Table I. The profile of EBVaGC donors.

Donor	Sex	Age	Anatomical subsites[a]	Macroscopic type	Histopathological grading	Staging[b]
GC1	F	69	ML	Borr.2	Well	IV
GC2	M	80	UM	Borr.2	Poorly (solid type)	IV
GC3	M	70	ML	Borr.3	Moderately	IIIb
GC4	M	58	U	0-IIc	Poorly (non-solid type)	Ib

[a]Anatomical subsites: U, upper third; M, middle third; L, lower third (Japanese Classification of Gastric Carcinoma, 13th edition, Japanese Gastric Cancer Association). [b]Stage grouping (TNM Classification of Malignant Tumors, 6th edition, International Union against Cancer).

well. On days 14, 21 and 28, the cultures were stimulated with irradiated FH01-LCL (responder:APC = 4:1), and fresh medium containing interleukin (IL)-2 (25 U/ml) (28,29) was added. Effector cells were assessed for cytotoxic activity after 3 rounds of restimulation.

Generation of $LMP2_{419-427}$ peptide-induced CTLs. PBMCs were separated by adherence to a plastic tissue-culture flask to enrich monocytes. The monocyte-enriched population was then cultured in the presence of 1,000 U/ml granulocyte/macrophage colony-stimulating factor (Genzyme, Minneapolis, MN) and 1,000 U/ml of IL-4 (Genzyme) in RPMI 1640 containing 5% human AB serum (Japan Red Cross Society). After 7 days culture, the cytokine-generated DCs were loaded with 20 μg/ml of $LMP2_{419-427}$ peptide (TYGPVFMCL) (synthesized and provided by Takara Bio, Shiga, Japan) derived from LMP2 in the presence of 3 μg/ml of ß2-microgloblin (Lee BioSolutions, St. Louis, MO) for 4 h and were irradiated (50 Gy). The autologous LCL (FH01-LCL) was also used as APC after $LMP2_{419-427}$ peptide loading (10 μg/ml) and irradiation (100 Gy). FH01-PBMCs ($2x10^6$) were co-cultured with $LMP2_{419-427}$ peptide-loaded DCs and $LMP2_{419-427}$ peptide-loaded FH01-LCL in 24-well tissue culture plates (responder: LCL:DC = 40:2:1). On days 7 and 14, fresh medium, IL-2 (100 IU/ml, Shionogi, Osaka, Japan), and $LMP2_{419-427}$ peptide-loaded (10 μg/ml) FH01-LCL (irradiated) were added to the culture (responder: stimulator = 10:1).

Flow cytometric analysis. Phenotypic characterization of CTLs was carried out by flow cytometry using a FACSCalibur (Becton Dickinson, San Jose, CA) and CellQuest software (Becton Dickinson). Immunofluorescence staining was performed using the following FITC-conjugated monoclonal antibodies (mAbs): anti-CD3 mAb, anti-CD4 mAb, and anti-CD8 mAb (Dako A/S, Denmark).

Measurement of CTL responses in vitro. CTLs were tested for killing activities by standard ^{51}Cr release assay described previously (17). Peptide-pulsed target cells were prepared by incubating the cells with $LMP2_{419-427}$ peptide (20 μg/ml) overnight at 37°C. Target cells were labeled with 200 μCi of ^{51}Cr (Amersham, Piscataway, NJ) per $3x10^6$ cells for 1 h at 37°C, washed in phosphate-buffered saline (PBS), and added to 96-well U-bottom plates at $1x10^4$ cells in a final volume of 200 μl per well. After a 5-h incubation period at 37°C, the

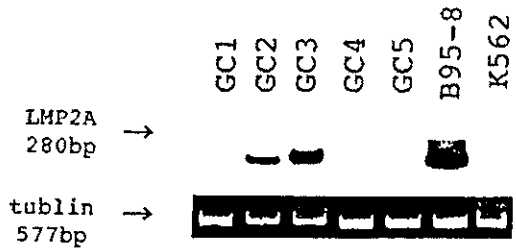

Figure 2. Detection of LMP2A mRNA in gastric carcinoma tissues. Total cellular mRNA was subjected to RT-PCR for LMP2A mRNAs followed by Southern hybridization with ^{32}P-labeled probes. B95-8 and K562 were used as positive and negative controls. Human ß-tubulin mRNA was stained with ethidium bromide. Arrows indicate the specific PCR products with their sizes.

radioactivity in the supernatants was determined using Cobra Model 5002 Gamma Counter (Packard, Meriden, CT). All tests were conducted in triplicate, and the percentage of specific cytotoxicity was defined by the formula: [(experimental ^{51}Cr release - spontaneous ^{51}Cr release)/(maximum ^{51}Cr release - spontaneous ^{51}Cr release)] (17,30).

For interferon (IFN)-γ release, CTLs were co-cultured with target cells for 12 h at 37°C. Culture supernatants were collected for measurement of human IFN-γ release using specific ELISA kits according to the manufacturer's instructions (Biosource, Camarillo, CA).

Results

LMP2 expression in patients with EBV-associated gastric carcinoma. EBV specific region was amplified from genomic DNA extracted from tumors in 4 (GC1-GC4, 3 men and 1 woman) out of 18 patients, indicating that 22% of patients with gastric cancer showing high titer of EBV IgG (viral capsid antigen) were identified to be EBVaGC (Fig. 1, Table I). LMP2 mRNA was detected in 3 of the 5 tumors examined by RT-PCR (Fig. 2). This pattern of the EBV gene expression in gastric carcinoma tissues was consistent with results reported previously (31).

Primary culture of tumor cells obtained from patients. Primary cultures of LMP2 mRNA-positive tumor cells derived from EBV-positive patients (GC2 and GC3) were obtained by the

Figure 3. Microscopic state of GC3 cells. GC3 was derived from resected stomach tissue from a patient with moderately differentiated adenocarcinoma. GC3 cells had a flat structure (bar, 100 μm).

above described method. As shown in Fig. 3, morphological examination indicated that these cells were epithelioid and irregular in size, with large hyperchromatic vesicular nuclei suggesting malignant features of cells. Furthermore, these cells were stained with anti-LMP2 antibody by immunohistochemical study (Fig. 4A). FACS analysis showed that GC3 cells were positive for HLA-A24. 888mel stably transfected with LMP2 cDNA (LMP2-888mel) was also stained by anti-LMP2 antibody (Fig. 4B and C). Thus, GC3 and LMP2-888mel were used as target cells expressing LMP2 endogenously for CTL assay in the following experiments.

Generation of CTLs specific for LMP2. To induce CTLs specific for LMP2, we first cocultured PBMCs with irradiated autologous EBV-transformed LCL (LCL-induced CTL). LCL is known to be a potent APC which can generate effector CTLs by stimulating resting memory $CD8^+$ T cells. After 3 rounds of stimulation, LCL-induced CTLs efficiently lysed autologous LCL (FH01). Although the recognition of tumor cells expressing LMP2 (LMP2-888mel) was observed, its activity was relatively low (Fig. 5A). Since LCL does not seem to induce primary CTL response because of the lack of second signals through the costimulatory molecules, we next used DCs as an APC to make CTL potent enough to kill tumor targets. A mixture of DCs pulsed with $LMP2_{419-427}$, a HLA-A24-restricted peptide derived from LMP2, and LCL was used as a stimulator in this experiment. T cells induced by these APCs (TYG-LCL-induced CTLs) were then restimulated with peptide-loaded LCLs. Phenotypic analysis of effector cells obtained after two rounds of restimulation showed that the percentage of $CD3^+$, $CD8^+$ and $CD4^+$ cells were 93.3±3.9, 70.5±6.2 and 21.6±9.7 respectively. TYG-LCL induced CTLs had 1.5 times higher cytotoxic activity against both peptide-pulsed cells and targets expressing LMP2 endogenously (LCL, LMP2-888mel) (Fig. 5B).

Recognition of primary cultured tumor cells by CTLs. Since established cell lines showing autonomic growth may behave differently from the primary cultured cells, it seems to be

