] Furthermore, patients with Barrett's esophagus often exhibit pathologic levels of acid reflux, even during therapy with PPIs in doses that completely eliminate the symptoms and signs of GERD.[21,22] Clearly, conventional medical therapy for GERD does not abolish acid reflux in most patients with Barrett's esophagus.

Nocturnal acid breakthrough can be eliminated in most patients on PPI therapy by the addition of a histamine H_2-receptor blocker at bedtime.[23] Thus, with polypharmacy that includes PPIs in high doses, it is possible to abolish acid reflux in patients with Barrett's esophagus. I do not recommend this approach at this time for the following reasons: (1) Perfect control of acid reflux clearly is not necessary to effect the healing of reflux esophagitis. Indeed, elimination of the symptoms and signs of GERD can be achieved in most patients who are treated with a PPI taken at a conventional dosage only once each day.[18] (2) The evidence to support the notion that complete elimination of acid reflux reduces the risk of cancer in Barrett's esophagus is indirect and weak at best. It may not be appropriate to extrapolate the results of studies performed in the artificial environment of organ culture to the clinical situation. Furthermore, some experimental data even suggest that elimination of acid reflux may not be desirable. In an experimental model of esophageal adenocarcinoma involving rats treated with a carcinogen, for example, exposure of the esophagus to gastric juice *protected* against the development of cancer.[24] (3) Complete elimination of acid reflux would entail considerable inconvenience and expense, because of both the high doses of the multi-

ple medications required and the esophageal pH monitoring studies necessary to document the efficacy of therapy in controlling acid reflux. Presently, I feel that the available data only support the administration of medications in dosages that will eliminate the symptoms and endoscopic signs of GERD for patients with Barrett's esophagus. The general guidelines established for the medical treatment of GERD[4,25] are applicable, irrespective of the presence of Barrett's esophagus.

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Reversal of Barrett's Esophagus With Electrocoagulation and Laser

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Contemporary medical and surgical therapies are successful in controlling the reflux symptoms and healing erosions in patients with Barrett's esophagus. However, complete reversal of Barrett's esophagus with these therapies in an effort to eliminate the potential for the development of adenocarcinoma is distinctly unusual. Many endoscopic modalities have been used in an effort to reverse Barrett's esophagus. All have been combined with acid reduction with pharmacologic therapy or fundoplication.

ELECTROCOAGULATION

Table I lists the series of patients in whom reversal of Barrett's esophagus was achieved with multipolar electrocoagulation. The series range from no residual intestinal metaplasia,[2,3] to isolated glands of intestinal metaplasia underlying new squamous epithelium,[1] to 1 to 2 cm of residual macroscopic Barrett's esophagus.[4] An average of 1.6 to 3.7 treatment sessions were necessary to reverse the Barrett's esophagus. Transient odynophagia, dysphagia, and chest pain were the most common side effects. One patient had recurrence of a prior stricture.

LASER

Table II lists the series in which different forms of laser energy were used. Berenson et al.[6] conducted a model study in which 0.25 to 4 cm^2 areas were treated with argon laser. Squamous reepithelialization occurred in 93% of areas with three squamous borders compared to 29% with one squamous border, suggesting spread from contiguous squamous epithelium. Nd:YAG laser after fundoplication reversed Barrett's esophagus in 11 patients; interestingly, two patients had gastric intestinal metaplasia—it is not clear whether this was present prior to therapy.[7] Potassium-titanyl-phosphate (KTP) laser has been used with varying results.[9,10] Barham et al.[9] demonstrated a unique pattern of squamous epithelium within Barrett's glands as well as intestinal metaplasia underlying new squamous epithelium. Transient mild retrosternal pain was reported in these series.

Fig. 1 suggests the theoretical depth of injury using different endoscopic reversal techniques. Techniques with deeper injury also have the potential for more severe complications such as esophageal strictures. In the series of electrocoagulation and laser therapy listed in Tables I and II, no perforations were reported. Deeper injury might prove to be appropriate for a patient with high-grade dysplasia who might have intramucosal cancer that was not detected by endoscopic biopsy.

ISSUES TO BE DEFINED

The level of acid suppression necessary for reversal of Barrett's esophagus is not clear. Some series report reversal with persisting abnormal esophageal pH acid exposure,[5] whereas others report the need to increase the dose of proton pump inhibitor to achieve reversal or recurrence of Barrett's esophagus with discontinuation of medication.

Technical advances in the probes or the administration of energy would theoretically lead to reversal in fewer treatment sessions and more even depth of injury so as to avoid residual underlying intestinal metaplasia. Ultimately, the critical issue is the impact of reversal on the incidence of adenocarcinoma.

EXPERIMENTAL NATURE OF BARRETT'S ESOPHAGUS REVERSAL

Ultimately, reversal techniques have to be independently validated. Efficacy, safety, and duration of reversal have to be documented. Validation of biologic markers to define the risk of adenocarcinoma is important for selection of candidates for reversal therapy.

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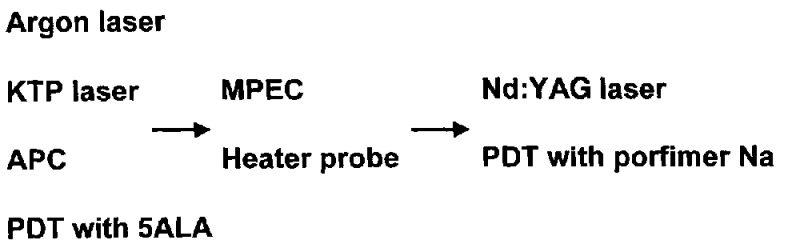

Fig. 1. Theoretic depth of ablation. MPEC = multipolar electrocoagulation; APC = argon plasma coagulation; PDT = photodynamic therapy; 5-ALA = 5-aminolevulinic acid.

Table I. Electrocoagulation reversal

Reference	No. of patients	Mean follow-up (mo)	Residual intestinal metaplasia
1	11	24	3
2	14	19.3	0
3	8	12	0
4	21	12	2
5	27	4.5	5
6	54	Post therapy	5

Table II. Laser reversal

Reference	Laser	No. of patients	Mean follow-up (mo)	Residual intestinal metaplasia
7	Argon	10 (40 areas)	N/A	17
8	Nd:YAG	11	26	2 gastric cardia
9	Nd:YAG	19	3	9
10	KTP	13	3-18	11/9
11	KPT	10	11	0

The development of an index of demographic, endoscopic, histologic, and biologic characteristics identifying the highest risk patients for the development of cancer would facilitate clinical application.

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Antireflux Surgery in the Management of Barrett's Esophagus

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Barrett's esophagus is a complication of gastroesophageal reflux disease characterized by the presence of intestinal metaplasia in an esophagus lined by cardiac-type mucosa; alternatively it is described as a columnar-lined esophagus with intestinal metaplasia or Barrett's mucosa. The clinical significance of this finding is that it confers a greater than 30 to 125-fold risk of progression to esophageal adenocarcinoma, which emerges at a rate of about one to two cancers per 100 patient-years of follow-up.[1-3]

It was previously thought that Barrett's esophagus developed quickly to its full extent, with little subsequent increase in length.[4] This was based on data from patients with only long segments of Barrett's mucosa within the tubular esophagus (i.e., >3 cm). It is now accepted that intestinal metaplasia occurs in segments of cardiac-type mucosa that are less than 3 cm in length and that intestinal metaplasia can even occur in extremely small segments of cardiac-type mucosa located just below the squamous epithelium of an endoscopically normal-appearing gastroesophageal junction.[5-7]

It has been shown that the presence of cardiac-type mucosa at the gastroesophageal junction is almost always associated with an inflammatory infiltrate and increased esophageal acid exposure on 24-hour esophageal pH monitoring.[7] Consequently this finding is referred to as carditis and is one of the early signs of gastroesophageal reflux disease. The length of the inflamed cardiac-type mucosa has been shown to be related to the degree of esophageal acid exposure, the level of lower esophageal sphincter pressure, and the length of the sphincter exposed to the abdominal pressure environment.[8] It is now known that the segments of cardiac-type mucosa can show intestinal metaplasia on biopsy and that the prevalence of this finding is directly related to the length of the segment.[8] On the basis of these studies, it is hypothesized that Barrett's esophagus spreads progressively upward in a stepwise fashion. Initially, short segments of inflamed cardiac-type mucosa develop below an endoscopically normal-appearing gastroesophageal junction. The inflammation causes a loss in the pressure and length of the lower esophageal sphincter resulting in greater esophageal acid exposure with extension of inflamed cardiac-type mucosa into the distal 3 cm of the esophagus. With further loss of sphincter pressure and length, the process extends higher into the esophageal body. With time and under proper conditions, intestinalization of the cardiac mucosa can occur with a prevalence that is related to the duration and functional severity of the disease. It is the occurrence of intestinalization that is associated with a cancer risk and is required for the diagnosis of Barrett's esophagus. Understanding the disease in this way has strong implications regarding its therapy.

Based on the preceding information, there are three goals in the management of Barrett's esophagus: (1) prevent the development of the metaplastic epithelium by stopping reflux early in the disease process; (2) promote or induce healing or regression of the metaplastic epithelium such that the cancer risk mucosal change (intestinal metaplasia) is eliminated; and (3) induce a quiescence of the intestinalized metaplastic epithelium and halt its progression to dysplasia and cancer. The goal of therapy for Barrett's esophagus according to the American College of Gastroenterology is to "control the symptoms of gastroesophageal reflux disease." The College states that "symptom relief is an appropriate end point for the therapy of Barrett's esophagus."[9] This approach has been shown to be ineffective in that the eradication of symptoms cannot be equated with elimination of reflux, nor has it been able to reliably achieve the second and third management goals.[10-14]

In a study by Hameeteman et al.[1] from The Netherlands, 50 patients with a columnar-lined esophagus were treated medically and followed from 1.5 to 14

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years (mean 5.2 years). Of these 50 patients, initially only 34 had intestinal metaplasia on biopsy of the columnar mucosa. At the completion of the study 37 patients had intestinal metaplasia, suggesting that three patients developed the cancer risk epithelium during the 5-year study period. In addition, at the start of the study, six patients had low-grade dysplasia and one patient had high-grade dysplasia. By the end of the 5-year study period, 10 patients had low-grade dysplasia, three had high-grade dysplasia, and five had adenocarcinoma.[1] Similarly, Sharma et al.[15] followed 32 medically treated patients with short-segment Barrett's esophagus (mean length 1.5 cm) for a mean of 36.9 months and found a 5.7% annual incidence of progression to dysplasia. During the 98 patient-years of follow-up in their series, two patients developed high-grade dysplasia, and one of these patients had progression to cancer. Recall that the expected rate of cancer is 1 per 100 patient-years of follow-up. All patients in the study by Sharma et al. were treated with omeprazole, ranitidine, and/or promotility agents. These investigators commented that most patients developed dysplasia while on acid suppression medication, and they concluded that medical treatment does not prevent the development of dysplasia.

Recently Lagergren et al.[16] reported that the risk of esophageal adenocarcinoma was increased nearly eightfold among persons in whom heartburn, regurgitation, or both occurred at least once a week compared to persons without these symptoms. Of interest, they noted that the risk of esophageal adenocarcinoma was three times higher among patients who used medication to relieve the symptoms of reflux compared to those who did not take any antireflux medication.

Does Antireflux Surgery Prevent the Development of Barrett's Esophagus?

Since Barrett's esophagus is associated with gastroesophageal reflux disease, it would seem logical that stopping reflux by means of a surgical fundoplication should effectively prevent the development of Barrett's esophagus in patients who have gastroesophageal reflux without Barrett's esophagus. Antireflux surgery has been shown to restore lower esophageal sphincter function and abolish reflux of gastric contents, acid or bile, into the esophagus.[17] Consequently an antireflux operation ends the repetitive injury to the esophageal mucosa. Randomized clinical studies have confirmed superior control of reflux following antireflux surgery compared to medical therapy, and have shown the surgical antireflux procedure to be safe and durable.[13,18-20] Furthermore, recent advances in minimally invasive surgical technology have shown that the procedure can be performed laparoscopically with the same outcome, less morbidity, a shorter hospital stay, and a more rapid complete recovery.[21-23]

Very few authors have recorded the presence of Barrett's metaplasia following antireflux surgery when it was absent preoperatively. The long-term study of Luostarinen et al.[24] managed to perform endoscopy 20 years after Nissen fundoplication in 21 patients. In 15 the fundoplication appeared to be intact, and in six it appeared to be defective. Two of the 15 with intact fundoplications were found to have Barrett's esophagus on long-term follow-up, but the authors admit that these two patients did not have preoperative biopsies, and hence it cannot be concluded that Barrett's esophagus developed (Table I). In contrast, five of the six patients with defective fundoplications developed Barrett's esophagus during the follow-up period. This finding emphasizes that the patient with an intact fundoplication had the potential to develop Barrett's esophagus and did not, and that the benefits of surgery are dependent on performing an effective and durable repair.

A review of studies with endoscopic follow-up of patients treated by antireflux surgery showed that most concentrate on the healing of esophagitis. Reports by Thor and Silander[25] and Johansson et al.[26] both mention healing of esophagitis, and neither report identifies a single case of Barrett's esophagus developing within 5 years after an antireflux operation, which was not present before the operation (see Table I).

Table I. Development of Barrett's esophagus following antireflux surgery

Reference	No.	No. with CLE	Follow-up yr	No. developing Barrett's esophagus
Luostarinen et al.[24]	15*	0	20	2†
Thor and Silander[25]	31	4	5	0
Johansson et al.[26]	33	4	5	0
	79	8	range 5-20	2†

CLE = columnar-lined epithelium.
*All intact fundoplications.
†No preoperative biopsy in these patients to exclude Barrett's esophagus.

Despite the limitation of these studies, it appears that the *de novo* development of Barrett's esophagus is exceedingly rare in patients who have had effective antireflux surgery. This is in marked contrast to long-term medical treatment. A recent report from Austria found that up to 34% of patients on long-term acid suppression developed Barrett's esophagus while on therapy.[27] Seven (58%) of 12 patients on continuous omeprazole therapy developed Barrett's esophagus. Consequently a policy of correctly performing an effective fundoplication early in the course of the disease would likely reduce the incidence of Barrett's esophagus in the future. Risk factors for Barrett's esophagus, such as young age of onset, long duration of symptoms, persistent esophagitis, defective lower esophageal sphincter, and mixed duodenogastric reflux, should encourage early operation.

Does Antireflux Surgery Promote or Induce Healing or Regression of the Metastatic Epithelium Such That the Cancer Risk Epithelium (Intestinal Metaplasia) Is Eliminated?

There are conflicting reports about whether Barrett's mucosa regresses following antireflux surgery. Brand et al.,[28] in 1980, was the first to describe complete regression of all metaplastic epithelium in 4 of 10 patients with Barrett's esophagus following an antireflux procedure. Subsequently most reports have demonstrated that although some regression of the length of Barrett's esophagus is common, complete regression after an antireflux procedure occurs only occasionally, but progression in the length of Barrett's esophagus rarely occurs. A review of the English language literature since 1977 documents follow-up of 340 patients after antireflux surgery. Complete regression occurred in only 13 patients, whereas in 256 (75%) of the 340 patients the Barrett's epithelium remained unchanged.[29] Thus regression of traditional Barrett's esophagus cannot be reliably predicted or anticipated following antireflux surgery.

In contrast to the unreliable regression of the traditional (>3 cm) segments of Barrett's esophagus, we recently showed that 73% of patients with intestinal metaplasia at the gastroesophageal junction had complete regression of the intestinal metaplasia component of the Barrett's mucosa (i.e., the at-risk mucosa) following antireflux surgery.[30] By comparison, only 4% of patients with a long segment of Barrett's esophagus had loss of intestinal metaplasia after an antireflux procedure. Recently Low et al.[31] also reported the loss of intestinal metaplasia in two patients with short-segment (<3 cm) Barrett's esophagus.

Theoretically the ideal treatment for a patient with Barrett's esophagus is one that will restore the normal squamous mucosa and eliminate the cancer risk associated with the intestinalization of the cardiac-type mucosa. Currently neither medical nor surgical therapy reliably offers this. However, a number of experimental techniques for mucosal ablation show promise. In animal studies and limited clinical trials, some of these ablation techniques have been successful in removing the columnar mucosa and allowing subsequent squamous reepithelialization. The elimination of gastroesophageal reflux is critical to the success of squamous reepithelialization. Consequently, persistent or recurrent areas of intestinal metaplasia plague most series of mucosal ablation in which patients take proton pump inhibitors in an effort to stop reflux. In contrast, Salo et al.[32] recently reported successful ablation of Barrett's epithelium with the use of Nd:YAG laser after antireflux surgery. They followed 11 patients for a mean of 26 months after the last laser treatment and noted complete squamous regeneration in all patients. There was no residual metaplastic epithelium except in two patients with persistent intestinal metaplasia at the gastroesophageal junction. Ablation when combined with an antireflux procedure is likely to be the most reliable method to remove the metaplastic mucosa and allow squamous regeneration.

Does Antireflux Surgery Induce Quiescence of the Barrett's Mucosa and Halt the Progression to Dysplasia and Cancer?

Clinical experience has shown that patients with Barrett's esophagus can have progression to low-grade dysplasia, high-grade dysplasia, and eventually adenocarcinoma while undergoing medical treatment. In contrast, clinical evidence is mounting that in patients with Barrett's esophagus, surgical therapy is associated with a reversal of the first step in this process. In our series of 60 patients with intestinal metaplasia of the esophagus or esophagogastric junction, we found preoperatively low-grade dysplasia in 10 patients.[30] In 7 of the 10 patients the low-grade dysplasia reverted to Barrett's esophagus without dysplasia following an antireflux procedure. Similarly, Low et al.[31] noted that 4 of their 14 patients had low-grade dysplasia, and in all four patients it regressed to Barrett's esophagus without dysplasia after antireflux surgery.[31] In our opinion, preventing reflux with a properly constructed antireflux procedure stops the continual irritation of the metaplastic mucosa and allows the cells to become quiescent. If low-grade dysplasia persists after antireflux surgery, consideration should be given to mucosal ablation without acid suppression therapy.

Perhaps of greater importance is whether Barrett's esophagus progresses to high-grade dysplasia or cancer after surgical treatment of reflux disease. McCallum et al.[33] prospectively followed 181 patients with Barrett's esophagus. Twenty-nine had antireflux surgery while the remaining 152 patients were treated medically. After a mean follow-up of 5 years in the surgical group and 4 years in the medical group, there was a significant difference in the incidence of dysplasia and adenocarcinoma. Dysplasia was found in 3.4% of the surgical group compared with 19.7% in the medically treated group. No patient in the surgically treated group developed adenocarcinoma of the esophagus, whereas this did occur in medically treated patients. These investigators concluded that compared to medical therapy an antireflux operation in patients with Barrett's esophagus was significantly associated with a reduction in the incidence of dysplasia and cancer. Similarly Katz et al.[34] followed 102 patients with Barrett's esophagus for a mean of 4.8 years. By 3 years, approximately 8% of the medically treated patients had high-grade dysplasia. In contrast, patients treated by antireflux surgery had a significantly reduced risk of developing dysplasia. The 9-year dysplasia and cancer-free survival was 100% for 15 patients after an antireflux procedure and 50% for 82 patients treated with medical therapy ($P = 0.03$).

One factor complicating any analysis of progression of Barrett's esophagus to high-grade dysplasia or cancer after antireflux surgery is that the cellular and genetic alterations leading to the development of high-grade dysplasia and adenocarcinoma may have already occurred prior to performance of the antireflux procedure. It has been estimated to take up to 6 years for adenocarcinoma to develop within Barrett's esophagus with low-grade dysplasia, and thus some cancers, particularly those that present during the first few postoperative years, probably do not represent progression of disease after surgery. McDonald et al.[35] made this point in a study from the Mayo Clinic. They found invasive adenocarcinoma in two patients and high-grade dysplasia in one patient during surveillance after antireflux surgery, but they noted that no patient developed carcinoma or high-grade dysplasia after 39 months despite a median follow-up of 6.5 years, and a maximum follow-up of 18.2 years.

Review of the English language literature since 1975 revealed 11 series and a total of 346 patients with Barrett's esophagus followed after fundoplication.[29] Patients were found to have esophageal adenocarcinoma after antireflux surgery in only 7 of the 11 reports. Apart from these series, four isolated reports were found describing adenocarcinoma developing in Barrett's esophagus after an antireflux operation. Although the length of follow-up was not always available, 11 (58%) of the 19 cancers developed within 3 years of fundoplication, and 15 (79%) developed within 5 years of fundoplication. The remaining four cancers developed from 5 to 10 years after fundoplication, but in each case the patients had recurrent reflux on the basis of symptoms or positive 24-hour pH monitoring. Thus a functioning fundoplication seems to provide protection from progression of Barrett's esophagus to adenocarcinoma in patients where this process has not started prior to the surgical procedure.

In summary, the evidence as it exists today would support the notion that the best treatment for the prevention of Barrett's esophagus is an early antireflux procedure in patients who manifest the risk factors associated with Barrett's esophagus. The best treatment of a patient with established Barrett's esophagus is an appropriately selected and well-constructed antireflux procedure and yearly surveillance for the first 5 years with progressively longer intervals thereafter. If during surveillance low-grade dysplasia persists or develops, mucosal ablation should be considered. If high-grade dysplasia or intramucosal carcinoma develops without a visible lesion, a vagal-sparing esophagectomy should be considered.[36] If a visible lesion develops, an en bloc esophagectomy provides the best chance for cure.[37,38]

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Photodynamic Therapy in the Management of Barrett's Esophagus With Dysplasia

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Photodynamic therapy (PDT) involves the systemic use of a photosensitizing drug that is activated by a light of proper wavelength and power to produce singlet oxygen and subsequent cell death. PDT also results in ischemic necrosis of tissue, further enhancing the injury. Clinical use has focused on the use of PDT in human malignancies since cancers have preferential concentration of the photosensitizers. This characteristic allows some selectivity in treating malignant tissue while, to a degree, sparing normal tissues.[1,2] The primary use for PDT in gastroenterology has been in the treatment of esophageal cancer,[3-5] but trials are now underway to evaluate treatment effects in Barrett's esophagus with dysplasia and superficial cancers.

Porfimer sodium (Photofrin, QLT PhotoTherapeutics, Vancouver, B.C.) is the photosensitizer most used in clinical trials. It is now approved for use in certain types of esophageal cancer in the United States, Canada, The Netherlands, Japan, and France. Photofrin is also undergoing clinical trials in head and neck cancer, brain tumors, and Barrett's esophagus. Second-generation drugs currently being tested in esophageal disease include 5-aminolevulinic acid (5-ALA), metahydroxyphenylchlorin (mTHPC).[6,7] Benzoporphyrin derivative may be used in clinical trials in the near future.

With Photofrin-PDT for Barrett's esophagus with high-grade dysplasia, the drug is injected intravenously followed by endoscopy within 40 to 50 hours. In the current multicenter clinical trial, a 2.5 cm diameter 3, 5, or 7 cm windowed esophageal centering balloon is used to deliver dye-laser red light (630 nm) to the targeted esophageal disease. A cylindrical 2.5 cm diffuser attached to a fiberoptic probe also can be used to supplement the light dose to any esophageal nodular tissue. Light delivery can be repeated in 48 hours if needed to achieve additional mucosal destruction. Patients are usually treated in the outpatient setting.

As expected, with the deeper injury associated with Photofrin-PDT, chest pain and nausea commonly occur following treatment but resolve within several days. Fever of 100 to 101° F can also occur. Perforation is very unlikely but esophageal strictures occur in 25% to 34% after Photofrin-PDT in current studies.[8] Depending on clinical needs, more than one PDT session can be administered. Photofrin remains in body tissues for approximately 1 month necessitating that the patient avoid direct sunlight exposure during that period.[1,2]

Photofrin-PDT for Barrett's esophagus with dysplasia or early esophageal cancer has been used most extensively by Overholt and Panjehpour.[8] Mucosal destruction with Photofrin-PDT treatment is marked. Healing in the presence of proton pump inhibitor therapy (a "no-acid" environment) resulted in replacement of 75% to 80% of the treated Barrett's epithelium with normal squamous epithelium. In their first 100 patients, high-grade dysplasia was eliminated in 88% of 73 patients, low-grade in 92% of 14 patients, and T1 cancers in 77% of 13 patients. When combined with thermal ablation (contact YAG laser) of small residual islands of Barrett's epithelium persisting after PDT, complete elimination of Barrett's esophagus was also accomplished in 43 patients. Strictures occurred in 34% of patients but all responded to dilation. Repeated PDT sessions with overlapping areas of mucosal injury appear to be associated with a higher incidence of stricture formation, approximating 50%.[9] Utilizing four-quadrant biopsies every 2 cm of treated mucosa, subsquamous glandular mucosa was found in only 2 of the 100 patients, indicating a high incidence of complete ablation of Barrett's mucosa. Two other patients developed small nodules of

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Table I. Photodynamic therapy in Barrett's esophagus with high-grade dysplasia

Reference	No. of patients	Drug	HGD response (%)	Barrett's eradication (%)	Subsquamous Barrett's (%)	Additional therapy	Stricture (%)
Overholt and Panjehpour[8]	73	Photofrin	88	43	2-4	YAG	34
Wang[10]	26	HpD	88	35	NR		27
Gossner et al.[6]	10	ALA	100	0	? All	mTHPC (for carcinoma)	0
Barr et al.[7]	5	ALA	100	0	? All		0

HGD = high-grade dysplasia; mTHPC = meta-hydroxyphenylchlorin, ALA = aminolevulinic acid; HpD = hematoporphyrin derivative; NR = not reported.

high-grade dysplasia that were successfully retreated. One additional patient developed a small subsquamous adenocarcinoma 6 months after PDT that was also successfully retreated. The authors believed that the tumor was present at the time of the initial PDT and had not been fully destroyed.[8]

Wang,[10] expanding on earlier work,[11] has reported in tabulated form results in 26 patients using hematoporphyrin derivative for ablation in dysplastic Barrett's mucosa. Elimination of high-grade dysplasia was achieved in 88% and Barrett's mucosa in 35%. Strictures occurred in 27%. No information on the incidence of subsquamous Barrett's mucosa was provided.

In contrast, with 5-ALA–PDT, the injury is less as the photoactivating compound, and hence the injury, is confined to mucosa.[6,7] As such, whether ALA-PDT will be sufficient for destruction of Barrett's dysplasia is uncertain since current studies indicate a high percentage of residual subsquamous Barrett's mucosa in patients treated with ALA-PDT.[6,7] The reality is that to totally eliminate Barrett's mucosa, mucosal destruction must be significant and probably must be relatively deep.

Gossner et al.[6] have used 5-ALA–induced protoporphyrin IX PDT to treat 10 patients with severe dysplasia with elimination of dysplasia in all 10. Malignancies greater than 2 mm deep required mTHPC PDT for deeper tissue effect. Barr et al.[7] have reported on five patients treated with 5-ALA–PDT. In both studies, persistence of subsquamous Barrett's mucosa occurred and reepithelialization with squamous mucosa was only partial following PDT indicating an incomplete long-term response.

In conclusion, PDT appears to hold considerable promise as an endoscopic therapy for Barrett's esophagus with high-grade dysplasia (Table I). Under current techniques, deeper mucosal therapy with Photofrin-PDT produces better long-term results with elimination of Barrett's esophagus and subsquamous Barrett's mucosa but at the risk of a relatively high incidence of strictures. The more superficial treatment with 5-ALA is reported to eliminate dysplasia but persistence of Barrett's esophagus and the occurrence of post-therapy subsquamous Barrett's mucosa is high, thereby continuing the risk of adenocarcinoma. No deaths have been reported in more than 100 Barrett's patients treated with PDT. Currently Photofrin-PDT for Barrett's dysplasia is being studied in a multicenter international trial. However, earlier studies predict the significant promise that PDT will be an effective endoscopic therapy for Barrett's esophagus with dysplasia.

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High-Grade Dysplasia in Barrett's Esophagus: Surveillance or Operation?

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Barrett's esophagus is an acquired metaplasia of the distal esophagus associated with chronic gastroesophageal reflux. Periodic endoscopic surveillance is indicated because the incidence of adenocarcinoma increases significantly.[1-6] Even Drewitz et al.,[7] who prospectively found one of the lowest incidences of adenocarcinoma in patients with Barrett's esophagus (1 in 208 patient-years follow-up), recognized that there is about a 50-fold increase in adenocarcinoma in patients with this condition (480 cases per 100,000 population) compared to all esophageal carcinomas (8.5 cases per 100,000 population). If a positive diagnosis of adenocarcinoma is made, there is general agreement that resection of the esophagus is indicated. By contrast, the diagnosis of high-grade dysplasia without any evidence of cancer poses a clinical challenge: should such a patient undergo esophagectomy? On the one hand, it is known that some of these patients will harbor, at this time, a small curable adenocarcinoma. Furthermore, it is also known that they are at an even higher risk of developing adenocarcinoma than patients with Barrett's esophagus and no dysplasia. On the other hand, most of these patients do not have and may never have cancer. Furthermore, close follow-up of patients with high-grade dysplasia is designed to detect the development of cancer at an earlier and still curable stage. Thus it is important to examine objectively the evidence on both sides of the issue.

EVIDENCE FOR RESECTION

From 1983 to 1999, fifteen studies have reported on the frequency with which adenocarcinoma was found in patients operated on with a diagnosis of high-grade dysplasia.[2-5,8-17] As shown in Table I, of the 184 patients reported to have undergone esophagectomy with a diagnosis of high-grade dysplasia, 80 patients (43%) were found to have adenocarcinoma. The frequency with which an unsuspected adenocarcinoma was found, however, varies from 0% to 73%. This high variability may, in part, be explained by the fact that all but two of these studies were retrospective reviews, and the majority included only a handful of patients. Concern about the way these reports dealt with endoscopic findings and the biopsy protocol used has also been expressed, and further review is warranted.

Endoscopy

Seven of these 15 studies give no information on the way preoperative endoscopy was performed or on the findings.[3,8,9,11,13-15] Details such as the length of the columnar-lined mucosa, and the presence of mucosal abnormalities that are known to relate to the chance of harboring adenocarcinoma[4,5,16,17] are disregarded. On the other hand, eight of these reports[2,4,5,10,12,16-18] give detailed information concerning the endoscopic findings, the number of endoscopies performed, and the correlation between mucosal abnormalities and the final pathologic diagnosis. Two of the studies focused on the relationship between mucosal abnormalities and adenocarcinoma. Both found these abnormalities with equal frequency in patients who eventually were found to have dysplasia only and those who were found to have cancer.[4,17] Nodularity, ulceration, stricture, or erosions of the epithelium in patients with Barrett's esophagus should always alert the endoscopist to the potential presence of a neoplasm, and these should be biopsied, but their positive predictive value appears to be relatively low. Of similar interest, and not clear from these studies, is the frequency with which adenocarcinoma was found in patients with high-grade dysplasia whose mucosa was perfectly flat and had no mucosal abnormalities whatsoever.

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Table I. Missed adenocarcinoma and operative mortality in all of the patients with high-grade dysplasia only on preoperative esophagoscopy, who underwent an esophagectomy

Reference	Year	No. of patients	Missed adenocarcinoma		Mortality (No. %)
			No.	(%)	
Hamilton and Smith[9]	1987	5	3	60	0
Altorki at al.[10]	1991	8	3	38	0
Pera et al.[11]	1992	18	9	50	0
Levine et al.[5]	1993	7	0	0	1/14
Rice et al.[12]	1993	16	6	38	1/6
Streitz et al.[2]	1993	9	2	22	0
Wright et al.[18]	1994	15	7	47	0
Peters et al.[4]	1994	9	5	55	0
Edwards et al.[13]	1996	11	8	73	0
Heitmiller et al.[14]	1996	30	13	43	1/3.3
Ferguson and Naunheim[15]	1997	15	11	73	0
Cameron and Carpenter[16]	1997	19	2	10	0
Falk et el.[17]	1999	12	4	33	0
Other		10	7	70	0
TOTAL		184	80	43	3/2

Biopsy Protocol

Seven of these studies did not report the use of a standardized biopsy protocol. Most authors today agree that in order to increase the yield (identification of adenocarcinoma), biopsies should be carried out in all four quadrants at specific intervals (1 or 2 cm) and in *all areas that are irregular or friable within the Barrett's epithelium.* On the other hand, seven studies adhered to this commonly accepted biopsy protocol.[2,4,5,10,12,16,18] Even with the utilization of a standard biopsy protocol, 31% of adenocarcinomas were missed. Furthermore, one study[17] compared the yield between the regular biopsy forceps and the jumbo biopsy suggested by Levine et al.[5] Falk et al.[17] found that the jumbo forceps did not increase the yield (they found that in 33% of patients adenocarcinomas were missed by the jumbo forceps technique and 38% were missed by the standard forceps). This may, in fact, be due to the small size of the cancer focus in a large surface area of high-grade dysplasia and Barrett's esophagus in these patients, as Cameron and Carpenter[16] had pointed out in a histologic study of esophagectomy specimens from patients with high-grade dysplasia.

This evidence supports the concept that because cancer is missed by endoscopy in a large proportion of patients with high-grade dysplasia, an esophagectomy is indicated in all of these patients. The risk of leaving nearly half of the patients with an untreated cancer outweighs the risk that they will die from the operation (combined mortality rate 2% [range 0% to 14%]).

EVIDENCE FOR SURVEILLANCE

The rationale for surveillance in patients with high-grade dysplasia stemmed from the hypothesis that frequent examination and multiple biopsies of columnar epithelium would allow the physician to diagnose cancer at an early, and thus curable, stage while averting an esophagectomy in most patients. The largest study published to date included a group of 50 patients with Barrett's esophagus referred to the University of Washington.[5] Some of these patients had a diagnosis of high-grade dysplasia and/or early cancer. After repeating the endoscopy, 21 patients underwent esophagectomy (7 with the diagnosis of high-grade dysplasia only—all 7 had high-grade dysplasia only and no cancer was found in the resected specimen) and 29 patients entered a study that included periodic biopsies until such time as a positive diagnosis of cancer was made. Of the 29 patients who were followed with the diagnosis of high-grade dysplasia under the protocol, seven developed adenocarcinoma. All seven underwent esophagectomy. In five the tumor was limited to the mucosa and two had submucosal extension; none had lymphatic metastasis. None died at operation. The remaining 22 patients were followed with surveillance for a mean of 18 months and did not show any evidence of cancer. None of these patients died of esophageal cancer.

This prospective study highlights many important aspects of the natural history of high-grade dysplasia. First, using an aggressive endoscopic protocol, high-grade dysplasia can be differentiated from cancer in the great majority of patients. Second, 25% of pa-

tients (7 of 29) followed for 18 months—a relatively short period—developed an adenocarcinoma, and in two of them there was submucosal extension of the tumor at the time of the operation. On the other hand, 22 patients were spared an esophagectomy.

Schnell and Sontag[19] have reproduced the findings from the University of Washington group. Indeed they reported—albeit only in abstract form—on 42 patients with high-grade dysplasia who were placed on a surveillance protocol.[19] Over a follow-up period of 7.5 years, eight patients (19%) developed an esophageal adenocarcinoma, which was reported by the authors as "at a curative stage" (no further details available in the abstract). Of greater interest, however, is the fact that none of the remaining 34 developed a tumor or died.

The mortality rate for patients who developed esophageal cancer while under surveillance and underwent esophagectomy has been zero in these two studies.[5,19] Furthermore, these studies and others[4,16,17] have all emphasized the importance of appropriate and systematic mapping. They also show that 20% to 25% of patients with high-grade dysplasia will eventually develop cancer of the esophagus.

Because endoscopy and biopsy have their shortcomings and since a positive diagnosis would be desirable, are there other ways to identify early cancer in patients with high-grade dysplasia? Unfortunately neither DNA flow cytometry nor p53 mutations (both of which are more common in patients with cancer) can be used as the sole indicator for the development of neoplasia, as they are also present in many patients with dysplasia but without Barrett's esophagus.[5,20-23] On a more promising note, Falk et al.[24] have shown that the addition of brush cytology adds some information about malignancy to the standard or the jumbo biopsy protocol. Whether or not endosonography of the esophagus may help identify cancer in patients with high-grade dysplasia is still a matter of debate.[16,25-27]

CONTRASTING THE EVIDENCE

On the surface, the evidence presented favors performing an esophagectomy. Indeed 14 of the 15 reports emphasize that tumors are sometimes missed by endoscopy and biopsy. The problem is that, by and large, those papers are observational studies, and many disregarded appropriate endoscopic protocols designed to increase the yield of a positive diagnosis in patients with occult adenocarcinomas. Thus the issue of "missing a tumor" may be overemphasized. The two studies that support surveillance were carried out prospectively, under strict protocols, which strengthens their value. Unfortunately the study by Levine et al.[5] had a relatively short follow-up—only 18 months—and results of a more recent follow-up have not yet been published. The study by Schnell et al.[19] is only available in abstract form, and thus the information provided is limited. A follow-up report on Levine's patients and a full report on Schnell's study would go a long way toward clarifying these issues.

APPROPRIATE MANAGEMENT OF PATIENTS WITH HIGH-GRADE DYSPLASIA

Given the risks of esophagectomy and the sequelae of the operation versus the risk of ignoring a curable esophageal cancer, what should be the appropriate approach today? First, one must never accept an initial diagnosis of high-grade dysplasia until a pathologist who is well versed in this disease agrees. Second, we place all initially diagnosed patients on a very tight antireflux regimen to include the administration of proton pump inhibitors at twice the recommended dose for 8 weeks. This is not with the intention to reverse Barrett's esophagus but to reverse the inflammatory changes commonly seen in these patients, and thereby facilitate the interpretation of the biopsies. Third, after this period of treatment, a very careful endoscopic examination is carried out noting any irregularities associated with the segment of Barrett's esophagus as well as its total length. Multiple biopsies are taken of each quadrant every 2 cm, and every irregularity of the mucosa is independently biopsied. Endoscopic ultrasonography is performed and may identify further areas for biopsy. When all of these steps are taken, the clinical challenge posed by the initial diagnosis frequently no longer exists: some patients are going to be diagnosed as having carcinoma, some will be diagnosed as having no dysplasia, and a few will remain with high-grade dysplasia. Patients with a positive diagnosis of cancer should be offered an esophagectomy; those with no dysplasia should be returned to periodic surveillance (with consideration of an antireflux operation). Those who have high-grade dysplasia are engaged in a discussion that includes the endoscopic findings, their overall state of health, their age, and their ability to undertake the risk of esophagectomy. Within the group, subsets of patients can be identified for whom an operation or surveillance may be more appropriate. For example, a 10 cm long segment of Barrett's esophagus in a young person with high-grade dysplasia should probably be treated with an esophagectomy, whereas a short segment with high-grade dysplasia and no mucosal abnormalities in an older person may be watched.

In conclusion, high-grade dysplasia must first be positively diagnosed, every effort should be made us-

ing present-day technology to rule in (or out) a carcinoma, and once that is done, appropriate consideration of the individual's ability to handle risk and the institutional resources available should dictate the appropriate treatment.

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Telomerase Reverse Transcriptase Expression Is Increased Early in the Barrett's Metaplasia, Dysplasia, Adenocarcinoma Sequence

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Barrett's esophagus is a multistage polyclonal disease that is associated with the development of adenocarcinoma of the esophagus and esophagogastric junction. Telomerase activation is associated with cellular immortality and carcinogenesis, and increased expression of the telomerase reverse transcriptase catalytic subunit (hTERT) has been used for the early detection of malignant diseases. To identify biomarkers associated with each stage of the Barrett's process, relative mRNA expression levels of hTERT were measured using a quantitative reverse transcription–polymerase chain reaction method (ABI 7700 Sequence Detector (TaqMan system) in Barrett's intestinal metaplasia (n = 14), Barrett's dysplasia (n = 10), Barrett's adenocarcinoma (n = 14), and matching normal squamous esophagus tissues (n = 32). hTERT expression was significantly increased at all stages of Barrett's esophagus, including the intestinal metaplasia stage, compared to normal tissues from patients without cancer (intestinal metaplasia vs. normal esophagus, $P < 0.0001$; dysplasia, $P = 0.001$; adenocarcinoma, $P = 0.007$; all Mann-Whitney U test). hTERT expression levels were significantly higher in adenocarcinoma tissues than in intestinal metaplasia tissues ($P = 0.003$), and were higher in dysplasia compared with intestinal metaplasia tissues ($P = 0.056$). hTERT levels were also significantly higher in histologically normal squamous esophagus tissues from cancer patients than in normal esophagus tissues from patients with no cancer ($P = 0.013$). Very high expression levels ([hTERT ×100: β-actin] >20) were found only in patients with cancer. These findings suggest that telomerase activation is an important early event in the development of Barrett's esophagus and esophageal adenocarcinoma, that very high telomerase levels may be a clinically useful biomarker for the detection of occult adenocarcinoma, and that a widespread cancer "field" effect is present in the esophagus of patients with Barrett's cancer. (J GASTROINTEST SURG 2000;4:135-142.)

KEY WORDS: Telomerase, telomerase reverse transcriptase, hTERT, hTRT, telomere, Barrett's esophagus, esophageal neoplasms, esophageal adenocarcinoma

Barrett's esophagus is a disease in which the normal squamous lining of the distal esophagus is replaced with columnar epithelium in response to chronic gastroesophageal reflux disease.[1,2] It is a multiclonal, multistage disease in which Barrett's intestinal metaplasia progresses in some patients to low-grade dysplasia (LGD), high-grade dysplasia (HGD), and eventually adenocarcinoma.[3,4] The autopsy prevalence of Barrett's esophagus is approximately 400 per 100,000,[5] an estimated 700,000 Americans have this disease,[6] and both Barrett's esophagus and Barrett's-associated adenocarcinomas of the esophagus and esophagogastric junction are rapidly increasing in incidence.[7-10]

Unfortunately, current tests are unable to accurately identify either the stage of disease or the likeli-

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hood of disease progression in individuals with Barrett's esophagus. As a result, cancers are frequently not detected at an early stage and, because HGD can progress to cancer, patients with HGD are currently advised to undergo esophagectomy to remove the at-risk mucosa and any occult cancers.[11] Identification of biomarkers that are significantly associated with each Barrett's stage and with an increased risk of progression to cancer would therefore greatly benefit the selection of patients with this disease who should undergo surgical resection or other interventions.

Telomeres are specialized structures at the ends of eukaryotic chromosomes that have important roles in maintaining chromosome integrity and genomic stability.[12,13] In humans, telomeres consist of a simple hexameric sequence (TTAGGG) repeated for many kilobases.[14] Because of an inherent inability in normal eukaryotic cells to replicate the ends of linear molecules such as chromosomes, termed the "end replication problem,"[15,16] telomere length shortens with each cell division.[17] Loss of telomere length is associated with limitation of replicative ability (the "Hayflick limit," mortality stage M1), after which cellular senescence with irreversible growth arrest occurs.[18] Telomere shortening is thus an important factor in cellular[19,20] and probably whole-organism[21,22] aging.

Telomerase is a ribonucleoprotein enzyme complex that is capable of restoring and maintaining telomere length by providing an RNA template on which DNA polymerases can extend DNA replication, thus overcoming the "end-replication problem."[18,23] Most normal human somatic cells lack detectable telomerase, have progressive telomere shortening, and have a limited life span. Cells that are not subject to replicative senescence, such as germline cells, some stem cells, cancer cells, and other immortalized cells, are able to maintain telomere length. Most immortalized cells maintain telomere length because of telomerase activation in these cells,[24] although alternative lengthening of telomere (ALT) mechanisms is also important.[20,25] The catalytic subunit of human telomerase is a reverse transcriptase enzyme (hTERT or hTRT; human telomerase reverse transcriptase) that was cloned in 1997.[26-29] Direct evidence of the central importance of hTERT and maintenance of telomere length in the control of proliferation and cellular life span was provided by studies that showed that introduction of exogenous hTERT into telomerase-negative cells resulted in induction of telomerase activity and escape from cellular senescence.[18,23] Telomerase activation has been reported in more than 80% of human tumor types,[30] and for many of these tumors there are associations between telomerase activity levels and clinically important factors such as early cancer detection and cancer staging.[24,30-34]

In the only previous article on telomerase in Barrett's esophagus and esophageal adenocarcinoma, Morales et al.[35] used the in situ hybridization method to investigate the presence of telomerase RNA (hTER or hTR) in formalin-fixed, paraffin-embedded esophageal tissues from 48 patients with Barrett's esophagus and 11 patients with esophageal adenocarcinoma. These authors found that telomerase RNA was detectable at weak or moderate levels in 70% of intestinal metaplasia tissues, at moderate levels in 90% of LGD tissues, and at high levels in 100% of Barrett's HGD and adenocarcinoma specimens. Advantages of the in situ hybridization method are that archival tissues can be used and the presence of telomerase RNA can be localized within tissues, so that, for example, areas of HGD and LGD within an individual tissue section can be compared. The study by Morales et al.[35] suggests that telomerase activation is involved in the development and progression of Barrett's esophagus and adenocarcinoma, but expression of the telomerase catalytic enzyme subunit (hTERT) was not measured in their study. Assessment of hTERT expression is important because synthesis of the hTERT subunit, but not telomerase RNA (hTER), seems to be the rate-limiting determinant of telomerase activity.[36-39] Furthermore, hTER expression is widespread, whereas hTERT expression is restricted to cells or tissues with telomerase activity.[40] In the present study we assessed the prevalence and timing of hTERT activation in the Barrett's intestinal metaplasia, dysplasia, adenocarcinoma sequence. We undertook this study to investigate the role of telomerase in the development and progression of this disease, and to investigate the potential clinical usefulness of hTERT measurement in the management of patients with this disease.

