出國類別：進修

赴美國哈佛大學醫學院研習內分泌外科及內視鏡外科報告

服務機關：高雄榮民總醫院外科部
出國人　職稱：主治醫師
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出國地區：美國
出國期間：89 年 8 月 1 日 至 90 年 7 月 31 日
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報告名稱:

赴美國哈佛大學醫學院附設醫院從事「內分泌外科學之研究」

主辦機關:

行政院輔導會高雄榮民總醫院

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出國類別: 研究

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關鍵詞:　內分泌外科，內視鏡外科

內容摘要: 職於二零零零年八月一日奉准赴美國麻塞諸塞州波士頓市貝絲-以色列-狄可尼斯醫學中心(Beth-Israel-Deaconess Medical Center)及麻省總醫院(Massachusetts General Hospital)外科部一般外科進修，為期一年；這兩個醫院均為哈佛大學醫學院(Harvard Medical School)主要的綜合教學醫院，研習的科目為內分泌外科學及腹腔鏡外科學，臨床學習除參與手術外並以「影響腹腔鏡闌尾切除術轉成開腹手術的因素」(Factors Associated with Conversion to Laparotomy in Patients Undergoing Laparoscopic Appendectomy)為題，於2001年美國消化系外科醫學會年會(Annual Meeting of Surgical Society of Alimentary Tract, 2001, Atlanta)發表論文，此文並已獲美國外科醫學會雜誌(Journal of American College of Surgeons)接受，即將刊登(附件一)。在基礎研究方面，亦以「腸細胞對於缺血的反應與其分化狀態有關」(Enterocyte Response to Ischemia is Dependent on Differentiation State) 為題，於2001年消化系疾病週(Digestive Disease Week, 2001)發表研究成果(附件二)，這一年學習的過程庶可以指導教授Dr.Hodin為職撰寫的信函作一註腳(附件三)。

本文電子檔已上傳至出國報告資訊網

出國進修報告　　劉絜穎

摘要：

職於二零零零年八月一日奉准赴美國麻塞諸塞州波士頓市貝絲-以色列-狄可尼斯醫學中心(Beth-Israel-Deaconess Medical Center)及麻省總醫院(Massachusetts General Hospital)外科部一般外科進修，為期一年；這兩個醫院均為哈佛大學醫學院(Harvard Medical School)主要的綜合教學醫院，研習的科目為內分泌外科學及腹腔鏡外科學，臨床學習除參與手術外並以「影響腹腔鏡闌尾切除術轉成開腹手術的因素」(Factors Associated with Conversion to Laparotomy in Patients Undergoing Laparoscopic Appendectomy)為題，於 2001 年美國消化系外科醫學會年會(Annual Meeting of Surgical Society of Alimentary Tract, 2001, Atlanta)發表論文，此文並已獲美國外科醫學會雜誌(Journal of American College of Surgeons)接受，即將刊登(附件一)。在基礎研究方面，亦以「腸細胞對於缺血的反應與其分化狀態有關」(Enterocyte Response to Ischemia is Dependent on Differentiation State)為題，於2001年消化系疾病週(Digestive Disease Week, 2001)發表研究成果(附件二)，這一年學習的過程庶可以指導教授Dr.Hodin為職撰寫的信函作一註腳(附件三)。

目的：

內分泌外科學及腹腔鏡外科學研習。

過程：

職於二OOO年八月一日奉准赴美國麻塞諸塞州波士頓市貝絲-以色列-狄可尼斯醫學中心外科部一般外科進修，為期一年，貝絲-以色列-狄可尼斯醫學中心是哈佛大學醫學院數家主要的綜合教學醫院中之一，其他較著名的如麻省總醫院、波士頓兒童醫院、布里根及婦女醫院和麻省耳鼻喉科醫院均座落在波士頓市區中.與職同時在當地進修的還有本院小兒科陳珠瑾大夫在波士頓兒童醫院，放射科黃哲勳大夫在麻省總醫院，長庚牙科葉素嬌大夫在哈佛大學牙醫學院及彰化基督教醫院胸腔外科鄭清源大夫也同時在麻省總醫院進修，貝絲-以色列-狄可尼斯醫學中心以外科基礎研究著稱，每年有數量眾多且素質甚佳之文章發表在重要醫學期刊中，其一般外科擁有近十五位主治醫師，均具哈佛醫學院教職，每年單只一般外科向包括美國國家衛生署(NIH)及其他研究機構所申請的研究經費高達數百萬美金，因此科內的研究風氣非常興盛。我在台灣時依據本身的興趣及科內的需要申請至貝絲-以色列-狄可尼斯醫學中心進修，主要是因為其外科部副主任Hodin教授不僅是美國知名的內分泌外科及腹腔鏡外科專家，在腸道細胞對於人體各種賀爾蒙及其他外在刺激素所致的基因變化亦有卓越的成果，其文章曾見於包括「自然」及「科學」等世界一流期刊，在幾經E-mail與Hodin教授本人聯繫後，終於二OOO年七月底飛抵波士頓，並於八月一日正式參與醫院的工作，Hodin教授在第一天就交付我Research Project，主要是研究有關腹腔鏡闌尾切除病患手術失敗的原因，希望能就大規模病患資料的回顧找出影響手術結果的因素，藉以選擇合適的病患接受腹腔

鏡手術。這些病歷收集及整理分析的工作都得在閒暇的時間去完成、因為大部分的時間得與Hodin教授開刀及查房，在參與他們實際的工作後給我最深的感觸是他們工作之勤奮與專業實在是我們在台灣的醫護人員應該努力學習的，每天早上六點總醫師就會帶著所有住院醫師及實習醫師查房，而後主治醫師於七點開始查房、結束之後方開始一天的開刀或門診的工作。Hodin教授的手術以內分泌外科及腹腔鏡外科為主，非常幸運的他也准許職參與手術的進行擔任第一或第二助手，所以在這一年當中，職有機會參與非常多內視鏡甲狀腺切除、副甲狀腺切除、腹腔鏡膽囊切除、腎上腺切除、胰臟切除、脾臟切除、闌尾切除甚或腸道切除手術，就甲狀腺切除而言，大致與本國手術方式無甚差異，但是由於美國患者多早期就醫，所以手術進行起來較諸本國病患似乎是容易一些，但是在參與手術的過程職亦學習到與之前老師教導不同的手術技巧，例如甲狀腺上極(upper pole)血管的處理，上喉神經外分支的保存，副甲狀腺體的保存等均有新的體會，在副甲狀腺腫瘤及腺體增生的治療方面，則可以看到目前最新的治療方向：例如術前核子醫學試劑的定位合併小傷口或腹腔鏡輔助下的腫瘤摘除、術中副甲狀腺素濃度的測定以決定腫瘤是否完全摘除等等，均為目前治療副甲狀腺疾患的新方向，可惜的是在台灣該類核子醫學試劑未納入健保給付範圍、至於術中副甲狀腺素的測定則因本院所購置的儀器公司尚未提供可測定的模組，故仍無法進行。在腹腔鏡膽道手術方面，職則可以大膽的說本院在莫主任引進是項手術後的臨床經驗與美方相較毫不遜色，特別對於急性膽囊炎及總膽囊結石以腹腔鏡手術處理較諸他們更是不遑多讓，對於目前在本院較少以腹腔鏡手術處理之胰臟、脾臟及腎上腺疾病的處理，由於病患數量眾多，職則是學習到不易獲