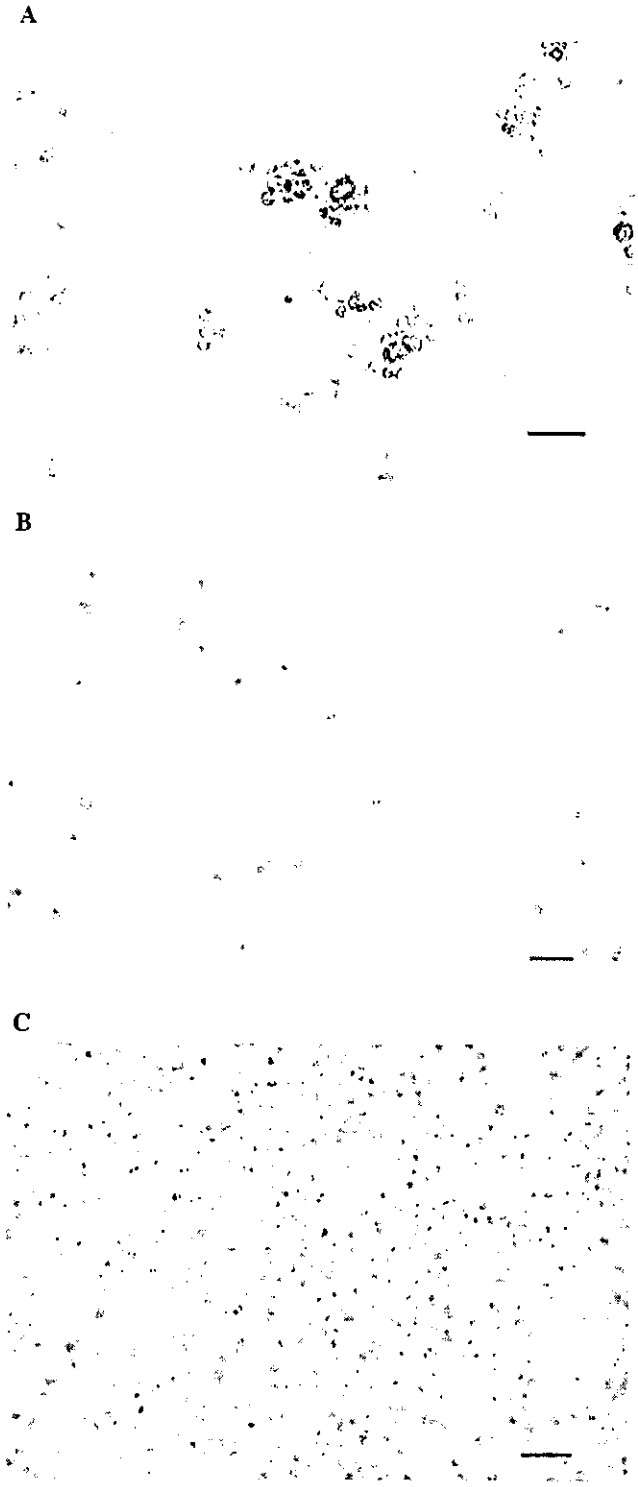

Figure 4. Immunohistochemical staining of LMP2 (bar, 100 μm). GC3 cells were showing weakly positive LMP2 staining at the cell membrane (A). 888mel-LMP2 was positive staining of LMP2 (B), but 888mel was negative (C).

important to investigate the recognition of tumor cells freshly isolated from patients by T cells. Therefore, we tested the recognition of GC3 cells obtained and cultured from surgically resected specimens of patients with EBVaGC, by TYG-LCL-

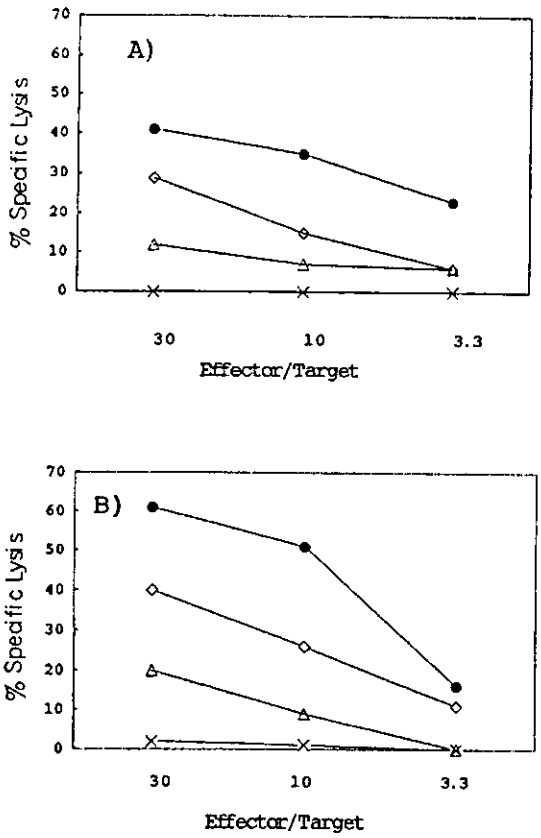

Figure 5. The use of LCL and peptide-pulsed DCs as APCs augments the cytotoxic activity of CTLs. (A), T cells were primed with autologous LCL. After 3 rounds of restimulation, effector cells were tested for cytotoxic activity against FH01-LCL (●), 888mel (×), LMP2-888mel (◇), and $LMP2_{419-427}$ peptide loaded 888mel (Δ). (B), T cells were induced by autologous LCL and $LMP2_{419-427}$ peptide-pulsed DCs. Effector cells were tested for cytotoxic activity against FH01-LCL (●), 888mel (×), LMP2-888mel (◇), and $LMP2_{419-427}$ peptide loaded 888mel (Δ).

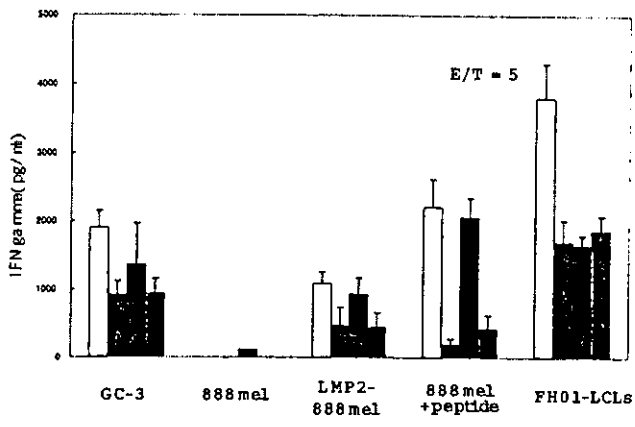

Figure 6. The recognition of TYG-LCL-induced CTLs against the cells of GC3 by IFN-γ determination assay. CTLs were stimulated *in vitro* with target cells with or without (open bar) monoclonal antibodies specific for HLA class I (hatched bar), class II (dotted bar) or HLA A24 mAb (solid bar). Culture supernatant were harvested overnight and evaluated for IFN-γ levels by ELISA (in pg/ml; mean ± SEM of triplicate samples). Error bars show the standard deviation.

induced CTLs. As demonstrated in Fig. 6, the TYG-LCL-induced CTLs produced ~1,900 pg/ml of IFN-γ in response to GC3 cells as well as 888mel-LMP2 whereas it produced <50 pg/ml in response to 888mel (LMP2 negative). The production of IFN-γ was partially abolished by anti-class I and anti-HLA-A24 mAbs in the blocking assay (Fig. 6). In contrast, the recognition of peptide-pulsed 888mel was blocked almost completely by anti-HLA-A24 mAb.

Discussion

The goal of this study was to assess the possibility of LMP2-targeting immunotherapy for EBVaGC by measuring the cytotoxicity of CTLs induced by LCL and peptides derived from LMP2 against primary cultured gastric cancer cells as well as tumor cell lines, which express LMP2 antigen. Principally, strong CTL responses toward EBNA3A, 3B, and 3C subset of virus proteins will occur in the healthy virus carriers (16,17). However, if one considers many EBV-associated malignancies, only a limited group of antigens such as EBNA1 and LMP2 are known to be expressed on tumor cells. Because LMP2 is the only EBV transcript expressed by B cells from EBV-positive healthy donors, it is reasonable to use LCL as APCs for inducing CTL competent for killing tumors (32). Indeed, quite a few epitope peptides have been identified by stimulating T cells with peptide-loaded LCLs (20). It has also been shown that cross-priming of CTLs using DCs loaded with dead LCLs induced an expansion of $CD8^+$ T cells specific for EBNA3A and LMP2. Consistent with these previous reports, our study showed that *in vitro* sensitization of PBMCs by LCL gave rise to $CD8^+$-dominant CTLs showing cytotoxic activity against LCL. These CTLs recognized tumor cells transfected with LMP2 cDNA (LMP2-888mel) (Fig. 5), whereas 888mel, non-transfectant for LMP2, was not lysed by them. Since LCL has the potential to generate T cells able to kill tumors, vaccination with LMP2-expressing cells mimicking LCL is considered to be a useful strategy to induce tumor immunity *in vivo*.

Peptide-based approach has also been investigated in EBV-related tumors. $LMP2_{419-427}$, a 9-mer peptide derived from LMP2, has been identified to be an epitope for $CD8^+$ T cells (29). A mixture of LCL and DCs pulsed with the peptide was used as a stimulator in this setting. Generated CTLs (TYG-LCL-induced CTLs) showed 1.5 times higher activity not only to LCL but also to LMP2-transfected tumor (LMP2-888mel) as compared to CTLs induced by LCL alone. This result suggests that the use of an epitope peptide in addition to LCL is one of the options to get a more robust CTL response. Because LCL is considered to be able to stimulate only resting memory cells due to the lack of second signal through costimulatory molecules, the use of DCs as professional APCs might enhance CTL response by priming naive T cells specific for peptide. However, since peptide alone (peptide-pulsed DC without LCL) did not exert full response (data not shown) comparable to that induced by LCL, further examinations into the role of the peptide in generating CTL in this method are needed.

Although there have been several reports regarding the generation of CTLs able to recognize autologous tumor cells, they are limited to certain types of tumors including melanoma (33), breast cancer (34,35) and ovarian cancer (35). There have

been no reports that indicated the recognition of primary cultured autologous gastric cancer cells by CTLs so far, presumably because it is difficult to get a primary tumor cell culture and T cells at the same time. In the present study, TYG-LCL-induced CTLs produced substantial amount of IFN-γ in HLA-A24 restricted manner when they were incubated with a peptide-pulsed target. This CTL could also recognize cells expressing LMP2 endogenously (LMP2-888mel). Furthermore, they recognized primary cultures of tumor cells (GC3) obtained from patients with EBVaGC. Since this recognition was not blocked by HLA-A24 mAb completely, TYG-LCL induced CTLs may include certain clones which are specific for some different epitopes endogenously expressed on LCL other than that specific for $LMP2_{419-427}$ peptide. Indeed, FH01, the donor for PBMC, shares the same HLA-class I molecules as B52 besides A24 with 888mel (24). Thus, some unknown epitopes restricted by B52 might be involved in this recognition of tumor by T cells.