MATERIAL AND METHODS

Tissue Samples

Seventy tissue samples obtained at endoscopy and at operation from patients with Barrett's esophagus or adenocarcinoma of the esophagus or gastroesophageal junction were collected and immediately frozen in liquid nitrogen. Endoscopic biopsy specimens were obtained according to a protocol that required biopsy at 2 cm intervals from each quadrant (anterior, posterior, right, and left lateral positions) of the visible length of Barrett's mucosa and an additional biopsy from the normal-appearing squamous mucosa of the esophagus. Normal esophagus biopsy samples were taken from the proximal margin of the operative re-

section specimens or from an area at least 4 cm proximal to the macroscopically abnormal epithelium on endoscopy. Part of the specimen or an adjacent specimen was fixed in formalin and paraffin for histopathologic examination by one of two pathologists considered experts on Barrett's esophagus. Frozen-section examination of the study tissue was performed if the diagnosis was uncertain.

Cancers were classified as esophageal if the epicenter of the tumor was above the anatomic esophagogastric junction, and as esophagogastric junction (syn. cardia) cancers if the epicenter was at or within 1 cm distal to the esophagogastric junction. Specimens were classified as intestinal metaplasia if intestinal metaplasia but not dypslasia or cancer was present. Specimens were classified as dysplastic if either LGD or HGD was present. Dysplastic tissues were not divided into HGD and LGD groups because areas of LGD and HGD were commonly present in the same specimen.

Only the highest grade pathologic lesion from each patient and a specimen of normal squamous epithelium were included in the study. Thus Barrett's dysplasia and intestinal metaplasia tissues from patients with adenocarcinoma were not included, and intestinal metaplasia tissues from patients with dysplasia were not included.

Using these criteria, the following tissue samples were analyzed for hTERT expression: Barrett's intestinal metaplasia (n = 14), Barrett's dysplasia (n = 10), esophagus or esophagogastric junction adenocarcinoma (n = 14), and matching normal esophagus tissues from 32 patients. Approval for this study was obtained from the institutional review board of the University of Southern California School of Medicine, and written informed consent was obtained from participating patients.

RNA Extraction and cDNA Synthesis

A guanidinium thiocyanate method of mRNA isolation[41] was used (QuickPrep Micro mRNA Purification Kit, Amersham Pharmacia Biotech Inc., Piscataway, N.J.). Isolated mRNA was dissolved in 50 μl of 5 mmol/L Tris-HCl (pH 7.5). For cDNA synthesis, 20 μl 5× Moloney murine leukemia virus (MMLV) buffer (containing 250 mmol/L Tris-HCl [pH 8.3], 375 mmol/L KCl, and 15 mmol/L $MgCl_2$; Life Technologies, Gaithersburg, Md.), 10 μl dithiothreitol (100 mmol/L; Life Technologies), 10 μl dNTP (each 10 mmol/L; Amersham Pharmacia Biotech), 0.5 μl random hexamers (50 OD dissolved in 550 μl of 10 mmol/L Tris-HCl [pH 7.5], and 1 mmol/L EDTA; Amersham Pharmacia Biotech), 2.5 μl bovine serum albumin (3 mg/ml in 10 mmol/L Tris-HCl [pH 7.5]; Amersham Pharmacia Biotech), 2.5 μl RNAse inhibitor (5× 1000 units; Amersham Pharmacia Biotech), and 5 μl MMLV reverse transcriptase (200 U/μl; Life Technologies), added to a total volume of 50.5 μl.

Polymerase Chain Reaction Quantification of mRNA Expression

Quantitation of hTERT cDNA and an internal reference cDNA (β-actin) was done using a fluorescence detection method (ABI PRISM 7700 Sequence Detection System [TaqMan], Perkin-Elmer Applied Biosystems, Foster City, Calif.) as previously described.[42,43] In brief, this method uses a dual-labeled fluorogenic oligonucleotide probe that anneals specifically within the forward and reverse primers. Laser stimulation within the capped wells containing the reaction mixture causes emission of a 3′ quencher dye (TAMRA) until the probe is cleaved by the 5′ to 3′ nuclease activity of the DNA polymerase during polymerase chain reaction (PCR) extension, causing release of a 5′ reporter dye (6FAM). Production of an amplicon thus causes emission of a fluorescent signal that is detected by the system's charge-coupled device detection camera, and the amount of signal produced at a threshold cycle within the purely exponential phase of the PCR reaction reflects the starting copy number of the sequence of interest. Comparison of the starting copy number of the sequence of interest with the starting copy number of the reference gene provides a relative gene expression level.

The PCR reaction mixture consisted of 600 mmol/L of each primer (Table I), 200 nmol/L probe (Table I), 5 U AmpliTaq Gold Polymerase, 200 μmol/L each dATP, dCTP, dGTP, 400 μmol/L dUTP, 5.5 mmol/L $MgCl_2$, 1 U AmpErase uracil N-glycosylase, and 1× TaqMan Buffer A containing a reference dye, to a final volume of 25 μl (all reagents Perkin-Elmer Applied Biosystems). Cycling condi-

Table I. PCR primers and probes

Forward primer: hTERT-3081F [21 bp]
Sequence: CGTACAGGTTTCACGCATGTG
Reverse primer: hTERT-3162R [19 bp]
Sequence: ATGACGCGCAGGAAAAATG
TaqMan probe
Name: hTERT-3107T [28 bp]
Sequence: CAGCTCCCATTTCATCAGCAAGTTTGGA
Amplicon length: 82 bp

bp = base pairs.

tions were 50° C for 2 minutes, 95° C for 10 minutes, followed by 40 cycles at 95° C for 15 seconds and 60° C for 1 minute. Significant contamination with genomic DNA was excluded by amplifying non–reverse-transcribed RNA.

Statistical Analysis

hTERT expression levels in adenocarcinoma, Barrett's dysplasia, intestinal metaplasia, and normal squamous esophagus tissues were compared using the Kruskal-Wallis test to show significant differences in hTERT expressions within all histopathologic groups. The Mann-Whitney U test was then used to compare the expressions levels between two different groups. Only adenocarcinoma tissues from patients with adenocarcinoma were included in this analysis. Normal squamous esophagus tissues from patients with adenocarcinoma (n = 12) were not included in this analysis. hTERT expressions in histologically normal squamous esophagus tissues from patients with Barrett's esophagus but no cancer (n = 20) were compared with the expressions in histologically normal squamous esophagus tissues from patients with adenocarcinoma (n = 12) in a second analysis that used the same statistical methods. Expression levels in dyspla-

Table II. Comparison of hTERT expression levels in different Barrett's tissues and normal esophagus tissues*

Pathology	No. of tissues	hTERT Median	hTERT Range	Tissues compared	*P* value
Adenocarcinoma	14	8.45	0.42-330	Adenocarcinoma vs. normal esophagus	<0.0001
				Adenocarcinoma vs. dysplasia	0.17
				Adenocarcinoma vs. IM	0.003
Barrett's esophagus with dysplasia	7	1.89	0.95-17.5	Dysplasia vs. normal esophagus	0.001
				Dysplasia vs. IM	0.056
Intestinal metaplasia	13	0.9	0.26-11.38	IM vs. normal esophagus	0.007
Normal esophagus	20	0.32	0.04-3.6		

IM = intestinal metaplasia.

*Only adenocarcinoma tissues from patients with adenocarcinoma were used for this analysis; the hTERT expression levels measured in Barrett's dysplasia (n = 3), intestinal metaplasia (n = 1), and normal esophagus (n = 12) tissues from patients with cancer were excluded from the analysis. Intestinal metaplasia tissues from patients with dysplasia were not analyzed for hTERT expression.

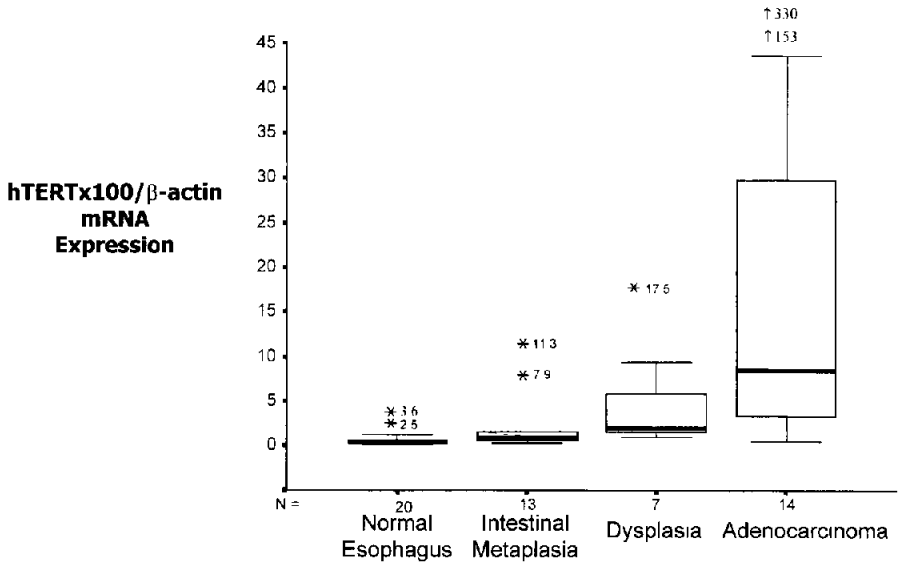

Fig. 1. Box and whisker plots of relative hTERT expression levels for specimens of each histologic type. The boxes show the twenty-fifth and seventy-fifth percentile (interquartile) ranges. Median values are shown as a horizontal bar within each box. The whiskers show levels outside the twenty-fifth and seventy-fifth percentiles but exclude far outlying values, which are shown above the boxes. The zero mark for relative hTERT expression on the vertical axis has been elevated to allow the lower limits of the boxes and whiskers to be seen.

sia and intestinal metaplasia tissues from patients with cancer were not compared with the expressions in these types of tissues from patients without cancer because of the small numbers of tissues in the cancer patient groups.

RESULTS

The median values and ranges of hTERT expressions in tissues with adenocarcinoma (n = 14), and in tissues with Barrett's dysplasia (n = 7), Barrett's intestinal metaplasia (n = 13), and squamous esophagus epithelium from patients without adenocarcinoma are shown in Table II. Premalignant and normal esophagus tissues from patients with Barrett's cancer were not included in this analysis because of the possibility that these values might be affected by the presence of cancer. As shown in Table II and Fig. 1, telomerase expression was significantly upregulated in all of the groups of Barrett's tissues, including intestinal metaplasia tissues, compared to the group of normal esophagus tissues from patients without cancer.

hTERT expression levels were significantly higher in adenocarcinoma tissues than in intestinal metaplasia tissues ($P = 0.003$), and were higher in dysplasia compared with intestinal metaplasia tissues, almost reaching statistical significance ($P = 0.056$) (see Table II). Very high telomerase expression levels ([hTERT $\times$ 100/β-actin] greater than 20) were only detected in patients with adenocarcinoma. These very high levels were found in 4 of the 14 patients with adenocarcinoma, including one patient with very high values in all tissue specimens examined.

The hTERT expression levels in the group of histologically normal squamous esophagus tissues from patients with adenocarcinoma (n = 12, median expression 1.53) was significantly higher than the levels found in normal esophagus tissues from patients with Barrett's esophagus only (n = 20, median expression 0.25, $P = 0.013$; Mann-Whitney U test).

DISCUSSION

This study demonstrates that telomerase reverse transcriptase (hTERT) expression is upregulated in Barrett's esophagus and Barrett's adenocarcinoma. There was considerable variation in hTERT expression levels in tissues at each Barrett's stage, but analyzing the grouped results for each histopathologic stage shows that there is a significant increase in expression at all Barrett's stages compared with normal esophagus tissues from patients without cancer. hTERT expressions were especially high in many of the dysplasia and adenocarcinoma tissues. Activation[18,23] and deactivation[27] of the telomerase ribonucleoprotein complex is limited by expression of the telomerase reverse transcriptase subunit. This study thus indicates that telomerase is activated in this disease, and that telomerase activation has a role in the development of Barrett's esophagus and its progression to cancer. Recent studies have shown that telomerase activation alone is not sufficient for cellular immortalization and the development of malignancy,[44-46] and other genetic alterations including *Rb*, *p16*,[46] and *p53* inactivation[30,47] are required. Inactivation of *p53* and *p16* occurs frequently in Barrett's adenocarcinomas,[4,48-58] and loss of heterozygosity studies suggest that *Rb* may also commonly be lost.[59] A study that directly assesses telomerase activity in Barrett's tissues using the telomere repeat amplification protocol assay[24,60] is in progress at this institution.

The finding that very high hTERT expression levels were found only in patients with cancer indicates that hTER expression measurement might be a clinically useful biomarker for the early detection of malignancy in patients with Barrett's esophagus. Some patients with cancer had very low hTERT levels, however, so that the predictive power of a low or only moderately elevated hTERT level is limited. For this reason, molecular diagnosis and staging of Barrett's patients who have low or moderately high hTERT levels will probably require assessment of other genes or, as is most likely, a panel of genes. Studies from this institution and elsewhere suggest that there are at least several other genes that have significantly different expressions or mutation frequencies at different Barrett's stages.[30,61-64] Analysis of these genes might be included in a panel of molecular tests useful for monitoring patients with Barrett's esophagus.

As in breast, lung, head and neck cancers, some hepatocellular cancers, and sun exposure–related skin cancers,[65,66] but unlike many other common cancers,[65] telomerase activation occurs early in the Barrett's multistage carcinogenesis process, with significant hTERT upregulation at the intestinal metaplasia stage. This finding indicates that Barrett's esophagus, even at its earliest stage of intestinal metaplasia without dysplasia, is a genetic disease with involvement of tumor-associated genes.

hTERT expression levels in this study were significantly higher in the group of histologically normal squamous esophagus tissues from patients with cancer compared with the group of histologically normal squamous esophagus tissues from patients without cancer. This indicates that genetic changes precede the appearance of morphologic changes in this disease. The normal esophagus biopsies were from areas that were well separate from macroscopic disease, indicating further that there is probably a very widespread oncogenic "field" effect in the esophagus in cancer patients. The presence of a field effect should theoretically increase the usefulness of gene expres-

sion measurements as biomarkers or cancer detection in Barrett's esophagus. The reason for this is that only a small proportion of the Barrett's epithelium is usually biopsied at endoscopy in routine clinical practice, so that small areas of HGD or cancer may not be biopsied and thus may go undetected (sampling error). The finding of a very high "cancer-level" hTERT expression level in any area of the esophagus, even in histologically normal squamous epithelium, might indicate that occult cancer is present. Alternatively, it might suggest that a patient with Barrett's esophagus was at increased risk for progression to cancer. In either case, such a patient should undergo repeat biopsy within a relatively short period.

Other studies have shown that hTERT expression may be present at low levels in normal tissues from patients without malignancy.[66] In particular, activated lymphocytes and cells from highly proliferative normal tissues such as the breast, the endometrium, the basal layer of the epidermis, and intestinal crypt cells are known to express low levels of telomerase activity. These cell types are unable to maintain telomere length, but in some of these cells telomere shortening occurs at a slower rate than in normal somatic telomerase-negative cells.[67,68] All of the patients in our study had either Barrett's esophagus or adenocarcinoma, so the conclusion that hTERT expression is present in the normal esophagus in humans is suggested by our study but needs to be confirmed by analysis of normal tissues from individuals with no esophageal pathology.

Findings similar to ours, and to those of Morales et al.,[35] who detected telomerase RNA (hTER) expression at all Barrett's stages, have been reported in studies of esophageal squamous cell carcinoma and its precursor lesion squamous dysplasia. Telomerase activity is increased in esophageal squamous cell carcinoma cells and tissues,[69-71] this activation probably occurs early, as shown by the detection of telomerase RNA in areas of squamous dysplasia,[72] and telomerase activity is found in normal esophagus tissues from patients with squamous cell cancer.[70,71]

The validity of using the quantitative RT-PCR fluorogenic detection (TaQman) system for measurement of telomerase RNA expression has been reported.[73] This present study demonstrates that this highly sensitive method is also suitable for quantification of hTERT expression. Compared to previous methods of RNA expression measurement, this method has the advantages of being quantitative rather than semiquantitative, rapid, and nonradioisotope dependent. The finding of hTERT expression in all of the tissues examined in this study may at least partly result from the sensitivity of this system for detecting cDNA messages.

CONCLUSION

Telomerase reverse transcriptase expression, and thus presumably telomerase activity, is significantly increased in Barrett's esophagus and Barrett's adenocarcinoma tissues. This increase in expression occurs at the earliest (intestinal metaplasia) stage of this disease, but expression levels are higher in later-stage (dysplasia and cancer) tissues than in tissues with intestinal metaplasia only. The presence of cancer is associated with an extensive field effect in the esophagus. All patients with very high hTERT expressions had cancer, suggesting that hTERT quantification may be clinically useful for the detection of occult cancer in patients with Barrett's esophagus. The TaqMan RT-PCR assay used is a highly sensitive, quantitative, non–radioisotope-dependent method for measuring relative hTERT mRNA expression.

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Effect of Laparoscopic Fundoplication on Gastroesophageal Reflux Disease–Induced Respiratory Symptoms

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Laparoscopic fundoplication controls heartburn and regurgitation, but the effects on the respiratory symptoms of gastroesophageal reflux disease (GERD) are unclear. Confusion stems from difficulty preoperatively in determining whether cough or wheezing is actually caused by reflux when reflux is found on pH monitoring. To date, there is no proven way to pinpoint a cause-and-effect relationship. The goals of this study were to assess the following: (1) the value of pH monitoring in establishing a correlation between respiratory symptoms and reflux; (2) the predictive value of pH monitoring on the results of surgical treatment; and (3) the outcome of laparoscopic fundoplication on GERD-induced respiratory symptoms. Between October 1992 and October 1998, a total of 340 patients underwent laparoscopic fundoplication for GERD. From the clinical findings alone, respiratory symptoms were thought possibly to be caused by GERD in 39 patients (11%). These 39 patients had been symptomatic for an average of 134 months. They were all taking H_2-blocking agents (21%) or proton pump inhibitors (79%). Seven patients (18%) were also being treated with bronchodilators, alone (3 patients) or in combination with prednisone (4 patients). Median length of postoperative follow-up was 28 months. In 23 patients (59%) a temporal correlation was found during 24-hour pH monitoring between respiratory symptoms and episodes of reflux. Postoperatively heartburn resolved in 91% of patients, regurgitation in 90% of patients, wheezing in 64% of patients, and cough in 74% of patients. Cough resolved in 19 (83%) of 23 patients in whom a correlation between cough and reflux was found during pH monitoring, but in only 8 (57%) of 14 of patients when this correlation was absent. Cough persisted postoperatively in the two patients who did not cough during the study. These data show that pH monitoring helped to establish a correlation between respiratory symptoms and reflux, and it helped to identify the patients most likely to benefit from antireflux surgery. Following laparoscopic surgery, respiratory symptoms resolved in 83% of patients when a temporal correlation between cough and reflux was found on pH monitoring; heartburn and regurgitation resolved in 90%. (J GASTROINTEST SURG 2000;4:143-149.)

KEY WORDS: Gastroesophageal reflux disease, esophageal manometry, prolonged pH monitoring, respiratory symptoms, laparoscopic fundoplication

Laparoscopic fundoplication controls heartburn and regurgitation in approximately 90% of patients,[1,2] but the effects on respiratory symptoms are less predictable.[3,4] The uncertainty stems from difficulty in establishing preoperatively whether cough and wheezing are actually caused by reflux when reflux is found on pH monitoring. The goals of this study were to determine the following: (1) the value of pH monitoring in establishing a correlation between respiratory symptoms and reflux; (2) the predictive value of pH monitoring on the results of surgical treatment; and (3) the outcome of laparoscopic fundoplication on gastroesophageal reflux disease (GERD)–induced respiratory symptoms.

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PATIENTS AND METHODS

Between October 1992 and October 1998, a total of 340 patients underwent laparoscopic fundoplication at the University of California San Francisco Medical Center for GERD. Heartburn and/or regurgitation were present in all patients. In 39 patients (11%), heartburn and regurgitation were accompanied by cough or wheezing that was attributed on clinical grounds to the GERD. Of these 39 patients with respiratory symptoms, 19 were men and 20 were women. The mean age was 59 years (range 32 to 77 years). Symptoms had been present for an average of 134 months.

All patients had been treated with acid-reducing medications (H_2-blocking agents, 21%; proton pump inhibitors, 79%), alone or in combination with prokinetic agents (36%). Seven patients (18%) were also being treated with bronchodilators, alone (3 patients) or in combination with prednisone (4 patients). All patients underwent a detailed preoperative examination that included symptom evaluation, barium swallow test, and esophagogastroduodenoscopy. The severity of heartburn, regurgitation, cough, wheezing, and hoarseness was scored by the patients before and after the operation using a five-point scale ranging from 0 (no symptoms) to four (disabling symptoms). The preoperative complaints included heartburn in 35 patients (90%) (score 3.3 ± 0.8), regurgitation in 30 patients (77%) (score 2.8 ± 1.1), cough in 39 patients (100%) (score 2.4 ± 1.0), wheezing in 14 patients (36%) (score 2.3 ± 1.1), and hoarseness in 13 patients (33%) (score 2.1 ± 0.9).

Results of barium swallow were normal in 28% of patients and showed a hiatal hernia in 72% of patients.

Thirteen percent of patients had no visible signs of esophagitis, 42% had grade I and II esophagitis, and 45% had grade III to IV esophagitis. No strictures were present.

Ambulatory 24-Hour pH Monitoring

Acid-suppressing medications were discontinued 3 days (H_2-blocking agents) to 14 days (proton pump inhibitors) before the study. Ambulatory pH monitoring was performed using a probe with two antimony sensors spaced 15 cm apart, 5 and 20 cm above the upper border of the manometrically determined lower esophageal sphincter.[5,6] The probes were calibrated in a standard buffer solution at pH 1 and 7 before and after monitoring. During the test, the patients consumed an unrestricted diet and took no medications that could interfere with the results. In addition, each kept a diary detailing any episodes of coughing. The 24-hour pH tracings were searched for a temporal relationship between the onset of an episode of cough and an episode of reflux (signified by a drop in pH to <4). An episode of coughing was considered to be induced by reflux if it occurred within 3 minutes of an episode of reflux in the distal or the distal-and-proximal esophagus.[5] Therefore, based on the relationship between cough and episodes of reflux, the patients were divided into three groups as follows: *Group A* = no correlation between cough and episodes of reflux, 14 patients (36%) (Fig. 1); *Group B* = correlation present between cough and episodes of reflux in the distal esophagus only, 13 patients (33%) (Fig. 2); *Group C* = correlation present between cough and episodes of reflux in the distal *and* proximal esophagus, 10 patients (26%) (Fig. 3). Two patients (5%) did not cough during the study. Esophageal acid exposure was calculated independently for the distal and the proximal esophagus (Table I).

Postoperatively, pH monitoring was offered to all patients, whether they were symptomatic or not. Eleven patients (28%) consented to have the test. All tracings were reviewed using the same criteria as for the preoperative tracings.

Table I. Ambulatory 24-hour pH monitoring

	Group A		Group B		Group C	
	Distal	Proximal	Distal	Proximal	Distal	Proximal
No. of reflux episodes	215 ± 142	79 ± 59	164 ± 68	38 ± 36	203 ± 176	39 ± 36
No. of reflux episodes >5 min	13 ± 13	3 ± 4	6 ± 4	0.5 ± 1.0	10 ± 8	4 ± 1
Longest reflux episode (min)	53 ± 53	23 ± 21	22 ± 24	3 ± 3	53 ± 101	11 ± 25
% Time pH <4 (total)*†	27 ± 23	7 ± 7	12 ± 4	1 ± 1	18 ± 15	2 ± 3
Upright position†	29 ± 21	8 ± 9	15 ± 6	2 ± 3	22 ± 15	3 ± 3
Supine position†	25 ± 28	6 ± 6	10 ± 7	0.6 ± 1	14 ± 17	1 ± 2
Reflux score (normal <15)†	85 ± 81	42 ± 41	49 ± 17	8 ± 7	54 ± 37	10 ± 10

*P <0.05 between groups A and B. There was no difference among the other groups for any other parameter.

†P <0.05 between groups A and B, and groups A and C. There was no difference between groups B and C.

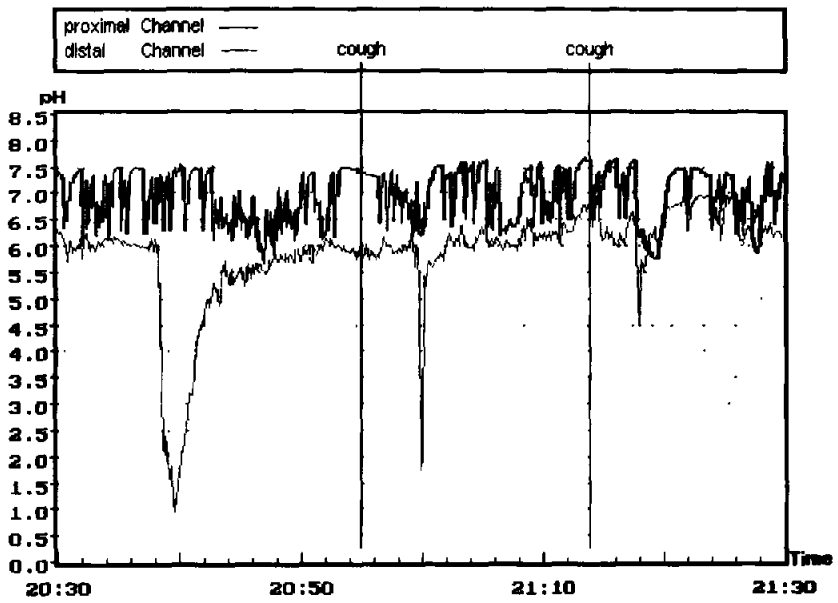

Fig. 1. Dual-sensor pH monitoring. *Light line*, acid reflux 5 cm above the lower esophageal sphincter; *dark line*, acid reflux 20 cm above the lower esophageal sphincter. There is no correlation between cough and episodes of reflux.

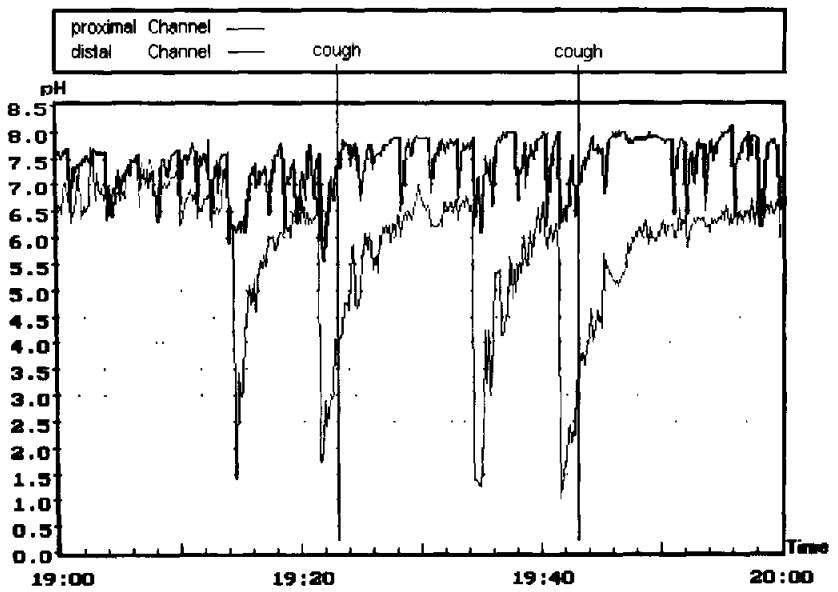

Fig. 2. Dual-sensor pH monitoring. *Light line*, acid reflux 5 cm above the lower esophageal sphincter; *dark line*, acid reflux 20 cm above the lower esophageal sphincter. A correlation exists between cough and reflux in the distal esophagus.

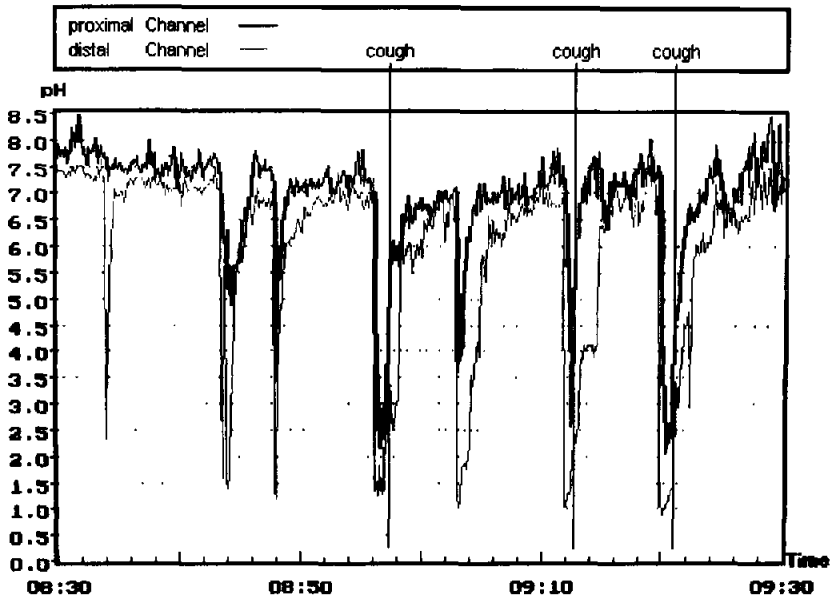

Fig. 3. Dual-sensor pH monitoring. *Light line*, acid reflux 5 cm above the lower esophageal sphincter; *dark line*, acid reflux 20 cm above the lower esophageal sphincter. A correlation exists between cough and reflux in the distal and proximal esophagus.

Table II. Esophageal manometry

	Group A	Group B	Group C
LES pressure (mm Hg)	10 ± 5	11 ± 4	10 ± 4
LES length (cm)	2.1 ± 1.0	2.1 ± 0.9	2.4 ± 0.5
DEA (mm Hg)	54 ± 31	70 ± 30	66 ± 38
PEA (mm Hg)	41 ± 21	53 ± 29	47 ± 22
NSEMD (% patients)	71	46	70
UES pressure (mm Hg)	104 ± 68	78 ± 34	66 ± 33

LES = lower esophageal sphincter; DEA = distal esophageal amplitude; PEA = proximal esophageal amplitude, NSEMD = nonspecific esophageal motility disorder; UES = upper esophageal sphincter.
There were no statistical differences among the groups in any category

Table III. Symptoms before and after laparoscopic fundoplication

	Group A		Group B		Group C	
	Preop	Postop	Preop	Postop	Preop	Postop
Heartburn	3.3 ± 0.9	0.5 ± 0.6	3.6 ± 0.5	0.2 ± 0.6	2.8 ± 0.8	0.3 ± 0.7
Regurgitation	3.0 ± 1.0	0.2 ± 0.4	2.3 ± 1.2	0	2.9 ± 1.0	0.1 ± 0.3
Cough	2.7 ± 1.1	0.6 ± 0.9	2.4 ± 1.1	0.7 ± 1.4	2.1 ± 1.0	0.4 ± 1.3
Wheezing	2.5 ± 1.0	0.3 ± 0.5	2.5 ± 1.3	1.2 ± 1.5	1.7 ± 0.9	0.5 ± 1.0
Hoarseness	3.3 ± 0.9	0.5 ± 0.6	1.5 ± 0.7	0	2.6 ± 0.6	0.1 ± 0.3

Each symptom was rated by the patient on a scale of 0 to 4, with 4 being most severe

Esophageal Manometry

The patients were studied after an overnight fast using techniques previously described.[5] Medications that might interfere with esophageal motor function (i.e., metoclopramide, cisapride, nitrates, β-agonists, calcium channel–blocking agents) were discontinued at least 48 hours before the study. The following variables were assessed: (1) length and resting pressure of the lower esophageal sphincter; (2) amplitude and propagation of the peristaltic waves in the distal and proximal esophagus; and (3) pressure of the upper esophageal sphincter (Table II). There were no differences in any of these variables among the three groups.

Surgical Treatment

Laparoscopic fundoplication was performed according to techniques previously described.[2,7] Twenty patients (51%) with normal esophageal peristalsis underwent a total (360-degree) fundoplication, and 19 patients (49%) with abnormal esophageal peristalsis (amplitude in the distal esophagus <40 mm Hg; >20% segmented waves; triple peaked or dropped waves or both) underwent a partial (240-degree) fundoplication.

Follow-Up

All patients were seen in follow-up 2 and 8 weeks postoperatively. Subsequently they were interviewed by phone at 3-month intervals. Median duration of follow-up was 28 months.

Statistical Analysis

Student's *t* test, Wilcoxon signed-rank test, and analysis of variance were used for statistical evaluation of the data. All results are expressed as mean ± standard deviation. Differences were considered significant at $P < 0.05$.

RESULTS

All operations were completed laparoscopically, and there were no operative or postoperative complications. The patients were allowed an unrestricted diet after 21 ± 9 hours, and left the hospital after 36 ± 17 hours (59% within 23 hours).

Table III shows the effects of the operation on symptoms. Heartburn resolved in 91% of patients, regurgitation in 90%, hoarseness in 82%, cough in 74%, and wheezing in 64%. The outcome in relation to cough was as follows: *Group A* = cough resolved in 8 (57%) of 14 patients, and heartburn resolved in 12 (92%) of 13 patients; *Group B* = cough resolved in 10 (77%) of 13 patients, and heartburn resolved in 12 (92%) of 13 patients; *Group C* = cough resolved in 9 (90%) of 10 patients, and heartburn resolved in 7 (88%) of 8 patients.

Four patients had residual reflux by ambulatory 24-hour pH monitoring. Two were asymptomatic and two had persistent symptoms. The wrap was found to be disrupted in one of these latter two patients; his symptoms resolved after a second fundoplication.

Cough persisted in the two patients who did not cough during the study, and both are still being treated with bronchodilators and prednisone. Cough improved in two patients in group A, but they still require treatment with inhalers. One of these two patients had an essentially normal pH monitoring result (DeMeester score 17) and was asymptomatic during the study. Two patients in group B with persistent cough had normal findings on pH monitoring postoperatively. There was no temporal correlation between episodes of coughing and the small (i.e., normal) amount of reflux in these patients.

One patient in group C became asymptomatic and was able to discontinue proton pump inhibitors, inhalers, and prednisone. Postoperative pH monitoring showed a normal score.

DISCUSSION

These results show that (1) pH monitoring was valuable in establishing a correlation between respiratory symptoms and reflux, (2) pH monitoring helped identify patients most likely to benefit from antireflux surgery, and (3) following laparoscopic fundoplication, heartburn resolved in 90% of patients, and cough resolved in 83% of patients when a temporal correlation between cough and reflux was found on pH monitoring.

Gastroesophageal Reflux and Respiratory Symptoms

GERD can produce typical symptoms, such as heartburn and regurgitation, and atypical symptoms, such as cough and wheezing.[8,9] The diagnosis of GERD-induced respiratory symptoms is not always easy, as some patients may not experience heartburn or have visible signs of esophagitis on endoscopy.[5] In addition, the pathogenesis of GERD-induced respiratory symptoms is multifactorial. They may be due to (1) activation by the refluxed material of a vagal reflex with consequent bronchoconstriction or (2) microaspiration of gastric contents. Although the former mechanism would only require reflux from the stomach into the distal esophagus, the latter would require

reflux into the proximal esophagus and spillage into the tracheobronchial tree.

The most proximal extent of reflux seems to be more important than previously thought. For instance, Johnson et al.[11] found that the pulmonary disease in progressive systemic sclerosis was a complication of gastroesophageal reflux and occult aspiration. More recently Tobin et al.[12] demonstrated that patients with idiopathic pulmonary fibrosis often have increased esophageal acid exposure, mostly at night and frequently into the proximal esophagus. Such findings suggest that control of reflux might halt progression of the pulmonary disease.

Prolonged pH monitoring should be the best way to establish a correlation between reflux and respiratory symptoms.[5,9,11,12] We compared the pH tracings with entries in the patient's concurrent diary, and considered cough to be due to reflux only when the two occurred within 3 minutes of each other. A pH probe was used with two sensors located 15 cm apart to measure the amount and extent of reflux. An acid pH as far as the upper esophageal sphincter would indicate more proximal reflux and presumably would enhance the likelihood that the respiratory symptoms were due to aspiration of the refluxate.[5] This finding might influence the choice of therapy.

Predictive Value of 24-Hour pH Monitoring

The results of the pH monitoring study identified which patients with respiratory symptoms were more likely to benefit from antireflux surgery. Cough resolved in 19 (83%) of 23 patients when a correlation between cough and reflux was detected during pH monitoring, but in only 8 (57%) of 14 of patients when no correlation was seen. Furthermore, cough resolved in 77% of patients who had a correlation between cough and acid in the distal esophagus (see Fig. 2), but it resolved in 90% of patients when the cough was associated with acid in the proximal as well as the distal esophagus (see Fig. 3).

Others have reported that reflux as far as the proximal esophagus is a predictor of a good response to medical antireflux therapy in patients with asthma.[8] In addition, our data suggest that the correlation between symptoms and proximal reflux foreshadows a good response to surgical therapy. In fact, even though patients in group A had more proximal reflux, patients in groups B and C had a better correlation between symptoms and reflux. The better results of fundoplication in group C suggest that aspiration caused their symptoms, and aspiration was stopped by the operation.

Effect of Laparoscopic Fundoplication on GERD-Induced Respiratory Symptoms

Most candidates for laparoscopic fundoplication have heartburn and have been treated with acid-reducing medications. Symptoms have been shown to be an unreliable indicator of reflux,[13] and endoscopy is imprecise in diagnosing nonerosive esophagitis.[14] Therefore esophageal manometry and pH monitoring should usually be performed preoperatively to define the presence and magnitude of reflux. Laparoscopic fundoplication eliminates heartburn and regurgitation in approximately 90% of patients selected in this way.[2]

The effects of fundoplication on respiratory symptoms is less predictable. Before antireflux surgery was done laparoscopically, there were few objective data describing the effects of fundoplication on respiratory symptoms.[10,15] For instance, Pellegrini et al.[15] performed Nissen fundoplications in five patients thought to have GERD-induced aspiration. Respiratory symptoms resolved in all. In recent years the number of patients undergoing laparoscopic antireflux surgery has increased greatly, which has allowed the question to be examined more thoroughly.[1,2] Hunter et al.[1] performed laparoscopic fundoplication in 300 patients, 87 of whom (29%) had respiratory symptoms. Resolution or improvement of respiratory symptoms occurred in 76 patients (87%). One group suggested that respiratory symptoms are more often relieved by antireflux surgery when esophageal motor function is normal than when it is abnormal.[3] In our experience the results were similar after total or partial fundoplication, and the outcome was unrelated to the presence or magnitude of esophageal motor dysfunction.

CONCLUSION

During the past 10 years major progress has been made in understanding the pathophysiology of GERD-induced respiratory symptoms. Prolonged pH monitoring has emerged as the key test to establish this correlation and is useful in predicting the outcome of therapy. Although typical and atypical symptoms respond satisfactorily to medical therapy in some patients,[8,16] others clearly benefit from surgery.[4] The two approaches differ in important ways, however. Acid-reducing medications change the pH of the refluxate, but reflux persists. Antireflux surgery, on the other hand, permanently corrects reflux altogether. The challenge is to identify which patients will benefit from surgery and to operate before the lungs become irreversibly damaged.

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Nonsteroidal Anti-Inflammatory Drugs Attenuate Proliferation of Colonic Carcinoma Cells by Blocking Epidermal Growth Factor–Induced Ca^{++} Mobilization

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Numerous studies suggest that nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit colorectal carcinogenesis. We have previously reported that NSAIDs, in human colonic carcinoma cells (Caco-2), attenuate epidermal growth factor (EGF)–induced cellular proliferation through a process independent of their inhibitory effects on prostaglandin synthesis. Furthermore, separate studies have also suggested that NSAIDs inhibit EGF-induced store-operated Ca^{++} influx. Thus we developed the hypothesis that NSAIDs may limit the activity of EGF by altering intracellular Ca^{++} ($[Ca^{++}]_i$) mobilization. Serum-deprived Caco-2 cells were employed for all experimentation. $[Ca^{++}]_i$ was measured with Fluo-3 and extracellular Ca^{++} influx was monitored by quenching Fluo-3 fluorescence with Mn^{++}. Proliferation was quantitated with two assays: cellular nucleic acid and total protein content. Caco-2 cells exposed to EGF demonstrated an initial increase in $[Ca^{++}]_i$ which was blocked by neomycin, an inhibitor of IP_3 generation, and the phospholipase C inhibitor U73122 but not U73343 (inactive control). This was followed by sustained extracellular Ca^{++} influx, which was attenuated with calcium-free buffer ($-Ca^{++}$), the store-operated Ca^{++} channel blocker lanthanum, indomethacin, ibuprofen, and aspirin. In subsequent studies, cells were treated with either serum-free media or EGF ± the aforementioned inhibitors, and again serum starved. Cells exposed to EGF ± the inactive phospholipase C inhibitor U73343 demonstrated a significant increase in nucleic acid and protein. However, proliferation induced by EGF was not observed when $[Ca^{++}]_i$ elevation was prevented by blocking either internal Ca^{++} store release via phospholipase C/IP_3 or sustained Ca^{++} influx through store-operated Ca^{++} channels. Sustained $[Ca^{++}]_i$ elevation, as induced by EGF, appears to be required for mitogenesis. These data support our premise that one mechanism whereby NSAIDs may attenuate colonic neoplasia is by blocking EGF-induced Ca^{++} mobilization. (J GASTROINTEST SURG 2000;4:150-161.)

KEY WORDS: Caco-2 cells, calcium, epidermal growth factor, colorectal cancer, NSAIDs

Recent laboratory, animal, epidemiologic, and clinical investigations support the concept that nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit both the initiation and proliferation of colorectal tumors.[1-4] Although several studies have suggested that endogenous prostaglandins play a large role,[5-8] a growing body of evidence suggests that NSAIDs may attenuate colorectal carcinogenesis independent of their inhibitory effect on prostaglandin synthesis.[9-11] Thus, despite significant research, the exact mechanism(s) whereby NSAIDs limit colonic neoplasia remain elusive.

We have recently investigated the effect of NSAIDs on cellular proliferation in human colonic carcinoma cells (Caco-2). Although NSAID treatment (indomethacin, ibuprofen, or aspirin) did not inhibit growth in cells treated with only serum-free medium, NSAID treatment did significantly attenuate protein and nucleic acid synthesis induced by the mitogen epidermal growth factor (EGF) through a

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process that appeared to be independent of prostaglandin synthesis inhibition.[12] Furthermore, separate studies have also suggested that NSAIDs inhibit EGF-induced store-operated Ca^{++} influx (SOCI). Thus we have developed the hypothesis that NSAIDs may limit the activity of EGF by altering intracellular Ca^{++} ($[Ca^{++}]_i$) mobilization. The connection, however, between EGF-induced Ca^{++} mobilization and EGF-induced cellular proliferation remains poorly defined. The major objectives of the current study were to investigate the mechanisms whereby EGF elicits changes in $[Ca^{++}]_i$ and to determine if these effects account for its mitogenic action.