致的技術、特別值得一提的是以腹腔鏡手術處理發炎性腸病變(Inflammatory bowel disease)所造成之腸阻塞等併發症，Hodin教授採用Pfannestiel切口，不僅傷口亦美觀，亦能加速病患術後之恢復，值得引進採用於其他腸道疾病之病患。除了平日上刀手術外，職便以工作之餘時間收集病歷完成Hodin教授所交付之任務、在前六個月的時間，職一共收集整理了七百多位因急性闌尾炎至貝絲-以色列-狄可尼斯醫學中心就診的病患的病歷資料、其中有595位乃以腹腔鏡執行闌尾切除，為了解造成手術失敗的原因、將所有相關的病患特性、臨床症狀、實驗室數據、放射線診斷發現及手術醫師因素收集齊全後進行單因子及多重變異分析，結果我們發現病患年紀、是否有腹膜炎症狀、電腦斷層檢查之嚴重程度及醫師經驗乃是影響手術成敗的因素，藉由這樣的發現，嗣後將可在術前篩檢適宜的病患進行腹腔鏡手術，不僅可以降低手術失敗的比例，更可減少醫院因腹腔鏡手術不成須開腹所造成之開刀房使用時間的浪費、器材的耗損及人員工作時間的延長.有助於降低醫院支出的耗損。這項研究在經過整理之後，職於2001年5月赴美國喬治亞州亞特蘭大市美國消化系外科醫學會中報告，之後更將之撰成文章投稿於美國外科醫學會雜誌，日前已獲接受，即將刊出。於2001年四月，Hodin教授轉赴麻省總醫院任職，擔任腸胃道外科主任，於是職亦跟隨他轉至麻省總醫院，如大家所知麻省總醫院乃全美歷史最久.最負盛名的醫學中心，職在轉至麻省總醫院之後主要的工作是在Hodin教授的實驗室中進行腸道細胞對不同的刺激因子基因所產生的變化，Hodin教授較早期的研究重點在於甲狀腺素對腸道細胞的作用，當時的研究成果見於「自然」、「科學」等世界頂尖的雜誌，晚近的興趣則轉至腸缺血或其他細胞間素(cytokines)對基因的影響、職的工作主要是

以細胞培養、核糖核酸萃取及北方墨點檢視發炎性腸病變時体內會上升之細胞間素及缺血對人類腸癌細胞株分化或生長基因的影響，初步的成果包括發現腸細胞對於缺血之反應與其分化狀態有關，這也顛覆了過去認為腸細胞的凋亡純粹是因缺血所引起的想法，有關這方面的研究也於今年5月於美國消化系醫學週中發表，此外有關細胞間素對腸細胞分化狀態的影響的研究方面，初步發現Interferon-gamma可促進腸細胞的分化，這個發現目前亦由Hodin教授的實驗室進一步探究其機轉，期望能對腸細胞的分化與生長能有更深一層的了解。在經過一年的異國生涯，職終於2001年七月底離開波士頓返回台灣，Hodin教授在職要離開之前為職撰寫的信函正可為此一年的進修作出最後的註腳。

心得：

在這一年當中，職有機會參與非常多內視鏡甲狀腺切除、內視鏡副甲狀腺切除及腹腔鏡膽囊切除、腎上腺切除、胰臟切除、脾臟切除、闌尾切除甚或腸道切除手術。就傳統甲狀腺切除而言，大致與本國手術方式無甚差異，但是由於美國患者多早期就醫，所以手術進行起來較諸本國病患似乎是容易一些，但是在參與手術的過程職亦學習到與之前老師教導不同的手術技巧，例如甲狀腺上極(upper pole)血管的處理，上喉神經外分支的保存，副甲狀腺體的保存等均有新的體會，在貝絲-以色列-狄可尼斯醫學中心除了傳統甲狀腺切除之外，亦從事頗多內視鏡甲狀腺切除，該院所採取的是無氣式的內視鏡甲狀腺切除，這樣的方法已經甚多其他醫學中心採用，成為內視鏡甲狀腺切除的主流方法，職在美所學習的經驗亦已在本院開始實施，希望來日可在某些

良性病患中取代傳統甲狀腺切除；在副甲狀腺腫瘤及腺體增生的治療方面，則可以看到目前最新的治療方向，例如術前核子醫學試劑的定位合併小傷口或腹腔鏡輔助下的腫瘤摘除、術中副甲狀腺素濃度的測定以決定腫瘤是否完全摘除及內視鏡副甲狀腺切除等等，均為目前治療副甲狀腺疾患的新方向，可惜的是在台灣該類核子醫學試劑未納入健保給付範圍、至於術中副甲狀腺素的測定則因本院所購置的儀器公司尚未提供可測定的模組，故仍無法進行，至於內視鏡副甲狀腺切除對於單一腫瘤為一可行的方法，如遇適當病例將可一試。在腹腔鏡膽道手術方面，本院經驗豐富，與美方相較並無遜色之處，特別對於急性膽囊炎及總膽囊結石以腹腔鏡手術處理較諸他們更是不遑多讓；對於目前在本院較少以腹腔鏡手術處理之胰臟、脾臟及腎上腺疾病的處理，由於病患數量眾多，職則是學習到不易獲致的技術；特別值的一提的是以腹腔鏡手術處理發炎性腸病變(Inflammatory bowel disease)所造成之腸阻塞等併發症，Hodin教授採用Pfnestiel切口，不僅傷口亦美觀，亦能加速病患術後之恢復，值得引進採用於其他腸道疾病之病患。在腹腔鏡闌尾切除術方面、根據職所發表的論文，嗣後將可在術前篩檢適宜的病患進行腹腔鏡手術，不僅可以降低手術失敗的比例，更可減少醫院因腹腔鏡手術不成須開腹所造成之開刀房使用時間的浪費、器材的耗損及人員工作時間的延長.有助於降低醫院支出的耗損。

建議：

一、研習醫院最好能與本院有相當程度的聯繫.如此可使得研習對方能重視我方派去人員，並且事先就研習項目做一完整規劃，俾能以最少時間獲致最大成效。

二、研習時間應有彈性，某些科目可能僅需幾個月時間，若欲從事一完整的基礎研究、則可能需數年的時間始能克盡其功。如職所從事之基礎研究僅能完成整體計劃之一部份，故僅能有初步的報告，較為可惜。

Factors Associated with Conversion to Laparotomy in Patients Undergoing Laparoscopic Appendectomy

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We certify that the authors have no commercial, proprietary, or financial interests in the products and companies described in this article.

Brief title: Conversion in Laparoscopic Appendectomy

ABSTRACT:

Background: Laparoscopic appendectomy (LA) has been increasingly adopted for its advantages over the open technique, but there is a possibility of conversion to open appendectomy (OA) if complications occur or the extent of inflammation prohibits successful dissection. This study is aimed to identify the preoperative predictors for conversion from laparoscopic to open appendectomy.

Study Design: Medical records of 705 consecutive patients with suspected appendicitis were reviewed retrospectively. LA was attempted in 595 patients by 25 different surgeons. Factors evaluated were age, sex, body mass index, previous abdominal surgery, previous appendicitis attack, pain, nausea, vomiting, fever, duration of symptoms, local or diffuse tenderness, leukocyte count and surgeon's experience in LA.