In conclusion, LMP2-expressing tumor cells including primary cultured gastric cancer cells from EBVaGC were successfully recognized by CTLs induced *in vitro* with LCL and HLA-A24-restricted peptide derived from LMP2. EBVaGC is suggested to be susceptible to the LMP2-targeting immunotherapy. Therefore, vaccination using cells endogenously expressing LMP2 along with the peptide or adoptive immunotherapy using T cells induced by LCL and the peptide are promising approaches for the treatment of HLA-A24 positive EBVaGC.

Acknowledgements

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免疫学

新しい癌の体外循環治療技術の開発

New technology of direct hemoperfusion for cancer therapy

背景

担癌患者の血中には癌の進展に伴い種々の特異的・非特異的免疫抑制物質が増加し，それに伴い抗腫瘍細胞性免疫能は低下する[1]．これらの免疫抑制物質を十分に除去した後に免疫療法あるいは化学療法を行えば，治療効果を増強しうることが期待できる．このような理論的背景に立脚し，1976年以降1980年代にかけて欧米およびわが国において，進行癌患者に対する治療として血漿交換による免疫抑制物質除去療法が試みられた[2-4]．本療法は一定の治療効果が認められたにもかかわらず，血漿置換液として大量に使用される血液製剤に起因する感染の危険性，有用な血漿成分の破棄，コストの問題などが山積し，普及には至らなかった．また，当時の癌免疫療法と化学療法の発達程度の未熟さが，この治療法のさらなる改良への気運を妨げてきた．

免疫抑制物質吸着性極細繊維カラムを用いた，血漿交換を伴わない癌体外循環治療技術の開発

近年の樹状細胞療法を中心とした癌の免疫細胞療法の進歩は著しく，また一方，癌の進展に伴う免疫抑制動態の詳細も明らかにされた．すなわち，癌患者の血中に増加してくるTGF-β，IL-6，VEGFなどのサイトカイン(表1)は，じかに癌の進展に関与するのみならず，細胞性免疫能を抑制することにより癌の進展をさらに助長する[5-9]．また，患者に悪液質をもたらし，化学療法の副作用増強にも関与し，その用量規定因子にもなっている．

著者らはこれら免疫抑制性サイトカインの制御を目的として，血漿交換を伴わない血液吸着療法としての癌体外循環治療技術の開発を行っている(図1)．すでにTGF-β吸着剤として特定のアミノ基を官能基として固定化した多孔質のポリスチレン系極細繊維を開発・同定し，それを充填した体外循環治療カラムが，他治療を組み合わせない単独治療においても担癌ラットの腫瘍増殖を有意に抑制し生存期間を延長することを明らかにした[10]．また，ヒト癌性胸腹水を用いた吸着実験で，同吸着剤がTGF-β以上にVEGF，IL-6を強く(90%以上)吸着除去することを明らかにしている．

本開発技術は，繊維工学，高分子化学の癌治療への応用であり，繊維の製造工程において抗サイトカイン抗体などの薬剤を使用せず，安全で簡便な体外循環治療を繰り返し施行できるという利点を有している．

臨床応用へ向けて

この現在開発中の癌治療用体外循環カラムを“免疫繊維カラム”と著者らは仮称しているが，現在継続中の動物実験における有効性確認，安全性確認を経た後に，近々臨床試験を開始する予定である(図2)．それには，表2に列挙したように種々の目的，用途が考えられる．癌治療用医療機材として

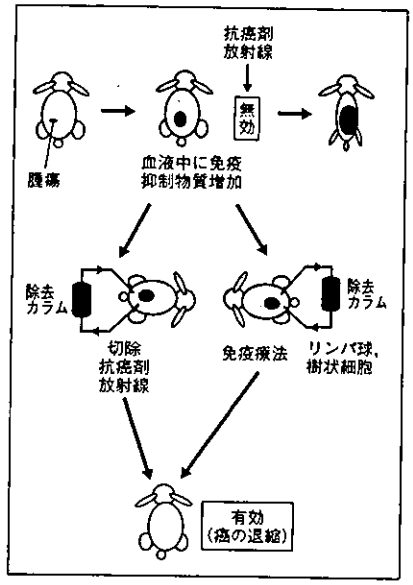

図1　免疫抑制物質除去カラム(免疫繊維カラム)を用いた血漿交換を伴わない癌体外循環治療技術
除去カラムはアミノ基含有ポリスチレン繊維の不織布を充填したもの．

表1　担癌の進行に伴い血中に増加する免疫抑制物質

transforming growth factor-β (TGF-β)
immunosuppressive acid protein (IAP)
interleukin-6 (IL-6)
prostaglandin E_2 (PGE_2)
vascular endothelial growth factor (VEGF)
etc.

表2　免疫繊維カラムの目的・用途(進行固形腫瘍患者を対象として)

① 細胞免疫療法との連動による効果増強
② 化学療法との併用による効果増強⟶難治性癌(膵癌，スキルス胃癌など)を対象とした癌治療用医療器材としての承認
③ 末期癌患者の悪液質(QOL)改善
④ 患者血清の *ex vivo* 処理による細胞培養用自己血清の調製

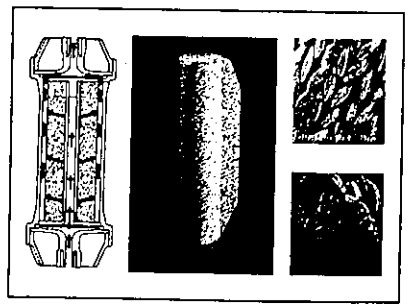

図2　免疫繊維カラム

の承認を最終目標とするため，進行癌患者を対象としてその単独治療による免疫賦活効果(血中免疫抑制性サイトカインの除去と細胞性免疫能の回復)を確認した後に，膵癌，スキルス胃癌などの難治性固形癌(これらの癌の進展にはTGF-β，VEGFがともに深く関与)を対象疾患とし，化学療法との併用による臨床試験を展開していければと考えている．

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癌・腫瘍学

アポトーシス誘導抗ErbB-2抗体による癌治療

Apoptosis inducing anti-ErbB-2 antibody for cancer therapy

癌に対する治療法としてモノクローナル抗体を用いた治療が注目を集めており，血液疾患に対する抗体治療はすでに臨床のなかで重要な位置を占めつつある．しかし，固形癌に関しては乳癌に対するtrastuzumab以外にはいまだFDAで認可された抗体は存在していないという現状である．

このような情況のなかで，著者らが独自に作成した消化器癌に過剰発現を多く認める受容体型チロシンキナーゼであるErbB-2に対するモノクローナル抗体CH401が，これまで報告されている抗ErbB-2抗体による抗腫瘍効果の作用機序とはまったく異なるアポトーシスの誘導であることが明らかになった．さらに，このアポトーシスに至る機序にMAPキナーゼの関与が示唆されるデータが出つつある．

本稿ではこのモノクローナル抗体CH401の作用機序解析を通して消化器癌に対して，CH401を用いる有用性について述べたい．

ErbB-2と癌

ErbB-2はレセプター型のチロシンキナーゼファミリーに属する癌遺伝子産物であり，遺伝子の増幅，蛋白の過剰発現は種々の癌において報告されている．乳癌では遺伝子増幅は10～33%，過剰発現は17～37%，卵巣癌では前者が20～26%，後者が32%，胃癌においてはそれぞれ8～25%，12～55%，大腸癌では3～7%，27～56%などと報告されている．

現在まで，ErbB-2に対する阻害剤や特異的抗体が種々開発され，治療研究が行われており，これらの薬剤による抗腫瘍効果が報告されてきている．実際，これまでにさまざまな抗ErbB-2抗体が作製され，そのひとつのtrastuzumabは現在FDAで認可している唯一の固形癌に対する抗体である．これらの抗ErbB-2抗体は抗体単独で，抗腫瘍効果を発揮するが，その作用機序はおもにErbB-2のダウンレギュレーションであることがすでに示されている[1]．

抗ErbB-2モノクローナル抗体CH401によるアポトーシスの誘導

著者らもこれまで数種類の抗ErbB-2モノクローナル抗体を作製してきた．そのうちの1種CH401がErbB-2過剰発現癌細胞にアポトーシスを誘導することを明らかにしてきた[2](図1)．このことはこれまで報告されているtrastuzumabを含むほとんどの抗ErbB-2抗体による抗腫瘍効果の機序とはまったく異なるものであった．このため，つぎにこのアポトーシスの機序に関する検討を行った．

ErbB-2からの増殖シグナルはおもにMAPキナーゼとPI3キナーゼ-Aktの経路に伝達されることが明らかになっている．そこで，MAPキナーゼおよびPI3キナーゼ-Aktに与える影響を検討した．さらに，アポトーシスの実行分子であるcaspaseの活性に与える影響を検討した．

MAPキナーゼ(ERK/JNK/p38)およびPI3キナーゼ-Akt経路の活性変化

まず，MAPキナーゼについてその活性の変化について検討した．増殖に関与するErkに関して，その活性はCH401添加8時間後より低下を認め，一方，アポトーシスに関与するJNKおよびp38については抗体添加2時間後から著明な活性上昇が認められた．以上よりアポトーシスの誘導に，各MAPキナーゼの関与が想定された．さらに，PI3キナーゼ-Aktの経路については，CH401添加後，Aktの活性は低下し，この変化もアポトーシスの誘導に関与していることが明らかになった．

つぎに，caspaseについて検討を行った．代表的な3種類のcaspase(caspase-3,8,9)に関して，CH401処理後の活性変化について検討を

Case Report

Complication of Jejunal Pouch Interposition after Proximal Gastrectomy: Case Report

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KEY WORDS:
Jejunal pouch; Proximal gastrectomy; Complication; Pouch stasis

SUMMARY

Interposition of a jejunal pouch after proximal gastrectomy is a popular reconstruction method in Japan, because it produces a good quality of life soon after surgery. Many reports have described its usefulness. However, there are few reports describing its complications. We report here for the first time a case of pouch stasis needing surgery.

