The Value of Consensus

John L. Cameron, M.D., Keith A. Kelly, M.D., Co-Editors

The "truth" in science is based largely on experimentation. Hypotheses are proposed, experiments designed and conducted, data collected and subjected to statistical analysis, and hypotheses supported or refuted based on the data. The truth emerges.

The truth in medical science is derived, in general, from a similar pathway. The management of individual patients with individual diseases, however, must often be done with incomplete data supporting major management decisions. The "truth" may not be known for an individual patient. Judgment, reason, and personal experience must then be employed. Consultation among various physicians is frequently sought, and a consensus achieved about the management of the patient. While imperfect, the approach usually works to the patient's benefit.

In this day and age, unlike a decade or more ago, most alimentary tract problems require a multidisciplinary approach. Gone are the days when a gastrointestinal surgeon managed a patient with an alimentary tract problem alone. Input from gastroenterologists, radiologists, interventionists, medical oncologists, radiotherapists, and individuals from other disciplines is usually required now. Today the alimentary tract surgeon not infrequently consults daily with gastroenterologists, relies heavily on radiologists, and particularly interventionists, and requires input from medical oncologists and radiotherapists when managing a patient. Consensus is part of today's practice.

Obtaining a consensus among experts, the Delphian method, has helped to set directions not only for individual patients but also on a broader scale. The consensus conferences held by the National Institutes of Health (NIH) are a prime example. Laparoscopic cholecystectomy for symptomatic gallstones, operations directed at morbid obesity, and the role of *Helicobacter pylori* in the etiology, pathogenesis, and treatment of peptic ulcers have gained acceptance in our practices, in part, because of the conclusions and recommendations of recent NIH consensus conferences. The consensus of experts continues to have great value for exploring a variety of pertinent topics and then setting state-of-the-art guidelines for all of us to consider.

Our Society, The Society for Surgery of the Alimentary Tract (SSAT), has recently sponsored consensus conferences at our annual meeting during Digestive Disease Week (DDW). Digestive Disease Week is an ideal setting for employing the Dephian approach in gastrointestinal diseases. To start with, numerous experts from the SSAT (surgeons), the American Gastroenterological Association (gastroenterologists), the American Association for the Study of Liver Diseases (hepatologists), and the American Society of Gastrointestinal Endoscopy (endoscopists) are already in one city, at one meeting, at one time. In addition, other experts in other clinical and basic disciplines related to gastrointestinal surgery are also present. Moreover, immediate public response to the presentations of the experts and the consensus formulated by them can take place.

The first such consensus panel sponsored by the SSAT is published in this issue of THE JOURNAL. The panel explored the topic, "Treatment of Hepatic Metastases From Colorectal Cancer." Several papers relevant to the topic were presented by the panelists, the papers were discussed by the panel, and the public responded. A consensus statement was ultimately synthesized by the panel. This represents the first inclusion of this type of material in the JOURNAL OF GASTROINTESTINAL SURGERY. We plan to continue and expand this practice, providing our readers respond positively.

Many areas exist in the management of diseases of the alimentary tract where current concepts of diagnosis and management are in a state of flux. We believe alimentary tract surgeons will benefit from periodic reviews of these areas by experts in the field. The recommendations of multidisciplinary panels on relevant topics in gastrointestinal surgery should be of interest to you, our readers. We welcome feedback regarding the value of consensus.

Treatment of Hepatic Metastases From Colorectal Cancer

*The SSAT, AGA, ASLD, ASGE, AHPBA Consensus Panel**

General Summary

1. The natural history of hepatic metastases from colorectal cancer is dismal without treatment, although occasional, prolonged survival is seen even without treatment.
2. Only a relatively small percentage of patients with hepatic colorectal metastases are eligible for local ablative therapy.
3. New therapeutic modalities should be evaluated by appropriately designed clinical trials when feasible, after initial uncontrolled reports indicate promise:
   a. The treatment of hepatic metastases from colorectal cancer is currently at this stage (i.e., awaiting the outcome of controlled trials).
   b. The need for scientific rigor must be balanced against the individual patient's right to choice and the physician's primary responsibility to the patient's needs. It is important, however, that this caveat not be abused by a zealous, albeit well-meaning physician to obtain consent that is not truly intellectually informed.
4. A preponderance of accumulated *uncontrolled* evidence strongly suggests that carefully selected patients often benefit from local hepatic resection. No such evidence clearly supports any other form of therapy for this condition. However, there is an insufficient *quality* and quantity of data to discriminate which patients should benefit from local hepatic resection or whether a combination of treatment should be chosen. At present there is not a *definitive* picture of the value or lack of value of any of these therapies in the asymptomatic patient, especially when the natural history and the stage of the tumor at the time of proposed intervention are taken into account.
5. There are insufficient data to form a consensus as to how to discriminate those patients who will benefit from complete resection from those who will not substantially benefit. However, *contraindications* for treatment include (a) incomplete control of the primary (colorectal) lesion; (b) extrahepatic metastatic disease (except under extraordinary circumstances); (c) systemic or local factors that render a particular patient at excessive risk for such treatment; and (d) the informed preference of the patient. Moreover, it is clear that there is no evidence that *incomplete* resection is of benefit to the asymptomatic patient. Numerous other prognostic factors do not reliably predict survival and currently available data do not provide the basis for a consensus as to whether or not a particular lesion or pattern of lesions should be resected. Currently, surgical resection is the only method of local ablative therapy with extensive but largely uncontrolled evidence that appears to indicate it is of benefit to these patients. Any alternative form of therapy should be offered as an adjunct to surgery and should be compared with resection alone in those who are eligible for resection.
6. A clear requirement for any further progress, including the design of trials, is a uniformly accepted staging/classification system for such hepatic metastatic disease that takes into account multiplicity, bilaterality, location, surgical accessibility, and other factors to provide a basis for standardization of trials. The Panel recommends the formation of a multidisciplinary study group to address the design of such a classification/stratification system, and subsequently of controlled clinical trials, at the earliest convenience.
7. It is recommended that, whenever possible, all patients treated with local ablative therapy for hepatic metastasis from colorectal cancer should be treated only in the setting of a controlled clinical trial.

Local Hepatic Resection

1. The panel agrees that, given the current state of knowledge, a single, accessible, metachronous metastasis in a good-risk patient with adequate control of the primary lesion and no evidence of extrahepatic disease should be

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*The Society for Surgery of the Alimentary Tract, American Gastroenterological Association, American Society for Liver Diseases, American Society for Gastrointestinal Endoscopy, and American Hepato-Pancreato-Biliary Association.

treated with local hepatic resection, whether or not it is symptomatic. At the other end of the spectrum, local hepatic resection for multiple, inaccessible, asymptomatic lesions in the face of systemic disease is not recommended. It remains unclear, however, where the threshold for surgical excision should be set within this broad spectrum. This requires formal evaluation by controlled clinical trials based on uniform standards of staging.
2. Surgical resection should not be undertaken except at a center where there is sufficient experience to document acceptable mortality and morbidity. By 1996 standards, mortality should be less than 5%.
3. Before a decision is made regarding resection, patients should be assessed, preoperatively and intraoperatively, by state-of-the-art imaging/localization studies to optimally delineate the extent of hepatic and potentially extrahepatic disease.

Cryosurgery

1. There are inadequate data to support a consensus that cryotherapy *alone* is either safe or effective. Such an approach should therefore be employed *only* as a component of a controlled clinical trial.
2. The role of cryotherapy *adjunctive to surgical resection*, although promising, remains to be established by controlled trials. (This is also true for other forms of nonresective local ablative therapy.)

Infusion Pump Regional Hepatic Chemotherapy

1. Although current data show a significant response rate for infusion pump chemotherapy *alone* in the treatment of unresectable metastases limited to the liver, an overall significant improvement in survival has *not* been demonstrated. Therefore this approach is *not* recommended.
2. Emerging evidence from ongoing controlled trials suggests that there may well be a role for infusion pump chemotherapy as *an adjunct to surgical resection*. However, substantial morbidity and even mortality may be associated with this form of therapy.

Systemic Chemotherapy

1. Although it is clear that systemic chemotherapy *alone* is associated with a 10% to 20% clinical, objective (≤50%) response rate, there has been no clear benefit demonstrated with respect to survival or quality of life.
2. Likewise, the benefit of systemic chemotherapy *as an adjunct to surgical resection* of hepatic metastases is also unclear but is currently being evaluated by a number of appropriately designed, prospective, randomized clinical trials.

Recommendations

There is a striking heterogeneity of criteria for classification of patients with hepatic metastases to provide the basis for comparison of trials, or the stratification of patients within a trial. Because many of the standard criteria for classification, including size, multiplicity, bilaterality, and accessibility among others, do not reliably predict prognosis, better criteria are needed, perhaps based on the biology of the tumor.

It is of the utmost importance that a universally applicable classification/staging system be established as soon as possible. Pursuant to this goal, the Consensus Panel recommends the formation of a multidisciplinary, international study group to define such a system. Major funding agencies should be approached for support of what should be a very cost-effective effort. This study should use as its primary end points overall survival, quality of life during the survival period, and the cost-effectiveness of the treatments.

This Consensus Conference represents the initiation as opposed to the completion of an effort to bring a rigorous, evidence-based scientific approach, unfettered by personal, parochial, or economic bias, to a potentially lifesaving therapeutic approach to patients who have heretofore been thought to be incurrable. This potential will be realized quickly and efficiently only by such a multidisciplinary and comprehensive approach.

Natural History of Liver Metastases From Colorectal Carcinoma

Jarle Norstein, M.D., William Silen, M.D.

Colorectal cancer is the fourth most common malignant disease in civilized countries, ranking behind cancer of the lung, breast, and prostate. In the United States it is estimated that 133,500 cases will be diagnosed in 1996. However, in numeric terms, colorectal cancer is the second most lethal malignancy after lung cancer. The number of deaths that will be attributable to colorectal cancer is estimated to be 55,000 in 1996 in the United States.[1] Metastasis to the liver is one of the most common sites of spread in patients who die of the disease. Knowledge of the natural course of the disease is of paramount importance in determining the proper indications for and contraindications to local ablative or systemic treatment of hepatic metastases, especially in elderly and frail patients. Hepatic metastases from colorectal cancer are asymptomatic until late in the course of the disease. There is thus little to palliate, and the only aim of surgical intervention is improved survival, while imposing the least possible suffering on the patient. Moreover, because many of the reports of "success" with local ablative therapy have been in uncontrolled studies, it is important to document the baseline (i.e., without treatment) status of this patient population.

EPIDEMIOLOGY OF LIVER METASTASIS IN COLORECTAL CANCER

Synchronous or Metachronous Metastases

Synchronous metastases are defined as metastases that are present in the liver at the time of the initial diagnosis of colorectal carcinoma, or at the time of resection of the primary cancer. Metachronous metastases are defined as metastases appearing at any time after the operation on the primary tumor. Metachronous metastases are believed to have been present in the liver prior to the primary operation, or seeded during the primary operative procedure,[2] or subsequently from extrahepatic tumor tissue. This implies that the proportion of synchronous or metachronous metastases in a given series will depend on the sensitivity and timing of the diagnostic methods that are used.

Synchronous Metastases

When assessed only by intraoperative palpation of the liver, approximately 15% to 25% of patients are found to have synchronous metastases.[3,4] The widely differing reported incidences, particularly in the older literature,[5] reflect differences in patient selection and possibly in the degree of effort expended to diagnose metastases during palpation and inspection, particularly through a lower abdominal incision. In hospital-based studies from departments of surgery, the incidence of synchronous hepatic metastases will be influenced by referral patterns and by the diagnostic efficiency or screening efforts utilized in the patient base. Population-based studies, which also include patients with advanced disease treated in departments of oncology or diagnosed only at autopsy, will reveal a higher incidence of synchronous liver metastases. Results from three recent population-based studies show remarkably similar incidences of synchronous metastases: 16.3% (Norstein et al., unpublished data), 18.1%,[6] and 19.4%.[7]

Thorough intraoperative inspection and palpation at the primary operation have in some studies been supplemented with autopsy findings in patients dying within 31 days of operation. In Goligher's study[8] from

From the Department of Surgery, Beth Israel Hospital, Boston, Mass.
Reprint requests: J. Norstein, M.D., Department of Surgery B, The National Hospital, N-0027 Oslo, Norway.

St. Mark's Hospital, 5 of 31 patients with rectal cancer, in whom the hepatic surface had been found to be smooth and free of evident secondary deposits at the primary operation, were found to have hepatic metastases at autopsy. Goligher speculated that approximately one in six such patients might have concealed liver metastases. This finding was not corroborated by Hogg and Pack[9] from Memorial Sloan-Kettering Cancer Center, New York, who found that only 5 of 100 patients who died postoperatively had previously unrecognized metastases at autopsy. In three of these five patients the pathologist could not identify the metastases on gross inspection and after sectioning of the liver, but subsequent microscopic study revealed diffuse metastatic cancer. However, none of 45 patients with *colorectal* cancer had concealed metastases in that study. In a study of similar design from Melbourne, Gray[10] found concealed metastases in 6 (7.7%) of 78 patients who died in the early postoperative period but who were believed to be free of hepatic tumor. The majority of these patients had colorectal cancer, but other intra-abdominal primary cancers were included in the study. Newer diagnostic methods such as CT during arterial portography and intraoperative ultrasonography have been reported to detect additional intrahepatic metastases that are not palpable.[11,12] However, CT arterial portography has a high rate of false positive findings because of perfusion defects that may be incorrectly identified as metastases, potentially adversely influencing the treatment of the primary tumor or of hepatic metastases if they are indeed present.[13] The precise accuracy of these techniques will never be validated at autopsy because of the low postoperative mortality (<5%) experienced today. Furthermore, it is possible that lesions might be overlooked even at autopsy if there is insufficient sampling of all portions of the liver. Therefore there is currently no "gold standard" for assessing the incidence of synchronous hepatic metastases.[14]

GROWTH RATE AND AGE OF HEPATIC METASTASES

Metastases, like primary tumors, are believed to grow exponentially in their early phases of tumor proliferation, with a relatively constant time for cell doubling. In experimental systems the rate of growth slows as the tumor enlarges. The concept of a decreasing growth rate modulating the exponential function is described as Gompertzian growth (after the equation originally described by Benjamin Gompertz in 1825).[15,16] Decreasing rate of growth is believed to be relevant for tumors larger than 1 gram (10^9 cells), possibly affecting the majority of *detectable* tumors. In spite of the large number of patients who have hepatic metastases, and the apparent prognostic importance of tumor growth rate, it is surprising that only one study has attempted to establish the growth rate of untreated lesions.[17] In this study the growth of hepatic metastases was assessed from serial CT scans, in most cases from only two observations. The tumor doubling times were found to vary from 48 to 321 days (mean 155 days) in clinically overt metastases, that is, those detected by palpation at operation, and from 30 to 192 days (mean 86 days) in "occult" metastases, that is, those that were not seen nor palpated intraoperatively but were detected by CT scan or ultrasonography postoperatively. Assuming that the growth of the metastases followed a Gompertzian curve, the age of the metastases was calculated. The mean age of the clinically overt metastases was estimated to be 3.7 ± 0.9 years (± standard error of the mean [SEM]) and that of occult metastases to be 2.3 ± 0.4 years (±SEM). (Actually, standard deviation [SD] would be a more appropriate measure of variation than SEM in this setting. Laudably, Finlay et al.[17] have published the original data in the article, permitting us to calculate the standard deviation. The mean age of overt metastases was 3.7 ± 3.1 years [± SD] and that of occult metastases 2.3 ± 2.0 years [± SD].) The difficulties and likely inaccuracy of this method are also illustrated by the estimated age of two metastases in the liver of one patient, one metastasis computed to be 1.3 years old and the other 11.4 years. The latter metastasis, as a single outlier, contributes 0.7 years to the mean age of overt metastases. If the 95% confidence interval of the estimates of tumor age would have been computed, the confidence interval would have included a tumor age of less than zero because of the skewed distribution of data, making a log transformation of the data necessary. Computations on log-transformed data show that the 95% confidence interval of tumor age for the overt metastases was 0.6 to 14.4 years and the 95% confidence interval of tumor age for the occult metastases was 0.4 to 8.5 years. The calculations of tumor age[17] show clearly that there is a prohibitively large variation in the growth pattern of hepatic metastases and predictions with regard to the initial time of development in individual patients will be quite imprecise. The efforts to estimate the age of metastases by observations late in the course of tumor development, which are bound to be imprecise, have been criticized by other authors.[18] Recently the assumption that tumors grow in a reasonably predictable manner according to the Gompertzian curve has been challenged in breast can-

cer.[19] A reappraisal of the actual growth rate, and therefore of tumor age, may significantly influence the attitude toward the election for hepatic resection or observation. Such information should be solicited through appropriately designed studies.

Metachronous Metastases

During the first 5 years after a curative operation for the primary tumor, metachronous hepatic metastases will appear as the initial site of failure in approximately 15% of patients (Norstein et al., unpublished data), but up to 40% of all patients initially thought to be rendered tumor free have been shown to eventually develop hepatic secondary lesions.[20] Therefore the apparent incidence of metachronous metastases will be influenced by the ability to detect synchronous metastases, since initially undetected synchronous metastases will later appear to be metachronous. In one study in which sequential imaging with CT and ultrasonography in the immediate postoperative period was used, 24 of 71 patients with no signs of metastasis at the primary operation were found to have "occult"metastases.[21] However, lesions in 8 of these 24 patients suspected to be synchronous metastases by CT or ultrasonography were reported by the authors to have been found to be benign 4 months postoperatively.[21] In addition to the 16 patients in whom metastases were diagnosed by imaging, one patient who died postoperatively was found at autopsy to have hepatic metastases. Thus 17 (24%) of 71 patients had occult (i.e., synchronous) hepatic metastases shortly after the primary operation. Only three of the remaining patients (4.2%) later developed true metachronous hepatic metastases. This demonstrates the difficulty in designating whether a metastasis occurring relatively soon after operation (within weeks or months) is in actuality a missed synchronous metastasis or a rapidly growing metachronous one.

Measurement of carcinoembryonic antigen (CEA) in gallbladder bile[22] at the primary operation has been proposed as a method for identifying early on those patients who are at great risk for development of subsequent hepatic metastasis. Although the method appears to be sensitive, it has been criticized because it lacks specificity.[23] Changes in the hepatic perfusion pattern[24] at the time of the primary operation indicate that it might be possible to classify patients who will or will not have subsequent metachronous metastases with reasonable accuracy. It might thus seem that the designation of metachronous metastases could be greatly reduced if the perioperative imaging methods were good enough. In the absence of a perfect perioperative imaging system, the follow-up program that is employed will clearly affect the incidence of metachronous metastases at any given point in time. In a prospective study of patients who had no evidence of synchronous metastases on preoperative ultrasonography and intraoperative palpation (Norstein et al., unpublished data), 30% of the metachronous liver metastases that eventually occurred by 5 years were diagnosed within the first 6 months using analyses of CEA levels and ultrasonography.

RISK FACTORS FOR DEVELOPMENT OF SYNCHRONOUS AND METACHRONOUS LIVER METASTASES

Stage of the Primary Tumor

Tumor stage is a strong risk factor for hepatic metastases. Whereas no patients with primary tumor in situ will have liver metastases,[25] the incidence of hepatic metastases increases with increasing depth of infiltration of the primary tumor, as well as with the presence and extent of lymphatic metastases.[20,26]

Venous Infiltration

Tumor infiltration into veins is associated with an increased risk of hepatic metastases.[27,28] However, venous infiltration is somewhat dependent on tumor stage, and controversy exists as to whether venous infiltration is an independent risk factor or merely an expression of advanced primary tumor.[29]

Tumor Location

Several studies have suggested that a higher percentage of patients with colon cancer have synchronous liver metastases as compared to patients with rectal cancer. In a population-based study from Melbourne comprising 1140 patients with colorectal cancer, 20.5% of them had colonic primary lesions, whereas 17.8% of patients with rectal cancer had liver metastases.[7] In a study from Cote d'Or, France, among 1986 cases, the incidence of metastases from colon and rectal cancers was 15.4% and 13.5%, respectively.[6] Although the trend toward an increased incidence of metastases from colon cancer was the same, none of these differences were statistically significant. In a large autopsy study Pickren et al.[30] found that when the cancer occurred in organs where blood is drained by the portal circulation there was a higher incidence of liver metastasis. In their study 242 (56.8%) of 426 patients with cancer of the colon at autopsy had hepatic metastases, whereas 141 (45.9%)

of 307 patients with cancer of the rectum had metastatic spread to the liver ($P < 0.005$). The presumptive biologic explanation for this difference is the variation in venous drainage from colonic and low rectal cancers, with low rectal cancers draining partly to the systemic circulation.

Absence of Metastases to Cirrhotic Livers

A puzzling finding is the virtual absence of metastasis to cirrhotic livers,[31,32] effectively eliminating cirrhosis as a contraindication to hepatic resection. Uetsuji et al.,[32] in a series of 250 patients with colorectal cancer, found no cases of cirrhosis among 40 patients with liver metastasis, but cirrhosis was present in 46 (21.9%) of 210 patients without metastases ($P < 0.001$). It was speculated that the reduced portal blood flow might be responsible for this phenomenon.

NATURAL COURSE OF THE DISEASE: PROGNOSIS AND PROGNOSTIC FACTORS

Resection of the Primary Tumor: Existence of Extrahepatic Disease

The median (which was not much different from the mean) survival time for patients with synchronous liver metastases is 6 to 12 months after diagnosis at the primary operation (Table I). There has been longstanding disagreement as to whether removal of the primary lesion significantly influences survival in those patients who have synchronous metastases. Most studies show that patients whose primary tumors have been resected have a better prognosis than those who have not received treatment directed at the primary lesion. Some authors have argued that since resection of the primary tumor is more likely to be carried out in patients with less extensive hepatic involvement, the improved survival in patients whose primary tumors have been removed is only an expression of this selection bias, whereas the prognosis is actually determined by the extent of hepatic metastasis.[33,34] Early studies attempting to control for extent of hepatic involvement failed to demonstrate a survival benefit for primary tumor resection. More recent studies utilizing multivariate techniques, however, have indicated that such a benefit exists[35] (Norstein et al., unpublished data). Extrahepatic disease in patients having had a locally radical operation for the primary tumor has been shown to have an independent unfavorable effect on survival, when the percentage of hepatic replacement by tumor is taken into account,[36] and in multivariate analyses.[35,37]

Number of Hepatic Metastases and Proportion of Liver Involved

The patient's prognosis is largely dependent on the number of hepatic metastases and the proportion of liver replaced by tumor.[35-39] In patients with solitary or unilobar metastases, the median survival is 10 to 21 months[37,40,41] (Norstein et al., unpublished data; see Table I). Very few patients with unresected liver metastases live longer than 3 years after diagnosis of the presence of hepatic metastases, irrespective of the degree of liver involvement. Survival beyond 5 years, although very unusual, is described both in untreated patients[42] and in patients treated with chemotherapy.[43] Histologic verification is crucial for the credibility of such reports of long-term survivors, however. In one study, which reported five patients (not histologically verified) who survived for more than 5 years with untreated, synchronous hepatic metastases, the diagnosis was not substantiated in two of them at subsequent laparotomy up to 69 months later.[44] A much-cited preliminary report from a prospective study conducted by Wood,[45] published in 1984, claimed that 16% of prospectively followed patients with solitary metastases were alive 5 years after diagnosis. However, this figure was apparently derived from 2 of 15 patients. No further publications have emerged from this study, which has since been abandoned.[41] In contrast to patients who have undergone liver resection, where several recent studies show that the survival curve reaches a plateau,[46-48] the small number of unresected true 5-year survivors most often die of their disease shortly thereafter. The longest surviving patient who has undergone resection of the primary tumor but had no treatment of the hepatic metastases was a well-documented case who lived for more than 10 years before he died of disease.[42] Another patient lived 14 years with chemotherapy.[49]

Resectability

Only five studies[40,41,46,50] (Norstein et al., unpublished data; Table II) have related prognosis to perceived resectability of the hepatic metastases in patients who did not undergo resection. Potentially resectable cases had a median survival of 14 to 24 months in these studies, which was significantly longer than the survival in cases judged to have unresectable hepatic metastases.

Laboratory Tests and Tumor Markers

Alkaline phosphatase level has, in most but not all[37] studies, emerged as an independent prognostic factor

Table I. Survival in some studies of synchronous liver metastases

Reference	No. of patients	Metastatic load/procedure	Median* survival (mo)	Survival rate (%) 1 yr	3 yr	5 yr	Actual survivors at 5 yr
Bacon and Martin (1964)[42]†	110	Any					
	25	Colostomy	6.7				
	25	No resection	6.5				
	10	Bypass	9				
	50	Palliative resection	15				
Jaffe et al. (1968)[33]‡	177	Any	**5**				
	14	Exploration	**1.5**				
	80	Palliative diversion	**4.5**				
	60	Primary resection	**5.5**				
Bengmark and Hafstrom (1969)[4]§	38	Any	**5.7 (7.8†)**				
Cady et al. (1970)[69]	269	Any	**13**				
Abrams and Lerner (1971)[34]§	58	Any	**5.9**				
Nielsen et al. (1971)[53]†§	103		NS				
Primary resected	7	Multiple	**5**				
	5	Several	**9**				
	20	Few	**18**				
Palliative/exploratory operation	50	Any	**5**				
No operation	15	Any	**1**				
Baden and Andersen (1975)[44]‖¶	105	Any	10				5¶
Wood et al. (1976)[40]	113	Any	**6.6**				
	87	Widespread	**3.1**	5.7	0		
	11	Unilobar	**10.6**	27	9.9		
	15	Solitary	**16.7**	60	13.3	8	1
Morris et al. (1977)[27]‖	49	Any	11.4	45	NS		1
Wanebo et al. (1978)[50]†	217						
	149	Nonresectable	7	25	2		
	18	Liver resectable	19	72	17		
	25	Liver resection	36	84	36	28	
Boey (1981)†	14	Not operated	1				
	63	Primary resection	7.5				
	5	Liver resection	15				
Bengtsson et al. (1981)[70]§	25	Any	4.5 (6.1†)				
Goslin et al. (1982)[54]‡§#	125	Any	12.5				
	87	≥4 mets	10				
	38	<3 mets	24				
Lahr et al. (1983)[71]‡§	175	Any	6.1				
	110	≥4 mets	4.1	19			
	19	2-4 mets	12.7	58			
	28	Solitary	11	46			
	4	Liver resection	4.5				

NS = not stated.
*Mean survival in bold face.
†Operative mortality excluded.
‡Synchronous and metachronous metastases.
§Including patients where the primary tumor was not resected.
‖All patients had their primary tumor removed.
¶Twenty-six cases with uncertain metastases, 5-year survivors without histologic proof of metastases, two 5-year survivors proven not to have liver metastases.
#Only nine patients did not receive chemotherapy.

Table I. Survival in some studies of synchronous liver metastases—cont'd

Reference	No. of patients	Metastatic load/procedure	Median* survival (mo)	Survival rate (%)			Actual survivors at 5 yr
				1 yr	3 yr	5 yr	
Wagner et al. (1984)[41]‖	252	Any	NS				
	182	Widespread	NS				
	31	Multiple, unilateral	15				
	39	Solitary	21	20			
	70	Liver resectable	NS				
Wood (1984)[45]	76	Widespread	NS		0		
	13	>1, unilobar	NS		0		
	15	Solitary	NS		16	2	
Finan et al. (1985)[38]	90	Any	10.3				
	65	Multiple	8.0				
	21	1-2 mets	15.5				
Palmer et al. (1989)[72]	30	Any	12				
Scheele et al. (1990)[46]	921	Any	6.9				
	62	Liver resectable	14.2				
Stangl et al. (1994)[37]†‡	484	Any	7.5	31	3	1	
	320	≥8	5.9	21	1	0	
	90	2-7 mets	12.5	52	7	4	
	74	Solitary	10.8	29	6	0	
Rougier et al. (1995)[35]‡	544	Any	NS				
	420	≥3	6.0				
	110	1-2	9.9				
Norstein et al. (1996)	177	Any	6				
	161	Multiple	6	22	0	0	
	10	Liver resectable	18	80	0	0	
	6	Liver resection	25	83	33	33	

Table II. Studies of natural history of patients with resectable metastases that were not resected

Reference	No. of patients	Metastatic load	Median* survival (mo)	Survival rate (%)			Actual survivors at 5 yr
				1 yr	3 yr	5 yr	
Wood et al. (1976)[40]	11	Unilobar	**10.6**	27	9.9		
	15	Solitary	**16.7**	60	13.3	8	1
Wanebo et al. (1978)[50]†	18	Liver resectable	19	72	17		
Wagner et al. (1984)[41]	31	Multiple, unilateral	15				
	39	Solitary	21	20			
Scheele et al. (1990)[46]	62	Liver resectable	14.2				
Norstein et al. (1996)	10	Liver resectable	18	80	0	0	

*Mean survival in bold face.
†Operative mortality excluded.

for survival in patients with unresected hepatic metastases. The degree of elevation of the CEA level at the time of diagnosis of hepatic metastases has also been identified as an independent prognostic factor in univariate analysis in most studies but has failed to achieve significance in multivariate analysis in one study.[35]

NATURAL COURSE OF METASTATIC DISEASE WHEN METASTASES ARE DETECTED WITH IMAGING METHODS: SELECTION BIAS

With newer diagnostic methods allowing earlier diagnosis, the survival from the time of diagnosis of hepatic metastases may be artificially increased by lead time bias. This will affect both untreated and resected patients. The apparent survival benefit of hepatic resection has been questioned because of this bias.[51] Few recent studies applying modern diagnostic methods have followed the natural course of unresected but technically resectable metastases[35,37] (Norstein et al., unpublished data). The true natural course today is thus difficult to tell, "with so little left to nature."[52] Furthermore, concomitant with the advent of more accurately estimated extents of hepatic involvement by modern diagnostic methods, hepatic resections have also become safer and more prevalent, thus selecting away favorable cases and blurring the assessment of prognostic factors. In the recent large study by Stangl et al.,[37] *untreated* patients with *solitary* metastases paradoxically had a worse survival than patients with two to seven metastases. The most likely explanation for this finding is that patients with solitary metastases had already been selected for resection, leaving only the most unfavorable patients in the natural history group. In a large prospective study of unresected synchronous and metachronous metastases,[35] the survival for patients with one or two metastases reported as a single group (survival of solitary metastases was not reported separately) was a median of 9.9 months, which was worse than that of patients with one or two metastases in previous studies.[38,40,41,53,54] This clearly demonstrates the effect of selection bias when patients with the most favorable prognosis undergo hepatic resection and analyses of natural history are carried out only on the remaining (unresected) patients.

OPERATIVE RISK AND EXTENT OF RESECTION

For patients with a reasonable operative risk, resection seems to provide a definite survival benefit. In most recent reports the operative mortality following resection of hepatic metastases is less than 3%, and the 5-year survival is greater than 25% as compared to only occasional 5-year survivors in studies of unresected cases.[42] These risk figures would probably be changed greatly if the required resection was a trisegmentectomy. Although the most recent reports from expert centers show operative mortality rates for trisegmentectomy approaching 0%,[55] operative mortality within 30 days of that procedure has exceeded 6.3% until recently in the same centers.[56] Other centers reported figures as high as 25% in a recent series,[57] and an even higher postoperative mortality (3 of 4) was previously reported in a small study.[58] Traditional criteria for operative mortality are no longer appropriate in the age of intensive care units, however, where death may be delayed well beyond the 30-day limit and can still be attributable to the surgical procedure. Iwatzuki and Starze[56] have thus attributed five "late" deaths (within 60 days) to trisegmentectomy, raising the operation-related mortality rate from 6.3% to 9.9%. The operation-related mortality in the most recent study in which trisegmentectomy was studied was increased from 25% at 30 days to 50% within 65 days.[57] Five-year survival after trisegmentectomy is in some series at 0%,[57] and in no series does it exceed 21%.[55] No studies of the natural course of liver metastasis have specifically analyzed patients who would have required a trisegmentectomy. Serious doubt exists as to the utility of this procedure, and the indications should be very restricted with regard to selection of patients as well as performing centers.

PROGNOSTIC SIGNIFICANCE OF GENOTYPIC OR PHENOTYPIC CHARACTERISTICS OF HEPATIC METASTASES

The prognostic significance of tumor genotypic or phenotypic characteristics has not been extensively investigated in patients with unresectable lesions because of their generally unfavorable prognosis. Ploidy has been proposed and investigated as a possible prognostic factor in unresectable metastases but no definite conclusion has been reached.[59]

PERFORMANCE STATUS AND QUALITY OF LIFE

Some studies have introduced performance status of the patient as a factor that affects the natural course of unresected disease, with significantly better prognosis in terms of survival if the performance status is favorable.[35,54] However, performance status is affected both by the metastatic process and by the patient's general health. Although this parameter may be of

great value in the assessment of prognosis (i.e., in terms of length of survival) in technically unresectable cases, it should be used with caution to provide guidance for selecting patients who might benefit from resective treatment. For example, a good performance status would indicate a relatively favorable prognosis if the patient were left untreated, but as an acceptable operative risk who stands to benefit from resection; the patient is also at risk for postoperative morbidity, mortality, and a diminished quality of life as a result of the operation. Conversely, a poor performance status could be due to advanced but resectable tumor (unlikely but possible), and thus poor performance status could result in denial of an operation from which a particular patient might benefit.

A formalized quality-of-life assessment has been claimed to be a better predictor of survival than tumor size[60] in patients with unresectable disease. A poor quality of life with poor survival is hypothesized to reflect the influence of a putative tumor product.[60] A recent study of hepatic artery chemotherapy infusion in patients with unresectable metastases concluded that 95% of *control* patient survival time was spent with normal quality-of-life scores.[61] This underscores the fact that the goal of nonresectional (as well as resectional) therapy is to achieve added survival time while maintaining good quality of life.

DYNAMICS OF THE METASTATIC PROCESS: THE TEST OF TIME

In most patients with liver metastases, the liver is not the only site of tumor tissue. When a patient is considered for hepatic resection, it is of the utmost importance to identify extrahepatic tumor and to determine whether the part of the liver destined to remain after resection might harbor cancer tissue. As an alternative to extensive and costly investigations with limited sensitivity, some surgeons have chosen to wait several months after identifying a resectable metastasis to see whether further metastases in the liver or extrahepatic tumor deposits might appear.[52,62] The philosophy behind this expectant attitude is to some extent based on Gompertzian kinetics. Within months, smaller and initially undetectable tumors, growing rapidly and possibly exponentially, will have time to catch up with the larger and more slowly growing index metastases. Thus the practice of waiting for several months could spare the patient the risk of operative death or complications of a futile operation. The obvious disadvantage of this approach is that the index lesions may also grow, thus making the surgical procedure more risky and less likely to yield safe margins. Moreover, tumor tissue may spread as metastases from hepatic metastases (tertiary metastases,[52,63] cascade spread[64,65]) to hilar glands, to the lungs, or to more remote locations during the waiting period. Two recent developments may result in the abandonment of this practice of observation in the near future. With the advent of the concept of hepatic reresections, subsequently appearing tumors in the liver may yet be removed,[66] with survival prospects approaching those reported after the initial hepatic resection for metastases. Extrahepatic (as well as intrahepatic) tumor may be identified with greater accuracy by means of positron emission tomography scanners, which are not yet widely available.[67]

CONCLUSION

Hepatic resection is technically feasible in approximately 10% of patients with synchronous metastases and in 20% to 25% of patients with metachronous metastases. The natural course of the disease is thus relevant for most patients. Survival of patients with hepatic metastatic disease beyond 5 years in the absence of liver resection has been reported but is very rare. Only a handful of cases are described in the world literature. The survival curve following hepatic resection levels off at approximately 25% to 30% in most recent studies.[46,68] The vast majority of patients surviving 5 years, and all patients surviving 5 years free of disease will thus consist almost entirely of those who have been resected. On the other hand, 70% to 75% of patients undergoing liver resection will die within 5 years, and the lives of some patients will have been shortened or made more uncomfortable by the operation.

In this predominantly elderly population, great care should be taken not to impose undue risks on the patients. The natural course of the disease in potentially resectable patients is such that the operative mortality rate must be low, probably not exceeding 3% to 4%, and the expected survival curve should be significantly different from that determined by the natural course. This implies that the general condition of the patients should predict a life expectancy of at least 18 to 24 months if hepatic resection is contemplated. For elderly patients the prospect of a 25% 5-year survival may be less attractive if there is a considerable risk of immediate death or suffering. Palliation is seldom an issue, liver metastases generally being asymptomatic until late in the course of the disease, and death is in most instances less painful than other cancer deaths. The fatigue experienced after major liver surgery may further diminish the quality of life during a period of life where the marginal utility of a patient's life may be rapidly decreasing as a result of increasing disability and competing causes of death.

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Resection of Colorectal Liver Metastases Revisited

Johannes Scheele, Claudia Rudroff, Annelore Altendorf-Hofmann

Surgical resection has evolved into the treatment of choice for colorectal liver metastases.[1] Since 1980, single-institution series exceeding 100 patients have reported 5-year survival rates ranging from 22% to 48%,[2-13] and rates from collected series have ranged from 21% to 33% (Table I).[14-16] In our own experience of 376 patients who underwent potentially curative resections, the 5-year survival rate was 39% with 34% of patients free of disease (Fig. 1).[17]

Optimized results in recent years may be the result of improved preoperative imaging techniques, increasing operative expertise, and optimal tailoring of the procedure to each patient's individual needs. Certainly a great deal of selection bias is also involved. The development of indications for and contraindications to resection of metastatic colorectal cancer has, nevertheless, shown a continuous liberalization of proposed selection criteria. Therefore a current viewpoint should address general inclusion and exclusion guidelines, appropriate preoperative workup, timing of liver resection, therapeutic options in cases of tumor recurrence, and the balance between operative risks and prognostic benefits.

INDICATIONS FOR LIVER RESECTION

Historical Aspects

When the technique of liver resection for colorectal metastases was first introduced in early anecdotal reports, the indication was restricted to true solitary metastases.[18,19] At that time, when the liver was still regarded as the "no man's land of surgery,"[20] it seemed to make perfect sense that advanced age, extrahepatic disease, and any resection requiring more than a right hemihepatectomy should probably preclude surgical treatment.

During the 1980s, however, resection of multiple lesions became increasingly accepted.[21] One of the first studies, published by the Mayo Clinic in 1984, demonstrated identical survival curves in 104 patients resected for solitary lesions and 37 patients who had multiple lesions removed with 5-year survival rates of 25% and 18%, respectively.[3] Even in the late 1980s, however, many reports were still reluctant to advocate surgery for bilateral disease.[3,22-26] In our own series, several analyses since 1990 have consistently failed to demonstrate any prognostic significance when solitary, multiple unilateral, and multiple bilateral metastases were compared[1,5,6] (Fig. 2). Because many of the conflicting results in major series may have been due to differences in statistical analysis rather than variations in clinical results, a consideration of appropriate principles of statistical evaluation may be of value for further discussion.

Principles of Current Statistical Evaluation

Besides the total number of patients involved, the median and minimum follow-up times and the number of patients lost to follow-up must be presented. Similarly, the number of patients in various subgroups is essential. Because 5-year (tumor-free) survival seems to be a major criterion of therapeutic success after surgical treatment of colorectal metastases, the number of patients with a follow-up period of 5 years or more should be balanced against those who have actually survived (without cancer relapse) this period of time (i.e., actual survival).