METHODS

Cells

Caco-2 cells were obtained from American Type Culture Collection (Rockville, Md.) at passage 15. Cells were maintained at 37° C in an atmosphere of 5% CO_2 and 100% relative humidity and were split on a weekly basis at a ratio of 1:6 on reaching confluency. Cells were detached using 0.5 g porcine trypsin and 0.2 g EDTA tetrasodium per liter of Hank's balanced salt solution (HBSS), and then plated onto either 24- or 48-well plates (Costar, Cambridge, Mass.) for experiments or into 150 cm^2 flasks for propagation. Cell passage was maintained between 50 and 65 and media were changed every 2 to 3 days. Caco-2 media consisted of Eagle's minimum essential medium (MEM) with nonessential amino acids supplemented with either 20% fetal bovine serum or 1% fetal bovine serum (serum-free medium), 100 μg/ml penicillin, 100 μg/ml streptomycin, and 0.25 μg/ml amphotericin B. Serum deprivation was initiated at 80% confluency, and all experiments were performed following 48 hours of serum starvation.

Solutions

Prior to all experiments assessing changes in $[Ca^{++}]_i$, media were aspirated and replaced with HBSS plus 10 mmol/L HEPES (H 8264, Sigma, St. Louis, Mo.; consisting of the following: 137 mmol/L NaCl, 5.7 mmol/L $NaHCO_3$, 5.3 mmol/L KCl, 1.26 mmol/L $CaCl_2$, and 0.8 mmol/L $MgSO_4$). Experiments involving Ca^{++}-free buffer used HBSS plus 10 mmol/L HEPES and 2 mmol/L BAPTA (HBSS ($-Ca^{++}$); H 6648, Sigma; consisting of 137 mmol/L NaCl, 5.7 mmol/L $NaHCO_3$, and 5.3 mmol/L KCl). All test compounds were dissolved in either HBSS or HBSS ($-Ca^{++}$). Epidermal growth factor (EGF), neomycin sulfate (neomycin), lanthanum chloride (La^{+++}), indomethacin, acetylsalicylic acid, and ibuprofen were obtained from Sigma. The aminosteroids U73122 and U73343 were purchased from Calbiochem (La Jolla, Calif.). Treatment with the preceding antagonists/inhibitors involved a 20-minute preincubation time followed by the addition of the respective inhibitor to all subsequent solutions within treatment groups.

Measurement of $[Ca^{++}]_i$ and Extracellular Ca^{++} Influx

Changes in intracellular Ca^{++} concentration ($[Ca^{++}]_i$) were quantitated as previously described[13] using the single wavelength Ca^{++} indicator Fluo-3 (Fluo-3, AM; Molecular Probes, Eugene, Ore.). Prior to loading with Fluo-3, cells were washed twice with HBSS. Fluo-3 was initially dissolved in Pluronic F-127 (20% solution in dimethylsulfoxide; Molecular Probes) to make a 1 mmol/L working solution and subsequently added to HBSS plus 1% fetal bovine serum for a final loading concentration of 4 μmol/L. Cells were then loaded with Fluo-3 for 50 minutes at 25° C in an atmosphere of 5% CO_2 and 100% relative humidity.

Caco-2 cells were then washed three times to ensure removal of all unloaded Fluo-3 and control and test solutions were added to the respective wells. At each time point, intracellular Ca^{++} concentration was calculated using the following equation:

$$[Ca^{++}]_i \text{ (nmol/L)} = K_d \frac{(F-F_{min})}{(F_{max}-F)}$$

where $F_{min} = 1.25\ F_{MnCl_2} - 0.25\ F_{max}$ and $K_d = 400$ nmol/L.[14] The maximum fluo-3 signal, or F_{max}, was determined by permeabilizing Caco-2 cells with 50 μmol/L digitonin (Sigma). The Fluo-3 signal was quenched to obtain F_{MnCl_2} using 2 mmol/L $MnCl_2$ and 50 μmol/L digitonin. Tetrakis (2-pyridylmethyl) ethylenediamine (TPEN; 50 μmol/L, Molecular Probes) was used in all solutions as a heavy metal scavenger.[15]

It is well accepted that manganese (Mn^{++}) can be used as a Ca^{++} surrogate to estimate extracellular Ca^{++} influx through the plasma membrane.[16] In separate experiments, Mn^{++} uptake was monitored by quenching Fluo-3 fluorescence with the addition of 2 mmol/L $MnCl_2$ to all solutions (control and experimental). Data are presented as mean relative fluorescence.

Continuous fluorescent signals during both protocols were quantitated using a CYTOFLUOR II fluorescent multiwell plate reader (PerSeptive Biosystems, Framingham, Mass.) employing 485 nm and 530 nm as the excitation and emission spectra, respectively.

Cells were maintained throughout the experiments at a temperature of 37° C with a heated stage.

Measurement of Cellular Proliferation

Cellular proliferation was estimated by measuring nucleic acid and, in separate samples, protein. A CyQUANT proliferation assay kit (C 7026; Molecular Probes) was employed to measure total nucleic acid. This assay relies on a green fluorescent dye, which exhibits strong fluorescent enhancement when bound to cellular nucleic acids. Protein levels were quantitated with a NanoOrange protein quantitation kit (N 6666; Molecular Probes). This assay is based on the binding of a fluorescent dye to the detergent coating and hydrophobic regions of proteins. For both assays, a CYTOFLUOR II fluorescent multiwell plate reader (PerSeptive Biosystems) was utilized employing the following spectra: 485 nm (excitation) and 530 nm (emission) for CyQUANT or 485 nm (excitation) and 590 nm (emission) for NanoOrange. Data for both protein and nucleic acid are presented as mean relative fluorescence.

Experimental Design

We initially verified our in vitro model of cellular proliferation and compared nucleic acid and protein synthesis induced by EGF to 20% fetal bovine serum. The next experiment was designed to determine the optimal time duration of EGF treatment required to elicit cellular proliferation. In the third and fourth experiments, we investigated the mechanism(s) whereby EGF elicits changes in $[Ca^{++}]_i$. The role of phosphoinositide-specific phospholipase C (PLC) and subsequent inositol 1,4,5-trisphosphate (IP_3) generation was determined using the aminosteroid U73122, an inhibitor of PLC catalyzed hydrolysis of phosphatidylinosotol 4,5-bisphosphate (PIP_2), its inactive analogue U73343, and neomycin (an inhibitor of IP_3 generation).[17,18] The mechanism of sustained influx of extracellular Ca^{++} was investigated with Ca^{++}-free buffer and the store-operated Ca^{++} channel (SOCC) blocker lanthanum (La^{+++}).[16] Inhibition of EGF-induced SOCI by NSAIDs was also verified employing the current experimental conditions. In subsequent studies, cells were serum starved (48 hours), pretreated (20 minutes) with either HBSS or the aforementioned inhibitors, treated (30 minutes) with either serum-free medium (MEM) or EGF, and again serum starved (48 hours) to determine what effect EGF-induced Ca^{++} mobilization may have on EGF-induced nucleic acid and protein synthesis. Finally, to rule out the possibility that any inhibition of EGF-induced mitogenesis may be a result of inhibitor activity unrelated to EGF-induced Ca^{++} mobilization, cells were serum starved (48 hours), treated (30 minutes) with either serum-free medium (MEM) or EGF, subsequently treated (20 minutes) with the aforementioned inhibitors, and again serum starved (48 hours).

Statistics

Statistical evaluation was performed by analysis of variance with a Scheffe post hoc test. Data ($n = 8$ to 12 per group) are reported as mean ± standard error of the mean. A P value <0.05 was taken to represent statistical significance.

RESULTS

EGF-Induced Cellular Proliferation

Preliminary data suggested that concentrations of EGF ranging from 10 to 1000 ng/ml elicited elevated but similar growth rates in treated Caco-2 cells, whereas lower EGF concentrations (1 ng/ml) did not appear to induce cellular proliferation (data not shown). Thus the lowest mitogenic concentration of EGF (10 ng/ml), a concentration considered to be physiologic, was employed for all subsequent experimentation. Caco-2 cells serum deprived (1% fetal bovine serum) for 48 hours demonstrated a plateau of nucleic acid and protein levels. Subsequent treatment with EGF (10 ng/ml) initiated a significant increase in both nucleic acid and, at a later time point, protein synthesis when compared to cells subsequently treated with only 1% fetal bovine serum. However, cells subsequently treated with 20% fetal bovine serum demonstrated higher nucleic acid and protein levels when compared to cells exposed to EGF. These data are shown in Fig. 1, *A* and *B*, and suggest that although EGF is a potent mitogen for quiescent cells, greater proliferation is induced with 20% fetal bovine serum.

Effect of Treatment Duration

In separate studies, Caco-2 cells were serum deprived for 48 hours, treated with EGF for variable time periods, and again serum deprived for a time period of 48 hours minus the EGF treatment duration. Cells treated with EGF for less than 20 minutes demonstrated nucleic acid and protein levels similar to those of control cells. Interestingly, cells treated for longer incubations (30 minutes to 48 hours) achieved increased and similar growth rates. These data are depicted in Fig. 2 and demonstrate that the signal whereby EGF elicits mitogenesis occurs within a relatively short period of time.

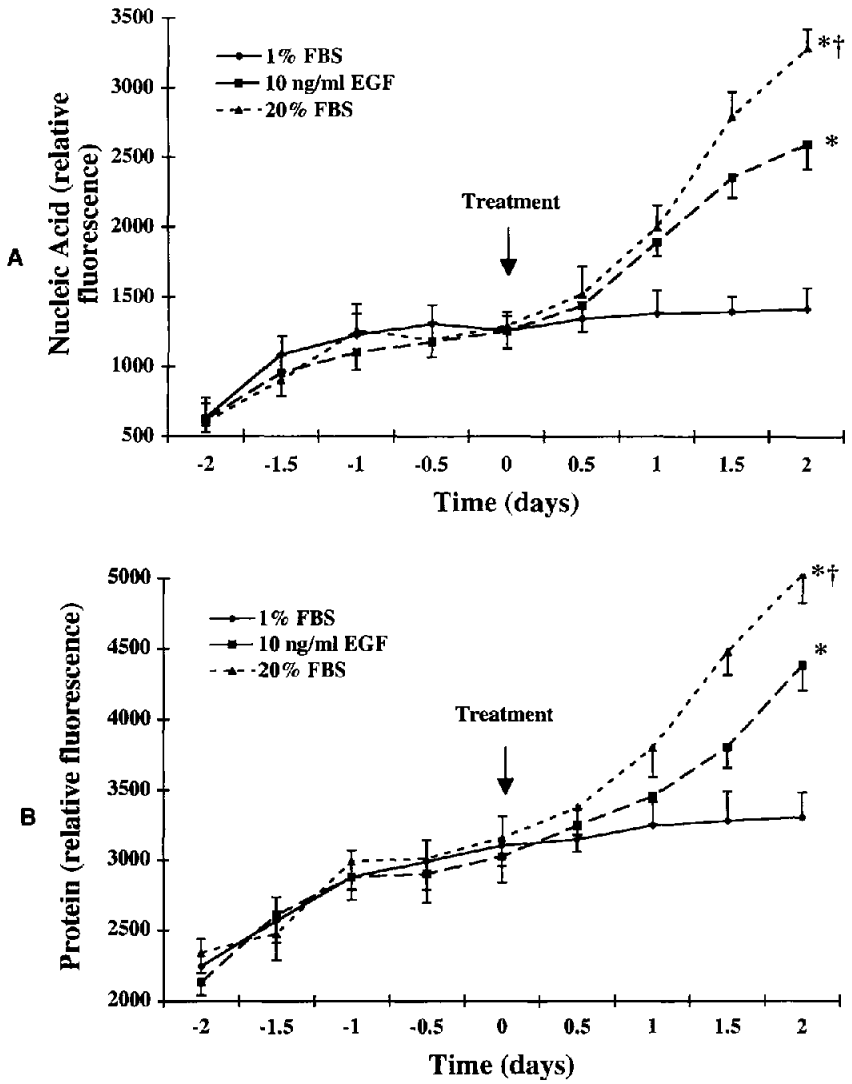

Fig. 1. Nucleic acid **(A)** and protein **(B)** levels in Caco-2 cells following 48 hours of serum deprivation and subsequent treatment with either 1% fetal bovine serum *(FBS)*, epidermal growth factor *(EGF)*, or 20% FBS (* = $P < 0.01$ vs. cells treated with MEM alone; † = $P < 0.01$ vs. cells treated with EGF; n = 12 per group).

EGF-Induced Ca^{++} Mobilization (Intracellular Ca^{++} Stores)

Previous data have suggested that EGF may initially increase $[Ca^{++}]_i$ through the release of intracellular Ca^{++} stores. Caco-2 cells, following 48 hours of serum deprivation, exposed to 10 ng/ml EGF demonstrated an initial increase in $[Ca^{++}]_i$ present within 2 to 3 minutes. Cells pretreated with neomycin (100 μmol/L; 20 minutes) or U73122 (1 μmol/L; 20 minutes), and subsequently treated with EGF, displayed no such elevation in $[Ca^{++}]_i$ and their levels were noted to be similar to control values. Cells pretreated with the inactive analogue U73343 (1 μmol/L; 20 minutes), however, and subsequently exposed to EGF, demonstrated no difference with regard to changes in $[Ca^{++}]_i$ when compared to cells treated with only EGF. These data are shown in Fig. 3 and demonstrate that the initial increase in intracellular Ca^{++} content induced by EGF involves the release of intracellular Ca^{-++} stores via a PLC- and IP_3-related mechanism.

EGF-Induced Ca^{++} Mobilization (SOCI)

Following the initial increase in $[Ca^{++}]_i$, Caco-2 cells exposed to EGF demonstrated a sustained elevation lasting up to 20 minutes (data not shown). Pre-

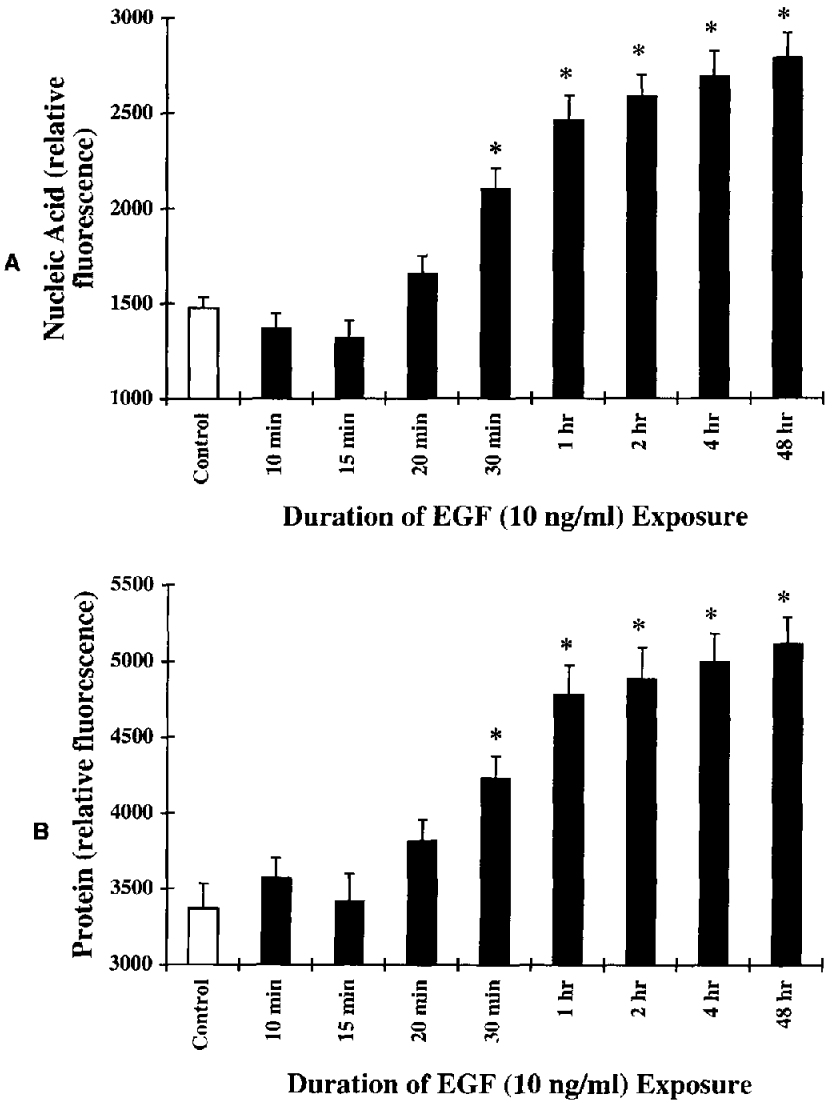

Fig. 2. Effect of epidermal growth factor *(EGF)* treatment duration on nucleic acid **(A)** and protein **(B)** levels in Caco-2 cells following 48 hours of serum deprivation (* = $P < 0.01$ vs. control; n = 6 to 12 per group).

liminary data have suggested that this plateau is a result of the influx of extracellular Ca^{++} through SOCCs.[19] Sustained intracellular Ca^{++} elevations (4 to 12 minutes) were not observed in cells treated with 10 ng/ml EGF in the absence of extracellular Ca^{++}. Quenching of Fluo-3 fluorescence by Mn^{++} was first evident 8 minutes after EGF exposure, which suggests the influx of extracellular Ca^{++} over this time period. Pretreatment of Caco-2 cells with the SOCC blocker La^{+++} (25 μmol/L; 20 minutes), alone, did not significantly affect intracellular Ca^{++} levels (data not shown) but did inhibit both the sustained intracellular Ca^{++} plateau and Mn^{++} uptake following EGF treatment. Pretreatment of cells with nondamaging, physiologically appropriate concentrations of indomethacin (5 μmol/L; 20 minutes), ibuprofen (10 μmol/L; 20 minutes), and acetylsalicylic acid (20 μmol/L; 20 minutes) equally inhibited the influx of extracellular Ca^{++} as determined by both $[Ca^{++}]_i$ measurements and Mn^{++} influx. These data, depicted in Fig. 4, are consistent with the concept that the sustained Ca^{++} elevation, as induced by EGF, is mediated by the influx of extracellular Ca^{++} through SOCCs. Furthermore, EGF-induced SOCI appears to be inhibited by NSAIDs in a manner very similar to the SOCC blocker La^{+++}.

Effect of Ca^{++} Signaling on EGF-Induced Mitogenesis

Caco-2 cells, following 48 hours of serum deprivation and subsequent pretreatment (20 minutes) with or without the aforementioned inhibitors, were then exposed to either 1% fetal bovine serum or 10 ng/ml

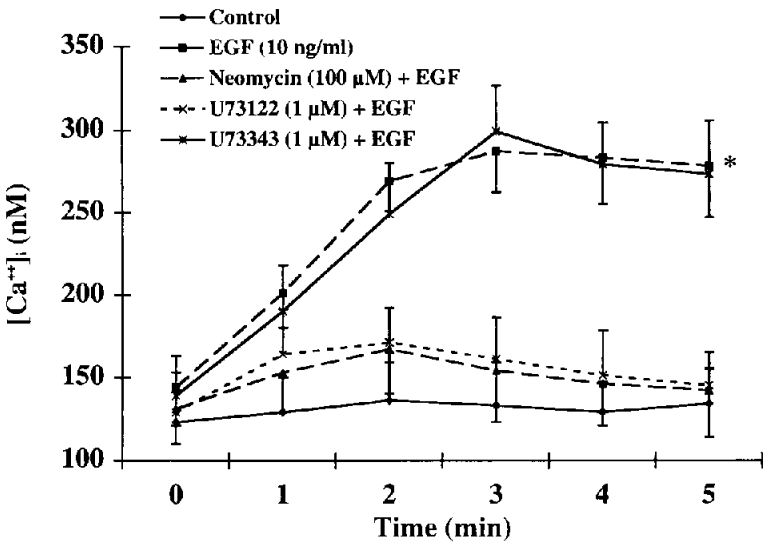

Fig. 3. Changes in intracellular calcium content in response to epidermal growth factor *(EGF)* alone or following pretreatment with neomycin (an inhibitor of IP_3 generation), the PLC inhibitor U73122, or its inactive analogue U73343 (* = P <0.01 vs. control; n = 6 to 12 per group).

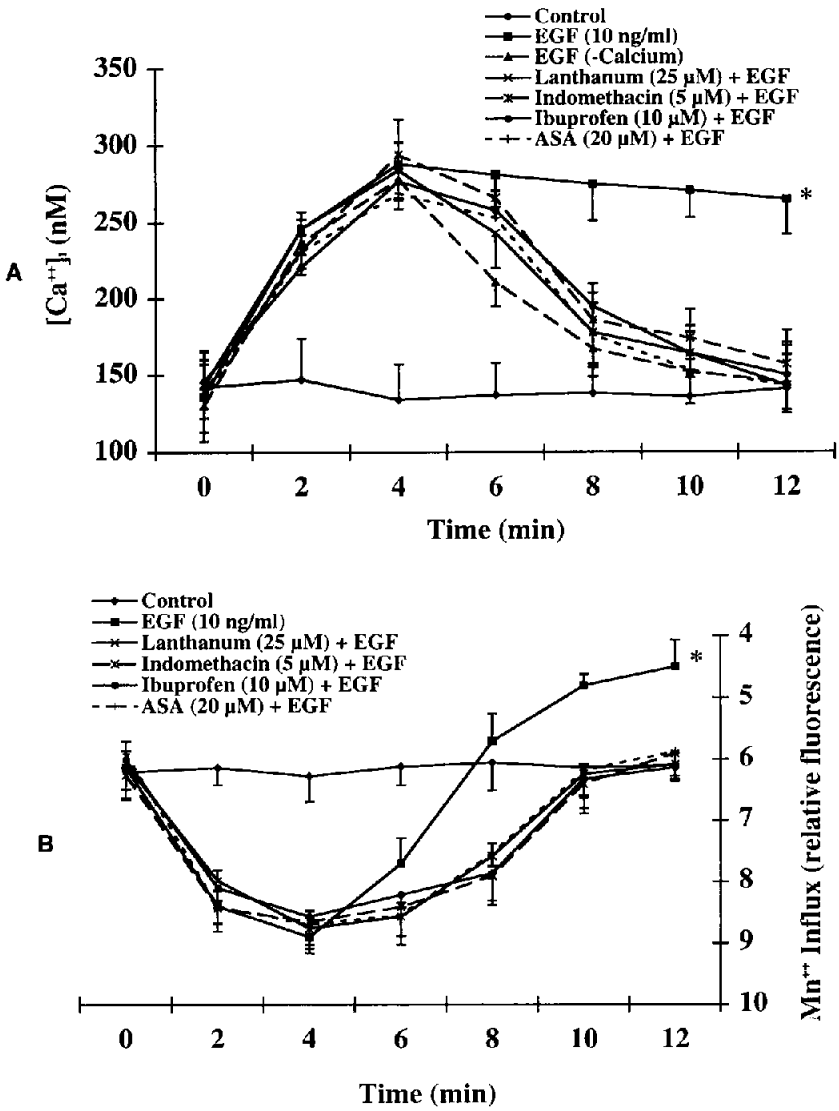

Fig. 4. Effect of pretreatment with either La^{+++} or various NSAIDs on changes in intracellular calcium concentration **(A)** or manganese influx **(B)** induced subsequently by epidermal growth factor *(EGF)* (* = P <0.01 vs. control; n = 6 to 12 per group).

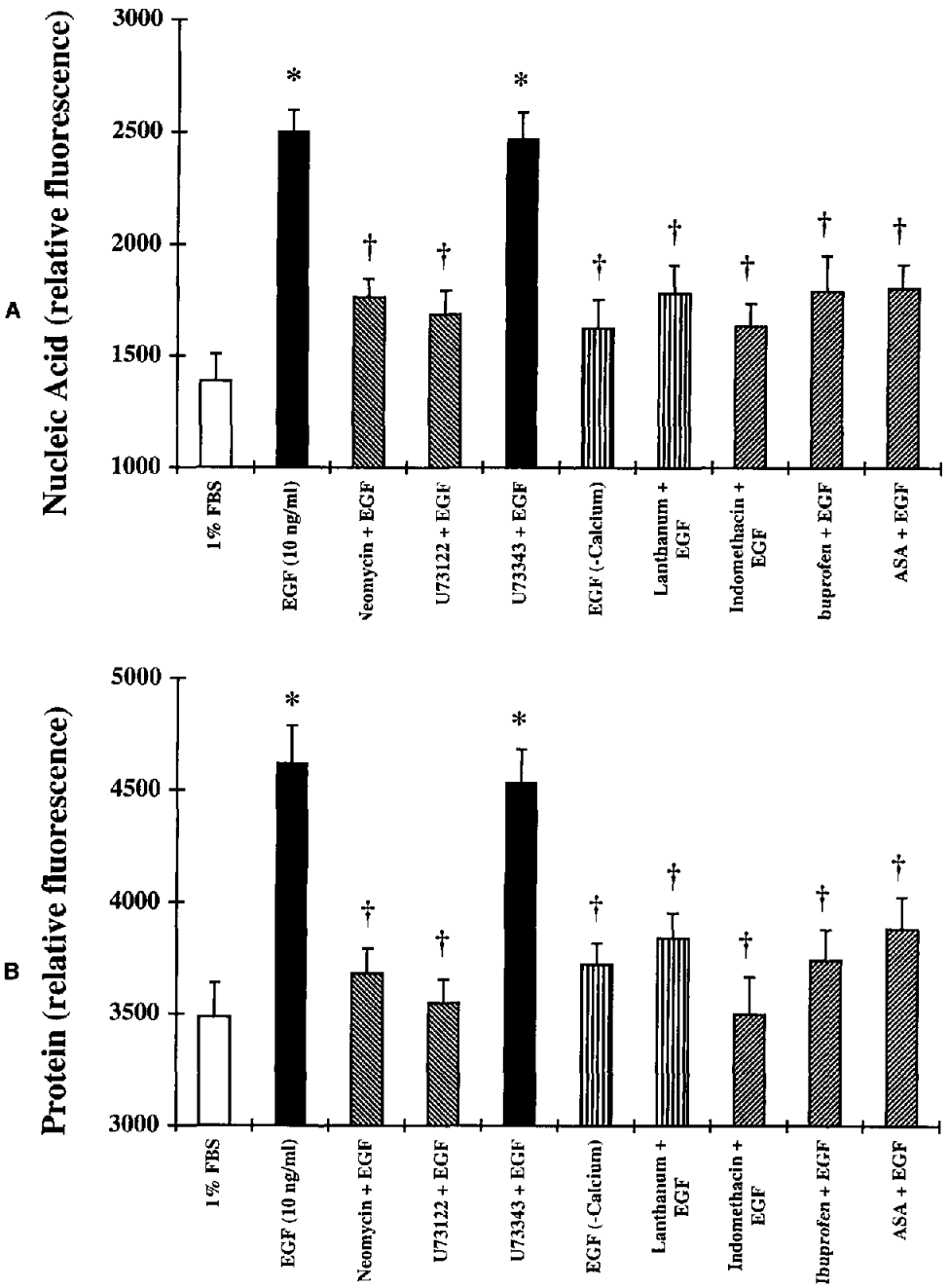

Fig. 5. Effect of pretreatment with either neomycin (an inhibitor of IP_3 generation), the PLC inhibitor U73122 or its inactive analogue U73343, La^{+++}, or various NSAIDs on nucleic acid **(A)** and protein **(B)** synthesis induced by epidermal growth factor *(EGF)* following 48 hours of serum deprivation (* = $P < 0.01$ vs. 1% fetal bovine serum *(FBS)* treatment; † = $P < 0.01$ vs. EGF treatment; n = 12 per group).

EGF. Cells exposed to EGF, with or without pretreatment with the inactive PLC inhibitor U73343, demonstrated a significant increase in nucleic acid and protein levels when compared to cells subsequently treated with only 1% fetal bovine serum. In contrast, EGF-induced nucleic acid or protein synthesis was not observed when any of EGF's effects on changes in intracellular Ca^{++} were prevented: internal store release via PLC and IP_3 (neomycin or U73122 pretreatment) or sustained extracellular Ca^{++} influx through SOCCs (Ca^{++}-free buffer, La^{+++}, indomethacin, ibuprofen, or acetylsalicylic acid pretreatment). These data are depicted in Fig. 5, *A* and *B*.

Finally, Caco-2 cells were serum starved (48 hours), treated (30 minutes) with either 1% fetal bovine serum or 10 mg/ml EGF, subsequently treated (20

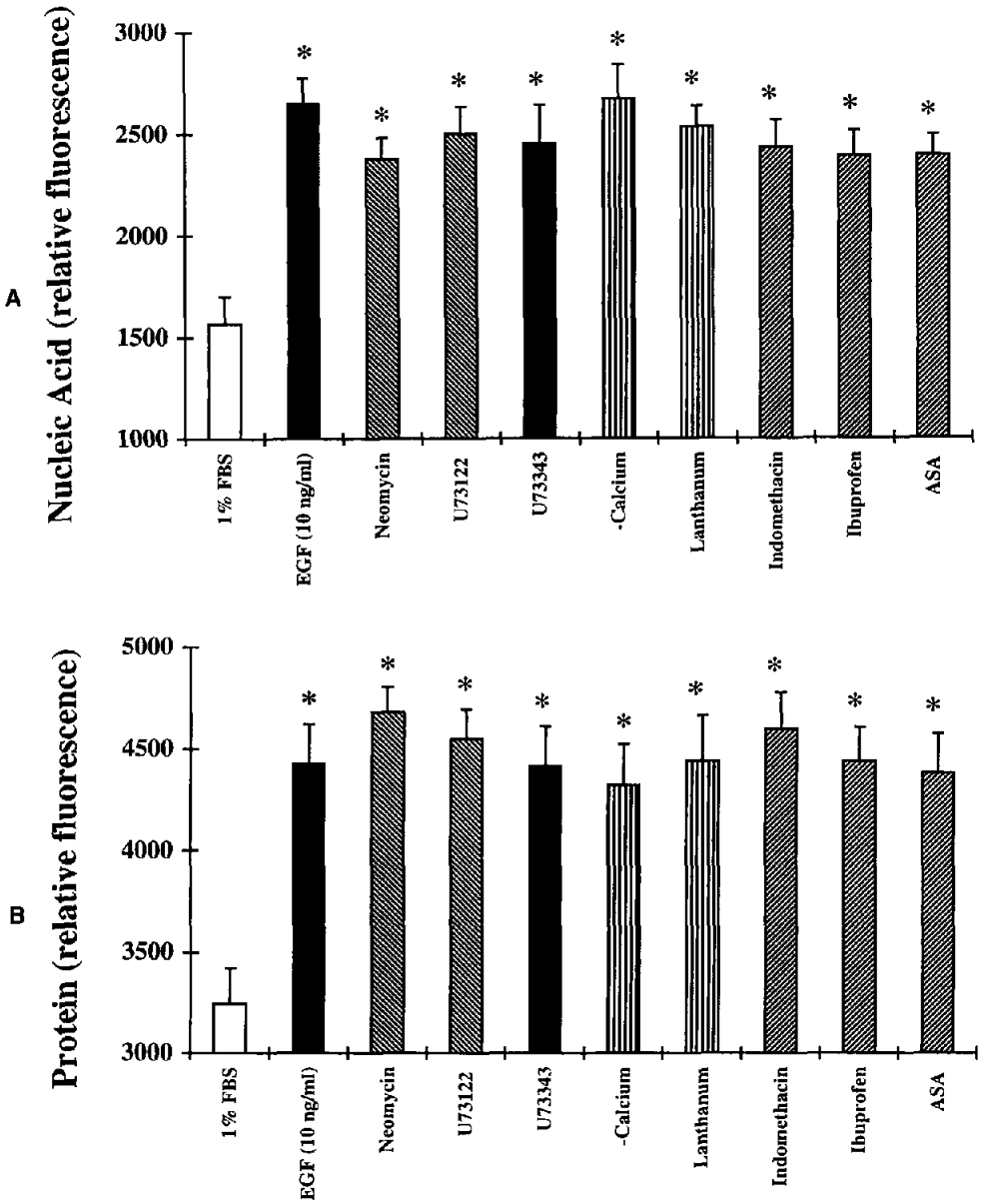

Fig. 6. Effect of posttreatment with either neomycin (an inhibitor of IP_3 generation), the phospholipase C inhibitor U73122 or its inactive analogue U73343, La^{+++}, or various NSAIDs on nucleic acid **(A)** and protein **(B)** synthesis induced by epidermal growth factor *(EGF)* following 48 hours of serum deprivation (* = $P < 0.01$ vs. 1% fetal bovine serum *(FBS)* treatment; n = 12 per group).

minutes) with the aforementioned inhibitors, and again serum starved (48 hours). Cells treated with EGF, as previously shown, demonstrated significantly increased nucleic acid and protein synthesis when compared to cells treated with only MEM. However, when cells were exposed to EGF and subsequently exposed to the aforementioned inhibitors of EGF-induced Ca^{++} mobilization, we observed no difference with regard to either nucleic acid or protein levels when compared to cells treated with EGF alone. These data, depicted in Fig. 6, suggested that inhibition of EGF-induced mitogenesis, as shown in Fig. 5, was a result of inhibitor activity directly related to EGF-induced Ca^{++} mobilization.

DISCUSSION

Previous studies from our laboratory have suggested that NSAIDs inhibit EGF-induced mitogenesis by blocking EGF-induced Ca^{++} signaling. Thus we speculated that EGF-induced Ca^{++} mobilization was a prerequisite for subsequent cellular prolifera-

tion. The current report strongly suggests that changes in Ca^{++} concentration, as induced by EGF, is required for mitogenesis. Several lines of evidence support this contention. The signal whereby EGF initiates the process of proliferation occurs within a relatively short period of time (30 minutes). This early signal appears to involve the initial release of Ca^{++} through the release of intracellular Ca^{++} stores via PLC activation and IP_3 generation followed secondarily by the sustained influx of extracellular Ca^{++} through SOCCs. The prevention of increases in $[Ca^{++}]_i$ with various inhibitors of Ca^{++} signaling, which in and of themselves appear to have little effect on EGF-induced cellular proliferation, significantly inhibits EGF-induced mitogenesis. Finally, the manner whereby NSAIDs inhibit EGF-induced mitogenesis is very similar to other conditions in which EGF-induced SOCI is blocked (Ca^{++}-free media or the use of the SOCC blocker La^{+++}). Thus the current study supports our premise that NSAIDs may attenuate colonic neoplasia by inhibiting EGF-induced Ca^{++} mobilization.

Caco-2 cells, derived from a human colonic carcinoma, were employed in the current study because these cells have been shown to possess EGF receptors[20] and have previously been shown to demonstrate physiologic responses to EGF.[21] Although these cells, when grown to postconfluency, have the unique ability to polarize, differentiate, and develop morphologic characteristics of normal enterocytes,[22] Caco-2 cells are more typical of colonic carcinoma cells early after plating. For these reasons, all experimentation was initiated when cells were 80% confluent. To date, we have not investigated the actions of EGF in other human colonic carcinoma cell lines.

Our observations regarding the mechanism whereby EGF elicits changes in $[Ca^{++}]_i$ are consistent with the literature. Several reports have suggested that EGF, after receptor binding, activates its tyrosine-specific protein kinase activity, which results in the subsequent phosphorylation of several substrates including PLC. Meisenhelder et al.[23] investigated the responses of both quiescent 3T3 mouse fibroblasts and A431 human epidermoid cells to EGF. They observed that EGF treatment resulted in the rapid phosphorylation of PLC tyrosines and serines and a subsequent increase in phosphatidylinositol turnover, suggesting that PLC is a substrate for EGF receptors. Prior work with mouse intestinal epithelial cells[24] and pancreatic AR42J[25] cells has demonstrated that the initial increase in $[Ca^{++}]_i$, as induced by EGF, was blocked by the PLC inhibitor U73122, but not the inactive agent U73343. These data confirm our observations and further suggest that EGF elicits an increase in PLC activity.

Following an initial increase in $[Ca^{++}]_i$, an effect that is likely the result of PLC stimulation followed by IP_3 generation and the release of intracellular Ca^{++} stores, EGF elicits a prolonged phase of increased intracellular Ca^{++} levels that appears to involve the influx of extracellular Ca^{++} through voltage-independent Ca^{++} channels (or SOCCs). Magni et al.[26] reported that the EGF-induced sustained elevation in $[Ca^{++}]_i$ in NIH-3T3 fibroblasts, was blocked by the imidazole derivative SC 38249, an SOCC blocker, in a manner very similar to cells tested after extracellular Ca^{++} was chelated with excess EGTA. Zhang et al.[27] also observed that the entry of extracellular Ca^{++} in hepatocytes, following EGF treatment, was not mediated by voltage-dependent Ca^{++} channels. In support of the current study, both Magni et al.[26] and Zhang et al.[27] also reported that the mitogenic effect of EGF in these two cell lines was inhibited when EGF-induced SOCI was prevented.

Previous work has also suggested that NSAIDs may inhibit Ca^{++} translocations involved with early cellular signaling. Abramson et al.[28] reported that indomethacin (30 μmol/L), piroxicam (50 μmol/L), or sodium salicylate (3 mmol/L) inhibited Ca^{++} uptake and increases in cytosolic Ca^{++} in human neutrophils stimulated by fMet-Leu-Phe through a process independent of any alteration of the affinity of fMet-Leu-Phe binding. Canesi et al.[29] investigated the EGF-activated signal transduction pathway in isolated digestive gland cells from mussels. They observed that indomethacin (20 μmol/L) blocked both EGF-induced Ca^{++} influx and subsequent mitogenesis.

Thus results from our laboratory and others suggest that Ca^{++} signaling, as induced by EGF, may play a role in stimulating quiescent (G0) cells to progress through G1 to DNA synthesis in S phase. Barbiero reported that BALB/c3T3 fibroblasts, following serum deprivation, exposed subsequently to fetal bovine serum demonstrated increases in $[Ca^{++}]_i$.[30] Furthermore, the addition of an extracellular Ca^{++} chelator (3 mmol/L EGTA) or the SOCC blocker SKF-96365A caused a significant reduction in fetal bovine serum–induced proliferation. Estacion et al.[31] observed that either low extracellular Ca^{++} or the SOCC blocker La^{+++}, both of which blocked platelet-derived growth factor (PDGF)–activated Ca^{++} influx, also inhibited PDGF-induced DNA synthesis.

Although there appears to be a linkage between elevations in $[Ca^{++}]_i$ and mitogenesis, this mechanism is not well understood. One explanation may be that Ca^{++} influxes play an important role in activating mitogen-activated protein (MAP) kinases, which appear to be essential for the proliferative response of cells. Agents such as EGF, associated with proliferation,

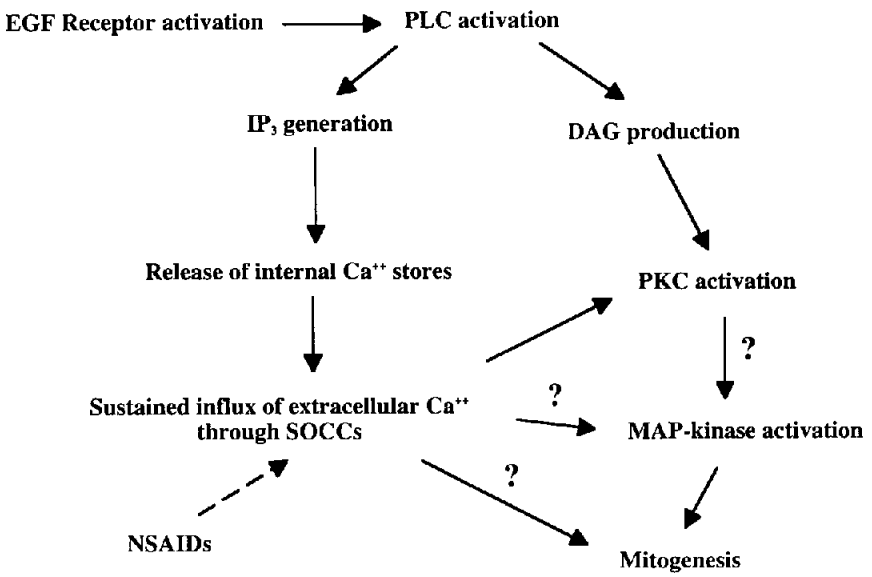

Fig. 7. Proposed schematic of one mechanism whereby nonsteroidal anti-inflammatory drugs *(NSAIDs)* may inhibit mitogen-stimulated cellular proliferation (PLC = phospholipase C; DAG = diacylglycerol; IP_3 = inositol 1,4,5-trisphosphate; PKC = protein kinase C; SOCC = store-operated calcium channel; MAP kinase = mitogen-activated protein kinase).

produce short-lived increases in MAP kinases, whereas those that cause differentiation produce longer lived elevations, which are sustained for hours.[32] Kavanagh et al.[33] investigated the role of Ca^{++} fluxes in the activation of MAP kinases induced by low doses of ionizing radiation in A431 cells. They reported that the addition of either the intracellular Ca^{++} chelator BAPTA/AM, the Ca^{++} antagonist TMB-8, or the PLC inhibitor U73223 blocked radiation-induced Ca^{++} oscillations and inhibited MAP kinase stimulation. Interestingly, quiescent fibroblasts grown in nominal Ca^{++} (0.1 mmol/L) and then briefly exposed to 1 mmol/L Ca^{++}, in the absence of other protein growth factors, demonstrated MAP kinase activation in a manner quite similar to that of cells exposed to other mitogens such as EGF.[34] Protein kinase C may also play an important role in initiating the MAP kinase cascade. Diacylglycerol, generated as a product of PLC activity, in combination with an elevation in $[Ca^{++}]_i$, is known to activate various protein C kinase isoforms.[35] Soltoff[36] reported that both $[Ca^{++}]_i$ elevations (via IP_3) and diacylglycerol-activated MAP kinase in PC12 cells in a EGF receptor–dependent manner.

Despite numerous investigations, the exact mechanism(s) whereby EGF stimulates DNA synthesis in any cell type remains unknown. However, evolving data suggest that an early elevation in $[Ca^{++}]_i$ may be a critical step in the cellular physiologic response characterized by a cascade initiated by substrate activation through the activation of kinases and phosphatases that link mitogenic receptor activation to the proliferative response. We propose that one mechanism whereby NSAIDs may ultimately attenuate colonic neoplasia is by blocking at least one step in this cascade–mitogen-induced Ca^{++} mobilization (Fig. 7).

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Discussion

Dr. B. Warner (Cincinnati, Ohio). Why did you choose the Caco-2 cell line? As you know, it is a differentiated colon cancer cell line. Have you examined other cell lines? It seems that the tyrosine kinase cascade to phospholipase C is a generalized mechanism, which may begin with other types of growth factors. Did you study growth factors other than EGF in terms of inhibition of proliferation? Finally, relevant to your overall hypothesis, you are suggesting that NSAIDs lessen metastasis or lessen the risk for cancer by inhibiting growth factor–induced proliferation. I am un-

aware of data to suggest that there is increased EGF receptor signaling in colon cancer or a correlation between EGF receptor quantity and colon cancer biology.

Dr. F. Kokoska. We investigated Caco-2 cells because they were well differentiated, and I think the less differentiated cell lines may be less responsive to growth hormones or mitogens. We have not investigated other cell lines. I want to stress that serum deprivation was initiated when cells were 80% confluent and that the cells were 2 to 3 days post confluent when the experiments ended. We wanted to work with the colorectal carcinoma side of Caco-2 so we did not allow cells to become post confluent 7 to 10 days, at which time they take on characteristics of small bowel epithelium. We have not looked at other growth factors. Many but not all other growth factors elicit calcium signaling similar to EGF, so it would be interesting to see if those that do not behave in a different manner. Finally, I think that this is just one small piece of the puzzle with regard to the effect of NSAIDs on colon carcinogenesis. There are data suggesting that NSAIDs may be involved with apoptosis and other work looking at the immunomodulating effects of NSAIDs. But we think that cellular antiproliferation is one of the nonsteroidal effects.

Dr. B. Bass (Baltimore, Md.). I am interested in your choice of measurements of growth, namely, nucleic acids and proteins. Have you looked at the more conventional measures of growth such as cell counts and tritiated thymidine uptake?

Dr. Kokoska. I like to avoid radioactivity if I can, and I have found these assays to be extremely sensitive. Both assays were used previously to quantitate nucleic acids and proteins for gel loading, and I found them to be extremely sensitive and fairly easy to perform. I have not correlated them with other measures of proliferation.

Hepatic Resection Using Intermittent Vascular Inflow Occlusion and Low Central Venous Pressure Anesthesia Improves Morbidity and Mortality

Herbert Chen, M.D., Nipun B. Merchant, M.D., Mukund S. Didolkar, M.D.