A 23-year-old man underwent proximal gastrectomy with interposed jejunal pouch for traumatic strangulated diaphragmatic hernia. Three years later, he complained of persistent vomiting. Since surgery, he had eaten as much as other young people. An upper gastrointestinal series showed dilatation of the jejunal pouch and stasis of contrast medium. Since conservative therapy was not effective, surgery was performed. In the operative findings, the jejunal pouch was extremely dilated, the remaining stomach had become atrophic, and moreover, the anastomosis was severely distorted. It was considered that frequent excessive ingestion caused irreversible dilatation of the jejunal pouch, resulting in pouch stasis. Even though the jejunal pouch is interposed for reconstruction, it is very important to give nutritional guidance to patients, especially young patients, to prevent pouch stasis caused by excessive food ingestion.

INTRODUCTION

Recently, jejunal pouch interposition has been in general use for reconstruction after proximal gastrectomy in Japan. It has been reported that this method prevents postoperative reflux esophagitis and allows adequate food ingestion soon after surgery leading to the best possible quality of life (1-3). Therefore, we have also performed proximal gastrectomy interposing a jejunal pouch between the esophagus and the gastric remnant for early gastric cancer and other benign diseases in the upper part of the stomach.

However, the complications of this reconstruction have been reported in only a few papers, describing the development of an ulcer in the jejunal pouch (4,5). Although pouchitis and pouch stasis have been reported in the ileal pouch-anal anastomosis (6), no case in the jejunal pouch for gastric substitute after proximal gastrectomy has ever been reported. There are no reports about the long-term follow-up of jejunal pouch interposition after proximal gastrectomy. Therefore, it is still not clear whether this method is free of serious problems and provides a better quality of life than other methods.

We report here for the first time a patient, who suffered from pouch stasis due to extreme dilatation of the jejunal pouch and required surgery. We also discuss the complications of jejunal pouch for gastric substitute after proximal gastrectomy.

CASE REPORT

A 23-year-old man was admitted to Wakayama Medical University Hospital in May 2001 with persistent vomiting and body weight loss. Three years before admission, he underwent proximal gastrectomy and reconstruction of an interposed jejunal pouch for traumatic strangulated diaphragmatic hernia. At that time, the operation procedure was as follows: after proximal gastrectomy preserving pyloric branches of the vagal nerve, the jejunum was divided 15cm distal to the ligament of Treitz and a 30-cm-long jejunal segment with vascular pedicles was prepared as a substitute for the pouch and brought up posterior to the transverse colon. Sacrifice of the mesenteric arcade was kept to a minimum in order to preserve the autonomic nerve and blood flow in the mesentery. Two cut edges of this segment were placed against the side of the gastric remnant. From the cut edges, a liner stapling device was inserted in order to construct the U-shaped jejunal pouch by side-to-side jejunostomy. An end-to-end anastomosis was made between the upper edge of the jejunal pouch and the esophagus using a circular stapling device. Then, an end-to-end anastomosis was made between the lower edge of the jejunal pouch and the remnant stomach. Our method is almost identical to others which have been reported (2,3).

There were no complications in the postoperative course, and he could eat as much as other young people soon after surgery. After he had been followed up for 6 months without any complications, he did not consult our hospital.

In January 2001, he complained of persistent vom-

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iting and body weight loss (14kg/5 months). Blood examinations showed hyponatremia (132mEq/L) and hypochloremia (97mEq/L) due to persistent vomiting and low serum levels of albumin (3.4g/dL), triglyceride (38mg/dL) and cholesterol (97mg/dL) due to hyponutrition. An upper gastrointestinal series showed extreme dilatation of the jejunal pouch and stasis of the contrast medium in the pouch **(Figure 1a, b)**. Gastrointestinal endoscopy showed that the jejunal pouch was extremely dilated and the anastomosis bent sharply, and therefore, it was impossible to examine the remnant stomach **(Figure 2a, b)**.

Since conservative therapy was not effective, surgery was performed. The jejunal pouch was hypotonic and remarkably dilated. The remnant stomach was atrophic and the anastomosis was obviously bent. The jejunal pouch and the remnant stomach were resected **(Figure 3a, b)**, followed by Roux-en-Y double tract reconstruction (7,8). Pathological findings showed that the autonomic nerves were not degenerated and that the muscle layer had become hypertrophic associated with the dilatation.

Two months after the operation, he had gained 13kg in weight and all data of the laboratory investigations were normalized, although frequent meals per day were needed.

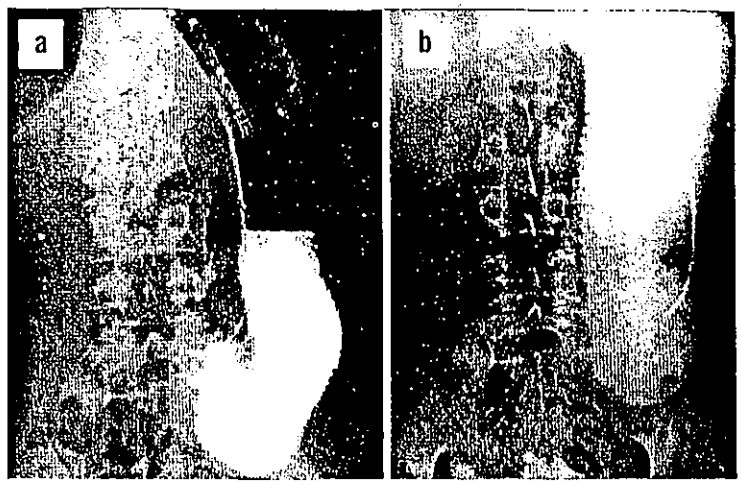

FIGURE 1 Radiograph of the jejunal pouch on admission showing **(a)** immediately after the intake of water-soluble contrast medium, and **(b)** 150 minutes later.

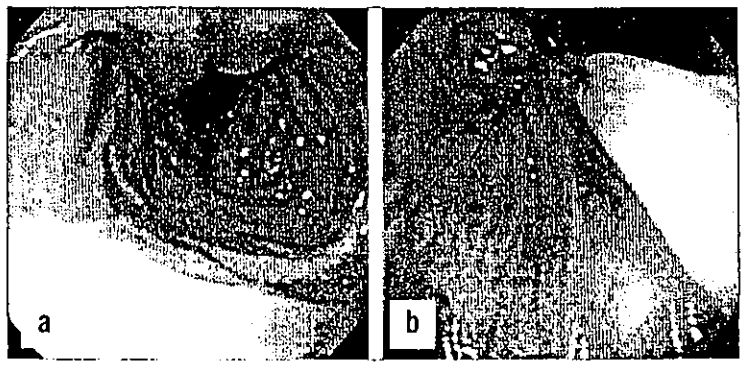

FIGURE 2 Endoscopic findings showing **(a)** the dilated jejunal pouch and **(b)** bending of the anastomosis (arrow).

DISCUSSION

The most popular methods of reconstruction after proximal gastrectomy were previously esophagogastrostomy (Mikulics) or jejunal interposition (Merendino). However, the postoperative course of these reconstruction methods was not favorable, due to the problems such as reflux esophagitis, inadequate food ingestion at any one time and difficulty in endoscopically examining the remnant stomach.

In 1993, Kameyama reported a new method of reconstruction, interposed jejunal pouch, to resolve these problems (9). Since then, several modified methods have been reported (1-3,10) and this method has been evaluated as superior to esophagogastrostomy or single jejunal interposition because of reducing postoperative disturbance such as alkaline reflux esophagitis and inadequate food ingestion, and it has become the most popular method for reconstruction after proximal gastrectomy in Japan. However, there have been two reports mentioning complications with interposed jejunal pouch (4,5). They reported the presence of a marginal ulcer at the interposed jejunal pouch and discussed the possibility of hypergastrinemia after proximal gastrectomy as the cause. However, there have been no studies with long-term follow-up after jejunal pouch interposition, and therefore, it is still to be clarified whether this reconstruction procedure is really superior for proximal gastrectomy.

Our report is the first case of extreme dilatation of the interposed jejunal pouch after proximal gastrectomy resulting in passage disturbance. The length of the jejunal pouch is related to the passage status of food (11), and long pouch reconstruction after total gastrectomy tends to increase reflux and delay emptying time. However, a 15-cm-long pouch, which was made in this case, seems to be standard according to other reports (1-5,9,10). Preserving the pyloric branch of the vagal nerve is important to maintain adequate passage of the food from the remnant stomach to the duodenum (2). Since lymph node dissection was not performed at all in this case because of benign disease, the hepatic branch and celiac branches were completely preserved. In fact, a smooth passage was observed by examining a postoperative barium meal after the first operation.

The problem in this case was that we could not continue to advise the patient about appropriate food ingestion because he stopped attending the hospital 6 months after surgery and, being young, he tended to ingest excessively at one time. We considered that frequent overingestion caused irreversible dilatation of the jejunal pouch resulting in passage disturbance and, finally, the anastomosis was distorted between the dilated jejunal pouch and the remnant stomach. The advantage that interposed jejunal pouch enables the patients to ingest sufficiently at one time soon after surgery became an unexpected pitfall in this case.