The predictive value of various factors involved in patient survival following hepatic resection shows great variation among different publications. When a univariate approach is used, if the sample of patients is too small, either in total or in a specific subgroup, it may be inadequate to assess the impact of potential prognosticators. In turn, as the number of persons involved increases, a growing proportion of factors will appear to be significant without actually having an independent impact on the prognosis. This basic statis-

From the Department of Surgery, Friedrich-Schiller-University Jena, Germany.
Reprint requests: Prof. J. Scheele, Department of Surgery, Friedrich-Schiller-University, Bachstr. 18, 07740 Jena, Germany.

Table I. Prognosis after resection of colorectal liver metastases

Reference	Year	R class	No. of patients	5-year survival (%)	Median survival (mo)
Institutional series					
Adson et al.[3]	1984	NS	141	25	21
Ringe et al.[4]	1990	0	119	27	35.1
Doci et al.[7]	1991	0	100	30	—
Herfarth and Hohenberger[8]	1992	NS	161	20-30	28
Rosen et al.[9]	1992	NS	280	25	—
Sugihara et al.[10]	1993	0	109	47.9	—
Gozzetti et al.[11]	1994	NS	108	28.0	—
Gayowski et al.[12]	1994	NS	204	32	33
Scheele et al.[17]	1996	0	376	39	41.3
Collected series					
Hughes et al.[14]	1988	NS	859	33	—
Nordlinger et al.[15]	1992	NS	1818	26	—
van Ooijen et al.[16]	1992	NS	118	21	—

R class = resection classification.
NS = not specified, including nonradical resections.

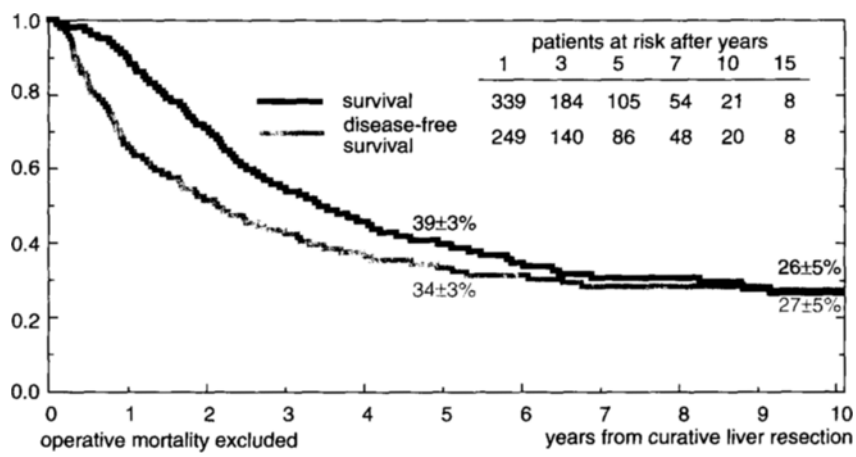

Fig. 1. Survival and disease-free survival in 376 consecutive patients undergoing "curative" (R0) resection of colorectal liver metastases between 1961 and 1993, with complete follow-up until January 1, 1996. (Adapted from Scheele J, Altendorf-Hofmann A, Stangl R, Schmidt K. Chirurgische Resektion kolorektaler Lebermetastasen: Goldstandard für solitäre und resektable Herde. Swiss Surg 1996;Suppl 4:4-17.)

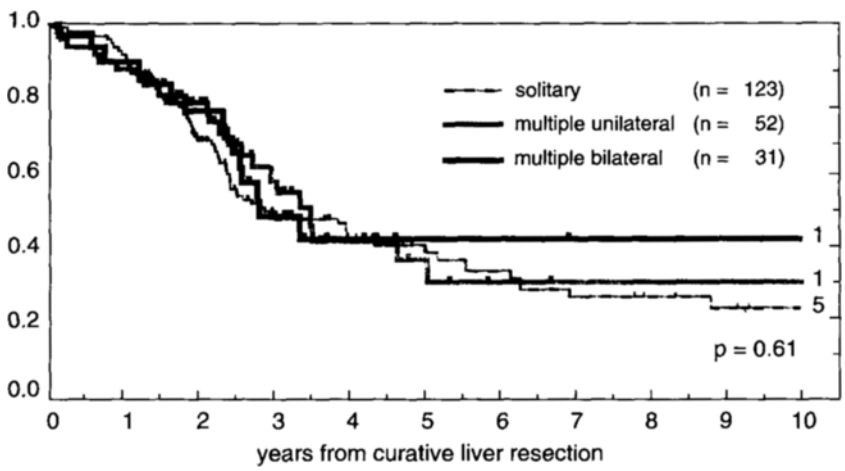

Fig. 2. Influence of number and distribution of colorectal liver metastases on survival in 206 consecutive patients undergoing "curative" (R0) resection between 1961 and 1988, with complete follow-up until January 1, 1990. (Adapted from Scheele J, Stangl R, Altendorf-Hofmann A, Gall FP. Indicators of prognosis after hepatic resection for colorectal secondaries. Surgery 1991;110:13-29.)

tical rule mandates a multivariate analysis but also requires a clear definition of which patients should and, more important, which ones should not be enrolled in a particular analysis.

In assessing the *overall effect* of a particular therapeutic approach, such as resection of colorectal liver metastases, actual and actuarial (tumor-free) survival figures, as well as median survival times, should be represented by "intent to treat" and should include operative deaths. This most accurately reflects the risk:benefit ratio of this strategy. In assessing the *impact of various factors on the prognosis* of patients who have undergone curative resection, however, this group definition needs to be modified. The radicalness of the procedure (R0 vs. R1/2 resection) is the overwhelming predictor of prognosis in that an R0 resection is an almost absolute prerequisite for 5-year survival.[1,4,10,12] Therefore any nonradical resection should be excluded from this particular analysis, as should operative deaths.[27,28] Otherwise, assessment of the biologic behavior of a particular tumor variable might be obscured by surgical failure.

Indicators of Prognosis

Demographic Features. Most authors would agree that sex does not significantly affect the outcome.[4-6,17] A few series report superior results for female patients,[3,29,30] whereas others report prolonged (disease-free) survival in men.[12,31] Age may influence the operative risk as well as patient selection but is unlikely to influence survival in those who make it past the early postoperative period.[1,4,17,32-34]

Primary Tumor. Location in the rectum,[17] mesenteric lymph node metastases,* and poor differentiation[1,17] are all associated with inferior results. As the effects of staging and location are predominantly apparent in synchronous metastases,[6,17,37] differences in the proportion of metachronous lesions may explain inconsistencies in results among various large series.† Other investigators may have missed the effect of mesenteric lymph node involvement on synchronous lesions by summarizing those patients as group "Dukes' D" with no further subcategorization according to the pN classification.[32]

Metastases. Multiplicity of lesions[3,7,34-36,42] and the intrahepatic tumor distribution[6,27] both seem to be of minor importance. Variations in results among reports addressing this aspect may often be due to inclusion of nonradical procedures. It is fair to assume that nonradical resections become more frequent as the number of lesions resected increases and when bilateral lesions are present. Satellite metastases proved particularly important in the Jena/Erlangen experience[1,6,17] and were also significant in other series.[9,27,43] They may indicate an adverse tumor biology with a higher potential for vascular invasion leading to spread, irrespective of whether the main lesions are single or multiple.[17] Interestingly, the proportion of subsequent pulmonary metastases was doubled in patients with satellite nodules.[1] This observation however, conflicts with data from Cobourn et al.[44] and Langer,[45] who found satellite nodules to be insignificant when they accompanied otherwise solitary metastases. In contrast to the number of metastases, the maximum diameter is usually a reliable indicator of prognosis,[4,17,46-49] as is the percentage of liver volume replaced by tumor, with no 5-year survivors among four[4] and five patients[27] with more than 50% of liver volume replaced by tumor, respectively. There are, however, some series that failed to demonstrate a significant influence of maximum tumor diameter on survival.[10,27,32,42,47]

Some authors have reported extremely poor results in patients resected for synchronous liver lesions and therefore either generally question the value of resecting synchronous metastases[50] or restrict the indication to solitary lesions.[8] Others have found similar* or slightly inferior results in comparison to metachronously detected lesions,[1,5,6,10,17] with 5-year survival rates ranging from 13.6% to 32%.[1,17,42,52-54] In one large series, synchronous presentation of metastases was associated with a favorable prognosis.[9] After all, the timing of metastasis detection should not determine the principal therapeutic approach.

The importance of the disease-free interval is still under discussion. Some authors found improved survival with an extended time interval between primary tumor resection and detection of liver metastases.[36] In some of these reports, however, patients with synchronous metastases are assigned to the "short interval" group.[12,15] If only metachronous metastases are included, no prognostic significance was detected in the Jena/Erlangen series comparing disease-free intervals of 12 months or less, 13 to 24 months, and more than 24 months, respectively.[17] There were also no such differences in other reports comparing disease-free intervals of more than or less than 12 months.[14,32]

Therapeutic Approach. The optimal mode of liver resection remains controversial. Most authors failed to detect any significant difference between anatomic and nonanatomic procedures, whereas others reported superior results for minor resections usually performed in a nonanatomic manner.[32] In the

*References 3, 6, 7, 15, 25, 34-36.
†References 1, 4, 17, 27, 31, 38-41.

*References 7, 24, 29, 34, 35, 42, 51.

Jena/Erlangen experience the anatomic approach reduced the incidence of histologically nonradical (R1) liver resections.[55-57] Even within the R0 group, however, survival using this technique was significantly better if it was compared with that in nonanatomic resections. Among anatomic procedures, there was no difference between standard resections and more sophisticated segment-oriented resections.[1,17]

Operative blood loss and subsequent hypotension have both been reported to result in significantly lower survival rates.[9,32,58] Only one series, however, proved to have an independent impact on prognosis applying a multivariate analysis.[16] Others found no effect even with the use of a univariate approach.[59] It is therefore unclear whether excessive blood transfusions, per se, impair the prognosis or simply reflect a more complex procedure facing a greater tumor load, which indicates a less favorable "tumor biology." In the Erlangen series,[17] a blood loss of 2000 ml or more was associated with decreased survival using univariate analysis, whereas blood transfusions had no significant influence (Scheele J, unpublished data).

Survey of Potential Contraindications

In their 1986 landmark publication, Ekberg et al.[27] proposed three general contraindications to liver resection for metastatic colorectal cancer: (1) the presence of four metastases or more, (2) additional extrahepatic disease, and (3) a resection margin of less than 1 cm.

One of the problems with this report was its inclusion of nonradical procedures. Nevertheless, the "Ekberg criteria," occasionally referred to even before the report was published,[24,31] have been frequently cited by subsequent authors.[14,27,31,35,60] In turn, the presence of one to three liver metastases, irrespective of unilateral or bilateral distribution, that can be removed with a safety margin of 1 cm or more is now generally advocated as a clear indication for liver resection, as long as extrahepatic disease is not present at the same time.

With respect to the maximum number of metastases, several authors have since reported long-term success in patients with four, five, or even more independent hepatic lesions. Nordlinger et al.[42] described 14 patients in whom five or more metastases were removed with clear margins. Their survival followed the same course as that seen in 36 patients who had two, three, or four metastases resected. Although less than 15% of the patients in each group were alive at 5 years, survival of more than 10 years was observed in some patients who had five or more lesions removed. In the 1989 update of the collected series of Hughes et al.,[36] 5-year survival and tumor-free survival were both reduced after resection of four or more metastases (18% and 14%, respectively), if compared to one or two lesions but were better than the rates after removal of three nodules (8% and 0%, respectively).[36] Recently Kawasaki et al.[61] described five patients with between 3 to 12 bilateral metastases who underwent liver resection after right portal vein embolization. Four patients remained alive and free of disease 36 to 74 months after surgery. In our series no difference was found in comparing one to three vs. four or more independent metastases that were completely removed (R0 resection). The group with four or more metastases comprised 35 patients, 15 of whom have survived 5 years, 14 without recurrent disease (Fig. 3).[17]

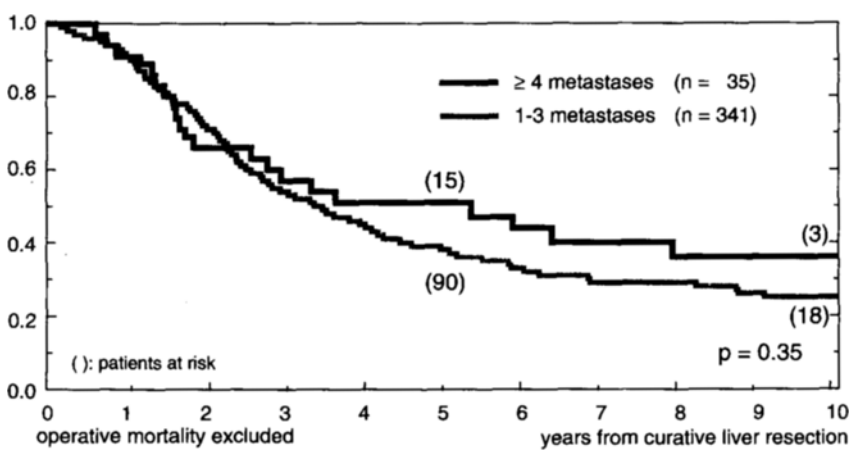

Fig. 3. Survival after R0 resection of four or more independent liver metastases does not differ from results in patients who had one to three metastases removed. (Adapted from Scheele J, Altendorf-Hofmann A, Stangl R, Schmidt K. Chirurgische Resektion kolorektaler Lebermetastasen: Goldstandard für solitäre und resektable Herde. Swiss Surg 1996;Suppl 4:4-17.)

There is certainly a technical limit to resectability, particularly if randomly distributed lesions are considered. In articles addressing resection of more than 10 metastases, it is occasionally difficult to assess the actual number of lesions in the long-term surviving subset, or long-term survival is restricted to patients with smaller numbers of lesions.[61,62] In a recent article by Sugihara et al.,[10] 15 patients with five or more metastases underwent "curative" resection, of whom 11 were alive at the end of the study, seven of them without recurrent disease. Of this disease-free group, five patients had surpassed the 4-year mark and two had survived longer than 10 years. There was, however, only one patient with 12 metastases at 55 months and one additional patient with six metastases at 43 months. All other patients, in particular the 5-year survivors, had five metastases resected.[10] In our series only two of 404 patients undergoing a curative resection had eight or more metastases resected; one of them became a long-term survivor (Scheele J, unpublished data).

More refined preoperative imaging techniques and the increasing application of intraoperative ultrasonography have improved diagnostic accuracy in recent years such that a redefinition of guidelines for future therapeutic decision making is needed. These guidelines should take into account the size, site, number and distribution of lesions in the liver, the presence of other factors known to indicate less favorable outcome, and obvious factors such as age and general condition. This may foster a situation where international, multicenter prospective trials of adjuvant and alternative treatment modalities can be more easily standardized. Any final assessment of the importance of an upper limit of lesions defining resectability would still be premature. It should be recognized that the number of patients who actually undergo curative resection of four or more metastases is still small but 5-year disease-free survival is at least possible.

Less attention is usually paid to satellite metastases. This may reflect their relatively low incidence of 14% to 24%[17,27,63] and the problem of unequivocally classifying a given lesion as being either independent or a satellite. In analyzing our material, a satellite metastasis was defined as (1) positioned in the same Couinaud segment[64] *or* less than 2 cm from the larger nodule, and (2) presenting with a diameter of less than 50% *and* less than 4 cm, even if associated with a giant main lesion.[6] In fact, all but one of our satellite metastases measured less than 1 cm in size. On the basis of this definition, satellite metastases have, in our more recent analysis, proved to be the most important single adverse prognosticator. However, there are meanwhile some long-term survivors despite satellitosis, and therefore even this criterion may not serve as an absolute contraindication to resection.[1,17]

The most generally accepted contraindication to liver resection is the presence of extrahepatic disease.* One of the very early reports that addressed this issue was presented in 1984. Extrahepatic disease precluded 5-year survival and lowered the actual 2- and 3-year survival rates to 36% and 11%, respectively, which was significantly lower than the figures of 72% and 63%, respectively, for patients without extrahepatic involvement. In the collected series of Hughes et al.,[36] patients with extrahepatic disease had a 20% 5-year survival rate but only 4% were free of disease at that time.

Our group has repeatedly reported more encouraging results. Although significantly inferior with respect to both survival and disease-free survival, 9 of 35 patients with extrahepatic disease and a follow-up time of more than 60 months have become 5-year disease-free survivors and 5 of 20 additional patients with follow-up periods ranging from 24 to 59 months are presently alive and free of recurrent tumor (Fig. 4).[17] Looking at them in greater detail, all these successfully treated patients had either local recurrent disease, direct tumor invasion of adjacent structures, or a solitary pulmonary metastasis as the site of extrahepatic tumor (Scheele J, unpublished data). Elias et al.[68] reported a 5-year disease-free survival of 33% among 11 patients in whom either lung metastases (n = 5) or pedicular colonic nodes (n = 6) were removed along with the hepatic resection. Gough et al.[69] presented one anecdotal 5-year disease-free survivor among nine patients with metastases removed from both organs. A similar combination of liver plus lung metastases, or liver plus locally recurrent disease, has been found acceptable in some recent articles on repeat hepatic resection, if both operations are looked at together.[17,47,70] Extrahepatic disease is rarely completely resectable; however, some highly selected patients may still be considered for surgical treatment.

The factor most influenced by the surgical approach is the margin of clearance. There is a general agreement that a margin of 1 cm or more is preferable,[31,36,71] and there is a growing consensus that this judgment must not be based on palpation of the liver but should instead be guided by routine application of operative ultrasonography.[72] With respect to the operative approach, a preference for anatomic procedures such as segmentectomies, according to the Couinaud terminology, rather than nonanatomic wedge excisions is advisable since these procedures are more likely to achieve satisfactory margins and reduce

*References 8, 9, 12, 16, 65-67.

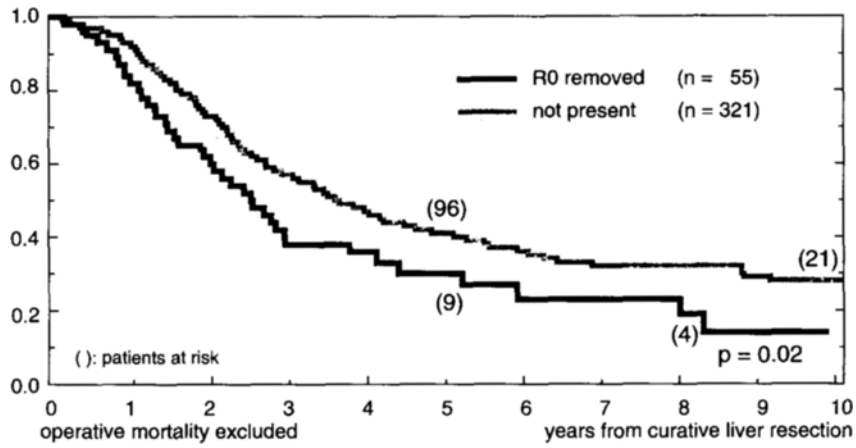

Fig. 4. The prognosis of R0 resection of liver metastases with additional extrahepatic disease is significantly worse compared to results in patients with metastases to the liver only, but still carries some long-term (disease-free) survival. (Adapted from Scheele J, Altendorf-Hofmann A, Stangl R, Schmidt K. Chirurgische Resektion kolorektaler Lebermetastasen: Goldstandard für solitäre und resektable Herde. Swiss Surg 1996;Suppl 4:4-17.)

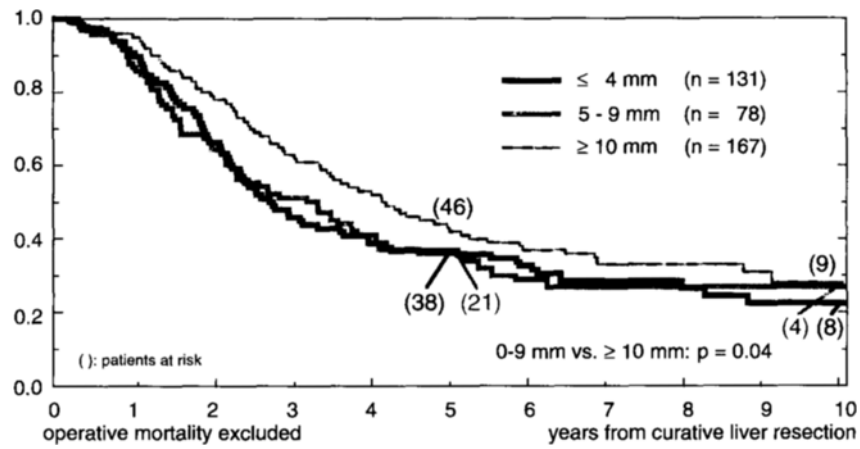

Fig. 5. Survival after R0 resection of colorectal metastases according to the margin of clearance. The prognosis is significantly better with a margin of 10 mm or more, but even with very limited margins (in contrast to R1 resections) long-term (disease-free) survival is observed. (Adapted from Scheele J, Altendorf-Hofmann A, Stangl R, Schmidt K. Chirurgische Resektion kolorektaler Lebermetastasen: Goldstandard für solitäre und resektable Herde. Swiss Surg 1996;Suppl 4:4-17.)

the incidence of inadvertently nonradical procedures.[57]

Reports that distinguish between nonradical operations (so-called R1 resections) and procedures with a clear resection margin, even of minimal extent, have demonstrated long-term survival only in the latter subgroup[12,17] (Table II). In the Erlangen series,[17] despite significantly better results with a margin of 10 mm or more, 38 five-year survivors have been observed among 131 patients with resection margins of 0 to 4 mm only, and an additional 21 five-year survivors had a resection margin of 5 to 9 mm (Fig. 5). These narrow margins were partially a result of limited surgical resection, predominantly in the earlier years. In the majority of cases, however, preoperative workup had already clearly demonstrated that only a minimal margin would be possible. In the large series by Elias et al.,[73] even the width of the resection margin failed to significantly influence prognosis as in the analysis by Nakamura et al.[47] In the recently published Pittsburgh series,[12] 95 patients with resection margins more than 1 cm had only slightly better survival than 92 patients with margins up to 1 cm, which closely matches the Erlangen results. The differences in survival (42% vs. 32%) and disease-free survival

Table II. Impact of resection classification on prognosis after resection of colorectal liver metastases

Reference	Year	R class	No. of patients	5-year survival (%)	Median survival (mo)
Ringe et al.[4]	1990	0	119	27	35.1
		1/2/X	38	0	18.4
Sugihara et al.[10]	1993	0*	109	47.9	—
		1	17	0	17†
Henne-Bruns et al.[71]	1993	0	66	37	31
		1/2	16	0	—
Gayowski et al.[12]	1994	0	187	37‡	—
		1/2	17	0	—
Rees et al.[46]	1996	0	89	37	—
		1/2	18	6	—
Scheele et al.[17]	1996	0	376	39	41.3
		1/2	65	2	14.8

*R0, no extrahepatic disease.
†Estimated from graph.
‡Calculated from tables.

Table III. Absolute contraindications to resection of colorectal liver metastases

No possibility of radical (R0) resection (but occasionally justified for symptomatic palliation)
Lymph node metastases at the liver hilum (but anecdotal success reported by Nakamura et al.[47])
Extrahepatic tumor (except for local recurrence, direct invasion of adjacent structures, or solitary (1 to 3?) lung metastasis)

(29% vs. 25%) at 5 years were only minor, whereas actual tumor involvement of the resection line precluded 5-year survival and lowered the 3-year figure to 12%. The lesson learned from these data is that one should keep removing metastases that, for reasons of size, number, or location, do not allow a 1 cm clear margin, as long as they can be removed with a complete rim of unaffected tissue (R0 resection).

CRITERIA FOR EXCLUSION

The absolute contraindications to resection of colorectal liver metastases are presented in Table III. As for any surgical procedure, there are patients and situations in which the risk of the procedure must be considered too high to balance the potential benefit. Apart from this, liver resection is of questionable value in terms of prognosis if not all of the tumor can be removed. This was consistently the case in the Erlangen series, where just one 5-year survivor and no 6-year survivors were observed among 104 patients who either had gross tumor left behind or had histologic evidence of tumor invasion at the cut surface.[17] Similar results have recently been reported by the Pittsburgh group[12] and by other authors.* An incomplete tumor debulking may very occasionally be justified in the rare situation where a patient is symptomatic as a result of large metastatic lesions.[4,18,27] Even considering the selection bias involved, median survival time may be improved in this particular subset of patients in whom an extreme tumor load is reduced to minimal or only microscopically detectable residual disease.[1,17]

A second absolute contraindication may be the presence of (advanced ?) lymph node metastases at the liver hilum.[9,14,27,66] This was a rare finding in the Erlangen series[17] with only three such patients, as well as in the collected material of Hughes et al.[14] with 24 out of 859 patients. In the Pittsburgh series[12] the incidence of positive nodes was slightly higher with 6 of 104 patients undergoing hilar dissection, whereas Ekberg et al.[27] (6/31 = 19%), Fortner et al.[34] (x/y = 31%), and Nakamura et al.[47] (6/22 = 27%) have reported significantly higher proportions of positive nodes. In Nakamura's series two patients with positive hilar lymph nodes remained disease free at 49 and 66 months, respectively. Both had undergone repeat liver resection.[47] It was not specified whether these metastases were macroscopically visible tumors or they were detected by more thorough microscopic examination, but the much higher incidence of lymph node involvement, aside from a systematic lymph

*References 4, 10, 24, 27, 46, 48, 71.

node dissection, makes a sophisticated histologic technique quite likely.

The presence of extrahepatic disease is commonly regarded as being a third absolute contraindication to resection.[8,12,16,65-67] We would modify this to include discontinuous extrahepatic tumor with the exception of a resectable local recurrence and a single (and possibly up to three) lung metastasis. Tumor at any other site, such as an adrenal metastasis, an omental deposit, nodules on the small bowel, and limited peritoneal spread, is rarely amenable to R0 resection. Despite occasional apparent R0 removal, however, it has always resulted in early recurrence of nonresectable disease in our experience.[17] Direct tumor infiltration usually affects the diaphragm or, rarely, the retrohepatic vena cava, the extrahepatic bile duct, and other adjacent structures such as the omentum, transverse colon, or stomach. Such a finding is quite often associated with other negative prognostic factors such as a large tumor mass or poor differentiation. By avoiding any attempt at taking down what initially may appear to be adhesions rather than infiltration, and by following an aggressive policy of wide en bloc excision of any adherent tissue, long-term disease-free survival may still be attainable.

PREOPERATIVE WORKUP

The preoperative diagnostic workup must address the primary tumor site, the extent of liver involvement, and the possibility of extrahepatic disease. The following are the basic methods used to achieve these goals:

1. Endoscopy of the colon, digital examination of the anastomotic site after low anterior resection accompanied by endoluminal ultrasonography, and CT scan of the pelvis after abdomino-perineal resection
2. Ultrasound, CT scan, and increasingly CT arterial portography[74] or MRI[75] of the liver
3. Bidirectional chest x-ray examination supplemented by spiral CT scan of the lungs in equivocal cases
4. Determination of carcinoembryonic antigen and CA 19-9 levels to provide a baseline for follow-up

Additional investigations may include cytologic study of bone marrow aspirate and, in patients with clinical abnormalities, imaging of other body areas such as the brain and spine. In borderline cases, where in effect more than half of the functional hepatic parenchyma must be resected, CT volumetry may optimize decision making. It could mandate either complete avoidance of any liver resection that carries an unacceptable risk or performance of preoperative portal vein embolization on the side to be resected to induce hypertrophy of the contralateral liver remnant, thereby avoiding postoperative hepatic insufficiency.[76] The value of immune scintiscans, and even single positron emission computed tomography (SPECT), has yet to be defined. With more specific antibodies they may hold some future value, particularly in the detection of disease in the extrahepatic abdomen and pelvis.[77-82]

From a practical point of view, identical results using external ultrasound and intravenous contrast-enhanced CT scans seem to balance the need for diagnostic accuracy regarding the intrahepatic tumor load, with the logistic and current financial constraints of medicine in most Western countries. If one of these techniques is inadequate for technical reasons, or if the findings are contradictory, CT portography and Endorem-MRI may serve as supplementary diagnostic tools. Any other modality should presently be reserved for prospective trials.

The preoperative diagnostic workup must be supplemented by thorough intraoperative assessment.[83,84] Besides clinical evaluation of the abdomen including inspection and palpation of the liver, the most important method is intraoperative ultrasonography.[66,85,86] In roughly 5% to 15%, this alters the surgical approach.[72] Additional metastases may preclude resection, and the interrelation between tumor edge and important intrahepatic vascular structures may change the resection strategy. Occasionally a patient whose disease appears to be unresectable after the initial surgical exploration is, in fact, shown to be resectable.

TIMING OF TREATMENT

There are two basic positions regarding the timing of treatment. One emphasizes the importance of early resection once the diagnosis is established, since the already existing liver metastases may become the source of lymphatic spread to the liver hilum and other perihepatic lymph nodes, or hematogenous spread to the lungs.[27,34,38] On the other hand, a considerable number of articles addressing this point advocate instead a policy of applying a "test of time" ranging from a few weeks to 6 months.[30,31] They argue that within this time a curable tumor is unlikely to become unresectable, since even at autopsy 12% of patients still present with solitary lesions and no extrahepatic spread.[87] Since no conclusive data are available to substantiate either of these positions unequivocally, this allows us to state our own personal position.

We would resect any metastasis that has exceeded 4 cm in diameter because this tumor has already passed a "test of time." It has grown to this size without be-

coming obviously diffuse or unresectable. In turn we would advocate a waiting period for very small lesions, which might be detected by increasingly sensitive diagnostic techniques, especially if they are multiple from the beginning. This is particularly true for a tumor distribution within the liver that would require resection of more than 50% of liver parenchyma and therefore expose the patient to a low but not insignificant operative risk. This position is supported by the poor prognosis of small multiple metastases in comparison to larger multiple lesions in some series.[3,27]

A particularly relevant issue in this context is the treatment of patients with synchronous metastases. The Jena/Erlangen policy was to combine the two procedures quite liberally. This would not increase the operative risk as long as certain basic requirements are met. We would combine any liver resection with a right-sided colon resection, and we would combine any colorectal procedure with removal of two Couinaud segments of the liver or less. If left-sided colorectal resections must be combined with an operation more serious than a right hemihepatectomy, this would be done simultaneously only in a very fit patient, after a thorough discussion of the risks and benefits. A low anterior resection under these circumstances would be covered by a diverting loop ileostomy. The general guidelines for the timing of liver resections as previously discussed are very strictly adhered to in those patients with synchronous metastases.

If the decision is made to delay the liver resection, a needle biopsy of the liver lesion should not be performed because tumor cell spillage and subsequent needle tract or localized peritoneal seeding may turn an otherwise promising situation into a condition that is incurable.[88] In most cases the nature of those tumors is completely clear based on either the operative appearance or the pre- and intraoperative imaging procedures.

RISK OF TREATMENT

The 30-day mortality rate for elective major liver resections in noncirrhotic patients currently ranges from 0% to 5% (Table IV).* Series with a particularly aggressive policy from both an oncologic and technical viewpoint usually do not have a zero mortality. But despite such an aggressive stance, mortality rates in some series, for instance in the Erlangen series,[17] have decreased from approximately 7% in the 1960s and 1970s to roughly 3% since 1990. Mortality after combined procedures may be slightly higher,[56] but this merely reflects the addition of two operative risks at a single point in time.

Morbidity of liver resection has also decreased considerably as a result of the application of the more refined techniques of parenchyma transection and hemostasis. It can be assumed that nowadays 10% to 15% of patients may develop significant liver-related complications, particularly a substantial right-sided pleural effusion, which occurs in approximately half of these patients.[1,17]

RESULTS OF HEPATIC RESECTION

Liver resection is the only therapeutic modality that carries a significant chance of long-term survival. After 5 years, approximately 20% to 40% of patients in a major series were still alive, and roughly 80% of them were free of recurrent disease at that time (see Table I). Significantly better results of up to 52%[89] have not been confirmed by subsequent analyses of an increased number of patients from the same center with longer and more complete follow-up,[12] or improved results have been achieved by excluding less favorable patients in the entire R0 group from the analysis, for instance, patients with extrahepatic disease.[10] Ten-year survival figures, if recorded at all, are rarely based on a large enough sample of patients. In the most recent analysis of the Jena/Erlangen series, survival after 10 years was 21%, and was based on 21 patients, 20 of whom remained free of recurrent disease. Several authors have meanwhile reported survival periods of more than 20 years,[5,90,91] and some patients are just approaching the 30-year mark.[17] The median survival and disease-free survival times are in the range of 40 and 30 months, respectively. Based on these data, the earlier discussion about whether the long-term results after liver resection are indeed an effect of treatment or simply the result of selection bias[22,92,93] can be decided in favor of there being a real prognostic effect of surgery.[4,5,20,34,94,95]

Tumor relapse after liver resection, as is the case after resection of primary colorectal cancer, occurs mainly within the first 2 years. The disease-free survival curve then flattens out and becomes almost identical to the survival curve after 7 years[1,12] (see Fig. 1). The Erlangen experience includes 196 patients who survived the initial 30 days after a curative liver resection more than 7 years ago. No definite tumor recurrence was ever observed in the 48 patients who had been free of disease after that time.[17]

In patients with recurrent disease and subsequent reresection, multi-institutional series have reported 5-year survival rates of 32% and 16%, respectively, after the second liver resection[15,96] (Table V). In the Erlan-

*References 3, 6, 7, 20, 24, 25, 50, 78, 79.

Table IV. Perioperative mortality and morbidity of liver resection for colorectal metastases

Reference	Year	Period	No. of patients	Mortality		Morbidity
				30-day	Total	
Adson et al.[3]	1984	1948-1982	141	—	4	—
Ekberg et al.[27]	1986	1971-1984	72	2.8	5.6	36
Hohenberger et al.[25]	1988	1981-1987	82	4.8	—	—
Holm et al.[29]	1989	1977-1986	35	0	0	46
Ringe et al.[4]	1990	1976-1987	157	4.5	—	10.2
Doci et al.[7]	1991	1980-1989	100	—	5	11
Herfarth et al.[8]	1992	1981-1991	161	—	3.1	31
Rosen et al.[9]	1992	1960-1987	280	—	4*	—
Henne-Bruns et al.[71]	1993	1985-1991	89	—	3.4	13.4
Sugihara et al.[10]	1993	1978-1989	109	1.8	—	—
Gozzetti et al.[11]	1994	1981-1991	108	0.9	—	21.4
Fong et al.[103]	1994	1985-1991	25	0	0	28
Gayowski et al.[12]	1994	1981-1991	204	0	1	—
Hananel et al.[54]	1995	1980-1990	26	0	0	66.6
Wanebo et al.[32]	1996	1978-1994	72	—	8.3	38
Rees et al.[13]	1996	1986-1995	150	0.7	3	23
Scheele et al.[17]	1996	1960-1993	471	4.5	6.4	16
		1990-1993	155	2.6	3.9	7

*Sixty-day mortality.

Table V. Perioperative mortality, morbidity, and prognosis after hepatic reresection for recurrent colorectal liver metastases

Reference	Year	No. of patients	Mortality (%)	Morbidity (%)	Survival (%)			Median survival (mo)
					3 yr	4 yr	5 yr	
Institutional series								
Stone et al.[107]	1990	10	0	—	—	—	—	—
Bozzetti et al.[100]	1990	11	9	40	—	—	—	18*
Vaillant et al.[98]	1993	15	0	40	57	—	30	33
Elias et al.[101]	1993	28	—	—	33†	—	—	—
Fong et al.[103]	1994	25	0	28	—	—	—	30*
Que and Nagorney[108]	1994	21	5	—	—	43	—	41
Scheele et al.[70]	1995	24	3‡	18	—	—	46	50
Scheele et al.[17]	1996	26	2§	—	—	—	57	60
Collected series								
Nordlinger et al.[15]	1992	144	—	—	30	—	16	—
Nordlinger et al.[104]	1994	130	0.8	25	33	—	—	—
Fernandez-Trigo et al.[96]	1995	170	—	—	45	—	32	34
Wanebo et al.[127]	1996	536	1.8	26	—	—	—	24‖

*Mean survival time.
†Estimated from graph.
‡Based on 34 reresections in 31 patients.
§Based on 44 resections in 36 patients.
‖Mean of reported median survival times.

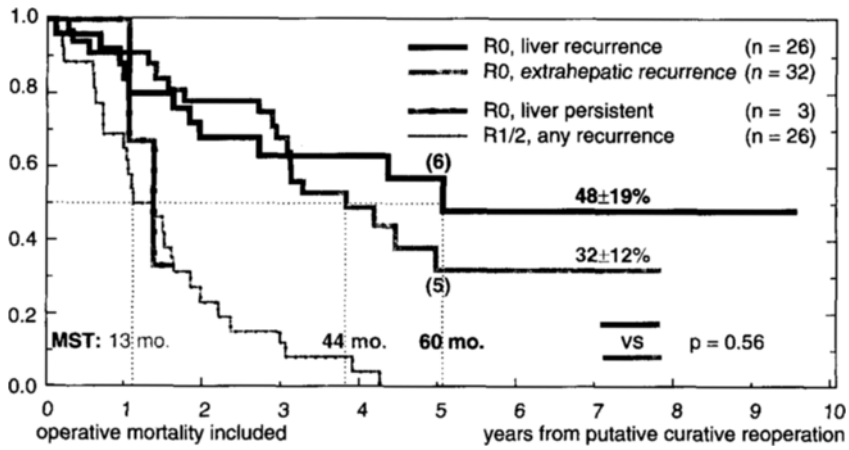

Fig. 6. Survival after a presumed "curative" reintervention for recurrent disease. If the initial liver resection was R0 (curative), a second R0 resection of intrahepatic and extrahepatic recurrent disease, respectively, resulted in 57% and 32% 5-year survival from the time of reintervention. In contrast, a nonradical reoperation carried a poor prognosis, as did R0 reintervention in three patients who had a nonradical initial liver resection. MST = median survival time. (Adapted from Scheele J, Altendorf-Hofmann A, Stangl R, Schmidt K. Chirurgische Resektion kolorektaler Lebermetastasen: Goldstandard für solitäre und resektable Herde. Swiss Surg 1996;Suppl 4:4-17.)

gen experience, survival and disease-free survival rates were 44% and 38%, respectively, if tumor recurrence within the liver is considered.[70] More recently, survival increased to 57% for hepatic recurrences (dropping to 48% shortly after 5 years) and to 32% if extrahepatic disease was removed (Fig. 6).[17] This prognostic success after resection for subsequent extrahepatic cancer included local recurrences as well as pulmonary metastases but no other sites of tumor relapse.[70]

Reresection is possible in approximately 20% of patients with a cancer recurrence after an initial R0 liver resection. Mortality and morbidity are similar to the rates for the initial liver resection, as is survival from the time of reintervention.[17,26,70,96-108] This justifies a policy of close follow-up because symptomatic recurrences are unlikely to be suitable for reresection.[70]

QUALITY OF LIFE

Quality of life after liver resection may be slightly impaired during the early postoperative period of 1 to 2 months. When the patient recovers and the liver has regenerated, as is normally seen in noncirrhotic patients, quality of life has been shown to be unchanged from the preoperative status.