Results: Conversion to OA occurred in 58 patients (9.7%). The most common reason for conversion was dense adhesions due to inflammation, followed by localized perforation and diffuse peritonitis. Significant factors associated with conversion to OA were age ≥ 65 (Odds ratio 3.78, 95% CI of 1.11-12.84), diffuse tenderness on physical examination (Odds ratio 11.32, 95% CI of 1.32-96.62), and a surgeon with less experience in LA (≤10 operations, odds ratio 3.38 and 95% CI of 1.02-11.17). In 261 patients evaluated by CT scan preoperatively, the presence of significant fat stranding associated with fluid accumulation, inflammatory mass or localized abscess also significantly increased the possibility of conversion (Odds ratio 5.60, 95% CI of 2.48-12.65).

Conclusions: Identifying the potential factors for conversion preoperatively will assist

the surgeons in making decisions concerning the management of patients with appendicitis and in the judicious use of LA.

KEYWORDS: Appendicitis

Laparoscopy

Computerized Tomography

INTRODUCTION:

Open appendectomy (OA) has been the standard operation for the treatment of acute appendicitis for more than a century (1-2). It is generally a simple operation with low morbidity and a near zero mortality (3). Excellent results have been reported employing early operative intervention, perioperative antibiotics, and a systematic surgical approach (4). Since first introduced in 1983 (5), laparoscopic appendectomy (LA) has become an increasingly popular treatment modality (6). Although a clear consensus favoring laparoscopic appendectomy has not been established (7-8), meta-analyses of randomized, controlled trials suggest that laparoscopic appendectomy has several distinct advantages over the open technique, including less postoperative pain, shortened hospital stay, faster recovery and lower wound infection rates (9-11). In addition, the cosmetic satisfaction is higher for laparoscopic patients (12-13). However, in many series, laparoscopic appendectomy has been associated with longer operative times and higher cost (11-13).

Laparoscopic appendectomy may need to be converted to open appendectomy if intraoperative complications arise or if the severity of disease prohibits a safe laparoscopic dissection. Conversion from laparoscopic to open appendectomy incurs the added costs of equipment and operative time, along with losing the benefits of the laparoscopic approach. Therefore, it may be helpful to develop preoperative criteria that can be used to decide the ideal operative approach for individual patients. The present study was designed to evaluate preoperative indicators that may prove useful in predicting conversion from laparoscopic to open appendectomy.

PATIENTS AND METHODS:

Between July 1996 and June 2000, 705 consecutive patients underwent operation for appendicitis at the Beth Israel Deaconess Medical Center, Boston, Mass. Hospital records were reviewed retrospectively. Open appendectomy (OA) was performed in 110 patients (16%). Laparoscopic appendectomy (LA) was attempted in 595 patients (84%), and only those patients were included in the analysis.

The following factors were analyzed to determine an association with the need for conversion from LA to OA: age, gender, body mass index (BMI), history of abdominal surgery, previous appendicitis attack, pain, nausea, vomiting, T_{max} (highest preoperative body temperature), duration of symptoms, local or diffuse tenderness on physical examination, total leukocyte count, and surgeon. For the purpose of this study, age was categorized as dichotomous a variable, and patients younger than age 65 years were compared with those age 65 years or older. Patients with BMI of 30 or more were categorized as "obese" (14-15) versus BMI of less than 30. History of abdominal surgery was categorized as "none" (including patients who had previous inguinal hernia repairs) versus any intra-abdominal procedure. Patients with T_{max} higher than 37.5°C were considered to have fever. The duration of symptoms was categorized into "≥5 days", "<5 days", and "no symptoms" for patients admitted for interval appendectomy. Patients with total leukocyte count of higher than 20000/mm^3 were compared to patients with lower leukocyte count. In this study period, laparoscopic appendectomies were performed by 25 different surgeons. Surgeons performing 10 or fewer laparoscopic appendectomies during the study period were compared to surgeons performing more than 10 LAs.

Statistic analyses were performed by SPSS statistical software (SPSS Inc., Chicago, Illinois). Data are presented as a proportion or as the mean ± SD. Student's t test was applied when appropriate. A p value of < 0.05 was considered statistically significant. Univariate analysis was first performed using Pearson's chi-square test to determine which clinical predictors were significantly associated with conversion from LA to OA. The value and 95% confidence interval (CI) of the odds ratio were obtained. Fisher's exact test was computed when a table had a cell with an expected frequency of less than 5. From 14 clinical variables, those identified of potential significance from the univariate analysis were chosen for forward stepwise logistic regression. Of the 595 patients, data for leukocyte count were not available in 13 patients. To evaluate whether deletion of those patients with incomplete data would result in bias, univariate analysis was repeated in 582 patients with complete data, and the result was compared with that of the entire patient population. Each predictor was also examined by comparison between patients with missing data and patients with complete data. These tests suggested that deleting 13 patients with missing data would not significantly change the result of subsequent multiple stepwise logistic regression. Therefore, 582 patients with complete data remained for multivariate analysis. Correlations between individual predictors were also evaluated before subsequent analysis. Multivariate stepwise logistic regression was then conducted, and independent predictors identified. This model was further validated in both the subgroups of patients with (n=261) or without (n=321) preoperative CT evaluation. Finally, patients with preoperative CT evaluation were used to establish the complete logistic model with CT grading as well as clinical predictors included. CT scans were done with a single detector spiral CT prior to Jan.

1999. After Jan. 1999, scans were done on multidetector scanner (High Speed Advantage and Qxi/Lightspeed, GE Medical Systems, Milwakee, WI). Intravenous and oral contrast material were given as a routine preparation protocol (16).

RESULTS:

Laparoscopic appendectomy was attempted in 595 patients (329 females and 266 males) known or suspected to have appendicitis. The average age of patients was 34±13 years with a range from 16 to 91 years. Final diagnosis, as confirmed at surgery and by pathological examination, and the incidence of conversion are shown in Table 1. 507 patients (85.2%) had a final diagnosis of appendicitis, 53 of which (10.5%) were perforated. The incidence of conversion from LA to OA was much higher ($p<0.05$) in patients with perforated appendicitis (26.4%) as compared to patients with non-perforated appendicitis (8.1%). Appendiceal tumor was found in 5 patients, including 3 mucinous cystadenoma, 1 adenocarcinoma and 1 carcinoid; 3 of these cases were converted. Both patients with intestinal malrotation were converted due to the unexpected operative findings. One of 46 patients with no specific cause of abdominal pain and one of 4 patients with focal fat necrosis were converted due to uncontrollable hemorrhage during dissection of mesoappendix and pericecal fatty tissue respectively. The overall conversion rate was 9.7%.

Table 2 summarizes the reasons for conversion from LA to OA. The most common reason for conversion was dense adhesions due to acute inflammation (36.2%), followed by localized perforation (12.1%) and diffuse peritonitis (10.3%). Five conversions (8.6%) occurred because of severe inflammation or necrosis at the appendiceal base, prohibiting safe application of the laparoscopic stapler. Four patients had to be converted due to bleeding from an abdominal wall vessel, appendiceal artery or periappendiceal fatty tissue. In three patients with appendiceal tumor, the operations were converted. Only one of them had the tumor identified intraoperatively, and the

other two patients were converted to OA for localized perforation and base inflammation, respectively. One patient had only part of his inflamed appendix removed laparoscopically and the operation was converted to remove the remaining appendiceal base. Thermal injury of small bowel adhered to the inflamed appendix occurred in one patient. The perforation was identified intraoperatively and was repaired in an open fashion. Other reasons noted for conversion included retrocecal position of appendix, inability to identify the appendix, suspected malrotation of the intestine, inability to maintain an adequate pneumoperitoneum, and hypotension due to Trendelenburg's position.