This is not a rare situation. Once patients are away from the influence of doctors, they can ingest as much as they want, which could result in a similar outcome

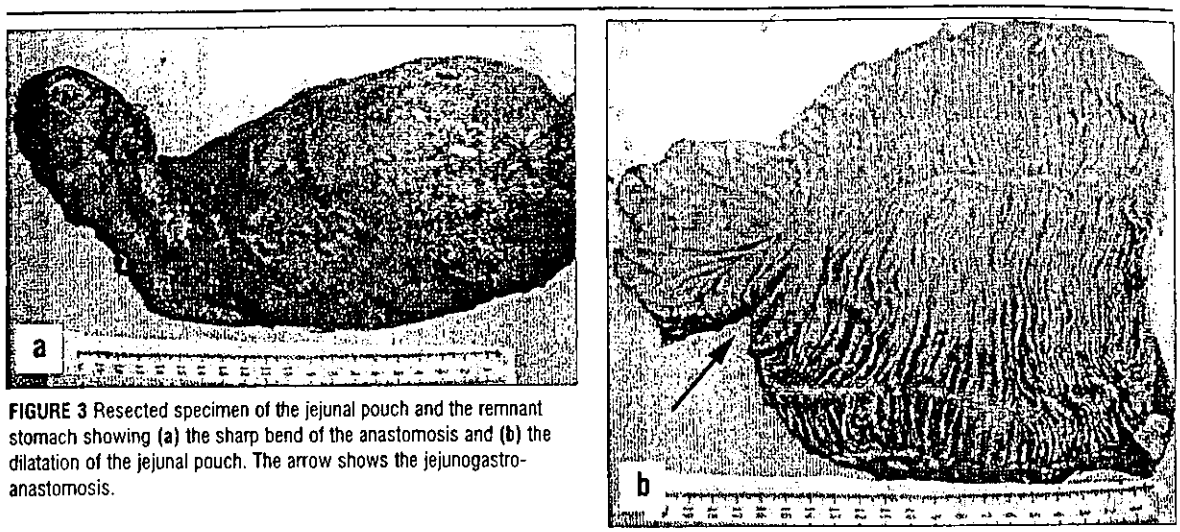

FIGURE 3 Resected specimen of the jejunal pouch and the remnant stomach showing **(a)** the sharp bend of the anastomosis and **(b)** the dilatation of the jejunal pouch. The arrow shows the jejunogastro-anastomosis.

to this case. Therefore, long-term follow-up is necessary, advising patients to avoid excessive ingestion.

In conclusion, the interposed jejunal pouch seems to be superior to other methods of reconstruction after proximal gastrectomy in terms of nutritional benefits and reducing number of meals per day. However, it is very important to give nutritional guidance to patients, especially to the young, for a long time after surgery to prevent pouch stasis caused by excessive food ingestion. A long-term follow-up study is necessary to evaluate whether jejunal interposition is really superior to other methods after proximal gastrectomy.

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Case Report

Virus-associated Hemophagocytic Syndrome and Hemorrhagic Jejunal Ulcer caused by Cytomegalovirus Infection in a Non-compromised Host; A Case Report of Unusual Entity

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SUMMARY
A 76-year-old man was admitted to our hospital with abdominal pain, nausea, and vomiting. The patient was diagnosed as ileus by abdominal radiography, which showed an enlarged bowel and an air-fluid level. Computed tomography of the abdomen showed a thickened intestinal wall. His general status suddenly worsened, and he was placed on a respirator and catecholamines to prevent acute respiratory distress syndrome, septic shock, and disseminated intravascular coagulation. He had continuous fresh anal bleeding. Total colonoscopy showed bloody stool originating from the ileum. Emergency operation was performed for hemorrhagic shock under general anesthesia. Intraoperative jejunal endoscopy revealed deep linear ulcers with bleeding in the jejunum, and 30cm of the jejunum was resected. Histopathologic examination revealed cytomegalic cells with intranuclear inclusion bodies in the tissues surrounding the ulcers, and it was diagnosed as cytomegaloviral enterocolitis with hemophagocytic syndrome in a non-compromised adult.

KEY WORDS:
Cytomegalovirus; Jejunal ulcer; Gastrointestinal hemorrhage; Virus-associated hemophagocytic syndrome; Disseminated intravascular coagulation

ABBREVIATIONS:
Acute Respiratory Distress Syndrome (ARDS); Disseminated Intravascular Coagulation (DIC); Cytomegalovirus (CMV); Virus-Associated Hemophagocytic Syndrome (VAHS); Gastrointestinal (GI)

INTRODUCTION

Cytomegalovirus (CMV) infection and gastrointestinal CMV disease is well recognized in severely immunocompromised patients with acquired immunodeficiency syndrome (AIDS), organ transplants, and malignant disease (1-4). Gastrointestinal lesions may present as either part of a systemic, or as a localized infection (5,6). It is difficult to treat the CMV infection, and the prognosis is usually poor in immunocompromised patients (1,7). On the other hand, healthy persons generally have an acquired permanent immunity against CMV. However, there have been some reports of severe CMV infection even in healthy adults (8,9). Virus-associated hemophagocytic syndrome (VAHS) is a reactive disorder of the phagocytic system, characterized by marked hemophagocytosis (10). There has been one previous case report of VAHS associated with CMV enterocolitis in a healthy adult (11). The patient was treated with steroids and anti-viral drugs.

We herein report a non-compromised case with VAHS and severe enterocolitis, which caused a hemorrhagic jejunal ulcer by CMV, requiring emergency operation.

CASE REPORT

A 76-year-old man was admitted to Wakayama Medical University Hospital with abdominal pain nausea, and vomiting. He had undergone distal gastrectomy with Billroth I reconstruction for early gastric cancer, and Y-graft replacement for abdominal aortic aneurysm 8 years earlier. He has not utilized immunosuppressive anticancer drugs, had no signs of recurrence, and had a good quality of life with social activities.

He had a low-grade fever, epigastrial tenderness, and was admitted on April 2nd, 2000. Blood pressure was 151/60 mmHg and the pulse was 120/min. Several laboratory findings were as follows; white blood cell count 1,000/mm^3, platelet 104,000/mm^3, C-reactive protein 8.0mg/dL, creatinine kinase 824 IU/L, amylase 1,160 IU/L, creatinine 3.1mg/dL, and urea nitrate 40mg/dL.

He was diagnosed as ileus and the abdominal radiography showed an enlarged bowel and an air-fluid level. Two hours after admission, his vital status suddenly worsened. Respiration ceased, and systolic blood pressure was 40 mmHg in spite of administration of cathecolamins. Therefore, we performed extended intensive care with respirator support and hemodynamic drugs for acute respiratory distress syndrome (ARDS), septic shock, acute pancreatitis, and disseminated intravascular coagulation (DIC).

Computed tomography of the abdomen showed a thickened intestinal wall. The intestinal mucosa was enhanced by contrast media, suggesting enterocolitis without ischemia rather than strangulating obstructions **(Figure 1)**.

His general condition improved with intensive care,

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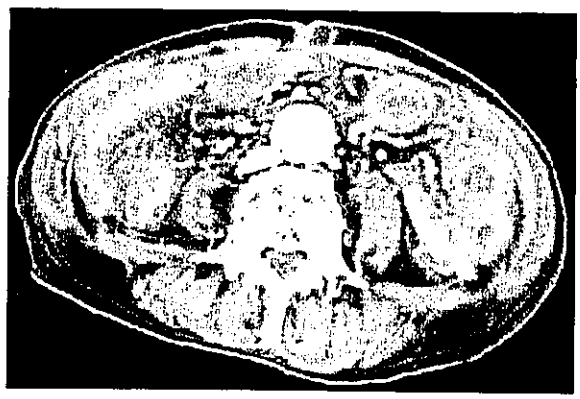

FIGURE 1 The computed tomography on admission.
The computed tomography on admission showed the thickness of the intestinal wall, however, the blood supply to the mucosa was normal.

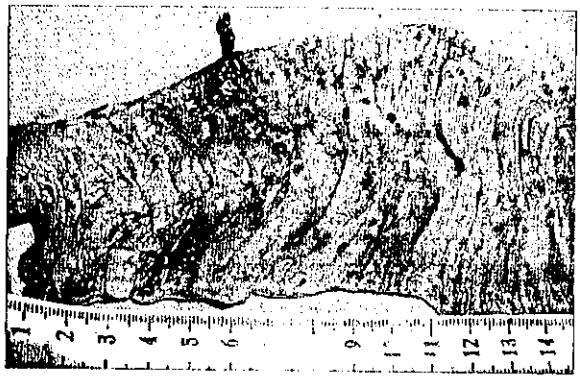

FIGURE 2 Jejunal ulcer.
The deep linear ulcers with bleeding in the jejunum.

and the ileus was cured by conservative treatment. However, he had continuous fresh anal bleeding from the 17th hospital day of admission. Upper gastrointestinal endoscopy showed normal findings. Total colonoscopy showed the entire colon was filled with bloody stool, supplied from the ileum. As his general condition was poor, we chose interventional treatment. However, angiography revealed no apparent hemorrhagic lesion.

Emergency operation was performed for hemorrhagic shock under general anesthesia. Laparotomy revealed a small amount of clear ascites. Macroscopically, the serosa and the mesenterium were normal and the hemorrhagic lesion was not identified. Intraoperative jejunal endoscopy inserted from the ileum revealed deep linear ulcers with bleeding in the jejunum. Thirty centimeters of the ileum was resected and we performed end-to-end jejunal anastomosis **(Figure 2)**.