COSTS

The costs of liver surgery must be calculated on the basis of an operative procedure that requires 3 to 5 hours for major liver resection and 1 to 2 hours of operating time for minor procedures. In general, 1 or 2 days in the intensive care unit will be required after resection of 50% or more of the liver parenchyma, followed by a total hospital stay of 1 to 2 weeks. After minor procedures, no time in the intensive care unit will be necessary, and the total hospital stay may be reduced to a few days. If adjuvant treatment is considered, there are additional costs that will depend on the method chosen and these are not discussed in this report.

UNANSWERED QUESTIONS AND PROPOSAL FOR TRIALS

At the present time, there is a strong feeling that (neo)adjuvant treatment should be implemented either to improve the general outcome of hepatic resection for metastatic disease or to possibly downstage patients to make them suitable candidates for resection.[26,50,109-112] There are, however, no convincing data available demonstrating a prognostic benefit when a noncurative resection is supplemented by any medical treatment, and only scant data are emerging that local destruction of minimal residual disease by laser, argon beam coagulation, or cryotherapy may allow for some long-term success.[112,113] Despite such anecdotal observations, there is still no effective therapeutic concept that can predictably achieve long-term survival after a nonradical procedure. Therefore adequate surgery remains the cornerstone in the cur-

rently available therapeutic armamentarium used to treat secondary colorectal liver metastases.

Following an R0 resection, there is still no strong indication that any commonly used chemotherapy regimen, or radiotherapy, given in an adjuvant fashion is likely to improve the results. However, new chemotherapeutic agents, refined immunotherapy protocols, preoperative chemoembolization, radioimmuno-guided surgery, ex situ and ante situ resection, or the combination of resection and cryotherapy all need to be evaluated in multicenter, multidisciplinary trials. Moreover, less invasive therapeutic alternatives such as MRI-guided laser destruction, percutaneous radionuclide injection, or laparoscopic interventions may complement, or even replace, conventional surgery in selected patients.[114,115] Hopefully, in the years ahead, such trials will establish the value of these various therapeutic modalities and help to further improve the results of surgical treatment of colorectal hepatic metastatic disease.[109,111,116-126]

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Hepatic Artery Infusion as Treatment of Hepatic Metastases From Colorectal Cancer

M. Margaret Kemeny, M.D., F.A.C.S.

Each year more than 30,000 patients in the United States develop metastases from colorectal cancers, only to the liver. Treatment of these patients is the challenge of surgical and medical oncologists. A large number of these patients, probably more than 70% of them, have unresectable disease and must be treated by means other than surgery. However, approximately 30% have potentially resectable disease. For these patients resection is appropriate, but questions concerning the role of chemotherapy remain to be answered.

Continuous hepatic artery infusion using a totally implantable pump that delivers chemotherapy directly to the liver has been studied for more than 20 years. At the present time, there are two main areas being investigated with regard to the use of hepatic artery infusion: one is treatment of unresectable hepatic metastases and the other is treatment after resection of hepatic metastases.

CLINICAL TRIALS

In the late 1970s and throughout the 1980s, several groups conducted randomized trials comparing continuous hepatic artery infusion of floxuridine (FUDR) with systemic therapy for unresectable liver metastases. The largest studies in the United States were conducted by Kemeny et al.[1] at Memorial Sloan-Kettering Cancer Center and by the Northern California Oncology Group (NCOG).[2] The Memorial Sloan-Kettering study included more than 163 patients and found a highly significant difference in response rates with 52% of patients having an objective response to intrahepatic therapy vs. 20% responding to systemic therapy. The systemic therapy used in that study was continuous intravenous infusion of FUDR. This study allowed a crossover of patients from the systemic arm to the intrahepatic arm resulting in no survival difference within the two groups. It is interesting to note that 20 of the 51 patients in the systemic group did not cross over to receive intrahepatic treatment generally because the ports to their hepatic arteries were clotted. Among patients who never received hepatic artery infusion and only received systemic therapy, the average survival time was 7 months, which is significantly different from the 17-month survival in patients who received intrahepatic therapy. In the NCOG study, again there was a significant difference in tumor responses but no difference in survival because patients were allowed to cross over from the systemic arm to the intrahepatic arm. The North Central Cancer Treatment Group (NCCTG) conducted a small noncrossover study comparing intrahepatic treatment with intravenous treatment.[3] In this study there was no difference in survival between the systemic arm and the intrahepatic arm, but again there was a statistically significant difference in responses. The NCCTG study was flawed, however, in that half of the 35 patients in the intrahepatic arm did not receive either the complete course of intrahepatic therapy or any intrahepatic therapy.[4,5] Thus it is very difficult to draw any definitive conclusions from a study involving only 17 analyzable patients. For the patients who did receive the complete course of therapy, there was a survival advantage. There are two studies from Europe, both of them large noncrossover studies and both of which demonstrated significant survival advantages for intrahepatic therapy. The problem with both of these studies was that in the English study the nonintrahepatic arm was a no-treatment control arm and in the French study the therapy in the systemic arm was left to the discretion of the physician; thus there was no uniformity of treatment. The recently published meta-analysis of hepatic artery infusion reviewed six of the seven trials of hepatic artery infusion, which included more than 654 patients.[6] The tumor response rate was 41% for patients receiving hepatic artery infusion vs. 14% for those given intravenous chemo-

From the Division of Surgical Oncology, North Shore University Hospital, Manhasset, N.Y.

therapy ($P = 10^{-10}$). The first site of treatment failure was the liver in 39% of patients who received hepatic artery infusion vs. 74% of those who were treated with intravenous chemotherapy. The patients who were assigned to receive intravenous chemotherapy first had a mean survival time of 12 months. The patients who received continuous hepatic artery infusions had a mean survival of 16 months. This difference was not significant. However, the majority of patients in the intravenous groups had been crossed over to receive intrahepatic therapy. If all of the trials are taken into account—that is, those in the intravenous chemotherapy arm and those in the ad libitum control arm, the hepatic artery infusion group did demonstrate significantly improved survival ($P = 0.0009$).

Because almost all of the trials conducted thus far have had a crossover arm, there is a need for a new, well-controlled randomized study. Such a protocol has recently been instituted in the Cancer and Leukemia Group B and will compare the best systemic treatment currently available, which consists of 5-fluorouracil plus leucovorin, with continuous hepatic artery infusion of FUDR, leucovorin, and Decadron using an Infusaid pump (Infusaid Corp., Norwood, Mass.). This trial will not only examine differences in tumor response and patient survival but will also analyze costs and quality of life. Approximately 350 patients will be required to answer these questions sufficiently, and the study should be completed within 3 years.

COMPLICATIONS OF HEPATIC ARTERY INFUSION

The principal toxiferous effects that accompany hepatic artery infusion are chemical hepatitis, gastritis and duodenitis, and biliary sclerosis.[7,8] The reported incidence of duodenitis, gastritis, and ulcers in various studies ranges from 17% to 56% but is less than 20% in most of them. Chemical hepatitis, which is an elevation of transaminase levels, has been reported to occur in 10% to 17% of patients, but in many studies the rate approaches 45% to 50%. This complication is reversible with temporary cessation of intrahepatic therapy. Biliary sclerosis, which is the most serious complication, is defined as a narrowing of the bile ducts especially at the hepatic duct bifurcation. It has been reported in anywhere from 5% to 29% of the patients in some of the original studies, but because some of the dosages have been modified it is now reported to occur much less frequently and should be seen in no more than 10% of patients being treated with intrahepatic infusion. Cholecystitis occurred in almost one third of the patients until cholecystectomy became part of the implant procedure.

Trials at Memorial Sloan-Kettering conducted in an attempt to decrease biliary sclerosis included adding Decadron to the FUDR in the infusion pump. This resulted in a decrease in biliary sclerosis to a level of 8% and in fact when Decadron and Leucovorin were added to the FUDR in the pump, the incidence of biliary sclerosis dropped to 3%. The same trials at Memorial Sloan-Kettering showed that increased response rates could be obtained when other drugs were added to the FUDR in the pump. The best response came from the addition of Leucovorin and Decadron to the FUDR with an objective response rate of 78% and a 2-year survival of 57%. As a result of this study, the new intergroup study will include the combination of FUDR, Leucovorin, and Decadron as the intra-arterial arm.

ROLE OF HEPATIC ARTERY INFUSION FOLLOWING LIVER RESECTION

For patients with resectable hepatic metastases, results of many institutional studies, especially within the past 15 years, have shown that resection of one to three metastases will yield a 5-year survival of greater than 30%. In light of these data, this has become the standard treatment in patients with one to three hepatic metastases. Studies analyzing areas of recurrence show that for the approximately 70% of patients that do have a recurrence in the liver, approximately 20% to 40% have a recurrence in the liver only, and another one third of patients will have recurrences in the liver and in extrahepatic sites. A randomized trial conducted at the City of Hope studied patients treated with infusional chemotherapy after resection.[9] This was a single-institution study with small numbers but interesting data were obtained. Of the 11 patients with solitary metastases, the five who were treated with resection plus hepatic artery infusion had a longer median survival of 37 months vs. 28 months for those treated with resection only. They also had a significantly longer median time to failure (30 months) in comparison to those who underwent resection only (8.7 months). Furthermore, it was noted that among the 15 patients who were treated with resection plus continuous hepatic artery infusion, not one of them had a recurrence in the liver. In 9 of the 15 patients who did have a recurrence, the lesions were in the lungs, bones, and other areas. Patients who had multiple resectable lesions who were randomized to pump-only therapy had a 64% complete response rate

and another 29% had a partial response. Thus more than 80% of the patients had an objective response to continuous hepatic artery infusion.

As a result of this study, an intergroup national study was initiated comparing treatment of one to three resectable metastases using surgery alone in one arm and surgery plus infusional intrahepatic chemotherapy in the other arm. That trial is almost completed and the data should soon be available on the usefulness of that modality.

CONCLUSION

There are data to suggest that infusional chemotherapy is more effective than systemic chemotherapy for liver-only metastases. The definitive trial is now in progress. There are also data to suggest that hepatic artery infusion after liver resection decreases the incidence of recurrent or additional liver metastases. Again, a national trial is in progress that should provide definitive answers.

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Cryosurgery in the Treatment of Liver Metastasis From Colorectal Cancer

T.S. Ravikumar, M.D., F.A.C.S., Ramon Sotomayor, M.D., Ravi Goel, B.A.

The practice of using ultrasound in the operating room has allowed resections for primary and metastatic liver cancer to be performed with increased safety and has permitted the accurate delineation of surgical margins—important concepts in achieving localized tumor control with improved patient outcome. Further, ultrasound enables the identification of unsuspected bilobar liver disease and demonstrates the proximity of tumors to critical vascular structures.[1] Although the operative mortality of major hepatic resection is now in the range of less than 5%, there are clearly patients who will not benefit from resection or simply will not tolerate resection because of poor hepatic reserve. A recent article by Wanebo et al.[2] reviewed 74 patients with colorectal metastases over a 14-year period; patients with Dukes' B primary colon cancer, one to three lesions, and unilobar disease had 5-year survival rates of 36%, 24%, and 26%, respectively. These patients were demonstrated to benefit from hepatic resection. However, these same investigators reported a very high mortality rate in 12 patients who underwent trisegmentectomy or extended resection.[2] The treatment of unresectable liver metastases has traditionally relied on systemic chemotherapy. Since systemic therapy did not change the survival or patterns of failure, several regional therapy options (chemotherapy and ablative approaches) have been explored.

There are various options for treating inoperable liver tumors, with cryotherapy being one of them. Percutaneous injection of 95% alcohol, although useful for treating small hepatocellular carcinomas, particularly in cirrhotic patients, has not found success in colorectal liver metastasis.[3] Various forms of intra-arterial chemotherapy and embolization of branches of the hepatic arterial supply to the tumors are useful in unresectable liver tumors.[3] Cytoreduction and sequential resection is another strategy that has been reported. A series from the Liver Cancer Institute in Shanghai reported on 72 patients with hepatocellular carcinoma who were resected out of a group of 663 patients deemed unresectable on initial exploration.[4] These patients were subsequently treated with hepatic artery ligation and hepatic artery infusion of chemotherapy before the second operation several months later. Survival in the resected group at 5 years was 62%.[4] Data on such an approach for colorectal liver metastasis are lacking.

CRYOSURGERY—BIOLOGIC CONSIDERATIONS

Cryosurgery or cryoablation is a technique that involves the use of extremely low temperatures to destroy tumors, which are then left in place to be reabsorbed. It is a focal therapy that allows treatment of lesions with preservation of normal tissue. Cryosurgery has been used in other organs such as the skin and entails direct contact with liquid nitrogen at low temperatures. This was the initial form of therapy used in the liver and involved application of liquid nitrogen on the surface of the liver. Modern liquid nitrogen delivery systems and vacuum-insulated recirculating nitrogen probes have now been developed that allow this technique to be used to treat tumors in virtually any segment of the liver. Real-time ultrasound permits accurate placement of probes and monitoring of the extent of freezing; the ice ball or "cryolesion" can actually be seen as it develops, along with the margin of normal tissue frozen around the

From the Department of Surgery and Cancer Institute of New Jersey, University of Medicine and Dentistry of New Jersey–Robert Wood Johnson Medical School, New Brunswick, N.J.
Reprint address: T.S. Ravikumar, M.D., F.A.C.S., Chief of Surgical Oncology, Cancer Institute of New Jersey, 195 Little Albany St., New Brunswick, NJ 08901.

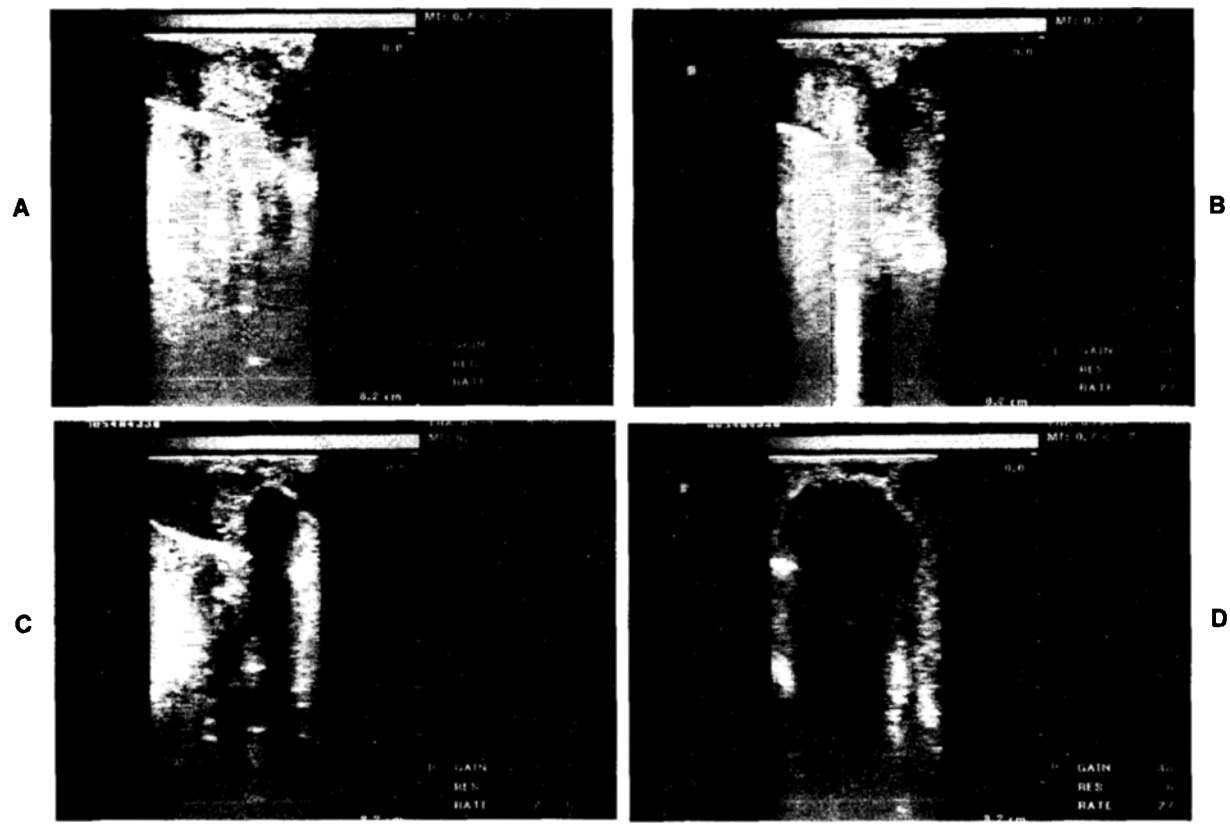

Fig. 1. Demonstration of real-time intraoperative ultrasound monitoring of hepatic cryosurgery. **A** and **B,** Cryoprobe placement in the center of the liver tumor. **C** and **D,** Demonstration of the freezing process. Hyperechoic rim and postacoustic shadowing are evident.

tumor, thus utilizing the same principle of surgical resection[5,6] (Fig. 1).

Cryosurgery works by destroying cells directly as a result of physicochemical effects and indirectly by affecting the structure of vascular channels. The process begins when liquid nitrogen circulates at −196° C through a probe that is placed in direct contact with the tissue to be treated; in this case the probe is placed under ultrasonographic guidance through the liver substance into the tumor. As ice forms, electrolytes and organic compounds are excluded from the crystal. A hyperosmolar environment is created in the extracellular compartment, which draws water from inside the cells. As a result the tissue shrinks, the cell membranes are disrupted, and intracellular protein is denatured, thus destroying cell function. Freezing propagates from one cell to another through communication channels. As more and more tissue crystallizes when small ice crystals grow together with large crystals, a grinding action is created that mechanically disrupts the tissue. Rapid freezing and slow thawing enhances tissue damage. As the treated area warms, water passes into the cells, leading to an increase in volume and bursting of the cell membranes. This process is repeated more than once so that any remaining viable tumor cells are destroyed on the second or third cycle.[6,7] Animal experiments confirmed the destruction of tumor cells in in vivo and in vitro studies.[7] The effectiveness of cryosurgery to control experimental colorectal liver metastasis was investigated in a rat model. Rat livers were implanted with tumor cells on one of the lobes of the liver; the animals were randomly assigned to one of three groups: no therapy, resection, or cryotherapy. The animals randomized to the cryotherapy group did just as well as those undergoing resection.

Cryotherapy also causes obliteration of tumor vessels leading to hypoxia and necrosis. This occurs in small-caliber arteries and veins as they undergo thrombosis as a result of direct contact with subzero temperatures. Fortunately, large vessels such as the hepatic veins and portal veins are protected from this effect because the flow of warm blood makes them act as thermal sinks, thus protecting their intima and media. To achieve the maximum effect, the temperature should be lowered rapidly to at least −35° C and

slowly warmed. It has been shown that one cycle is not sufficient to achieve adequate lysis of tumors and that two or three freeze-thaw cycles are best.[7]

Cold injury affects different tissues in different ways. In the liver, a temperature of −15° C is generally lethal; in the skin, however, much lower temperatures are required to destroy tumors. Also, the rate at which the tissue freezes is known to be an important factor. In studies using cellular suspensions of hepatocytes, it was found that intermediate rates of freezing were most lethal. In the liver itself, slow cooling results in formation of long structures of ice along blood vessels and the hepatic sinusoids, with surrounding tissue dehydration. When the liver is frozen at intermediate and high rates of cooling, significant intracellular ice formation occurs with minimal hepatocyte dehydration. Freezing to −40° C will result in complete destruction of hepatocytes, bile ducts, and connective tissues, whereas freezing to −10° C will spare normal hepatic parenchyma.

Once cryosurgery has been performed, the tumor undergoes necrosis and is left in place. A cellular repair process begins immediately and may last several months. Collagen scar formation takes place in the treated area; most lesions treated successfully will tend to show gradual shrinkage on follow-up with computerized axial tomography, with focal hepatic atrophy. Some lesions, particularly the small ones, may undergo full resorption and disappear completely.[8]

It has also been suggested that destruction of tumors by means of cryotherapy may somehow affect immunologic function and improve survival.[9] Tumors were implanted in rat livers and the animals were treated using one of the following four methods: hepatic artery ligation, resection, cryoablation, or liver mobilization only (sham treatment). In this study, although there were no differences in local tumor control, the animals treated with cryotherapy survived longer.[9] Clinically these effects, which are thought to be immunologic, have not been proved.[7]

TECHNICAL ASPECTS OF CRYOTHERAPY

Freezing is accomplished by using hollow metallic probes through which liquid nitrogen circulates. They are insulated except at the end of the shaft, which is the area that comes in contact with the tumor. Disc-type probes can be used for superficial lesions, whereas deeper lesions require the use of trocar-type probes. The size and shape of the ice ball are determined by the diameter of the probe (3 to 10 mm) and the length of the freezing zone (2 to 4 cm). Freezing begins from the probe outward, that is, from the center of the tumor to the periphery. The process can be effectively monitored using intraoperative ultrasonography, and the ice ball is created up to the desired margin (about 1 cm) around the lesion.

Cryotherapy requires a laparotomy under general anesthesia, although new instrumentation allows the use of a laparoscope in select cases. In general, the same principles that apply to liver resection apply to cryosurgery. Preoperative studies include an imaging module such as CT scan, MRI, or ultrasonography to note the number and size of the lesion or lesions and their proximity to critical structures.[5] The patient should be given adequate hydration since myoglobinuria with acute tubular necrosis has been reported early in the experience, particularly with large-volume freezing.[10] Exposure of a large volume of the liver to subfreezing temperatures may lead to significant hypothermia in a patient with an open body cavity who is under general anesthesia. Use of the Bair Hugger warming system (Augustine Medical, Inc., Eden Prairie, Minn.) in addition to standard maneuvers, was recommended by Onik et al.,[11] after they found significantly lower core body temperatures in 28 patients who had undergone surgery without this device.[11]

The abdomen is entered through a standard incision that allows adequate visualization and exposure. The abdomen is thoroughly explored, and the liver is mobilized and palpated bimanually. The liver is then carefully scanned using ultrasound to assess the number of lesions, the size, and the location in relation to vascular structures. A number of ultrasound transducers are commercially available including handheld linear-array transducers with a variety of configurations including I and T shapes; generally 5.0 or 7.5 MHz transducers are used. With the aid of ultrasonography, the shortest and least traumatic path to the tumor is chosen for cryoprobe placement that avoids major branches of the hepatic and portal veins and bile ducts. The surrounding viscera and diaphragm are packed out of harm's way to protect them from accidental injury during the freezing process. Adequate mobilization of the liver should be practiced before the probe is placed to allow adequate access to the liver in case of bleeding.[5] The size of the lesion will determine the size of the cryoprobe to be used. Performance of a Pringle maneuver may help enlarge the ice ball for a given probe if needed.

The goal of cryosurgery for liver tumors should be similar to modern standards for hepatic resection. Adequate margins of normal tissue freezing should be obtained; 1 cm margins are recommended by most authors. As the ice forms from the center outward, there is a central area of rapid freezing, an intermediate area of moderate freezing, and an area of slow cooling in the periphery. Thermal gradients of up to 10° C for every millimeter of tissue are seen depending on the efficiency of the probe. Therefore this

must be considered if adequate tumoricidal temperatures (i.e., −35° C) in the periphery of the ice ball are to be ensured.[7]

There are characteristic ultrasonographic features of the advancing cryolesion. The freeze front is seen in most cases as a hyperechoic rim with postacoustic shadowing beyond this rim (see Fig. 1). After thawing, the normal frozen liver (which is treated as a margin) appears hypoechoic when compared with normal unfrozen liver. The treated tumor remains hyperechoic after thawing; this creates a halo effect of hypoechoic normal liver tissue around a hyperechoic cryolesion, which helps ensure adequacy of margins.[12,13] To achieve optimal tumor destruction, two to three freeze-thaw cycles are recommended.[7,14] After completion of the freeze-thaw cycles, the probe is rewarmed and gently rotated on its axis before it is removed to avoid potential cracking of the liver and hemorrhage. There is usually some bleeding from the tract of the probe, which is controlled by packing with a hemostatic agent. The abdomen is then closed in standard fashion. The use of drains is not mandatory. Postoperative care is usually straightforward and patients are usually discharged home by the fifth or sixth postoperative day.

Follow-up of tumors treated with cryosurgery is carried out using CT scans and tumor markers. Necrosis of the tumor can be demonstrable with gas bubbles in the tumor, which can be of no clinical significance; hepatic abscess is rare. Within 3 to 6 months, the lesions shrink, and in most patients an area of fibrosis and architectural distortion persists.[8] The kinetics of tumor markers (e.g., carcinoembryonic antigen [CEA]) have been studied in patients treated with cryotherapy. This was studied in 11 patients with metastatic colorectal cancer to the liver in whom CEA levels were determined before and after surgery. CEA levels fell in all but two patients treated with cryosurgery and were found to return to pretreatment levels when these patients developed recurrences.[15] CEA levels usually fall between 4 and 8 weeks after surgery if cryotherapy is effective.

EXPERIENCE WITH CRYOSURGERY

Cryosurgery has been used throughout the world for a variety of liver tumors, both primary and metastatic. The worldwide experience with cryotherapy reflects the patterns and prevalence of hepatobiliary tumors in different parts of the world. Investigators in China have dealt almost exclusively with early and advanced hepatocellular carcinomas, whereas those in the United States, Britain, and Australia have dealt with metastases to the liver from colorectal primary lesions and some neuroendocrine tumors. Zhou et al.[16] reported the initial experience in China with 35 patients with primary liver tumors; this experience was then expanded to 107 patients,[17,18] 86% of whom had cirrhosis. Five- and 10-year survival rates were 22% and 8.2%, respectively, for the whole series, whereas in 32 patients with small tumors (<5 cm) 5- and 10-year survival rates were 48% and 17%, respectively. This was accomplished with no operative deaths and no serious complications such as rupture of tumor, delayed bleeding, or biliary fistula.[18]

In the United States a pilot study of 10 patients with colorectal metastases was initially reported in 1987.[13] The first five patients underwent resection after cryoablation with pathologic examination of the resected specimen showing coagulative necrosis of the tumor.[13] In a subsequent communication, among 20 patients with liver tumors treated with cryosurgery, 16 patients with colorectal metastasis showed a gradual decrease in CEA levels and two patients with liver lesions from neuroendocrine tumors became asymptomatic with normalization of their tumor markers (5-hydroxyindoleacetic acid and glucagon).[19] The usefulness of ultrasound in cryosurgery was demonstrated in a review of 110 consecutive patients who underwent exploration for hepatic tumors.[20] Ultrasound identified 21 patients not suitable for resection who were then treated with cryotherapy. At 14 months, the median follow-up in this study, 52% of recurrences were noted in the liver, whereas 24% were systemic recurrences. It was concluded that cryosurgery was effective in controlling some of the unresectable tumors but that regional or systemic therapy was needed as an adjunct to cryoablation.[20]

To evaluate the long-term response, Ravikumar et al.[13] evaluated their 5-year experience with cryosurgery to treat unresectable hepatic tumors. In this study comprising 32 patients, 75% had colorectal liver metastases. There were no postoperative deaths and only two major complications. Overall survival was 62% and disease-free survival 24% using actuarial analysis. Among the patients who had a recurrence, 12 (54%) of 22 were in the liver and extrahepatic sites; 32% had recurrence in the untreated remaining liver, whereas 14% developed disease in the lung, peritoneum, and bone. Among the entire group of 32 patients, however, only 3 (9%) of 32 developed a recurrence at the cryosurgery-treated site. Onik et al.[10] reported similar findings in 18 patients with colorectal metastases with a 22% long-term remission rate at a median follow-up of 28.8 months. This group also reported on 53 patients over 4 years including patients with metastatic lesions from ovarian, carcinoid, and head and neck primary tumors, as well as hepatomas and leiomyosarcomas.[21] An average of four tumors were frozen per patient with up to 16 lesions treated in one patient. Approximately half of their patients underwent at least a wedge resection of the liver in

addition to cryotherapy, and in several patients cryotherapy was used to treat a close margin. Long-term survival data were reported recently by these investigators from Pittsburgh, with an expected overall 5-year survival of approximately 20% (Society of Surgical Oncology, Chicago, March 7-21, 1997). Data from Australia showed long-term remission in 18 of 170 patients, whereas in the New England Deaconess experience, 25% disease-free survival was reported.[7] Nine cases were reported by a group in Hawaii with no deaths; all patients were alive at 11-months' follow-up.[22]

Another long-term series reported by Steele[23] evaluated 70 patients, 56 of whom had colorectal liver metastases. The medial survivals for patients who had residual disease at the end of the cryosurgery vs. those who had no gross residual disease were 24 months and 42 months, respectively. The author concluded that the survival for metastatic colorectal cancer cryoablation was analogous to that for resection in the Gastrointestinal Tumor Study Group multi-institutional series.

Based on worldwide data concerning colorectal metastases to the liver and primary hepatic tumors, it seems that long-term control is possible in approximately 20% of the cases. Although the experience with other metastatic tumors of the liver is small, experience with neuroendocrine tumors from the gastrointestinal tract should grow over the next few years, especially for amelioration of symptoms from endocrine activity.[7]

A question to be answered in the future is whether cryotherapy will become an alternative to resection. Although this question will be difficult to answer, there are now reports of the use of cryotherapy as an adjunct to resection. Welling and Lamping[24] described a technique in which the cryoprobe is used as a "handle" to assist in segmental resections of the liver. This technique was initially described in 10 patients, but there was little information on outcome. Recently the group at Memorial Sloan-Kettering Cancer Center reported on 16 tumors in 13 patients.[25] The cryoprobe was inserted into the tumor under ultrasound guidance and freezing was performed up to a 1 cm margin. The ice ball was maintained, and the probe was used for traction on the specimen while a segmental resection was performed in standard fashion. This technique was found to be useful for wedge resections in the dome of the liver and in patients who required a liver resection for a unilateral lesion and had a small contralateral lesion. There was one postoperative death. The authors suggest that this may allow excision of small tumors with adequate margins and maximal preservation of liver function.[25]

Many studies have documented the safety of cryoablation of liver tumors in addition to the long-term response in tumor control. There have been very few operative deaths and few major complications among more than 300 cases reported in the literature. The largest studies from Australia and Pittsburgh (each with more than 100 patients) report 0% and 4.5% mortality, respectively. Complications have included pleural effusion in most patients (of little clinical significance), hemorrhage resulting from liver cracking, hepatorenal syndrome, and acute tubular necrosis ascribed to myoglobinuria. Bile leaks and fistulas have also been reported, as well as subphrenic and intrahepatic abscesses.[5,6,10] Other important issues that pertain to the development of cryosurgery are the reduced length of hospital stay and minimal need for blood transfusion. The length of hospital stay for cryosurgery is approximately 6 days, whereas it averages 10 to 12 days for major hepatic resection.

IMPROVEMENTS IN CRYOSURGERY TECHNOLOGY AND COMBINED INFUSION APPROACHES

Although cryotherapy has expanded the possibilities of tumor control and disease progression in patients with inoperable colorectal hepatic metastasis, limitations still exist. In most circumstances a laparotomy is still required for adequate performance of the procedure. A new area of exploration is the use of laparoscopic surgery to perform these procedures. The second major limitation is the persistently high rate of failure in the untreated liver, presumably because of micrometastatic disease. A combination of cryotherapy and regional infusion chemotherapy may improve control in the liver.

Although laparoscopy has been used to perform many abdominal procedures, it is not currently applicable to major hepatic resection. However, technology has been developed that allows ultrasound examination of the liver through a laparoscope. A 10/11 port is necessary and adequate sagittal and transverse views of the liver can be obtained by changing the position of the transducer. Prospective studies of laparoscopic ultrasonography followed by open ultrasound examination have shown that laparoscopic transducers can accurately delineate hepatic anatomy, localize occult tumors, and delineate the relationship of tumors to major vessels and bile ducts.

The cryoprobes developed for laparoscopy are a modification of the probes used for conventional open surgery. We have used a 4.8 mm diameter probe that fits through a 5 mm laparoscopic port and is 40 cm long. As in open surgery, it is necessary to have available probes of various diameters and lengths depend-

ing on the size of the lesion. However, in contrast to open surgery, placement of the laparoscopic trocars must be individualized in accordance with the location of the lesion. It is often necessary to define the trajectory of the cryoprobe before inserting the trocars rather than using standard trocar sites. Once this is defined using ultrasonography, the tumor is localized with a long needle that can be passed percutaneously or through one of the ports. A J-type guidewire is passed, the needle is removed, and a dilator with a sheath is passed over the guidewire. The cryoprobe is then passed through the sheath. The sheath used for laparoscopic cryosurgery is stiffer than usual so that it does not collapse or bend. The sheath is then pulled back to the edge of the liver surface and cryoablation is completed under laparoscopic visualization and ultrasound monitoring. The sheath is then pushed up to the frozen tumor and the cryoprobe is removed. The tract of the probe can then be packed with a hemostatic material in the same way as the open technique to minimize bleeding. The pneumoperitoneum is well maintained with this technique.[6] Experience with the laparoscope and cryosurgery has been limited, and they have been used with solitary tumors in accessible locations; a group from Scotland has reported laparoscopic cryoablation in 18 patients.[26] Complications were similar to those seen with the standard approach, but data on longer term outcome is not yet available.

As outlined earlier, recurrence in the untreated liver after cryotherapy poses a significant problem. As in other sites, it is then necessary to search for alternative or adjunctive therapies as part of a multimodality treatment. Systemic chemotherapy has been notoriously ineffective in the management of colorectal liver metastases. Regional chemotherapy (particularly fluorodeoxyuridine [FUdR]), administered through pumps placed in the hepatic artery, has improved the response rate in comparison to systemic therapy, but this has not resulted in improved survival when tumors are advanced.[27] In regional infusion studies, patients with low-volume liver tumor burden (<20%) and those with relatively normal liver function tests benefited the most.[27] At the present time, intrahepatic chemotherapy is also being tested as an adjunct to resection. This is based on the assumption that recurrences in the liver are due to micrometastatic deposits that go undetected at the time of treatment. The same principle has been applied to tumors treated with cryotherapy. This has been studied by Morris et al.[28] and Horton[29] in Australia. In their experience with unresectable colorectal metastases, 11 patients were treated with cryotherapy alone and 38 patients were treated with cryotherapy and hepatic artery infusion of 5-fluorouracil (5-FU) and folinic acid in a 4-day regimen repeated monthly. They concluded that patients with cryotherapy alone were three times as likely to die of disease as patients treated with cryotherapy and intra-arterial 5-FU. Median survival for the initial group was 245 days compared with 570 days for the treated group. However, this was not a randomized trial; actually all 38 patients had pumps placed with the intention of administering intra-arterial chemotherapy. The 11 patients in the cryotherapy-only group were those in whom the delivery system failed or those who for some other reason could not be given intra-arterial chemotherapy.[30]

A trial at the Cancer Institute of New Jersey is ongoing to evaluate the combination of cryotherapy and regional hepatic arterial chemotherapy. It is based on the rationale that in randomized trials of intrahepatic chemotherapy, patients with a lesser liver tumor burden and lower lactate dehydrogenase and CEA levels

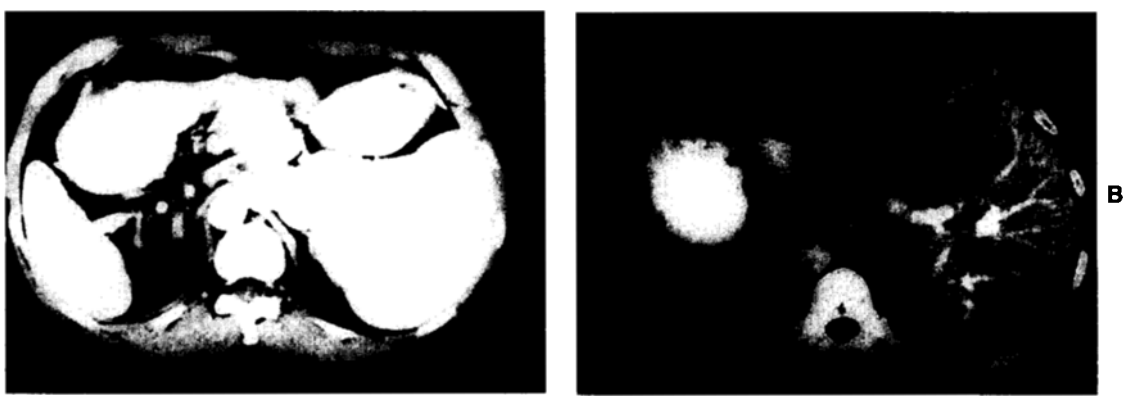

Fig. 2. Cryoablation and hepatic artery infusion for multifocal liver metastasis from rectal cancer. CT scans immediately after cryotherapy and at 3 years 9 months follow-up.

had a significant benefit in terms of tumor response and time to progression when compared to systemic chemotherapy.[26] This is a phase I/II trial in which 5-FU and FUdR are being used. In this trial we have shown that these two modalities can be administered safely without increasing hepatic toxicity (phase I component). Preliminary results in 12 patients show a remission rate of 67% at 2-year median follow-up compared with the 20% to 25% that has been achieved with cryotherapy alone (Fig. 2). The protocol will continue to accrue 20 patients with follow-up data, and incorporating a quality-of-life assessment protocol before consideration of a multi-institutional phase II or phase III study in the near future.

CONCLUSION

Cryotherapy is a treatment modality that can offer reasonable hope to patients with unresectable metastatic colorectal cancer to the liver and those with poor hepatic reserve or comorbid conditions that preclude major resection. It can be used for more than one liver lesion, for bilobar disease, and as an aid in segmental resections. We believe that the data in the literature provide confirmation of phase I (safety) and single-institution phase II (response) criteria. Multi-institutional phase II or phase III randomized studies may yet be warranted. The high rate of recurrence in multifocal liver tumors after cryotherapy suggests the need for effective adjuvant therapies. One such option appears to be the addition of intrahepatic infusion of cytotoxic drugs. Minimally invasive techniques for ultrasound-guided cryoablation will continue to evolve.

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Transcriptional Activation of the Human Villin Gene During Enterocyte Differentiation

Richard A. Hodin, M.D., Amy Shei, B.S., Shufen Meng, M.D.

Enterocyte differentiation occurs along the crypt-villus axis and is generally thought to involve the transcriptional activation of cell-specific genes, among which is the brush-border structural protein villin. We have examined the molecular mechanisms of villin induction using both in vivo and in vitro systems. Total RNA was purified from rat tissues or cultured cells by the guanidinium thiocyanate method and Northern blot analyses carried out using radiolabeled complementary DNA probes specific for villin or the actin control. Transient transfection (calcium/phosphate method) assays were performed using a luciferase reporter gene containing 2 kb of the human villin gene 5′-flanking region. We have found that the villin mRNA was expressed at high levels in the small intestine, to a lesser degree in the colon, and was not detected in the brain or liver. In HT-29 cells, villin mRNA levels increased 2.5-fold ($P < 0.001$) after 24 hours of sodium butyrate treatment, consistent with the process of enterocyte differentiation. Similarly, villin gene expression was induced in Caco-2 cells during postconfluence differentiation. Transient transfection assays demonstrated marked reporter gene activation (fourfold, $P < 0.001$) in response to sodium butyrate in HT-29 cells, but no activation in the liver cell line HepG2. The effects of sodium butyrate were dose dependent, reaching a maximum at a concentration of 5 mmol/L. We conclude that a 2 kb region of the human villin gene is able to mediate its transcriptional activation during HT-29 cell differentiation. This DNA regulatory region appears to function in a cell type–specific (gut) manner. (J GASTROINTEST SURG 1997;1:433-438.)