Hepatic resection results in significant morbidity and mortality primarily related to intraoperative blood loss. Intermittent vascular inflow occlusion (VO) and low central venous pressure (CVP) during hepatectomy have been used to reduce blood loss. To determine the benefit of VO and low CVP, we reviewed the outcomes of 168 consecutive patients who underwent liver resection. The results of 78 patients who had undergone hepatic resection between 1993 and 1998 with the use of VO and low CVP (post-VO/CVP) were compared to the previous 90 patients who had undergone hepatectomy without VO and low CVP (pre-VO/CVP) between 1979 and 1992. Hepatectomies were performed for metastatic disease (65%), hepatoma (20%), and benign tumors (15%). Resections included 18 trisegmentectomies, 67 lobectomies, and 83 segmental resections. Patients in both groups were similar with regard to extent of resection. Post-VO/CVP patients had significantly lower median estimated blood loss (725 ml vs. 2300 ml, $P < 0.001$), less postoperative morbidity (10.3% vs. 22.2%, $P = 0.038$), lower in-hospital mortality (2.6% vs. 10%, $P = 0.050$), fewer days in the intensive care unit (1.6 ± 0.1 days vs. 5.6 ± 1.2 days, $P = 0.003$), and shorter overall length of stay (8.0 ± 0.5 days vs. 15.0 ± 1.6 days, $P < 0.001$) than pre-VO/CVP patients. These data suggest that VO and low CVP during liver resection may improve patient outcomes. (J GASTROINTEST SURG 2000;4:162-167.)

KEY WORDS: Hepatic resection, central venous pressure, hepatic vascular inflow control, blood loss

Liver resection remains the only potentially curative treatment for hepatocellular carcinoma[1] and liver metastases from colorectal cancer[2-4] and other primary tumors.[5,6] Early experience with resection of these tumors resulted in significant morbidity and mortality.[7,8] Improved understanding of hepatic physiology and segmental anatomy of the liver has led to advances in surgical and anesthetic technique for hepatic resection. These improvements have resulted in lower mortality rates ranging from 2% to 5% in most modern series of liver resections. The morbidity associated with hepatic resection, however, remains high, ranging from 25% to 50%.[9-13] Recently several techniques such as total hepatic vascular exclusion, intermittent vascular inflow occlusion (VO), low central venous pressure (CVP) anesthesia, and acute isovolemic hemodilution have been shown to reduce blood loss during hepatic resection.[14-18]

We have been using a combination of VO and low CVP anesthesia during all hepatectomies since 1993 in an effort to reduce intraoperative blood loss and lower the risk of severe hemorrhage. Intermittent vascular inflow occlusion, or the Pringle maneuver, minimizes hepatic arterial and portal blood flow to the liver during parenchymal dissection. However, a substantial cause of blood loss during liver resection can result from hepatic venous or inferior vena caval injury. Low CVP facilitates control of bleeding from hepatic veins by reducing the pressure gradient that promotes bleeding through inadvertent extrahepatic venous injuries as well as hepatic venous bleeding during parenchymal dissection. Recent studies have

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shown that low CVP anesthesia can be safely used and results in reduced blood loss compared to other reported series.[16,19] However, the effect of VO in conjunction with low CVP anesthesia on morbidity and mortality after liver resection has not been clearly demonstrated. Thus, to determine the benefit of VO and low CVP anesthesia, we reviewed the outcomes of 168 consecutive patients who underwent liver resection between 1979 to 1998, comparing patients who underwent liver resection by means of these techniques to those who did not.

MATERIAL AND METHODS

Patients

Between 1979 and 1998, 168 consecutive patients undergoing liver resection were identified from a prospective database. One surgeon (M.S.D.) performed more than 90% of the operations. The most recent 78 consecutive patients (1993 to 1998) had undergone hepatic resection with the use of VO and low CVP anesthesia (post-VO/CVP) and were compared to the previous 90 patients (1979 to 1992) who had undergone hepatectomy without VO and low CVP anesthesia (pre-VO/CVP). Patient demographics, type of liver resection, estimated blood loss, overall morbidity, and in-hospital mortality, as well as the length of stay in the intensive care unit (ICU) and in the hospital, were compared between the two groups of patients.

Technique of Liver Resection

After induction of general anesthesia, a CVP line was placed in the right internal jugular vein. A midline or bilateral subcostal incision was used. Intravenous fluid administration was minimized to maintain a CVP of less than 5 mm Hg and a urine output of at least 25 ml/hr. After dissection of the hilar plate, inflow control was obtained by encircling the contents of the hepatoduodenal ligament with umbilical tape. The patient was placed in the 15-degree Trendelenburg position to improve venous return and minimize the risk of air embolism. For major hepatic resections, the hepatic veins were controlled extrahepatically. Before transection of the liver parenchyma, small branches from the vena cava were controlled. Intermittent vascular inflow occlusion (Pringle maneuver) was achieved using a Rammel tourniquet for 10 minutes with 1- to 2-minute intervals of reperfusion during parenchymal transection. The liver parenchyma was divided by means of either a clamp-crush technique or a Cavitron ultrasonic surgical aspirator (Valleylab, Inc., Boulder, Colo.). Structures within the liver parenchyma were clipped or ligated. CVP was maintained below 5 mm Hg until the specimen was removed and adequate hemostasis was achieved. Additionally, a Valsalva maneuver was used to check hepatic vein bleeding prior to closure of the abdomen. Euvolemia was then achieved with crystalloid and colloid fluid resuscitation. Cell Saver (Haemonetics Corp., Braintree, Mass.) was used to reinfuse lost blood in several cases. Packed cells and/or autologous units of blood were transfused to maintain a hemoglobin level between 8 and 10 g/dl.

Definition of the extent of liver resection is as previously described.[7,20,21] A right lobectomy refers to resection of segments V, VI, VII, and VIII as per the Couinaud classification. A left lobectomy involves resection of segments II, III, and IV. A right trisegmentectomy, also referred to as an extended right lobectomy, includes resection of segments IV, V, VI, VII, and VIII, whereas a left trisegmentectomy includes the resection of segments II, III, IV, V, and VIII. Segmental resections refer to resection of one or two individual segments as defined by the Couinaud classification.

Statistical Analysis

Analysis of variance, log-rank, and chi-square tests were used to determine differences between the two groups. Statistical significance was defined as a P value of <0.05.

RESULTS

Patient Data

The mean age of all 168 patients who underwent hepatic resection was 60 ± 1 years, and 87 (52%) were women. Hepatectomies were performed for metastastic disease in 109 patients (65%), hepatoma in 34 patients (20%), and benign disease in 25 patients (15%). A substantial number of patients had comorbid conditions: 28 patients (17%) had chronic obstructive pulmonary disease, whereas 28 (17%) had athlerosclerotic disease. Concomitant liver diseases including cirrhosis, alcoholism, and hepatitis were present in 13 (8%), 30 (18%), and five (3%) patients, respectively.

When comparing pre- and post-VO/CVP patients, both groups were similar with regard to age, sex, and the prevalence of athlerosclerotic disease, alcoholism, and hepatitis (Table I). More pre-VO/CVP patients had undergone liver resection for malignancy (90% vs. 77%, $P = 0.021$) and more had chronic obstructive pulmonary disease (22% vs. 11%, $P = 0.038$). In contrast, post-VO/CVP patients had a higher prevalence of cirrhosis (14% vs. 2%, $P = 0.004$).

Table I. Patient data

	Pre-VO/CVP	Post-VO/CVP	*P* value
Age (mean ± SEM)	59 ± 1 yr	61 ± 2 yr	NS
Female sex	52%	45%	NS
Malignant tumor	90%	77%	0.021
Cirrhosis	2%	14%	0.004
Athlerosclerotic disease	17%	18%	NS
Chronic obstructive pulmonary disease	22%	11%	0.038
Alcoholism	17%	19%	NS
Hepatitis	3%	4%	NS

VO/CVP = intermittent vascular inflow occlusion and low central venous pressure anesthesia; SEM = standard error of the mean; NS = not significant.

Table II. Types of liver resection performed*

	Pre-VO/CVP	Post-VO/CVP	Total
Trisegmentectomy	10 (11%)	8 (10%)	18 (11%)
Lobectomy	44 (49%)	23 (30%)	67 (40%)
Segmental/wedge resection	36 (40%)	47 (60%)	83 (49%)
TOTAL	90	78	168

VO/CVP = intermittent vascular inflow occlusion and low central venous pressure anesthesia.
*P – not significant.

Table III. Median estimated blood loss

	Pre-VO/CVP (ml)	Post-VO/CVP (ml)	*P* value
All patients	2300 (range 50-2000)	725 (range 50-6000)	<0.001
By extent of hepatic resection			
Trisegmentectomy	6750 (range 3400-17,000)	1400 (range 1000-1900)	<0.001
Lobectomy	2600 (range 500-20,000)	1000 (range 100-6000)	<0.001
Segmental resection	900 (range 50-15,000)	500 (range 50-2000)	<0.001

VO/CVP = intermittent vascular inflow occlusion and low central venous pressure anesthesia.

Type of Liver Resections

Hepatic resections included 18 trisegmentectomies (11%), 67 lobectomies (40%), and 83 segmental resections (49%). When comparing pre- and post-VO/CVP patients (Table II), there was a trend toward a higher proportion of lobectomies in the pre-VO/CVP group (49% vs. 30%) and a higher number of segmental resections in the post-VO/CVP group (60% vs. 40%). However, these differences were not statistically significant.

Blood Loss

Post-VO/CVP patients had a significantly lower median estimated blood loss compared to pre-VO/CVP patients (725 ml vs. 2300 ml, P <0.001). Since blood loss often is related to the extent of liver resection, and because there was a trend toward more major resections in the pre-VO/CVP group, we then stratified blood loss according to the particular hepatic resection (Table III). Patients in the post-VO/CVP group had significantly lower estimated

Table IV. Complications

	Total (%)	Pre-VO/CVP (%)	Post-VO/CVP (%)	*P* value
Wound infection	10 (6)	6 (7)	4 (5)	NS
Postoperative bleeding	8 (5)	5 (6)	3 (4)	NS
Respiratory failure	8 (5)	6 (7)	2 (3)	NS
Renal failure	7 (4)	5 (6)	2 (3)	NS
Reexploration	7 (4)	5 (6)	2 (3)	NS
Pneumonia	3 (2)	1 (1)	2 (3)	NS
Abdominal abscess/bile leak	7 (4)	6 (7)	1 (1)	NS

VO/CVP = intermittent vascular inflow occlusion and low central venous pressure anesthesia; NS = not significant.

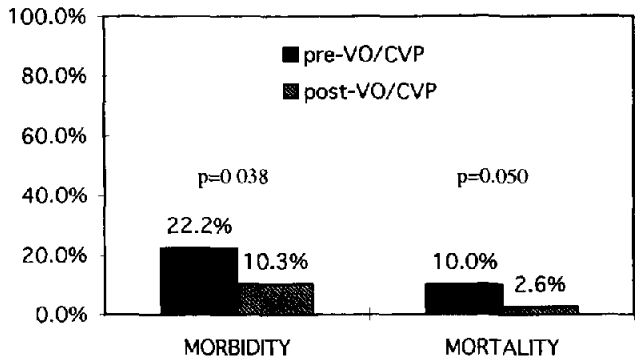

Fig. 1. Liver resection using intermittent vascular inflow occlusion and low central venous pressure anesthesia (VO/CVP) resulted in significantly lower morbidity and mortality compared to liver resection without VO/CVP.

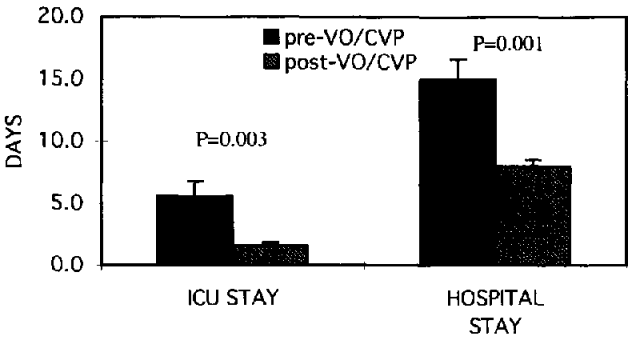

Fig. 2. Liver resection using intermittent vascular inflow occlusion and low central venous pressure anesthesia (VO/CVP) resulted in significantly shorter stays in the intensive care unit and hospital compared to liver resection without VO/CVP.

blood loss compared to pre-VO/CVP patients whether they underwent trisegmentectomy, lobectomy, or segmental resection.

Morbidity and Mortality

The overall complications in this series of 168 patients are listed in Table IV. Although individual complications were not significantly different between the two groups, the pre-VO/CVP group had an overall morbidity rate of 22.2% compared to 10.3% for the post-VO/CVP group ($P = 0.038$) (Fig. 1). The in-hospital postoperative mortality rate was also significantly lower in the pre-VO/CVP patients compared to the post-VO/CVP patients (2.6% vs. 10%, $P = 0.05$).

Length of Stay

Lengths of stay in the ICU and hospital were also compared between the two groups (Fig. 2). Liver resection with VO and low CVP anesthesia resulted in significantly shorter stays in the ICU and hospital. The mean lengths of stay in the ICU for pre- and post-VO/CVP patients were 5.6 ± 1.2 days and 1.6 ± 0.2 days, respectively ($P = 0.003$). The mean total hospital lengths of stay for pre- and post-VO/CVP patients were 15.0 ± 1.6 days and 8.0 ± 0.5 days, respectively ($P = 0.001$).

DISCUSSION

Intraoperative hemorrhage remains a major risk of performing hepatic resection and correlates directly with postoperative morbidity.[8] In this report we have shown that the routine use of VO and low CVP anesthesia significantly reduces blood loss and lowers morbidity and mortality following hepatic resection. Furthermore, the use of these techniques shortens the ICU and total hospital lengths of stay.

The use of inflow control and maintenance of low CVP during hepatic resection affords several advantages. Controlling the portal venous and hepatic arterial blood inflow to the liver during parenchymal dissection allows for a bloodless field of dissection. This facilitates easy identification and control of intrahe-

patic structures resulting in a meticulous dissection of the liver along proper anatomic and segmental planes. In addition, visualization and ligation of smaller vessels and bile ducts within the liver parenchyma also decreases postoperative complications.

Once hepatic inflow of blood is controlled, the risk of bleeding is primarily from hepatic veins during parenchymal dissection, mobilization of the liver from the inferior vena cava, or extrahepatic venous control. Maintenance of a CVP below 5 mm Hg significantly reduces the pressure gradient across the hepatic veins and inferior vena cava resulting in a several-fold decrease in the blood loss associated with injuries to these vessels. With a low CVP, however, openings in these veins can increase the risk of air embolism. Therefore patients are placed in a 15-degree Trendelenburg position. This reduces the risk of air embolism and also improves venous return to the heart, which may be compromised with a low CVP.

Other reports on the use of intermittent inflow occlusion and low CVP anesthesia have shown similar results.[15,16,19] Cunningham et al.[19] showed in 100 consecutive hepatic resections in which these techniques were used that estimated blood loss was 450 ml, 1000 ml and 1300 ml for segmental resections, lobectomies, and trisegmentectomies, respectively. The overall morbidity in their study was 24% with an operative mortality of 3%.

Melendez et al.[16] recently described their experience with 496 major liver resection using low CVP anesthesia. They reported an in-hospital mortality rate of 3.8%, a median blood loss of 645 ml, and not a single episode of postoperative renal failure secondary to the maintenance of a low CVP intraoperatively. Their results are very similar to the mortality rate of 2.6% and median blood loss of 725 ml seen in the post-VO/CVP patients in our study. The incidence of renal insufficiency in our study was actually lower in the post-VO/CVP patients compared to the pre-VO/CVP patients (3% vs. 6%), and no patients had any permanent renal dysfunction.

Arnoletti and Brodsky[15] reported a series of 34 patients who underwent major liver resection for metastatic disease using total inflow occlusion and clamp-crush technique, and compared their outcomes to those of 15 patients who did not have inflow occlusion. They found that patients who had hepatectomy with inflow occlusion had a lower median blood loss, fewer administered units of transfused blood, and shorter stays in the ICU and hospital. These authors, however, found no differences in morbidity or mortality between the two groups in their small number of patients.

To date, there has not been a randomized control study comparing low CVP anesthesia with standard anesthetic management. All of the reported series in which these techniques were used for liver resection are reports of outcomes alone. Our study compares two groups of patients, with and without the use of inflow control and low CVP over different time periods. Clearly, improvements in anesthetic and ICU management could have had a significant impact on postoperative morbidity and mortality. However, these prospective data do suggest that the use of low CVP anesthesia and inflow control may improve patient outcomes after hepatectomy.

Several other techniques aimed at reducing blood loss during hepatic resection have also been described. Total vascular isolation of the liver during liver resection has been used in a number of small series.[17,22] Although this allows blood-free transection of liver parenchyma, there is still the potential for bleeding when flow is restored. Futhermore, total vascular isolation has been associated with increased operative times, increased hepatic ischemic times, and increased morbidity, and has not been shown to reduce blood loss and/or transfusion requirements compared to intermittent inflow occlusion.[16,17,22] In addition, a prospective controlled trial comparing total vascular isolation to portal triad clamping showed no differences in operative blood loss between the two techniques.[17] Acute isovolemic hemodilution has also been used in small series of patients[14,23,24] with some success. Acute isovolemic hemodilution consists of the collection of the patient's blood after induction of anesthesia and simultaneous replacement with crystalloid solution to maintain an isovolemic state. The patient is maintained at a hematocrit of 20% to 25% during surgery. The patient's blood is administered intraoperatively after all surgical blood loss has ceased or in response to a hemodynamic necessity. Theoretically, blood lost during the operation is diluted, resulting in a lower net loss. Although acute isovolemic hemodilution has been used with some success to decrease transfusion requirements, it has not led to a reduction in operative blood loss.[14] This technique is also time consuming and cumbersome. Other techniques including intraoperative Cell Saver, preoperative autologous blood donation, erythropoietin administration, and intraoperative hypovolemia have also been shown to decrease transfusion requirements following hepatic resection.

CONCLUSION

The combination of intermittent vascular inflow occlusion and low CVP anesthesia is a safe, effective technique to reduce the intraoperative blood loss that

occurs during liver resection. Patients who undergo hepatectomy with intermittent inflow occlusion and low CVP have significantly lower blood loss, lower morbidity, and lower mortality compared to patients who do not. Furthermore, the reduction in blood loss and morbidity translates into shorter ICU and total hospital lengths of stay. Therefore the data favor the routine use of intermittent inflow occlusion and low CVP anesthesia during hepatic resection.

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Isolated Right Segmental Hepatic Duct Injury: A Diagnostic and Therapeutic Challenge

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Biliary leaks and injuries are not an uncommon occurrence following laparoscopic cholecystectomy. Bile leaks associated with the biliary anatomic variant of a low-inserting right segmental hepatic duct can be particularly difficult to diagnose in that results of endoscopic retrograde cholangiography (ERC) are usually interpreted as "normal" with no leaks demonstrated. The aim of this study was to describe a single institution's experience with nine patients with biliary leaks associated with this anatomic variant and to discuss their management. A retrospective analysis of the hospital records of all patients with bile duct injuries managed at a single institution between 1980 and July 1998, inclusive, was performed. Nine patients were identified as having an isolated right segmental hepatic duct injury associated with a biliary leak. Seven (78%) of the nine patients had undergone a laparoscopic cholecystectomy, whereas the remaining two patients (22%) had undergone an open cholecystectomy. All of the patients had undergone endoscopic retrograde cholangiography at outside institutions, the results of which had been interpreted as normal with no apparent leaks. The median interval from the time of cholecystectomy to referral was 1.4 months. All patients were managed with initial percutaneous access of the involved right segmental biliary system, with placement of a percutaneous transhepatic stent. After the biliary leak was controlled, all patients underwent Roux-en-Y hepaticojejunostomy to the isolated biliary segment. All patients had an uncomplicated postoperative course. There were no postoperative anastomotic leaks. Postoperative stenting was maintained for a mean of 8 months. Six (67%) of the nine patients had a long-term successful outcome with minimal or no symptoms. In three patients, recurrent symptoms with pain and/or cholangitis developed at a mean of 34 months. All three patients underwent percutaneous cholangiography, which demonstrated an anastomotic stricture, and all were managed with percutaneous balloon dilatation with a successful outcome. Currently eight (89%) of the nine patients are asymptomatic, with a mean follow-up of 70.4 months (range 12 to 226 months). One patient had intermittent right upper quadrant pain with normal liver function tests but has not required intervention. Isolated right segmental hepatic ductal injury with biliary leakage is an uncommon complication following laparoscopic cholecystectomy. A diagnostic dilemma is created by the presence of a bile leak with a normal endoscopic retrograde cholangiogram. Management begins with percutaneous access of the transected isolated ductal system followed by reconstruction as a Roux-en-Y hepaticojejunostomy. (J GASTROINTEST SURG 2000;4:168-177.)

KEY WORDS: Bile duct injury, cholecystectomy, biliary fistula

Injuries to the bile duct can occur during either open or laparoscopic cholecystectomy. Although laparoscopic cholecystectomy has significant advantages compared to the open procedure, there is an increased incidence of biliary injuries associated with this procedure.[1-3] As experience in the management of laparoscopic bile duct injuries has been gained, the mechanisms of injury have been established.[3-5] Misidentification of anatomy appears to be the most common cause of laparoscopic bile duct injury. In the "classic" bile duct injury, the common bile duct is mistaken for the cystic duct, clipped, and divided.[4] Because most bile duct injuries are not recognized at the time of laparoscopic cholecystectomy, this injury usually results in a bile leak presenting in the early postoperative period as either bile ascites or a loculated "biloma." The algorithm for management of patients presenting in the postoperative period with evidence

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of a bile duct injury involves early cholangiography to demonstrate the injury.[5-8]

Abnormal anatomy, although often used as an explanation for an injury, is rarely documented in patients with laparoscopic bile duct injuries. An important anatomic variant, however, does appear to be associated with injury and also creates a diagnostic dilemma at the time of presentation. The low insertion of a right segmental hepatic duct, either into the hepatic duct or even the cystic duct, places this structure in a position at risk to be injured during laparoscopic cholecystectomy. The diagnostic dilemma associated with this injury is that frequently endoscopic retrograde cholangiography (ERC) will not demonstrate a leak, and may give a "normal" appearance with demonstration of hepatic bifurcation and filling of both the right and left hepatic lobes. It is often not recognized that only one of two segments of the right lobe is being visualized. The aim of this report was to review our institutional experience with the management of patients presenting with bile duct injuries associated with this anatomic variant and to describe their management and long-term outcome.

METHODS

A retrospective review was performed of the hospital records of all patients with bile duct injuries managed at the Johns Hopkins Hospital between 1980 and July 1998, inclusive. All patients identified as having an isolated right segmental hepatic duct injury associated with a bile leak were included in this analysis. Records were reviewed to determine patient demographics, the nature of the original procedure, presentation, and management both prior to referral and at the Johns Hopkins Hospital. Finally, the results of surgical reconstruction, both short-term and long-term, were reviewed with follow-up data obtained up to April 1999.

Management at the Johns Hopkins Hospital generally followed a standard protocol beginning with review of outside radiologic studies including cholangiograms and sinograms obtained through operatively or percutaneously placed drains. Prior interpretation of all endoscopic retrograde cholangiograms had not demonstrated the site of the bile leak and in all cases a "bifurcation" of the right and left system was demonstrated. Careful review of these studies, however, revealed a relative absence of complete filling of the right hepatic ductal system, suggesting a "missing" segment. Based on the ERC and sinography, selected percutaneous transhepatic cholangiography was performed into the isolated segment of the liver (usually hepatic segments VII and VIII). In all cases the segmental ductal system was accessed with demonstration of free extravasation into the peritoneal cavity. A percutaneous transhepatic catheter was placed into the segment to allow external drainage of the transected biliary tree. If present, abdominal fluid collections were percutaneously drained. After allowing 2 to 4 weeks for clinical improvement, all patients underwent surgical reconstruction with creation of a Roux-en-Y hepaticojejunostomy to the isolated segment. To identify the transected duct at the time of laparotomy, the interventional radiologist was asked to advance the biliary catheter well below the area of transection into the subhepatic space, where it could be easily identified at the time of exploration.

Postoperative management included the use of long-term biliary stents. The duration of stenting was directed by the surgeon's preference, based on the patient's clinical course and the cholangiographic appearance.

RESULTS

Nine patients were identified with isolated right segmental hepatic duct injuries. There were eight women and one man. The mean age of the patients was 50.1 years (range 35 to 67 years). Seven patients (78%) had undergone a laparoscopic cholecystectomy, whereas the two remaining patients (22%) had undergone an open cholecystectomy. Two of the nine patients presented with a free flow of bile into the peritoneal cavity (bile ascites), whereas seven of the nine patients had an identifiable collection of bile (biloma) (Fig. 1). Three of the patients had undergone percutaneous drainage of bile collections prior to transfer. All patients had undergone ERC prior to referral with no evidence of a "visible bile leak" and were thought to have normal biliary anatomy (Fig. 2). In two patients sinograms performed through existing drainage catheters had demonstrated communication with the isolated segment (Fig. 3). The median interval from cholecystectomy to referral to the Johns Hopkins Hospital was 1.4 months (range 0.8 to 3.5 months).

Percutaneous cholangiography was performed at the Johns Hopkins Hospital in all patients, which demonstrated a transected isolated right hepatic ductal system, with free extravasation in the peritoneal cavity (Fig. 4). A percutaneous biliary catheter was placed in all patients into the undrained segment with control of the biliary fistula and prompt resolution of all signs of biliary sepsis. The median length of preoperative biliary drainage was 17 days. Prior to laparotomy for biliary reconstruction, the biliary catheter was advanced through the transected duct into the subhepatic space to facilitate identification of the involved duct (Fig. 5).

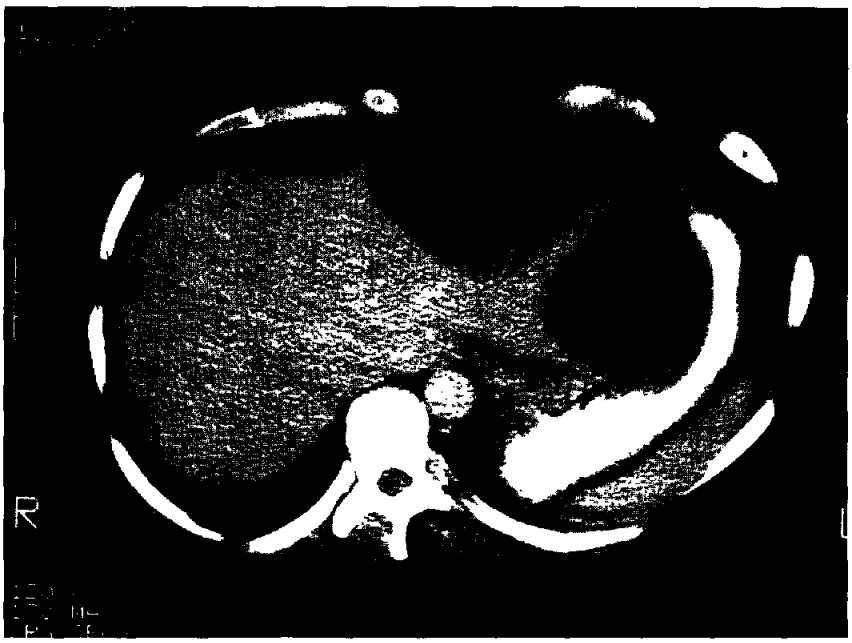

Fig. 1. CT scan demonstrating a bile collection or "biloma" following isolated hepatic duct injury.

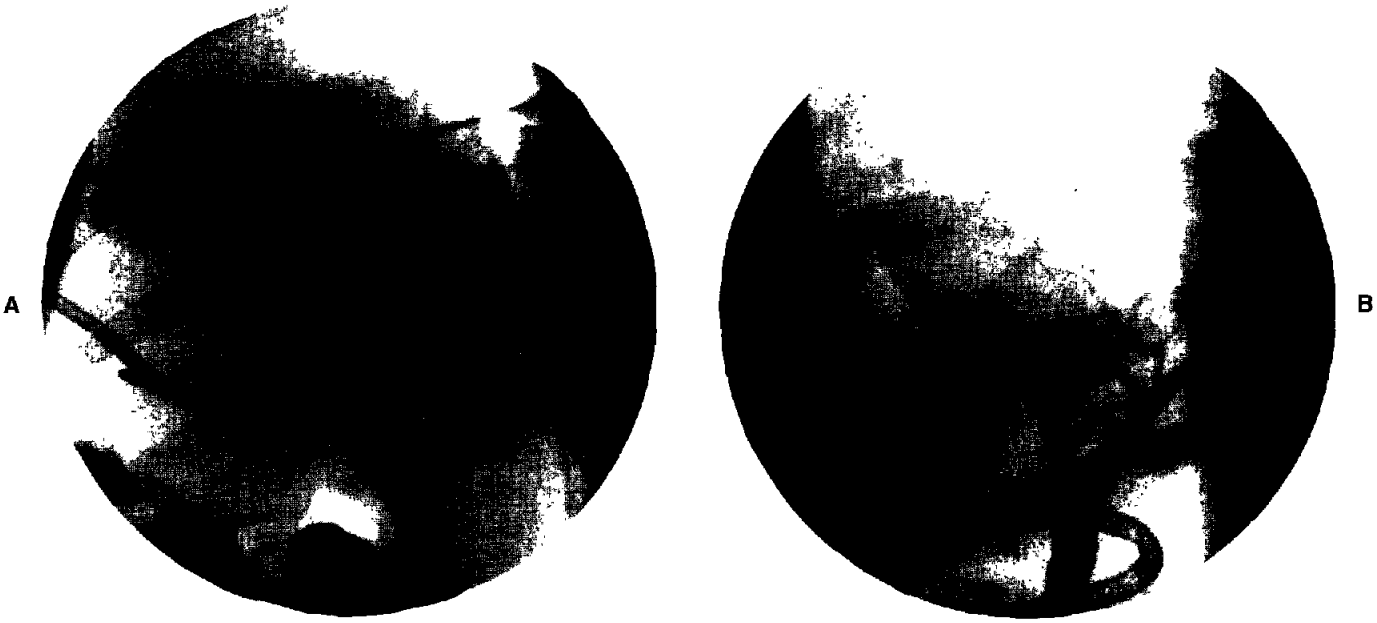

Fig. 2. A, Endoscopic retrograde cholangiogram showing no evidence of a biliary leak and an "intact" hepatic bifurcation. **B,** Close-up view of the same patient demonstrating the hepatic duct bifurcation.

Fig. 2, cont'd. C, Endoscopic retrograde cholangiogram from a patient with an isolated right segmental hepatic duct injury. There is no evidence of contrast leakage, and an apparent bifurcation is present.

Fig. 3. Sinogram from a patient with a percutaneously placed drainage catheter demonstrating filling of the transected isolated biliary system.

Fig. 4. Percutaneous transhepatic cholangiogram demonstrating the extensive hepatic biliary duct system drained by the transected right segmental hepatic duct.

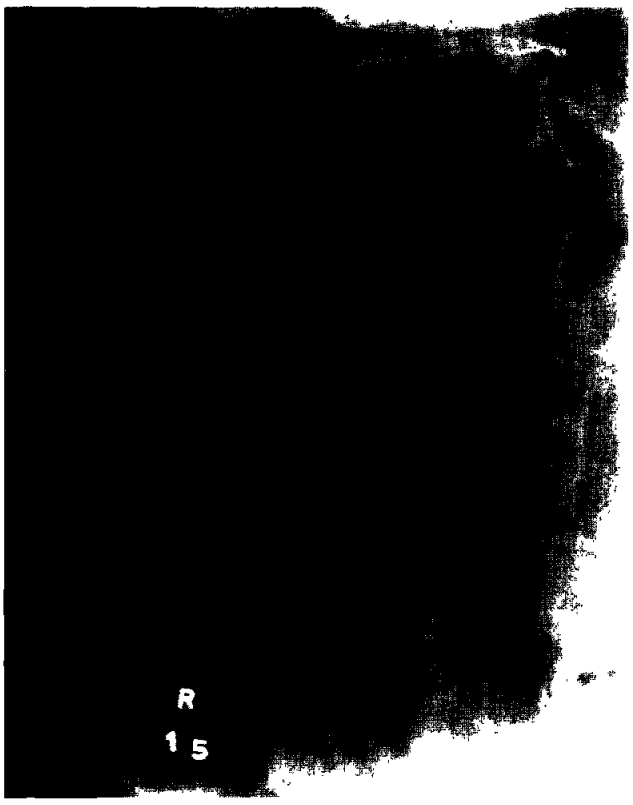

Fig. 5. Final percutaneous transhepatic biliary catheter placement prior to laparotomy. Note the presence of the catheter coiled well into the subhepatic space.

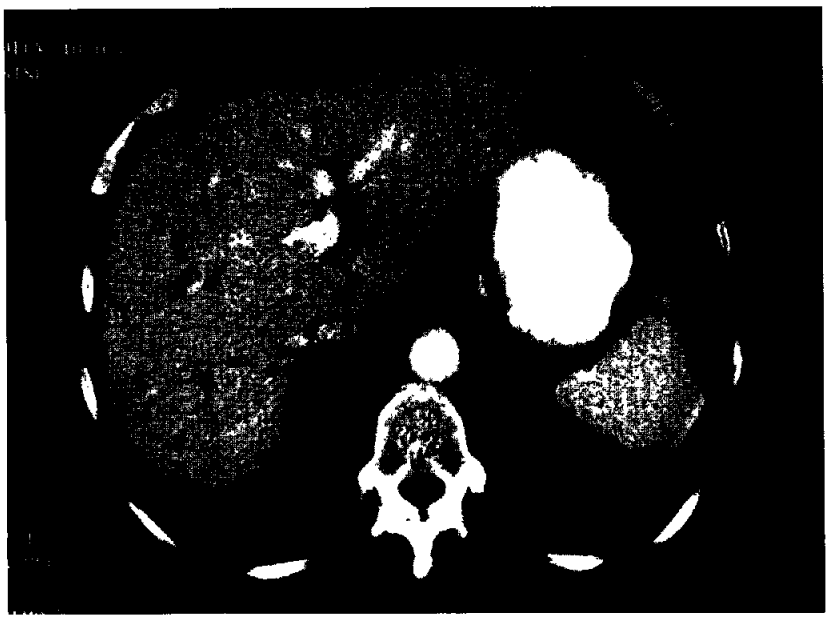

Fig. 6. CT scan demonstrating dilated biliary tree of the posterior segment of the right hepatic lobe of the liver due to postoperative anastomotic stricture.

Surgical management consisted of a Roux-en-Y hepaticojejunostomy to the isolated hepatic duct in all cases. All anastomoses were constructed over Silastic biliary stents. The remainder of the biliary duct system was normal and was not involved in the reconstruction. The postoperative course was uncomplicated in all patients, and in no case was there a postoperative biliary anastomotic leak. The duration of postoperative stenting ranged from 3 to 18 months with a mean of 8 months.

On follow-up, six of the nine patients have reported no significant postoperative symptoms and have not required invasive procedures for evaluation or treatment since initial stent removal. Three of the nine patients reported symptoms of right upper quadrant pain with intermittent fever and chills consistent with cholangitis. Computed tomography demonstrated obstruction of the same biliary segment (Fig. 6) and repeat percutaneous cholangiography was performed, which demonstrated an anastomotic stricture. In all three patients balloon dilatation was performed with a successful outcome. The interval from initial repair to development of symptoms of a recurrent stricture was 19, 30, and 54 months (mean 34 months). The length of stenting following percutaneous dilatation of the recurrent stricture was 7, 7, and 13 months. In all cases the catheters have been removed, and two of the three patients are currently asymptomatic. The remaining patient continues to report intermittent mild right upper quadrant pain without cholangitis and with normal liver function tests. Currently all nine patients have their biliary stents removed, at a mean follow-up of 70.4 months (median 36 months, range 12 to 226 months).

DISCUSSION

Injuries to the bile duct can occur following both open and laparoscopic cholecystectomy. The incidence of injury during laparoscopic cholecystectomy appears to be higher than that after open cholecystectomy and is currently estimated at 0.4% to 0.6%.[1-3] As experience in the management of laparoscopic bile duct injuries has been gained, the common mechanisms of injury have been established.[3-5] Misidentification of anatomy appears to be the most frequent cause of laparoscopic bile duct injury. In the most common scenario, described by Davidoff et al.,[4] the "classic" injury involves mistaking the common bile duct for the cystic duct. The common bile duct is then clipped and divided. Further retraction of the gallbladder leads to a second, higher injury, with division of the common hepatic duct, often as it approaches the bifurcation. If the proximal hepatic duct is not adequately secured with clips, a bile leak develops. This injury occurred in 63% of patients in a series reported by Branum et al.[5] from Duke University. Regardless of the nature of the injury, most biliary injuries are not recognized at the time of initial laparoscopic cholecystectomy.[5,6]

The management of laparoscopic bile duct injuries presenting in the postoperative period has been well

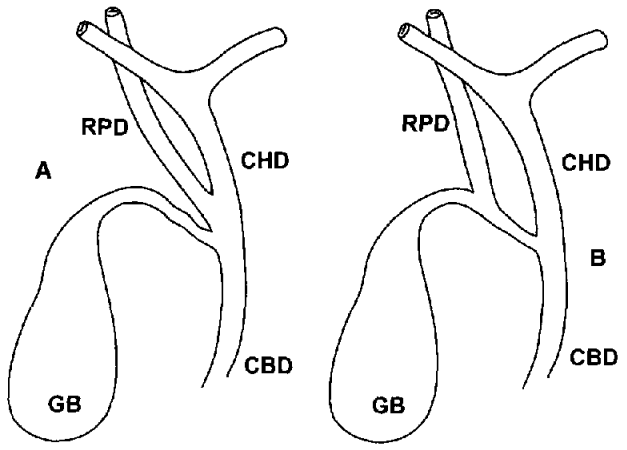

Fig. 7. A, Aberrant hepatic ductal anatomy showing low insertion of a right posterior segmental hepatic duct into the common hepatic duct. *RPD*, right posterior duct; *CHD*, common hepatic duct; *GB*, gallbladder; *CBD*, common bile duct. **B,** Insertion of a right posterior segmental hepatic duct into the cystic duct.

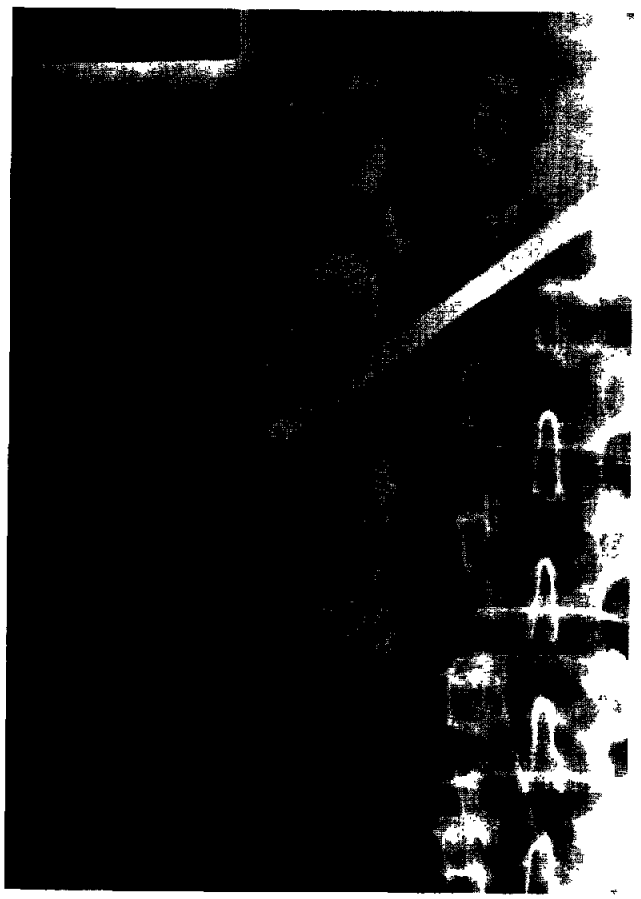

Fig. 8. Intraoperative cholangiogram from a patient who was eventually found to have an isolated right segmental hepatic duct injury. Note the apparent hepatic bifurcation seen, suggesting that normal anatomy is present, allowing "safe" performance of a laparoscopic cholecystectomy.

defined by a number of authors.[3-9] The importance of preoperative biliary imaging has been emphasized in a report by Stewart and Way.[9] In their analysis, failure to define the biliary anatomy preoperatively ultimately led to failure in 96% of patients undergoing surgical reconstruction. ERC is a readily available technique used to evaluate patients with a suspected biliary injury. However, in the classic injury involving bile duct transection, the retrograde cholangiogram will only show a "cutoff" of the common bile duct below the cystic duct. It is essential, therefore, that percutaneous transhepatic cholangiography be peformed to define the exact location and nature of the injury, as well as to demonstrate the anatomy of the proximal biliary tree, which will be used for reconstruction.

Although abnormal biliary anatomy is frequently used as an explanation for injuries, documentation of aberrant or variant anatomy is unusual. The anatomic variant of a low-inserting right segmental hepatic duct, however, is of significant concern with respect to both an increased likelihood of injury and difficulty in management. This duct, which usually drains the right posterior segment of the liver (segments VII and VIII), may insert low into the common hepatic duct or even the cystic duct (Fig. 7). This insertion places this duct in jeopardy for injury during laparoscopic cholecystectomy. This anatomic variant may go unrecognized with intraoperative cholangiography (Fig. 8) and also creates a dilemma with respect to diagnosis in the postoperative period. In this setting the ERC may be interpreted as normal with no evidence of a biliary leak and an apparent normal hepatic duct bifurcation. In many cases the bile leak may be attributed to minor ducts from the gallbladder fossa, namely, the ducts of Luschka. However, awareness of this biliary anatomic variant is important to lead to recognition and to ensure a suitable outcome for patients sustaining this injury.

The importance of this anatomic variant was first emphasized by Meyers et al.[10] in a report of 14 patients with injuries involving this ductal segment. In six patients the injury to the segmental duct occurred in combination with a classic biliary injury, necessitating complex reconstruction of multiple transected ducts. In seven patients, however, the segmental duct injury was isolated with the remainder of the biliary tree intact. Five of the patients presented with a bile leak, whereas two patients presented with problems resulting from obstruction with fevers, chills, and an elevated alkaline phosphatase. In this report the authors stressed the importance of awareness of this variant. They predicted that with a presumed incidence of low insertion of a segmental hepatic duct of 0.3%, with more than 600,000 cholecystectomies performed per year, surgeons would encounter this vari-

ant 18,000 times. In the Duke series of approximately 100 cases of major bile duct injuries, the isolated hepatic segmental duct injury accounted for 7% of all cases.[10]

In the current series we identified nine patients over an 18-year period with such injuries. Seven of these injuries have been seen in the last 8 years and have occurred during laparoscopic cholecystectomy. The other two injuries occurred in the prelaparoscopic era. Analysis of a recent series of 89 biliary injuries referred to our institution included five isolated hepatic duct injuries, which represents 5.6% of all injuries seen at our institution.

The key to management of an isolated segmental hepatic duct injury is recognition. ERC will not demonstrate the site of the leak and will demonstrate the presence of a hepatic bifurcation with ductal branches in both the right and left lobes of the liver (see Fig. 2). It must be recognized, however, that there is an absence of complete filling of the right biliary ductal system. This absence of complete segmental hepatic ductal filling (on the right), in the presence of a major bile leak, should suggest an injury to an isolated right segmental hepatic duct. Sinography performed via a drainage catheter, placed operatively or percutaneously into a bile collection, may allow retrograde filling of the injured segment to also confirm the diagnosis. Based on interpretation of endoscopic retrograde cholangiograms and sinograms the interventional radiologist can access the involved hepatic segment percutaneously and place a percutaneous transhepatic biliary catheter into the segment. External drainage should allow prompt control of the biliary fistula, eliminate sepsis, and allow optimal timing of elective reconstruction. Prior to surgical exploration, the interventional radiologist should confirm that the biliary catheter protrudes well through the transected duct into the subhepatic space. In this position it becomes easily identifiable by the surgeon to facilitate dissection of the involved duct to be used in reconstruction. Surgical reconstruction can then be performed to the isolated duct as a Roux-en-Y hepaticojejunostomy. The percutaneously placed catheter can be converted to a soft Silastic catheter at the time of laparotomy and is used for long-term stenting.