Histopathologic examination revealed cytomegalic cells with intranuclear inclusion bodies in the tissue surrounding the ulcers. Immunopathological study with CMV antibody confirmed the presence of CMV infection **(Figure 3)**. The serum antibody to CMV was serologically examined as follows; anti CMV-IgG was 432.8 GI compared to 46.4 GI on the third hospital day, and anti CMV-IgM was 1.9 MI.

The gastrointestinal bleeding improved without antiviral agents including ganciclovir. He had respiratory distress syndrome after the operation and required a respirator for one month. Then, he was discharged and was able to resume social activities.

FIGURE 3
Histological examination.
Cytomegalic cells with intranuclear inclusion bodies were found in the tissue around the ulcers.

DISCUSSION

CMV is a member of the herpes virus family, and from 40 to 100 percent of adults acquire antibody to CMV (12). Primary CMV infection in immunocompetent individuals is usually asymptomatic or nonspecific, similar to many acute viral infections. After initial infection, the virus resides in multiple cellular reservoirs and begins a lifelong latent phase (13).

In immunocompromised individuals, however, CMV is a common pathogen and frequently becomes serious. Gastrointestinal CMV disease either part of a systemic, or localized infection is often seen in severely immunocompromised patients including AIDS, organ transplant recipients, malignancies, immunosuppressive therapy, and sometimes in inflammatory bowel disease (1,14). Clinically apparent CMV disease is most often caused by reactivation with compromising of T lymphocyte-mediated immunity (15) but may also be caused by primary infection by transmission of CMV positive organs or blood to CMV negative recipients (2,16). There are also some reports of severe CMV infection in healthy adults with no identifiable immunosuppression (8,9). Indeed, the present case had been healthy before this episode, and showed no symptoms of immunosuppressive diseases including AIDS, malignancies, and inflammatory bowel disease.

Symptoms of gastrointestinal CMV disease include abdominal pain, diarrhea, vomiting, nausea, and gastrointestinal (GI) hemorrhage (3). Any part of the alimentary tract can be affected (1). Serious cases may cause ileus, perforation and major GI hemorrhage (3,15,17). Its endoscopic features are nonspecific and highly variable, ranging from the presence of multiple deep linear ulcers to diffuse erosions and erythema (5,14,18). Pseudomembranes are also reported as rare endoscopic findings of CMV enterocolitis (4). In the present case, there was a jejunal ulcer caused by CMV, but no colic CMV lesions were observed. Jejunal CMV lesion is rare compared with colic, esophageal, or stomach lesions (9).

The pathogenesis of CMV-associated ulcerations is a complex process involving vascular endothelial CMV infection with subsequent ischemic mucosal injury (7).

Occlusion is caused by invasion of the infected endothelium into the lumen of capillaries and venules. Unfortunately, endoscopic findings, serologic analysis, and radiologic findings, including angiography are not conclusive tools for final diagnosis, and histological examination or viral culture is required.

The present case was diagnosed as CMV enterocolitis by histological examination of ulcerated lesions. Moreover, viral culture and DNA analysis was useful for the differential diagnosis (19-22).

It is suggested that he had VAHS developed by CMV. VAHS is a reactive disorder of the phagocytic system, characterized by marked hemophagocytosis (23). Proliferation of histiocytes is caused by hypercytokinemia, particularly Interleukin (IL)-2, interferon (IFN)-γ, and IL-6 which is activated by viral infection (10,24,25). Epstein-Barr virus is most common in VAHS, however, there are some reports of VAHS developed by CMV infection. Since CMV enterocolitis was not expected before the pathological examination of the resected lesion, we have insufficient data to fulfill the criteria of VAHS, but no other etiology was apparent to explain his course. There was one previous case report of CMV enterocolitis associated with VAHS in a healthy adult (11). The patient was successfully treated for DIC, pancytopenia, and enterocolitis, with antiviral agents and steroids. In our case, although there was no evidence of immunosuppression on admission, it is considered that he had systemic CMV disease, including severe enterocolitis and VAHS. He recovered after the operation without treatment with antiviral agents.

It was emphasized that CMV is a causative virus of gastrointestinal hemorrhage and VAHS, and the outcome of CMV enterocolitis and VAHS has been reported to be better in patients with no obvious evidence of immunocompromised state (10,26). The mortality rate of CMV disease is much higher in immunocompromised hosts. In our case, systemic CMV infection and VAHS in an immunocompetent individual caused severe symptoms. The therapy for CMV infection and VAHS requires further investigation.

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Original Paper

Treatment of Chronic Hepatitis C: Our Experience

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KEY WORDS: Chronic hepatitis C; Treatment; Comparison of interferon α monotherapy and interferon α/ribavirin combination

ABBREVIATIONS: Hepatitis C Virus (HCV); Hepatitis C (HC); Hepatocellular Carcinoma (HCC); Interferon α (INF α); Hepatitis C Virus Ribonucleic Acid (HCV RNA); Alanine Aminotransferase (ALT); Hepatitis A Virus (HAV); Hepatitis B Virus (HBV); Human Immunodeficiency Virus (HIV); Million Units (MU); Three Times a Week (TIW)

ABSTRACT

Background/Aims: While an optimal treatment of chronic hepatitis C has not yet been established, it has been demonstrated that the interferon α/ribavirin combination is more effective than interferon α monotherapy.

Methodology: One hundred and forty-three patients with chronic hepatitis C received the following treatment: eighty patients an 18-month monotherapy (3-month follow-up) and sixty-three patients a 12-month combined therapy (6-month follow-up). Therapeutic efficacy and adverse effects were compared.

Results: In 80 patients in the monotherapy group, complete response was achieved in 49.2%. This was reduced to 27.5% three months after therapy. Significant differences were observed in HCV 3 genotype where complete response was achieved in 12 out of 14 patients ($p=0.01$). With the combined therapy administered to 63 patients, complete response was achieved in 54.5%. This was reduced to 43.2% after 6 months of follow-up. Among the responders or partial responders, significant differences were observed with regard to age ($p=0.0047$) and subtype 1b ($p=0.012$). Comparing the groups of naive patients and relapsers, a statistically significant difference ($p=0.027$) was found in therapeutic efficacy.

Conclusions: In the treatment of chronic hepatitis C, combined therapy proved more effective than monotherapy. This is, however, not yet a satisfactory solution.

INTRODUCTION

Chronic hepatitis C (HC) presents a major health problem throughout the world (1). The hepatitis C virus (HCV) was discovered in 1989 and was classified within the *Flaviviridae* family (2). It is estimated that approximately 170 million people of the world population are infected with HCV. The average infection prevalence in Europe is 1%, ranging from 0.5% in northern European countries to 2% in Mediterranean countries. The latest studies have revealed high prevalence in eastern countries (0.7-5%) (3). The chronic form of the disease, which is non-symptomatic in the majority of cases, develops in 85% of the infected subjects. Chronic HC is an insidious disease that can have different manifestations, reaching from non-symptomatic chronic infection with normal liver enzymes to serious progressive chronic hepatitis resulting in cirrhosis and development of hepatocellular carcinoma (HCC). It is estimated in industrial countries that HCV accounts for 20% of acute hepatitis cases, 70% of chronic hepatitis cases, 40% of liver cirrhosis cases, 60% of HCC cases and 30% of liver transplant cases (4,5). Cirrhosis due to chronic HC is becoming the leading cause of liver transplant. The annual incidence of HCC is 1-4% in patients with overt cirrhosis (4-7). In the EUROHEP study (8), 384 patients with compensated cirrhosis due to HCV infection were followed for a period of five years. The five-year risk of decompensation was 18%, of HCC 17% and of death due to liver disease 9% (21% in ten years). The virus is mainly transmitted through blood and blood products. Other possible paths of transmission include sexual contacts or close contacts between family members and perinatal and nosocomial infection (9-12). Chronic HC is associated with a number of extrahepatic diseases (mixed cryoglobulinemia, membranoproliferative glomerulonephritis, panarteritis nodosa and sicca syndrome) (13).

METHODOLOGY

The prospective, comparative study was carried out to compare the efficacy of IFN α monotherapy and IFN α/ribavirin combination therapy in chronic hepatitis C.

The first group of patients treated with IFN α (Shering-Plough, USA; Hoffmann-La Roche, Switzerland) monotherapy included 80 patients (54 men and 26 women). The average age of the patients was 39 years (range 13-60). Thirty-four patients reported possible parenteral infection (23 had undergone transfusion, 4 were intravenous drug abusers, 4 healthcare workers who experienced inadvertent punctures, and 3 were tattooed). Sexual transmission was likely in 4 patients and in 1 patient intrafamilial spread was the

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Original Paper

Timing of Laparoscopic Cholecystectomy for Acute Cholecystitis with Cholecystolithiasis

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KEY WORDS: Early laparoscopic cholecystectomy; Acute cholecystitis; Hospital stay; Conversion rates; Endoscopic retrograde cholangiography (ERC)

ABBREVIATIONS: Laparoscopic Cholecystectomy (LC); Computed Tomography (CT); Ultrasonography (US); Endoscopic Retrograde Cholangiography (ERC); Magnetic Resonance Cholangiography (MRC); C Reactive Protein (CRP); Glutamate Pyruvic Transaminase (GPT); Total Bilirubin (T-Bil); Percutaneous Transhepatic Gallbladder Drainage (PTGBD)

ABSTRACT

Background/Aims: Laparoscopic cholecystectomy is now used in the treatment of acute cholecystitis. The aim of this study is to define the optimal timing for laparoscopic cholecystectomy treated with cholecystolithiasis in patients with acute cholecystitis.