The mammalian small intestine is lined with an epithelium in which undifferentiated, pluripotent crypt stem cells give rise to four lineages of differentiated cells including enterocytes, goblet, enteroendocrine, and Paneth's cells. The transition from the undifferentiated, renewable crypt cell to the terminally differentiated villus enterocyte appears to involve the transcriptional activation of a number of cell type–specific genes including the enzymes, transporters, and structural proteins that reside within the apical microvilli.[1,2] Among the markers of enterocyte differentiation is the actin binding protein, villin. Villin is the major structural component of the brush-border membrane and its expression is limited to the epithelia of the small and large intestine as well as the proximal renal tubule.[3,4] The molecular mechanisms responsible for the tissue-specific pattern of villin expression are not well known.

HT-29 and Caco-2 cells are human colon cancer–derived cell lines that have been used extensively as in vitro models for the intestinal epithelium. Both cells appear to most closely resemble small intestinal crypt cells, but each has the capacity to differentiate into a more villus-like phenotype under various experimental conditions. For example, HT-29 cells have been shown to differentiate in response to sodium butyrate (NaBu) treatment[5] or prolonged culture in glucose-free media,[6] whereas Caco-2 cells differentiate when grown under postconfluent conditions.[7] In all cases cellular differentiation has been documented by both morphologic (tight junctions or microvilli among others) and biochemical (brush-border enzyme expression) criteria.

In a previous study from this laboratory,[8] we examined the induction of villin gene expression in NaBu-treated HT-29 cells. We determined that villin in-

From the Department of Surgery, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Mass.
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Reprint requests: Richard A. Hodin, M.D., Department of Surgery, Beth Israel Hospital, 330 Brookline Ave., Boston, MA 02215.

duction occurs at the messenger RNA level and is entirely dependent on the synthesis of new proteins. In addition, the maximal effects on villin gene expression were seen at a NaBu concentration of 5 mmol/L, with lesser effects at both lower and higher doses. In the present study we employed both in vivo and in vitro systems to further elucidate the molecular mechanisms that govern villin gene regulation.

MATERIAL AND METHODS

Tissue Harvest

Adult Sprague-Dawley rats were maintained on standard chow in wire cages in accordance with the guidelines set forth by the Beth Israel Hospital Animal Use Committee. The animals were anesthetized with intraperitoneal pentobarbital, and harvested tissue samples were immediately frozen in dry ice and stored at −80° C. The anatomic distinction between intestinal segments was as previously described.[9]

Cell Culture

HT-29, Caco-2, and HepG2 cells were purchased from American Type Culture Collection (Rockville, Md.) and grown in 160 cm^2 plastic flasks at 37° C/5%CO_2 in Dulbecco's modified Eagle's media (Gibco, Grand Island, N.Y.) supplemented with 10% fetal bovine serum (Sigma, St. Louis, Mo.), 2 mmol/L L-glutamine, and penicillin/streptomycin (100 U/ml). The media were changed every 3 days and the cells were separated via trypsinization when they reached confluence. The media were changed 24 hours prior to the start of each experiment and treatments with NaBu were carried out as indicated.

Northern Blot Analysis

Total RNA was extracted from rat tissues or cells in culture using the guanidinium thiocyanate method.[10] Northern blot analyses were performed by loading 20 μg of RNA in each lane of an agarose/formaldehyde gel, separating through electrophoresis, then transferring to nitrocellulose membranes, and baking for 2 hours at 80° C. Examination of ethidium bromide–stained gels verified equal loading of RNA in each lane. Complementary DNA probes were labeled with ^{32}P by the random primer method,[11] typically to a specific activity of 5×10^8 cpm/μg DNA. The villin probe is a 530 bp fragment from the human cDNA and was provided by Dr. M. Arpin.[12] The actin probe is a 1.0 kb PstI fragment derived from mouse β-actin cDNA.[13] Conditions for hybridization were as follows: 5X standard saline citrate (SSC)/50% (volume/volume) formamide/1% (weight/volume) sodium dodecyl sulfate (SDS) at 42° C. Washing conditions were 2X SSC/0.1% SDS at 50° C. Relative changes in mRNA levels were determined by laser densitometry of autoradiograms and normalized for actin mRNA. Statistical analyses were carried out using Student's unpaired t test; $P < 0.05$ was considered significant.

Nuclear Run-On Assays

Nuclei were isolated from the cells in culture using standard NP-40 lysis and stored at −70° C. The run-on transcription reaction was performed using thawed nuclei (approximately 10^7) in the presence of ^{32}P uridine triphosphate (100 μCi). Following DNA and protein digestion, the radiolabeled RNA was precipitated with trichloracetic acid and purified. The RNA was then hybridized to nitrocellulose filters containing linearized cDNA plasmids corresponding to villin, intestinal alkaline phosphatase (IAP), and actin. Hybridization and washing conditions were similar to those used for Northern blot analyses.

Transient Transfection

HT-29 or HepG2 cells were transferred to 100 mm^2 dishes and grown to 80% confluence. The villin reporter plasmid contains approximately 2 kb of the human villin 5′-flanking region linked to the luciferase structural gene and was kindly provided by Dr. S. Robine.[14] Ten to 15 μg of DNA was transfected in each experiment, along with 2 μg of a thymidine kinase–human growth hormone (TK-GH) plasmid that was used to control for transfection efficiency. The DNA to be transfected was precipitated with ethanol and resuspended in $CaCl_2$/HEPES buffer and then added directly to the culture dish. After 24 hours, the media were changed and the cells incubated for 24 hours with NaBu as indicated. The cells were then washed with phosphate-buffered saline solution and extracts prepared using a freeze-thaw method. Protein assays were done by the Bradford method[15] and luciferase activity in relative light units was determined by standard luminometry.

RESULTS

Villin Gene Expression in Rat Tissues

Villin mRNA levels were examined in a variety of intestinal and nonintestinal rat tissues by Northern blot analyses to verify the tissue-specific pattern of villin gene expression (Fig. 1). No villin mRNA was detected in the forestomach, which is an anatomic continuation of the esophagus and is lined with stratified squamous epithelium. So, too, villin mRNA was

absent from the glandular stomach, which is lined with columnar cells that lack microvilli. In contrast, abundant villin mRNA was seen along the length of the small intestine, with lesser amounts as one moves from the proximal colon to the rectum. A single villin mRNA band is detected in these rat tissues, which is in agreement with findings in previously reported studies.[4] In nonintestinal tissues, that is, liver and brain, no villin mRNA was evident.

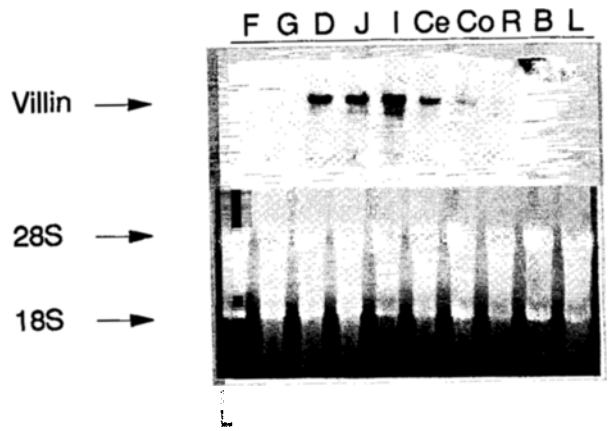

Fig. 1. Tissue distribution of villin gene expression. Northern blot analyses were performed on total cellular RNA derived from various rat tissues and probed with the human villin cDNA. Twenty micrograms of RNA was loaded in each lane with equal amounts verified by ethidium bromide staining as shown in the bottom panel. F = forestomach; G = glandular stomach; D = duodenum; J = jejunum; I = ileum; Ce = cecum; Co = colon; R = rectum; B = brain; and L = liver.

Villin Expression During Enterocyte Differentiation In Vitro

Northern blot analyses of RNA derived from HT-29 and Caco-2 cells revealed basal villin mRNA levels and dramatic induction as a function of the differentiation process (Fig. 2). In HT-29 cells 48 hours of NaBu treatment caused a 2.5-fold increase ($P < 0.001$) in villin mRNA levels, as we have previously reported.[6] In Caco-2 cells villin levels increased threefold ($P < 0.001$) at 14 days of postconfluent growth. Two human villin mRNA species are seen, 4.0 and 3.2 kb, and these have previously been shown to arise by an alternative choice of polyadenylation signals.[16] In contrast to its expression in these intestinal cells, villin mRNA was not detected in the liver cell line HepG2 under either basal or NaBu-treated conditions (not shown).

Nuclear Run-On Assays

As shown in Fig. 3, the rate of villin transcription increased with 24 hours of NaBu treatment, whereas the actin control was unchanged. For control purposes a second enterocyte marker, IAP, was also seen to be induced by NaBu at the level of gene transcription.

Transient Transfection Studies

The villin-luciferase reporter plasmid was transfected into HT-29 cells and treatments were carried

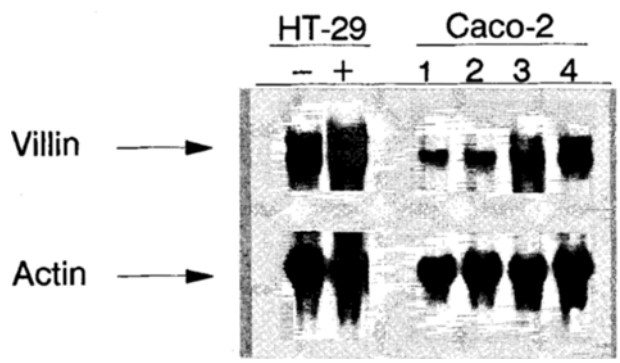

Fig. 2. Villin gene expression as a function of enterocyte differentiation. HT-29 cells were treated with either saline solution (–) or 5 mmol/L NaBu (+) for 48 hours and Caco-2 cells were harvested at *(1)* 80% confluence, *(2)* 100% confluence, *(3)* 7 days post confluence, or *(4)* 14 days post confluence. Twenty micrograms of RNA was loaded in each lane with equal amounts verified by ethidium bromide staining and probing with the actin control (bottom panel). Increases in villin mRNA levels in both models of enterocyte differentiation were statistically significant ($P < 0.001$).

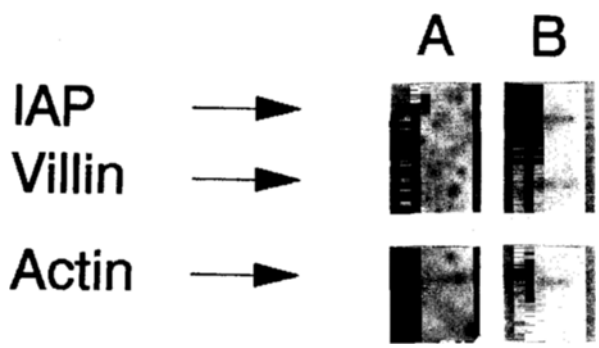

Fig. 3. Villin transcriptional regulation. Autoradiograms of nuclear run-on assays are depicted (n = 3) and demonstrate the increases in both villin and intestinal alkaline phosphatase *(IAP)* gene transcription in HT-29 cells by NaBu. There is no change in the actin control.

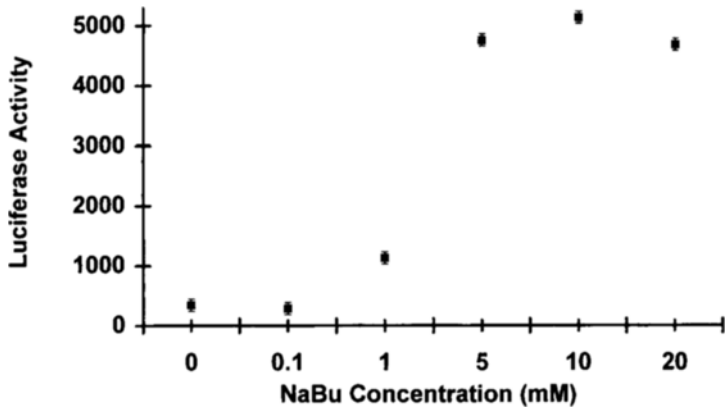

Fig. 4. Transactivation of villin reporter gene by NaBu. HT-29 cells were transfected with a villin-luciferase reporter plasmid and treated for 24 hours with increasing concentrations of NaBu. Data represent the mean ± standard error of the mean from four independent experiments and refer to relative light units, as determined by standard luminometry. As a control for transfection efficiency, 2 μg of TK-GH plasmid was cotransfected and luciferase activity divided by growth hormone levels in the serum.

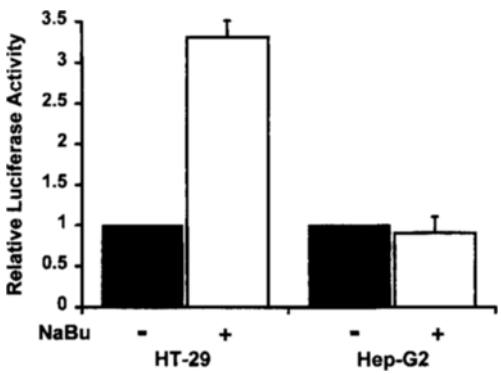

Fig. 5. Transient transfections in HT-29 and HepG2 cells. HT-29 or HepG2 cells were transfected with a villin-luciferase reporter plasmid and treated for 24 hours with 5 mmol/L NaBu. Data represent the mean of three independent experiments and refer to relative light units (arbitrarily defined as 1 for both HT-29 and HepG2 cells) in NaBu-treated cells compared to untreated cells. As a control for transfection efficiency, 2 μg of TK-GH plasmid was cotransfected in each experiment.

out with various doses of NaBu. As seen in Fig. 4, modest reporter gene activation occurred at 1 mmol/L NaBu, with higher levels at 5 mmol/L NaBu. The high level of reporter gene activation was maintained at 10 and 20 mmol/L NaBu. A promoterless luciferase plasmid was used for control purposes and showed only background activity under both control and NaBu-treated conditions.

Depicted in Fig. 5 are the results of transfections in HT-29 cells compared to HepG2 cells. An approximate 3.5-fold induction in luciferase activity was seen in the case of the HT-29 cells, whereas no induction by NaBu was seen in HepG2 cells. In another set of experiments there was marked (threefold) villin reporter gene induction with NaBu treatment seen in Caco-2 cells but minimal induction (~1.5-fold) in the breast cancer cell line MCF-7 (not shown).

DISCUSSION

Villin is a calcium-regulated, actin binding protein that is a major component of the enterocyte brush border and appears to play a critical role in the establishment of apical microvilli. For example, transfection of the human villin cDNA into fibroblasts causes a redistribution of F-actin and growth of microvilli,[17] whereas villin antisense expression impairs the assembly of the brush border in Caco-2 cells.[18] In the intact gut, low levels of villin are present in undifferentiated crypt cells, whereas high levels exist in the differentiated villus cells. In addition to the intestinal epithelia, villin is also expressed in the proximal tubular epithelium of the kidney, cells that also contain an apical brush border.

Our studies in the intact rat serve to verify the pattern of tissue-specific villin expression and show this to be due to regulation at the level of gene expression. The findings of villin mRNA induction by NaBu in HT-29 cells and in postconfluent Caco-2 cells have not been previously reported and indicate that the regulation of villin expression in vitro is also primarily at the mRNA level. These results are consistent with the increased villin mRNA levels previously re-

ported in HT29-18 cells in which glucose deprivation was used as the differentiation stimulus.[4] The nuclear run-on assays demonstrate that this increase in villin occurs at the transcriptional level, as predicted from previous work on villin gene regulation.[14] The possibility remains, however, that changes in mRNA half-life also contribute to the induction of villin mRNA by NaBu.

Robine et al.[14] have shown that 2.0 kb of human villin 5′-flanking DNA was capable of mediating the increase in HT-29 cell villin expression on differentiation in glucose-free media. Since we found that the identical reporter plasmid was activated by NaBu, it is possible that a common mechanism might underly villin transcriptional activation during HT-29 cell differentiation. The precise mechanism by which NaBu alters cellular growth and differentiation has not been established. NaBu is known to block histone deactylase leading to hyperacetylation[19] and a link between histone acetylation and gene transcription has recently been identified.[20] It is not known whether glucose deprivation similarly alters the state of histone acetylation. Further work will be needed to identify the exact DNA *cis*-regulatory element(s) and *trans*-acting factor(s) that function to regulate villin gene expression in the context of crypt-villus differentiation.

The dose-dependent effects of NaBu appear to be different in the transfection studies compared to the endogenous villin gene. That is, we have previously shown in HT-29 cells that the maximal effects of NaBu on villin mRNA levels were at the 5 mmol/L concentration, whereas the effects were almost completely lost at the 20 mmol/L concentration. This unusual dose-response curve was also seen in the case of IAP, another marker of differentiation.[8] In contrast, recent studies from our laboratory have revealed that the induction of the cell cycle inhibitor, p21, was seen to plateau at 5 mmol/L NaBu, with similar induction at 20 mmol/L NaBu (unpublished observations). This latter dose response is the same as that seen in the present transfection studies in which only transcriptional activation through the villin 5′-flanking DNA is being tested. It is possible, therefore, that transcriptional activation continues at the higher NaBu concentration but that a separate mechanism comes into play in the case of villin and IAP (but not p21), which leads to a decrease in mRNA levels, for example, decreased mRNA half-life. Further studies will be needed to explain this apparent discrepancy in the NaBu dose response between the endogenous gene regulation and the reporter gene activation.

The present studies add to our understanding of villin gene regulation, demonstrating tissue specificity both in vivo and in vitro. The dramatic difference in villin reporter gene transactivation in the intestinal vs. the nonintestinal cell lines provides the basis for future studies aimed at elucidating the mechanisms underlying gut-specific gene expression.

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Discussion

Dr. R. Fitzgerald (Palo Alto, Calif.). I know that in glucose-deprived induction of differentiation in HT-29 cells, there are microvillar inclusions that contain villin. I wondered in your butyrate differentiation model whether you observed microvillous inclusions and whether that could account for some of your increased transcription of villin mRNA?

Dr. R.A. Hodin. We have not yet examined this very extensively, but with electron microscopy, we do see changes with butyrate, as has been reported by others. These changes usually require more than just a couple of days. As you probably know, the glucose deprivation model usually requires weeks of incubation. That also appears to be the case with butyrate, where changes require a number of days, or even weeks, to occur.

Dr. T. Ko (Galveston, Tex.). Have you looked at other intestinal epithelial cell lines that do not differentiate in tissue culture? Are you using HepG2 as your control? Have you looked at IEC-6 or Caco-2 cells? Do they lack villin?

Dr. Hodin. The only other intestinal cell line that we have looked at is the IEC-6 line, which does not contain any villin mRNA by Northern blot analyses. We have not actually done transfection studies in those cells to see whether they have the machinery to turn on the villin gene.

Dr. B. Schirmer (Charlottesville, Va.). How specific is this model physiologically for butyrate? Is it a physiologic substance that is necessary, or is this simply something that is necessary in the in vitro model?

Dr. Hodin. I think it is really an in vitro system we are talking about. Butyrate has a number of effects on different cell lines. I think it is difficult to look at the in vitro systems and try to draw any parallels with the in vivo system concerning what short-chain fatty acids might be doing. It is true, however, that butyrate is present in the human colon at concentrations similar to those used in vivo.

Dr. Schirmer. Can you achieve the same incubation with another substance, or is butyrate specific to this in vitro system?

Dr. Hodin. The only other thing that has really worked in HT-29 cells, as one of the questioners mentioned, is this glucose deprivation model. If you take these HT-29 cells out of glucose-containing media and put them in galactose-containing media, over a period of weeks the cells will appear to differentiate.

Small Bowel Transplantation: Effects on Function of Nonadrenergic, Noncholinergic Nerves

Michel M. Murr, M.D., Michael G. Sarr, M.D.

Previous work from our laboratory showed that spontaneous contractile activity of jejunal smooth muscle increases after small bowel transplantation. Our aim was to determine whether small bowel transplantation alters the function of nonadrenergic, noncholinergic (NANC) nerves. Seven groups of rats (n ≥7 in each group) were studied as follows: 1 week after sham celiotomy and 1 week and 8 weeks after 45 minutes of ischemia/reperfusion (IR1 and IR8), jejunal and ileal transection and reanastomosis (TR1 and TR8), or orthotopic small bowel transplantation (TX1 and TX8). Contractility of jejunal circular muscle strips was studied in vitro. Spontaneous contractile activity increased in the IR1, TR1, and TX1 and TX8 groups ($P < 0.01$). Under NANC conditions, spontaneous activity increased in TR1 and in both TX1 and TX8 ($P < 0.01$) despite the lack of an increase in the frequency of contractions in TX1. Electrical field stimulation inhibited contractile activity at low frequencies, but under NANC conditions this inhibition persisted at higher frequencies. The calculated equieffective frequency (F_{100}) that produced a response equal to baseline contractile activity was similar in all groups, but under NANC conditions was greater in TX1 ($P < 0.025$). Functional alterations of NANC nerves are partly responsible for the increase in spontaneous activity in rat jejunal circular muscle strips after a limited ischemia/reperfusion injury, after selective disruption of enteric neural continuity (transection/reanastomosis), and after small bowel transplantation. These findings may provide important insight into graft dysfunction after small bowel transplantation in humans. (J GASTROINTEST SURG 1997;1:439-445.)

Our understanding of the enteric function of the transplanted gut lags behind both the technical advances and our current knowledge of the immunobiology of small bowel transplantation (SBTx). Despite seemingly adequate engraftment, dysfunction is not uncommon in intestinal grafts and may be the result of derangements brought on by the transplantation procedure itself (denervation, ischemia/reperfusion injury) or by immune phenomena.[1]

Although vascular and enteric continuity are restored by SBTx, extrinsic neural reinnervation to the gut does not occur.[2] Extrinsic denervation of the gut has rather insignificant functional impact on global patterns of motility[3,4]; however, neither its acute or chronic effects on smooth muscle contractility nor the adaptive process that occurs in response to permanent extrinsic denervation have been elucidated. Extrinsic nerves project to ganglia in the enteric nervous system (ENS) and not directly to the smooth muscle cells. The ENS has a major regulatory effect on intestinal function,[5] and its inhibitory effect on the inherently excitable myocytes is mediated, in large part, through intrinsic nonadrenergic, noncholinergic (NANC) nerves and extrinsic adrenergic nerves.[6,7] Our previous work demonstrated that SBTx does not result in hypersensitivity to cholinergic and adrenergic agonists, and the increase in spontaneous contractile activity may be the result of an alteration in the tonic output of NANC nerves.[8,9] The current study was designed to extend this work by determining which of the nonimmunologic perturbations necessitated by SBTx (nonspecific effects of anesthesia, extrinsic denervation, ischemia/reperfusion, or interruption of the continuity of the ENS) affects spontaneous or stimulated output of the enteric NANC nerves.

From the Department of Surgery and the Gastroenterology Research Unit, Mayo Clinic and Mayo Foundation, Rochester, Minn.
Supported by United States Public Health Service grant DK39337 from the National Institutes of Health and by the Mayo Foundation.
Correspondence: Michael G. Sarr, M.D., Professor of Surgery, Gastroenterology Research Unit, Mayo Clinic, 200 First St. SW, Rochester, MN 55905.

MATERIAL AND METHODS

Animal Preparations

Procedures and subsequent animal care were performed according to the guidelines of the Animal Care and Use Committee of the Mayo Foundation in accordance with the guidelines of the National Institutes of Health and the United States Public Health Service Policy on the Humane Use and Care of Laboratory Animals. All animals used were male Lewis rats (Harlan Sprague Dawley, Inc., Indianapolis, Ind.) weighing 250 to 300 g. General anesthesia was achieved by intraperitoneal injections of 30 to 50 mg/kg sodium pentobarbital (Ampro Pharmacy, Arcadia, Calif.). Animals were divided into the following groups: sham celiotomy (SC), ischemia/reperfusion (IR), intestinal resection/reanastomosis (TR), and intestinal transplantation (TX).

Sham Celiotomy. The abdominal cavity was opened via a midline incision, and the intestine was manipulated. Thirty minutes later the abdominal wall and skin were closed in layers. Rats were studied 1 week later (n = 8).

Ischemia/Reperfusion. After a midline celiotomy, the vascular arcades were ligated at the mesenteric side of the proximal jejunum and terminal ileum, and the intestinal lumen was clamped at these two points to prevent backflow. Rats were then given 1.5 ml of heparinized Ringer's lactate (10 U/ml) intravenously and the superior mesenteric artery was occluded for 30 to 35 minutes, thereby stopping all vascular inflow to the entire jejunoileum. The jejunoileum was kept outside the abdomen and immersed in chilled Ringer's lactate and ice. Core temperature was monitored by means of a rectal thermometer. After unclamping, 2 to 3 ml of warmed Ringer's lactate was given intravenously. Rats were studied 1 week (IR1, n = 9) and 8 weeks (IR8, n = 7) postoperatively.

Intestinal Transection/Reanastomosis. The continuity of the intrinsic (enteric) nervous system between the jejunoileum and the duodenum was disrupted by transecting the proximal jejunum and terminal ileum followed by immediate reanastomosis, thereby neurally isolating the intramural ganglia and plexuses of the ENS in the jejunoileum from the proximal gut pacemaker without any ischemic event but maintaining extrinsic innervation. Rats were studied 1 week (TR1, n= 8), and 8 weeks (TR8, n = 8) postoperatively.

Intestinal Transplantation. Inbred Lewis rats were used as donors and recipients to avoid immunosuppression and immune reactions. Total orthotopic small intestinal *iso*transplantation was performed using standard microvascular techniques.[8,10] In brief, after flushing both the intestinal lumen with a chilled 1% neomycin solution and the graft vasculature with 2 to 3 ml of chilled heparinized (10 U/ml) Ringer's lactate, the jejunoileum was removed and stored in chilled Ringer's lactate while the recipient was being prepared. The graft was revascularized by end-to-side, aorta-aorta, and portal vein–inferior vena cava anastomoses (warm ischemia, 30 to 60 seconds; cold ischemia, 40 to 50 minutes). Subsequently, a native enterectomy was performed, and intestinal continuity was reestablished by means of a jejunojejunostomy and ileoileostomy. Recipients received 1 ml/hr of warmed Ringer's lactate intravenously during the procedure. The animals were allowed immediate access to water and rat chow. Animals that died within 4 days were considered technical failures. None of the surviving animals developed intestinal obstruction. The animals were weighed every 2 days for the first week after SBTx and once a week thereafter. Animals were studied 1 week (TX1, n = 9) and 8 weeks (TX8, n = 8) after SBTx.

Conduct of Experiments

Rats were anesthetized as previously described, and a standardized segment of proximal jejunum was removed, opened, and pinned in chilled modified Krebs-Ringer's bicarbonate solution (composition in mmol/L: NaCl, 118.3; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 25.0; calcium disodium edetate, 0.26; and glucose, 11.1). Full-thickness strips (1 to 2 mm wide) were cut in the direction of the circular muscle layer, suspended in eight separate 25 ml tissue chambers at 37.5° C, filled with modified Krebs-Ringer's bicarbonate solution, and continuously bubbled with 95% oxygen and 5% carbon dioxide (Puritan-Bennett Corp., Overland Park, Kan.). The strips were suspended vertically between a fixed point in the chamber and a noncompliant force transducer (Kulite Semiconductor Products, Inc., Leonia, N.J.) to measure isometric force. Contractile activity was monitored on an eight-channel strip-chart recorder (Grass 7D Polygraph, Grass Instrument Co., Quincy, Mass.) and converted to digital signals by a computerized data acquisition system (Biopac Systems, Inc., Goleta, Calif.). The digital signals were displayed and stored on a computer (Reason 486, Reason Technology, Inc., Minneapolis, Minn.) for online analysis using specialized software (AcKnowledge, Biopac Systems, Inc.).

Length/Tension-L_o. Spontaneous activity stabilized after an initial 45 minutes of equilibration in tissue chambers with washout every 15 minutes. Each strip

was incrementally stretched to its optimal length (L_o) beyond which further stretching did not increase the amplitude of spontaneous contractions. Equilibration for 5 to 10 minutes was allowed after each stretch. All subsequent measurements and experiments were done at L_o. Strips that did not develop spontaneous contractile activity (1%) were excluded. All strips were allowed to equilibrate at L_o for 10 to 20 minutes before further interventions.

Electrical Field Stimulation. Trains of square-wave stimuli were generated by means of an SD9 stimulator (Grass Instrument Co.) and applied within the tissue chambers through two parallel platinum electrodes. Stimulus time was kept constant at 10 seconds by means of a computerized timer (Gralab S45, Dimco Gray Co., Dayton, Ohio). After equilibration at L_o, a standard stimulus (20 V, 4 msec duration, 20 Hz) was applied every 2 to 3 minutes until spontaneous phasic activity was abolished or a total of four stimuli were applied. Subsequently, stimuli with varying frequencies (1, 2, 5, 10, 20, and 50 Hz) were applied every 2 to 3 minutes with no intervening periods of washout.

Nonadrenergic, Noncholinergic Conditions. Experiments were carried out after incubation at L_o for 45 minutes with atropine (10^{-7} mol/L), propranolol (5×10^{-6} mol/L), and phentolamine (10^{-5} mol/L). Tetrodotoxin (10^{-6} mol/L) was added to the chambers after the conclusion of the experiment to verify the neural vs. myogenic nature of the response to EFS.

Measurement of Weight. At the conclusion of each experiment, all strips were washed thoroughly, blotted twice on No. 1 filter paper, and weighed (Mettler AC 100, Mettler Instrument Corp., Greifensee, Switzerland).

Drugs and Chemicals

DL-Propranolol hydrochloride, phentolamine hydrochloride, atropine sulfate salt, and tetrodotoxin were purchased from Sigma Chemical Company (St. Louis, Mo.). All concentrations are expressed as the final molar concentration in the tissue chamber.

Data Analysis

Baseline spontaneous contractile activity, as measured by the integral of the force generated (*g*-5 min), was determined at L_o before any interventions were made. The integral (area under the curve) rather than the mean or peak amplitude of contraction was measured to account for the frequency, duration, and both phasic and tonic contractions. The frequency of contractions was determined separately. The response to EFS was quantitated (integral of force) for the exact 10-second interval of stimulation, avoiding the "off-contraction" that occurs when the stimulus is terminated. The force generated (*g*-5 min) was standardized per wet weight of the respective strip (*g*-5 min/mg). We calculated two parameters during EFS. First, the equieffective frequency (F_{100}) was a calculated value extrapolated from frequency-response curves as the frequency that would result in a response equal to the baseline spontaneous activity. The values of F_{100} were compared to determine whether there was a shift in the frequency-response curves between groups. If the F_{100} differed from that in the SC group, we also calculated the difference in the force of contractions at the maximal inhibitory frequency in SC (2 Hz) and at the maximal excitatory frequency in SC (20 Hz) in an attempt to compare the relative response to EFS as a function of NANC nerves.

Statistical Analysis. One way analysis of variance was used to compare the means of all groups. If a significant F value (<0.05) was obtained, means were compared using Student's *t* test with Bonferroni's correction where appropriate. All data are mean ± standard error of the mean.

RESULTS

Spontaneous Contractile Activity

The force of spontaneous contractile activity was increased ($P \leq 0.008$) in IR1, TR1, and in both TX1 and TX8 when compared to SC (Table I). Spontaneous activity was 2.7 ± 0.3, 2.8 ± 0.3, 3.4 ± 0.3, and 2.6 ± 0.4 *g*-5 min/mg in IR1, TR1, TX1, and TX8, respectively, as compared to 1.1 ± 0.2 *g*-5 min/mg in SC. The frequency of contractions did not differ between groups indicating that the amplitude of contractions was increased.

Under NANC conditions, increases in spontaneous activity persisted in TR1, TX1 (Fig. 1), and TX8 when compared to SC (5.5 ± 0.5, 4.4 ± 0.3, and 4.7 ± 0.7 *g*-5 min/mg, vs. 2.2 ± 0.3 *g*-5 min/mg, respectively; $P \leq 0.01$). The increase in spontaneous activity was secondary to an increase in the amplitude of contractions; this increase was especially evident in TX1 in which the frequency decreased in comparison to SC (11 ± 1 vs. 23 ± 2; $P < 0.008$). NANC conditons also increased the spontaneous contractile activity in the SC, IR8, TR1, TX1, and TX8 groups and increased the frequency of contractions in all groups except IR1, TR1, TX1, and TX8 in comparison to baseline conditions.

Table I. Spontaneous contractile activity of jejunal circular muscle strips in vitro

	Baseline conditions		NANC conditions	
Group	Contractile force*	Frequency†	Contractile force*	Frequency†
SC	1.1 ± 0.2	13 ± 2	2.2 ± 0.3‡	23 ± 2‡
IR1	2.7 ± 0.3§	14 ± 1	3.0 ± 0.3	19 ± 3
IR8	1.5 ± 0.2	16 ± 1	2.7 ± 0.4‡	24 ± 2‡
TR1	2.8 ± 0.3§	17 ± 2	5.5 ± 0.5‡,§	20 ± 3
TR8	1.8 ± 0.3	10 ± 1	2.6 ± 0.2‡	21 ± 3‡
TX1	3.4 ± 0.3§	12 ± 1	4.4 ± 0.3‡,§	11 ± 1§
TX8	2.6 ± 0.4§	11 ± 1	4.7 ± 0.7‡,§	18 ± 3

Values are mean ± standard error of the mean (n ≥7 rats).
*g-5 min/mg.
†Contractions/min.
‡Differs from baseline; $P \leq 0.04$.
§Differs from SC group; $P \leq 0.008$.

Table II. Calculated equieffective frequency* (F_{100}) in jejunal circular muscle strips

Group	Baseline conditions	NANC conditions
SC	5 ± 1	19 ± 4
IR1	4 ± 1	14 ± 2
IR8	5 ± 1	17 ± 2
TR1	3 ± 2	36 ± 7
TR8	4 ± 1	16 ± 2
TX1	4 ± 1	34 ± 4†
TX8	5 ± 1	21 ± 4

*Hz; values are mean ± standard error of the mean (n ≥7 rats).
†Differs from SC group; $P = 0.02$.

Electrical Field Stimulation

EFS elicited a frequency-dependent inhibition of spontaneous activity at low frequencies (1 to 5 Hz) and a frequency-dependent increase in force generated at higher frequencies (5 to 50 Hz) in all groups (see Fig. 1). A higher frequencies the muscle strips exhibited predominantly tonic contractions. The calculated equieffective frequency, F_{100}, that resulted in a response equal to 100% of baseline activity did not differ between groups (Table II).

Under NANC conditions, however, EFS caused a distinct inhibition of all phasic activity at both low and high frequency in all groups. The muscle strips responded to EFS at lower frequencies (≤5 Hz) with a short-lived decrease in baseline tone, reflecting a relaxation of the circular smooth muscle of the strip. At higher frequencies (>20 Hz) EFS caused a small increase in force as a result of a more tonic contraction. The F_{100} also increased in all groups under NANC conditions and was greater 1 week after SBTx (TX1) compared to SC (34 ± 4 vs. 19 ± 4 Hz; $P = 0.02$). When the effect of EFS was examined at 2 Hz, the relative inhibitory effect (when compared to spontaneous activity) was greater in TX1 than in SC (data not shown; $P < 0.01$). Similarly, at 20 Hz the effect of EFS on the SC group was a net increase in contractile activity, whereas in TX1 the net effect of EFS was an inhibitory effect ($P < 0.01$).

DISCUSSION

Graft dysfunction after gut transplantation continues to be a problem in the clinical setting.[11] This dysfunction is poorly understood and may result from immune reactions and immunosuppressive drugs, or it may be a consequence of the transplantation procedure itself, related to the extrinsic denervation, interruption of enteric neural continuity of the graft with the proximal gut, ischemia/reperfusion injury, or lymphatic interruption. Indeed, all of these alterations are known to affect several aspects of gut function.[1]

In previous studies we demonstrated that isogeneic SBTx increases spontaneous contractile activity in the rat jejunum in vitro[8,9] by mechanisms that involve the ENS but not via a hypersensitivity to cholinergic or adrenergic agonists. Our previous work and the current study specifically addressed a model of total orthotopic syngeneic SBTx to avoid any confounding immune reactions or immunosuppression. These findings prompted us to investigate the effect that NANC nerves exhibit on contractile activity after SBTx, not only under baseline conditions but also after direct stimulation of the NANC nerves by EFS. In addition, we used different experimental groups to selectively control for the physiologic changes that

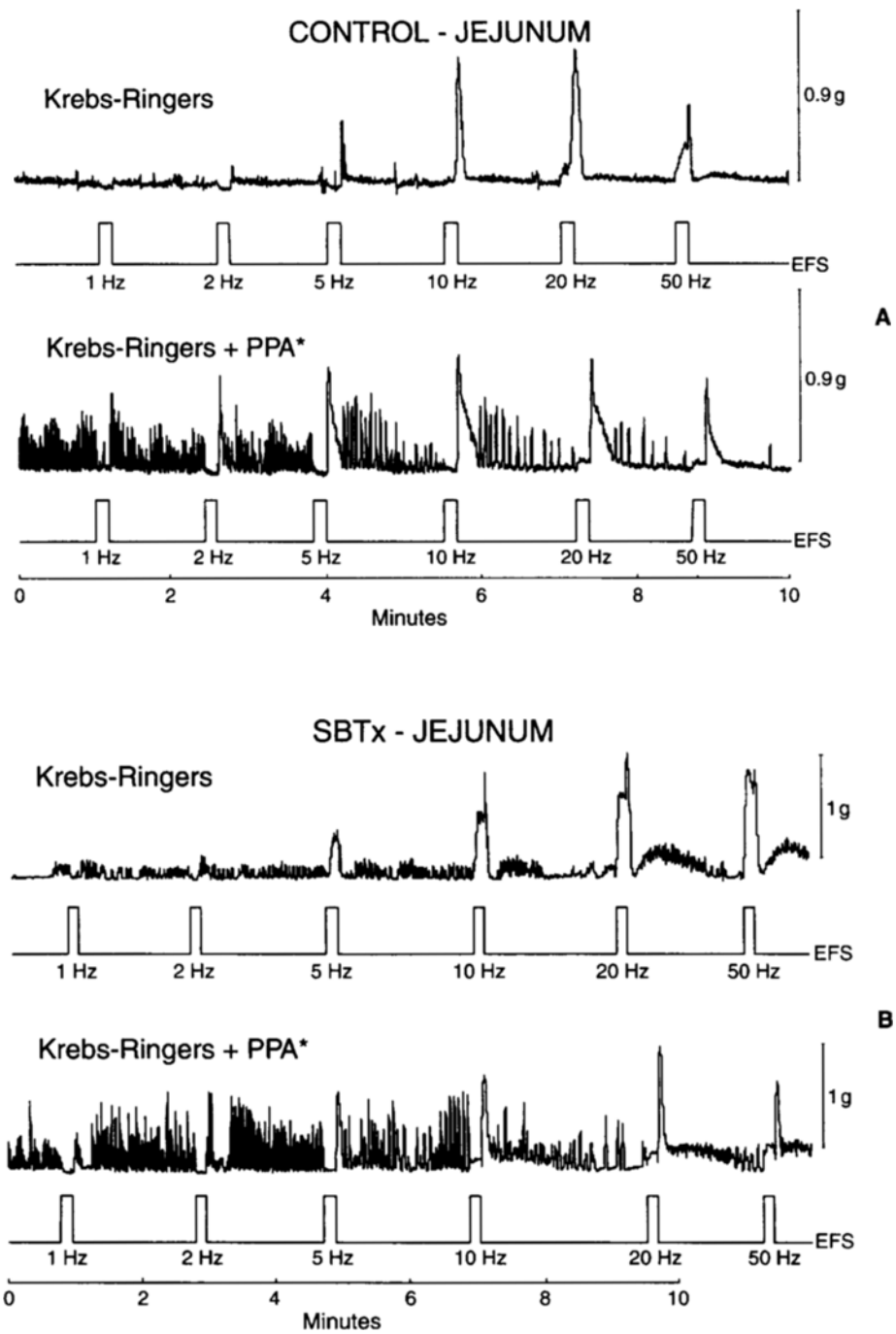

Fig. 1. Effect of electrical field stimulation (20 V, 4 msec) on rat circular muscle strips at different frequencies. **A,** Neurally intact control tissue. **B,** Muscle strips 1 week after orthotopic, isogeneic small intestinal transplantation. *PPA = propranolol (5×10^{-6} mol/L), phentolamine (10^{-5} mol/L), atropine (10^{-6} mol/L).

occur as necessitated by the transplantation procedure itself. The use of sham-operated control rats served another purpose in demonstrating that the anesthetic agents used or the effects of the celiotomy itself had no appreciable effect on smooth muscle or NANC nerve function 1 week after the operation.