The operation time for patients converted to OA was 114±47 minutes, whereas those patients successfully treated laparoscopically had an operation time of 62±24 minutes ($p<0.01$). The post-operative hospital stay was also significant higher for converted patients (4.3±3.3 vs. 1.6±1.7 days, $p<0.01$). Complications occurred in 40 (6.7%) patients, including post-operative ileus (12), intra-abdominal abscess (12), pneumonia (3), wound infection (3), intraperitoneal bleeding/hematoma (4), wound dehiscence (1), pulmonary edema (1), acute renal failure (1), sepsis (1), trocar site bleeding (1) and intestinal obstruction (1). Patients converted to OA had a higher complication rate compared to the laparoscopic cases (20.7% vs. 5.2%, $p<0.01$). There was no mortality in this series.

In univariate analysis (Table 3), three statistically significant factors for conversion ($p<0.05$) were identified: age≥65, presence of diffuse tenderness on preoperative exam, and surgeon's inexperience (performing 10 or fewer LAs). The factors "duration of symptoms" ($p=0.067$) and "fever" ($p=0.077$) were also selected to avoid excluding

potentially important factors in initial screening. Correlation analysis showed that there was no significant association between those 5 potentially important factors, and therefore they were all included in further multivariate analysis. Multiple stepwise logistic regression was next performed to evaluate those potentially important predictors. Criteria of entry and removal of predictors were p value of 0.05 and 0.1, respectively. The results showed that age≥65 (Odds ratio= 5.79, CI= 2.19 to 15.33), presence of diffuse tenderness (Odds ratio= 10.56, CI= 2.46 to 45.34) and surgeons with less experience in LA (Odds ratio= 4.97, CI= 2.16 to 11.43) were significantly associated with higher risk of conversion to OA. Factors excluded in multivariate logistic regression were "duration of symptoms" and "fever" (p>0.1).

In this study, 261 patients (44%) were evaluated by CT scan preoperatively. The decision to perform a CT scan was made by the evaluating physician (either ER doctor or Surgeon). The findings based on the entire 582 patients by initial multivariate analysis were validated in the subgroup of patients in whom CT scan was performed preoperatively (n=261) and those without preoperative CT scan (n=321). In stepwise logistic regressions, both subgroups of patients yielded exactly the same three independent predictors as found in full patient population. This result suggests that there are no differences in clinical characteristics between these two subgroups of patients. It also allowed subsequent analysis, in which the CT grading was included as an independent variable to predicting outcome, using only the subgroup of patients with CT scan.

Patients with preoperative CT scans (n=261) were analyzed by stepwise logistic regression. Two radiologists who were not aware of any clinical information or whether

the operations were converted performed the assessment of CT grading retrospectively. The CT finding was graded (17) as 0 if there was no abnormality (n=18), as 1 if question abnormal finding such as slightly thickened appendix with or without enhanced wall was visualized (n=7), as 2 if a thicken appendix with greatest transverse diameter larger than 6 mm was found (n=56), as 3 if there was periappendiceal fat stranding (n=130), as 4 if the periappendiceal fat stranding was associated with fluid accumulation (n=32), or as 5 if there was an inflammatory mass or abscess (n=18). The conversion rate in patients with CT grades equal or greater than 4 was significant higher than that in patients with lower CT grades (32% vs. 14.8%, $p<0.001$). In the stepwise selection of significant predictors, the difference of –2LogLikelihood values between models with 4 parameters (CT grade included) and 3 parameters (CT grade not included) was found to be 16.61 with a p value of less than 0.001. This result suggests CT grading significantly improved the prediction for conversion from LA to OA and, therefore, was included in the final model of this study. The four significant independent predictors for conversion identified in the final model are summarized in Table 4. The Hosmer-Lemeshow Goodness-of-Fit test (18) statistics is 2.09 (degree of freedom=2) with p value of 0.35, indicating that the observed and expected frequencies by decile are not significantly different.

Using the final logistic model, patients can be categorized into 16 groups based on the presence or absence of the 4 predictors. The predicted probabilities of conversion to OA of the 16 groups are shown in Table 5. Patients without any of the 4 predictors had a probability of conversion of 5.6%, while patients with all 4 predictors had a 98.0% probability of conversion.

DISCUSSION:

The recent development of laparoscopic techniques and improvements in operative instrumentation have made laparoscopic cholecystectomy the procedure of choice in the management of gallstone disease (19-20). Although the current response to laparoscopic appendectomy has not been uniformly favorable (21-22), comparative studies (23-24) and meta-analyses of randomized controlled studies (9-11) have found that laparoscopic appendectomy has several advantages over the open technique, including reduced postoperative pain, fewer wound infections, and shorter convalescence. In some patients, the cosmetic benefit is a strong reason to choose laparoscopic rather than open appendectomy. The only disadvantages of laparoscopic appendectomy are a longer operative time (in some series) and the use of disposable products, which may result in an increased overall cost (11, 21).

The rates of conversion reported in the literature are variable (23, 25-30) and may be attributed to a variety of patient, surgeon, and technical factors. Based upon this study and others, it appears that the conversion to the open technique lengthens the operative time and leads to even higher hospital cost. Therefore, understanding the factors that are associated with increased likelihood of conversion may be helpful not only for patients to have a better informed decision about their operation, but also for the surgeons to appropriately select patients for laparoscopic appendectomy.

The 9.7% conversion rate in this study is in accordance with other published studies (23, 26, 28-30), although lower conversion rates (0 to 3.3%) have been reported (27, 31-32). All of these retrospective studies are subject to selection bias in regard to which patients initially undergo an attempt at laparoscopy. In this series, 25 surgeons

were involved in the treatment of patients with appendicitis. We were not able to differentiate the rates of conversion between those surgeons who attempt laparoscopic appendectomy for all patients and those surgeons with highly selective criteria for laparoscopic appendectomy. Interestingly, however, we did find that surgeons performing fewer laparoscopic appendectomies had a higher incidence of conversion to open appendectomy. As such, it is possible that increasing experience with laparoscopic appendectomy will lead to lower conversion rates.

Of the clinical parameters evaluated, age of 65 and older was found to be an independent predictor of conversion. Elderly patients often present with unusual, non-classical symptoms and signs (33), and have delayed surgical intervention, perhaps explaining the increased risk of conversion in these patients.

Although patients with diffuse peritonitis on physical examination constituted only a small percentage (1.3%) of the study population, multivariate analysis indicated that these patients carried a high risk (odds ratio=11.32) of conversion. Conversions to laparotomy in these patients were often made in the early phase of the operation, suggesting that surgeons were concerned that incomplete irrigation laparoscopically could result in post-operative abscess formation. In the future, with increasing experience and better suction-irrigation instruments, some of those operations may be completed laparoscopically.

In a study of laparoscopic cholecystectomy, obesity was reported to be a moderate predictor of conversion to open cholecystectomy (34). In our study, however, obese patients with a BMI of 30 or higher showed no increased risk of conversion to open appendectomy. It is possible that the availability of longer trocars and increasing

practice of open insertion of the umbilical port have overcome the problems rendered by obese body habitus. Other clinical factors not associated with increased risk of conversion are gender, previous abdominal operation, previous attack of appendicitis, RLQ pain, nausea, vomiting, fever, local tenderness on physical examination, duration of symptoms and total leukocyte count.