With the use of this technique, the nine patients in this series had an uncomplicated postoperative course with no biliary anastomotic leaks. Stenting was maintained postoperatively for a mean of 8 months. In all patients the catheters were eventually removed. The incidence of late stricture of this anastomosis, however, was 33%. This incidence is significantly higher than that noted in a recent report from our institution evaluating the short-term outcome following repair of major bile duct injuries.[6] In that report of 52 patients completing treatment after surgical reconstruction, the incidence of recurrent stricture was only 8% at a mean follow-up of 33.4 months. The mean follow-up in this current series of nine patients is significantly longer and may partially account for this difference. However, it would appear that this reconstruction performed to a small peripheral duct is at significant risk for late stricture. Secondary treatment with balloon dilatation of the recurrent stricture has proved successful, leaving all nine patients with their stents removed and a satisfactory result.

CONCLUSION

Isolated right segmental hepatic duct injuries with a biliary leak are an uncommon complication following laparoscopic cholecystectomy, accounting for approximately 5% of all major ductal injuries. These injuries can be associated with what appears to be a normal endoscopic retrograde cholangiogram leading to the conclusion that the biliary leak is coming from minor biliary ducts (ducts of Luschka). Recognition, however, of incomplete biliary duct filling of the right hepatic ductal system is the key to diagnosis. Percutaneous access of the transected isolated ductal system is essential for management for both identification of the injury and control of the biliary fistula and associated sepsis. A percutaneous biliary catheter also facilitates reconstruction, which can be completed as a Roux-en-Y hepaticojejunostomy. Although the incidence of failure following initial surgical repair is higher than expected following biliary reconstructions for other major bile duct injuries, secondary balloon dilatation can prove successful in maintaining long-term anastomotic patency and good results.

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Discussion

Dr. L. Stewart (San Francisco, Calif.). Did you note in your analysis of bile duct injuries persons who had complete transections of the right hepatic duct as opposed to a segmental duct? And if so, could you give me an estimate of how many of these patients there were? Similarly, what was the incidence of right hepatic artery injury in your population? Among our patients who have right hepatic duct injury or right segmental duct injury of the class IV type, a number of them also have right hepatic artery injury and some of them have specific ligations. Also, was there an increased incidence of hepatic abscesses in this group compared to other persons with bile duct injuries?

By way of comment, its important to note that these injuries can be missed on intraoperative cholangiography. The only way to prevent these injuries is to use meticulous technique dissecting out the triangle of Calot.

Dr. K. Lillemoe. Drs. Stewart and Way shed light on this topic several years ago when they showed that patients who either did not undergo cholangiography or, more important, had incomplete cholangiography were at high risk for failure after biliary reconstruction following bile duct injury. In answer to your questions about our series, there were 5 of a total of 89 patients with bile duct injuries who fell into this category for an incidence of approximately 6%. There were at least two other patients whom we recognized as having had the right duct injured separately from the main hepatic duct. Many of those patients had already undergone some form of reconstruction before they came to us, however, so we do not know what the original anatomy was. I do not have information on right hepatic arterial injuries. One patient had a major infarction that required debridement after referral, but since we do not perform arteriography on a routine basis, the incidence of arterial injury is unknown. Finally, these patients are at increased risk for hepatic abscess due to late obstruction; however, none of these nine patients developed hepatic abscess.

Dr. D. Nagorney (Rochester, Minn.). I am confused about the ductal anatomy. The anatomy that was previously described by Meyers et al.,[10] that is, segments 7 and 8 coming off the right lateral sector, is incredibly unusual. Usually it is segments 6 and 7. Second, you had a 33% early failure rate and you do not yet have any long-term follow-up data. Why not just remove the right lateral sector and never worry about this again? The liver will compensate.

Dr. Lillemoe. Meyers et al.[10] referred to hepatic segments 7 and 8. In our series most of the injuries involved the posterior segment of the right lobe using the older terminology. In terms of performing a resection because of the high risk of failure, this may be a substantial resection in many of these patients. Currently all nine of our patients are basically asymptomatic with their livers intact, and they have no significant problems. So I think that if you can get by with reconstruction, that is still the way to go.

Dr. M. Callery (Worcester, Mass.). An interesting point made by Meyers et al.,[10] which you cite in your report, is that the prevalence rate of this anomaly would suggest that it would be encountered 18,000 times across America this year. But it only represents about 5% to 7% of bile duct injuries.

In the preoperative workup, is there a role for CT cholangiography or magnetic resonance cholangiography? With respect to minimizing your 33% long-term late stricture rate, is there any way you can improve on that by changing the timing of the operation? Do you treat the sepsis prior to surgery?

I would cast one last vote for resection as an option. Once the involved segments of the liver have been removed, patient should not need anything else. Patients still have the same incision, and they have 8 fewer months of external drainage.

Dr. Lillemoe. Your point about the incidence emphasizes the importance of careful dissection. It is essential to dissect enough of the triangle of Calot to make sure there is no aberrant duct.

We did not use magnetic resonance cholangiography in these patients because it was not yet available. I think it would be ideal for demonstrating this anatomy, and I certainly would use it if I had a new patient with such an injury now. With respect to the timing of our repair, we tend to not operate on bile leaks urgently. We first achieve control of the patient's fistula with a percutaneous catheter. We then allow the fistula to close and oftentimes the external drains can be removed. The biloma resolves and the surgery is scheduled at a point that is optimal for the patient. The mean time to operation in this series was about 3 weeks after referral. I appreciate your comments concerning resection, but I continue to hold this as the last option.

Dr. B. Langer (Toronto, Ontario, Canada). In response to the recommendation that resection might be a good early option, I think resection is the operation of last resort for this type of injury. I would like to remind everyone that a segmental duct can be ligated without causing any harm whatsoever to the patient, as long as the duct is not infected. I think that is a good strategy. I have used it in pa-

tients who had very small peripheral ducts. I have used it in patients who had a moderately large segment of liver involved in the injury. If one finds that a tiny duct is not suitable for use in an anastomosis, I think ligation is the first step. One can always come back and reconstruct the duct after it dilates if the patient is symptomatic. If an infection occurs and cannot be controlled, a resection can then be performed.

Dr. Lillemoe. At the time of laparoscopic cholecystectomy, if the patient is recognized to have this injury and the duct is smaller than 3 mm, ligation is probably the course. All of our patients had ongoing fistulas at the time of referral and we felt uncomfortable using simple ligation because of concern about infection.

Dr. L. Way (San Francisco, Calif.). Undetected ligation is one variant of this particular injury that sometimes has consequences but often does not. We have seen a number of completely occluded ducts without fistula that were silent.

Dr. S. Strasberg (St. Louis, Mo.). I agree that these are difficult injuries to diagnose. We have treated approximately 15 of these patients during the past 5 or 6 years and there are two things we have noticed. One is that it is sometimes possible to determine that an injury is present by looking at the comparative size of the right and left ducts at the origin. If the right duct seems small, this suggests that an injury is present. But because the shape of the liver can differ from one patient to another, an overlay comparison between the CT scan and the percutaneous transhepatic cholangiogram is useful. Magnetic resonance cholangiography will become the "gold standard" because it picks up the injury on one film.

Dr. Lillemoe. The use of the term "normal" cholangiogram throughout the presentation and manuscript reflects the fact that these films were often initially read as unremarkable. An experienced radiologist or surgeon, however, will note the subtle points that Dr. Strasberg suggested and realize that the films are not truly normal, and therefore suspect the injury.

Anatomic Segmental Hepatic Resection Is Superior to Wedge Resection as an Oncologic Operation for Colorectal Liver Metastases

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Hepatic wedge resection for colorectal liver metastasis has been reported to have a high incidence of positive surgical margins. Anatomic segmental resection is now widely practiced, although there are few data comparing segmental and wedge resection in terms of tumor clearance or long-term outcome. There were 267 patients who underwent liver resection for metastatic colorectal cancer between July 1985 and October 1998 at our institution who had either a wedge (n = 119) or segmental (n = 148) resection. Patient, tumor, and treatment data were compared, actuarial survival was determined, and prognostic factors were analyzed. Anatomic segmental resection was associated with similar blood loss, operative time, and complications as wedge resection. Segmental resection had a significantly lower rate of positive margins (2% vs. 16%) compared to wedge hepatectomy ($P < 0.001$). On univariate analysis, segmentectomy resulted in longer survival with a median of 53 months vs. 38 months for wedge hepatectomy ($P = 0.015$). Preoperative carcinoembryonic antigen level, positive margin of resection, and the presence of extrahepatic disease independently predicted survival on multivariate analysis. Anatomic segmental resection is a safe procedure and is superior to wedge resection as an oncologic operation for colorectal liver metastasis because it results in better tumor clearance and improved survival. (J GASTROINTEST SURG 2000;4:178-184.)

KEY WORDS: Colorectal metastases, hepatectomy, wedge resection, segmentectomy, outcome

Hepatic wedge resection is a widely accepted treatment for colorectal liver metastases. Although removal of a hepatic tumor with an adequate margin of surrounding normal tissue may appear straightforward, the margin of excision is involved by tumor in up to 30% of cases.[1] Our own experience is that wedge hepatectomy results in a 17% rate of positive margins.[2] Two factors contribute to inadequate tumor clearance following nonanatomic wedge resection. First, traction on the specimen during division of the liver parenchyma tends to produce a fracture at the interface of the fragile soft liver tissue and the hard colorectal metastasis. Second, because of limited exposure and the lack of vascular control, hemorrhage commonly occurs at the base of the wedge resection. Bleeding may obscure the plane of the intended parenchymal transection and consequently compromise the final margin.

A better understanding of liver anatomy and improvements in radiologic imaging and anesthetic techniques have led to the widespread use of segmental liver resections.[3-12] In fact, segmentectomy now comprises approximately 20% of our hepatic resections.[4] Segmental hepatectomy based on the anatomic descriptions of Couinaud[13] is appealing for several reasons. First, vascular inflow is often controlled prior to transection of the liver. The resultant demarcation of the parenchyma indicates the boundaries of excision and ensures an adequate margin of normal tissue throughout the procedure. Next, segmentectomy may be used to preserve liver substance in cases that would otherwise require a lobar resection. For this reason,

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segmental resections have been especially useful for patients with cirrhosis who have bilobar or recurrent hepatocellular carcinoma. Moreover, segmental resection is suitable for the treatment of colorectal liver metastases since removal of the tumor with an adequate margin is all that is needed because intrahepatic metastases (i.e., tumor satellites) from an established colorectal liver metastasis are rare.[14]

There are few data comparing the results of wedge and segmental liver resections for colorectal metastases in terms of margin status and survival. We postulated that segmental liver resection achieves better tumor clearance than wedge resection, and herein we report our experience in 267 patients who had either a wedge or segmental hepatectomy for metastatic colorectal adenocarcinoma.

METHODS

Patients

There were 267 patients who underwent unilobar wedge resection(s) or sublobar segmental resection for colorectal metastases at Memorial Sloan-Kettering Cancer Center from October 1985 to October 1998 identified from the Liver Resection Database of the Department of Surgery. Patients who had a lobar hepatectomy as part of their resection or a combined wedge and segmental resection were excluded. Information was obtained from the database, medical records, patients, family, and physicians.

The clinical risk score[15] (0 to 5 points) was calculated by assigning one point for each of the following: positive nodal status of the primary colorectal tumor, disease-free interval from the resection of the primary tumor to the first liver metastasis of less than 1 year, preoperative carcinoembryonic antigen (CEA) level greater than 200 ng/ml, more than one liver tumor, and largest tumor greater than 5 cm. The clinical risk score is based on an analysis of 1001 patients who had a partial hepatectomy for colorectal metastases at our institution, and the score predicts survival as patients with 0, 3, or 5 points had a 5-year survival of 60%, 20%, and 14%, respectively.

Resection Technique

Intraoperative ultrasonography was used to confirm the anatomic relationship of the tumors to vascular and biliary structures.[16] Wedge hepatectomy was generally performed with the intention of including at least a 1 to 2 cm rim of normal liver tissue. The surface of the liver was scored with electrocautery. The parenchyma was transected by means of a clamp crushing technique to allow visualization of portal structures and hepatic vein tributaries that were clipped or ligated. Our techniques of segmental resection have been described in detail.[4] The liver segments are as described by Couinaud.[13] Frequently the procedure was initiated by obtaining vascular inflow control. Pedicle ligation based on the anatomic distribution of Glisson's capsule was performed for right-sided segmental resections including sectorectomies.[17,18] In some cases temporary control of the hepatic vein outflow was also established prior to division of the liver. The liver tissue was divided with a crushing technique using a Kelly clamp as with wedge resection.

Statistics

Actuarial survival was calculated from the time of the liver resection according to the method of Kaplan-Meier. Prognostic factors for survival were examined by univariate analysis using log-rank and multivariate analyses with a Cox regression model. Variables were compared by means of chi-square analysis using Fischer's exact test (two-sided) or with the independent Student's t test when appropriate. A P value of 0.05 was considered significant.

RESULTS

Clinicopathologic Data

A total of 267 patients with colorectal metastases to the liver underwent either a wedge or segmental hepatectomy at Memorial Sloan-Kettering Cancer Center during the period from October 1985 to October 1998. There were 155 men (58%) and 112 women (42%). The median age was 65 years (range 28 to 87 years). The primary colorectal adenocarcinoma was located in the colon in 196 patients (73%) and in the rectum in 71 (27%). At the time of the initial colorectal resection, 160 patients (60%) had metastases to regional lymph nodes and 60 (22%) had synchronous liver metastases. The median disease-free interval from the time of the resection of the primary colorectal tumor to the development of liver metastasis was 12.5 months (range 0 to 82 months). The disease-free interval was less than 1 year in 130 patients (49%). The CEA level exceeded 200 ng/ml in 18 patients (7%) at the time of liver resection. The preoperative clinical risk score was 0 or 1 (out of 5 possible points) in 122 patients (46%).

There were 214 patients (80%) who had a single colorectal liver metastasis and only seven patients had more than three tumors. The median size of the largest metastasis was 3.0 cm (range 0.4 to 19 cm). The greatest dimension of the largest tumor was less than 5 cm in 200 patients (75%). Extrahepatic disease was present in 14 individuals (5%) and included three

Table I. Comparison of the clinicopathologic features of the wedge resection and segmental resection treatment groups (n = 269)

Variable	Wedge resection (n = 119)		Segmental resection (n = 148)		*P* value
	No.	Percent	No.	Percent	
Sex					0.62
Female	52	44	60	41	
Male	67	56	88	59	
Age					0.41
<70 yr	83	70	110	74	
>70 yr	36	30	38	26	
Primary tumor site					0.78
Colon	86	72	110	74	
Rectum	33	28	38	26	
Primary nodal status					0.079
Node negative	55	46	52	35	
Node positive	64	54	96	65	
Disease-free interval					0.014
<12 mo	68	57	62	42	
≥12 mo	51	43	86	58	
Carcinoembryonic antigen					0.81
<200	84	90	101	92	
≥200	9	10	9	8	
Number of tumors					0.88
1	96	81	118	80	
>1	23	19	30	20	
Largest tumor size					0.003
<5	100	84	100	68	
≥5	19	16	48	32	
Extrahepatic disease					0.60
No	111	93	141	95	
Yes	8	7	7	5	
Clinical score[15]					0.54
<2	57	48	65	44	
≥2	62	52	83	56	

with direct extrahepatic extension into fat, two with omental metastasis, two with involved portal/celiac nodes, two with abdominal wall nodules, one with diaphragm invasion, two with pelvic metastasis, one with a retroperitoneal metastasis, and one with ovarian metastasis.

Of the 267 patients, 119 (45%) had a wedge resection of the liver and 148 (55%) had a segmental resection. The comparison between the two treatment groups with respect to clinicopathologic variables is shown in Table I. The sex distribution, age, primary tumor site, primary nodal status, preoperative CEA level, number of liver tumors, and the presence of extrahepatic disease were similar. In the segmental resection group, there were significantly more patients who had a disease-free interval greater than or equal to 1 year (58% vs. 43%, $P = 0.014$) and a tumor size greater than or equal to 5 cm (32% vs. 16%, $P = 0.003$). The clinical risk scores[15] prior to hepatectomy were similar in the two groups.

Treatment

The hepatic resections are listed in Table II. The 148 segmental resections included 43 left lateral segmentectomies, 29 right posterior sectorectomies, 17 caudate resections, nine right anterior sectorectomies, eight segment IV resections, six segment III resections, and one central resection (segments IV, V, and VIII). There were 21 patients (8%) who received an intra-arterial hepatic pump for postoperative chemotherapy, seven of whom were treated with wedge resection.

Table II. Types of liver resection (n = 269)

Liver resection	No.	Percent
Wedge	119	45
Segmental	148	55
Left lateral segmentectomy (II and III)	43	16
Right posterior sectorectomy (VI and VII)	29	11
Caudate (I)	17	6
Right anterior sectorectomy (V and VIII)	9	3
Segment IV	8	3
Segment III	6	2
Other	36	14

Table III. Outcome analysis of wedge vs. segmental liver resection for colorectal metastasis

Variable	All patients (n = 267)	Wedge resection (n = 119)	Segmental resection (n = 148)	*P* value
Mean operative time (min)	195 ± 6	189 ± 10	198 ± 8	0.46
Mean blood loss (ml)	513 ± 50	456 ± 90	531 ± 60	0.48
Median hospital stay (days)	8 (range 1-30)	9 (range 4-26)	8 (range 1-30)	NS
30-day mortality	1 (0.4%)	1 (0.8%)	0	NS
Complications	45 (17%)	15 (13%)	30 (20%)	0.10
Positive margin	22 (8%)	19 (16%)	3 (2%)	<0.001
Survival				0.015
1 year	94%	91%	95%	
3 year	62%	52%	72%	
5 year	43%	37%	49%	
Median (mos)	50	38	55	

NS = not significant.

Surgical Outcome

The mean operative time and the mean blood loss were similar between the wedge resection and segmental resection groups (Table III). The median hospital stay was 9 days after wedge resection and 8 days after segmental resection (P = NS). There was only one operative death in this series, which occurred after a wedge resection. The complication rate was higher in the segmental resection group (20% vs. 13%), but this did not reach statistical significance. In the wedge resection group, there were two patients with pulmonary embolus, one with cardiac arrhythmia, two cases of pneumonia, one enteric fistula, one gastrointestinal hemorrhage, and two patients with ileus or partial small bowel obstruction. In the segmental resection group, there were two patients with pulmonary embolus, one with cardiac arrhythmia, four with intra-abdominal collections, two with pneumonia, one with biliary fistula, and six with ileus or partial small bowel obstruction.

The rate of positive resection margins was significantly higher after wedge resection than with segmentectomy (16% vs. 2%). Only three patients had a positive surgical margin out of the 148 undergoing segmental liver resection. In contrast, 19 patients (16%) had a positive margin after a wedge resection. The rate was even higher in the 23 patients with more than one tumor who underwent a wedge resection as six of them (26%) had a positive margin.

Survival

At a median follow-up of 25 months (range 1 to 140 months) and a mean follow-up of 33 months, there are 132 patients (49%) without evidence of disease, 32 (12%) are alive with disease, 99 (37%) died of disease, and four (2%) died of other causes. There were 59 deaths in the wedge resection group, which represents 50% of the group, and there were 40 deaths in the segmental resection group, which was 27% of that subset. The overall 1-, 3-, and 5-year survival rates were 94%, 62%, and 43%, respectively, and the median was 50 months. The patients who underwent segmental liver resection had sig-

Table IV. Univariate analysis of prognostic factors for survival

Variable	No.	5-year survival (%)	Median survival (mo)	*P* value
Sex				0.29
Female	112	39	45	
Male	155	45	52	
Age				0.14
<70 yr	193	46	52	
>70 yr	74	36	46	
Primary tumor site				0.75
Colon	196	43	49	
Rectum	71	42	53	
Primary nodal status				0.55
Node negative	107	45	53	
Node positive	160	43	46	
Disease-free interval				0.34
<12 mo	130	39	47	
≥12 mo	137	49	56	
Carcinoembryonic antigen				0.0005
<200	185	48	56	
≥200	18	14	27	
Number of tumors				0.12
1	214	47	55	
>1	53	28	42	
Largest tumor size				0.25
<5	200	44	53	
≥5	67	41	41	
Resection margin				0.0002
Negative	245	45	53	
Positive	22	28	22	
Extrahepatic disease				0.0028
No	252	46	53	
Yes	15	18	29	
Type of resection				0.015
Wedge	119	37	38	
Segmental	148	49	55	

nificantly longer survival ($P = 0.015$) (Fig. 1). The median survival after segmental resection was 55 months compared to only 38 months for wedge resection.

Prognostic Factors for Survival

The characteristics of the entire population of 269 patients were analyzed to determine predictors of survival (Table IV). Sex, age, primary tumor site, nodal status of the primary tumor, disease-free interval, number of liver metastases, and the size of liver metastases had no impact on survival. By univariate analysis, survival was significantly decreased with a preoperative CEA level greater than 200 ng/ml ($P = 0.0005$), positive resection margin ($P = 0.0002$), the presence of extrahepatic disease ($P = 0.0028$), and a wedge hepatectomy ($P = 0.015$). CEA level, resection margin, and extrahepatic disease were independent predictors of survival on multivariate analysis (Table V).

Table V. Multivariate analysis of prognostic factors for survival

Variable	Hazard	*P* value
Segmental resection		0.27
Positive margin	2.2	0.011
Extrahepatic disease	3.4	0.0025
Carcinoembryonic antigen >200 ng/ml	2.9	0.0006

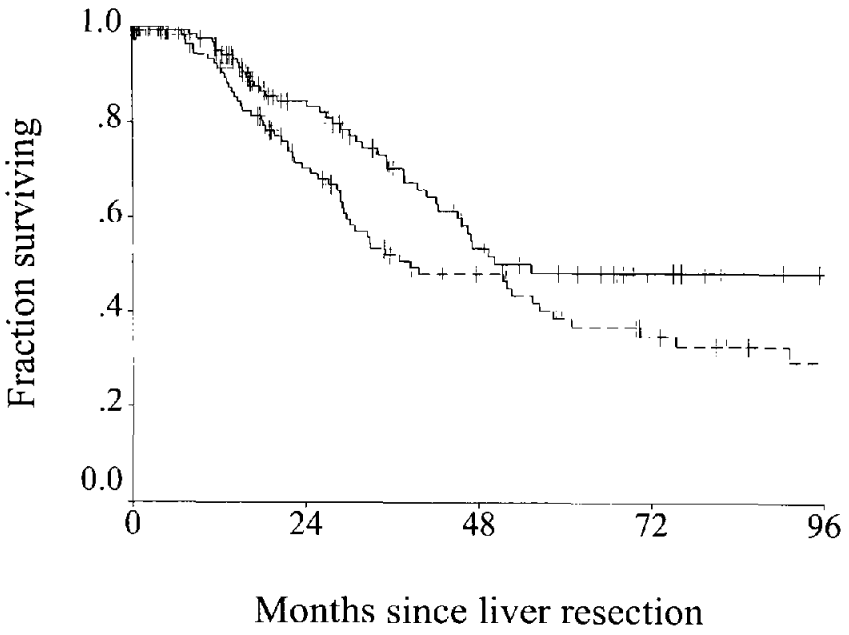

Fig. 1. Disease-specific survival following segmental (n = 119, *solid line*) and wedge (n = 148, *broken line*) liver resection for colorectal liver metastases ($P = 0.015$).

DISCUSSION

Surgical resection offers the only potential of cure for patients with metastatic colorectal adenocarcinoma to the liver. Hepatectomy is now performed with a perioperative mortality rate of less than 5% and results in a 5-year survival rate of 25% to 38%.[2,19-22] Our recent analysis of 1001 consecutive patients who underwent partial hepatectomy for colorectal liver metastases over a 13-year period at Memorial Sloan-Kettering Cancer Center showed a 5-year survival of 37%.[15]

Although it is established that surgical resection provides a survival benefit for patients with liver metastases from colorectal cancer, the optimal margin of resection is not well defined. What is clear is that the goal of resection is to obtain a negative pathologic margin. The presence of a positive margin has been shown to be an independent predictor of survival as it was in this study.[15,21-24] In fact, others have found that patients with a positive surgical margin have similar survival as unresected patients.[1] Several investigators have demonstrated that outcome is improved when the margin is greater than 1 cm.[22-26] This is substantiated by a careful pathologic analysis in which occult invasive cancer was found frequently in normal liver lying within 1 cm of a colorectal liver metastasis.[27] Therefore, in this study, it is likely that the true rate of positive margins was even higher than 16% following wedge resection.

The type of hepatic resection that is performed for a particular patient depends on the extent of the margin that is desired as well as the size, number, and location of the metastatic deposits and the amount of normal parenchyma that may be sacrificed. Hepatectomy may be categorized broadly as extended (trisegmentectomy), lobar, segmental (sublobar), or wedge resection. In the past, wedge resections were commonly performed for colorectal metastases that did not require a lobar resection. However, wedge resection was associated with a 30% rate of positive margins.[1] In addition, it resulted in decreased survival as patients who had a wedge resection did significantly worse compared to those who had an anatomic resection (which at the time comprised mostly lobar resections).[22,28,29] For patients with tumors smaller than 4 cm, the 5-year survival after wedge resection was 31% compared to 52% after anatomic resection.[28] A similar difference existed for tumors larger than 4 cm.

Because of the high rate of positive margins and decreased survival following nonanatomic wedge resections for colorectal liver metastases, we began to favor segmental resection over wedge resection. Our change in philosophy is evident when comparing two time periods during this study. Prior to 1992, there were 81 wedge resections and 53 segmental resections (ratio 1.5 to 1), whereas since 1992 there have been 38 wedge resections and 95 segmentectomies (ratio 1 to 2.5).

Our results with 267 patients in this study show that segmental liver resection for colorectal metastasis is safe and effective. There was no statistical difference in operative time, blood loss, mortality, postoperative complications, or length of hospital stay compared to wedge resection. In fact, there were no perioperative deaths following segmental resection in this series. The principal difference between the two types of resections was the rate of positive margins. There were only three patients out of 148 who had a positive surgical margin after segmentectomy. In contrast, there was a 16% rate of positive margins with wedge resection. The margin status was an independent determinant of survival. Although this is not a randomized study, it is not surprising that segmental resection provides better tumor clearance. This may be partly a result of resecting more liver parenchyma, but more important it is due to a more controlled dissection carried out along anatomic planes, which preserves the margin during the performance of the resection.

Wedge hepatectomy for the treatment of colorectal metastases should not be completely abandoned. It still has a role for the removal of very small peripheral lesions in which adequate margins are easily attained. Cryo-assisted wedge resection may be useful when preservation of normal liver precludes segmentectomy.[30] Wedge resection may also be used to confirm the diagnosis of metastasis in patients with unresectable liver disease.

Segmental resection is therefore superior to wedge resection as an oncologic operation for colorectal liver metastases since it markedly reduces the rate of positive margins and is associated with longer survival. Segmentectomy is now preferred to wedge resection and should be widely adopted.

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Inflammatory Cytokines Alter Human Gallbladder Epithelial Cell Absorption/Secretion

Robert V. Rege, M.D.

Gallbladder inflammation is an early feature of gallstone formation in animal models. The inflammatory response is associated with increases in myeloperoxidase and interleukin (IL)-1 activities in the gallbladder wall. The present studies were designed to determine whether inflammatory cytokines directly affect gallbladder epithelial cell absorptive function. Studies were performed using cultured human gallbladder epithelial cells derived from a well-differentiated gallbladder carcinoma. Confluent monolayers were exposed to interleukin-1 (IL-1α), IL-1α plus its specific receptor inhibitor IL-1ra, tumor necrosis factor (TNF-α), lipopolysaccharide, or prostaglandin E_2. Unidirectional sodium and chloride fluxes were measured and used to calculate net ion fluxes. Compared to control monolayers, lipopolysaccharide, prostaglandin E_2, IL-1α, and TNF-α decreased mucosal-to-serosal and net sodium and chloride fluxes and increased serosal-to-mucosal movement of sodium and unmeasured ions. The effects of IL-1α were completely inhibited by its specific receptor antagonist IL-1ra. Similar to the proinflammatory agents lipopolysaccharide and prostaglandin E_2, the inflammatory cytokines IL-1α and TNF-α directly affected gallbladder epithelial cell absorptive function. Because normal gallbladder absorptive function is protective against gallstone formation, alterations in absorptive function due to inflammation in the gallbladder wall may play a role in gallstone pathogenesis. (J GASTROINTEST SURG 2000;4:185-192.)

KEY WORDS: Gallbladder absorption, gallbladder secretion, inflammatory cytokines, interleukin-1, tumor necrosis factor

Gallbladder epithelial cells actively absorb sodium chloride, bicarbonate, and water-concentrating bile 5- to 10-fold and acidify common duct bile from a pH of 7.4 or greater to a pH of 6.0 in fully concentrated gallbladder bile. Classically, absorption was attributed to a carrier-mediated, passive, apical sodium chloride cotransport driven by an energy-dependent, basolateral Na/K-ATPase.[1,2] However, more recent studies support parallel Na^+/H^+ and $Cl^-/HCO3^-$ ion exchange across the apical membrane of the gallbladder cell.[3-6] Under some circumstances including inflammation, the gallbladder secretes, rather than absorbs, fluid.[7-10] Gallbladder secretion may be mediated via prostaglandins and leukotrienes,[7-9,11-14] intracellular calcium,[15] cystic fibrosis transmembrane conductance regulator (CTFR),[16] and nitric oxide.[17]

Inflammation in the gallbladder wall is an early feature of animal models of gallstones.[18-23] Inflammation is noted as soon as crystals appear and before stones form in the gallbladder lumen. We have shown that inflammation during gallstone formation is associated with increased myeloperoxidase and interleukin (IL)-1 activity in the gallbladder wall.[24-27] Moreover, recent work in our laboratory demonstrates that human cultured gallbladder cells express messenger RNA for the IL-1 and TNF-α receptors IL-1 RI, TNF-α RI, and TNF-α RII (unpublished observations). Therefore we explored the effects of the inflammatory cytokines IL-1α and TNF-α on gallbladder epithelial cell absorption.

METHODS

Studies were performed using cultured human gallbladder (HCGF) cells derived from a well-differentiated human gallbladder carcinoma.[28] They maintain

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characteristics of normal gallbladder epithelial cells, especially the ability to absorb sodium chloride, bicarbonate, and water in Ussing chambers in vitro via an amiloride-dependent process. They also exhibit cytokeratin markers consistent with biliary epithelial cells, develop polar orientation with clear apical villi, maintain a normal potential difference and tissue resistance in a Ussing chamber, and secrete mucus when stimulated with prostaglandin. HCGE cells grow to confluent monolayers in 6 to 9 days on polycarbonate matrices and are easily mounted in the Ussing chamber where they maintain a potential difference of approximately 1 to 2 mV across the monolayer. Similar to gallbladder membranes, the monolayers of gallbladder epithelial cells exhibit amiloride-dependent absorption and acidify mucosal solutions. Cells have been maintained in Richmond, Virginia for the past 15 years and maintain characteristics of gallbladder epithelial cells until passage 110.[28] Cells employed for the present study ranged from passage 80 to 87.

Cells were subcultured from frozen cells in Eagle's minimum essential medium (no L-glutamine) plus 10% fetal calf serum. Penicillin/streptomycin and sodium pyruvate were added. Cells (1×10^6) were then placed into wells with polycarbonate matrices placed at the bottom. Confluent monolayers were mounted in Ussing chambers. Monolayer confluency was verified visually and by the ability of the monolayer to maintain a potential difference in the Ussing chamber.

HCGE monolayers were mounted between two halves of a Plexiglas Ussing chamber with insert adapters filled on both sides with Krebs-Ringer's solution (control monolayers) or Krebs-Ringer's plus test substances. Monolayers were continually gassed with 95% oxygen–5% carbon dioxide. After the potential difference and short-circuit current (Isc) stabilized, cytokines or control solutions (saline-solubilizing cytokines) were added to the mucosal chamber. Potential difference and Isc resistance were monitored every 10 minutes during the experiment. After a 10- to 20-minute period of stabilization, net sodium (J^{Na}) or Cl^- (J^{Cl}) fluxes were determined by measuring mucosal-to-serosal (Jms) and serosal-to-mucosal (Jsm) fluxes of trace amounts of ^{22}Na (across one monolayer) and ^{35}Cl (across a paired monolayer). Jms and Jsm were measured during short circuit conditions (voltage clamp) by adding radioisotope to one chamber and measuring appearance in the opposite chamber every 10 minutes for three periods. Between measurement of unidirectional fluxes, chambers were emptied, washed gently, and refilled with solutions. Flux in the opposite direction was then measured by a similar technique. Net flux was calculated as the difference between mucosal-to-serosal and serosal-to-mucosal fluxes using the following formula: Jnet = Jms + Jsm.

By charge and mass balance at steady state, the number of cations and anions transported across the monolayer must be equal. Therefore $J^{Na} + J^{R+} = J^{Cl} + J^{R-}$, where J^{R+} and J^{R-} are other cations and anions transported across the monolayer. J^{R+} and J^{R-} represent essentially hydrogen and bicarbonate ions in this system. It is not possible to differentiate between hydrogen ion transported in one direction and bicarbonate ion transported in the opposite direction. The equation above can then be rewritten as follows: $0 = J^{Na} - J^{Cl} + J^{R}$, where J^{R} equals the net flux of all other ions.

Control monolayers were exposed to Krebs-Ringer's solution alone. A set of control tests was performed in parallel with each test group to ensure that the monolayers were transporting normally. Positive control tests were performed by exposing monolayers to either 50 or 100 μg/ml of the proinflammatory agent lipopolysaccharide (from *Escherichia coli*; Sigma Chemical Co., St. Louis, Mo.) or to 0.1 mmol/L 16,16-dimethylprostaglandin E_2 (99% pure by thin-layer chromatography; Sigma Chemical). Test monolayers were exposed to either 10, 50, or 125 pg/ml of human recombinant interleukin-1 (IL-1α; R & D Systems, Minneapolis, Minn.), 125 pg/ml IL-1α in the presence of 250 to 1000 pg/ml of human recombinant IL-1 receptor antagonist (IL-1ra; R & D Systems), or 10 and 125 pg/ml of human recombinant tumor necrosis factor (TNF-α; R & D Systems). Data were calculated as the mean of five experiments and is reported as mean ± standard error of the mean (SEM). Differences between test groups and specific control groups were statistically analyzed using Student's *t* test. Differences between multiple groups were analyzed by means of analysis of variance.

RESULTS

Net sodium chloride transport occurred across all monolayers from the mucosal to the serosal reservoirs of the Ussing chambers. As is the convention, ion flux in this direction is depicted as positive numbers. Potential differences ranged from −0.8 to −1.4 millivolts and Isc from 101 to 215 milliamperes. Net sodium (8.1 to 10.7 mEq/hr-cm^2) and chloride (8.1 to 11.1 mEq/hr-cm^2) fluxes across control monolayers varied from day to day, but on any given day, net sodium and chloride fluxes were similar to each other. Therefore, the calculated net flux of other ions, J^R, was close to 0, ranging from only 0.4 to −0.4 mEq/hr-cm^2. As expected, net mucosal-to-serosal fluxes of sodium and chloride were largely due to high

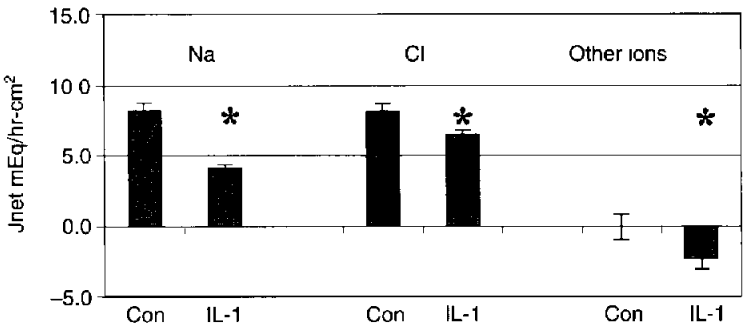


Fig. 1. Effects of IL-1α on the flux of sodium and chloride ions. In each case, ion flux for a given ion is compared to its paired control *(Con)* value. Note the significant decreases in net sodium and chloride flux compared to control values. The two bars on the right side of the graph illustrate the calculated effects of IL-1α on "other" ions. * = statistical significance at $P < 0.05$.

values of Jms and low Jsm (see control values in Figs. 1-5) These results are consistent with active sodium chloride absorption by HCGE monolayers and are similar to values of net sodium and chloride flux observed with intact gallbladder membranes placed into Ussing chambers.[1-6,29]

Effects of Interleukin-1α on HCGE Monolayer Ion Absorption/Secretion

Monolayers were first exposed to three doses of IL-1α, 10, 50, and 125 pg/ml, and net sodium flux was examined. Net sodium flux decreased from 8.5 ± 0.6 mg/hr-cm² in control specimens to 7.6 ± 0.5, 6.0 ± 0.5 ($P < 0.07$), and 5.1 ± 0.8 ($P < 0.005$) for 10, 50, and 125 pg/ml of IL-1, respectively. More extensive experiments examine both sodium and chloride fluxes were then performed at 125 pg/ml.

Fig. 1 illustrates the effects of 125 pg/ml IL-1α on sodium and chloride movement across HCGE cell monolayers. IL-1α significantly ($P < 0.002$) decreased net sodium flux from 8.1 ± 0.6 mEq/hr-cm² in control monolayers to 4.0 ± 0.3 mEq/hr-cm² in the test monolayers. Likewise net chloride flux decreased from 8.1 ± 0.5 to 6.4 ± 0.4 mEq/hr-cm² ($P < 0.02$). The calculated flux of other ions J^R increased significantly ($P < 0.04$) from −0.03 ± 0.9 mEq/hr-cm² to −2.4 ± 0.5 mEq/hr-cm² (net serosal-to-mucosal movement).

Examination of unidirectional fluxes in Fig. 2 further delineates alterations in sodium and chloride fluxes. Il-1α caused a significant ($P < 0.005$) decrease in mucosal-to-serosal flux from 10.9 ± 0.9 to 9.2 ± 0.7 mEq/hr-cm². Chloride flux also decreased from 11.5 ± 0.7 to 9.5 ± 0.8 mEq/hr-cm², but this decrease in chloride flux did not reach statistical significance (Fig. 2, *A*). The increase in serosal-to-mucosal movement of sodium in monolayers treated with IL-1α (−2.8 ± 0.3 vs. −5.2 ± 0.6 mEq/hr-cm²) was even more significant ($P < 0.004$), while serosal-to-mucosal chloride flux (−3.3 ± 0.3 and −3.0 ± 0.5 mEq/hr-cm², respectively) did not change (Fig. 2, *B*).

Monolayers were exposed to IL-1α in the absence and presence of its specific receptor antagonist IL-1ra to determine whether the effects of IL-1α were specific and receptor mediated. Results are shown in Fig. 3. Note that 125 pg/ml IL-1α alone again decreased net sodium absorption significantly ($P < 0.001$). The addition of 250 pg/ml IL-1ra increased net sodium flux slightly but not significantly. The 500 and 100 pg/ml doses of IL-1ra, however, completely reversed the effects of Il-1α so that the net sodium fluxes of 9.2 ± 0.5 and 8.3 ± 0.6 mEq/hr-cm² were not statistically different from the control value of 9.3 ± 0.8 mEq/hr-cm² and were statistically higher ($P < 0.001$ and 0.002, respectively) than the value (4.7 ± 0.3 mEq/hr-cm²) observed in the presence of IL-1α alone.

Effects of Tumor Necrosis Factor on HCGE Cell Monolayer Ion Absorption/Secretion

TNF-α was first examined at two doses, 10 and 125 pg/ml. Net sodium flux decreased from 10.2 ± 0.8 mEq/hr-cm² in control solutions to 7.7 ± 0.7 ($P < 0.03$) and 4.7 ± 0.3 ($P < 0.0001$) mEq/hr-cm² for 10 and 125 pg/ml, respectively. More in-depth studies were then performed with 125 pg/ml and TNF-α.

Similar to IL-1α, 125 pg/ml TNF-α (Fig. 4) significantly decreased net sodium absorption from 10.2 ± 0.8 to 4.7 ± 0.3 mEq/hr-cm² ($P < 0.000$) and net chloride secretion from 9.7 ± 0.9 to 6.3 ± 0.2 mEq/hr-cm² ($P < 0.002$). The calculated net flux of all other ions changed from 0.4 ± 1.1 to −1.6 ± 0.4

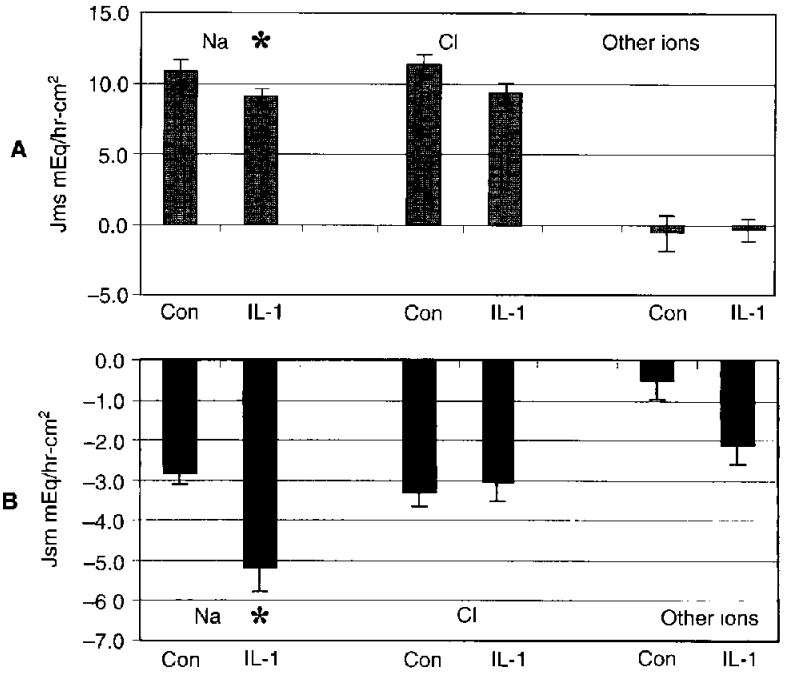


Fig. 2. Unidirectional fluxes of ions across the monolayers. Sodium *(Na)* flux is depicted on the left, chloride *(Cl)* in the middle, and "other" ions on the right. **A,** Interleukin-1 significantly decreased mucosal-to-serosal flux of sodium and chloride, but "other" ions were not significantly different. **B,** Serosal-to-mucosal sodium, but not chloride, flux was significantly decreased. Thus there was a significant increase in the serosal-to-mucosal flux of "other" (unmeasured) ions. * = Statistical significance at $P < 0.05$.

Fig. 3. Effects of IL-1ra. IL-1α again significantly decreased net sodium flux. When increasing doses of IL-1ra from 250 to 1000 pg/ml were added, there was a progressive inhibition of IL-1α effects. Sodium flux was not statistically different from control *(Con)* values at the two highest doses of IL-1ra. * = Statistical significance at $P < 0.05$.

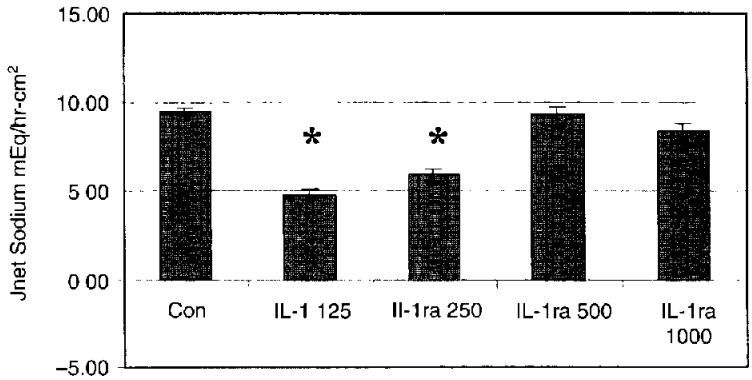
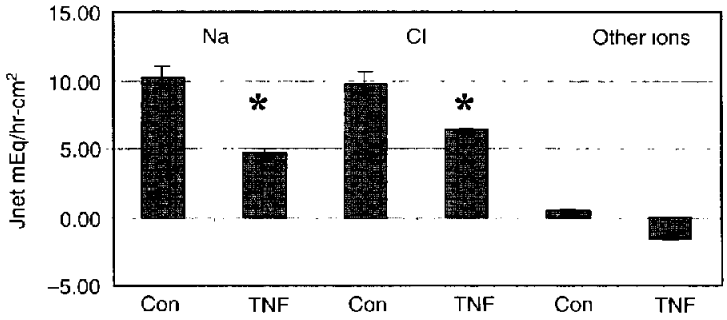


Fig. 4. Effects of TNF-α (125 pg/ml). Sodium *(Na)* flux is depicted by the two bars to the left, chloride *(Cl)* flux in the middle, and calculated fluxes of "other" ions at the right. TNF-α caused effects similar to those of IL-1α, but significant changes in the flux of other ions were not seen. * = Statistical significance at $P < 0.05$.