The ENS contains intrinsic neurons, both cholinergic and peptidergic, which can be excitatory or inhibitory.[12] The ENS also receives direct innervation from extrinsic nerves, both vagal and sympathetic. The latter adrenergic nerves serve, in large part, an inhibitory function. Our past and current observations of an increase in spontaneous contractile activity after SBTx support the concept that extrinsic innervation serves as the brake on spontaneous, phasic, myogenic contractile activity. SBTx necessitates extrinsic denervation, and since it is known that reinnervation of the gut wall does not occur,[2,13] inhibition of smooth muscle contractile activity after SBTx may be mediated, in large part, by NANC nerves. This concept prompted our interest in studying the functional output of NANC nerves by measuring muscle contractility. Our experimental preparation evaluates the nerve-muscle components of the intestinal wall as one functional unit.

Our previous work did not demonstrate any hypersensitivity of jejunal smooth muscle strips to muscarinic cholinergic or adrenergic agonists[8,9]; thus we concluded that the increase in spontaneous contractile activity after SBTx may be secondary to an alteration in the contractile mechanism within the smooth muscle cell itself or secondary to changes in effective neural output from the ENS. We hypothesized that the injury related to ischemia/reperfusion and/or simple intestinal transection (disruption of enteric/intrinsic neural continuity with the proximal gut) may result in a further reduction in tonic inhibitory output from the ENS to the jejunal smooth muscle. The present study showed that contractile activity was increased at both 1 week and 8 weeks after SBTx partly as a result of a change in the functional output of NANC nerves. It is interesting that ischemia/reperfusion (IR1) and interruption of the continuity of the ENS (TR1) resulted in the same phenomena 1 week postoperatively, but this effect did not persist at 8 weeks (IR8 and TR8). Ischemia/reperfusion injury certainly affects mucosal structure and function[14] and may *directly* affect the function of the ENS and/or intestinal smooth muscle; these effects appear to be largely transient without long-term sequelae. Sprouting and reinnervation of enteric nerves across the anastomosis[15] may explain the temporal difference in the TR group, but it points to different mechanisms that may be at work in the IR, TR, and TX groups to produce a similar end result of increased contractile activity, especially since the frequency of contractions differed between groups. These mechanisms may not be mutually exclusive, because spontaneous activity was also increased after ischemia/reperfusion possibly, in part, through a modest hypersensitivity to bethanechol.[9]

Study of spontaneous contractile activity under NANC conditions was used in an attempt to abolish the tonic input of cholinergic and primarily adrenergic nerves. In muscle strips from control animals, contractile force showed a significant increase primarily via a prominent increase in the frequency of contractions. Similar effects were seen in IR8 and TR8. IR1 failed to demonstrate an increase in either force or frequency, whereas TX1 and TX8 showed an increase in force with no change in frequency. The lack of an effect early after selective ischemia/reperfusion (IR1) without extrinsic denervation suggests that ischemia/reperfusion injury may, at least early postoperatively (1 week), either alter adrenergic function or downregulate NANC nerve output, but this effect does not persist at 8 weeks postoperatively. The persistence of an increase in contractile force in the SBTx groups (both at 1 week and 8 weeks) suggests a primary effect on NANC nerve function because all adrenergic input is necessarily absent after SBTx.

EFS under baseline conditions stimulates all intrinsic nerves with the release of many neurotransmitters (both excitatory and inhibitory) resulting in either a net inhibitory or net excitatory effect on muscle activity. Selective blockade of adrenergic and cholinergic receptors unmasks the spectrum of NANC nerves in response to EFS; under these conditons both low (<5 Hz) and high (>5 Hz) frequencies resulted in inhibition of phasic activity as compared to inhibition of phasic activity only at low frequency (EFS) under baseline conditions. The rightward shift of F_{100} in the TX1 group suggests either an upregulation of inhibitory NANC nerves, a downregulation of excitatory NANC nerves, or a primary effect on the smooth muscle itself. This study does not allow any differentiation among these possibilities; evidence from histologic studies by others supports the hypothesis of an upregulation of NANC neurons because NADPH-diaphorase activity (an indirect marker for nitric oxide production) and gene expression of vasoactive intestinal polypeptide, an inhibitory transmitter, are increased after SBTx.[16,17] Similar work using a different methodology and addressing longitudinal muscle (rather than circular muscle) to study contractile activity found that the NANC component of the response to EFS was increased at 1 month and 1 year after SBTx.[18]

One additional consideration must be acknowledged. Although our experiments were focused on net

NANC nerve output, we are unable to fully exclude primary alterations in myogenic activity by the different surgical perturbations introduced into the experimental setup independent of any neural influence. We are assuming that the responses to EFS are secondary to nerve stimulation with release of neurotransmitters, both excitatory and inhibitory; however, EFS may alter smooth muscle contractility independent of neural stimulation. Although we tried to control for this by studying tissues at different frequencies of EFS and under differing experimental conditions (baseline, NANC conditions, and previously with bethanechol and norepinephrine), it remains possible that ischemia/reperfusion, intestinal transection, and SBTx may alter the smooth muscle excitability, contractility, and response to EFS independent of neural influences.

CONCLUSION

Interruption of the ENS (transection/reanastomosis) alone or in combination with extrinsic denervation, as with SBTx, increases spontaneous contractile activity in the rat jejunal circular smooth muscle by altering the functional output of NANC nerves under baseline and stimulated conditions. The changes in response to EFS in TX1 are not apparent in TX8, whereas changes in spontaneous activity are persistent in both TX1 and TX8, suggesting that different subgroups of NANC nerves may be affected differentially by SBTx and the mode of stimulation. These findings may have important implications for understanding and treating graft dysfunction after SBTx in humans.

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Does Extended Pancreaticoduodenectomy Increase Operative Morbidity and Mortality vs. Standard Pancreaticoduodenectomy?

Calogero Iacono, M.D., Luca Bortolasi, M.D., Enrico Facci, M.D., Giovanni Falezza, M.D., Gaetano Prati, M.D., Gerardo Mangiante, M.D., Giovanni Serio, M.D.

The poor prognosis of pancreatic carcinoma after resection is related to distant metastases and local recurrence that is characterized by a strong tendency to infiltrate the retroperitoneal tissue and spread along the neural plexuses and lymph nodes. Thorough clearance of these tissues around the celiac and mesenteric axes, aorta, and inferior vena cava from the diaphragm to the inferior mesenteric artery (extended pancreaticoduodenectomy may lower the rate of local recurrence, but the procedure has been criticized for its higher morbidity and mortality. Our aim was to compare extended pancreaticoduodenectomy (EPD) with standard pancreaticoduodenectomy (SPD) in terms of postoperative morbidity and mortality. Data from 47 patients who underwent either EPD (n = 24) or SPD (n = 23) between November 1992 and October 1995 were retrospectively analyzed. Preoperative laboratory findings, operative risk (according to the American Society of Anesthesiologists classification), type of operation (classic Whipple vs. pylorus-preserving Whipple), operative time, intraoperative blood and plasma transfusion, postoperative morbidity and mortality, and postoperative hospital stay were scrutinized. The results showed that all of the parameters considered were similar in the EPD and SPD groups (intraoperative blood transfusion 800 ± 490 ml vs. 700 ± 586 ml, postoperative mortality 0% vs. 4.3%, overall morbidity 45.8% vs. 47.8%, surgical morbidity 37.5% vs. 34.7%, and postoperative hospital stay 16 ± 8.1 days vs. 17 ± 13.1 days. These two groups differed only in the operative time, which was significantly longer for EPD than for SPD (360 ± 68.9 minutes vs. 330 ± 66.9 minutes, $P = 0.02$). Although the operative time is increased with EPD, there does not appear to be an increase in intraoperative complications, postoperative morbidity and mortality, or postoperative hospital stay with this procedure. However, definitive confirmation of these results can only be provided by a prospective randomized study. (J GASTROINTEST SURG 1997;1:446-453.)

Although a century has elapsed since Codivilla[1] performed the first pancreaticoduodenectomy in Italy in 1898, this remains a complex operation that carries a substantial operative risk. Perioperative mortality, which was high up until the 1970s,[2-4] is now less than 5% in specialized treatment centers,[5-10] and some surgeons have recently reported no operative deaths in large personal series.[8,11] Postoperative morbidity, on the other hand, remains high.[4,6,9,12,13]

In an attempt to improve survival in patients with pancreatic carcinoma, Fortner[14] introduced an approach termed "regional pancreatectomy." Later, Japanese surgeons proposed a technique referred to as "extended pancreaticoduodenectomy," which combined pancreaticoduodenectomy with extended lymphadenectomy and clearance of the neural plexuses and connective tissue of the celiac trunk, superior mesenteric axis, aorta, and inferior vena cava from the diaphragm to the inferior mesenteric artery.[15,16] This proposal was based on a detailed clinicopathologic study of the manner in which pancreatic cancer spreads.[15,16]

The aim of the present study was to assess whether extended pancreaticoduodenectomy (EPD) increases postoperative morbidity and mortality as compared to standard pancreaticoduodenectomy (SPD).

From the Department of Surgery, Division of General Surgery C, University of Verona, Verona, Italy.
Supported in part by a grant from MURST (Italian Ministry of the University and Scientific and Technological Research), Rome, Italy.
Reprint requests: Calogero Iacono, M.D., Department of Surgery, Division of General Surgery C, University Hospital, 37134 Verona, Italy.

PATIENTS AND METHODS

We reviewed the hospital records of 47 consecutive patients undergoing pancreaticoduodenectomy between November 1992 and October 1995 in the Division of General Surgery C, Department of Surgery, University of Verona.

EPD was performed in 24 patients (group A); there were 18 men and six women whose median age was 60 years (± 9.1 years standard deviation [SD]). SPD (group B) was performed in 23 patients (11 men and 12 women) whose median age was 58 years (± 11.7 years SD).

All patients with benign disease underwent SPD, whereas those with malignant disease were not selected but consecutively treated in the early period (November 1992 to December 1993) by SPD and in the ensuing period (January 1994 to October 1995) by EPD after the adoption of this operation as our standard procedure for surgical treatment of pancreatic and periampullary carcinoma.

The indications for extended and standard pancreaticoduodenectomies are listed in Table I. H_2 blockers and antibiotics were administered preoperatively. Somatostatin analogues were not used prophylactically.

Standard Pancreaticoduodenectomy

SPD was carried out in the usual manner. We performed the pancreatic resection at the neck level for benign disease and at the body level for pancreatic and periampullary carcinomas. In the group with malignant disease, two patients had a segmental and tangential resection, respectively, with reconstruction of the portal mesenteric axis because of vascular involvement. A pylorus-preserving technique was used in all patients with benign disease, as well as in one patient with intraductal papillary mucinous carcinoma and one with pancreatic carcinoma (which was recognized postoperatively after thorough pathologic examination of the specimen); all other malignant lesions in this group were treated with hemigastrectomy.

Table I. Indications for extended and standard pancreaticoduodenectomy

	EPD	SPD
Pancreatic carcinoma	16	15
Periampullary carcinoma	7	0
IPMcm	1	1
Cystic tumor	0	4*
Chronic pancreatitis	0	2
Duodenal cystic dystrophy	0	1

IPMcm = intraductal papillary mucinous carcinoma with mucinous invasion pattern.
*Two serous cystadenomas, 1 acinar cystic tumor, and 1 mucinous cystadenoma.

Extended Pancreaticoduodenectomy

We performed EPD via a different approach from the translateral one proposed by the Japanese authors.[15,16] The gallbladder was dissected from its bed and the lymphatic, neural, and connective tissue of the hepatoduodenal ligament was resected along its entire length; this maneuver was facilitated by early transection of the common hepatic duct near its bifurcation. Either hemigastrectomy or the pylorus-preserving technique was used in this group, and selections were made on the basis of randomized criteria. When the pylorus-preserving technique was performed, the pyloric lymph nodes were excised. The pancreatic body was dissected and clearance of the retropancreatic and periceliac tissue was completed with skeletonization of the common and proper hepatic artery and of the splenic artery 2 to 3 cm beyond the level of the pancreatic resection. Transection of the pancreatic body was performed on the left margin of the aorta and a portion of the resected margin was sent for frozen section analysis. The pancreatic head and uncinate process were dissected from the mesenteric vessels; meticulous retroportal and perimesenteric clearance of the adipose, neural, and lymphatic tissue was carried out and these were resected en bloc with the pancreaticoduodenal specimen. Once the specimen was removed, skeletonization of the aorta from the diaphragm to the inferior mesenteric artery was then performed (Fig. 1).

In both extended and standard pancreaticoduodenectomies, the pancreaticojejunostomy was performed in an intussusceptive end-to-end fashion; the common hepatic duct was anastomosed end to side with the jejunum, to avoid impinging on the intestinal mucosa with the interrupted absorbable stitches. The operation was completed using a side-to-side gastrojejunostomy or an end-to-side duodenojejunostomy.

All anastomoses were performed on the same loop according to the modified Child's technique[17] and were not stented. Two closed suction drains were placed close to the pancreatic and biliary anastomoses.

We compared patients undergoing EPD (group A) and SPD (group B) taking into consideration the preoperative values for hematocrit, hemoglobin, serum bilirubin, albumin, and total protein, operative risk according to the American Society of Anesthesiologists (ASA) classification, Whipple or pylorus-preserving procedures, operative time, intraoperative blood and plasma transfusions, postoperative mortality and morbidity, and postoperative hospital stay.

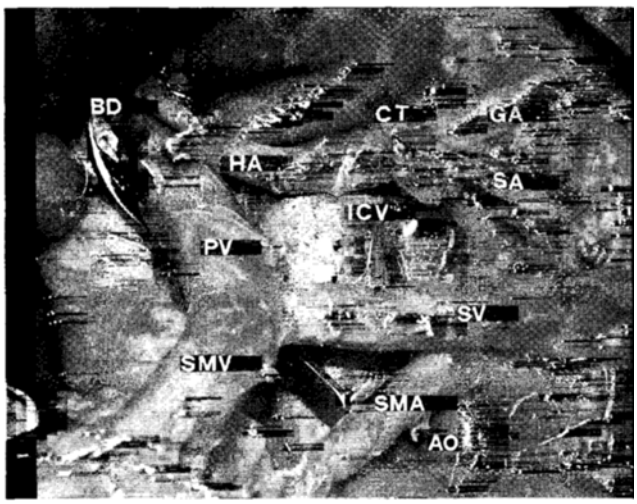

Fig. 1. Intraoperative findings after extended pancreaticoduodenectomy. BD = common hepatic duct; PV = portal vein; SMV = superior mesenteric vein; SV = splenic vein; ICV = inferior vena cava; HA = hepatic artery; CT = celiac trunk; SMA = superior mesenteric artery; SA = splenic artery; GA = gastric artery; AO = aorta; P = pancreas.

Statistical analyses were performed using Student's *t* test, Mann-Whitney U test, Fisher's exact test where appropriate, and chi-square test; *P* values <0.05 were considered statistically significant. Values were expressed as median ± standard deviation (SD) when the data were not normally distributed.

A diagnosis of pancreatic fistula was made when the daily peripancreatic drain output exceeded 10 ml and the amylase and lipase activity of this fluid was three times higher than plasma levels on or after postoperative day 4.[18]

Operative mortality was defined as death within 30 days of the operation.

RESULTS

Preoperative laboratory parameters, operative risk, and types of gastrointestinal reconstruction (hemigastrectomy with side-to-side gastrojejunostomy or pylorus-preserving technique with end-to-side duodenojejunostomy) were similar in groups A and B (Tables II and III). The only statistically significant dif-

Table II. Preoperative laboratory findings and operative risk according to American Society of Anesthesiologists classification in extended vs. standard pancreaticoduodenectomy

	EPD	SPD	*P* value
Hematocrit (%)	39 (±3.7)*	37 (±4.8)*	NS
Hemoglobin (g/dl)	12.9 (±1.4)*	12.5 (±1.6)*	NS
Serum bilirubin (mg/dl)	3.1 (±6.0)*	1.8 (±9.2)*	NS
Serum albumin (g/L)	37 (±4.7)*	40.6 (±5.1)*	NS
Serum total protein (g/L)	65.7 (±7.4)*	70 (±7.6)*	NS
ASA I (No. of patients)	7	9	NS
ASA II (No. of patients)	14	11	NS
ASA III (No. of patients)	3	3	NS

NS = not significant.
*Data are expressed as median ± standard deviation.

Table III. Type of operation (Whipple or pylorus-preserving), operative time, and blood and plasma transfusion in extended vs. standard pancreaticoduodenectomy

	EPD	SPD	*P* value
Pylorus-preserving pancreaticoduodenectomy (No. of patients)	7	9	NS
Whipple procedure (No. of patients)	17	14	NS
Operative time (min)	360 (±68.9)*	330 (±66.9)*	0.02
Intraoperative blood transfusion (ml)	800 (±490)*	700 (±586)*	NS
Intraoperative plasma transfusion (ml)	800 (±641)*	600 (±526)*	NS

NS = not significant.
*Data are expressed as median ± standard deviation.

ference was in operative time: median operative time in group A was 360 ± 68.9 minutes compared to 330 ± 66.9 minutes in group B (P = 0.02; Table III). Transfused volumes of blood and plasma were similar in the two groups (Table III).

The overall operative mortality rate was 2.1% with death occurring in 1 of 47 patients as a result of multiorgan failure following hemorrhagic shock. In this patient from group B, bleeding occurred on postoperative day 11 as a result of hepatic artery pseudoaneurysm rupture close to the ligature of the gastroduodenal artery. The patient underwent angiographic embolization, but on postoperative day 16 the bleeding recurred. At emergency laparotomy a necrotic left lateral segment of the liver was detected; the bleeding hepatic artery was ligated and a left lateral segmentectomy was performed. The patient died of multiorgan failure 2 days later.

The overall morbidity was 46.8% (22 of 47 patients) and rates were similar in the two groups: 45.8% (n = 11) in group A and 47.8% (n = 11) in group B. Surgical complications were present in nine (37.5%) and eight (34.7%) patients in groups A and B, respectively (P = NS) (Tables IV and V).

Pancreaticojejunal anastomosis leak was the most frequent surgical complication, with an incidence of approximately 17% in both groups. All pancreatic leaks were treated nonoperatively with maintenance of the peripancreatic drains placed during surgery, administration of the somatostatin analogue octeotride, and total parenteral nutrition.

There were three biliary leaks in group A vs. none in group B, but the difference was not statistically significant (Fisher's exact test, P = 0.23); all healed with conservative treatment.

Delayed gastric emptying occurred in only three cases: two in the EPD group (1 pylorus-preserving and 1 partial gastrectomy pancreaticoduodenectomy) and one in the SPD group (pylorus-preserving pancreaticoduodenectomy).

Table IV. Postoperative mortality, morbidity, and hospital stay in extended vs. standard pancreaticoduodenectomy

	EPD	SPD	*P* value
Postoperative mortality (No. of patients)	0 (0%)	1 (4.3%)	NS
Overall morbidity (No. of patients)	11 (45.8%)	11 (47.8%)	NS
Surgical complications (No. of patients)	9 (37.5%)	8 (34.7%)	NS
Postoperative hospital stay (days)	16 (±8.1)*	17 (±13.1)*	NS

NS = not significant.
*Data are expressed as median ± standard deviation.

Table V. Postoperative individual complications in extended vs. standard pancreaticoduodenectomy

	EPD	SPD	*P* value
Overall complications	11/24 (45.8%)	11/23 (47.8%)	NS
Pancreatic fistula	4 (16.6%)	4 (17.3%)	NS
Biliary leak	3 (12.5%)	0 (0%)	NS
Digestive leak	0 (0%)	1 (4.3%)	NS
Intra-abdominal hemorrhage	0 (0%)	1 (4.3%)*,†	NS
Intra-abdominal abscess	1 (4.1%)†,‡	0 (0%)	NS
Intra-abdominal fluid collection	1 (4.1%)	1 (4.3%)	NS
Sepsis	0 (0%)	1 (4.3%)	NS
Hepatic abscess§	1 (4.1%)†,‡	0 (0%)	NS
Delayed gastric emptying	2 (8.3%)	1 (4.3%)	NS
Venous cathether sepsis	1 (4.1%)	0 (0%)	NS
Pleural effusion and pneumonia	4 (16.6%)	5 (21.7%)	NS

NS = not significant.
*Operative mortality.
†Relaparotomy.
‡These complications were present in the same patient.
§Caused by preoperative percutaneous transhepatic biliary drainage for jaundice.

Relaparotomy was performed in two patients, one in each group. The group A patient had had preoperative percutaneous biliary decompression, which caused a hepatic hematoma. On postoperative day 30 after EPD, the patient had fever related to sepsis; ultrasound and CT scans demonstrated an abscess in the area of the hematoma and a separate intra-abdominal fluid collection. Percutaneous drainage was attempted but proved unsuccessful, and the patient underwent surgical drainage of the abscess. The group B patient was the one who died of multiorgan failure following hemorrhagic shock, as mentioned previously.

Length of postoperative hospitalization was similar in the two groups: a median of 16 ± 8.1 days in group A and 17 ± 13.1 days in group B (Table IV).

DISCUSSION

Pancreaticoduodenectomy is the treatment of choice for cancer of the pancreatic head, neck and uncinate process and for periampullary carcinoma. Because the mortality rate is less than 5%, it is also indicated for selected benign and inflammatory pancreatic diseases.[19] Overall survival rates in patients undergoing pancreaticoduodenectomy for pancreatic carcinoma[5,20-22] are reported to be lower than those in patients with periampullary carcinoma and particularly carcinoma of the papilla of Vater[5,6,13,23,24]; although some authors report 5-year survival rates higher than 20% for pancreaticoduodenectomy in pancreatic cancer.[8,10,25,26]

In an attempt to improve the poor prognosis of pancreatic carcinoma, more aggressive procedures such as total pancreatectomy[27,28] and regional pancreatectomy[29] have been proposed. Nevertheless, total pancreatectomy has failed to yield a better survival rate than pancreaticoduodenectomy in patients with pancreatic cancer.[30,31] Regional pancreatectomy also fails to improve the long-term survival rate and carries a high operative mortality.[29,32] Recently, however, Fortner et al.[33] have reported a 5.3% operative mortality rate (3.8% for subtotal regional pancreatectomy) and 33% 5-year survival after regional pancreatectomy for pancreatic lesions smaller than 2.5 cm.

In the early 1980s Japanese surgeons adopted EPD,[15,16] which owes its popularity to the fact that the poor prognosis of pancreatic cancer after resection is related to local recurrences and distant metastases.[34-36] Local recurrences are attributed to the fact that there is a strong tendency for pancreatic carcinoma to infiltrate the retroperitoneal connective tissue and spread along peripancreatic neural plexuses and locoregional lymph nodes.[37] Although EPD cannot prevent distant metastases,[38,39] it permits a more radical local resection, possibly decreasing the risk of local recurrence; it requires, in addition to pancreaticoduodenectomy, thorough clearance of the retroperitoneal lymphatic tissue, peripancreatic neural tissue, and connective tissue around the celiac axis and its branches, the mesenteric vessels, aorta, and inferior vena cava from the diaphragm to the inferior mesenteric artery.[15,16] This technique appears to reduce the rate of local recurrence[16] and increase overall survival in the Japanese experience. Ishikawa et al.[16] reported 3- and 5-year survival rates of 38% and 27%, respectively, in patients undergoing EPD vs. 13% and 9% in patients undergoing standard lymphadenectomy. Nagakawa et al.[40] reported similar results, with a 4.8% 5-year survival rate for standard resection vs. a 29.7% rate for extended resection. The main limitation of these retrospective studies, however, is that the patients were not randomized.

Although survival of patients with T_1 stage I disease was similar after standard and extended resection in one multicenter study, patients with T_1 stage II tumors had significantly higher 3- and 5-year survival rates after extended vs. standard pancreaticoduodenectomy (32.5% and 32.5% vs. 27.8% and 11.1% respectively; $P < 0.05$).[41] Some believe that these results can be achieved only when EPD is combined with intraoperative radiotherapy.[42]

However, other Japanese investigators and certainly most Western observers do not share this optimistic view. Nakao et al.[43] reported a 5-year survival rate of only 5% in 90 extended pancreatic resections (48 total and 42 pancreaticoduodenectomies). Geer and Brennan[10] report a median survival of 22 months for EPD vs. 18 months for SPD with no statistical difference ($P = 0.08$). Yeo and Cameron[44] believe that reports in the literature have failed to demonstrate a significant advantage in terms of survival for radical resection over standard resection, and they report a 21% actuarial 5-year survival rate with SPD at Johns Hopkins Hospital.[26]

Considering that EPD is a more complicated operation than SPD, we retrospectively analyzed postoperative morbidity and mortality in these two groups of patients.

In our series there were no postoperative deaths in the EPD group, whereas there was only one death in the SPD group (4.3%). This compares favorably with most of the recent reports in the literature where mortality can be as high as 17%.[16,40-42,45] However, there is a consistent trend toward higher mortality in the patients compared to those undergoing SPD.[5-10]

The explanation for the low postoperative mortality rates reported by some authors is related to the high hospital and surgical volumes in the specialized centers where these resections were performed.[25,46,47]

Many of the Japanese reports deal with the pathologic and prognostic aspects of EPD[43,48-51] but fail to consider postoperative complications, with the exception of Nagakawa et al.[40] who reported a 32.8% incidence of postoperative complications in EPD.

The results of our retrospective study show a 45.8% overall morbidity rate for EPD, which is not different from the 47.8% rate for SPD.

Surgical complications were observed in 9 (37.5%) of 24 patients undergoing EPD and 8 (34.7%) of 23 patients undergoing SPD. These findings are similar to those reported in the literature[4-6,9,12,13] for SPD.

One of the most frequent surgical complications in our experience, as has been the case in other reports,[2,5,6,12,52] is pancreatic fistula, which occurred with a similar frequency in our groups of patients, suggesting that it is unrelated to the extent of resection. However, this complication, which up until only a few years ago entailed a relaparotomy with increased postoperative mortality, is now treated conservatively with maintenance of the drains, or with percutaneous drainage and medical treatment.[9,52-54]

We observed three cases of biliary leak (12.5%), all in the EPD group, in the early part of the series (not statistically significant compared to the SPD group). This rate is higher than what has been reported in the literature for SPD.[2,5,9,12,54] We believe that the cause of this complication may be devascularization of the common hepatic duct due to excessive skeletonization of the hepatoduodenal ligament. To avoid this problem we now resect the hepatic duct very close to the bifurcation. Bile leaks are also treated conservatively.[9]

Intra-abdominal hemorrhage is another serious complication. In our experience it caused the death of a patient in the SPD group as a result of rupture of a pseudoaneurysm of the hepatic artery close to the ligature of the gastroduodenal artery. Similar cases are reported in the literature on both extended and standard pancreaticoduodenectomies.[55] One cause of intra-abdominal bleeding may be the proteolytic action of an enzyme-rich intra-abdominal fluid collection on the vessel wall.[52,55] Although during extended lymphadenectomy the regional vessels are extensively dissected and skeletonized, there is no convincing evidence that the risk of vascular injury and bleeding is higher,[45,55] and our findings corroborate this. Some surgeons suggest wrapping the exposed, denuded vessels in residual omentum, falciform ligament,[55] or a mixture of cyanoacrylate and gum.[56]

The incidence of delayed gastric emptying was low in our series, as it has been in other reports.[5,7,12,57] We had two cases (8.3%) in the EPD group and one (4.3%) in the SPD group.

The low incidence of relaparotomy (4.1% in the EPD group and 4.3% in the SPD group) is most probably due to the increasing success of nonoperative management of postoperative complications that would have necessitated surgical intervention in the past.

CONCLUSION

The two groups of patients undergoing extended and standard pancreaticoduodenectomies were similar in terms of preoperative laboratory parameters, operative risk, and types of gastrointestinal reconstruction. Intraoperative blood and plasma transfusions, postoperative morbidity and mortality, and length of hospital stay were also similar in the two groups. EPD was different from SPD only in that it required a significantly longer operative time. This was to be expected because EPD involves meticulous dissection of the connective, lymphatic, and neural tissue.

Possible limitations of this retrospective study include the risk of a beta-type error (sample size) and lack of randomization. However, our findings suggest that EPD may increase the operative time but is not associated with increased intraoperative complications, postoperative morbidity and mortality, or postoperative hospital stay. Definitive confirmation of these results can only be provided by a prospective randomized study.

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Efficacy of p120 Antisense–Mediated Therapy for Pancreatic Cancer

James W. Freeman, M.D., William E. Strodel, M.D., Patrick C. McGrath, M.D.

p120 Antisense oligodeoxynucleotides were used to determine whether they inhibited cell growth of MIA PaCa-2, a highly tumorigenic human pancreatic carcinoma cell line. Growth inhibition assays were determined in vitro by the ability of these oligomers to inhibit DNA synthesis and cell growth. For in vivo studies, nude mice were injected with cells and palpable tumors were found in 16 of 20 animals by day 14. Sixteen animals (8 in each group) were then treated daily (25 mg/kg intraperitoneally) for up to 40 days with nonsense control oligomers or p120 antisense oligomers. p120 Antisense oligomers inhibited the in vitro proliferation of MIA PaCa-2 cells in a dose-dependent manner, and optimal growth inhibition of greater than 90% was achieved at an antisense oligomer concentration of 100 μmol/L. The tumor volume was calculated for antisense- and nonsense-treated animals. Fifteen days after the beginning of treatment, control animals had a significantly greater ($P = 0.0035$) tumor volume (425 ± 244 mm^3 above baseline) as compared to p120 antisense–treated animals (166 ± 116 mm^3). Seven of the eight control animals formed tumors that had a volume greater than 1200 mm^3 45 days after treatment was begun, whereas only three of eight p120 antisense–treated animals had tumors that were this large. Two of the latter three animals had relatively large, palpable tumors (>150 mm^3) prior to treatment. Twenty days after treatment was stopped (day 60), all animals had tumors larger than 1200 mm^3. p120 Antisense oligomers were effective for inhibiting in vitro growth of the pancreatic cancer cell line MIA PaCa-2. In preliminary studies, p120 antisense oligomers appeared to inhibit the rate of growth in nude mice; however, no cures were achieved. The most effective response was seen in animals with initial low tumor burden. (J GASTROINTEST SURG 1997;1:454-460.)

Carcinoma of the exocrine pancreas is the fifth leading cause of cancer deaths in Western society and one of the leading causes of cancer deaths world wide.[1-3] There has been little improvement in patient survival in this century; the 5-year survival rate of patients with this disease is less than 5%, and the only cure is surgical resection.[1,2] Although surgery is considered the only curative modality, less than 10% of the patients have a localized tumor and thus are candidates for surgical resection. Despite advances in chemotherapy, radiation therapy, and surgical procedures, the overall impact of conventional therapeutic modalities has been minor in the management of pancreatic cancer.

Newer approaches now being considered are the use of neoadjuvant therapy and surgery combined with biologic markers to better predict and specify the behavior of these tumors. Other approaches include identifying genetic differences between normal malignant tissues with the intention of using gene therapy to replace tumor supressor genes or to inhibit the expression of oncogenes or other growth regulatory molecules expressed by tumor cells. A number of such molecules exist in pancreatic cancer including activated oncogenes and the overexpression of growth regulatory molecules.[4-6] The selective inhibition of growth regulatory molecules could potentially reverse the malignant phenotype, inhibit growth, and/or induce cell death. One approach for the selective inhibition of gene expression has been the use of antisense oligodeoxynucleotides.[7-12] Antisense oligodeoxynucleotides are short nucleotide sequences that are com-

From the University of Kentucky Medical Center, Division of General Surgery, Lexington, Kentucky.
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Reprint requests: James W. Freeman, Department of Surgery, University of Kentucky Chandler Medical Center, 800 Rose St., Lexington, KY 40536-0084.

plementary to messenger RNA and to one strand of DNA. These types of antisense molecules can inhibit specific gene expression by several different mechanisms including transcriptional arrest, inhibiting initiation or elongation of transcription, inhibiting splicing, and promoting messenger RNA degradation via activation of RNAse H.

One potential growth regulatory molecule that appears to be overexpressed in tumor tissues and represents a downstream target of oncogenic changes is the p120 protein.[13-15] p120 Is a proliferation-associated nucleolar protein that is temporally expressed at a specific time during mid-G_1.[16] Studies have indicated that p120 expression is required for ribosomal biogenesis punitively through its role as an RNA methyltransferase.[17] Antisense-mediated specific inhibition of p120 expression has been shown to inhibit the growth of breast cancer cell lines[13] and to block G_1 to S-phase transition in mitogen-stimulated lymphocytes.[18] The present study was undertaken to determine the efficacy of p120 antisense oligodeoxynucleotide therapy for inhibiting the growth of pancreatic cancer cells. For this initial study, a highly tumorigenic pancreatic carcinoma cell line, MIA PaCa-2, was selected. MIA PaCa-2 cells have multiple genetic mutations including *ras*, p53, and the tumor growth factor-β type II receptor gene.[19,20] This cell line is growth factor independent, grows well in soft agar, and rapidly forms tumors in nude mice.[20]

MATERIAL AND METHODS

The MIA PaCa-2 pancreatic carcinoma cell line was purchased from American Type Culture Collection (Rockville, Md.), and cells were cultured using standard conditions as previously described.[20]

Synthesis of Oligomers

Two separate 15 mer antisense oligodeoxynucleotides were synthesized. One oligonucleotide was prepared as a phosphodiester (p120 antisense-sj). This oligomer was directed against a region of the gene that overlaps the eleventh splice junction. The second oligonucleotide was directed against the AUG-transcriptional start site of the p120 gene (p120 antisense-aug) and was prepared as a phosphorothioate. The nonsense oligomer for in vitro studies was a 15 mer phosphodiester oligodeoxynucleotide with the same nucleotides in the p120 antisense-sj but in a random "nonsense order." The nonsense control for the in vivo studies was a phosphorothioate oligodeoxynucleotide (Isis-1082) that has been previously described.[21]

Proliferation Assays

Cell proliferation was measured by the ability of ^{3}H-thymidine to be incorporated into DNA as previously described.[15] Cells were plated in six replicates in 96-well plates at 5×10^3 cells per 100 μl. The next day cells were left untreated or antisense or nonsense oligomers were added and the cells were cultured for an additional 24 hours. At that time an additional 50 μl of medium containing 1 mCi of ^{3}H-thymidine was added to each well for an additional 4 hours incubation, and the cells were then harvested and thymidine incorporation determined by liquid scintillation counting. Growth was also determined by the ability of viable cells to metabolize 3-[4,5-dimethylthiazol-2-yl]-2,5-diphentetrazolium bromide (MTT) metabolic labeling assay.[22] For this assay, cells were plated as above and treated with antisense or nonsense oligomers, and MTT assays were performed daily over a 7-day period. MTT was added at a final concentration of 0.5 mmol/ml. After 2 hours' incubation at 37° C, the medium was removed and cells were lysed in dimethyl sulfoxide. Color intensity was measured with a spectrophotometer at 590 nm. Results in the untreated group of cells were not significantly different from those in nonsense-treated cells and are therefore not shown.

Western Blot Analysis

Treated and control cells were washed and resuspended in Laemmli buffer at a concentration of 2×10^7 cells/ml. An equivalent of 10^5 cells were subjected to electrophoresis in each lane of a 7.5% polyacrylamide gel containing 0.1% sodium dodecylsulfate. The p120 protein was detected following Western transfer by immunoalkaline phosphatase as previously described.[16]

Therapeutic Effects of p120 Antisense

The p120 antisense directed against the AUG transcriptional start site (p120 antisense-aug) was used for all in vivo testing. This oligomer was chosen since it was prepared as a phosphorothioate and may therefore have a longer in vivo half-life than phosphodiester oligomers.[21] For these assays, MIA PaCa-2 cells were inoculated (2×10^6 cells per mouse) into the flank of NCr nu/nu nude mice. Animals were monitored and tumors were apparent by day 14, at which time treatment was begun. Animals were given intraperitoneal injections daily at a concentration of 25 mg/kg. This concentration of phosphorothioate oligodeoxynucleotides was chosen as an optimal nontoxic dose based on previous experiments using ro-

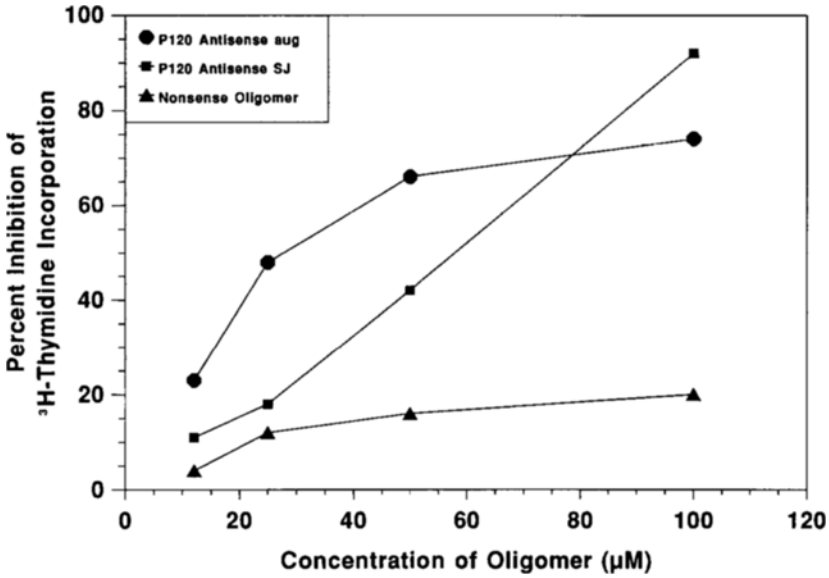

Fig. 1. The effect of p120 antisense oligomers on DNA synthesis was determined by ^{3}H-thymidine incorporation assay. The percentage of inhibition represents the ^{3}H-thymidine counts per minute of treated cells divided by the ^{3}H-thymidine counts per minute of untreated control cells × 100. The results were plotted as the mean of six replicates and the standard deviation was less than 13% for each mean.

dent models.[23] Animals were treated with oligomers for up to 40 days. The tumor volume was estimated following measurements of the length and width using the method of Shin.[24] A statistical comparison was made by means of Student's *t* test between the control and treated groups. Animals were killed after they developed tumors larger than 1200 mm^3.