Methodology: A retrospective analysis of 73 patients with acute cholecystolithiasis who were treated by either early laparoscopic cholecystectomy within 72 hours after initial onset or initial conservative treatment followed by delayed laparoscopic cholecystectomy 4 days later.

Results: There were 31 patients in the early group and 42 in the delayed group. There was no significant difference in the rate of conversion from laparoscopic to open surgery (6.4% *vs.* 20.0%), postoperative complications. However the early group had significantly shorter operation time (103 *vs.* 135 min, $p<0.01$) and shorter postoperative hospital stay (6.2 *vs.* 9.6 days, $p<0.01$).

Conclusions: We advocate early laparoscopic cholecystectomy within 72 hours of onset of symptoms to decrease conversion rates from laparoscopic to open surgery. This decreased conversion rate results in decreasing the length of operation time and postoperative and total hospital stay.

INTRODUCTION

Laparoscopic cholecystectomy (LC) has increasingly been accepted as the procedure of choice for the standard treatment of symptomatic cholecystolithiasis, gallbladder polyps, adenomyomatosis and chronic cholecystitis since its introduction in 1989 (1). A successful LC is associated with a less painful postoperative course, a lower analgesic requirement, a shorter hospital stay, and less cosmetic disfigurement (2). Recently, LC is increasingly being used as well for the treatment of acute cholecystitis (2). In the early reports, LC was considered to be a contraindication for acute cholecystitis (2), however, with increasing experience in laparoscopic surgery many surgeons have reported that LC has been used for acute cholecystitis (3). In an attempt to perform LC in acute cholecystitis, it remains controversial whether early LC should be selected or whether delayed LC should be selected after the acute inflammation has subsided. The potential advantage of early LC during the period of acute phase is associated with an earlier recovery and shorter hospital stay (4,5). On the other hand, initial conservative treatment (delayed LC) may lead to a technically easier and lower conversion rate (6). In the present study, we retrospectively compared the early and delayed laparoscopic treatment of patients with acute cholecystitis. Our assessment of the results of attempted LC for acute cholecystitis paid particular attention to the interval from the onset of symptoms to the time of operation.

METHODOLOGY

Over a 10-year period (1991-2000), 702 cholecystectomies were performed in the Second Department of Surgery at the Wakayama Medical University. Six hundred and twenty-five patients (89%) were operated laparoscopically and 77 patients (11%) by the open method. The study included 73 patients with acute cholecystitis; the diagnosis of acute cholecystitis was based on the combination of a compatible clinical sign (right upper quadrant pain and tenderness, temperature greater than 37.5°C, white blood cell count greater than 12,000/μL) and ultrasonographic evidence (presence of gallstones or debris in a thickened and edematous gallbladder, positive sonographic Murphy sign and pericholecystic fluid collections (9).

Patients were excluded from this study if they had: 1) previous upper abdominal surgery; 2) coexisting choledocholithiasis; 3) the gallbladder with perforated or penetrated other organs; or 4) performed percutaneous transhepatic gallbladder drainage (PTGBD). In the early group (n=31), LC was performed within 72 hours, whereas in the delayed group (n=42), LC was performed after conservative treatment with intravenous fluid and antibiotics for over 5 days. In both the early and delayed group, LC was performed by one surgeon (K.U) who has performed more than 500 laparoscopic cholecystectomies.

All patients were routinely examined by computed tomography (CT) and ultrasonography (US) preoperatively. We routinely performed endoscopic retrograde

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cholangiography (ERC) or magnetic resonance cholangiography (MRC) for all the patients with acute cholecystitis in the preoperative period to exclude choledocholithiasis and to show the biliary tract anatomy. Laboratory data were studied on admission and after LC; in particular, WBC, C reactive protein (CRP), glutamate pyruvic transaminase (GPT), and total bilirubin (T-Bil). Data were collected retrospectively and included operative findings, conversion to open cholecystectomy, operating time, length of postoperative stay and postoperative complications.

All the data are shown as mean ±standard deviation. Statistical analysis was performed with the chi-square test and Student's *t*-test. Probability differences of 0.05 or less were considered significant.

RESULTS

In the early group (31 patients), there were 17 males and 14 females with a mean age of 52.8 years (range 32-72). Of the 42 patients in the delayed group, there were 22 males and 20 females with a mean age of 62.8 years (range 29-74) **(Table 1)**. There was no significant difference between the gender ratio and the age of patients in both groups. In laboratory findings on admission, the values of WBC were equivalent in the two groups **(Table 1)**.

One of 31 patients (3.2%) in the early group and eight of 42 patients (19.0%) in the delayed group required conversion to open surgery ($p<0.05$) **(Table 2)**. Technical difficulties including inflammatory adhesion and uncontrolled bleeding were the main reasons for conversion. The mean duration of surgery in patients in the early group was 103±23 minutes *versus* 135±42 minutes in the delayed group ($p<0.01$). If converted patients were excluded, mean operation time in the early group was 101±20 min compared with 120±31 min in the delayed group ($p<0.01$). **Table 2** shows intraoperative accidents or complications with LC for patients with acute cholecystitis. There were no major complications during the operation such as the injury of biliary tract or other organs. There were 11 patients (35.5%) in the early group and 17 patients (40.5%) in the delayed group with intraperitoneal spillage of bile. Intraoperative blood loss was 93±56mL in the group and 138±72mL in the delayed group ($p<0.01$) and there were no cases with blood infusion.

Table 3 compares the postoperative course and outcome of the two groups. There were no major complications in the study population. Ten complications occurred in 10 patients (32.3%) in the early group, whereas 13 complications occurred in 12 patients (28.6%) in the delayed group. There was no statistically significant difference between the two groups. Common complications included disturbance of liver function, prolonged fever up (over 37.5°C, during 3 days after operation) and wound infection.

Mean preoperative hospital stay was 2.2 and 13.9 days in the early and the delayed groups, respectively ($p<0.01$) **(Table 4)**. Postoperative hospital stay was 6.2 *versus* 9.6 days in the two groups ($p<0.01$). Total hospital stay in the early group was 8.3 days and in the delayed group 22.3 days ($p<0.01$).

DISCUSSION

Laparoscopic cholecystectomy (LC) is first introduced for elective treatment of cholecystolithiasis, and acute cholecystitis is increasingly managed by LC (6,7). Acute cholecystitis had been considered to be a contraindication for LC in the past and there have been many reports advocating LC for acute cholecystitis (8-10), because the conversion to open surgery is more frequent in acute than chronic cholecystitis and the edematous and inflammatory process found at acute cholecystitis changes the vascular and biliary anatomy, sometimes leading to more complications (7). The conversion rate of LC for acute cholecystitis

TABLE 1 Background of Patients with Acute Cholecystitis

	Early group	Delayed group
No. of patients	31	42
Age Mean ±SD	52.8±15.9	56.8±18.9
(range)	(32-71)	(29-74)
Gender male/female	17/14	22/20
Data of admission WBC (/μL)	14,230±1,250	14,540±1,820

TABLE 2 Intraoperative Course of LC

	Early group (n=31)	Delayed group (n=42)
Surgical duration (min)	103±23*	135±42
Cases requiring conversion	1**	8
Surgical duration exclusion conversion cases (min)	101±20* (n=30)	120±31 (n=34)
Injury of biliary tract	0	0
Injury of other organs	0	0
Spillage of bile	11	17
Blood loss (mL)	93±56*	138±72
Blood transfusion	0	0

*Student's *t* test, $p<0.01$.
**χ^2 test, $p<0.005$.

TABLE 3 Morbidity and Mortality after LC

	Early group (n=31)	Delayed group (n=42)
Death	0	0
Bile leak	0	0
Intra-abdominal abscess	0	0
Intra-abdominal bleeding	0	0
Disorder of liver function	2	4
Prolonged fever*	6	6
Wound infection	2	3
Patients with complications (rate)	10 (32.3%)	12 (28.6%)

*over 37.5°C, during 3 days after surgery.

TABLE 4 Perioperative Hospital Stay

	Early group (n=31)	Delayed group (n=42)
Preoperative hospital stay (days)	2.2±0.6*	13.9±7.5
Postoperative hospital stay (days)	6.2±2.7*	9.6±3.2
Total hospital stay (days)	8.3±3.2*	22.3±11.9

*Student's *t* test, $p<0.01$.

has been reported to range from 6% to 38% (4-6), which is significantly higher than the less than 5% rate reported for chronic cholecystitis (7). However, some authors have reported that early LC for the treatment of acute cholecystitis has no adverse effect on complication and conversion rates (8). In the prelaparoscopic era, prospective randomized studies demonstrated that the outcome for patients undergoing early open cholecystectomy within 7 days of the onset of symptoms was superior to delayed interval surgery (11,12). Garber *et al.* recommended LC within 5 days of the onset of symptoms because of a low incidence of positive bile cultures, a negligible percentage of postoperative complications and mortality, and a short hospitalization associated with lower costs (13). Pessaux *et al.* recommend LC for acute cholecystitis within 3 days of admission because it effectively reduces the length of hospital stay (14).