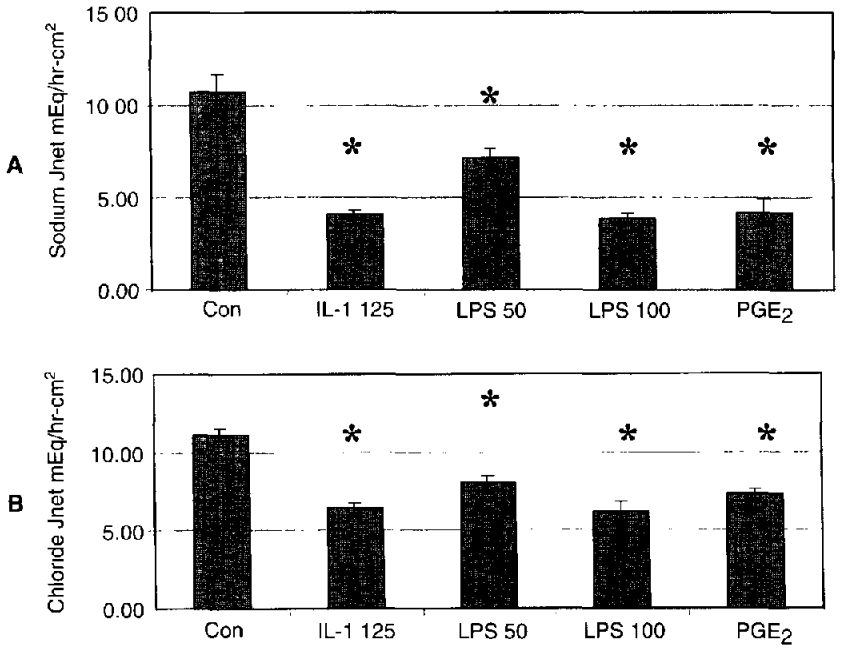


Fig. 5. Effects of IL-1α (125 pg/ml) compared to effects of lipopolysaccharide *(LPS)* (50 and 100 μg/ml) and prostaglandin E_2 *(PGE_2)* (0.1 mmol/L). **A,** Note that like IL-1α, LPS and PGE_2 decreased net sodium flux. The effects of LPS increased with the increased dose. **B,** Likewise LPS and PGE_2 decreased chloride flux to levels comparable to those observed with IL-1.

mEq/hr-cm², but these changes were not statistically significant. The changes in net ion flux were due to a significant ($P < 0.0001$) decrease in mucosal-to-serosal sodium flux and significant ($P < 0.0001$ and $P < 0.01$) increases in serosal-to-mucosal movement of sodium and chloride across the monolayer.

Effects of Lipopolysaccharide and Prostaglandin E_2 on HCGE Cell Ion Absorption/Secretion

The effects of 50 and 100 μg/ml lipopolysaccharide on HCGE monolayers is illustrated in Fig. 5. The effects of 125 pg/ml IL-1α are again included for comparison. Lipopolysaccharide at these doses significantly decreased net sodium 10.7 ± 1.0 to 7.1 ± 0.5 ($P < 0.006$) and 3.8 ± 0.3 ($P < 0.0001$) mEq/hr-cm², respectively (Fig. 5, *A*). Significant and progressive decreases in chloride flux also occurred from 11.1 ± 0.4 in control specimens to 8.1 ± 0.5 ($P < 0.001$) and 6.1 ± 0.7 ($P < 0.001$) mEq/hr-cm² (Fig. 5, *B*). The decreases in sodium and chloride fluxes with 100 μg/ml lipopolysaccharide were similar to those observed with IL-1α. Changes in net flux were due to significant decreases in mucosal-to-serosal sodium movement from 13.4 ± 0.8 mEq/hr-cm² to 10.6 ± 0.5 ($P < 0.02$) and 9.4 ± 0.5 ($P < 0.002$) mEq/hr-cm², decreases in mucosal-to-serosal chloride movement from 13.9 ± 0.6 mEq/hr-cm² to 10.9 ± 0.6 ($P < 0.004$) and 10.3 ± 0.5 ($P < 0.004$) mEq/hr-cm², and increases in serosal-to-mucosal movement of sodium from 2.7 ± 0.3 mEq/hr-cm² to 3.6 ± 0.3 ($P < 0.04$) and 5.6 ± 0.3 ($P < 0.001$) mEq/hr-cm². The net flux of other ions changed from −0.4 ± 0.5 mEq/hr-cm² to −1.0 ± 0.5 (not significant) and −2.3 ± 0.4 ($P < 0.002$) mEq/hr-cm².

Similar changes were observed with prostaglandin E_2. Prostaglandin E_2 decreased net sodium flux significantly from 10.2 ± 0.8 mEq/hr-cm² to 4.1 ± 0.7 ($P < 0.002$) mEq/hr-cm² and net chloride flux from 9.7 ± 0.7 to 7.3 ± 0.4 ($P < 0.01$) mEq/hr-cm². The observed changes resulted from significant decreases in mucosal-to-serosal sodium movement from 12.9 ± 1.0 mEq/hr-cm² to 8.6 ± 0.4 ($P < 0.001$) and increases in serosal-to-mucosal movement of sodium from 2.8 ± 0.3 to 4.4 ± 0.4 ($P < 0.007$) mEq/hr-cm². Again, the net flux of other ions changed significantly from 0.4 ± 0.1 to −3.17 ± 0.3 ($P < 0.002$) mEq/hr-cm². These results indicate that lipopolysaccharide and prostaglandin E_2 decrease active absorption of sodium chloride and increase serosal-to-mucosal movement of sodium and other ions.

DISCUSSION

The experiments presented herein were designed to determine whether inflammation and inflammatory cytokines in the gallbladder wall are capable of directly altering gallbladder absorption and secretion. Inflammatory cytokines also likely affect gallbladder epithelial function indirectly via stimulation of gallbladder nerves, white blood cells, and fibrocytes in the gallbladder wall, but these mechanisms were purposely not studied here. Cultured gallbladder epithelial cell monolayers are pure epithelial cells eliminating potential sources of cytokines other than IL-1α and TNF-α, chemokines, eicosinoids, and neural transmitters with the potential to alter gallbladder absorptive function.

HCGE cells have a potential disadvantage. They were derived from a well-differentiated gallbladder carcinoma and the potential exists that these cells may respond differently from normal gallbladder cells. As discussed in Methods, HCGE cells have been extensively studied and maintain characteristics and function indistinguishable from normal cells. Because suitable monolayers of normal cells are not available for study in the Ussing chamber, it was thought that these cells were adequate for probing the relationship between inflammation and gallbladder function.

The possibility remains that IL-1α and TNF-α stimulated HCGE cells to produce prostaglandins, which in turn altered sodium chloride transport by an autocrine effect. In separate experiments in our laboratory, increases in prostaglandin E_1 and E_2 concentrations in the media did not occur when HCGE cells were exposed to IL-1α and TNF-α. Moreover, the baseline levels of prostaglandin E_1 and E_2 were a magnitude lower (μmol/L vs. mmol/L) than the amount required for stimulation of the membranes in Ussing chambers. Therefore we believe that the effects of IL-1α and TNF-α observed in the present studies represent direct effects on gallbladder epithelial cells. A direct, receptor-mediated effect of IL-1α is also supported by the inhibition of IL-1α effects by its specific receptor inhibitor IL-1ra.

The composition of fluid in each compartment of the Ussing chamber was the same and experiments were performed under short circuit conditions to eliminate chemical and electrical gradients across the monolayer. Therefore net ion flux across the monolayer represents active transport of ions. IL-1α and TNF-α decreased net sodium chloride flux across HCGE cell monolayers and alterations in ion transport resulted largely from a decrease in mucosal-to-serosal sodium transport. In addition, there were significant increases in serosal-to-mucosal movement of sodium and other ions. However, the effects of IL-1α and TNF-α were not exactly the same. Although IL-1α increased movement of sodium and other ions into the lumen, TNF-α increased serosal-to-mucosal movement of sodium, chloride and, to a lesser extent, "other" ions.

The methodology employed here is standard but is not capable of determining which transporters facilitated the process. Current theory holds that under normal transport conditions, sodium chloride absorption is driven by energy-dependent, basolateral Na/K-ATPase.[1,2] Because nearly equal amounts of sodium and chloride are transported in vitro by the gallbladder, it was long thought that a single Na^+/Cl^- cotransporter was responsible for entry of sodium and chloride into the cell across the apical membrane. However, more recent evidence suggests that entry of sodium and chloride ions into the cell is due to passive parallel Na^+/H^+ and $Cl^-/HCO3^-$ ion exchangers.[3-6] Stimulation of HCGE cells by IL-1α and TNF-α caused not only a decrease in net sodium chloride absorption but also a significant disparity between transport of the two ions. This was demonstrated in our data as significant transport of "other" ions. The experimental conditions do not allow us to determine whether the observed changes in ion movement involved only alterations in function of the parallel antiport exchangers or if other transporters or ion channels were activated during exposure to the cytokines. The identity of "other" ions involved in the cytokine-induced alterations in gallbladder absorption/secretion are also not apparent from these studies, although considering the composition of Krebs-Ringer solution, the "other" ions are likely bicarbonate and/or hydrogen ions.

The changes in ion transport caused by IL-1α and TNF-α were very similar to those observed when monolayers were exposed to the proinflammatory agents lipopolysaccharide and prostaglandin E_2. These results indicate that the gallbladder epithelial cell's response to inflammation, whether mediated through cytokines, eicosinoids, or lipopolysaccharide, is similar. Elsewhere in the gastrointestinal tract, inflammation causes several "protective" effects including mucus secretion, secretion of fluid (to wash away noxious and infectious agents?), and increases in motility to clear the lumen of the responsible agents. We have shown that gallbladder epithelial cells respond to inflammation in a similar manner. Crystalline components of stones induce an inflammatory cascade in normal guinea pig gallbladder and inflammation alters mucus secretion and gallbladder absorption in vivo.[24-27] The present studies show that changes in absorption may in part be mediated through direct effects of cytokines on gallbladder epithelial cells.

Although chronic inflammation and fibrosis result from the presence of gallstones, inflammation is also an early event in gallstone formation that actually precedes the appearance of gallstones.[27] In fact, we have shown that early gallstone inflammation can be induced by biliary crystals present in bile before gallstones form.[24-27]

Inflammation may be of critical importance in the cascade of events leading to gallstone formation and gallstone-induced gallbladder disease. Although decreased absorption by an inflamed gallbladder might be protective by diluting and eliminating noxious luminal agents, we have shown that normal concentration and acidification of bile is protective against gallstone formation and growth.[6] Therefore early inflammation noted in the gallbladder wall may contribute to the formation and growth of gallstones by interfering with normal gallbladder function. After gallstones form, inflammation and fibrosis may occur. These inflammatory changes are thought to be due to intermittent obstruction of the cystic duct and/or chronic irritation of the mucosa by the gallstones. The role played by inflammation in the symptoms that patients have from their gallstones and late gallbladder dysfunction is not understood. Further studies are required to determine the exact events that occur in vivo and the mechanisms that lead to cytokine-induced alterations in gallbladder cell absorption and secretion.

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Discussion

Dr. H. Pitt (Milwaukee, Wis.). A recent study has suggested that exposing pancreatic cells to lipopolysaccharide is a model for acalculous acute cholecystitis. Are your findings perhaps more related to what occurs in the gallbladder during sepsis than to gallstone formation?

Dr. Rege. My interest has focused on gallstone-induced inflammation, but inflammation from any source would cause these changes. This has wider significance and would apply to patients in critical care units who have episodes of sepsis. It would also apply to patients with symptomatic cholecystitis who have bacteria in their bile.

Dr. B. Svanvik (Linkoping, Sweden). We studied the effects of prostaglandins many years ago. Adding prostaglandins to the mucosa will affect the transport of fluid and electrolytes in the same way as you have seen in the Ussing chamber.

Dr. Rege. Looking at your in vivo experiments in cats, it is clear that these substances can affect other cells in the gallbladder wall such as neurons, fibroblasts, or white cells. I think that these cells can produce substances that cause changes in absorption and secretion. The significance of this work, which is slightly different from what both of us have studied in vivo, is that it shows that these substances have direct effects on gallbladder epithelial cells, since no other cells are present in our monolayers. These cells also have receptors for cytokines.

Role of Cytosolic Phospholipase A_2 in Cytokine-Stimulated Prostaglandin Release by Human Gallbladder Cells

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Eicosanoids are involved in gallbladder inflammation, epithelial water transport, and mucous secretion. Phospholipase A_2 enzymes liberate arachidonic acid from membrane phospholipids for the synthesis of eicosanoids. The purpose of this study was to determine the effect of selective cytoplasmic and secretory phospholipase A_2 inhibitors on basal and stimulated arachidonic acid and prostaglandin E_2 release in gallbladder cells. Western immunoblotting was employed to evaluate both cytosolic and secretory phospholipase A_2 enzymes in human gallbladder cells. Cells were incubated for 22 hours with ^{3}H-labeled arachidonic acid. Arachidonic acid and prostaglandin E_2 release was then measured in the supernate after 2 hours of exposure to human interleukin-1β, alone or after pretreatment for 1 hour with the inhibitors. Unstimulated gallbladder cells express both 85 kDa cytosolic and 14 kDa secretory phospholipase A_2. The 85 kDa phospholipase A_2 was induced by interleukin-1β, whereas there was no apparent change in secretory phospholipase A_2 enzyme concentrations. Both the secretory phospholipase A_2 inhibitor *p*-bromophenylacyl bromide and the cytosolic phospholipase A_2 inhibitor arachidonyl trifluoromethyl ketone decreased basal and interleukin-1β–stimulated arachidonic acid release. In contrast, only inhibition of cytosolic phospholipase A_2 led to a decrease in interleukin-1β–stimulated prostaglandin E_2 release. Basal and interleukin-1β–stimulated arachidonic acid release appears to be the result of the activity of both cytosolic and secretory phospholipase A_2. Interleukin-1β–stimulated prostaglandin E_2 release appears to be dependent on the activity of cytosolic phospholipase A_2. (J GASTROINTEST SURG 2000;4:193-200.)

KEY WORDS: Gallbladder, phospholipase A_2, interleukin-1β, prostaglandins

Prostanoids have been implicated in the development of gallbladder disease. Cholecystitis has been associated with increased gallbladder prostaglandin E_2 (PGE_2) production.[1] PGE_2 has been shown to stimulate the production of mucin and other glycoproteins from gallbladder epithelium,[2,3] and an increase in synthesis of PGE_2 has also been associated with decreased biliary motility.[4] Arachidonic acid, the precursor molecule for eicosanoids, has also been implicated in the development of gallbladder disease. Elevated levels of free arachidonate have been found in the bile of patients with cholesterol crystals.[5] The addition of arachidonate has been shown to increase mucin production in gallbladder explants.[6]

The phospholipase A_2 (PLA_2) family of proteins is thought to provide the primary mechanism for the generation of free arachidonic acid. The PLA_2 proteins catalyze the hydrolysis of fatty acyl bond at the sn-2 position of phospholipids, leading to the liberation of free fatty acids, including arachidonic acid, and the generation of lysophospholipids.[7-11] Increased PLA_2 activity has also been associated with gallbladder disease. In ischemic models of acalculous cholecystitis, PLA_2 activity is increased.[12] Elevated levels of PLA_2 enzymes have been demonstrated in the bile of patients with gallstones.[13] In addition, treatment of patients with gallstone disease with ursodeoxycholate has been shown to decrease biliary levels of PLA_2 proteins.[14]

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Numerous enzymes have been demonstrated to display PLA_2 activity. Two groups of enzymes are thought to be responsible for most of the free arachidonic acid generated for subsequent formation of eicosanoids. One group consists of two proteins categorized as secretory PLA_2 enzymes ($sPLA_2$). The $sPLA_2$ enzymes consist of the closely related group II and group V PLA_2 enzymes. Both $sPLA_2$ enzymes are 14 kDa proteins that are secreted.[15,16] The $sPLA_2$ enzymes require millimolar concentrations of Ca^{2+} and bind to cell surface proteoglycans[17-19] to catalyze the release of fatty acids from the sn-2 position of cell membrane phospholipids. The $sPLA_2$ enzymes do not display substrate specificity as they liberate linolenic acid and arachidonic acid from cell membranes.[16] These $sPLA_2$ enzymes have clearly been demonstrated to be important for prostanoid formation in a number of cell systems.[20-22]

An additional PLA_2 enzyme that is important in prostaglandin production is the group IV cytosolic PLA_2 ($cPLA_2$) enzyme. The $cPLA_2$ enzyme is an 85 kDa intracellular protein that shares no sequence homology with the $sPLA_2$ enzymes.[23,24] In contrast to the $sPLA_2$ enzymes, $cPLA_2$ enzyme requires only micromolar concentrations of Ca^{2+} and selectively hydrolyzes phospholipids with arachidonyl residues at the sn-2 position.[25-27] A rise in intracellular calcium leads to translocation of the $cPLA_2$ enzyme to the nuclear membrane and the endoplasmic reticulum.[28] This translocation, as well as phosphorylation of the molecule, appears to be necessary for subsequent release of free arachidonic acid.[29-31] The $cPLA_2$ enzyme has also been clearly demonstrated to play an important role in prostanoid formation in some cell systems.[32,33]

The aim of the current study was to determine the role of $sPLA_2$ and $cPLA_2$ enzyme activity in the release of free arachidonic acid and PGE_2 in human gallbladder epithelial cells. We utilized *p*-bromophenylacyl bromide (BPB) to inhibit $sPLA_2$ enzyme activity. BPB alkylates a histidine residue on the $sPLA_2$ enzymes, which leads to inactivation.[34] BPB appears to have little effect on $cPLA_2$ enzyme activity.[35] Arachidonyl trifluoromethyl ketone ($AACOCF_3$) was employed to inhibit $cPLA_2$ enzyme activity. $AACOCF_3$ is a potent competitive inhibitor of $cPLA_2$ enzyme and appears to have little effect on the $sPLA_2$ enzymes.[36-38] Interleukin-1β (IL-1β) was chosen as a stimulant for this study as it stimulates PLA_2 enzyme activity in numerous cell lines.[39-41] Interleukin-1 also appears to be a relevant mediator of gallbladder inflammation in some animal models of gallbladder disease. Lipopolysaccharide has been shown to produce gallbladder inflammation and this response may be mediated in part by interleukin-1.[42] This stimulus may also be relevant in human disease, as interleukin-1 levels have been shown to be increased in diseased gallbladders.[43]

METHOD AND MATERIALS

Cell Culture

A human gallbladder epithelium cancer cell line was used for these experiments. The origin of the cells, cell freezing and thawing, and cell culture methods have been previously described.[44] Cells were maintained in Dulbecco's minimum essential medium (DMEM) with 10% fetal bovine serum, 1% nonessential amino acids (Sigma, St. Louis, Mo.), 5 ml/L antibiotics (10,000 units/ml penicillin, 10 mg/ml streptomycin), 1% sodium pyruvate (100 mmol/L), 5 ml/L glutamine (200 mmol/L), 2.5 ml/L amphotericin B, and 1 ml/L bovine insulin (100 μg/30ml). Cells were incubated at 37° C in 95% air: 5% carbon dioxide.

Western Blot Analysis

For Western blotting of PLA_2 proteins, cells were plated onto 12-well culture plates (Costar, Cambridge, Mass.) in 1 ml of DMEM. At 70% confluence the medium was changed. Those wells designated serum free received 1 ml of DMEM, whereas the others received 1 ml of DMEM with 10% fetal bovine serum. After 24 hours, cells were washed with Krebs-Ringer's–bicarbonate buffer (KRB; Sigma), and appropriate wells were exposed for 2 hours to IL-1β (Merck DuPont Laboratories, Glenolden, Pa.). The cells were scraped from the plates and centrifuged. The cell pellets were lysed for 10 minutes at room temperature with 0.9% NaCL, 20 mmol/L Tris-HCl, 20 mmol/L β-glycerophosphate, 0.1% sodium dodecyl sulfate (SDS), 10 mg/ml apoprotinin, 10 μg/ml leupeptin, and 10 μg/ml pepstatin A (all from Sigma). To remove particulate matter these samples were centrifuged for 20 minutes at 10,000*g* and the resultant supernate was collected. Cell protein content was determined[45] and 18 μg protein samples were electrophoresed on a 7.5% SDS-polyacrylamide gel. Each $cPLA_2$ protein gel was run with a molecular weight standard (Santa Cruz Biotechnology, Santa Cruz, Calif.), and the $sPLA_2$ protein gels were run with a human synovial $sPLA_2$ protein standard (Cayman, Ann Arbor, Mich.). Samples were transferred to nitrocellulose, and the membranes were exposed to either the $sPLA_2$ protein antibody (Upstate Biotechnology, Lake Placid, N.Y.) or the $cPLA_2$ protein antibody (Santa Cruz Biotechnology) for 2 hours.

The cPLA antibody is a rabbit polyclonal antibody produced by immunization with the amino terminal domain, amino acids 1 to 216, of human $cPLA_2$. It uniformly identified the human cPLA standard produced in *Escherichia coli* as the 35 kDa polyhistidine-tagged fusion protein (Santa Cruz Biotechnology). It does not cross-react with other phospholipases (Santa Cruz Biotechnology). The $sPLA_2$ antibody is a mouse-produced antibody with the immunogen being purified

human 14kDa $sPLA_2$. It does not react with other PLA_2 enzymes isolated from pancreas or snake venom, and the antibody will detect 0.1 ng $sPLA_2$ per 5 ng protein (Upstate Biotechnology). The antibody uniformly cross-reacted with the human 14kDa $sPLA_2$ standard.

The membranes were then treated for 30 minutes with peroxidase-conjugated antimouse immunoglobulin G (Santa Cruz Biotechnology) and then exposed using the enhanced chemiluminescence method and detection reagent (Amersham, Arlington Heights, Ill.) for 1 minute, after which they were exposed for 10 minutes to Kodak X-O Mat x-ray film (Kodak, Rochester, N.Y.) to detect the proteins recognized by the antibodies. Changes in PLA_2 enzyme content were estimated visually.

Measurement of Extracellular Arachidonic Acid Release

Cells were grown to confluence on 24-well cell culture plates (Costar). Cells were then labeled with 0.5 μCi/ml of ^{3}H-labeled arachidonic acid (Merck Dupont, Glenolden, Pa.) for 20 hours. Wells were washed three times at 5-minute intervals with Hank's buffered saline solution containing 0.1 mg/ml of bovine serum albumin (HBSS/BSA) to remove any unincorporated ^{3}H-labeled arachidonic acid. The cells were exposed to IL-1β in HBSS/BSA for 2 hours. Wells designated to receive the $cPLA_2$ enzyme inhibitor $AACOCF_3$ (Cayman) or the $sPLA_2$ enzyme inhibitor BPB (Sigma) were treated with these agents in HBSS/BSA for 1 hour prior to exposure to IL-1β. At the end of the treatment period, supernates were removed, cleared of detached cells by centrifugation, and assayed for radioactivity by liquid scintillation counting.

Measurement of Prostaglandin E_2 Release

Cells were grown to confluence on 24-well cell culture plates. The cells were then washed with KRB and exposed for 2 hours to IL-1β (50 units/ml) in KRB. Again, wells designated to receive the inhibitors were treated with these agents 1 hour prior to the addition of IL-1β. Supernates were collected and PGE_2 concentrations measured in duplicate by a competitive enzyme assay, which uses an acetylcholinesterase tracer (Cayman) as described previously.[14] The cells were washed and frozen for subsequent cell protein measurement. Cells were thawed, scraped into 1N NaOH, and protein content was determined using bovine serum albumin as a standard. PGE_2 release was expressed as picograms of PGE_2 per milligram of cell protein.

Statistical Analysis

All statistical analyses were performed by means of analysis of variance. Differences in mean values for each group were determined by Fisher's least significant difference. Significance was defined as any difference with a p value of less than 0.05.

RESULTS

Western Blot Analysis of Cytosolic and Secretory Phospholipase A

Significant levels of $sPLA_2$ and $cPLA_2$ enzymes were expressed in unstimulated gallbladder cells (Fig. 1). The $sPLA_2$ enzyme migrated at approximately the 14 kDa molecular weight size to the same point as the human synovial $sPLA_2$ standard. The 85 kDa $cPLA_2$ enzyme was identified at approximately 110 kDa as determined by molecular weight markers. Although the $cPLA_2$ enzyme has been determined to be an 85 kDa protein by analysis of its messengerRNA, it typically migrates to the 110 kDa position in SDS.[23,26] The addition of IL-1β did not appear to induce production of $sPLA_2$ enzyme; however, there did appear to be some induction of $cPLA_2$ enzyme by IL-1β as compared to cells incubated in serum-containing medium. Depriving the gallbladder cells of serum for 24 hours did not appear to affect basal $sPLA_2$ or $cPLA_2$ production.

Extracellular Arachidonic Acid Release

Both the $cPLA_2$ enzyme inhibitor $AACOCF_3$ and the $sPLA_2$ enzyme inhibitor BPB decreased basal extracellular arachidonic acid release (Table I). The addition of IL-1β increased extracellular arachidonic acid release in a concentration-dependent fashion. $AACOCF_3$ significantly decreased arachidonic acid

Table I. ^{3}H-labeled arachidonic acid release by gallbladder cells

	No treatment	IL-1β (10 units/ml)	IL-1β (50 units/ml)
Buffer	2506 ± 96	3341 ± 243*	3762 ± 270*
$AACOCF_3$ (10 μmol/L)	1440 ± 90*	2799 ± 68†	2929 ± 114†
BPB (10 μmol/L)	1570 ± 223*	3046 ± 110	3152 ± 137†

n = 7 to 8 reported as CPM/ml
*P < 0.05 vs. buffer alone
†p < 0.05 vs. IL-1β alone

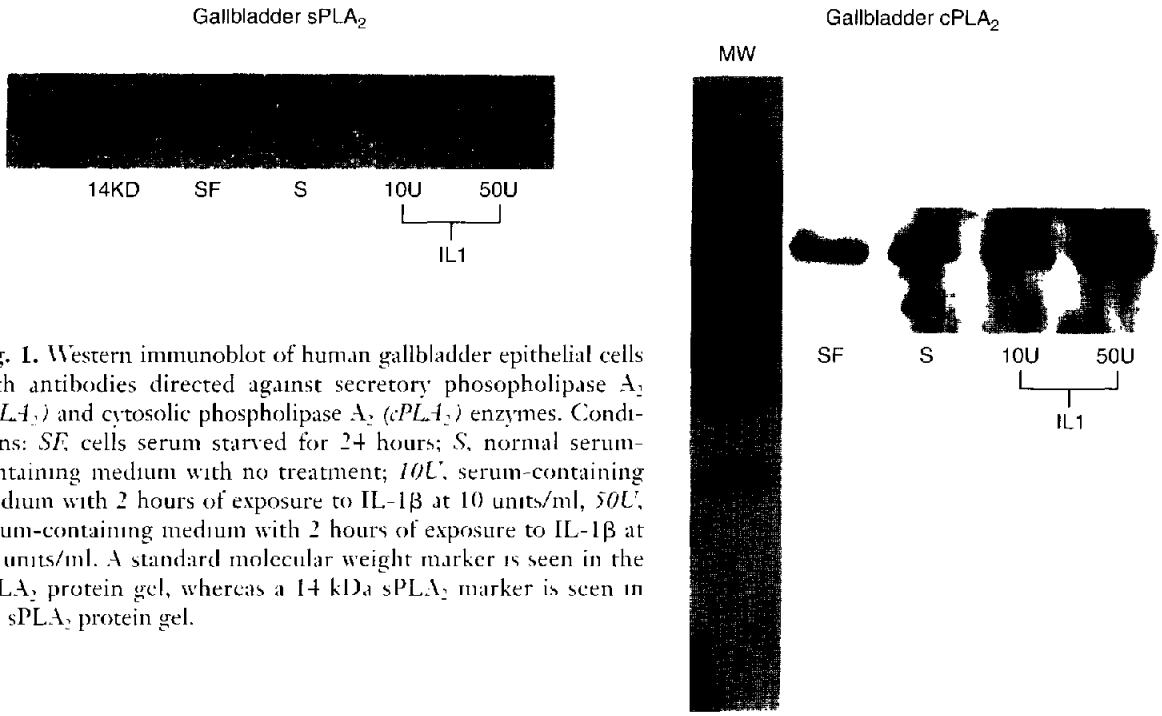

Fig. 1. Western immunoblot of human gallbladder epithelial cells with antibodies directed against secretory phosopholipase A_2 *(sPLA$_2$)* and cytosolic phospholipase A_2 *(cPLA$_2$)* enzymes. Conditions: *SF*, cells serum starved for 24 hours; *S*, normal serum-containing medium with no treatment; *10U*, serum-containing medium with 2 hours of exposure to IL-1β at 10 units/ml, *50U*, serum-containing medium with 2 hours of exposure to IL-1β at 50 units/ml. A standard molecular weight marker is seen in the $cPLA_2$ protein gel, whereas a 14 kDa $sPLA_2$ marker is seen in the $sPLA_2$ protein gel.

Table II. Prostaglandin E_2 release by gallbladder cells

	No treatment	IL-1β (50 units/ml)
Buffer	646 ± 112	5483 ± 432*
AACOCF$_3$ (10 μmol/L)	901 ± 70	993 ± 80†
BPB (10 μmol/L)	673 ± 117	6733 + 499†

*P <0.05 vs. buffer alone.
†p <0.05 vs. IL-1β alone.
n = 8 reported as picograms of PGE_2 per milligram of cell protein.

release in response to 10 units/ml and 50 units/ml of IL-1β. BPB also was able to decrease IL-1β–stimulated arachidonic acid release, and this effect reached statistical significance at an IL-1β concentration of 50 units/ml.

Prostaglandin E_2 Release

Treating unstimulated gallbladder cells with AACOCF$_3$ or BPB did not significantly change basal PGE_2 levels (Table II). The addition of 50 units/ml of 1L-1β significantly stimulated PGE_2 release in gallbladder cells. Treatment of the cells with AACOCF$_3$ decreased IL-1β–stimulated PGE_2 release to nearly basal levels. In contrast, treatment of cells with BPB significantly increased IL-1β–stimulated PGE_2 release above that of cells treated with IL-1β alone.

DISCUSSION

Inflammatory gallbladder disease continues to account for considerable morbidity and mortality. Prostanoids mediate many inflammatory processes and may contribute to gallbladder pathophysiology. The availability of free arachidonic acid is a limiting factor in the production of prostaglandins, and the generation of free arachidonic acid is dependent on PLA_2 enzyme activity in most systems that have been examined.

Interleukin-1β is an important mediator of inflammation. The relationship between IL-1β, the various PLA_2 enzymes, and prostanoid production appears to vary between cell lines. IL-1β has been shown to stimulate PGE_2 production through a $cPLA_2$ enzyme–dependent process in human lung fibroblasts,[46] human amniotic WISH cells,[47] human lung adenocar-

cinoma cells,[48] and murine osteoblasts.[49,50] In contrast, IL-1β has been demonstrated to stimulate PGE_2 production through an $sPLA_2$ enzyme–dependent mechanism in rabbit chondrocytes,[51] rat glomerular mesangial cells,[52,53] and rat fibroblasts.[54] In rat ovarian cells IL-1β has been shown to increase expression of both $sPLA_2$ and $cPLA_2$ enzymes.[55,56] It is apparent that IL-1β is capable of stimulating release of arachidonic acid through both PLA_2 enzyme pathways, and that the relative importance of these pathways may depend on the metabolic pathways of the target cell.

In our study we have demonstrated that the addition of the $cPLA_2$ enzyme inhibitor $AACOCF_3$ or the $sPLA_2$ enzyme inhibitor BPB significantly decreased basal arachidonic acid release from human gallbladder epithelial cells (see Table I). We have also demonstrated that the addition of IL-1β produces an increase in extracellular release of arachidonic acid in a dose-dependent manner and that this IL-1β–stimulated release of arachidonic acid can be decreased by the addition of BPB or $AACOCF_3$. Therefore basal and IL-1β–induced release of arachidonic acid into the extracellular medium involves the activity of both $cPLA_2$ and $sPLA_2$ enzymes.

In contrast to their effects on basal arachidonic acid release, neither inhibitor significantly affected basal PGE_2 release by the gallbladder cells (see Table II). The addition of IL-1β at a concentration of 50 units/ml increased PGE_2 release eightfold over control conditions. Treatment of the cells with the $cPLA_2$ enzyme inhibitor $AACOCF_3$ completely blocked the IL-1β–stimulating effects on PGE_2 production. In contrast, inhibition of $sPLA_2$ enzyme with BPB not only failed to block IL-1β–stimulated PGE_2 release, it actually appeared to slightly increase prostanoid release from the stimulated cells. Therefore IL-1β–stimulated PGE_2 release from human gallbladder epithelium is mediated through $cPLA_2$ enzyme activity.

If both $cPLA_2$ and $sPLA_2$ enzyme activity is involved in extracellular release of arachidonic acid, why does only inhibition of $cPLA_2$ enzyme activity lead to decreased PGE_2 release? One possibility is that the activation of $sPLA_2$ enzyme depends on the prior activity of the $cPLA_2$ enzyme. Dennis et al.[57–59] have demonstrated that lippopolysaccharide/platelet-activating factor stimulation of PGE_2 release from the macrophage-like $P388D_1$ cell line involves an early activation of $cPLA_2$ enzyme that is necessary for the subsequent activation of $sPLA_2$ enzyme. Although activation of $cPLA_2$ enzyme was required for maximal PGE_2 synthesis, it was actually the activity of $sPLA_2$ enzyme that provided most of the arachidonic acid for the cyclooxygenase enzymes in those studies. An interrelationship between $cPLA_2$ and $sPLA_2$ enzyme activity has also been found in Chinese hamster ovarian cells,[60] rat peritoneal macrophages,[61] and murine mast cells.[62] If $sPLA_2$ enzyme activation also requires prior $cPLA_2$ enzyme activity in gallbladder epithelial cells, then we may be directly inhibiting the IL-1β stimulation of $sPLA_2$ enzyme by treatment with BPB and indirectly inhibiting the activation of $sPLA_2$ enzyme with $AACOCF_3$. Therefore, even though it is possible that $sPLA_2$ enzyme may be primarily responsible for extracellular arachidonic acid release, both inhibitors could decrease this activity.

It is also possible that specific metabolic pathways exist for prostanoid production. The arachidonic acid pool produced by the $cPLA_2$ enzyme may be isolated intracellularly and specifically available for PGE_2 production. We have previously demonstrated in other cell lines that specific stimuli produce a prostanoid species through a specific cyclooxygenase pathway, and another prostanoid species through a different cyclooxygenase pathway.[63] Further studies are needed using the specific PLA_2 inhibitors to ascertain the effect of these agents on the formation of other prostanoid species as well as the effects of these agents in altering cytokine-produced gallbladder mucosal cell injury and mitogenesis.

Since our assay measured the release of extracellular arachidonic acid, and there was poor correlation between this effect and PGE_2 release, the relevance of the extracellular pool of arachidonic acid in these cells must be questioned. The $sPLA_2$ enzymes are in fact secreted enzymes that bind to the cell membrane to exert their effect.[16–18] Although numerous researchers have presented evidence that extracellular arachidonic acid is taken up and processed to prostanoids in some cell lines,[18,20–21] this may not hold true for all cell types. The extracellular arachidonic acid pool may be irrelevant to gallbladder epithelial prostanoid production. This certainly does not preclude the possibility that the extracellular arachidonic acid may be important for processing to eicosanoids by neighboring cells in an in vivo setting. In contrast to $sPLA_2$ enzymes, $cPLA_2$ is an intracellular enzyme that exerts its effect on the endoplasmic reticulum and the nuclear membrane.[27,28] Thus $cPLA_2$ enzyme may be the primary provider of arachidonic acid to cyclooxygenase enzymes in gallbladder epithelial cytoplasm. Additional research to determine the effect of IL-1β and these inhibitors on intracellular levels of free arachidonic acid will be required to evaluate these possibilities.

Further complicating the evaluation of the roles of $sPLA_2$s and $cPLA_2$ enzymes in cell culture is the presence of other enzymes with PLA_2 activity. An intracellular 80 kDa calcium-independent enzyme with

PLA_2 activity (iPLA$_2$–group VI) has been described.[64] Although this enzyme appears to function primarily as a housekeeping enzyme involved in phospholipid remodeling,[65] its PLA_2 enzyme activity has been shown to be inhibited by $AACOCF_3$.[66,67] It is therefore possible that some of the effects that we have ascribed to intracellular cPLA$_2$ enzyme activity may in fact be mediated through the intracellular iPLA$_2$ enzyme. Unfortunately no inhibitor specific for the iPLA$_2$ enzyme has been developed. In addition, another 14 kDa sPLA$_2$ (group X) enzyme has recently been isolated from human fetal lung.[68] The role of this enzyme in prostanoid production is unknown.

CONCLUSION

Interleukin-1β is able to stimulate both free arachidonic acid release and PGE_2 production from human gallbladder epithelial cells. The IL-1β–stimulated release of extracellular arachidonic acid appears to involve both sPLA$_2$ and cPLA$_2$ enzyme activity. In contrast, IL-1β stimulated PGE_2 release is only blocked by the inhibition of cPLA$_2$ enzyme activity. Based on the results of the present study and a previous report, human gallbladder mucosal cells produce PGE_2 in response to inflammatory stimuli primarily through the activity of the 85 kDa cytoplasmic PLA_2 and inducible cyclooxygenase 2 enzymes.[44]

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Predicting the Need for Colectomy in Pediatric Patients With Ulcerative Colitis

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Total colectomy is curative for ulcerative colitis. However, many pediatric patients are medically managed and may not require surgery. There are currently no available criteria to identify children who will benefit from early colectomy. The purpose of this review was to identify criteria associated with the need for colectomy. A 15-year review of patients at a major pediatric center with biopsy-proved ulcerative colitis was conducted. Age at the time of the first symptom, diagnosis, and surgery were recorded as well as steroid dependence, site of disease, extraintestinal manifestations, and family history. Seventy-three patients ranging in age from 1 to 18 years were identified. Thirty-seven patients (50.1%) required total colectomy before the age of 18. The average patient age at the time of the first documented symptom was 11.3 ± 0.5 years. Among patients who were steroid dependent and had pancolitis, 73% required colectomy. Patients with these factors failed medical management 77% (27 of 35) of the time, and colectomy was performed within 3 years of diagnosis. The combination of steroid dependence and pancolitis was associated with an increased need for colectomy. In pediatric patients with these factors, early colectomy may limit the need to endure prolonged courses of medications and the disability allied with this disease. (J GASTROINTEST SURG 2000;4:201-206.)

KEY WORDS: Pediatric, ulcerative colitis, colectomy

Ulcerative colitis (UC) is a significant problem in the pediatric population afflicting 2 of 100,000 children.[1] In contrast with adults, permanent disability in the form of attenuated linear growth may occur as a side effect of the underlying condition or the medications designed to treat this disease.[2,3] In addition, pediatric patients often have a more acute and severe course of UC (90%) when compared with adult patients (50%).[4,5]

Total abdominal colectomy, rectal mucosectomy, and restorative ileal pouch–anal anastomosis is curative and has evolved as the standard definitive therapy. The percentage of pediatric patients with UC who ultimately require operative therapy has been reported to range from 15% to nearly 50%.[1,6-9] This variability is likely related to variations in medical management,[4] as well as the local success of the various continence-preserving surgical procedures.[10,11]

The ability to identify pediatric patients early after the diagnosis of UC and predict those who are most likely to require operative therapy is a meaningful goal. Earlier identification of these high-risk patients may avoid the deleterious effects of long-term chronic disease and medical therapy on growth. In addition, more timely surgical intervention in these select patients may facilitate a more rapid return to a more normal quality of life. The purpose of this study therefore was to review the experience at a major center for both the medical and surgical management of pediatric UC and carefully characterize both groups of patients in order to identify criteria associated with the need for colectomy.

METHODS

A retrospective review of patients with pathologically proved UC treated at the Children's Hospital Medical Center (Cincinnati, Ohio) from 1975 through 1990 was carried out. All patients were followed until the time of surgery, until they reached the

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age of 18 years, or to the end of the available medical record.

Patients with Crohn's disease or indeterminate colitis were excluded from the study. Additional exclusion criteria encompassed patients with incomplete medical records or patients for whom the original diagnosis and most of the pre- or post-operative care were performed at an outside institution. The extent of disease was determined either by colonoscopy or by direct analysis of the colon specimen following surgery. For this study, disease location was classified as rectal, rectal with left colon involvement, or pancolitis.

Standard demographic data including sex, race, family history, and patient age at the time of the first symptom and at the time of diagnosis were recorded. At our institution, patients received consistent medical therapy from a single group of pediatric gastroenterologists. Generally the first line of treatment was sulfasalazine (a 5-ASA product), followed by the use of topical steroids and then systemic steroids. During the study period, few patients at our institution received additional immunosuppressive medications. In this review, steroid dependence was defined as the requirement of daily steroids for longer than 3 months during a year-long period.

Indications for surgery during the study period included the following: (1) microscopic evidence of dysplasia; (2) hemorrhage; (3) intractability; (4) steroid dependence; (5) intolerance or complications of steroid therapy; and (6) significant growth retardation.[12] When these indications were met, a total abdominal colectomy, rectal mucosectomy, and ileal pouch–anal anastomosis were performed as has been previously described.[13]

Differences in the proportion of patients with clinical characteristics or outcomes were assessed by use of the chi-square or Fisher's exact test. Summary statistics for continuously distributed variables are expressed as mean ± standard error of the mean (SEM). A significance level of 0.05 was used.

RESULTS

A total of 250 charts from our colitis database were reviewed and 73 patients with UC were included in the study cohort. The average patient age at the time of first documented symptom was 11.3 ± 0.5 years (range 1.2 to 18 years). Twenty-four of the patients (33%) analyzed were less than 10 years of age. As noted in previous studies, the age at diagnosis did not correlate with the need for surgery.[6]

Medical management failed to successfully control the disease and colectomy was required in 37 patients (50.1%). The demographic characteristics of the patients requiring colectomy and those treated medically are presented in Table I. As shown in this table, the groups were closely matched; however, there is a slight male predominance in the colectomy group as has previously been appreciated among both pediatric and adult patients.[7,14] Extraintestinal manifestations of UC were present in 15 children (21%). Most of these manifestations were in the form of arthralgias, and there was no correlation with disease severity or the need for surgery. Additionally, an overall 12% positive family history of inflammatory bowel disease was appreciated. Again, there was no correlation between this history and the success or failure of medical management.