RESULTS

p120 Antisense-Mediated Inhibition of Cell Proliferation and p120 Expression

The treatment of MIA PaCa-2 cells with p120 antisense oligodeoxynucleotides inhibited the in vitro growth of these cells. Cell growth was first measured by determining the effect of various concentrations of two different p120 antisense molecules and a p120 nonsense control molecule on DNA synthesis as measured by ^{3}H-thymidine incorporation. Both p120 antisense molecules inhibited DNA synthesis in a dose-dependent manner (Fig. 1). However, the kinetics of the inhibition differed between the two antisense molecules. The p120 nonsense control oligodeoxynucleotide showed a slight inhibition of DNA synthesis (<20% at 100 μmol/L). The p120 antisense-aug oligomer showed a greater growth inhibition at low concentrations (almost 50% at 25 μmol/L) than the p120 antisense-sj oligomer (17% at 25 μmol/L). There was a plateau effect on growth inhibition by the p120 antisense-aug oligomer at a concentration between 50 and 100 μmol/L. The p120 antisense-sj oligomer did not show a plateau at the concentrations of oligomer used and reached a maximum inhibition of DNA synthesis of greater than 90% at a concentration of 100 μmol/L. A single 100 μmol/L dose of either antisense oligomer completely inhibited any increase in cell numbers in MIA PaCa-2 cells cultured over a 7-day period (Fig. 2). The effect of treatment with p120 antisense-sj was analyzed for its ability to inhibit p120 protein expression. When compared to the nonsense control (Fig. 3, lanes 2, 4, and 6), the p120 antisense oligomer specifically inhibited p120 expression in a dose-dependent manner (Fig. 3, lanes 1, 3, and 5).

p120 Antisense-Mediated Inhibition of Pancreatic Tumor Growth in Nude Mice

The potential efficacy of p120 antisense oligomers to inhibit tumor growth in vivo was examined using a nude mouse model. Sixteen mice bearing palpable MIA PaCa-2 tumors (14 days after they were injected with cells) were randomly placed into two separate groups and treated for 40 days with either antisense (p120 antisense-aug) or nonsense control oligomers. The tumor volume for each treatment group is shown for days 1, 15, and 45 after treatment was initiated

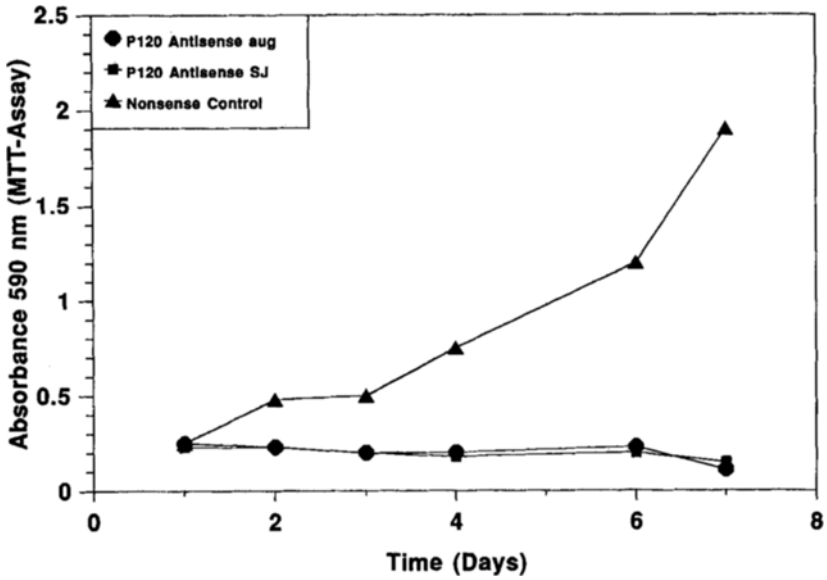

Fig. 2. The effect of a single treatment of the p120 antisense or nonsense oligonucleotide on the growth of MIA PaCa-2 cells was determined over a 7-day period. Growth was determined by a metabolic MTT assay using six replicate measurements. The standard deviation was less than 0.5 for each mean. Cells were treated on day 1 with a 100 μmol/L concentration of antisense or nonsense oligonucleotide. Metabolic labeling was recorded over a 7-day period.

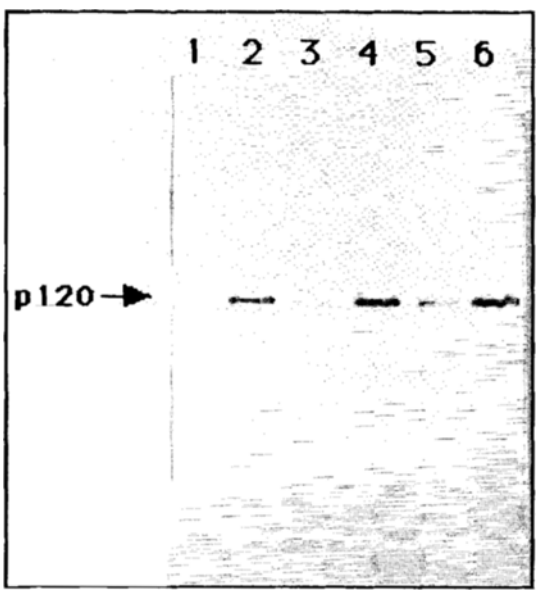

Fig. 3. The effect of p120 antisense treatment on the expression of p120 protein was determined by Western blot analysis. Lane 1, cells treated with 100 μmol/L p120 antisense oligomer; lane 2, cells treated with 100 μmol/L nonsense oligomer; lane 3, cells treated with 50 μmol/L p120 antisense; lane 4, cells treated with 50 μmol/L nonsense oligomer; lane 5, cells treated with 25 μmol/L p120 antisense oligomer; and lane 6, cells treated with 25 μmol/L nonsense oligomer.

(Fig. 4). On day 1 prior to treatment, the initial tumor volume was greater for the antisense treatment group (133 ± 101 mm^3) than the tumor volume in the nonsense treatment group (64 ± 26 mm^3). The increase in tumor growth over baseline (day 1 values; see Fig. 4, A) was calculated every 5 days. The results showed a statistical difference between the control group and the antisense-treated group. A graph for day 15 is shown in Fig. 4, *B*. Mice receiving the nonsense control oligomer grew significantly ($P = 0.0035$) larger tumors 15 days after treatment was started (average tumor volume = 425 ± 244 mm^3), as compared to p120 antisense–treated animals (average tumor volume = 166 ± 116 mm^3). In the nonsense-treated con-

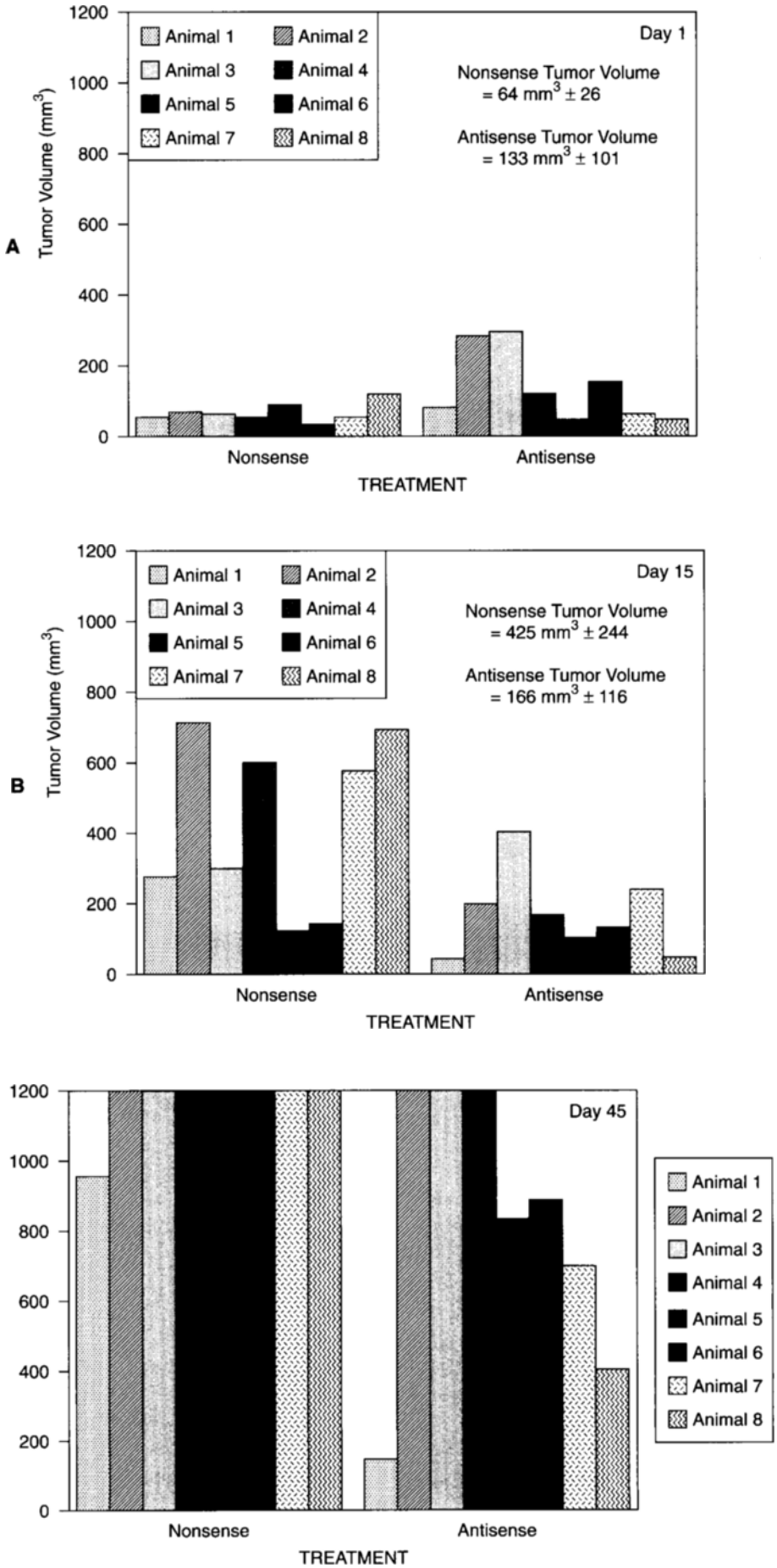

Fig. 4. For legend see opposite page.

trol group, seven of eight mice had tumors larger than 1200 mm^3 at day 45(see Fig. 4, *C*). At this same time, only three of eight mice from the p120 antisense–treated group showed tumors larger than 1200 mm^3. Interestingly, two of these three mice had rather large tumors (>150 mm^3) prior to treatment. By 60 days after treatment was started, all mice in both the control and p120 antisense–treated groups had tumors larger than 1200 mm^3.

DISCUSSION

The studies presented here evaluated the efficacy of antisense oligodeoxynucleotide therapy for inhibiting the growth of pancreatic tumor cells by blocking the specific gene expression of p120, a growth regulatory molecule. Studies using a variety of tumor types indicate the potential therapeutic efficacy of antisense oligonucleotides.[8-12]

Several potential problems exist in antisense approaches for therapy. These include the molecular target chosen and the properties of the oligonucleotide including stability, cellular uptake, and nonspecific toxicity.[7] Activated oncogenes such as *ras* are attractive targets in pancreatic cancer, but they are not expressed in all pancreatic cancers and these cancers may also have mutations of other oncogenes or tumor suppressor genes such as in p53.[19] A potentially more attractive target may be growth regulatory molecules that are downstream targets of oncogenic changes.[13,16] One such molecule having this feature is p120. p120 Plays a role in cell cycle regulation and represents a downstream target of oncogenic changes. Such oncogenic changes cause a decreased regulation in the expression of p120 at the transcriptional level.[16] Increased expression of p120 raises nucleolar function, promotes an increased proliferative potential, changes cellular morphology, and increases tumorigenic properties including a reduced dependence on normal physiologic growth regulators.[15] Conversely, a dramatic reduction in the level of p120 expression by specific antisense oligonucleotides may cause pancreatic cancer cells to undergo cell cycle arrest or cause cellular death by apoptosis or necrosis.

In this study we compared two separate p120 antisense oligodeoxynucleotides for their ability to inhibit the in vitro growth of the human pancreatic tumor cell line MIA PaCa-2. One of the antisense molecules (p120 antisense-sj) was a phosphodiester developed to overlap a splice junction of the p120 gene.[15,18] The other antisense molecule (p120 antisense-aug) was developed against the AUG transcriptional start site. The phosphodiester backbone of the latter oligomer was modified to form a phosphorothioate. These antisense molecules were first tested for their ability to inhibit DNA synthesis after a single cell culture treatment of 24 hours. Both antisense molecules inhibited DNA synthesis in a dose-dependent manner. The phosphorothioate p120 antisense aug molecule showed a greater level of inhibition at lower concentrations than the phosphodiester antisense molecule. However, the phosphodiester antisense, to the p120 splice junction, showed a somewhat greater inhibitory activity at the highest antisense concentration used (100 μmol/L). The reason for the difference in kinetics of the inhibitory activity of these two oligodeoxynucleotides is unclear from this study. These differences may reflect the difference in binding sites or the longer half-life that has been reported for phosphorothioate oligodeoxynucleotides compared to phosphodiester oligodeoxynucleotides.[7] Both antisense molecules were equally effective for inhibiting the growth of MIA PaCa-2 cells over a 7-day period. The ability of p120 antisense molecules to inhibit cell proliferation in MIA PaCa-2 cells is in agreement with our previous studies, which showed that treatment with these antisense molecules prevented cell cycle progression[13,18] and the growth of breast cancer cells in vitro.[13]

The phosphorothioate p120 antisense was chosen for in vivo studies since it showed the greatest inhibitory activity in vitro at lower concentrations and because phosphorothioates are reported to be more stable in vivo.[7,21] This antisense molecule was used in this study to determine whether it could inhibit the growth of established pancreatic tumors. The p120 antisense–treated mice showed an overall decrease in tumor size up to 45 days after treatment was begun. However, no cures were obtained and all mice developed large tumors by day 60. It is interesting to note that two of the three mice in the p120 antisense–treated group that formed large tumors at day 45 (>1200 mm^3 tumor volume) had initial tumor volumes greater than 150 mm^3. This suggests that p120 antisense may be more effective for inhibiting the growth of small tumors or micrometastasis. It remains to be determined whether an increased dose of antisense would be tolerated by the animals and whether in-

Fig. 4. Sixteen animals with palpable tumors were divided into two groups of eight animals each and treated with either nonsense or p120 antisense oligomers. Tumor volume is shown for day 1 **(A)**, day 15 **(B)**, and day 45 **(C)** after treatment was begun. The mean tumor volume is shown for days 1 and 15.

creasing the concentration of antisense would be more effective in inhibiting tumor growth. Furthermore, it is possible that multiple antisense molecules directed against other growth regulatory molecules or oncogene products would be a more effective strategy for inhibiting the growth of pancreatic tumors.

We thank Drs. Brett Monia and Frank Bennett, of Isis Pharmaceuticals, Carlsbad, Calif., for providing antisense oligomers and for helpful suggestions.

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Acute Isovolemic Hemodilution During Major Hepatic Resection—An Initial Report: Does It Safely Reduce the Blood Transfusion Requirement?

Herbert Chen, M.D., James V. Sitzmann, M.D., Catherine Marcucci, M.D., Michael A. Choti, M.D.

Surgical resection remains the mainstay of treatment for patients with hepatic tumors, despite the associated morbidity including the need for blood transfusion. Acute isovolemic hemodilution (AIH) has been shown to decrease the transfusion requirement for cardiac, urologic, and orthopedic procedures. However, the reported experience with AIH during hepatic resections is limited. Seven patients underwent major hepatic resection from July 1992 to June 1994 with standard AIH. Their clinical parameters were compared with those of nine matched control patients during the same time period. AIH and control patients had similar preoperative laboratory values (hematocrit, bilirubin, and coagulation studies), extent of liver resection, and pathologic diagnoses. Mean tumor diameters were larger in the AIH group (9.3 cm vs. 5.8 cm). Most important, patients managed with AIH required homologous blood transfusions significantly less often than the control group (14% vs. 67%; $P = 0.05$). Furthermore, if they did receive transfusions, AIH patients needed fewer units of red cells (0.1 ± 0.1 units vs. 1.7 ± 0.6 units). There was no morbidity associated with AIH. AIH can be safely performed in patients undergoing major hepatic resection for malignancy. AIH appears to reduce the number of patients requiring homologous blood transfusion as well as the number of units transfused per patient. This technique warrants further study in a larger prospective, randomized trial. (J GASTROINTEST SURG 1997;1:461-466.)

Hepatic resection is one of the few treatment options that offers the potential for cure in patients with liver malignancies. Despite the low mortality rates, which range from 2% to 5%, the associated morbidity remains high, ranging from 25% to 50% in some series including the substantial operative blood loss necessitating transfusion of blood products.[1-5] Several methods to minimize the need for transfusion of blood products after surgery are currently available and include the use of intraoperative autotransfusion using devices such as the Cell Saver (Haemonetics Corp., Braintree, Mass.), preoperative autologous blood donation, erythropoietin administration, and intraoperative hypovolemia.

One technique that has achieved significant success in reducing the requirement for postoperative blood transfusions is acute isovolemic hemodilution (AIH). AIH has been widely used in a variety of surgical procedures including cardiac, orthopedic, and urologic operations, when a significant operative blood loss is expected.[6-9] Briefly, AIH consists of the collection of the patient's whole blood after induction of anesthesia and simultaneous replacement with crystalloid solution to maintain an isovolemic state. The patient is maintained in a state of relative anemia during surgery, and the patient's whole 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. Experience with AIH in liver surgery is limited.[10,11] We have used this technique selectively at our institution, and this report reviews our initial experience with AIH in hepatic resections for neoplasms over a 2-year period (July 1992 to June 1994).

METHODS

Medical records from patients who underwent major hepatic resection for gastrointestinal neoplasms

From the Departments of Surgery (H.C. and M.A.C.) and Anesthesia (C.M.), Johns Hopkins Medical Institutions, Baltimore, Md.; and the Department of Surgery, Georgetown University Medical Center (J.V.S.), Washington, D.C.
Reprint requests: Michael A. Choti, M.D., Department of Surgery, Halsted 614, 600 N. Wolfe St., Baltimore, MD 21287.

between June 1992 and July 1994 were analyzed. Of these, seven patients had surgery with standard AIH and form the basis of this report. The decision to use AIH in these cases was made because of the surgeon's belief that a significant blood loss could be anticipated. The clinical parameters of these patients were compared with those of nine non-AIH patients during the same time period. Transfusion data included all homologous units of red cells received during surgery and during the entire postoperative hospitalization period.

The standard protocol for AIH at our institution has been previously described[7] and is illustrated in Fig. 1. Prior to surgery, all candidates for AIH were required to have a preoperative hematocrit value of 33% or greater. After induction of general anesthesia, whole blood was collected via central venous access using commercially available, blood bank–approved, anticoagulated autologous blood collection kits (Fenwal Laboratories, Deerfield, Ill.). One standard unit of whole blood (450 cc) was removed at a time, labeled with the patient's name, and stored in the operating room at 25° C until needed. In each patient, between two and five total whole blood units were removed to achieve an estimated hematocrit value of approximately 25%. Simultaneous replacement with an identical or greater volume of crystalloid was administered at a separate venous access site. Additional volume replacement was given later, as dictated by hemodynamic parameters determined by central venous and intra-arterial monitoring. AIH was generally completed prior to surgical incision and required approximately 30 minutes.

All patients underwent surgical exploration through a right subcostal incision extending to the sternum medially and into the right flank laterally. Major hepatic resections included extended or formal lobectomies or multiple (>4) nonanatomic wedge resections. All lobar resections were performed with control of the portal venous system, hepatic arterial inflow, and hepatic venous outflow before dissection of the parenchyma. Total vascular isolation was not routinely used during this time period. An ultrasonic aspirator (Cavitron, Boulder, Colo.) was used for all parenchymal dissections. Transfusion decisions were made intraoperatively by the anesthesia team and postoperatively by the attending surgeon based on clinical parameters. The volume of intravenous crystalloid and colloid solutions administered intraoperatively was left to the discretion of the anesthesia team. All remaining withdrawn whole blood units were transfused after liver resection was completed and hemostasis was obtained.

Statistical data were analyzed using the Mann-Whitney, rank-sum, and Fisher's exact tests. Significance was defined as $P \leq 0.05$.

RESULTS

Patient Data

Demographic data from the patients who had AIH vs. the non-AIH patients during the same time period

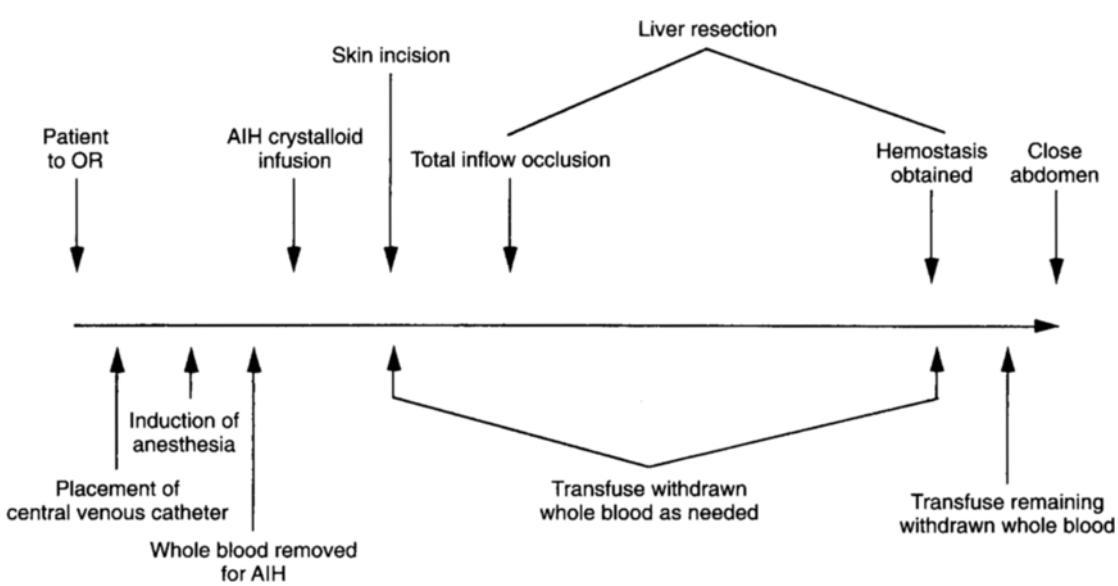

Fig. 1. Acute isovolemic hemodilution *(AIH)* protocol. Schematic of the standard protocol for AIH used at Johns Hopkins Hospital.

are shown in Table I. Patients in both groups were similar in age (mean 56.5 years vs. 58.5 years, respectively). The groups differed in their gender composition; AIH patients were predominantly female (86%), whereas non-AIH patients were mostly male (67%). The incidence of hepatitis and cirrhosis in each group was 28% vs. 22% and 0% vs. 11%, respectively. Previous abdominal surgery and preoperative therapy (hepatic radiation therapy and systemic chemotherapy) were comparable in the two groups.

Table II presents the pre- and postoperative laboratory data for AIH and non-AIH patients. Pre- and postoperative prothrombin and partial thromboplastin ratios (compared to control laboratory values) did not differ between AIH and non-AIH patients. Although no patients in the AIH group developed coagulation problems, one of nine non-AIH patients required treatment for postoperative coagulopathy. The two groups had similar mean and median preoperative hematocrit values (mean: AIH 40.0% ± 2.0% vs. non-AIH 40.5% ± 2.1%; median: AIH 38.5% vs. non-AIH 37.5%). Mean and median postoperative hematocrit values dropped in the AIH patients to 30.4% ± 2.0% and 32.9%, respectively, whereas in the non-AIH patients these values dropped to 32.3% ± 1.1% and 30.7%, respectively. These differences were not statistically significant. Preoperative mean bilirubin levels, which have been shown to be a preoperative predictor of morbidity,[1] were similar between AIH (0.7 ± 0.1 mg/dl) and non-AIH (0.8 ± 0.1 mg/dl) patients.

Operative Data

Hepatic resections were categorized into three groups: (1) type 1—multiple wedge resection and for-

Table I. Patient demographics*

	AIH	Non-AIH
Age (yr)		
Mean ± SEM	56 ± 5	58 ± 5
Median	55	60
Sex		
Male	1/7 (14)	6/9 (67)
Female	6/7 (86)	3/9 (33)
Hepatitis	2/7 (28)	2/9 (22)
Cirrhosis	0/7 (0)	1/9 (11)
Previous abdominal surgery	4/7 (56)	6/9 (67)
Previous radiation therapy	1/7 (14)	1/9 (11)
Previous chemotherapy	2/7 (28)	6/9 (66)

AIH = acute isovolemic hemodilution; SEM = standard error of the mean.
*Values in parentheses are percentages.

Table II. Laboratory data

	AIH	Non-AIH
Preoperative prothrombin time*		
Mean ± SEM	1.00 ± 0.00	1.04 ± 0.01
Median	1.00	1.00
Postoperative prothrombin time*		
Mean ± SEM	1.13 ± 0.05	1.14 ± 0.05
Median	1.10	1.10
Preoperative partial thromboplastin time*		
Mean ± SEM	0.93 ± 0.04	0.94 ± 0.03
Median	1.00	1.00
Postoperative partial thromboplastin time*		
Mean ± SEM	0.94 ± 0.04	1.01 ± 0.10
Median	0.90	1.00
Preoperative hematocrit (%)		
Mean ± SEM	40.0 ± 2.0	40.5 ± 2.1
Median	38.3	37.5
Postoperative hematocrit (%)		
Mean ± SEM	30.4 ± 2.0	32.3 ± 1.1
Median	32.9	30.7
Preoperative total bilirubin (mg/dl)		
Mean ± SEM	0.7 ± 0.1	0.8 ± 0.1
Median	0.7	0.8

Abbreviations as in Table I.
*Values compared to laboratory control values.

mal left lobectomy; (2) type 2—right lobectomy; and (3) type 3—trisegmentectomy, extended lobectomy, and complex resection. Among the AIH patients, four had type 1 resections, one had a type 2 resection, and two had type 3 resections (Table III). Patients in the non-AIH group had similar operations: five had type 1 resections, two had type 2 resections, and two had type 3 resections. Liver tumors in AIH patients were slightly larger, on average (9.3 cm vs. 5.8 cm), but this difference did not reach statistical significance. Pathologic diagnoses of all patients are shown in Table IV. Both AIH and non-AIH patients had similar disease processes. The most common indication for hepatectomy in these groups was metastatic colorectal carcinoma (n = 9) followed by hepatocellular carcinoma (n = 4).

Table III. Hepatic resections

	AIH	Non-AIH
Type 1 Multiple wedge resection, left lobectomy	4	5
Type 2 Right lobectomy	1	2
Type 3 Trisegmentectomy, extended lobectomy, complex resection	2	2

Table IV. Pathologic diagnoses

Liver histology	AIH	Non-AIH
Metastatic colorectal carcinoma	4	5
Hepatocellular carcinoma	1	3
Mucinous cystadenoma	1	0
Hepatic adenoma	1	1

Patient Outcome

Operative outcome and transfusion data in AIH and non-AIH patients are shown in Table V. Despite undergoing AIH, the AIH patients had comparable mean and median anesthesia times compared to the non-AIH group (mean: AIH 6.1 ± 0.5 hours vs. non-AIH 5.9 ± 0.5 hours; median: AIH 5.7 hours vs. non-AIH 6 hours). Mean and median operative times were also similar in the two groups (mean: AIH 5.0 ± 0.5 hours vs. non-AIH 4.9 ± 0.4 hours; median: AIH 4.3 hours vs. non-AIH 5.2 hours). None of these differences were statistically significant. AIH patients tended to receive a slightly higher average volume of intravenous fluids than non-AIH patients (6.9 ± 1.0 liters vs. 6.2 ± 1.0 liters), but this did not reach statistical significance. Mean and median estimated blood losses were virtually identical in the two groups (mean: AIH 1.2 ± 0.3 liters vs. non-AIH 1.4 ± 0.3 liters; median: AIH 1.2 liters vs. non-AIH 1.3 liters). Although they had similar estimated blood losses, patients managed with AIH required homologous blood transfusions significantly less often than non-AIH patients (14% vs. 67%, P value = 0.05). Furthermore,

Table V. Operative outcome and transfusion data

	AIH	Non-AIH	*P* Value
Anesthesia time (hrs)			
Mean ± SEM	6.1 ± 0.5	5.9 ± 0.5	NS
Median	5.7	6.0	
Operative time (hr)			
Mean ± SEM	5.0 ± 0.5	4.9 ± 0.4	NS
Median	4.3	5.2	
Intraoperative intravenous fluids given (L)			
Mean ± SEM	6.9 ± 1.0	6.2 ± 1.0	NS
Median	7.0	6.0	
Estimated blood loss (L)			
Mean ± SEM	1.2 ± 0.3	1.4 ± 0.3	NS
Median	1.2	1.3	
Red cells transfused (U)			
Mean ± SEM	0.1 ± 0.1	1.7 ± 0.6	P <0.05
Median	0.0	1.0	
Patients receiving blood transfusion (%)	14	67	P = 0.05

NS = not significant; other abbreviations as in Table I.

when they did need a blood transfusion, AIH patients required an average number of fewer units of red cells (0.1 ± 0.1 units vs. 1.7 ± 0.6 units; $P < 0.05$). There were no operative deaths in either group. Among the AIH patients, three (43%) of seven had infection-related morbidity, whereas in the non-AIH group six (67%) of nine did. There was no morbidity associated with AIH. However, there was also no direct transfusion-related morbidity in the non-AIH group.

DISCUSSION

Hepatectomy remains the mainstay of treatment for patients with hepatocellular carcinoma or isolated liver metastases from colorectal cancer. Improved surgical techniques and anesthesia practices have dramatically decreased the mortality rates of liver resections.[1-5,12] However, morbidity rates, including the need for blood transfusions, remain high. Although AIH has been shown to reduce transfusion requirements in cardiac, urologic, and orthopedic procedures,[6-9] there is limited experience with AIH during liver resection. This series constitutes the first report of AIH during hepatic resection where the results in AIH patients are compared directly with those in non-AIH patients during an identical time period. In this study we have shown that AIH is a safe technique that can reduce the percentage of patients requiring homologous blood transfusions (67% to 14%). Furthermore, when they were given blood transfusions, patients with AIH often required fewer units (0.1 units vs. 1.7 units). Although these results are encouraging, they are limited by the small number of patients in both groups. In addition, our study is not a randomized controlled trial. AIH was performed at the surgeon's discretion, probably for reasons of anticipated large estimated blood loss. Thus the patients with an expected greater blood loss may have been favored for AIH, and such a bias may, in fact, have underestimated the actual reduction in transfusion requirement attributed to AIH. In addition, patients with larger tumors (mean diameter 9.3 cm vs. 5.8 cm) underwent AIH. This bias could also have underestimated the benefit of AIH.

Although AIH has been shown to be beneficial in other operations, a limited number of reports describe the use of AIH during major hepatic resection. In 1984 Schaller et al.[10] reported on eight pediatric patients who underwent major liver resections with AIH during a 7-year period. Although these authors showed that the technique was safe and effective in reducing intraoperative blood loss in children, they did not directly compare their results with those from a control group of patients. Sejourne et al.,[11] in 1989, reported on 22 consecutive patients who received AIH during liver resection in an 11-month period compared to 22 patients who did not have AIH during a previous 14-month period. These authors concluded that AIH reduced the requirement for transfusion of all blood products significantly. Although they had more patients in both groups, Sejourne et al.[11] compared groups from different time periods. Improvements in surgical technique and postoperative care could have contributed to their results. Findings in both of these studies, however, are consistent with our results.

Morbidity from blood transfusion can be significant and may include infections, allergic reactions, and clerical errors resulting in donor-recipient mismatch. The risk of transmission of pathogenic viruses such as hepatitis B, hepatitis C, hepatitis D, hepatitis non-B, non-C, and human immunodeficiency virus-1 are not negligible. Moreover, in patients with cancer, blood transfusion has been associated with a decreased time to recurrence, as well as a decreased survival after resection of colorectal liver metastases.[13,14] Blood transfusion is also associated with significant costs including procurement by the Red Cross, processing, delivery, storage, and patient cross-matching of red cells. In addition, patient anxiety or religious beliefs (i.e., Jehovah's Witness) can limit the desire to be transfused with banked blood products. To minimize some of these risks, autologous preoperative blood donation is becoming the most popular method to reduce the need for homologous blood transfusions. However, the cost of this technique is often close to $800 per unit of autologous blood donated.

The morbid events of blood transfusion are in stark contrast to AIH. With AIH, although the possibility of bacterial or gross contamination is not completely eliminated, there is virtually no chance of acquired viral infections. In addition, the storage lesions (mild hemolysis, decreased pH, increased P_{CO_2}, and increased plasma K+) and decrement in product function that are recognized to occur in blood products that have been stored in a blood bank for a period of time are avoided. The chances of clerical error and the catastrophic consequences of a major hemolytic transfusion reaction are reduced, as well as the clinical sequelae of a transfusion reaction resulting from unrecognized minor antigen/antibody reactions. It must be noted, however, that AIH does have some potential disadvantages. Although equipment costs for AIH may be minimal and blood bank charges may be avoided or reduced, there is potential for incurring hidden costs in the additional operating room time necessary to collect several units of whole blood (approximately 30 minutes in this series). In this report, however, the mean and median anesthesia and operative times were not any longer in the AIH group.

Thus we estimate the total cost of AIH to be less than $75 per unit of autologous blood. Units removed during AIH that are not transfused will rarely be accepted for later use. Last, this technique may not be acceptable to the strictest practitioners of the Jehovah's Witness faith.

CONCLUSION

AIH during major hepatic resection is a safe and effective technique that reduces the need for homologous blood transfusions in selected patients. Although this initial report is encouraging, we recognize its limitations—that is, the fact that the patient groups were small and nonrandomized. However, we have shown that AIH can be safely performed during major hepatic resections with an apparent benefit. Furthermore, we estimate that the cost of AIH is one-tenth that of preoperative autologous donation. Thus we are currently pursuing a prospective, randomized study with AIH in patients undergoing major hepatic resections for malignancy.

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Epidermal Growth Factor and Neurotensin Induce Microvillus Hypertrophy Following Massive Enterectomy

Charlotte K. Ryan, M.D., Jen-nie H. Miller, M.S., Anna S. Seydel, M.D., Karen de Mesy Jensen, M.S., Harry C. Sax, M.D., F.A.C.S.

The compensatory hypertrophy that develops after massive enterectomy is rarely adequate to prevent the development of short bowel syndrome. Trophic hormones such as epidermal growth factor (EGF) and neurotensin (NT) may be useful in improving and accelerating this adaptive response. This study delineates the effects of NT and EGF on remnant small bowel at the microvillus cellular level, which is the prime determinant of surface area. New Zealand white rabbits (2 kg) underwent midgut transection (sham) or 70% jejunoileal resection. Alzet pumps containing saline solution (control), EGF (1.5 μg/kg/hr), or NT (900 μg/kg/day) were implanted in resected animals after which they underwent 1 week of infusion. A second group of EGF animals was killed 2 weeks after infusion completion to assess delayed effects (EGF-delayed). Proximal jejunum was fixed for light and electron microscopy; villus and microvillus parameters were read in a blinded fashion. EGF (2.17 ± 0.05 μm), EGF-delayed (2.26 ± 1.5 μm, and NT (1.96 ± 0.02 μm) animals had significantly increased microvillus heights compared to the control group (1.49 ± 0.04 μm). Calculated brush-border surface areas were increased in a similar fashion. EGF and NT failed to elicit increases in jejunal gross villus heights. EGF and NT induce enterocyte microvillus hypertrophy and increase absorptive surface area in remnant bowel after massive enterectomy. In addition, the trophic effects of EGF persist after cessation of infusion. These peptides may be useful in accelerating small bowel adaption and preventing the development of short gut syndrome. (J GASTROINTEST SURG 1997;1:467-473.)

Following massive enterectomy, the remaining bowel must undergo both morphologic and physiologic adaption and upregulation to prevent the development of short gut syndrome. Morphologically it has been shown that the remnant intestine undergoes both hypertrophy and hyperplasia[1] with possible triggering mechanisms being humoral factors, pancreaticobiliary secretions, and luminal nutrients. This compensation, however, is rarely adequate. Enhancement of this natural adaptation would possibly reduce the significant morbidity and expense of prolonged total parenteral nutrition (TPN). The aim of this investigation was to examine means of facilitating and accelerating morphologic adaption by increasing the absorptive surface area of the intestinal mucosa.

Exogenous infusion of gastrointestinal trophic hormones may potentially be the most useful way to achieve accelerated hypertrophy or hyperplasia. Candidate factors include epidermal growth factor (EGF), human growth factor, neurotensin (NT), bombesin, and plerocercoid growth factor. We chose two of these hormones for our investigative work, namely, EGF and NT. EGF, a 53-amino acid polypeptide with receptors along the entire length of small bowel, promotes DNA synthesis and the transcription of RNA, stimulates enterocyte proliferation, mediates repair of mucosal injury, and regulates crypt cell growth.[2] Direct mucosal application of EGF to jejunal mucosa upregulates glucose-coupled Na^+ absorption and microvillus surface area within 30 minutes.[3] Studies of

From the Departments of Pathology (C.K.R. and K. de M.J.) and Surgery (J.H.M., A.S.S., and H.C.S.), University of Rochester Medical Center, Rochester, N.Y.
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Reprint requests: Charlotte K. Ryan, M.D., University of Rochester Medical Center, Department of Pathology, 601 Elmwood Ave., Rochester, NY 14642-8410.

oral and parenteral EGF did not show mucosal hyperplasia or increased mucosal mass following resection.[4-6] Our laboratory has shown that EGF upregulates nutrient transport after 70% enterectomy.[6] EGF, however, in spite of enhancing nutrient uptake, does not increase villus height. Nutrient uptake may be dependent on the available absorptive surface area of the bowel mucosa. Although villus height is a factor in assessing mucosal surface area, the major determinants are microvillus height and cell density.[7] (Microvilli are the numerous parallel cylindrical processes that comprise the brush border of intestinal villi; they are visible only with electron microscopy.) Therefore a study of microvilli may actually be more pertinent in evaluating the trophic effects of peptides than merely gross villus heights.