During the last decade, CT scan has become increasingly used as a tool for the diagnosis of acute appendicitis (35). The most common findings of acute appendicitis on CT included pericecal inflammation, mesenteric fat stranding, visualization of an abnormal appendix and free fluid (36). A definitive CT diagnosis of acute appendicitis can usually be made when an abnormal appendix is identified or if a calcified appendicolith is seen in association with pericecal inflammation (37). Although interpretation of CT might not necessarily correlate with pathologic findings (38), perforated appendicitis is usually accompanied by significant pericecal phlegmon or abscess formation. Therefore, it is not surprising that the patients with CT grade 4 or 5 in our series were found to be an independent predictor of conversion.

The present study has identified four independent predictors of conversion: older age (≥65), diffuse peritonitis on physical examination, surgeons with less experience in laparoscopic appendectomy, and extensive abnormalities on preoperative CT scan. The overall goodness-of-fit of this model have been verified by Hosmer-Lemeshow test. As shown in Table 5, patients with none of the four predictors had a probability of conversion of 5.6%, and a combination of those four predictors increases the likelihood of failed laparoscopic appendectomy, patients with all four predictors carrying a 98.0% probability of conversion. This stratification may allow adequate discrimination

between patients with high and low risk of conversion, but the validity of the proposed model will need to be tested in a prospective manner.

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TABLE 1. Final Diagnosis and Incidence of Conversion in 595 Patients Suspected to Have Appendicitis

Final Diagnosis	Number of Patients	Conversion to OA (%)
Non-perforated appendicitis	454	37 (8.1%)
Perforated appendicitis	53	14 (26.4%)
Nonspecific abdominal pain	46	1 (2.2%)
Rupture or torsion of tubo-ovarian cyst	14	0 (0)
Tumor of appendix	5	3 (60%)
Diverticulitis of Colon	4	0 (0)
Endometriosis	4	0 (0)
Focal fat necrosis or hemorrhage	4	1 (25%)
Omental Infarction	3	0 (0)
Retrograde menorrhagea	3	0 (0)
Pelvic Inflammatory Disease	2	0 (0)
Malrotation	2	2 (100%)
Uterine fibroid with necrosis	1	0 (0)
Total	595	58 (9.7%)

Table 2. Reasons for Conversion from LA to OA

Reason	No. of Patients
Dense adhesion due to inflammation	21(36.2%)
Localized perforation	7 (12.1%)
Diffuse peritonitis	6 (10.3%)
Base inflammation or necrosis	5 (8.6%)
Retrocecal position	5 (8.6%)
Bleeding	4 (6.9%)
Unable to visualize appendix	3 (5.2%)
Unexpected diagnosis	2 (3.4%)
Tumor of appendix	1 (1.7%)
Remove only part of appendix in LA	1 (1.7%)
Bowel injury	1 (1.7%)
Unable to maintain adequate pneumoperitoneum	1 (1.7%)
Hypotension due to Trendelenburg's position	1 (1.7%)
Total	58 (100%)

Table 3. Clinical Predictors for Conversion to OA in Univariate Analysis

Predictor	No. of Patients	No. and % of Conversions	Odds ratio (95% CI)	p
Age				
≥ 65	21	7 (33.3%)	5.13 (1.98-13.28)	0.002
< 65	574	51 (8.9%)		
Gender				
Male	266	29 (10.9%)	1.27 (0.74-2.18)	0.393
Female	329	29 (8.8%)		
BMI				
≥ 30	83	9 (10.8%)	1.14 (0.54-2.44)	0.717
<30	512	49 (9.6%)		
Previous operations				
Yes	86	10 (11.6%)	1.26 (0.61-2.61)	0.525
No	509	48 (9.4%)		
Previous appendicitis				
Yes	50	3 (6.0%)	0.57 (0.17-1.89)	0.460
No	545	55 (10.1%)		
RLQ pain				
Yes	566	56 (9.9%)	1.48 (0.34-6.40)	1.000
No	29	2 (6.9%)		

Fever				
Yes	263	32 (12.2%)	1.63 (0.95-2.81)	0.077
No	332	26 (7.8%)		
Nausea				
Yes	362	40 (11.0%)	1.48 (0.83-2.66)	0.182
No	233	18 (7.7%)		
Vomiting				
Yes	189	19 (10.1%)	1.05 (0.59-1.87)	0.864
No	406	39 (9.6%)		
Duration of symptoms*				
≥ 5 days	35	7 (20.0%)		0.067
< 5 days	518	49 (9.5%)		
No symptom	42	2 (4.8%)		
Local tenderness				
Yes	514	52 (10.1%)	1.41 (0.58-3.39)	0.445
No	81	6 (7.4%)		
Diffuse tenderness				
Yes	8	4 (50.0%)	9.87(2.40-40.59)	0.004
No	587	54 (9.2%)		
WBC >20000†				
Yes	36	2 (5.6%)	0.51 (0.12-2.20)	0.565
No	546	56 (10.3%)		

Surgeon				
< 10 LA	32	10 (31.3%)	4.88(2.18-10.90)	<0.001
> 10LA	563	48 (8.5%)		

* Patients underwent interval appendectomy were categorized as having "no symptom"

Odds ratio was not calculated for parameters with 3 or more levels.

†White blood cell count available in only 582 patients.

Table 4. Significant Predictors for Conversion to OA in Final Multivariate Analysis

Predictor	Odds ratio	95% CI	p
Age ≥ 65 years	3.78	1.11-12.84	0.033
Diffuse tenderness	11.32	1.32-96.62	0.027
Surgeons with ≤ 10 LA	3.38	1.02-11.17	0.046
CT grade ≥ 4	5.60	2.48-12.65	<0.001

Table 5. The Predictive Risks of Conversion to OA Based on the Logistic Regression Model

Age ≥ 65	Diffuse tenderness	Surgeons ≤ 10 LA	CT grade ≥ 4	Predictive Risk (%)
No	No	No	No	5.6
Yes	No	No	No	18.4
No	Yes	No	No	40.3
No	No	Yes	No	16.8
No	No	No	Yes	25.0
Yes	Yes	No	No	71.9
Yes	No	Yes	No	43.3
Yes	No	No	Yes	55.8
No	Yes	Yes	No	69.5
No	Yes	No	Yes	79.1
No	No	Yes	Yes	53.0
Yes	Yes	Yes	No	89.6
Yes	Yes	No	Yes	93.4
Yes	No	Yes	Yes	81.0
No	Yes	Yes	Yes	92.7
Yes	Yes	Yes	Yes	98.0

附件二.

ENTEROCYTE RESPONSE TO ISCHEMIA IS DEPENDENT ON DIFFERENTIATION STATE

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ABSTRACT

Enterocytes at the tips of microvilli are more sensitive to an ischemic insult than those cells residing in the crypts, an effect thought to be due to a relative lack of collateral flow. We speculated that this increased cellular, sensitivity to ischemia may be an intrinsic feature of the cells related to their differentiated phenotype. In order to test this hypothesis, enterocyte response to ischemia was determined using both in-vivo and in vitro models. For the in-vivo studies, male Sprague-Dawley rats underwent laparotomy and small intestinal ischemia was induced by clamping the superior mesenteric artery (SMA) for 30 or 60 minutes after which reperfusion was allowed for various time points up to four days. Injury was assessed histologically as well as with northern blots probing for the enterocyte differentiation markers intestinal alkaline phosphatase (IAP) and lactase, as well as the gut-epithelial marker villin. The mucosal changes consistent with ischemia/reperfusion injury were evident: a rapid inflammatory response followed by progressive villus cell loss beginning at the tips and progressing to the crypts, depending on the degree of insult, with an eventual return to normal microanatomy. IAP and lactase were lost immediately after ischemia and returned with reperfusion, confirming that the differentiated cells are particularly sensitive to ischemic injury. The in-vitro studies employed two separate models of enterocyte differentiation: sodium butyrate (NB) treated HT-29 cells and Caco-2 cells maintained for 7 days post-confluence. In both models, undifferentiated and differentiated cells were subjected to treatment with 2-deoxyglucose and oligomycin-A (in-vitro model of ischemia) and apoptosis was assessed by FACS analysis. Differentiation of both cell lines resulted in a significantly greater apoptotic response to ischemia compared to undifferentiated cells exposed to an identical insult. We conclude that differentiated enterocytes may be inherently more sensitive to ischemia-induced injury than their undifferentiated counterparts. These findings call into question the popularly held belief that villus tip cells are more susceptible to ischemia because of their location relative to the microvascular anatomy.