Table I. Demographics of study population

	Medical management	Colectomy
No. of patients	36	37
Mean age ± SEM (yr)	10.4 ± 0.8	11.9 ± 0.7
Age range (yr)	2.2-17.9	1.2-17.9
Male sex	14 (39%)	21 (57%)
Ethnicity		
White	27 (75%)	31 (84%)
Black	6 (17%)	2 (5%)
Hispanic	0 (0%)	1 (3%)
Other	3 (8%)	3 (8%)
Family history	4 (11%)	5 (14%)

Table II. Medical treatment

Medication	Medical management	Colectomy	Total
Sulfasalazine	31 (86%)	26 (70%)	57 (78%)
Topical steroids	11 (31%)	21 (57%)	32 (44%)
Steroid (dependent)	15 (42%)	32 (86%)	47 (64%)
Cyclosporine	0 (0%)	1 (3%)	1 (1%)
6-Mercaptopurine	3 (8%)	1 (3%)	4 (5%)

The average length of follow-up was 5.4 ± 0.6 years (range 0.4 to 13.8 years) for patients whose disease could be controlled with medication. Nine patients (25%) were followed for less than 3 years, 17 (47%) were followed for 3 to 7 years, and 10 (28%) were followed for more than 7 years. Alternatively, those patients who required a colectomy averaged 2.7 ± 0.4 years (range 0.07 to 13.7 years) from diagnosis until definitive surgery. Most of the patients were operated on for chronic nonremitting symptoms, and no patients in this study had dysplasia or neoplasia as their indication for surgery. Sixty-five percent of patients required surgery within 3 years of

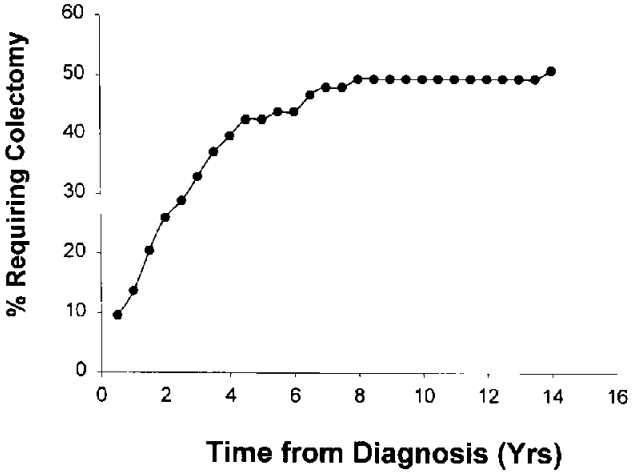

Fig. 1. Percentage of all patients reviewed (n = 73) requiring colectomy vs. time in years from initial diagnosis of ulcerative colitis.

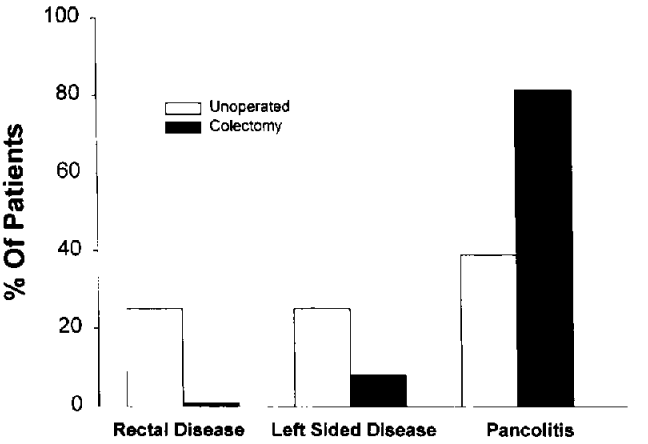

Fig. 2. Percentage of patients unoperated (medically managed; n = 36) or requiring colectomy (total abdominal colectomy, rectal mucosectomy, ileal pouch–anal anastomosis; n = 37). Disease distribution was divided into rectal disease, left-sided disease (rectal and left colon), and pancolitis.

diagnosis, and there was a relative leveling out of the number of children requiring colectomy beyond 4 years from the time of diagnosis (Fig. 1). There was no difference in the need for surgery based on age at diagnosis since a similar number of patients diagnosed before or after the age of 10 years required surgery.

The medical treatment among groups did not vary significantly (Table II). However, substantially more patients requiring colectomy were steroid dependent and required the use of systemic steroids. During the study period, only rarely did patients receive additional immunosuppressive medications.

The next variable compared between the two groups was the pattern of disease distribution. As shown in Fig. 2, the largest proportion of patients in both groups had pancolitis. Despite this increase in both groups, the most substantial increase in the percentage of pancolitis was among those requiring surgery. Less than 10% of patients requiring colectomy had a disease distribution other than pancolitis (Fig. 3).

Finally, when the percentage of patients who were steroid dependent and had pancolitis was examined, a significant difference was appreciated between patients requiring colectomy and those successfully managed with medication. Seventy-three percent of patients who eventually required colectomy compared to only 22% of those managed with medication had the combination of steroid dependence and pancolitis ($P < 0.05$ by chi-square analysis). Additionally, of the 35 patients with pancolitis and steroid dependence, 77% failed medical management and eventually underwent definitive surgery for disease control.

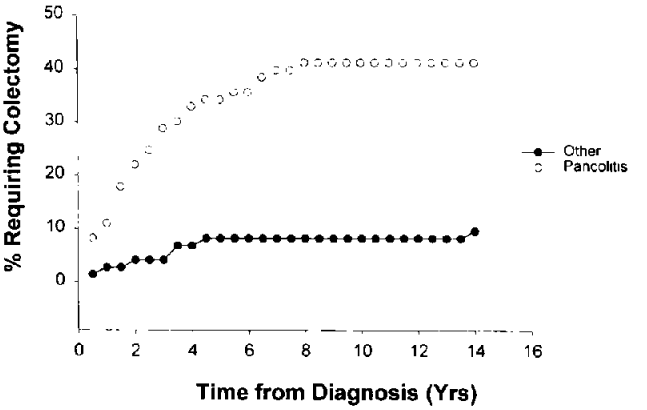

Fig. 3. Percentage of all patients reviewed (n = 73) requiring colectomy based on disease distribution, either pancolonic or other (rectal or left-sided disease), plotted over time from diagnosis in years.

DISCUSSION

The ability to predict the clinical course of UC in pediatric patients provides the clinician with the ability to more appropriately manage and counsel patients and their families. This review provides data for predicting which children are likely to fail medical management and therefore benefit from early operative therapy. This study retrospectively demonstrates that half of the children diagnosed with UC eventually required surgical management within 3 years of diagnosis. More significantly, a majority of these pa-

tients were steroid dependent and had pancolonic disease leading to medical failure.

Growth failure may complicate pediatric UC. Although chronic steroid use is common with severe UC, a direct association between steroids and growth failure is difficult to demonstrate. The goals of treatment of UC in children should therefore be aimed at controlling the disease while avoiding significant steroid toxicity to allow for normal growth. Unfortunately these goals cannot always be achieved with medical management alone. Recently Nicholls et al.[15] have shown that in 72% (13 of 18) of children undergoing colectomy for UC, catch-up growth or cessation of decreasing linear growth could be achieved.[15] In this same report, the importance of performing surgery prior to epiphyseal closure and the pubertal growth spurt was emphasized. Given the retrospective nature of the current study, detailed analysis of growth failure was not possible.

Early identification of pediatric patients with UC who require surgical treatment may also reduce the toxicity associated with long-term steroid or immunosuppressive therapy. Many pediatric and adult patients with UC endure chronic steroid use and may suffer many of the complications associated with such treatment including increased infections, hypertension, osteoporosis, nephrolithiasis, and cutaneous striae. Additionally, although steroid use may induce disease remission, the occurrence of steroid side effects has been reported to necessitate surgical treatment in more than 70% of such patients.[16]

Although there is increasing interest in alternative immunosuppressive therapy for patients with inflammatory bowel disease, only a few patients in this retrospective study received such treatment. This factor may account, in part, for our rate of colectomy. It is perhaps too early to predict the long-term effects that these drugs will have on altering the need for surgery. In two separate studies, cyclosporine was shown to induce clinical disease remission, but the high rate of relapse led to the need for colectomy in more than 70% of the patients, many within 1 year.[17,18] In another study, the addition of either azathioprine or 6-mercaptopurine resulted in a maintained disease remission in four of eight patients.[19] Recently it has been reported that both 6-mercaptopurine and azathioprine alone have been successful in reducing the need for steroids; however, that study failed to comment on the ultimate need for surgical intervention.[20] In a survey of pediatric gastroenterologists, the greatest concerns regarding the use of immunosuppressive therapy for inflammatory bowel disease were bone marrow and immune suppression and the increased risk of malignancy.[21] Infectious complications of cyclosporine including fatal *Pneumocystis carinii* pneumonia and disseminated aspergillosis have been reported.[22] Additionally, 46% of pediatric patients treated with azathioprine or 6-mercaptopurine had an adverse effect, with 18% requiring cessation of treatment secondary to hypersensitivity or infectious side effects.[23]

An additional concern with long-term medical management of UC is the risk of neoplasm. Although no patients in this study had a colon cancer identified, the risk after 20 years of disease in pediatric patients has been demonstrated in one study to be 8.4%, the majority of whom have a history of pancolitis.[6] In addition, the potential for increased extraintestinal neoplasms secondary to immunosuppressive therapy needs to be considered.[24] The risks of long-term medical management therefore need to be carefully considered in the pediatric population with expected long-term survival.

In this review, medical management failed to provide disease control in 37 children (50.1%), resulting in the need for definitive operative therapy. Sixty percent of patients reviewed had involvement of the entire colon, confirming the more extensive disease pattern seen in the pediatric population.[6,7,25,26] This increased extent of colonic involvement indicates UC that may be more resistant to medical management.[27] When the patients requiring surgery were specifically examined, pancolitis was noted in more than 80%, confirming earlier reports that more than 60% of children with pancolitis eventually require surgery for disease control.[7] This review used disease extent at the time of surgery or most proximally identified site of disease during any follow-up examination. In a review of 171 pediatric patients, Hyams et al.[9] evaluated patients based on the extent of disease at diagnosis. In that study, there was no significant difference based on the site of colon involvement and the need for surgical management.

In addition to the high percentage of medical failures associated with pancolitis, this study revealed an association between steroid dependence and the need for ultimate operative intervention. For this review, we defined steroid dependence as the requirement of daily steroids for longer than 3 months during a yearlong period. These patients generally required steroids to suppress symptoms and withdrawal of steroids led to a clinical recurrence. Similar definitions of chronic steroid use have been employed in other reviews.[9] Eighty-six percent of children requiring colectomy were steroid dependent at the time of surgery, implying increased disease severity. In an earlier review, disease severity at the time of diagnosis was also indicative of a greater 5-year risk of requiring surgical management.[9] In the current series, 77% of patients with both steroid dependence and pancolonic

involvement eventually required surgery. Given the fact that this is a retrospective study and that after the age of 18 years patients were transferred to an adult gastroenterologist, it is probable that we underestimated the number of children who eventually required surgery. This patient population therefore represents a high-risk group of children with UC who could not be controlled with medical management.

In this review, nearly half of the children who required surgery were identified within 3 years of diagnosis. The early identification of patients requiring colectomy is important, as Fonkalsrud[5] has discovered that there is an increased risk of complications from the surgical management of UC as the duration of medical management and the dosage of medication increase. Additionally, delayed surgical therapy results in increased morbidity, hospital stay, and cost, and a decreased functional result.[8] Hospital charges for a patient undergoing a curative total colectomy with ileal pouch–anal anastomosis have been reported to be approximately $18,000.[28] Alternatively, the average annual cost for medical management of UC is nearly $1500.[29] The cost is further increased in children with severe disease requiring hospitalizations and more expensive medical therapy. It therefore becomes evident that in children with pancolitis and steroid dependence, earlier surgical management may reduce health care costs.

Prompt surgical treatment results in good to excellent long-term functional outcome in 85% to 95% of children.[5,11] Quality-of-life assessments in adults with UC managed medically or surgically have revealed equal or improved quality following surgery.[30,31] In a review of pediatric patients following surgical management of UC, quality-of-life assessments revealed ratings equal to those of normal healthy age-matched counterparts.[32]

This retrospective review therefore provides further insight for identifying which children will require surgical management of their UC. As demonstrated here, 50% of pediatric patients followed will fail medical management and require proctocolectomy. The unique characteristic of this subgroup of patients is that the vast majority were both steroid dependent and had pancolitis leading to surgery. This information may be useful to inform these patients of the high likelihood that definitive operative therapy will be required. Earlier surgical intervention in these children can help prevent the long-term physical and psychological effects of this chronic disease, as well as remove any potential cancer risk. In view of this study, future studies designed to look at the outcome of early surgical management vs. immunosuppressant medical therapy for steroid-dependent or steroid refractory UC in pediatric patients are necessary.

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The Jejunal Pouch as a Rectal Substitute After Proctocolectomy

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Our hypothesis was that a jejunal pouch used as a rectal substitute after proctocolectomy would slow enteric transit, delay defecation, and decrease stool frequency compared to an ileal pouch so used. Twelve dogs underwent proctocolectomy; six had a jejunal pouch–distal rectal anastomosis and six had an ileal pouch–distal rectal anastomosis. After recovery, postprandial mouth-to-anus transit was slower in jejunal pouch dogs (253 ± 18 minutes [mean ± SEM]) than in ileal pouch dogs (112 ± 7.9 minutes; $P < 0.05$). Moreover, jejunal pouch dogs passed only 4.1 ± 0.3 stools during the 12 hours after eating, whereas ileal pouch dogs passed 6.3 ± 0.9 stools ($P < 0.05$). The mean frequency of proximal ileal pacesetter potentials after feeding was less in jejunal pouch dogs (12 ± 0.4 cycles/min) than in ileal pouch dogs (16 ± 0.3 counts/min; $P = 0.01$), and jejunal pouches had more action potentials (jejunal = 82% ± 4.3% of pacesetter potentials had action potentials, ileal = 61% ± 3.0%; $P < 0.05$). In contrast, gastric emptying and pouch motility, emptying, mucosal integrity, and bacteriologic and histologic properties were similar in the two groups of dogs. We concluded that the jejunal pouch operation slowed enteric transit, delayed defecation, and decreased postprandial stooling compared to the ileal pouch operation. (J GASTROINTEST SURG 2000;4:207-216.)

KEY WORDS: Jejunal pouch, ileal pouch, pouch-anal anastomosis, ulcerative colitis, restorative proctocolectomy

The ileal pouch–anal canal anastomosis has become the operation of choice for many patients undergoing proctocolectomy for chronic ulcerative colitis.[1] The operation involves complete excision of the diseased large intestine, yet transanal defecation and reasonable fecal continence are maintained, and an incontinent ileostomy is avoided. There are drawbacks to the operation, however. After 8 years, patients still have a mean of six stools per 24 hours.[2] Moreover, approximately half of the patients have persistent nocturnal fecal spotting, and almost half have had at least one bout of inflammation in the pouch.[1,2] In addition, using the ileum to make a pouch may impair the function of the ileum, decreasing its ability to absorb electrolytes, vitamin B_{12}, and bile salts, and to secrete hormones such as peptide YY.[3]

We wondered whether a jejunal pouch might not be a better rectal substitute than an ileal pouch. A jejunal pouch, with its larger diameter, should have a lower basal interpouch pressure, be more distensible, and still have cleansing contractions during fasting, thus reducing stasis and the possibility of "pouchitis." Moreover, a jejunal pouch might be inherently more resistant to inflammatory changes than an ileal pouch. The ileum shows chronic ileitis in approximately 10% of patients with chronic ulcerative colitis, whereas the jejunum is seldom involved. Also, not using the ileum for a pouch should preserve its absorptive and hormonal functions better than when it is used as a pouch. Indeed, we found in past tests that although most patients with ileal pouches have maintained an effective ileal hormonal "brake" on small bowel transit in the postprandial period, some have not.[4]

Other investigators have used "straight" segments of jejunum as rectal substitutes in humans,[5,6] but we hypothesized that a jejunal pouch would be better. A

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pouch would provide a larger reservoir. Tests on human cadavers have shown that a jejunal pouch can be constructed in humans so as to allow the pouch to reach to the anal canal for an anastomosis.[7]

We tested some of these hypotheses recently using in situ jejunal pouches as rectal substitutes in dogs.[8] We found that these pouches did indeed have a lower intrapouch pressure and were more distensible, and they did have more cleansing interdigestive migrating motor complexes than ileal pouches. Drawbacks appeared with these in situ pouches, however. Fistulas developed between the proximal jejunum and the pouches. The dogs experienced postoperative diarrhea and weight loss. Clearly, a better type of jejunal pouch was required.

In the present experiments we created a new type of jejunal pouch, an isolated jejunal pouch transposed to the pelvis and interposed between the terminal ileum and the anal canal. The aims of the present experiments were to compare the clinical, physiologic, bacterial, and anatomic properties of the new type of jejunal pouch to those of conventional ileal pouches used as rectal substitutes after proctocolectomy.

METHODS

Animal Preparation

Twelve healthy female mongrel dogs weighing from 16 to 26 kg were fasted overnight and given 500 mg of cephazolin intravenously. They underwent general anesthesia with methohexital sodium (12.5 mg/kg) and 1.5% isoflurane. Atropine sulphate (0.04 mg/kg intramuscularly) was given to prevent shortening of the bowel during the operation. Using a sterile operating technique, a midline celiotomy was made, and a colectomy and proximal proctectomy were accomplished. The proctectomy extended distally to a point 2 cm proximal to the pelvic floor.

In 6 of the 12 dogs, a J-shaped pouch was constructed from the distal jejunum. The small bowel was divided at a site one-half the distance between the ligament of Treitz and the ileocolic valve. The 30 cm of jejunum immediately proximal to the transection was used to form a J-shaped jejunal pouch. The 30 cm segment was folded into two 15 cm limbs and the pouch was constructed using two layers of 3-0 polyglycolic acid suture. The distal cut end of the proximal jejunum was anastomosed end to end to the proximal cut end of the ileum, whereas the distal cut end of the ileum was anastomosed end to end to the afferent limb of the jejunal pouch. The newly formed jejunal pouch was then anastomosed to the distal rectum (Fig. 1).

In the other six (control) dogs, 30 cm of terminal ileum was used to form a J-shaped ileal pouch, and this pouch was anastomosed to the distal rectum, as is done

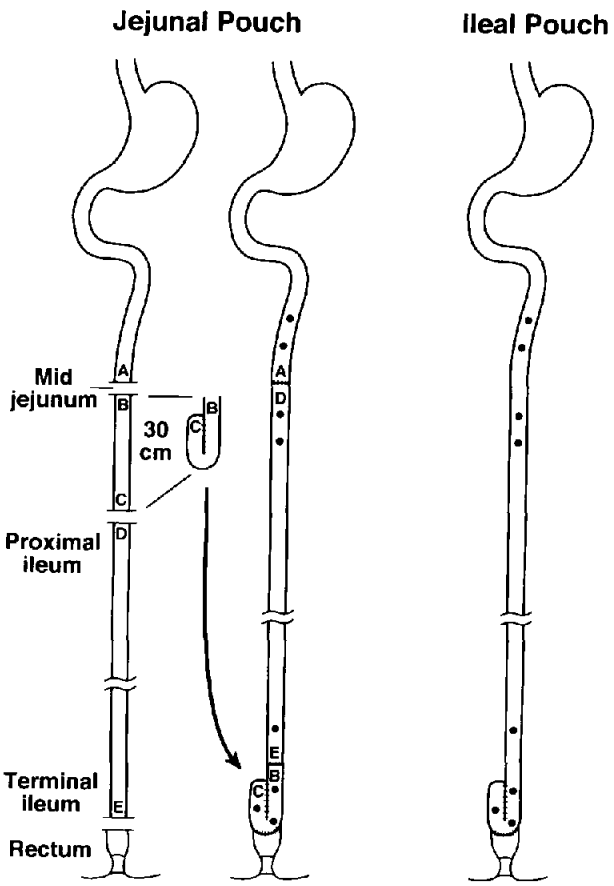

Fig. 1. Canine experimental preparation. *Left*, Jejunal pouch–distal rectal anastomosis. *Right*, Ileal pouch–distal rectal anastomosis. • = electrodes. Letters *A* through *E* identify specific areas of the bowel.

in a conventional ileal pouch–distal rectal anastomosis[1] (see Fig. 1). Both types of pouch were anastomosed to the distal rectum instead of the anal canal at the dentate line because of the greater ease of distal rectal anastomosis and to preserve completely the anal sphincter and its innervation. This study was designed to explore pouch function and not the fate of the retained distal rectal and proximal anal canal mucosa.

In both groups of dogs, eight enteric electrodes were applied as in Fig. 1. Electrodes 1 and 2 were placed on the midjejunum, 5 cm apart, beginning 5 cm proximal to the jejunoileal anastomosis. Electrodes 3 and 4 were placed on the proximal ileum, 5 cm apart, beginning 5 cm distal to the jejunoileal anastomosis. Electrode 5 was placed on the distal ileum 5 cm proximal to the ileal pouch anastomosis, and electrodes 6, 7, and 8 were placed on the pouch 5 cm, 10 cm, and 15 cm distal to the ileal pouch-rectum anastomosis. All electrodes were monopolar Ag-AgCl recording electrodes. The electrodes were connected by insulated copper wires to a multioutlet socket embedded in a

stainless steel cannula. The cannula was positioned in and anchored to the left anterior abdominal wall.

The animals were allowed to recover for 10 weeks, after which testing was begun.

Clinical Observations

The general health of the dogs, their eating habits, and their weight were carefully recorded each week after the operation. Mouth-to-anus transit time and patterns of defecation were measured as follows. After an overnight fast, the animals were fed 312 g of canned dog food (CNM, Purina, St. Louis, Mo.) to which was added 2 tablespoons of charcoal (activated carbon 6-14 mesh, Fisher Scientific, Pittsburgh, Pa.). The dogs were placed in the kennel and carefully observed over the ensuing 12 hours to note the time between eating and the first stool containing charcoal and the number of stools occurring between eating and the subsequent 12 hours. The weight of stools over the first 12 postprandial hours was also recorded. The experiment was performed twice in each dog.

Physiologic Observations

Myoelectric and Motor Tests During Fasting. Small intestinal and pouch electrical activity, pouch distensibility, pouch pressure-volume response, and pouch volume-pressure response were measured twice on different days during fasting in each conscious animal beginning 10 to 14 weeks after the operation.

Electrical activity was recorded after an overnight fast. The dogs were placed fully conscious in a Pavlov stand, and the electrodes were connected to an electrical amplifier/computer acquisition system. Using the stainless steel cannula as a ground, myoelectrical signals were amplified by means of Mayo custom-built analog amplifiers. The amplified analog signals were then converted to digital signals sampled at 100 Hz, which were displayed in real time on a VGA monitor while being simultaneously stored on magnetic media to be analyzed later.[9] The electrical recordings were continued for at least 4 hours.

Pouch distensibility was evaluated after an overnight fast by an electronic barostat.[8] A compliant rubber bag was attached to the distal end of a 14 F plastic tube, the proximal end of which was connected to rigid bellows containing air. The bag, catheter, and bellows formed a closed system. An electronically driven motor continuously adjusted the bellows to maintain the air pressure within the system at a value specified by the operator. The tube was inserted through the anus for approximately 15 cm, in order to locate the bag in the pouch. The tube was tied to the animal's tail to anchor the bag in the pouch. Air pressure within the system was set at 3 mm Hg to expand the bag and fill the pouch to the extent that the muscular tone of the pouch would allow. The volumes recorded by the barostat were measured for 2 hours. The barostat data were digitized and stored on disk for later computer analysis.

Using the same barostat device, the pressure-volume response of the pouch was evaluated twice in each dog by gradually increasing the pressure in the system. The bag was distended at the rate of 1 mm Hg/min until a pressure of 10 mm Hg was reached or until the dog showed signs of discomfort. Also, a volume-pressure response of the pouch was evaluated twice in each animal by gradually increasing the volume in the intrapouch bag by a rate of 20 ml every 2 minutes.

Myoelectric and Motor Tests During Feeding. On other days, electrical activity was recorded during feeding. After an overnight fast, the dogs were placed fully conscious in Pavlov stands, and the electrodes were connected to an electrical amplifier/computer acquisition system as already described. When the dogs were in phase I of the interdigestive migrating myoelectric complex (MMC), they were fed 312 g of canned dog food (CNM, Purina). The electrical recordings were continued for at least 2 hours. The experiment was performed twice in each animal. On still other days, pouch distensibility was evaluated for 2 hours after an overnight fast and ingestion of the canned dog food using an electronic barostat in the same way that was already described during fasting. The volumes recorded by the barostat were measured for 2 hours. The study was performed twice in each dog.

Gastric Emptying. After an overnight fast, the alert, conscious dogs were given a meal consisting of 60 ml of egg white to which 1.0 mCi of ^{99m}Tc sulfur colloid (Syncor International Corp., Phoenix, Ariz.) had been added. After the dogs consumed the meal, the head of a gamma camera (ELSCINT model 409 with a medium-energy collimator) was placed in contact with the right lateral surface of the abdomen and pelvis of the dog as the animal lay in the prone position in a Pavlov sling. Static lateral view scintigraphic images with 20% windows around the ^{99m}Tc energy peak (140 keV) were obtained for 60 seconds. A 60-second count was taken beginning immediately after ingestion of the meal (time 0), 5 minutes after ingestion, 10 minutes after ingestion, and then every 20 minutes until 2 hours after ingestion of the meal. The counts/minute (cpm) were recorded, corrected for decay, and expressed as a percentage of isotope remaining in the stomach. Scintigraphic data were stored and later analyzed to determine the $T_{1/4}$ (time for 25% of the isotope to empty from the stomach), $T_{1/2}$ (time for

50% of the isotope to empty), and $T_{3/4}$ (time for 75% of the isotope to empty).[10]

Pouch Emptying. After an overnight fast, the dogs were given a 100 ml, 154 mmol/L NaCl enema per anum to cleanse the pouch. After defecation of the saline solution, the animals were positioned in the Pavlov sling, and an instillate of 60 ml of egg white tagged with 1.0 mCi of ^{99m}Tc sulfur colloid was placed into the pouch via a 14 F catheter passed per anum. The catheter was withdrawn, and the head of the gamma camera was placed in contact with the left lateral surface of the dog's pelvis as the dog lay in the prone position in a Pavlov sling. Lead shielding was placed over the dog's pelvis to isolate the area of the pouch through a 15 cm port. A 60-second count was measured immediately and was considered time 0.

The dog was removed from the stand, placed in the kennel, and observed until the first bowel movement. The animal was then repositioned immediately in the Pavlov stand, and the procedure described above was performed again to count the isotope remaining in the pouch. The scintigraphic images were stored and analyzed later. The results were expressed as the percentage of isotope emptied from the pouch during the first bowel movement after instillation of the marker into the pouch. Each dog was studied twice.

Pouch Mucosal Integrity. Pouch mucosal integrity was measured once in each animal, at 12 to 14 weeks after the operation. Five unoperated healthy dogs served as controls. Mucosal integrity was assessed by measuring the ability of the mucosa to exclude a radioactive marker. A "leaky" mucosa would permit more of the marker to pass through the mucosa into the bloodstream and be excreted in the urine. The marker used was radioactive-labeled ethylenediaminetetraacetic acid (^{51}Cr-EDTA, size = 350 daltons, NEN Life Science Products, Boston, Mass.). At the time of the test, a stock solution of ^{51}Cr-EDTA (specific activity 17 to 75 MBq/mg) was diluted with isotonic saline solution, so that 4 ml had an activity of approximately 0.1 mCi (3.7 MBq). The 4 ml test solution was counted over 60 seconds in a gamma counter (LKB - 1272 Clinigamma–Automatic Gamma Counter, Long Island Scientific, Port Jefferson, N.Y.).

After an overnight fast, the dogs were given a 100 ml saline (154 mmol/L NaCl) enema per anum to cleanse the pouch. The conscious animals were placed in a Pavlov stand, a 12 F urinary Foley catheter was inserted into the urinary bladder, and a urinary collecting bag was connected to it. A second 12 F catheter was next passed transanally into the pouch, and a 2 ml isotonic saline solution was instilled into the pouch to ensure correct positioning of the catheter. The 4 ml radioactive test solution was then instilled, followed by an additional 4 ml of isotonic saline to flush residual radioactivity in the catheter into the pouch. The pouch catheter was then removed. The animals were given 500 ml of Ringer's lactate solution (Baxter International Inc., Deerfield, Ill.) intravenously to ensure adequate urine output. Urine was collected over the ensuing 4 hours, the volume was measured, and five 5 ml samples were taken and counted in a gamma counter in the same manner as the test solution. The mean of five samples was used to determine the amount of radioisotope in the 4-hour volume of urine. Permeability of the pouch mucosa was assessed as the percentage of the administered dose of ^{51}Cr-EDTA recovered in the urine; the greater the urinary recovery, the greater the permeability.[11]

Fecal Bacteriology

Quantitative aerobic and anaerobic measurements of bacterial flora of the pouch were performed. A sample from just-passed feces was immediately serially diluted by 10-fold increments with sterile Ringer's lactate to a dilution of 10^{-8}. Inoculation of culture plates was performed using a sterile pipette to place 0.1 ml of each dilution onto the culture plate. The inoculum was then spread on the plate with a hockey stick applicator. For aerobic incubation, each dilution was inoculated onto sheep blood agar. The 10^{-4}, 10^{-6}, and 10^{-8} dilutions were also inoculated onto eosin–methylene blue agar and colistin-nalidixic acid (Columbia base) agar. Similarly, for anaerobic incubation, each dilution was placed onto rabbit blood agar (*Brucella* base). The 10^{-4}, 10^{-6}, and 10^{-8} dilutions also inoculated onto gentamycin-vancomycin agar and phenylethyl alcohol agar. The anaerobic plates were incubated in an anaerobic jar. The aerobic cultures were incubated overnight. The anaerobic cultures were first assessed at 48 hours, then again at 5 to 7 days. The dilution with the best isolation was used for counting the number of colonies of each morphologic type. A Gram stain was done on each colony type, and the colonies were placed into one of four categories: gram-positive coccus, gram-negative coccus, gram-positive bacillus, and gram-negative bacillus. The total number of colonies in each classification was recorded. For detection of true anaerobes, a representative of each colony type was subcultured to sheep blood and chocolate agar plates and incubated aerobically and anaerobically in CO_2. Growth in CO_2 and/or O_2 disqualified a colony as anaerobic.

Anatomic Observations

After completion of the preceding tests (mean 33 weeks after the operation), all dogs were killed with

an overdose of pentobarbital. Their abdomens were opened and the areas of operation inspected. The pouch was removed, opened longitudinally, and its length and width measured. The pouch was carefully inspected and samples from the proximal, middle, and distal pouch were removed for histologic analysis, staining the tissues with hematoxylin and eosin. Villous blunting and mucosal atrophy were graded as none, mild, moderate, or severe.

Analysis of Data

Electrical data were processed by converting the analog signals from the electrode tracings to digitized signals. The digitized signals were analyzed using a VAX/VMS platform by the method previously described by our laboratory.[9] The mean frequency of pacesetter potentials (PPs) in each channel was determined using pacesetter potential event times in a single recording channel by inspecting 20 minutes of the 2-hour tracing after the first 30 minutes. The percentage of PPs with action potentials in each channel was calculated during the same time interval. The interdigestive MMCs were analyzed by inspecting the 4-hour tracings during fasting following the criteria of Code and Marlett.[12] The analysis was focused on four parameters: presence or absence of the MMCs, duration of the MMC cycles, the duration of the phase IIIs of the MMCs, and the velocity of propagation of phase IIIs.

The slopes of the pressure-volume curves and volume-pressure curves were obtained using linear regression for each run. Gastric emptying variables ($T_{1/4}$ and $T_{1/2}$) were calculated by fitting a power exponential curve to the data from each run using linear regression.[10]

The average of the two runs obtained for each variable was determined. Groups were compared using the two-sample *t* test unless otherwise noted. *P* values <0.05 were considered statistically significant. Summary values in the text are given as mean ± standard error of the mean (SEM).

RESULTS

Clinical Observations

General Health. The dogs remained healthy overall and ate well during the 16 to 56 weeks that they were followed after the operation (mean 33 weeks). During the first 6 postoperative weeks, the animals had watery, mushy stools. However, the stools became semisolid after that. The dogs lost about 10% of their body weight (jejunal pouch = 11.6% ± 1.6%, ileal pouch = 8.8% ± 1.5%; $P > 0.05$) in the first 2 weeks after the operation, but they subsequently regained all their lost weight (Fig. 2). They weighed slightly more at the time of sacrifice than they did initially (jejunal pouch = 1.4 ± 1.0 kg more vs. ileal pouch = 2.1 ± 1.0 kg more).

One jejunal pouch dog developed a bowel obstruction 4 days after the operation. The obstruction was due to adhesions caused by the wires attached to the electrodes. The animal underwent a laparotomy to relieve the bowel obstruction. No signs of bowel necrosis were observed. The dog recovered well after the second operation and underwent complete testing beginning 10 weeks after the operation. A second dog, an ileal pouch dog, developed abdominal distension and peritonitis, and died 46 weeks after the operation, by which time all tests had been completed. At autopsy, an erosion of the vessels in the midjejunal mesentery with a resulting hematoma,

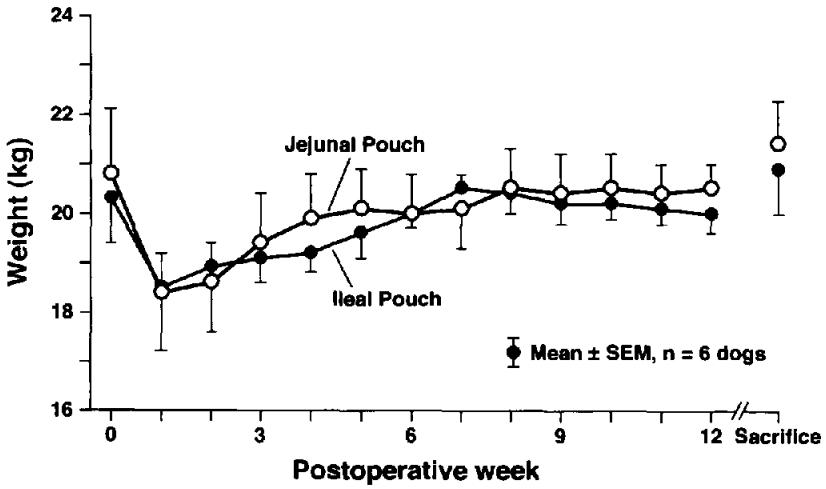

Fig. 2. Body weight against time in dogs with jejunal pouches and ileal pouches used as rectal substitutes.

bowel obstruction, and perforation of the affected segment was discovered. The erosion was caused by the electrode wires. A third dog, a jejunal pouch dog, also developed a bowel obstruction and died 16 weeks after the operation, again after all tests had been completed. Necrosis of two thirds of the small bowel below the jejunojejunal anastomosis had occurred due to volvulus.

Enteric Transit and Stooling. Mouth-to-anus transit after eating took longer in the jejunal pouch dogs (253 ± 18 minutes) than in the ileal pouch dogs (112 ± 7.9 minutes; $P < 0.05$). The number of stools in the first 12 hours after eating was less in the jejunal pouch dogs (4.1 ± 0.3 stools per 12 hours) than in the ileal pouch dogs (6.3 ± 0.9 stools per 12 hours; $P < 0.05$). However, the 12-hour weight of stool was similar for both groups (jejunal pouch 153 ± 11 g per 12 hours vs. ileal pouch 159 ± 18 g per 12 hours; $P > 0.05$).

Physiologic Observations

Myoelectric and Motor Activity During Fasting. The frequency of midjejunal PPs was similar in the jejunal pouch dogs and the ileal pouch dogs (jejunal pouch 17 ± 0.1 cpm vs. ileal pouch 17 ± 0.2 cpm), but the frequency was slower in the proximal ileum of the jejunal pouch dogs (jejunal pouch dogs 13 ± 0.3 cpm vs. ileal pouch dogs 16 ± 0.2 cpm) and in the distal ileum (jejunal pouch dogs 12 ± 0.6 cpm vs. ileal pouch dogs 15 ± 0.2 cpm; Fig. 3 and Table I). The frequency of PPs in the pouch was similar in both groups (jejunal pouch 13 ± 0.3 cpm vs. ileal pouch 13 ± 0.2 cpm). Action potentials occurred with 85% ± 3.0% of the jejunal pouch PPs during fasting, whereas the PPs from the ileal pouch had action potentials associated with PPs only 37% ± 3.9% of the time ($P < 0.05$). The duration of the MMC cycles, the duration of phase III, and the velocity of propagation of phase III were similar in the two groups of dogs (Table I). Phase IIIs did not propagate into the ileal pouches. We could not determine whether phase IIIs propagated into jejunal pouches because action potentials were occurring with almost all pouch PPs at all times, just as occurs with phase III.

The mean volume in the pouch over a period of 2 hours using the barostat was similar in both groups (jejunal pouch 40 ± 2.0 ml vs. ileal pouch 52 ± 10.2 ml), as were maximum and minimum volumes (Table II). However, the volumes in the pouch varied less in the jejunal pouches than in the ileal pouches ($P < 0.05$).

The mean slope of the pressure-volume curves for the dogs with jejunal pouches (0.16 ± 0.0 mm Hg/ml) was similar to the mean slope for the animals with

Table I. Small intestinal electrical activity during fasting after enteric pouch–distal rectal anastomosis (values are means ± SEM)

Site	Jejunal pouch (n = 6 dogs)				Ileal pouch (n = 6 dogs)			
		Interdigestive MMCs				Interdigestive MMCs		
	Frequency of PPs (cpm)	Duration of cycles (min)	Duration of phase IIIs (min)	Velocity of propagation of phase IIIs (cm/min)	Frequency of PPs (cpm)	Duration of cycles (min)	Duration of phase IIIs (min)	Velocity of propagation of phase IIIs (cm/min)
Midjejunum	17 ± 0.1	103 ± 4.6	3.5 ± 0.2	2.6 ± 0.4	17 ± 0.2	102 ± 14	4.2 ± 0.3	2.2 ± 0.1
Proximal ileum	13 ± 0.3*	103 ± 8.0	4.4 ± 0.5	2.2 ± 0.9	16 ± 0.2	106 ± 14	3.8 ± 0.1	2.7 ± 0.7
Distal ileum	12 ± 0.6*	88 ± 18	4.4 ± 0.3	3.7 ± 0.7	15 ± 0.2	103 ± 13	4.0 ± 0.2	2.7 ± 0.3
Pouch	13 ± 0.3	†	†	†	13 ± 0.2	‡	‡	‡

MMCs = migrating myoelectric complexes; PPs = pacesetter potentials.
*$P < 0.05$ compared to ileal pouch.
†No value available; phase III–like pattern present at all times
‡Phase IIIs not identified in ileal pouches.

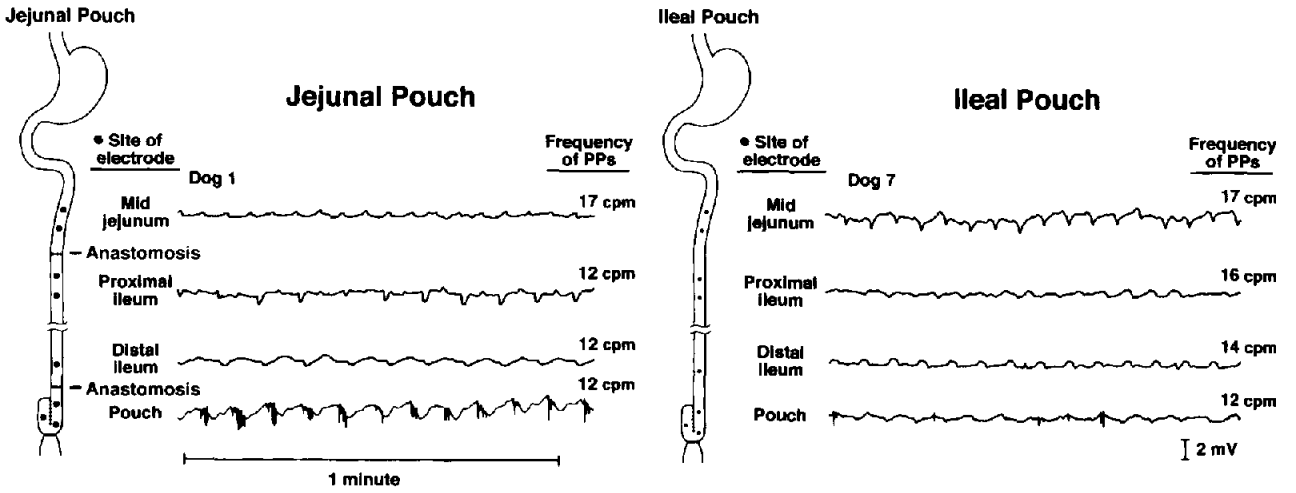

Fig. 3. Canine electrical tracings from midjejunum, proximal ileum, distal ileum, and pouch *(left)* after jejunal pouch–distal rectal anastomosis and *(right)* after ileal pouch–distal rectal anastomosis.

Table II. Intrapouch volume measured with a barostat in jejunal pouch and ileal pouch dogs (values are means ± SEM)

	Jejunal pouch (n = 6 dogs)		Ileal pouch (n = 6 dogs)*	
Intrapouch volume	Fasting	Fed†	Fasting	Fed†
Maximum (ml)	71 ± 1.3	61 ± 8.3	79 ± 8.4	67 ± 7.1
Mean (ml)	40 ± 2.0	36 ± 4.1	52 ± 10.2	54 ± 9.7
Minimum (ml)	21 ± 5.7	23 ± 1.9	23 ± 10	16 ± 6.9

*Values do not differ from jejunal pouch values, $P > 0.05$
†Values do not differ from fasting values; $P > 0.05$.

Table III. Small intestinal electrical activity after feeding in dogs with enteric pouch–distal rectal anastomoses (values are means ± SEM)

	Jejunal pouch (n = 6 dogs)		Ileal pouch (n = 6 dogs)	
Site	Frequency of PPs (cpm)	% of PPs with action potentials	Frequency of PPs (cpm)	% of PPs with action potentials
Midjejunum	17 ± 0.2	54 ± 3.6	17 ± 0.3	52 ± 3.0
Proximal ileum	12 ± 0.4*	64 ± 5.2	16 ± 0.3	59 ± 3.7
Distal ileum	13 ± 0.3*	48 ± 0.2	14 ± 0.3	40 ± 8.3
Pouch	13 ± 0.3	82 ± 4.3*	13 ± 0.1	61 ± 3.0

PPs = pacesetter potentials
*$P < 0.05$ compared to ileal pouch.

ileal pouches (0.14 ± 0.0 mm Hg/ml, $P > 0.05$; Fig. 4). Moreover, the slopes for the volume-pressure studies were also similar in the two groups (jejunal pouch 15 ± 1.0 ml/mm Hg vs. ileal pouch 15 ± 0.8 ml/mm Hg; $P > 0.05$).

Myoelectric and Motor Activity After Feeding. The frequencies of PPs in the small bowel and in the pouch during the fed state were similar to those in the fasting state. The jejunal pouch dogs had slower frequencies of PPs in the proximal ileum (12 ± 0.4 cpm) and the distal ileum (13 ± 0.3 cpm) than the ileal pouch dogs (16 ± 0.3 cpm and 14 ± 0.3 cpm, respectively [Table III]; $P < 0.05$). The pouches of the jejunal pouch dogs (82% ± 4.3%) had more action potentials than the pouches of the ileal pouch dogs (61% ± 3.0%; $P < 0.05$).

The mean barostat volume in the pouch after feeding over a period of 2 hours was similar in both

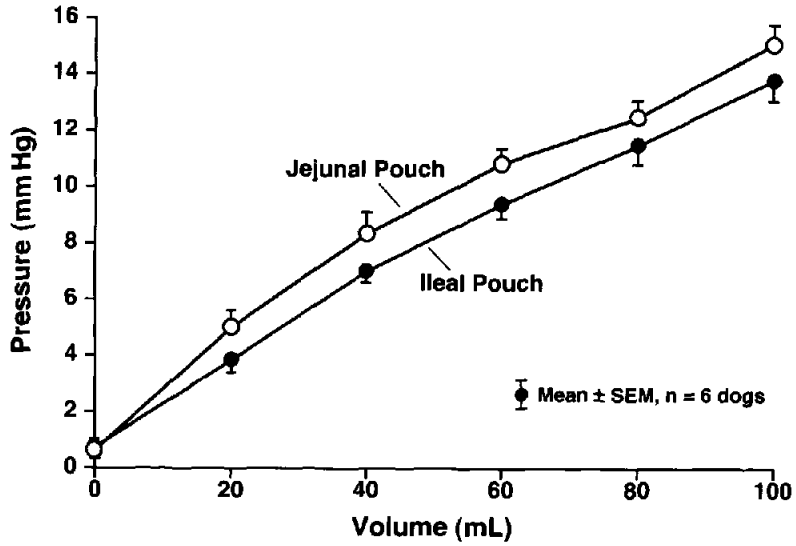

Fig. 4. Pressure-volume curves in jejunal pouch dogs and ileal pouch dogs.