NT is a 13-amino acid peptide that is mainly localized within the gut in the endocrine N cells of the ileum but is also found in the duodenum, jejunum and colon.[8] Like EGF, neurotensin has numerous secretory and motility effects on the gastrointestinal tract with the plasma levels of both hormones increasing following food uptake.[9] NT has been shown to prevent mucosal hypoplasia in rats fed an elemental diet[10] and to stimulate mucosal growth in rats fed normal laboratory chow.[11] Exogenous administration of NT in experimental animals after small bowel resection has resulted in increased jejunal villus height in some laboratories.[11,12] The effect of NT on nutrient absorption is unknown. Because both NT and EGF possess trophic properties, we tested the effects of both peptides in short gut animals on microvillus height (rather than merely gross villus height) and intestinal absorptive surface area.

MATERIAL AND METHODS

Animals

Male New Zealand white rabbits (Hazelton Research Products, Inc., Denver, Pa.) weighing 2 kg (N = 20) were housed in individual cages in light/dark–cycled, temperature-controlled rooms in accordance with institutional guidelines and the "Guidelines for the Care and Use of Laboratory Animals" (Department of Health and Human Services, National Institutes of Health). Animals were divided into five groups of four rabbits each and were fasted for 18 hours preoperatively and allowed access to 5% glucose water. Under ketamine (30 to 50 mg/kg) and xylazine (25 to 30 mg/kg) anesthesia, the rabbits underwent midjejunal transection (without removal of bowel) and reanastomosis (sham) or 70% midjejunoileal resection and anastomosis (all other groups) using 5-0 Vicryl sutures. Gentamycin (4 mg/kg) was administered intramuscularly immediately before surgery and on postoperative day 1. Banamine (1.0 mg/kg) was given intravenously immediately after surgery and on postoperative day 1. For the first 48 hours postoperatively, the rabbits were given 5% glucose water. Water and standard rabbit chow (Purina Mills, Inc., St. Louis, Mo.) were provided ad libitum, at a rate previously noted, to allow consumption of all food throughout the experiment. After 1 week, Alzet pumps (Alza Corp., Palo Alto, Calif.) were placed subcutaneously in the midscapular region using Innovarvet IM (0.25 ml/animal) for sedation and 1% lidocaine for local anesthesia. Rabbits were randomized to receive through pumps either EGF (Austral Biologicals, San Ramon, Calif.) at a rate of 1.5 μg/kg/hr, NT (Bachem Bioscience, Philadelphia, Pa.) at a rate of 900 μg/kg/day, or saline solution (controls). The infusions were stopped after 1 week, and rabbits in all study groups were weighed and killed with the exception of the EGF-delayed rabbits. These animals were killed 2 weeks after pump removal to assess whether possible stimulatory effects of EGF were more than transient.

Microscopy

The small bowel was rapidly removed and the first 1 cm of proximal jejunum was fixed in 10% buffered formalin, dehydrated, and embedded in paraffin for histologic sectioning. Additionally, a 10 cm segment of proximal jejunum was suspended from a 10 gm weight and weighed. Sections were stained with hematoxylin and eosin for morphometric analysis using an Olympus microscope (Olympus America, Inc., Lake Success, N.Y.) and micrometer to determine villus height. The 10 longest and most properly oriented villi per sample were measured from the base of the crypt to the villus tip, and the mean was calculated in a blind manner to prevent observer bias.

Transmission electron microscopy was employed to assess microvillus height, density, and width. A villus from the paraffin block was chosen based on perfect longitudinal orientation of the enterocytes. A bone marrow biopsy needle was used to core out a section of this villus. This core specimen was then deparaffinized, osmicated in 1.0% osmium tetroxide, dehydrated, and infiltrated with Spurr's epoxy resin (J.B. EM Services, Dorval, Canada). The tissue blocks were embedded in plastic molds and polymerized at 70° C. Two and one-half microsections were cut on a JB-4 type microtome, stained with basic fuchsin and methylene blue, and examined by light microscopy to select an optimally oriented midvillus focus for electron microscopic examination. Tissue was "popped off" according to a previously published method[13] and then sectioned. The thin sections were stained with

uranyl acetate and lead citrate and examined under a Hitachi 7100 electron microscope (Hitachi Denshi America, Ltd., Woodbury, N.Y.) Only well-oriented longitudinal microvilli from midvillus regions were assessed. The microvillus brush-border surface area was computed as previously described[14] employing the variable parameters of microvillus height, density, and width. The following formula was used: $A = (N/2)^2 (2\pi rl + \pi r^2)$, where N = number of microvilli along 2 μm of cell surface, r = mean radius of N microvilli, and 1 = mean length (height) of N microvilli. Ten duplicate measurements from each specimen were taken by a blinded observer and the mean was calculated.

Statistical Analysis

All results are reported as mean values ± standard error of the mean with significance determined as $P < 0.05$ by Student's *t* test or analysis of variance (with SNK post hoc) where appropriate.

RESULTS

Body Weights

There was a 4% to 7% increase in body weight in all groups of rabbits that had undergone small bowel resection. There was no statistical differences among NT, EGF, or control groups. Sham animals had increased their weight by 12%. Ten centimeter proximal jejunal weights were recorded as follows: sham (1.716 ± 0.174 g), control (2.212 ± 0.242 g), NT (3.240 ± 1.294 g), EGF (2.576 ± 0.230 g), and EGF-delayed (2.54 ± 0.747 g). The weights were all increased over the sham animals at the time of sacrifice with no statistical differences among NT, EGF, or control rabbits. By 4 weeks EGF-delayed animals had increased their body weight by 33%.

Gross Villus Heights

There were no statistical increases in the length of jejunal villi as determined by light microscopy in control (8.92 ± 0.74 mm × 0.1), sham (8.63 ± 0.55 mm × 0.1), NT (8.87 ± 0.73 mm × 0.1), EGF (9.40 ± 0.40 mm × 0.1), or EGF-delayed (8.34 ± 0.87 mm × 0.1) groups (Fig. 1). The morphologic appearance of all sections was normal with unaltered architecture and no evidence of enterocyte damage.

Brush-Border (Microvilli) Heights

Representative electron micrographs of brush borders in control, NT, and EGF animals from the jejunal midvillus regions are shown in Fig. 2. EGF and

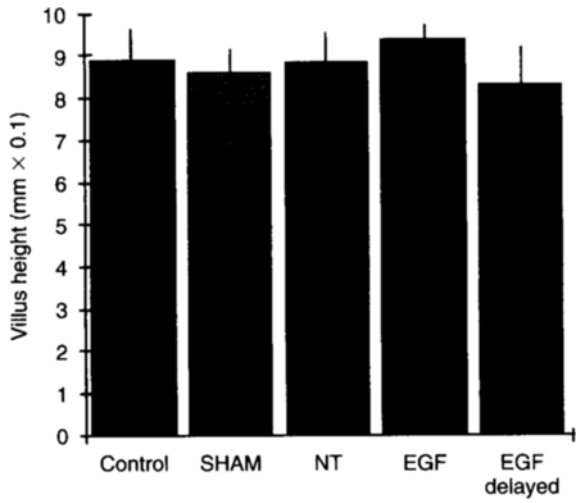

Fig. 1. Jejunal gross villus heights determined by light microscopy and measured from the base of the crypt to the villus tip in the following study groups: midgut transection (sham); saline (control), neurotensin (NT), epidermal growth factor (EGF), and EGF-delayed animals. Data are represented as mean ± standard error of the mean (SEM) (n = 4). There are no significant differences among the groups.

NT treatment elicited significant increases in height in comparison to control animals. Aside from changes in height, ultrastructure remains similar with no distortion, loss, damage, or other abnormalities noted in microvillus architecture. Figure 3 shows the actual microvilli heights for all experimental groups. EGF (2.17 ± 0.05 μm) and EGF-delayed conditions (2.26 ± 1.5 μm) produced significantly increased microvillus height in comparison to sham (1.63 ± 0.03 μm) and control (1.49 ± 0.04 μm) groups. The NT group also had significantly increased microvillus height in comparison to the control group (1.96 ± 0.02 μm).

Brush-Border Surface Areas

EGF (48.10 ± 2.57 μm^2) and EGF-delayed conditions (65.63 ± 8.40 μm^2) resulted in significantly increased surface areas compared to the sham (36.32 ±0.96 μm^2) and control (39.21 ± 1.24 μm^2) groups. Referring to Fig. 4, it is also noted that the NT group (47.78 ± 2.71 μm^2) has a significantly greater surface area than the sham group. In general, the greater surface areas were directly proportional to the increases in microvillus height in the treated animals. The width of the villi remained constant at 0.08 μm under all experimental conditions. There appeared to be an increase in cell density in the EGF-delayed group (21.60 microvilli per 2 μm of cell surface) compared to the EGF (18.89), NT (19.64), control (20.50), and

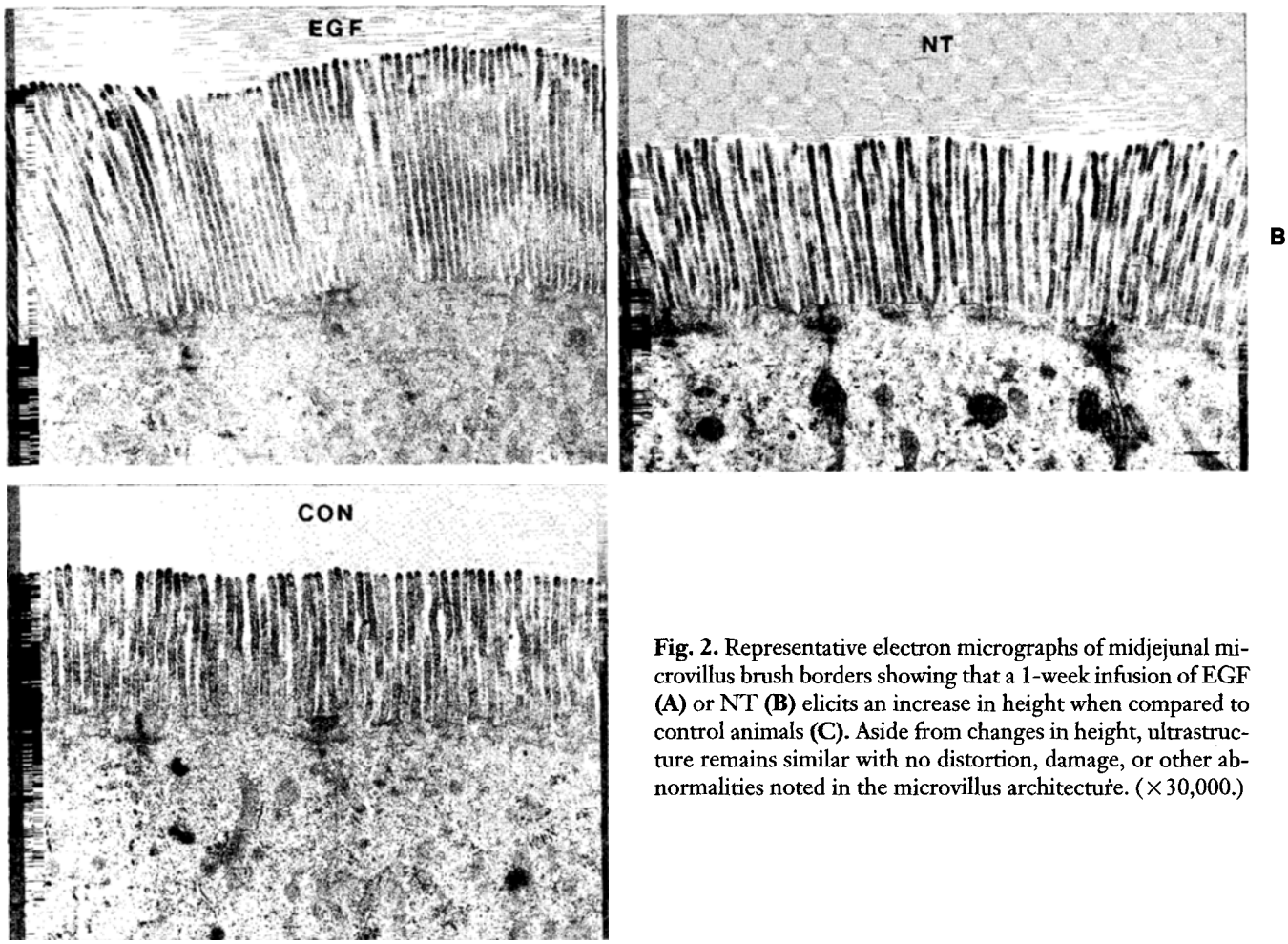

Fig. 2. Representative electron micrographs of midjejunal microvillus brush borders showing that a 1-week infusion of EGF **(A)** or NT **(B)** elicits an increase in height when compared to control animals **(C)**. Aside from changes in height, ultrastructure remains similar with no distortion, damage, or other abnormalities noted in the microvillus architecture. (×30,000.)

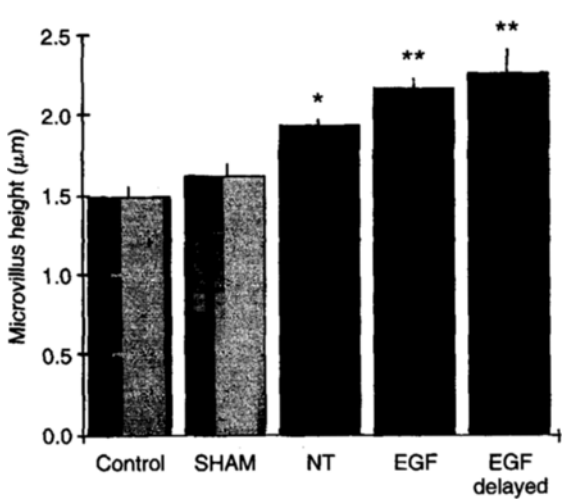

Fig. 3. Jejunal brush-border heights as determined by electron microscopy from midvillus region in sham, control, NT, EGF, and EGF-delayed study groups. Data are represented as mean ± SEM (n = 4). EGF and EGF-delayed groups have significantly higher microvillus height compared to sham and control (**) groups. The NT group has a significantly increased microvillus height compared to the control (*) group.

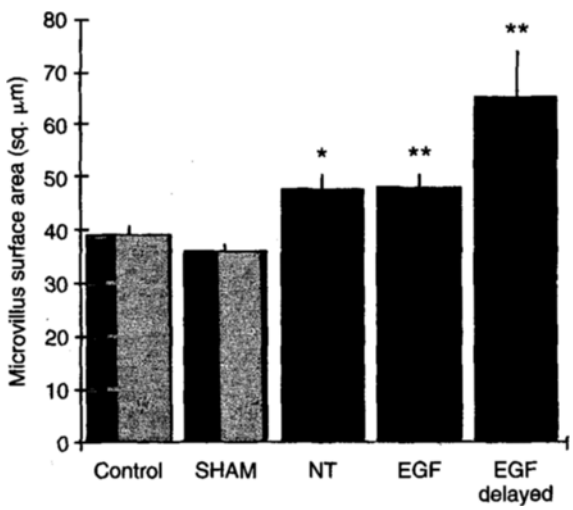

Fig. 4. Brush-border surface areas as determined by electron microscopy from midvillus regions of sham, control, NT, EGF, and EGF-delayed groups expressed in square micrometers. Data are represented as mean ± SEM (n = 4). EGF and EGF-delayed groups have significantly greater surface area compared to sham and control (**) groups. The NT group has a significantly greater surface area than the sham (*) group. The difference between the EGF-delayed and NT or EGF groups is not significant at the $P = 0.05$ level.

sham (18.75) groups; since this value is squared in the surface area formula, it contributed to the rather high value for the EGF-delayed group. However, this apparent difference was not found to be statistically significant at the $P = 0.05$ level.

DISCUSSION

Massive small bowel resection results in short bowel syndrome, a devastating medical condition with inherent physiologic ramifications that necessitate a lifelong dependence on TPN. Moreover, TPN itself, with its often concomitant complications of hepatobiliary dysfunction, sepsis, and vascular thrombosis, is a scenario best avoided if at all possible. To date, small bowel transplantation is not a viable option for most patients. Since bowel does attempt to adapt to massive resection, a major therapeutic goal would be to stimulate these responses in hopes of reducing TPN dependency and being able to initiate enteral nutrition. Compensatory villus hypertrophy[15-17] and bowel lengthening occurs after resection, but it is often an inadequate response. Several groups have attempted to surgically increase lost surface area but this has not yet proved to be efficacious.[18] The use of various gut peptides with their numerous trophic, secretory, and motility effects to achieve an adaptive response is a most attractive idea; the most advantageous hormone, however, along with its mode and timing of administration, still needs to be determined.

In this study we have investigated two gut peptides, EGF and NT, and found them both to be effective when administered parenterally in inducing increases in microvillus height and absorptive surface area. EGF seemed to elicit a slightly greater response than NT with significantly higher values than both sham and control animals in microvillus height and surface area. These trophic effects, however, caused no increase in gross villus height. We did see evidence of a generalized hypertrophy since segmental intestinal mass had increased in all groups including controls when compared to sham rabbits. There were no differences among experimental groups, and this compensatory hypertrophy is to be expected from the enterectomy alone, even in the absence of exogenous trophic hormones.

Studies in the literature reporting the effects of peptides in short gut animals on microvillus and villus heights are limited. Hardin et al.[3] infused rabbit jejunal loops with EGF and noted significant increments in brush-border surface area at both 30 and 120 minutes after infusion, which were directly related to increases in microvillus height. Our data showing increased microvillus heights and surface areas support these results, and although we administered EGF for a longer period of time (1 week), the intestinal mucosa was sampled immediately after cessation of infusion. We likewise elicited no change in gross villus parameters. There have been additional studies relating EGF to villus heights, but these are somewhat peripheral since the short gut model was not employed. Instead, small bowel mucosa in rats was either rendered atrophic by an elemental diet[19] or damaged by methotrexate[20] to determine whether EGF could reverse these insults. One week of EGF achieved an increase in gross villus parameters in the elemental diet mucosa but not in mucosa exposed to methotrexate. There have been, however, two reports specifically dealing with massive small bowel resection and NT. In 80% resected rats, 14 days of subcutaneous NT increased gross villus heights and mucosal DNA with a greater response in jejunum than ileum.[12] Izukura et al.,[21] likewise in rats, reported similar results after 7 days of NT with increases in mucosal weights, protein, and RNA content. We demonstrated evidence of augmented microvillus height and surface area with NT (as in EGF) but no evidence of amplified gross parameters, namely, villus length or intestinal mass. In part, some of these discrepancies in our own data may be attributed to differences in animal models and dosages. However, perhaps gross villus heights may be variable and too dependent on a multitude of conditions; changes in microvillus heights after peptide infusions may be more consistent markers of absorptive surface area.

EGF may acutely exert its influence on brush-border surface area by affecting the actin cytoskeleton of the microvillus. There is evidence that EGF receptors are associated with actin filaments, and these filaments can polymerize after EGF stimulation.[22] Moreover, the gain in brush-border surface area induced by mucosally administered EGF is not dependent on de novo protein synthesis[3,23]; membrane lipid composition and physical properties also do not change. These findings suggest that the increase in surface area is due to redistribution of existing microvillus plasma membrane from an intracellular source.[3] Whether these mechanisms can be applied to NT or other gastrointestinal hormones remains to be established.

Our results suggest that changes in the brush border, which are only visible by electron microscopy, occur soon after peptide administration and before less subtle parameters of mucosal hyperplasia such as changes in villus height, total wall thickness, or intestinal mass. Our microvilli determinations were performed on tissue taken immediately after termination of 1-week of infusion (except in EGF-delayed animals). It is quite possible, however, that increases in microvillus height occur even before 1 week's time.

Indeed, topical application of EGF to rabbit jejunal loops disclosed an increase in microvillus height after just 30 minutes,[3] and it was concluded that EGF acutely upregulated surface area in the absence of DNA replication or protein transcription. Hence it is not surprising that we saw no gain in gross villus parameters in spite of changes in microvillus height. These observations are consistent with the hypothesis that EGF and possibly NT and other peptides augment brush-border surface area by redistributing preformed enterocyte plasma membrane. Increases in gross villus height and intestinal mass connote architectural changes and DNA replication and may involve mechanisms other than surface membrane changes in individual cells.

After perceiving such a rapid effect of EGF and NT on microvillus histology, we did not expect our data to also reveal a persistent stimulation by EGF after a 2-week discontinuance of therapy. Two other laboratories, however, have reported delayed effects after an initial dose of EGF in massively resected rabbits. One group found that EGF markedly increased mucosal weight, glutamine uptake, and enzyme capacity of maltase and aminooligopeptidase up to 6 weeks after drug cessation.[24] Another study showed stimulation of maltase activity and upregulated glucose absorption beyond infusion completion.[5] This persistent effect may be due to enhanced transporter affinity, and induction of greater numbers of transporters per enterocyte or alterations in the geographic distribution of glucose-transporting enterocytes on the villus.[25] However, since enterocytes may travel up the villus and be sloughed within 2 to 4 days along with their newly acquired receptors or induced plasma membrane changes, perhaps it is the basal stem cells that are most significantly affected by EGF. An alternate explanation is that EGF induces a trophic hormonal factor that continues to be secreted after being initiated by EGF. There is some evidence that NT promotes mucosal growth indirectly by a stimulation of pancreaticobiliary secretions.[26] Moreover, significant increases in plasma enteroglucagon (a gut hormone) have been observed in resected rats treated with NT, suggesting that NT brings about the release of enteroglucagon.[12]

CONCLUSION

Both NT and EGF, by increasing microvillus height and absorptive surface area, augment the compensatory hyperplasia that occurs in remaining small bowel after massive enterectomy. In addition, the trophic effects of EGF persist after cessation of therapy. Administration of these peptides may be therapeutically useful to ameliorate short bowel syndrome and enhance small bowel adaptation, especially during the early stages after massive resection.

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Management of Advanced Duodenal Polyposis in Familial Adenomatous Polyposis

Claudio Soravia, M.D., Terri Berk, M.S.S.A., Gregory Haber, M.D., Zane Cohen, M.D., Steven Gallinger, M.D., M.Sc.

Patients with familial adenomatous polyposis (FAP) are at increased risk for the development of periampullary cancer. The aim of this study was to evaluate the roles of endoscopic and surgical therapy in the management of advanced duodenal polyposis in FAP. From 1990 to 1995, seventy-four FAP patients were enrolled in a prospective endoscopic surveillance protocol. Among these, 11 (14.8%) developed advanced duodenal polyposis and one had duodenal adenocarcinoma. Six patients underwent endoscopic resection of duodenal (n = 5) or ampullary adenomas (n = 1). The following operations were performed in the remaining six patients: ampullectomy in four, open polypectomy in one, and a Whipple procedure in one. There was one patient who died of acute pancreatitis following endoscopic ampullectomy. The patient with invasive duodenal cancer died of local recurrence. Small polyps were observed at the site of previous resection in all (9 of 9) patients undergoing repeat endoscopy during a mean follow-up of 18 months (range 4 to 34 months). An endoscopic and local surgical resectional approach to advanced duodenal polyposis in FAP is fraught with high recurrence rates, although recurrent polyps are small and may be amenable to retreatment in the future. Long-term follow-up is necessary to prove that deaths from duodenal or ampullary cancer are prevented with this strategy. (J GASTROINTEST SURG 1997;1:474-478.)

Duodenal polyposis develops in up to 90% of patients with familial adenomatous polyposis (FAP).[1] With the widespread acceptance of prophylactic colectomy for patients with FAP, these persons remain at increased risk for developing gastrointestinal cancer in the retained rectum[2] and in the duodenum.[3-7] Although the natural history of duodenal polyposis in FAP is not fully understood, a mean of approximately 22 years has been observed between the diagnosis of FAP and the development of duodenal or ampullary cancer.[8] However, there are no accurate predictive factors, either clinical, genetic, endoscopic, or histologic, to clearly identify patients who are prone to develop duodenal or ampullary cancer.

At present, our policy is to screen FAP patients regularly by means of repeated upper gastrointestinal (UGI) endoscopic examinations. Selective surgical or endoscopic treatment is advised for patients with advanced or progressive duodenal polyposis. This report presents our experience in 12 consecutive patients who had advanced duodenal polyposis along with a review of the literature.

PATIENTS AND METHODS

Between January 1990 and December 1995, a total of 74 FAP patients were prospectively enrolled in an UGI endoscopic surveillance protocol. Our method for staging duodenal polyposis, as well as our surveillance schedule and treatment recommendations, are outlined in Table I. All endoscopic examinations are performed with a side-viewing endoscope, and multiple biopsy specimens are taken from the gastric and duodenal mucosa, from the ampulla of Vater, and from visible lesions. Surgical or endoscopic resection is advised according to the resectability of the adenoma and the location in the duodenum. If cancer is diagnosed, radical surgery is advised. Among the entire group of 74 screened FAP patients, 11 (14.8%) developed advanced duodenal polyposis, and another patient developed duodenal cancer shortly after his first endoscopy at the age of 58 years. There were nine men and three women whose mean age was 47.4 years (range 33 to 62 years). Age at prophylactic colorectal surgery, duodenal therapy, and clinical outcome were reviewed.

From the Department of Surgery, and Steve Atanas Stavro Familial Gastrointestinal Cancer Registry, Mount Sinai Hospital, and the Department of Medicine (G.H.), Wellesley Hospital, University of Toronto, Toronto, Ontario, Canada.
Reprint requests: Steven Gallinger, M.D., Department of Surgery, Suite 1225, Mount Sinai Hospital, 600 University Ave., Toronto, Ontario, M5G 1X5, Canada.

Table I. Criteria for grading duodenal polyposis severity in FAP

Grade	Endoscopic morphology	Histology	Recommendation
1	Normal	Normal	EGD every 5 years
2	Normal or polyp 1-2 mm	Adenoma	EGD every 3 years
3	Polyp 2.1-10 mm	Adenoma	EGD every 6 months
4	Polyp >10 mm	Adenoma	Endoscopic/surgical resection
5	Any polyp or mass	Adenocarcinoma	Radical surgery

EGD = esophagogastroduodenoscopy.

RESULTS

The mean age at the time of the colorectal procedure was 29.3 years (range 18 to 47 years), and the mean elapsed time from colorectal surgery to treatment of duodenal polyps was 18.5 years (range 2 to 44 years). Mean time from advanced duodenal polyposis diagnosis to duodenal adenoma resection was 3.2 years (3 months to 9 years). Table II summarizes the demographic data of these 12 patients. According to our staging system, grade 4 or benign duodenal polyposis was observed in 11 patients and grade 5 or malignancy in one.

In the endoscopically treated group, one patient died following resection of an ampullary adenoma. This patient presented 2 hours after endoscopic ampullectomy with severe abdominal pain due to hemorrhagic pancreatitis. She died 3 days later of acute respiratory distress syndrome and multiple organ failure. The patient with invasive duodenal carcinoma underwent a local duodenal resection, as radical surgery was contraindicated because of his concomitant severe coronary artery disease. The only postoperative complication was a transient low-output duodenal fistula following open polypectomy.

Patient 8 was advised to have a Whipple operation because of the presence of numerous areas of flat but severely dysplastic mucosa throughout the duodenum.

Mean follow-up after the duodenal procedure was 18 months (range 4 to 34 years). Patient 6 died 19 months after duodenal tumor resection of peritoneal carcinomatosis. In the remaining patients who are still alive, side-viewing endoscopic surveillance has been ongoing beginning within 1 year following endoscopic or surgical resection. Patient 8, who had a Whipple operation, has not yet undergone endoscopic evaluation of his afferent loop but this is planned. Overall, polyp recurrence was observed in all nine of the surviving patients at the site of previous endoscopic polypectomy or surgical resection. Recurrent polyps are small (<2 mm) and to date are only mildly dysplastic.

DISCUSSION

The principal findings from this study are as follows: (1) 11 (15%) of 74 FAP patients prospectively enrolled in an UGI endoscopic surveillance protocol developed advanced duodenal polyposis; (2) selective endoscopic or surgical resection has proved feasible in removing large polyps, although recurrence rates are high; and (3) endoscopic ampullectomy resulted in a postprocedure death from hemorrhagic pancreatitis and we therefore do not support its use for removing ampullary adenomas.

Epidemiologic studies have demonstrated a general population incidence for duodenal/ampullary adenocarcinoma of 1%,[9] whereas in FAP registries an incidence of 4.5% is reported.[8] Offerhaus et al.[4] estimated that the risk of periampullary cancer in FAP patients is 100 to 300 times that of a control population. Recently, familial segregation has been demonstrated with respect to the severity of periampullary tumors in FAP kindreds.[10] These findings underscore the need for endoscopic surveillance, especially in high-risk families.[11-14] Side-viewing endoscopy together with multiple biopsies of normal and adenomatous mucosa is important to properly evaluate the duodenum.[6] At present, our recommendations include endoscopic examination every 3 to 5 years for patients with stages I and II disease; UGI endoscopic surveillance every 6 months for stage III duodenal polyposis, and endoscopic or surgical resection of stage IV duodenal adenomas.

Histologic studies support the hypothesis that an adenoma-to-carcinoma sequence occurs during the development of duodenal and ampullary cancer in patients with FAP.[5,15-18] Yet the natural history of periampullary malignancy in FAP is unclear. It is also controversial whether the adenoma-to-carcinoma sequence in the duodenum is similar to that in the colon and the rectum, since both similarities and differences exist in somatic molecular genetic alterations in neoplasms from the two sites.[19] The location of the adenomas in the ampullary and periampullary region

Table II. Clinical data and outcome in 12 FAP patients with advanced doudenal polyposis

Patient	Sex	Age at CR Rx (yr)	Age at D Rx (yr)	Stage	ER or surgery	Histology	Adenoma size (cm)	Dysplasia	Complications	Polyp recurrence
1	M	27	47	IV	ER	Tubullovillous	1	Moderate		Yes
2	M	47	63	IV	ER	Tubular	2	Moderate		Yes
3	F	25	48	IV	ER	Villous	1-2	Moderate	Death from AP (patient 3)	
4	M	19	38	IV	Ampullectomy	Villous	3	Severe	Transient fistula (patient 4)	Yes
5	M	45	47	IV	ER	Villous	3	Moderate		Yes
6	M	22	66	V	Polypectomy	Villous	3-4	Adenocarcinoma		Cancer death
7	M	37	41	IV	ER	Tubullovillous	1-1.5	Severe		Yes
8	M	18	42	IV	Whipple	Villous	2	Severe		Unknown
9	M	43	61	IV	Ampullectomy	Villous	2	Severe		Yes
10	M	37	51	IV	Ampullectomy	Villous	2-3	Moderate		Yes
11	F	35	52	IV	Ampullectomy	Villous	3	Moderate		Yes
12	F	35	57	IV	ER	Tubular	1-2	Moderate		Yes

CR Rx = colorectal procedure; D Rx = duodenal procedure; AP = acute pancreatitis; ER = endoscopic resection.

Table III. Review of the literature on management of duodenal polyposis in FAP patients

Reference	No. of patients	Mean age (yr)	Endoscopic treatment	Local surgery	Radical surgery	Complications	No. of recurrences (%)	Mean follow-up (mo)
Galandiuk et al.[30]	6	49	3	1	1 (Whipple)	NS	2 (33)	28
Beckwith et al.[32]	14	55	4	3	5 (Whipple)	1 death from pancreatic sepsis	NS	NS
Asbun et al.[24]	2	43	0	2		1 ileus	0 (0)	8.5
Penna et al.[26]	12	45	0	12		NS	12 (100)	13.3
Balladur et al.[27]	2	50	0	0	1 (Whipple) 1 (PPDP)	1 biliary fistula	0 (0)	24
Iwama et al.[25]	10	41	0	7	1 (Whipple)	1 anastomotic leak 1 cholangitis	0 (0)	18
Chung et al.[28]	4	49	0	0	4 (PSD)	1 pancreatic fistula	0 (0)	24
Present series	12	47	6	5	1 (Whipple)	1 duodenal fistula 1 death post endoscopic ampullectomy	9 (100)	18

NS = not stated; PPDP = pylorus-preserving duodenopancreatectomy; PSD = pancreas-saving duodenectomy.

suggests that bile might be partly responsible for the malignant transformation of the periampullary mucosa.[20] Jagelman et al.[8] estimated an average time of 22 years for the development of duodenal or ampullary malignancy following colectomy in FAP patients. In our series a mean elapsed period of 18.5 years was observed between prophylactic colorectal surgery and the duodenal procedure. There are differences between Japanese and Western FAP series with regard to location of UGI adenomas.[9,21-23] Japanese series have reported more gastric than duodenal adenomas in FAP patients and more gastric than duodenal cancers.[5] These differences may be explained by the variable phenotypic expression of FAP, and environmental factors such as diet may also play an important role.

Surgery is still our preferred method for removal of large adenomas (>3 cm), especially those located in the immediate ampullary region. Local surgery (ampullectomy) is probably appropriate in cases of nonmalignant adenoma, whereas radical surgery is necessary for duodenal or ampullary carcinoma.[24,25] Because extensive polypectomy carries a high recurrence rate,[26] radical surgery for benign adenoma might be contemplated if rapid growth is observed, in cases of large adenomas (>4 cm) located at the ampulla or in the periampullary region, or if the adenoma has a villous architecture and/or severe dysplasia.[5,27,28] Whenever invasive carcinoma is diagnosed, a Whipple procedure is advised.[29-32] Following more conservative surgical procedures, the recurrence rate for duodenal adenoma is high and ranges from 33% to 100% [24,26,30,32] (Table III). Endoscopic resection of adenomas is an attractive approach but requires considerable expertise[33] since complications from endoscopic resection can be disastrous.[34-36]

At least two conflicting conclusions may be drawn from this report. From a negative perspective, all patients in our series have developed recurrent polyps at previous sites of local resection. On the basis of the high recurrence rate alone, it can be suggested that our approach to duodenal polyposis in FAP is inappropriate and more radical surgery (perhaps a prophylactic classical Whipple operation or pylorus-preserving duodenopancreatectomy) is justified for these patients (Parc R, personal communication). However, a contrasting argument can be presented since, to date, recurrent polyps are very small and only mildly dysplastic. Given the unclear natural history of duodenal polyps in FAP, it seems reasonable to suggest that many years will pass before recurrent polyps progress to large, severely dysplastic lesions. Moreover, close endoscopic surveillance will be maintained for all these patients. Although morbidity and mortality rates for the Whipple operation are now acceptably low, little is known about the long-term effects of radical pancreaticoduodenectomy for young patients such as those in our FAP population. Furthermore, it is likely that recurrent polyps would develop in the afferent loop following the Whipple operation.

It is obvious that novel pharmacologic approaches are needed to prevent recurrence following resection, since drugs such as sulindac appear to have only minor efficacy in the UGI tract of patients with FAP.[37]

The outcome of patient 6 deserves special comment. Despite yearly UGI surveillance over a 5-year period, he was found to have invasive duodenal adenocarcinoma. It is important, however, to mention that he entered the UGI endoscopic surveillance protocol at a later age in comparison to the other patients. This may explain why, despite surveillance, he was found to have invasive duodenal carcinoma. We currently recommend initiating UGI endoscopic surveillance in FAP patients at 25 to 30 years of age.

This series demonstrates that a selective endoscopic or surgical approach to FAP patients with advanced duodenal polyposis may be worthwhile, although longer follow-up is needed to prove that deaths from cancer have been prevented. In the surgical group there were no procedure-related deaths, and morbidity was acceptable. In addition, the death of the patient who underwent postendoscopic ampullectomy indicates that this form of treatment is not advisable.

CONCLUSION

Although no uniform consensus exists among FAP registries for the management of advanced duodenal polyposis in patients with FAP, we believe that a tailored endoscopic or surgical resectional approach is feasible. However, prospective multicenter studies are needed to develop management protocols that are not only standardized but also cost-effective.

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Helicobacter pylori May Cause "Reflux" Gastritis After Gastrectomy

Yoshi Nagahata, M.D., Yasutomo Azumi, M.D., Norihisa Numata, M.D., Masafumi Yano, M.D., Teruyuki Akimoto, M.D., Yoichi Saitoh, M.D., F.A.C.S.

Patients with "reflux" gastritis after gastrectomy suffer from a variety of symptoms, and this type of gastritis may sometimes compromise the quality of life of these patients. Since *Helicobacter pylori* is considered to be one of the most important pathogenetic factors in gastritis, the association between *H. pylori* and reflux gastritis was investigated in this study. A total of 145 patients with gastrectomy were entered into the study. Five biopsy specimens from the gastric remnant were taken at upper gastrointestinal endoscopy. One specimen was examined pathohistologically, and the remaining four were examined for *H. pylori* infection. Fifty-two patients (36%) demonstrated *H. pylori* infection. The prevalence of *H. pylori* was significantly higher in patients who had a partial gastrectomy, and it was significantly lower in patients who had undergone gastrectomy more than 4 years previously. The histologic gastritis score in patients with *H. pylori* infection was significantly higher. Furthermore, *H. pylori* was eradicated in patients with some symptoms of gastritis and no bile reflux to the residual stomach at endoscopy; in these patients the symptoms were relieved and the histologic gastritis score decreased significantly. In conclusion, possible involvement of *H. pylori* is suspected in the pathogenesis of "nonreflux" gastritis after gastrectomy. (J GASTROINTEST SURG 1997;1:479-486.)

"Reflux" gastritis, which occasionally occurs after gastrectomy, is associated with various symptoms such as abdominal pain, nausea, emesis, and loss of appetite.[1] This gastritis may eventually compromise the patient's quality of life.[2] Reflux of intestinal fluid containing bile juice to the residual stomach has been considered a primary pathogenetic factor in this type of gastritis.[3] Medical treatment has been directed toward dietary manipulation, improved gastric emptying, reduction of hydrochloric acid secretion, and bile salt binding by cholestyramine. However, these measures have often been unsuccessful.[2,4,5] Surgery is sometimes performed to divert intestinal fluids away from the residual stomach. However, prevention of reflux by surgical means does not always bring relief from the gastritis symptoms. In fact, there may be pathogenetic factors other than the reflux that cause the gastritis.

Helicobacter pylori is considered one of the most important pathogenetic factors in gastritis and gastroduodenal ulcers and may be associated with nonulcer dyspepsia.[6-8] Eradication of *H. pylori* has been shown to improve gastritis and gastroduodenal ulcers.[9,10] The presence of *H. pylori* is closely associated with histologic gastritis, and the number of bacteria present is related to the severity of the gastritis.[11] Thus it is possible that *H. pylori* may cause reflux gastritis after gastrectomy. The association between *H. pylori* infection and this reflux gastritis was investigated in the present study.

PATIENTS AND METHODS

Patients

A total of 145 patients who underwent gastrectomy between May 1993 and March 1996 were enrolled in this study. Some patients, however, were excluded from participation for the following reasons: total gastrectomy, recurrence of gastric cancer, presence of stomal ulcers, and administration of drugs such as antibiotics, corticosteroids, nonsteroidal anti-inflammatory drugs, antacids, H_2 receptor antagonists, antisecretory agents, or sucralfate. Informed consent was obtained from all patients participating in the study.