Keywords: Small intestine, ischemia, apoptosis, differentiation.

INTRODUCTION

The small intestinal lumen is lined by a simple columnar epithelium which is continually regenerated and has a turnover time of three to six days (1). This constant renewal of the gut mucosa is critical for the maintenance of its structural and functional integrity. The rapid proliferation and replacement of sloughed cells is accomplished by the coordinated and highly regulated processes of replication, migration, differentiation and apoptosis (2,3). A population of rapidly dividing pluripotent stem cells located in the crypts of Leiberkuhn give rise to four distinct cell lineages, of which the enterocyte is the most common, comprising ~ 95% of the overall small bowel epithelial population (4). As these stem cells divide, they migrate upwar along the crypt-villous axis, withdraw from

the cell cycle, differentiate and eventually undergo apoptosis and are sloughed into the lumen (5). The mechanisms which govern this highly ordered progression from rapidly dividing pluripotent stem cell to terminally differentiated, apoptotic cell are not well understood, but are clearly innate characteristics of the intestinal epithelium which can be modified by a variety of physiologic and pathophysiologic stimuli(6-8).

Gut ischemia is commonly observed in several clinical settings, among which are acute occlusive or low flow states, transient intestinal ischemia associated with vascular bypass procedures requiring cross clamping.of the aorta and trauma patients being resuscitated from hemorrhagic shock. It is known that ischemia-induced injury to the gut begins in the mucosa and progresses outwardly to the serosa (9,10).

The cells at the tips of the villi are particularly sensitive to an ischemic insult, an observation thought to be the result of their location at the end distribution of a central arteriole which leads to a lower oxygen tension compared to the crypt (11). The gut epithelium has been shown to respond to ischemia by undergoing apoptosis rather than necrosis (12-14). Since, under normal conditions, differentiated enterocytes (or villus cells) are programmed to undergo apoptosis and these same cells are those which are most sensitive to an ischemic insult, we speculated that their increased sensitivity to ischemia may be an intrinsic property of the cells related to their differentiation state.
The present studies confirm the hyper-sensitivity of the villus enterocytes to an in-vivo ischemic insult. Furthermore, using in-vitro models of enterocyte differentiation we show that, in the complete absence of a microcirculation,differentiated gut epithelial cells are more likely to undergo ischemia-induced apontosis. These results suggest that villous tip cells are more sensitive to ischemia than their counterparts in the crypts as a result, at least in part, of their more differentiated phenotype, calling into question the popularly held belief that their increased sensitivity results from their location at the end distribution of the microvasculature.

MATERIALS AND METHODS

In-vivo:
Male Sprague-Dawley rats (200 - 250 gins) were obtained from Charles River (Wilmington, MA) and maintained on a standard chow diet with water ad libitum in accordance with the institutional guidelines set forth by the animal welfare committee of the Beth Israel Deaconess Medical Center. The animal were fasted with water ad libitum for one day prior to experiment. Anaesthesia was induced with an IP injection of pentobarbital (40mg/kg). After assuring an adequate level of anesthesia, laparotomy was performed and the superior mesenteric artery (SMA) occluded with a microvascular clamp (George Tiemann and Co., Happauge, NY) for a period of 30 or 60 minutes, after which the clamp was removed (time zero) and the incision closed with simple interupted 3 - 0 silk sutures. The animals were then allowed to recover and subsequently maintained on a standard chow diet with water ad libitum. At various time points, the animals were sacrificed with pentobarbital overdose (80mg/kg) and the small bowel harvested and fixed in 37% formaldehyde for histology or processed for northern blot analyses.
Histology: Segments of rat small intestine were imbedded in paraffin wax and then serially sectioned, fixed and stained with hematoxylin and eosin following standard histologic protocols. The crypt-villus height was determined by an independent observer in control and post-ischemia groups (n=10) from randomly selected sections (double blind design) at 100x using a calibrated ocular-grid eyepiece (Nikon, Tokyo, Japan).

Northern Blots:

Total RNA was extracted from rat small intestine using the guanidium thiocyanate method (15). Northern blot analyses were performed by loading 20ug of RNA per lane on an agarose-formaldehyde gel, separating by electrophoresis, transferring onto nitrocellulose membranes and baking for 2 hours at 80°C. Equal loading of RNA per lane was confirmed by examination of ethidium bromide stained gels. Complimentary DNA probes were 32^{P}-radiolabled to a specific activity of approximately $5x10^{8}$ cpm6gg DNA according to the technique of Feinberg and Vogelstein (16). The intestinal alkaline phosphatase (IAP) probe is a 1.9 kb PstI fragment derived from the human IAP cDNA (17) and the villin probe is a 530 bp fragment from the human cDNA and was provided by Dr. M. Arpin (18). The lactase probe is a 1.8 kb EcoRI/PstI fragment derived from the rat cDNA and was provided by Dr. Richard Grand (19). The actin probe is a 1.0 kb PstI fragment derived from the mouse actin cDNA (20). Hybridizations were carried out in 5x saline sodium citrate (1X SSC = 3M NaCl, 0.3M NaCitrate)/50% formamide/1% sodium dodecyl sulfate (SDS) (Sigma) at 42oC. The washing conditions were 2x SSC/ 0.1% SDS at 50°C. Cell Culture: HT-29 and Caco-2 cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA) and maintained in standard dulbeccos's modified eagles's media (Gibco BRL, Rockville, MD) with 10% fetal bovine serum (FBS), 2 mM glutamine and 100U/ml penicillin-streptomycin (Bio-Whittaker, Walkersville, MD) at 37oC and 5% C02. Cells were passaged and experiments performed at 70% confluence except, in the case of the post-confluent studies as indicated. Media was changed every three days and just prior to each experiment. Differentiation was induced in the HT-29 cells by adding sodium butyrate (NB, Siam, St. Louis, MO) to the media to a final concentration of 5 mM for 24 hours and in the Caco-2 cells by maintaining them for 7 days after they had reached confluence.

In-vitro Ischemia:

Cells were washed once with high-phosphate buffered ringers solution containing glucose (HPBR +, 5mM HEPES, 5mM KCI, 3.33mM NaH2P04, 0.83mM Na2HP04, 1mM MgC12, 135mM NaCl, lmM CaC12, lOmM Glucose, Sigma) and then incubated in HPBR+ for 30 minutes at 370C and 5% C02 after which they were washed once with high-phosphate buffered ringers solution without glucose (HPBR-) and divided into two treatment groups: non-ischemic controls and ischemic cells. The ischemia group was incubated with lOmM 2-deoxyglucoseand 1M oligomycin-A (Sigma) in HPBR- (OLI/DOG cocktail) for various time points while the control groups were incubated in HPBR+ for the same time periods, with all incubations at 37oC and 5% C02. This ischemia protocol has previously been shown to result in marked but reversible ATP depletion (21). After treatment, both groups of cells were washed three times in phosphate buffered saline solution (PBS, pH 7.4, Gibco) and returned to standard media until harvest for FACS analysis.