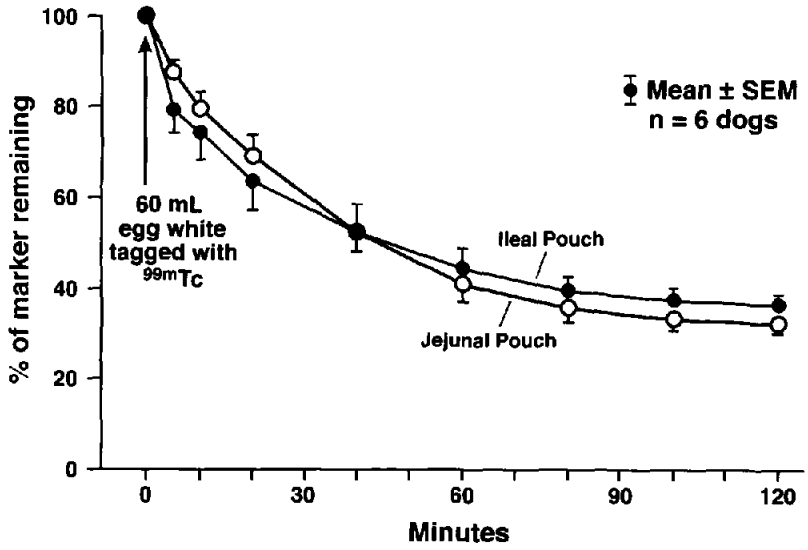

Fig. 5. Gastric emptying measured scintigraphically against time in jejunal pouch dogs and ileal pouch dogs.

groups (jejunal pouch 36 ± 4.1 ml vs. ileal pouch 54 ± 9.7 ml; $P > 0.05$). We also did not find differences in the maximum or minimum volumes. However, the mean, maximum, and minimum volumes again varied more in the ileal pouch dogs than in the jejunal pouch dogs ($P < 0.05$), just as in fasting. Pressure-volume curves and volume-pressure curves were not determined after feeding.

Gastric Emptying. Gastric emptying of the ^{99m}Tc-labeled egg white meal was similar in both groups (jejunal pouch $T_{\frac{1}{2}}$ 51 ± 6.0 minutes vs. ileal pouch $T_{\frac{1}{2}}$ 51 ± 11.2 minutes; $P > 0.05$). Proportionate emptying data computed from estimated parameters of the fitted-power exponential model[10] also showed no difference between the two groups (Fig. 5).

Pouch Emptying. The percentage of pouch instillate evacuated by the jejunal pouch dogs (84.3% ± 2.1%) was similar to the percentage evacuated by the ileal pouch dogs (85.9% ± 3.0%). The length of the pouch measured scintigraphically in the two groups of dogs was the same (jejunal pouch 15 ± 0.5 cm vs. ileal pouch 15 ± 0.5 cm). The time between instillation and defecation of the instillate in the jejunal pouch dogs (20 ± 4.9 minutes) was similar to that of the ileal pouch dogs (18 ± 5.2 minutes; $P > 0.05$).

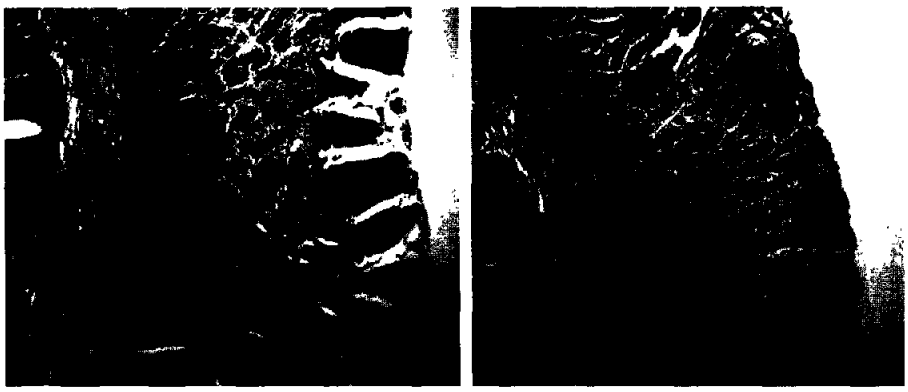

Fig. 6. Photomicrograph of a canine jejunal pouch *(left)* and a canine ileal pouch *(right)*. Villi are better preserved in the jejunal pouch. (Hematoxylin and eosin stain; original magnification ×40.)

Pouch Mucosal Integrity. Pouch mucosal integrity was also similar in both groups. In jejunal pouch dogs 4.8% ± 1.6% of isotope instilled in the pouch was found in the urine after 4 hours, whereas 5.8% ± 1.3% was found in ileal pouch dogs ($P > 0.05$) and 4.1% ± 2.0% in unoperated dogs with a healthy rectum ($P > 0.05$).

Fecal Bacteriology

Both groups of pouch dogs showed bacterial overgrowth in the feces. Jejunal pouch dogs had $8 \times 10^8 \pm 4 \times 10^8$ colony-forming units/gram of stool of aerobic bacteria and $18 \times 10^8 \pm 5 \times 10^8$ colony-forming units/gram of stool of anaerobic bacteria, whereas ileal pouch dogs had $9 \times 10^8 \pm 5 \times 10^8$ colony-forming units/gram of stool of aerobic bacteria and $15 \times 10^8 \pm 4 \times 10^8$ colony-forming units/gram of stool of anaerobic bacteria ($P > 0.05$).

Anatomic Observations

Three dogs (1 jejunal pouch dog and 2 ileal pouch dogs) had a localized chronic infection surrounding the electrode cannula that had been placed in the anterior abdominal wall. No peritonitis was present, however. The jejunal pouches were shorter (12.5 ± 0.7 cm) than the ileal pouches (14.7 ± 0.5 cm; $P < 0.05$), but the two types of pouches had similar widths (jejunal pouch 8.9 ± 0.2 cm vs. ileal pouch 8.8 ± 0.1 cm). No evidence of mucosal inflammation or ulceration was present on either gross or microscopic examination of any pouch. Mild villous blunting and mucosal atrophy were seen in some part of all five jejunal pouches and in all five ileal pouches examined histologically. Other areas of each pouch showed no villous blunting or atrophy. In contrast, areas of moderate villous blunting and atrophy were found in three of the ileal pouches but in none of the jejunal pouches (Fig. 6).

DISCUSSION

This article provides original observations on the use of an isolated transposed jejunal pouch as a rectal substitute after proctocolectomy. Jejunal pouches have been used extensively as substitutes for the stomach, but there have been few if any studies dealing with them used as substitutes for the rectum. This article shows that dogs with a jejunal pouch used as a rectal substitute recovered well from the operative procedure, gained weight back to and exceeding their preoperative weight, and remained healthy over at least a 6-month period. None of them developed jejunal pouch fistulas, as occurred in our past tests with in situ jejunal pouches. Clearly this new type of jejunal pouch can be used with success as a rectal substitute.

The new type of jejunal pouch, however, did not have a larger resting volume than the conventional ileal pouch, whereas the in situ jejunal pouch we used in past tests did have this property.[8] The explanation may be that the jejunal pouches we used here were actually smaller at autopsy than the ileal pouches, even though the two types of pouches were made from identical lengths of intestine and they seemed to be the same size on pouch scintigraphy. In spite of their smaller size, however, the new jejunal pouches had as great a resting volume and were as distensible as the larger ileal pouches.

Jejunal pouches had advantages over ileal pouches in these tests. Transit through the enteric tract in the dogs with jejunal pouches was slower. Because gastric emptying and pouch emptying were similar in jejunal pouch dogs and ileal pouch dogs, we reasoned that

the longer mouth-to-anus transit in the jejunal pouch dogs was due to slower small bowel transit and slower pouch filling. Indeed, we know from past tests that transection of the jejunum, as performed to form the jejunal pouch in these dogs, decreases the frequency of PPs, hence contractions in the bowel distal to the site of transection.[9] Ectopic pacemakers then appear in the distal bowel. These pacemakers pace the bowel proximal to them backward. The decrease in pacesetter potential frequency and the backward pacing slow transit through the bowel. The slow transit likely explains the longer time between eating and the first bowel movement and the smaller number of bowel movements experienced in the postprandial period.

Another possible explanation for the slow small bowel transit in the jejunal pouch dogs is that the ileum is likely protected from the effects of fecal stasis in them,[3] thus perhaps preserving the ability of the ileum to secrete hormones, such as peptide YY, that slow transit through more proximal bowel. We did not measure hormonal secretion in these tests, however.

The jejunal pouches had more fasting and postprandial action potentials, hence more contractions, than the ileal pouches. Nonetheless, the increased contractility of the jejunal pouch did not interfere with its ability to act as a reservoir. Indeed, the amount of stool stored in the jejunal pouches must have been greater than that stored in the ileal pouches. The total weight of stools passed postprandially by the jejunal pouch dogs was similar to that passed by the ileal pouch dogs, but the jejunal dogs had fewer stools. Thus the volume of each stool must have been larger.

A major unanswered question is whether jejunal pouches would be less susceptible to pouchitis than ileal pouches. Neither type of canine pouch showed loss of mucosal integrity and neither developed pouchitis in the postprandial period. Moreover, bacterial overgrowth in the pouches was the same in the jejunal and ileal pouches. Nonetheless, the jejunal pouches had greater contractility than the ileal pouches, and this may have contributed to their ability to empty. Stasis in pouches is one of the factors that may predispose them to the development of pouchitis.

Blunting of villi and mucosal atrophy may also make pouches more susceptible to pouchitis and even dysplasia.[13] We found mild mucosal atrophy in both types of pouch, as have others.[8,14] The atrophy was more severe in some ileal pouches, but the overall differences between the two types of pouch were not clear-cut. The greater contractility of the jejunal pouches may have protected them somewhat from mucosal atrophy, but this is unclear.

In summary, jejunal pouches, although they are more difficult to construct than ileal pouches, are as good a rectal substitute in dogs as ileal pouches and have the advantage of slowing intestinal transit, delaying defecation, and decreasing the number of bowel movements after meals compared to ileal pouches. Jejunal pouches may have clinical application in some patients who require excision of the large intestine for ulcerative colitis.

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Prospective Randomized Trial of Early Initiation and Hospital Discharge on a Liquid Diet Following Elective Intestinal Surgery

Kevin E. Behrns, M.D., Amanda P. Kircher, B.S.N., Joseph A. Galanko, Ph.D., Michelle R. Brownstein, M.D., Mark J. Koruda, M.D.

Length of hospital stay after elective intestinal surgery may be related to patient tolerance of a diet. We hypothesized that early initiation and discharge home on a clear liquid diet would decrease the length of hospital stay without increasing morbidity. The aim of this study was to determine if early initiation and discharge on a clear liquid diet decreases the length of hospital stay and is safe. Forty-four patients were randomly assigned to either a standard diet or a clear liquid diet. A standard diet (n = 17) was begun after the passage of flatus or stool, and consisted of clear liquids to a volume of approximately 750 ml, then three solid meals, and discharge thereafter. Patients randomized to a clear liquid diet (n = 27) received 30 ml/hr of clear liquids on postoperative day 2, unlimited clear liquids on postoperative day 3, and were dismissed on a clear liquid diet on postoperative day 4. All patients were followed by a daily telephone call and clinic visit. The primary outcome variable was length of hospital stay. The incidence of postoperative intestinal-related sequelae, complications, and readmission rates did not differ between groups. Postdischarge intestinal symptoms were common in both groups but tended to resolve faster in the patients on a standard diet. The length of hospital stay was decreased in the patients on a clear liquid diet compared to those on a standard diet (6.1 ± 1.1 days vs. 4.4 ± 0.2 days; $P = 0.09$), but total hospital costs did not differ. Early initiation and hospital discharge on a clear liquid diet after elective intestinal surgery decreases the length of hospital stay and is safe. (J GASTROINTEST SURG 2000;4:217-221.)

KEY WORDS: Postoperative diet, clear liquid diet, postoperative care, postoperative nutrition

The time-honored management of postoperative diet after elective intestinal surgery consists of awaiting the return of bowel function followed by initiation of a clear liquid diet, possibly a full liquid diet, and finally solid food. Few data exist to support this dietary regimen, and this approach often results in patients remaining on nothing by mouth for 2 to 3 days during ileus resolution. Surgeons fear that earlier initiation of a diet will induce nausea and vomiting necessitating placement of a nasogastric tube and will potentially increase the risk of anastomotic dehiscence. Recent emphasis on decreased length of postoperative stay has produced benchmark dismissal within 3 to 4 days of uncomplicated intestinal surgery,[1] but the safety and efficacy of this approach has not been established.

Physiologically, small bowel ileus resolves within hours of manipulation, but gastric and colonic motor function do not return until 48 to 72 hours postoperatively.[2] Several studies have demonstrated that nasogastric decompression is not required after elective intestinal surgery.[3,4] As an extension of these data, some authors have studied intake of solid food within 24 hours of operation, but these dietary regimens were either poorly tolerated in 14% of patients[5] or resulted in vomiting in 44% of patients.[6] Although these studies suggest that early resumption of oral feeding is tolerated in most patients, a substantial number of patients may have poor dietary compliance or complications.

We hypothesized that early initiation and hospital discharge on a clear liquid diet following elective intestinal surgery would decrease the length of hospital

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stay and be well tolerated. Resumption of a solid diet in the home environment may be more suitable to overall patient recovery.

MATERIAL AND METHODS

Patients undergoing elective intestinal surgery were candidates for the study if they did not require preoperative total parenteral nutrition or enteral nutrition. Only English-speaking study candidates were enrolled because telephone follow-up was mandatory following hospital discharge. An intensive care unit stay was permitted if the duration of ICU care was less than 48 hours and the stay was for monitoring purposes only and not because of hemodynamic instability. The study protocol was reviewed and approved by the University of North Carolina School of Medicine Committee on the Protection of the Rights of Human Subjects, and written informed consent was obtained from all patients preoperatively. Eligibility was determined at the time of the operation, and patients were randomized on postoperative day 2.

From 1995 through 1998, a total of 44 patients were randomized to either a standard diet (n = 17) or a clear liquid diet (n = 27). All patients had their nasogastric tubes removed at the conclusion of the operation. Postoperative analgesia was at the discretion of the patient and the anesthesiologist, and was delivered by either an epidural catheter or a patient-controlled analgesia pump. Patients randomized to the standard diet group received clear liquids only after passage of stool or flatus. Clear liquids were maintained in this group until a total volume of approximately 750 ml was consumed without evidence of nausea, vomiting, or abdominal distention. If liquids were well tolerated, patients were placed on solid food and dismissed from the hospital with dietary instructions after three solid meals had been consumed.

Patients randomized to the clear liquid diet group were begun on 30 ml of clear liquids per hour on postoperative day 2. On postoperative day 3 these patients received unlimited clear liquids and were discharged from the hospital on postoperative day 4 with dietary instructions, if there was no evidence of intestinal dysfunction. For the patients on the clear liquid diet, dietary instructions allowed institution of a solid diet immediately on their return to the home environment. At the University of North Carolina Memorial Hospital, a clear liquid diet provides 6 g protein, 150 g carbohydrates, no fat, and 624 kcal in a volume of approximately 800 ml. A typical solid meal consists of 20 g protein, 125 g carbohydrates, 28 g fat, and 830 kcal.

All patients were followed by a daily telephone call following dismissal from the hospital to ensure that the postoperative diet regimen was being tolerated. Specifically patients were asked about the presence of nausea, vomiting, constipation, diarrhea, low urine output, light-headedness, and dietary intolerance. Daily telephone calls ceased when the patients were asymptomatic and tolerating a regular diet or were readmitted to the hospital. All patients were followed up in the clinic within 10 days of operation.

Outcome Variables

The primary outcome variable was length of postoperative hospital stay. Secondary outcome variables included safety as measured by mortality, morbidity, and hospital readmission, weight change (preoperative weight minus postoperative weight at the time of clinic visit), postoperative intestinal symptoms, and hospital costs.

Statistical Analysis

The sample size of each group was calculated with an alpha value of 0.05 and a beta value of 0.9 with a mean length of stay of 7 days and a standard deviation of 2 days. If the reduction in the length of hospital stay was 1 day, then 85 patients would be required per group, but if the reduction in the length of stay was 2 days, then the sample size would be 22 patients per group. A 10% exclusion rate would yield a total sample size of 186 patients for a 1-day difference and 48 patients for a 2-day difference in length of stay. Interim analysis was performed with 44 total patients because no exclusions occurred.

For continuous variables, means ± standard error of the mean are presented. For categoric variables, counts and percentages are presented. For comparisons of continuous variables between the two groups, Wilcoxon two-sample tests were used because the data were not distributed normally. For comparisons of categoric variables, Fisher's exact test was used. A P value of less than 0.05 was considered statistically significant.

RESULTS

Patient Demographics, Diseases, and Operations

Seventeen patients were randomized to the standard diet and 27 patients to the clear liquid diet. Patients in each group were of similar age and sex (Table I). The most common diseases treated were Crohn's disease, ulcerative colitis, neoplasia, and diverticular disease. Although no statistical difference between groups was noted, the clear liquid diet group contained fewer patients with ulcerative colitis (see Table I). The percentage of patients undergoing small bowel resection (jejunal in one, ileal in 12), colon resection (right colectomy in 5, left colectomy in 2,

Table I. Patient demographics, diseases, and operations

	Standard diet (n = 17)	Clear liquid diet (n = 27)	*P* value*
Age (yr)†	47 ± 4	45 ± 3	0.72
Sex (% male)	41	56	0.54
Disease‡			
Crohn's	6 (35%)	10 (37%)	1.0
Ulcerative colitis	6 (35%)	3 (11%)	0.07
Neoplasia	3 (18%)	8 (30%)	0.49
Diverticular	1 (6%)	3 (11%)	1.0
Other	1 (6%)	3 (11%)	1.0
Operation‡			
Small bowel resection	6 (35%)	7 (26%)	0.52
Colon resection	9 (53%)	17 (63%)	0.55
Small bowel and colon resection	2 (12%)	3 (11%)	1.0

*$P < 0.05$ was considered statistically significant; Fisher's exact test and Wilcoxon two-sample test
†Mean ± standard error of the mean.
‡Number of patients.

Table II. Number of patients with intestinal-related adverse postoperative events, weight loss, and narcotic requirements

	Standard diet (n = 17)	Clear liquid diet (n = 27)	*P* value*
Nasogastric tube use	0 (0%)	2 (7%)	1.0
Ileus	1 (6%)	2 (7%)	1.0
Emesis	3 (18%)	2 (7%)	0.36
Enteral/parenteral nutrition	0	0	1.0
Weight loss (kg)†	1.9 ± 0.9	1.7 ± 0.8	0.72
Narcotic requirements (mg of morphine sulfate equivalent)			
Postoperative day 1	24 ± 6	30 ± 6	0.65
Postoperative day 2	22 ± 8	22 ± 5	0.41
Postoperative day 3	13 ± 3	10 ± 3	0.40

*$P < 0.05$ was considered statistically significant, Fisher's exact test and Wilcoxon two-sample test.
†Preoperative weight (kg) minus weight (kg) at time of initial postoperative clinic visit

transverse colectomy in one, sigmoid colectomy in 5, total abdominal colectomy in 8, and low anterior resection in 5), or combined small and large bowel resection was similar in both groups (see Table I).

Intestinal-Related Symptoms

No patients in the standard diet group required insertion of a nasogastric tube postoperatively, but two patients (7%) in the clear liquid diet group had nasogastric tubes placed (Table II). Symptomatic prolonged postoperative ileus and emesis were infrequent occurrences in both groups (see Table II). No patient in either group required postoperative enteral or parenteral nutrition, and the amount of weight loss (preoperative weight minus postoperative weight at clinic visit) was similar in the two groups (see Table II). The narcotic requirement on postoperative days 1 to 3, expressed in morphine sulphate equivalents, did not differ between groups.

Complications, Postdischarge Symptoms, and Readmission

The surgical complication rates were 29% and 19% ($P = 0.47$) in the standard diet and clear liquid diet groups, respectively (Table III). Complications included unexplained syncope in one, wound infection in two, abscess in one, and dehydration in one in the clear liquid diet group, and wound infection in two, abscess in two, and fever of unknown origin in one in the standard diet group. Postdischarge symptoms (nausea, vomiting, constipation, diarrhea, low urine output, light-headedness, and dietary intolerance) were present in the majority of patients in both groups, but decreased over the 3 days of telephone follow-up (see Table III). Patients in the standard diet group, however, tended to have quicker resolution of postdischarge symptoms than patients in the clear liquid diet group (see Table III). The readmission rate was 24% in the standard diet group vs. 11% in the clear liquid diet group; this difference did not achieve

Table III. Number of patients with postoperative complications, postdischarge intestinal symptoms, and readmission

	Standard diet (n = 17)	Clear liquid diet (n = 27)	*P* value*
No. with complication	5 (29%)	5 (19%)	0.47
Postdischarge symptoms			
Day 1	14 (82%)	15 (56%)	0.10
Day 2	5 (29%)	16 (59%)	0.07
Day 3	1 (6%)	8 (30%)	0.12
No. of readmissions	4 (24%)	3 (11%)	0.40

*$P < 0.05$ was considered statistically significant, Fisher's exact test and Wilcoxon two-sample test.

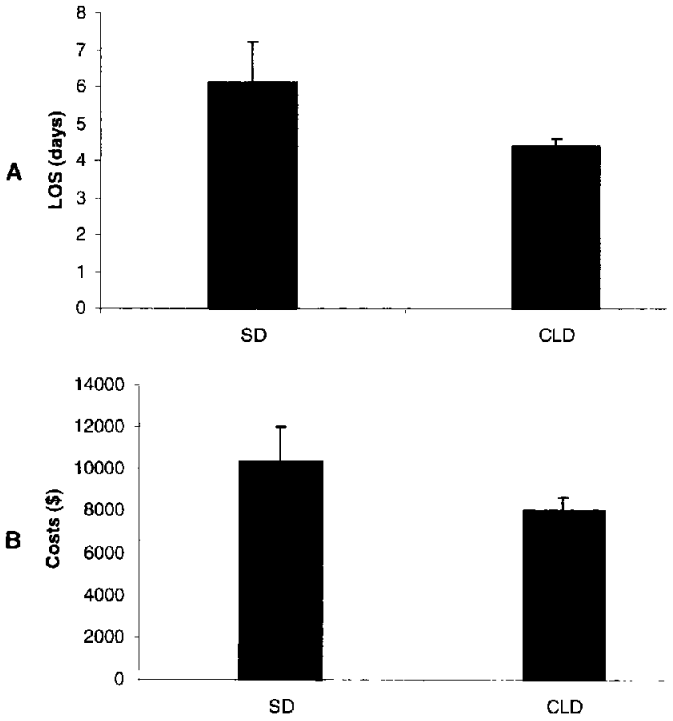

Fig. 1. Mean (± standard error of the mean) length of stay (LOS) **(A)** and hospital costs **(B)** are shown for patients receiving a standard diet *(SD)* or a clear liquid diet *(CLD)*. The decreased length of stay in the CLD group approached statistical significance ($P = 0.09$), but the hospital costs did not differ between groups ($P = 0.28$).

statistical significance ($P = 0.40$) (see Table III). One patient in each group was readmitted for a problem unrelated to the primary disease or operation (gout, tubo-ovarian abscess), and the other patients were readmitted for intestinal-related symptoms including nausea, vomiting, and dehydration.

Length of Stay and Hospital Costs

The postoperative length of stay was 6.1 ± 1.1 days in the standard diet group compared to 4.4 ± 0.2 days in the clear liquid diet group (Fig. 1, *A*). This difference approached statistical significance ($P = 0.09$). Although the hospital stay was shorter in the patients on a clear liquid diet, overall hospital costs did not differ substantially between the two groups (Fig. 1, *B*). The hospital costs were $10,332 ± $1644 in the standard diet group and $8049 ± $613 ($P = 0.28$) in the clear liquid diet group. Neither the length of stay nor the hospital costs were different between groups when the readmission length of stay and costs were included.

DISCUSSION

The aim of this study was to determine whether early initiation of a clear liquid diet following elective intestinal surgery resulted in a shorter hospital stay and was safe. We found that patients who received a clear liquid diet beginning on postoperative day 2 tended to be discharged from the hospital sooner than patients treated with standard dietary protocol. Early discharge from the hospital did not result in a higher rate of adverse events or an increased readmission rate. In fact, these patients had fewer complications and a lower readmission rate, although this was not statistically significant. Postdischarge intestinal symptoms were observed frequently in both groups of patients, but these symptoms resolved more rapidly in the patients on a standard diet. We conclude that early initiation and hospital discharge on a liquid diet result in a shorter postoperative length of stay and is safe.

Our reasons for choosing early initiation and hospital discharge on a clear liquid diet were multiple. Even though the caloric content of a clear liquid diet is generally low,[7] adequate fluid intake is readily achieved, thereby preventing the dehydration that may occur with a diet that consists primarily of solid food. Furthermore, adequate solid food intake to meet energy requirements after major surgery often does not occur for up to 14 days after surgery.[8,9] Previous studies have suggested that early initiation of a solid diet results in nausea, vomiting, and abdominal distention in 14% to 44% of patients.[5,6] Induction of these symptoms often leads to insertion of a nasogastric tube and prolonged hospitalization. Intolerance of solid food early after intestinal surgery may be related to alterations in gastrointestinal hormone secretion, motility, and transit. In fact, earlier studies have

shown that gastric emptying of liquids may be normal in patients within 24 hours of surgery.[10] We hypothesized that gastric emptying and intestinal transit of liquids may be less symptom-provoking than solid food, and thus better tolerated. Finally, patients may choose more palatable solid foods and have a more comfortable convalescence in the home environment.

A clinically significant trend toward a shorter hospitalization was achieved in patients who received a clear liquid diet. These patients were discharged from the hospital an average of 1.7 days earlier than patients in the standard diet arm. The shorter length of stay in the group receiving a clear liquid diet may be related to a "true" diet effect, that is, in these patients, the ileus is more quickly resolved and they can be discharged from the hospital within a shorter period of time. Alternatively, closer scrutiny of postoperative care of all patients in the study may have resulted in a "study effect" that hastened the discharge of patients receiving a clear liquid diet. Attending surgeons were not blinded to the randomization and participated in discharge planning. A "study effect," however, is less likely because patients in the clear liquid diet group did not have increased readmission rates compared to patients in the standard diet group. Pedersen et al.[11] demonstrated previously that a well-constructed plan for accelerated hospital dismissal for breast cancer patients resulted in decreased length of stay and reduced hospital costs. In our study, the reduction in the length of hospital stay in the clear liquid diet group did not translate into decreased hospital costs. This finding most likely reflects the multifactorial components of hospital costs, yet length of stay is often a primary determinant of total hospital costs. Our findings have implications for establishing dietary clinical care guidelines following elective intestinal surgery. These data support early initiation and hospital discharge on a clear liquid diet with resumption of solid food in the home environment. This practice did not lead to increased complications or higher readmission rates in this trial.

This study showed that a solid diet could be initiated safely in the home environment, but postdischarge gastrointestinal symptoms were frequently bothersome. The majority of patients in both study groups reported gastrointestinal symptoms on the first postdischarge day, but these symptoms tended to resolve more quickly in patients in the standard diet group. Although these symptoms did not result in an increased readmission rate in the patients on a clear liquid diet, these findings suggest that clinical practice guidelines that advocate discharge on a clear liquid diet after elective intestinal surgery require close follow-up and readily available access to a health care provider. Despite gastrointestinal symptoms following hospital discharge, most patients appeared satisfied with discharge on a liquid diet and earlier recovery in the "comfort" of their home.

The decreased length of stay in the clear liquid diet group was clinically but not statistically significant. This is likely because of the relatively small number of patients enrolled in the study. Because hospital discharge on a clear liquid diet was safe, and we considered a decreased length of stay of 1.7 days to be clinically significant, we felt that the study should be terminated at interim analysis. A statistical power of 0.9 could have been achieved if patient enrollment continued, but this would have required an additional 142 patients.

CONCLUSION

Early initiation and hospital discharge on a clear liquid diet following elective intestinal surgery decreases the length of hospital stay and is safe. Gastrointestinal symptoms occur frequently with home initiation of solid foods, but these symptoms do not generally require hospital readmission. Clinical practice guidelines that include early initiation and hospital discharge on a clear liquid diet following elective intestinal surgery require close patient follow-up and readily available access to a health care provider.

We thank Angela Glover and Ellen Hughes for their secretarial assistance.

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Laparoscopic Fundoplication for Dysphagia and Peptic Esophageal Stricture

To the Editors:

I read with great interest the article by Spivak et al. (J GASTROINTEST SURG 1998;2:555-560), and I have the following comments and questions for the authors:

1. In my experience in 185 patients with esophageal strictures, 95% of them had Barrett's esophagus; that is, specialized columnar epithelium was present at the level of and distal to the stricture. It is hard to believe that acid reflux causes a deep ulceration in esophageal squamous epithelium and a stricture is then formed, with squamous epithelium distal to the stricture. Therefore I would like to know whether in their 34 patients with peptic esophageal stricture, what type of epithelium was present at the level of and just distal to the stricture? Did all of them have esophageal squamous epithelium or did some have specialized columnar epithelium? If the endoscopist discovers a stricture and a biopsy is done proximal to it, squamous epithelium will always be present. But if the stricture is dilated and another biopsy is done just distal to the stricture, I can assure you that in the vast majority of cases, columnar epithelium will be found with or without intestinal metaplasia.
2. It seems very strange to me that the 40 patients with esophageal stricture had a normal lower esophageal sphincter pressure. In my experience, approximately 80% of these patients have an incompetent sphincter, with a resting pressure of less than 6 mm Hg and 90% will have an abdominal length of less than 10 mm.
3. In the two patients with "esophageal shortening," how far from the incisors (in cm) was the lower esophageal sphincter located?
4. What were the results of 24-hour pH studies in all of these patients before and after surgery? This must be known in order to have an objective parameter for effective control of reflux?
5. What were the results of manometric studies in these patients late after surgery?
6. Which were the endoscopic findings late after surgery (i.e., severity of stricture [diameter, length]), which were the results of biopsies done proximal and distal to the stricture late after surgery?
7. The mean follow-up was 1.5 years, which is too short for definitive results. I would be very interested to see later reports on these patients, perhaps 6 to 8 years after surgery. My personal feeling is that the recurrence rate in this particular group will be high if objective parameters are evaluated.
8. In the six patients with Shatzki's ring, what was the distance of this ring (in cm) from the incisors and how far was the lower esophageal sphincter (in cm) from the incisors?

I am asking these questions to gain a better understanding of the true origin of peptic esophageal stricture. I am also concerned that laparoscopic results of antireflux surgery in patients with esophageal disease with complications (Barrett's esophagus with stricture or ulcers) are combined with cases of "simple" non-Barrett's esophagus, and follow-up is usually short. I think we must await the real long-term results in all of these different groups of patients.

Attila Csendes, M.D., F.A.C.S.
Clinical Hospital University of Chile
Santiago, Chile

Reply

Dr. Csendes asks some very perceptive questions concerning our article. As was the case in his patients, most of our patients had columnar mucosa distal to the esophageal stricture. In some of these patients, including all of those with Shatzki's ring, the type of columnar epithelium found distal to the stricture was gastric-type epithelium. It is my belief that these latter patients do not truly have Barrett's esophagus. They merely have a stricture forming at the level of the gastroesophageal junction. In general, these patients represent a less severe form of reflux disease than those with dense fibrous strictures.

I think the problem Dr. Csendes alludes to relates to two different methods of reporting lower esophageal sphincter pressure. In our laboratory we use the Castell criteria, in which the lower limit of normal for lower esophageal sphincter resting pressure is 15 mm Hg. The mean of 12 mm Hg is certainly indicative of a hypotensive lower esophageal sphincter in most of our patients with strictures.

We have found endoscopic measurements of esophageal length to be quite unreliable. The endoscopic measurement of the gastroesophageal junction at less than 40 cm does not always indicate that the esophagus will be foreshortened. In the two patients requiring Collis gastroplasty, the distance between the incisors and the gastroesophageal junction was 34 cm in one patient and 38 cm in another.

We use 24-hour ambulatory pH monitoring selectively. Our indications for pH monitoring include patients without evidence of esophageal injury, patients with primarily atypical symptoms, and patients participating in a study. In patients with a peptic stricture, a hiatal hernia, and heartburn responsive to medical therapy, we find it unnecessary to perform 24-hour pH studies pre- or postoperatively. Thus most of the patients in this study did not undergo 24-hour pH pH monitoring.

Esophageal motility studies were not performed routinely in the postoperative period, and thus I have no way of specifically answering this question. Patients in our series were followed symptomatically. In two patients reporting

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no dysphagia, endoscopic surveillance for Barrett's esophagus revealed that the stricture had completely disappeared. In four patients with persistent dysphagia, endoscopy and dilation were performed. In two patients the stricture had improved from the preoperative status, and in two other patients there was no change.

We are in the process of following these patients on an annual basis for 10 years. It is still too early to report the results of late follow-up.

In patients with Shatzki's ring, the ring occurred at the squamocolumnar border. We did not particularly correlate this endoscopically measured distance from the distance of the high-pressure zone measured manometrically.

I share Dr. Csendes' sentiment that patients with advanced esophageal disease may require additional therapy, especially if the esophagus is foreshortened.

John G. Hunter, M.D.
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Atlanta, Georgia

Interval Appendectomy in the Laparoscopic Era

To the Editors:

In a recent paper Surg 1999 by Nguyen, Silen, and Hodin, the thrust of the article is summarized by the following quote:

"Given the minimal morbidity of the procedure, we believe that ILA [interval laparoscopic appendectomy] should be considered for most patients who initially present with a periappendiceal mass."

Thirty-five years ago, I wrote an article on recurrent appendicitis[2] stressing the need for interval appendectomy performed 6 weeks to 2 months after drainage if the periappendiceal inflammatory mass had not been managed completely with appendectomy. I quoted a significant incidence of recurrence, which was our experience at the time, and suggested that early interval appendectomy should be considered in this group. Many of the appendectomies that we subsequently performed were associated with either a stump of the appendix, a fibrinous band, or an appendiceal residue that did not pose a risk to the patient. Over the years it has become well recognized that interval appendectomy is not necessary in the majority of patients who have a periappendiceal inflammatory mass treated by antibiotics, drainage, and observation. One of the procedures that we have found to be most helpful in evaluating these patients is the barium enema. If after 6 weeks a barium enema reveals no residual appendix, then it is most appropriate not to perform subsequent surgery because the risk of recurrent appendicitis is so small. On the other hand, if there is a residual appendix demonstrated on barium enema films, paying careful attention to the filling cecum, then we believe the risk of appendicitis is real and consideration should be given to performing an interval appendectomy after a reasonable period of time, and we strongly agree that the laparoscopic approach is appropriate.

I believe that it is essential to comment on the article by Nguyen et al.[1] because the impression one has from reading it is that interval laparoscopic appendectomy is appropriate in general for patients with periappendiceal inflammatory masses. I do not believe that this is uniformly the case. I believe it is the case in the minority of the patients who have periappendiceal inflammatory masses and only those who have a residual appendix following the appropriate nonoperative intervention.

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Reply

Dr. Befeler suggests that interval appendectomy is not necessary in the majority of patients who initially present with a periappendiceal inflammatory mass treated by antibiotics, drainage, and observation. This issue remains controversial, since recurrence rates for appendicitis have been reported to be anywhere from 0% to 20%, and surgeons clearly differ in terms of their recommendations to these patients. We would certainly agree with Dr. Befeler that a nonoperative approach is reasonable, assuming one is fairly certain that the original presentation was not due to a neoplastic process and the patient understands the potential for recurrent problems in the future.

Dr. Befeler suggests that a barium enema is helpful in these patients; he believes that if the appendix does not fill with contrast medium, then there must be fibrous obliteration and, therefore, minimal if any risk of recurrent appendicitis. Although this is an interesting hypothesis, I wonder if Dr. Befeler is aware of any supporting data. Ironically, nonfilling of the appendix on barium enema has generally been taken as a sign indicative of the presence of appendicitis, usually in the acute setting but even in patients with more chronic symptoms. Clearly this question of the significance, if any, of appendiceal filling on contrast enema should be addressed in a rigorous, prospective manner.

Our main purpose in writing our paper on interval laparoscopic appendectomy was to communicate to other practitioners the fact that this is an extremely simple and safe procedure associated with minimal morbidity. Since the issue of interval appendectomy depends entirely on a risk/benefit ratio, we believe it is important to recognize that the "risk" side has probably been diminished in this new era of laparoscopy.

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Thirty-five years ago, I wrote an article on recurrent appendicitis[2] stressing the need for interval appendectomy performed 6 weeks to 2 months after drainage if the periappendiceal inflammatory mass had not been managed completely with appendectomy. I quoted a significant incidence of recurrence, which was our experience at the time, and suggested that early interval appendectomy should be considered in this group. Many of the appendectomies that we subsequently performed were associated with either a stump of the appendix, a fibrinous band, or an appendiceal residue that did not pose a risk to the patient. Over the years it has become well recognized that interval appendectomy is not necessary in the majority of patients who have a periappendiceal inflammatory mass treated by antibiotics, drainage, and observation. One of the procedures that we have found to be most helpful in evaluating these patients is the barium enema. If after 6 weeks a barium enema reveals no residual appendix, then it is most appropriate not to perform subsequent surgery because the risk of recurrent appendicitis is so small. On the other hand, if there is a residual appendix demonstrated on barium enema films, paying careful attention to the filling cecum, then we believe the risk of appendicitis is real and consideration should be given to performing an interval appendectomy after a reasonable period of time, and we strongly agree that the laparoscopic approach is appropriate.

I believe that it is essential to comment on the article by Nguyen et al.[1] because the impression one has from reading it is that interval laparoscopic appendectomy is appropriate in general for patients with periappendiceal inflammatory masses. I do not believe that this is uniformly the case. I believe it is the case in the minority of the patients who have periappendiceal inflammatory masses and only those who have a residual appendix following the appropriate nonoperative intervention.

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REFERENCES

1. Nguyen DB, Silen W, Hodin RA. Interval appendectomy in the laparoscopic era. J GASTROINTEST SURG. 1999;3:189-193.
2. Befeler D. Recurrent appendicitis. Arch Surg 1968;89:666-668.

Reply

Dr. Befeler suggests that interval appendectomy is not necessary in the majority of patients who initially present with a periappendiceal inflammatory mass treated by antibiotics, drainage, and observation. This issue remains controversial, since recurrence rates for appendicitis have been reported to be anywhere from 0% to 20%, and surgeons clearly differ in terms of their recommendations to these patients. We would certainly agree with Dr. Befeler that a nonoperative approach is reasonable, assuming one is fairly certain that the original presentation was not due to a neoplastic process and the patient understands the potential for recurrent problems in the future.

Dr. Befeler suggests that a barium enema is helpful in these patients; he believes that if the appendix does not fill with contrast medium, then there must be fibrous obliteration and, therefore, minimal if any risk of recurrent appendicitis. Although this is an interesting hypothesis, I wonder if Dr. Befeler is aware of any supporting data. Ironically, nonfilling of the appendix on barium enema has generally been taken as a sign indicative of the presence of appendicitis, usually in the acute setting but even in patients with more chronic symptoms. Clearly this question of the significance, if any, of appendiceal filling on contrast enema should be addressed in a rigorous, prospective manner.

Our main purpose in writing our paper on interval laparoscopic appendectomy was to communicate to other practitioners the fact that this is an extremely simple and safe procedure associated with minimal morbidity. Since the issue of interval appendectomy depends entirely on a risk/benefit ratio, we believe it is important to recognize that the "risk" side has probably been diminished in this new era of laparoscopy.

Richard A. Hodin, M.D.
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no dysphagia, endoscopic surveillance for Barrett's esophagus revealed that the stricture had completely disappeared. In four patients with persistent dysphagia, endoscopy and dilation were performed. In two patients the stricture had improved from the preoperative status, and in two other patients there was no change.

We are in the process of following these patients on an annual basis for 10 years. It is still too early to report the results of late follow-up.

In patients with Shatzki's ring, the ring occurred at the squamocolumnar border. We did not particularly correlate this endoscopically measured distance from the distance of the high-pressure zone measured manometrically.

I share Dr. Csendes' sentiment that patients with advanced esophageal disease may require additional therapy, especially if the esophagus is foreshortened.

John G. Hunter, M.D.
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Atlanta, Georgia

Interval Appendectomy in the Laparoscopic Era

To the Editors:

In a recent paper Surg 1999 by Nguyen, Silen, and Hodin, the thrust of the article is summarized by the following quote:

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Preoperative Biliary Drainage: Impact on Intraoperative Bile Cultures and Infectious Morbidity and Mortality After Pancreaticoduodenectomy

To the Editor:

I am writing to inform you of an apparent duplicate publication in JOURNAL OF GASTROINTESTINAL SURGERY. An article that appeared in the September/October 1999 issue[1] is very similar to one that was published in the August 1999 issue of *Annals of Surgery*.[2]

The authors are identical, as is the time span of the study. A major difference is that despite the same time span of study, the number of patients is larger in the series reported in *Annals of Surgery*. However, the majority of the patients are reported twice, the tables are very similar, and many sentences are repeated word for word. One paper was presented at the Society of Surgical Oncology and the other was presented at the American Gastroenterological Association.

I have also informed the editor of *Annals of Surgery* of this situation. I have no personal quarrel with anyone at Memorial Sloan-Kettering Cancer Center, nor do I seek publicity by having this letter published. I just feel that some of us have trouble getting *any* paper accepted by a prestigious journal. Duplicate publication obviously precludes someone else from reporting his or her work. Of course, the other problem with duplicate publication is that it leads to a perception by casual readers that a certain conclusion is supported by more data than really exist. I do not understand why such respected academic surgeons feel the need to present and publish the same material more than once.

James E. Barone, M.D.
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Stamford, Connecticut

REFERENCES

1. Povoski SP, Karpeh MS Jr, Conlon KC, Blumgart LH, Brennan MF. Preoperative biliary drainage: Impact on intraoperative bile cultures and infectious morbidity and mortality after pancreaticoduodenectomy. J GASTROINTEST SURG 1999;3:496-505.
2. Povoski SP, Karpeh MS Jr, Conlon KC, Blumgart LH, Brennan MF. Association of preoperative biliary drainage with postoperative outcome following pancreaticoduodenectomy. Ann Surg 1999;230:131-142.

Reply

Thank you for the opportunity to respond. We would certainly be concerned if we thought that we were, in any way, a party to "duplicate publication." Perhaps a closer view of the manuscripts would clearly resolve the issue.

Dr. Barone is correct that the authors of both papers are the same, as they are the group studying such problems and the patient group is the same—that is, patients undergoing pancreaticoduodenectomy. However, the association ends there. Perhaps the easiest way to describe this is to examine the data.

In the first paper, "Preoperative Biliary Drainage: Impact on Intraoperative Bile Cultures and Infectious Morbidity and Mortality After Pancreaticoduodenectomy," we examined 161 consecutive patients between January 1994 and January 1997. In the second paper, "Association of Preoperative Biliary Drainage With Postoperative Outcome Following Pancreaticoduodenectomy," we examined 240 consecutive patients. The total patient population, therefore, was similar, as was the time interval studied. The clearest difference, however, is the intentions of the papers. The first one focuses on examining the microorganisms found in the bile at the time of the operation with and without preoperative biliary drainage. The second paper focuses on the actual outcome in patients who have undergone preoperative biliary drainage. To confirm the major differences requires that we examine the data supplied in the tables of each manuscript.

In paper 1 there were eight tables. In paper 2 there were 12. Four of the 12 tables in paper 2 are similar in title to four of the eight tables in paper 1, as they describe demographics; the difference is that in the first paper there are 125 patients in Table II and in the second paper there are 175 in Table II. Similarly, in Table III of paper 1 there are 94 patients examined, and in paper 2 there are 126 patients examined. In Table IV there are 161 patients in paper 1 and 240 patients in paper 2 (Table I). In Table VI, there are 161 patients in paper 1 and 240 patients in paper 2 (Table V).

The remaining tables are *completely* different. Paper 1 focuses on microorganisms in Tables V, VII, and VIII. There is *no* table addressing the issue of microorganisms in paper 2. As it was the microorganisms that were the focus of the first paper, this would seem appropriate.

The second paper provides an extensive analysis of comorbid conditions, preoperative and interoperative variables associated with intra-abdominal abscess, postoperative complications, and postoperative death, along with analysis of patients who only had pancreatic adenocarcinoma. Table XII in paper 2 is an extensive review of the literature that addresses *this* subject.

Although we, like Dr. Barone, would view duplicate publication very seriously, we believe that a review of these two manuscripts would show great differences, and the extensive amount of data provided is quite, quite different. Perhaps one way to look at it is that if the two manuscripts had been combined, would any editor accept a manuscript with 16 tables including, for example, eight nonoverlapping tables that took up four pages of journal text?

In summary, we view Dr. Barone's allegations with great concern, but on this occasion it appears that they cannot be substantiated. We would encourage a more critical review of the two articles.

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