From the First Department of Surgery, Kobe University School of Medicine, Kobe, Japan.
Presented at the Thirty-Seventh Annual Meeting of The Society for Surgery of the Alimentary Tract, San Francisco, Calif., May 19-22, 1996.
Reprint requests: Yoshi Nagahata, M.D., First Department of Surgery, Kobe University School of Medicine, 7-5-2 Kusunoki-cho, Chuo-ku, Kobe 650, Japan.

Operative resection procedures were classified as either partial gastrectomy or subtotal gastectomy, according to whether the procedure involved cutting of the short gastric arteries at the greater curvature. Resection without this procedure was considered partial gastrectomy. The resectioned amount was approximately two thirds of the stomach in the partial gastrectomy and more than four fifths of the stomach in the subtotal gastrectomy.

Study Protocol

Upper gastrointestinal endoscopy was performed after an overnight fast. Five mucosal biopsy specimens were taken from the residual stomach using sterilized biopsy forceps. Endoscopes were cleansed, disinfected, and rinsed. Gastric biopsy specimens were taken from the lesser and greater curvature, within 2 cm from the gastroduodenostomy or gastrojejunostomy. Two of the five specimens were then sent for bacteriologic examination and two specimens were examined by the urease test. The remaining specimen was prepared for histologic examination. Since erythema without edema is often observed in the gastric mucosa after gastrectomy, endoscopic gastritis was diagnosed according to the Sydney system[12] at a distance of 2 cm or more from the anastomosis by the presence of two or more of the following conditions: edema, erythema, friability, exudate, erosion, hyperplasia, atrophy, visibility of the vascular pattern, intramural bleeding spots, or nodularity.

Bacteriologic Assessment

Two biopsy specimens were immediately transported to the laboratory and processed within 30 minutes of collection. The specimens were cultured using Skirrow plates made of blood agar and a Campylobacter-selective supplement. These plates were incubated at 37° C in a microaerophilic environment (5% oxygen) for 5 days. The remaining two biopsy specimens were immediately placed into the urea-containing well of the stat urease test (Asuka; PML Microbiologicals, Portland, Ore.).

Histologic Assessment

A formalin-fixed biopsy specimen was embedded in paraffin and sections were stained with hematoxylin and eosin. The degree of gastritis was best determined by the gastritis score devised by Rauws et al.[13] (Rauws' score), which consisted of the following four parameters: density of the inflammatory infiltrate in the lamina propria (0 to 2); density of polymorphonuclear leukocytes in the lamina propria (0 to 3); presence of intraepithelial polymorphonuclear leukocytes (0 to 3); and superficial erosion (0 to 2).

Table I. Patients

	H. pylori (+)	*H. pylori* (−)	Total
No. of patients	52 (36%)	93 (66%)	145
Sex (M:F)	39:13	61:32	100:45
Primary disease			
Gastric cancer	42 (39%)	65 (61%)	107
Gastroduodenal ulcers	9 (30%)	21 (70%)	30
Others	1 (13%)	7 (87%)	8

Eradication Assessment

Eighteen patients with *H. pylori* infection who had severe symptoms of gastritis and showed no obvious bile reflux on endoscopic examination were treated with amoxicillin, 1500 mg three times daily for 5 days; omeprazole, 20 mg once daily; and sofalcone, 300 mg three times daily for 4 weeks. Endoscopy was performed more than 3 months after the end of treatment, at which time bacteriologic and histologic assessments were also performed.

Gastric Acid Output, Pepsinogen, and Digestive Hormone Determination

Preoperative and postoperative gastric acid output was determined as basal acid output (BAO) and maximal acid output (MAO) using tetragastrin at a dosage of 4 mg/kg. Serum pepsinogen I and II, gastrin and secretin, and plasma somatostatin levels were also determined.

Statistical Methods

Values were expressed as mean ± standard deviation. Statistical analyses were performed by means of Student's t test, Fisher's exact test, and chi-square analysis. A P value <0.05 was considered significant.

RESULTS

Patients

Fifty-two (36%) of the 145 patients demonstrated *H. pylori* infection. *H pylori* prevalence was 39% in patients with gastric cancer and 30% in patients with gastroduodenal ulcers (Table I). The surgical procedures performed were subtotal gastrectomy in 45 and partial gastrectomy in 100 patients, respectively. *H. pylori* prevalence in patients with partial gastrectomy (42%) was significantly (P <0.05) higher than in those with subtotal gastrectomy (22%). Reconstruction procedures were gastroduodenostomy in 82 and gastrojejunostomy in 63 patients, respectively. *H. pylori* prevalence was 37% in patients with gastroduodenos-

Table II. Operative procedures

	H. pylori (+)	*H. pylori* (−)	Total
Resection			
Subtotal gastrectomy	10 (22%)	35 (78%)	45
Partial gastrectomy	42 (42%)	58 (58%)	100*
Reconstruction			
Gastroduodenostomy	30 (37%)	52 (63%)	82
Gastrojejunostomy	22 (35%)	41 (65%)	63

*P <0.05 vs. subtotal gastrectomy.

Table III. Gastric acid output

	Before operation (mEq/hr)	After operation (mEq/hr)	% Decrease
Subtotal gastrectomy (n = 23)			
BAO	2.5 ± 2.6	0.3 ± 0.4	88
MAO	8.9 ± 4.5	0.9 ± 0.7*	90
Partial gastrectomy (n = 39)			
BAO	2.7 ± 2.6	0.6 ± 0.5	78
MAO	8.6 ± 4.4	2.3 ± 2.0	73

BAO = basal acid output; MAO = maximal acid output (tetragastrin, 4 μg/kg).
*P <0.01 vs. partial gastrectomy.

Table IV. Association between endoscopic gastritis and *H. pylori* infection

	H. pylori (+)	*H. pylori* (−)	Total
Gastritis (+)	26 (38%)	43 (62%)	69
Gastritis (−)	26 (34%)	50 (66%)	76

tomy and 35% in patients with gastrojejunostomy, which was not significantly different (Table II).

H. pylori prevalence was considerably higher in patients in their fifties or younger. However, the prevalence decreased with age and was only 20% in patients in their seventies and 17% in those in their eighties (Fig. 1). When considering time elapsed since gastrectomy, *H. pylori* prevalence was 54% when it had been less than a year since surgery, 43% between 1 and 3 years, 29% between 4 and 10 years, and 17% when more than 10 years had passed since gastrectomy. The prevalence decreased significantly (P <0.05) with time elapsed since gastrectomy (Fig. 2).

Gastric Acid Output, Pepsinogen, and Digestive Hormones

As shown in Table III, the MAO value in subtotal gastrectomy was significantly (P <0.01) lower than in partial gastrectomy. There was no significant difference, however, in the BAO value between the two operative procedures after gastrectomy (Table III). Values for serum pepsinogen I and II after partial gastrectomy are shown in Fig. 3. There was no significant difference in serum pepsinogen I and II levels and in the pepsinogen I/II ratio between patients with and without *H. pylori* infection (see Fig. 3). Serum gastrin, secretin, and plasma somatostatin levels after partial gastrectomy were determined and were shown not to be significantly different between patients with and without *H. pylori* infection (Fig. 4).

H. pylori Infection and Endoscopic Gastritis

Twenty-six (38%) of 69 patients with endoscopic gastritis were infected with *H. pylori*, whereas 26 (34%) of 76 patients without endoscopic gastritis were infected with *H. pylori*. There was no significant difference in the rate of *H. pylori* infection between patients with and without endoscopic gastritis (Table IV).

Rauws' Score

Rauws' scores in patients with *H. pylori* infection (6.7 ± 1.6) were significantly (P <0.01) higher than in those without *H. pylori* infection (2.3 ± 1.5). For each parameter the score was significantly (P <0.01)

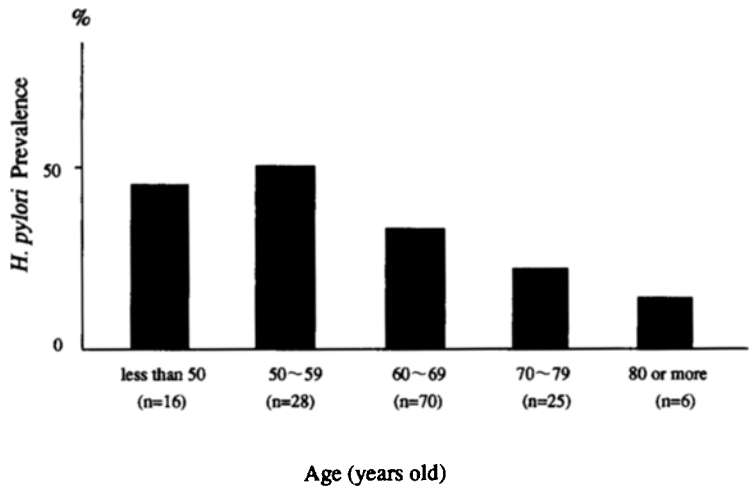

Fig. 1. Relationship between *H. pylori* prevalence and age. The prevalence decreased in patients in their seventies or older.

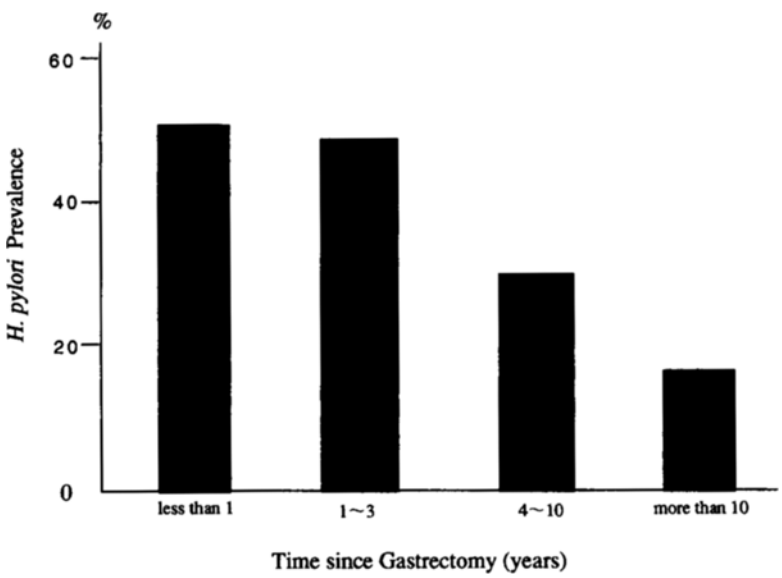

Fig. 2. Relationship between *H. pylori* prevalence and time since gastrectomy. The prevalence decreased significantly with time elapsed since gastrectomy ($P < 0.05$).

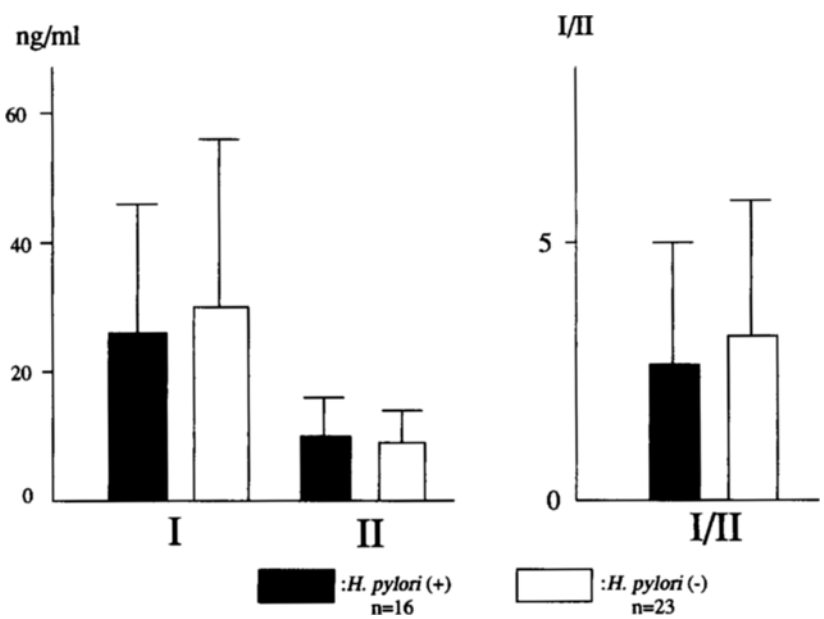

Fig. 3. Serum pepsinogen I and II *(left)* and I/II ratio *(right)* in patients with partial gastrectomy. Patients with subtotal gastrectomy were excluded.

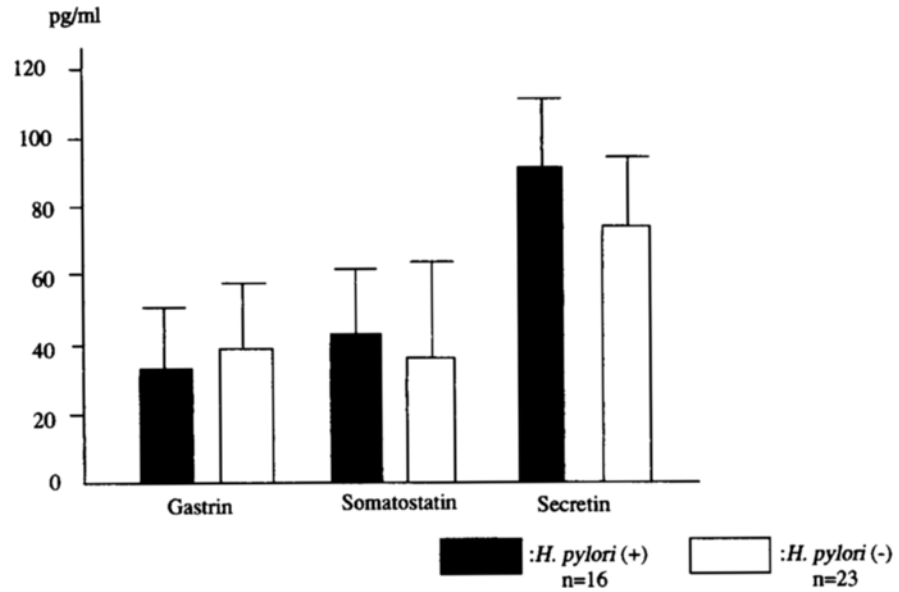

Fig. 4. Serum gastrin, secretin, and plasma somatostatin levels in patients with partial gastrectomy. Patients with subtotal gastrectomy were excluded.

Table V. Rauws' score

Parameter	*H. pylori* (+)	*H. pylori* (−)
Inflammatory infiltrate in the lamina propria	1.6 ± 0.5*	1.0 ± 0.7
Polymorphonuclear leukocytes in the lamina propria	2.4 ± 0.7*	0.7 ± 0.8
Intraepithelial polymorphonuclear leukocytes	1.9 ± 0.8*	0.3 ± 0.4
Superficial erosion	0.8 ± 0.7*	0.3 ± 0.5
TOTAL SCORE	6.7 ± 1.6*	2.3 ± 1.5

*P <0.01 vs. *H. pylori* (−).

Table VI. Change in Rauws' score after eradication

	Before eradication	After eradication
Rauws' score	6.3 ± 1.6	1.2 ± 1.0*

*P <0.01 vs. before eradication.

higher in patients with *H. pylori* infection than in those without *H. pylori* infection (Table V).

Eradication

Sixteen (89%) of the 18 patients with *H. pylori* infection had their infections successfully eradicated, after which their symptoms improved. Rauws' scores in these 16 patients decreased remarkably after eradication of *H. pylori*. The average Rauws' score after eradication (1.2 ± 1.0) was significantly (P <0.01) lower than before eradication (6.3 ± 1.6; Table VI).

DISCUSSION

The rate of *H. pylori* infection was reported to be 60% to 80% in patients with gastric cancer and 70% to 90% in patients with gastroduodenal ulcers.[9,14-18] The rate in patients with gastrectomy, however, was reported to be 40% to 56%[19-21] compared to the 36% in the present study. *H. pylori* prevalence was considerably lower after gastrectomy. With regard to the primary disease that necessitated gastrectomy,

H. pylori prevalence was 39% in patients with gastric cancer and 30% in patients with gastroduodenal ulcers. *H. pylori* prevalence was twofold greater in patients with partial gastrectomy compared to subtotal gastrectomy. The incidence of *H. pylori* infection was reported to be higher at the antrum pyloricum than at the corpus gastricum and showed a highly significant positive correlation with acid output of the stomach.[22] The decrease in the gastric acid output after gastrectomy was greater in the patients with subtotal gastrectomy compared to those with partial gastrectomy, as shown in Table III. These results are possible reasons for the decrease in the prevalence of *H. pylori* in the patients with subtotal gastrectomy. *H. pylori* prevalence was 54% when assessed less than a year after gastrectomy. The prevalence was 43% when 1 to 3 years had passed since gastrectomy, the rate dropped to 29% between 4 and 10 years, and reached the very low rate of 17% more than 10 years after surgery. This result may imply that *H. pylori* prevalence after gastrectomy decreases gradually with age in the present study, although the prevalence was reported to increase with age up to the seventies in patients with a whole stomach.[23-25]

Why then did the prevalence of *H. pylori* infection decrease after gastrectomy? The intragastric conditions changed after gastrectomy. These changes involved many factors such as the amount of gastric mucosa remaining, the distribution of *H. pylori* within the stomach, the intragastric pH, aggravation of mucosal atrophy, augmentation of intestinal metaplasia, and bile reflux to the residual stomach. Acid output correlated well with the occurrence of *H. pylori*. The prevalence of *H. pylori* was 10% in achlorhydria and rose to 100% in cases where acid output was greater than 30 mmol/hr.[22] The decrease in the *H. pylori* prevalence was accompanied by aggravation of the gastric mucosal atrophy and augmentation of intestinal metaplasia, which increased after gastrectomy.[26,27] The exact reason for the decline in the rate of *H. pylori* infection in patients with gastrectomy is still unknown; however, reflux of bile juice is a prime suspect because bile inhibits *H. pylori* growth.[28] Thus *H. pylori* could barely survive in the residual stomach. It may then be postulated that *H. pylori* can inhabit the stomach before gastrectomy, remain alive for some time after the surgery, and gradually decrease in the gastric remnant year by year. An exact conclusion has not been reached since *H. pylori* infection was not investigated in all patients before gastrectomy in this study. It is generally accepted that after gastrectomy some symptoms gradually abate after sufficient time has passed since the operation, and this phenomenon is known as postoperative adaptation. The decreasing prevalence of *H. pylori* more than 4 years after surgery may be a potential cause of this phenomenon.

Serum pepsinogen I and II levels and the I/II ratio are considered markers of the degree of atrophic gastritis, which is associated with *H. pylori* infection. Slightly higher levels of pepsinogen I and I/II ratio were seen in patients with *H. pylori* infection compared to patients without *H. pylori* infection; however, no significant differences were shown in the present study. *H. pylori* infection is believed to increase the serum gastrin level[29] and to affect the plasma somatostatin level.[30] However, there were no significant differences in serum gastrin, plasma somatostatin, and serum secretin levels between patients with and without *H. pylori* infection. In the present study, serum pepsinogen, serum gastrin, plasma somatostatin, and serum secretin were determined in approximately one third of the patients in total. The results in this study may reflect the selection bias of the patients or our sample may have been too small. Further investigations should continue with regard to these issues.

Only 38% of patients with endoscopic gastritis had *H. pylori* infection, although 34% of patients without endoscopic gastritis had *H. pylori* infection. These results may cause errors in the endoscopic diagnosis of gastritis. It was not possible to reliably predict the presence of histologic gastritis based on endoscopic appearance[31,32] so the Rauws' score, which is more accurate in determining the degree of gastritis,[13] was adopted. Rauws et al.[13] obtained four specimens randomly at upper gastrointestinal endoscopy; however, they did not discuss any significant differences in the gastritis scores among the four specimens. We obtained one biopsy specimen for histologic evaluation in this study, and there may be some limitations to generalizing gastric histologic findings based on a single specimen. Initially we obtained three biopsy specimens for histologic evaluation from the residual stomach at random, within 2 cm from the anastomosis, and determined the Rauws' score for each specimen in a preliminary study of 10 patients. An actual difference in Rauws' scores within each specimen from the same residual stomach at the same time was less than two in eight cases and was two in two cases. In no case was there a difference of more than two in Rauws' scores. From these preliminary results we took only one biopsy specimen at random within 2 cm from the anastomosis. Although gastritis may occur in any part of the residual stomach, this site seemed to adequately represent the gastritis. Rauws' scores in the patients with *H. pylori* infection were significantly higher compared to those without *H. pylori* infection.

The etiology of this gastritis, which was histologically proved, possibly involved *H. pylori* infection unless bile reflex to the residual stomach was seen. Although bile reflux is an important factor in the pathogenesis of gastritis, *H. pylori* infection also seems to be a pathogenetic factor inasmuch as prevention of the reflux cannot always cure the gastritis.[33] Further investigation of the consistent perioperative period, including preoperative investigation of *H. pylori* infection, should further clarify this issue.

Eighteen patients who complained of severe gastritis symptoms without bile reflux at endoscopy were treated for *H. pylori* eradication. In 16 of these patients the symptoms improved and the Rauws' score decreased with successful eradication. There are various regimens for *H. pylori* eradication[8,9,34] that produce successful eradication rates of 60% to 80%. Because bismuth salt had various adverse effects on either the gastrointestinal tract or the central and peripheral nervous system, our regimen consisted of a combination of amoxicillin, omeprazole, and sofalcone (2'-carboxymethoxy-4,4'bis [3-methyl-2-butenyloxy] chalcone),[35] which is effective in the treatment of gastric ulcers. The minimum inhibitory concentration of this drug was as low as that of bismuth salt,[36] and it also produced no severe adverse effects.[34] Amoxicillin and omeprazole are recognized as useful in eradicating *H. pylori*. A considerably high eradication rate of 89% was achieved in the present study. Reflux of bile juice usually occurs in patients with gastrectomy and the bile juice inhibited the growth of *H. pylori*.[28] Decreased acid output after gastrectomy may aid in the eradication of *H. pylori* since many antibiotics are effective and stable at middle pH.[36,37] The postoperative decrease in acid output itself may contribute to the high eradication rate. Furthermore, the maximal gastric acid output after subtotal gastrectomy was significantly lower than that after partial gastrectomy, and *H. pylori* prevalence in patients with subtotal gastrectomy was lower than in those with partial gastrectomy. This confirms the relationship between *H. pylori* infection and gastric acid output. With a low acid output *H. pylori* has difficulty surviving in the residual stomach. On these grounds a high eradication rate was achieved in the patients with gastrectomy.

Consequently the Rauws' scores in patients with *H. pylori* infection were significantly higher than in those without *H. pylori* infection, and eradication of *H. pylori* was associated not only with improvement in the symptoms of gastritis but also with a decrease in the Rauws' score. *H. pylori* is possibly involved in the pathogenesis of "nonreflux" gastritis after gastrectomy.

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Endorectal Mucosal Advancement Flap: The Preferred Method for Complex Cryptoglandular Fistula-in-Ano

Richard W. Golub, M.D., William E. Wise, Jr., M.D., Bruce A. Kerner, M.D., Karamjit S. Khanduja, M.D., Pedro S. Aguilar, M.D.

Cryptoglandular fistula-in-ano is a common affliction that usually responds well to conventional surgical procedures such as fistulectomy, fistulotomy, and seton placement. These procedures, however, can be associated with varying degrees of fecal incontinence. Endorectal mucosal advancement flap has been advocated as an alternative procedure that avoids this problem. This study was undertaken to determine the risks and benefits associated with endorectal mucosal advancement flap in the treatment of complex fistula-in-ano. One hundred sixty-four patients underwent 167 endorectal mucosal advancement flap procedures for complex cryptoglandular fistula-in-ano between January 1982 and December 1990. There were 126 men and 38 women whose mean age was 42.1 years (range 20 to 79 years). The majority of the patients (70%) had complex fistulas (transsphincteric, suprasphincteric, or extrasphincteric). Fifteen patients (9%) had an intersphincteric fistula. All patients were available for short-term follow-up (6 weeks). Postoperative morbidity was minimal and included urinary retention in 13 patients (7.8%) and bleeding in one patient. Healing time averaged 6 weeks. Long-term follow-up, ranging from 19 to 135 months, was carried out in 61 patients. There were two recurrences (3.28%). Nine patients (15%) complained of varying degrees of fecal incontinence. Six patients complained of incontinence to flatus and three patients complained of incontinence to liquid stool. No patient was incontinent of solid stool. Sixty patients (98%) rated their functional result as excellent or good. Endorectal mucosal advancement flap is a safe and effective technique for the treatment of complex cryptoglandular fistula-in-ano. It can be performed with minimal morbidity, no mortality, an acceptable recurrence rate, and little alteration in anorectal continence. (J GASTROINTEST SURG 1997;1:487-491.)

Cryptoglandular fistula-in-ano is a common affliction that usually responds well to conventional operative procedures such as fistulectomy, fistulotomy, and seton placement. These procedures, however, can be associated with varying degrees of fecal incontinence ranging from 6% to 45%.[1-3] The risk of fecal incontinence is particularly high with complex fistulas (transsphincteric, suprasphincteric, and extrasphincteric), and therefore these fistulas require careful preoperative evaluation and often alternative surgical techniques. One such technique is the endorectal mucosal advancement flap. First proposed by Noble[4] in 1902 for repair of a rectovaginal fistula, and later modified by Elting[5] and Laird,[6] this technique employs a broad-based flap of rectal wall to obliterate the origin of the fistula. In 1985 Aguilar et al.[7] proposed the use of an endorectal mucosal advancement flap to treat complex cryptoglandular fistula-in-ano. This report is a continuation of that series.

SURGICAL TECHNIQUE

Most of the patients undergoing an endorectal mucosal advancement flap procedure for cryptoglandular fistula-in-ano received a standard anorectal bowel preparation consisting of a clear liquid diet and a Fleet enema (C.B. Fleet Company Inc., Lynchburg, Va.) on the evening before and the morning of surgery. Intravenous antibiotics were employed only for endocarditis or prosthetic joint prophylaxis when indicated.

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Reprint requests: Richard W. Golub, M.D., F.A.C.S., State University of New York Health Science Center at Brooklyn, 450 Clarkson Ave., Box 40, Brooklyn NY 11203.

The technique of endorectal mucosal advancement flap has been described extensively elsewhere and is essentially a modification of the techniques originally developed by Noble[4] and Elting.[5] General, regional, or local anesthesia with intravenous sedation can be used. Intraoperative fluids are limited. Most patients are placed in the prone jackknife position with the buttocks taped apart, although the lithotomy position can be advantageous for posterior fistulas. Illumination with a headlamp can be helpful. The perineum and anal canal are prepared and irrigated with povidone-iodine solution. Anesthesia is supplemented with a perianal field block consisting of bupivacaine 0.25%, lidocaine 0.5% with 1/200,000 epinephrine, and 150 units of hyaluronidase (Wydase, Wyeth-Ayerst Laboratories, Philadelphia, Pa.). A Hill-Ferguson retractor is placed in the anal canal and a thorough anorectal evaluation is undertaken. Associated pathologic conditions are noted. Internal and external openings are located, and the course of the fistulous tract is carefully assessed by palpation and gentle probing.

The fistulous tract is removed subcutaneously from the external opening to the level of the external sphincter muscle. The residual tract and any granulation tissue is removed by curettage. Secondary tracts are noted and treated in a similar fashion. The perianal skin is widely saucerized. Using sharp scissors dissection, a flap consisting of anoderm, mucosa, and submucosa is raised from the intersphincteric groove to a point approximately 3 to 4 cm proximal to the internal opening. The configuration of the flap is trapezoidal with the base wider than the apex to ensure an adequate distal blood supply. Meticulous electrocautery hemostasis is maintained throughout the course of the dissection.

The opening in the internal anal sphincter is closed with interrupted 3-0 chromic sutures. The flap is advanced distally, without tension, to cover the defect in the internal sphincter and the excess mucosa (including the internal opening) is amputated. The distal edge is sutured to the internal anal sphincter at the level of the intersphincteric groove, and the lateral aspects are reapproximated to adjacent rectal mucosa using 2-0 or 3-0 chromic catgut sutures. Sterile dressings are applied to the wound. Packing, topical antibiotics, and suction catheters are not routinely used. Fecal diversion is avoided.

Repair of the external voluntary sphincter was not undertaken in this series of patients. However, in patients presenting with anteriorly placed fistulas, perineal body reconstruction and/or simple reefing of the anterior sphincter musculature can be added to the procedure if preoperative history suggests incontinence or physical examination demonstrates lax musculature or an attenuated perineal body.

Postoperatively the patient is started on a regular diet supplemented with bulk fiber agents and stool softeners. Oral analgesics, diazepam, and warm sitz baths are employed to promote local hygiene and to control pain and muscle spasms. Patients are discharged home when they are able to void without difficulty. Follow-up is conducted every 2 to 3 weeks until all wounds are healed.

RESULTS

One hundred sixty-four patients underwent 167 endorectal mucosal advancement flap procedures for cryptoglandular fistula-in-ano from January 1982 through December 1990. Fistulas secondary to inflammatory bowel disease, actinomycosis, foreign bodies, and malignancy were excluded from this study, as were rectovaginal and rectourethral fistulas. There were 126 men and 38 women whose mean age was 42.1 years (range 20 to 79 years). Sixty-two percent of patients had undergone prior anorectal surgery, including 18 patients (11%) who had previous fistula surgery. Perirectal pain and drainage were the most common presenting symptoms with a preoperative duration of 1 to 3 months. Five patients (3%) presented with an acute fistulous abscess. All patients underwent an endorectal mucosal advancement flap as their definitive surgical procedure.

One hundred fifteen patients (70%) were classified as having complex fistula-in-ano (transsphincteric, suprasphincteric, or extrasphincteric), whereas 15 patients (9%) had intersphincteric fistula. The majority of these 15 patients had anteriorly placed fistulas and were deemed at risk for incontinence with conventional surgical technique. The type of fistula was not recorded in 34 patients (Fig. 1). An internal opening was located in the posterior midline in 87 patients (53%). All 164 patients were available for short-term follow-up. Complications and healing rates are based on this group of patients. Healing time averaged 6 weeks for all patients. Ten patients (5.98%) experienced prolonged healing. All of these patients developed either small subcutaneous (7 patients) or superficial fistulas in the early postoperative period. Six patients were treated with incision and drainage, three patients had an in-office fistulotomy, and one patient required a repeat mucosal advancement flap. Postoperative morbidity also included urinary retention (13 patients) and bleeding (1 patient) (Table I). There were no operative deaths.

Long-term follow-up was carried out by office visit or telephone questionnaire in 61 patients (37%) and

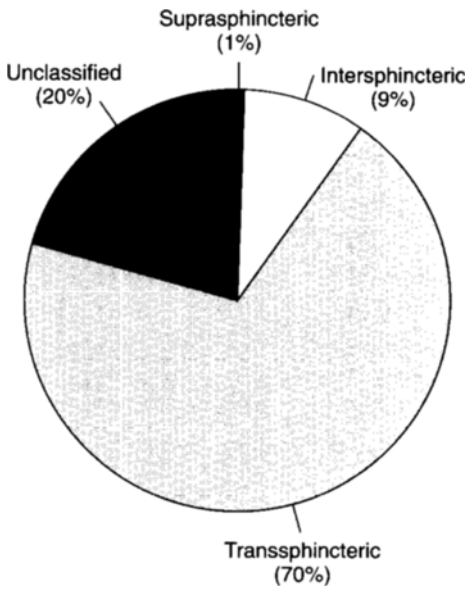

Fig. 1. Summary of fistula classifications.

Table I. Summary of early complications (N = 167)

Complication	No. of patients	%
Urinary retention	13	7.78
Bleeding	1	0.6
Abscess	7	4.2
Fistula	3	1.8

Table II. Summary of late complications (N = 61)

Complication	No. of patients	%
Fistula	2	3.28
Incontinence		
Flatus	6	9.8
Liquid	3	4.9
Ectropion	1	0.6
Anal stenosis	1	0.6

ranged from 19 to 135 months (mean 71.2 months). Long-term results regarding incontinence, recurrence, and functional outcome are based on this group of 61 patients. Ninety-eight percent of patients questioned (60 of 61) rated their outcome as either good or excellent. Two patients (3.28%) developed a fistula-in-ano at a site remote from their index fistula, requiring a second mucosal advancement flap for definitive treatment. These recurrences were at 33 and 47 months, respectively. One patient developed anal stenosis and one developed a mucosal ectropion. Nine patients (15%) complained of varying degrees of incontinence following repair. Six patients complained of incontinence to flatus, whereas three patients complained of incontinence to liquid stool. No patient was disabled by his or her incontinence and no patient complained of incontinence to solid stool (Table II). All complications developed in patients with either complex or unclassified fistulas.

DISCUSSION

Cryptoglandular fistulas-in-ano originate from an anal crypt at the dentate line and traverse varying amounts of the anal sphincter mechanism. Traditionally, simple fistulas (superficial, intersphincteric, and low transsphincteric) have been treated by fistulotomy with a low recurrence rate and minimal clinical alteration in continence. There is, however, a diminution in anal canal resting pressure,[8] and varying degrees of fecal incontinence have been reported.[1-3] More complex fistulas (high transsphincteric, suprasphincteric, and extrasphincteric) have been treated with seton placement and staged fistulotomy with similar alteration in continence.[9,10] Recently there has been renewed interest in the endorectal mucosal advancement flap because of continuing concerns regarding sphincter damage and incontinence following "conventional" fistula surgery. Presumed advantages of this procedure include less alteration in anatomic integrity, preservation of the sphincter mechanism, rapid healing, reduced pain, and avoidance of a diverting stoma. Additionally, the technique has the advantage that recurrence does not preclude a repeat advancement flap or a "traditional" surgical approach.

Evaluation of the surgical treatment of fistula-in-ano includes assessment of healing time, recurrence, and fecal incontinence. Healing time averaged 6 weeks in our series with 10 patients (5.98%) experiencing delayed healing of their surgical wounds. The majority of these patients had external wounds that either closed prematurely or developed subcutaneous fistula as a result of bridging at the site of the external wound. These patients all required reopening of the external wound or superficial fistulotomy. One patient required a repeat mucosal advancement flap procedure. The delayed healing in our series compares favorably with the 8.75% of patients whose wounds took longer than 26 weeks to heal in the report by Vasilevsky and Gordon.[2]

Recurrence rates range from 3.7%[11] to 40.6%[12] in the literature. Complex fistula, previous history of

anorectal abscess, and failure to identify all of the tracts at the index operation have all been implicated in the development of a recurrent abscess or fistula. The initial series from this institution reported a recurrence rate of 1.5%.[7] The recurrence rate of 3.28% in this series compares very favorably with all published reports. The two patients with recurrent abscess and fistula presented at 33 and 47 months postoperatively, and both had abscesses and fistulas remote from the index abscess. These may actually represent new fistulous tracts rather than recurrent disease.

Fecal incontinence is reported in almost any series published on the surgical treatment of fistula-in-ano. Comparison of various studies is difficult because of a lack of standard reporting techniques for patients with altered continence. In this series, six patients available for long-term follow-up complained of incontinence to flatus and three complained of incontinence to liquid stool. The mean follow-up in this group of 61 patients was 71.2 months. No patient complained of gross incontinence, and no patient was disabled by his or her incontinence. Reports in the literature demonstrate rates of incontinence ranging from 0.00%[13] to 30%.[14] These reports all rely on "conventional" surgical therapy with either fistulotomy or seton placement. Our incontinence rate in 61 patients followed for a mean of six years compares favorably with the rates quoted in the literature.

The endorectal advancement flap was originally used in the treatment of rectovaginal fistula, but there are now several reports documenting the success of this technique in the repair of complex cryptoglandular fistula-in-ano and rectourethral fistulas.[15,16] In the original series from this institution, Aguilar et al.[7] reported on 189 patients who underwent advancement flap for fistula-in-ano. An 80% follow-up, ranging from 8 months to 7 years, revealed a 90% asymptomatic group and a 10% group who had minor symptoms. Eight percent of the symptomatic patients had minor soiling; 7% were incontinent of gas and 6% were incontinent of loose stool. No patient was incontinent of solid feces. Three patients (1.5%) developed recurrent fistula-in-ano. Two of them had a second mucosal advancement flap performed with good results and the other patient declined further therapy. Similarly, in a study of 15 patients with high recurrent anal fistula, Oh[17] described satisfactory healing in 87% and Wedell et al.[18] reported complete healing in 29 of 30 patients.

Although it is generally accepted that anorectal surgery should be avoided in patients with Crohn's disease, some authors have recently begun using endorectal mucosal advancement flaps in selected Crohn's patients with encouraging results. In a retrospective study from the Cleveland Clinic, Ozuner et al.[19] reported on 101 patients undergoing advancement flap procedures including 47 with Crohn's disease. Overall recurrence was seen in 29 patients (29%); however, the etiology of the fistula did not statistically affect outcome. Failure was only influenced by previous number of repairs. In a smaller study of eight patients, six of whom had Crohn's disease, healing was achieved in six. Early recurrence occurred in one patient with acute Crohn's proctitis and in one patient with an idiopathic horseshoe track.[20]

In a larger series Kodner et al.[21] reported on 107 patients undergoing endorectal advancement flap over a 10-year period. The causes were obstetric injury in 48, cryptoglandular abscess fistula in 31, Crohn's disease in 24, and trauma in four. Complete primary healing was achieved in 90 patients (84%). However, of the 17 patients with persistent fistula, nine underwent successful secondary surgical correction (superficial fistulotomy or repeat advancement flap) for a final success rate of 94%. As with the previous reports, this technique worked well in patients with Crohn's disease. Seventeen (71%) of 24 patients healed after initial repair and 22 (92%) of 24 patients healed after more than one procedure.

Recently anorectal manometry was introduced in the evaluation of these patients in an attempt to determine the physiologic consequences of this procedure. Manometric evaluation in 10 patients both pre- and postoperatively found patients with cryptoglandular fistula-in-ano to have normal physiologic parameters and, most important, endorectal mucosal advancement did not statistically alter anorectal motor or sensory function in the postoperative period.[22] Other studies have reported similar results. In a series of 11 patients undergoing endorectal advancement flap for idiopathic, infralevator, transsphincteric fistulas, Finan[23] recorded successful healing in 10. One patient developed an abscess under the flap and the fistula was laid open in the standard manner. All patients remained continent. Additionally, pre- and postoperative manometric testing revealed no change in anal sensation, median maximal resting pressure, or the resting pressure profile of the anal canal.

CONCLUSION

Endorectal mucosal advancement flap is a safe, effective, and easily learned technique for the treatment of complex cryptoglandular fistula-in-ano. The advantages of this procedure include preservation of normal anorectal anatomy, avoidance of large perineal wounds, preservation of the anal sphincter mechanism, and avoidance of a diverting stoma. It can be performed with minimal morbidity, no deaths, and a

low recurrence rate. We believe that endorectal mucosal advancement flap should be considered the treatment of choice for complex cryptoglandular fistula-in-ano.

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Letters to the Editors

To the Editors:

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Once again, congratulations!

Michael J. Zinner, M.D.
Surgeon-in-chief
Brigham and Women's Hospital
Boston, Massachusetts

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