Apoptosis analysis:

Cells were seeded at a density of 1x 10^{6} per well in 6-well cluster plates (Becton-Dickinson, Franklin Lakes, NJ) and chemically-induced ischemia was administered as indicated. The cells were then returned to standard media for 24 hours after which they were collected

in trypsin (Bio-Whittaker), washed with PBS (pH 7.4) and fixed in 70% ethanol (Aaper, Shelbyville, KY) overnight. The following day, the cells were centrifuged at 1000 rpm for 10 minutes, resuspended in 50 g/ml propidium iodide (PI, Sigma, St. Louis, MO) in PBS and immediately subjected to flow cytometry optimized for PI using a FACS scan (Becton-Dickinson). Appropriate settings of forward and side scatter gates were used to examine 10,000 cells per sample. Results were analyzed with CellQuestt (Becton-Dickinson) and Modfit (Verity Software House, Topsham, ME) softwares.

Statistical analysis:

Statistical analyses were carried out by using the student t-test, with $p < 0.05$ considered statistically significant.

RESULTS

Villus tip cells are more sensitive to an ischemic insult.

The ischemia induced by SMA occlusion resulted in mucosal sloughing accompanied by an intense inflammatory infiltrate during the reperfusion period. The severity of injury was proportional to the degree of ischemic time (30 or 60 minutes) and was demonstrated by the progressive loss of the villi beginning at the tips and moving toward the crypt base. Figure IA shows the histologic damage in the jejunum seen at 6 hours of reperfusion after an ischemic insult of 60 minutes. By 24 hours, the microanatomy returns to normal. A quantitative analysis of the damage is depicted in Fiqure 1B. In both the jejunum and ileum, there is a significant decrease in the crypt-villus heights at 6 hours after an ischemic time of 30 minutes (16.8 and 25%, respectively, $p<0.005$) which is even more dramatic after a 60 minute insult (51.1 and 98.1%, respectively, $p<0.005$). After 24 hours of reperfusion, the crypt-villus heights return to pre-ischemia control values.

Differentiated enterocytes are more sensitive to an ischemia-induced apoptosis in-vitro.

To confirm that it is the loss of the differentiated enterocytes that results in the decrease in the crypt villus height, we subjected the post-ischemia small intestines to northern blot analyses. Figure 2 shows blots of RNA taken from rat small intestines harvested at 6 and 24 hours of reperfusion after an ischemic insult of 60 minutes. The control rats underwent a sham laparotomy which was closed after 60 minutes. The differentiation markers IAP and lactase are shown to be expressed in the control intestines (Lane C). These markers, which are only expressed by differentiated enterocytes, are lost at 6 hours of reperfusion as a result of the ischemia, indicating the loss of differentiated enterocytes from the mucosa. At 24 hours, these markers return to near normal levels of expression, paralleling the time course of the histologic damage. Villin expression, which is greater in differentiated enterocytes but also occurs in the undifferentiated cells, is significantly attenuated at 6 hours but not completely lost, indicating that viable enterocytes remain within the crypts. As was the case for IAP and lactase, villin expression also returns to normal by 24 hours. Actin is included as a control for equal loading of RNA in each lane.

Differentiated cells are more sensitive to ischemia-induced apoptosis in-vitro

It has been shown in-vivo that enterocytes respond to transient ischemia by undergoing apoptosis, an active process that requires new protein synthesis (12-14). By exposing cells in-vitro to increasing times of the chemically-induced ischemia, it was determined that an insult of 3 hours caused a significant increase in the rate of apoptosis in both the HT-29 an Caco-2 cell lines (data not shown), therefore we chose this degree of ischemia for further experiments. Ischemia-induced apoptosis was seen in both the undifferentiated (pre-

confluent) and differentiated (post-confluent) Caco-2 cells; however, the magnitude of apoptosis was much greater in the differentiated cells (7 fold vs 1.1 fold, $p<0.005$, Fique 3). Since the in-vitro model removes the cells from a microcirculation, this result suggests that the increased cellular sensitivity to ischemia may be an intrinsic feature of differentiated enterocytes.

Finally, we examined HT-29 cells using the differentiation agent sodium butyrate. Results with this second, independent in-vitro model system were similar to those seen with the Caco-2 cell model. Indeed, differentiated HT-29 cells were more sensitive to ischemia-induced apoptosis than their undifferentiated counterparts (14.6 fold vs 2.6 fold increases respectively, $p<0.005$, Figure 4). It should be noted that treatment with sodium butyrate itself caused a moderate increase in apoptosis independently of ischemia. We next compare ischemia-induced apoptosis in pre and post confluent HT-29 cells. Since post-confluence does not cause differentiation in these cells, this experiment was designed to test whether the sensitivity to ischemia in Caco-2 cells was truly related to the differentiation process as opposed to being a function of altered growth. Pre and post confluent HT-29 cells were equally sensitive to ischemia-induced apoptosis (Figure 3), supporting the conclusion that it is the differentiating effects of post-confluence in Caco2 cells that result in the increased sensitivity to ischemia-induced apoptosis.

DISCUSSION

The processes of gut mucosal epithelial cell growth arrest, migration, differentiation and apoptosis are critical for the maintenance of small intestinal structure and function. Perturbations of any of these processes can lead to the clinical sequelae of diarrhea, malabsorption, and impaired barrier function as commonly observed in the critical care setting. An understanding of the highly coordinated mechanisms that regulate gut mucosal turnover under normal conditions may provide insight into how these processes are modified in response to pathophysiological stimuli, and in turn may lead to specific interventions that could be used to preserve small intestinal structure and function in the face of a variety of insults.

Transient intestinal ischemia is commonly encountered in clinical practice, particularly in surgical critical care. The present studies confirm the mucosal damage that occurs in the gut in response to ischemia. That is, with increasing ischemic time, the small intestinal villi begin to slough beginning at the tips and moving toward the crypts. Cell loss results not from necrosis, as might be expected, but rather from apoptosis (12-14), an active process that requires new protein synthesis. The observation that enterocytes residing at the tips of the villi are the first to undergo apoptosis in response to transient ischemia coupled with the knowledge that these same cells are, under normal conditions, destined for programmed cell death as part of their normal life span, suggested that these differentiated enterocytes might be "primed" for an apoptotic response to stressful stimuli such as ischemia. We have shown that it is indeed the differentiated enterocytes that are preferentially lost due to an ischemic insult in-vivo. The differentiation markers IAP and lactase lost after ischemia, whereas the expression of villin, which is expressed by both differentiated and undifferentiated enterocytes, is just attenuated after the same amount of ischemia/reperfusion. Taken together, these findings indicate that it is the differentiated enterocytes that are lost while the undifferentiated cells remain to restore the villi to their normal microanatomy when perfusion is restored.

It has generally been assumed that the increased sensitivity to ischemia exhibited by the dfferentiated enterocytes is related to the factor that these cells reside at or near the villus tip at the end of the vasculature without collateral flow. By employing two in-vitro model systems, however, we were able to determine the response of cells to equivalent degrees of ischemia in the absence of a microcirculation. This approach removes the confounding effects of relative variations in blood flow based upon microanatomical location. Using the two independent models of enterocyte differentiation, we have shown that differentiated cells undergo a significantly greater apoptotic response to ischemia than undifferentiated cells. These results suggest that the enterocyte's response to an ischemic insult may depend, at least in part, on intrinsic cellular factors based upon differentiation state.

The two in-vitro models, Pre/Post confluent Caco-2 and butyrate treated HT-29 cells, have both been extensively characterized by our lab and others (22-23). In both models, a variety of differentiation characteristics are seen, including growth arrest, induction of cell cycle inhibitors followed by markers of differentiation and morphological features sash as microvill1i. For control purposes, we employed pre and post confluent HT-29 cells, since these cells do undergo growth arrest but do not differentiate. The fact that pre and post confluent HT-29 cells exhibited similar ischemia-induced apoptotic response strongly supports our hypothesis that it is the differentiation process that specifically makes the cells more sensitive to ischemia.

The present results indicate that the undifferentiated enterocytes are relatively more resistant to an ischemic insult, suggesting that a novel therapeutic approach to protect the small bowel from transient ischemia-induced damage might be possible (i.e., maintaining gut epithelial cells in an undifferentiated state prior to or during ischemia). Alternative y, future investigation may reveal the links between differentiation-induced and ischemiainduced apoptosis, providing potential targets to manipulate in order to protect the small bowel mucosa from ischemic damage. Such potential interventions would undoubtedly be valuable in settings such asvascular procedures or intestinal transplantation where gut ischemia is planned in advance.

CONCLUSION

Differentiated enterocytes are more sensitive to an ischemic insult due, at least in part, to their more differentiated phenotype. As such, this increased susceptibility to ischemiainduced apoptosis appears to be an intrinsic feature of the cells based on differentiation state rather than an entirely non-specific response based on relative oxygen tension. These results suggest that further investigation into the link(s) between enterocyte differentiation, apoptosis and ischemia-induced apoptosis may identify targets to manipulate in order to protect the small bowel from an ischemic injury.

ACKNOWLEDGMENTS

We wish to thank the laboratory of Dr. Jeffery B. Matthews for help in establishing the in-vitro model of chemically-induced ischemia.

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FLIGURE LEGENDS

Figure 1. Rat small intestinal histologic response to an ischemic insult of 60 minutes. At 6 hours of reperfusion, there is an intense inflammatory infiltrate and marked sloughing of the villus tips followed by the return to normal microanatomy at 24 hours (l A). The loss of the villus tips is demonstrated by the decrease in the crypt-villus height which also returns to pre-ischemia control values at 24 hours (1B). Shown are the average measurements (n=10) taken from representative sections of jejunum and ileum at 6 and 24 hours after ischemic times of 30 and 60 minutes.

Figure 2. Northern blot analysis of rat small intestinal mucosal RNA probing for the enterocyte differentiation markers intestinal alkaline phosphatase (IAP) and lactase as well as the gut epithelial marker villin. Both LAY and lactase are lost at 6 hours after an ischemic insult of 60 minutes. Villin, which is more highly expressed in the differentiated enterocytes, is significantly decreased but not lost. The markers return to pre-ischemia control values by 24 hours. Actin is included as a control for equal RNA loading.

Figure 3. Effect of differentiation on ischemia-induced apoptosis in Caco-2 cells. Undifferentiated (pre-confluent) cells exhibited a small apoptotic response to a transient chemically-induced ischemic insult of three hours. The baseline rate of apoptosis (controls) in the differentiated (post-confluent) cells is unchanged compared to the undifferentiated cells; however, differentiation results in a dramatic increase in ischemiainduced apoptosis (7 fold vs. 1.1 fold, $p<0.005$, $n=3$). Post-confluence, which does not cause differentiation in HT-29 cells, did not increase the baseline rate of apoptosis or result in a higher rate of ischemia-induced apoptosis in this cell line.

Figure 4. Effect of differentiation on ischemia-induced apoptosis in HT-29 cells. As was the case with the Caco-2 cell line, undifferentiated HT-29 cells demonstrated a relatively small apoptotic response to the transient ischemia. Differentiation with sodium butyrate caused an increased rate of apoptosis and resulted in a significantly large amount of ischemia-induced apoptosis (14.6 fold vs. 2.6 fold increase, $p<0.005$, $n=9$).

附件三

hodin.jpg (1630x2145x24b jpeg)

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Associate Professor of Surgery
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November 5, 2001

Re: Shiuh-Inn Liu, MD

To Whom It May Concern:

Dr. Shiuh-Inn Liu spent one year as a research fellow within the Department of Surgery at the Harvard Medical School in Boston, Massachusetts. He worked under my supervision from July 2000-July 2001, both at the Beth Israel Deaconess Medical Center and at the Massachusetts General Hospital. Dr. Liu performed in an outstanding fashion during this fellowship. He is extremely bright and has great insight into clinical surgery. He worked extremely hard and was quite productive both in regard to his clinical research as well as the basic science laboratory.

His major clinical project had to do with the topic of laparoscopic appendectomy. He reviewed a large consecutive serious of patients who underwent this procedure and assessed a variety of factors to determine those clinical features which would predict the need for a conversion from laparoscopic to open appendectomy. His work was done in a very careful fashion and required extensive reviews of both the medical records as well as the surgical literature. Dr. Liu was chosen to present this work at the 2001 Annual Meeting of the Society for Surgery of the Alimentary Tract. In addition, he wrote a manuscript, which is now In Press in the *Journal of the American College of Surgeons.*

In addition to this clinical research project, Dr. Liu also worked in our basic science research laboratory and again did an outstanding job. He was able to master several important techniques in molecular biology, including cell culture work, RNA purification, and northern blot analysis. His observations are quite interesting in that he was able to demonstrate significant effects of various cytokines (interferon-gama, TNF, interleukin-1) on the phenotype of intestinal epithelial cells in culture. This work is continuing in our laboratory and I believe it will have important implications regarding the gut mucosal dysfunction that occurs in the setting of various inflammatory conditions, including Crohn's disease, ischemia, etc.

It was indeed a pleasure to work with Dr. Liu in his one-year fellowship. He is completely honest and trustworthy and gained the respect of all of our colleagues in the Department of Surgery as well as collaborators in the Department of Radiology. He clearly has a bright future and I look forward to watching his progress.

Please feel free to contact me with any questions.

Sincerely,

Richard Hodin, MD

哈佛醫學院Hodin教授推薦信

致有關人士:

劉絜穎醫師在本人指導下,自2000年7月至2001年7月一年間在美國麻塞諸塞州波士頓市哈佛醫學院附設貝絲以色列狄可尼斯醫學中心及麻省總醫院進修,進修期間表現極為優異,劉醫師對於臨床工作極有心得而且極為勤奮,在臨床工作及基礎醫學研究都獲得重要成果。在臨床工作方面,他的主要研究方向是內視鏡闌尾切除術,他收集大量接受內視鏡闌尾切除術病患的臨床資料,分析可能造成內視鏡手術失敗的因子,這項研究成果曾於2001年美國消化系外科醫學會中發表,這篇文章也將於美國外科醫學會雜誌中發表。

在基礎研究方面,劉醫師以細胞培養及北方墨點等方法探索不同細胞間素對腸道細胞基因表現的影響,本實驗室亦延續他的研究成果.進一步探討腸細胞在人體遭遇不同疾病時變化的情形。

本人非常欣慰能在這一年中與劉醫師共同工作,他是一位極為誠實、值得信任的工作伙伴,並且得到本院外科部及放射線部曾與他共事的同仁的尊敬,本人相信並且期待他必有光明的前程。

如有任何問題請毋吝惜隨時與本人聯絡。

Richard Hodin