In the present study, we have retrospectively analyzed 73 patients who were operated laparoscopically for acute cholecystitis. Based on the operation timing the patients were divided into two groups. The early group comprised 31 patients who underwent LC within 3 days of onset of symptoms and the delayed group consisted of 42 patients who underwent LC after more than 4 days following onset of symptoms. The conversion rate from laparoscopic to open cholecystectomy was 12.3% in 73 patients with acute cholecystitis. The conversion rate for the early group was 6.5% as compared to 16.7% for the delayed group. Conversion was significantly less frequent in patients undergoing LC within 3 days of admission (early group) compared to those undergoing surgery beyond 4 days (delayed group). The average operation time for the early group was shorter than the delayed group. Ten patients (32.3%) had postoperative minor complications; however, there were no cases of major complications including injury to the biliary system and no perioperative death. The average intraoperative blood loss in the early group was little compared with the delayed group and the average postoperative hospital stay in the early group was shorter in the delayed group. This study shows that laparoscopic cholecystectomy can be performed safely in patients with acute cholecystitis and suggests that early timing of LC within 3 days of onset of symptoms tends to reduce the conversion rate and length of procedure, as well as the total and the postoperative hospital stay. In our expression, in the early phase of acute inflammation, adhesions are easily separated, and there is usually an edematous plane around the gallbladder to facilitate dissection. After a period of conservative treatment, the inflammation and edema are replaced by fibrotic adhesions between the gallbladder and surrounding structures, which occasionally render laparoscopic dissection extremely difficult.

Some authors reported that unsuspected choledocholithiasis was detected in 4-7% in patients with acute cholecystitis (15-17). Intraoperative cholangiography is controversial because it cannot always be performed routinely without risk of biliary injury under acute processes. We performed endoscopic retrograde cholangiography (ERC) or magnetic resonance cholangiography (MRC) for all patients with acute cholecystitis at the preoperative period of LC to exclude choledocholithiasis and to show the biliary tract anatomy without intraoperative cholangiography. Indeed, there were no patients with choledocholithiasis in the present study.

In conclusion, this study supports the view that early LC within 72 hours of onset of symptoms is safer and more feasible in the treatment of acute cholecystitis. Furthermore, the early procedure provides the economic advantage of a markedly reduced postoperative and total hospital stay.

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ORIGINAL ARTICLE

Clinicopathological Features of Malignant Intraductal Papillary Mucinous Tumors of the Pancreas

The Differential Diagnosis From Benign Entities

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Background: The accurate differential diagnosis of malignant intraductal papillary mucinous tumors (IPMTs) of the pancreas from benign IPMTs remains unclear.

Hypothesis: Predictive factors for differentiating malignant IPMTs from benign IPMTs can be documented.

Design: Retrospective study (1999-2003).

Setting: Wakayama Medical University Hospital, Wakayama, Japan.

Patients: Twenty-seven consecutive patients with IPMTs (11 with adenoma, 3 with dysplasia, 5 with adenocarcinoma, and 8 with invasive adenocarcinoma) who underwent surgery were retrospectively analyzed in terms of clinicopathological features.

Main Outcome Measure: Clinical data, preoperative imaging findings, cytology, and tumor marker level, including carcinoembryonic antigen (CEA) and carbohydrate antigen (CA19-9), in serum and pure pancreatic juice.

Results: In preoperative imaging findings, the mean tumor size for the malignant IPMT group (81±18 mm) was significantly larger than that for the benign IPMT group (31±4 mm) (P=.002). The mean mural nodule size for the malignant IPMT group (9.8±4.4 mm) was significantly larger than that for the benign IPMT group (3.3±5.7 mm) (P=.002). The CEA levels in pure pancreatic juice in the malignant IPMT group (3051±7556 ng/mL) were significantly higher than in the benign IPMT group (41±80 ng/mL) (P=.003), although no significant differences in cytologic analyses and CA19-9 levels in pure pancreatic juice were found between the 2 groups.

Conclusion: Our findings suggest that tumor size larger than 30 mm, mural nodule size larger than 5 mm, and CEA levels higher than 110 ng/mL in pure pancreatic juice were predictive factors for diagnosis of malignant IPMTs.

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SELECTION OF A SURGICAL PROcedure for treating intraductal papillary mucinous tumors (IPMTs) still remains controversial, because IPMTs show a wide spectrum of histological characteristics, ranging from hyperplasia to invasive carcinoma.[1-3] IPMTs are believed to have a favorable prognosis compared with ductal cell carcinoma.[3-5] However, IPMTs have a poor prognosis when invasive carcinoma derived from IPMTs has developed.[5-8] The biologic behavior of IPMTs remains unclear. Previous studies have been performed to differentiate malignant IPMTs from benign IPMTs by retrospective investigations of clinical data, imaging findings,[1,9-15] cytologic analyses in pure pancreatic juice,[16] or molecular analysis.[17] Consequently, no consensus concerning early diagnosis of malignant IPMTs has been attained as yet. Moreover, a simultaneous analysis of these factors has not been performed, to our knowledge. We simultaneously retrospectively analyzed clinical data, imaging findings, cytologic analyses, and tumor markers in pure pancreatic juice. The aim of the present study was to determine preoperative factors that are predictive for the early diagnosis of malignant IPMTs.

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METHODS

From January 1, 1999, to May 31, 2003, 27 patients with IPMTs were treated at Wakayama Medical University Hospital, Wakayama, Japan. Clinicopathologic data were reviewed to determine the age, sex, symptoms, and presence of other pancreatic disease. All patients underwent ultrasonography (US), computed tomography (CT), endoscopic US, and endoscopic retrograde pancreatography (ERP) for

preoperative imaging diagnosis. These imaging data were reviewed with respect to tumor location, tumor size, the largest diameter of the main pancreatic duct (MPD), and the presence of mural nodule. We retrospectively compared the preoperative imaging diagnostic data with histopathological findings to obtain sensitivity, specificity, and accuracy.

During ERP, brushing for cytologic analysis was performed, and then pancreatic juice was immediately collected for 10 minutes with a catheter inserted into the pancreatic duct after an intravenous injection of 50 U per body of secretin (Eisai Co Ltd, Tokyo, Japan). A sample was immediately put on ice and divided into 2 sections: one for cytologic examination and the other for measurement of carcinoembryonic antigen (CEA) and carbohydrate antigen (CA19-9) levels. The cytologic samples of collected pancreatic juice were immediately put on ice after adding heparin. After pancreatic juice was centrifuged, the cell pellet was smeared on glass slides, fixed in 95% ethanol, and stained with the Papanicolaou technique. For measurement of CEA and CA19-9 levels, the pancreatic juice was centrifuged and the supernatants were measured with CEA or CA19-9 immunometric chemiluminescent assay kit (Bayer Medical Co, Tokyo, Japan) according to the manufacturer's instructions. Serum levels of CEA and CA19-9 were also measured.

The resected pancreas specimens were fixed with 10% formaldehyde solution and serially cut into 5-mm intervals and embedded in paraffin. All tissue sections were stained with hematoxylin-eosin. Then all slides of the resected pancreas were reviewed by independent pathologists. Histologically, 14 patients had benign IPMTs (11 adenomas, including 2 MPD type, 5 branch duct type, and 4 combined type, and 3 dysplasias), and 13 patients had malignant IPMTs (5 adenocarcinomas, including 1 branch duct type and 4 combined type, and 8 invasive adenocarcinomas, including 2 MPD type and 6 combined type) according to the classification by Japan Pancreas Society.[18] We retrospectively reviewed clinicopathologic and preoperative imaging diagnostic data to identify indicative signs of malignant IPMTs.

The optimal cutoff levels for tumor size, mural nodule size, and CEA in pure pancreatic juice for differentiation between benign and malignant IPMTs were sought by constructing receiver operating characteristic (ROC) curves, which were generated by calculating the sensitivities and specificities of tumor size, mural nodule size, and CEA data at several predetermined cutoff points.[19] All values are expressed as mean±SD. The χ^2 test was performed to assess the difference in accuracy of imaging studies and to calculate differences in cases with and without mural nodule between benign and malignant IPMTs. All other statistical analyses were performed with the Mann-Whitney *U* test. Statistical significance was defined as $P<.05$.

RESULTS

CLINICAL CHARACTERISTICS

Clinical characteristics of the enrolled patients are given in **Table 1**. There were no significant differences in age, sex, and symptoms between the patients with benign and malignant IPMTs. Fourteen (52%) of 27 patients were asymptomatic. The reasons for performing imaging studies in asymptomatic patients were as follows: follow-up of other diseases, including diabetes mellitus, in 3 benign IPMT patients; annual medical examinations in 6 benign IPMT patients; other diseases in 4 malignant IPMT patients; and annual medical examinations in 1 malignant IPMT patient. The main symptom was pain in 11 (41%) of 27 patients. Only the malignant IPMT group experienced weight loss (15%). Twenty (74%) of 27 patients had no history of pancreatic disease. Seven (26%) of 27 patients had diabetes mellitus or chronic pancreatitis.

Table 1. Clinical Characteristics of 27 Patients With Intraductal Papillary Mucinous Tumors (IPMTs) of the Pancreas

Characteristic	Benign IPMT Group (n = 14)	Malignant IPMT Group (n = 13)
Age, mean ± SD, y	65 ± 3	72 ± 1
Sex ratio, M:F	1.3:1	1.7:1
Symptoms, No. (%)		
Asymptomatic	9 (64)	5 (38)
Abdominal pain	5 (36)	6 (46)
Weight loss	0	2 (15)
Pancreatic disease, No. (%)		
None	11 (79)	9 (69)
Diabetes mellitus	1 (7)	3 (23)
Chronic pancreatitis	2 (14)	1 (8)

PREOPERATIVE IMAGING FINDINGS

The number of IPMTs located in the total pancreas was 4 (31%) of 13 in the malignant IPMT group and none in the benign IPMT group. Tumors were located in the pancreatic head in 6 patients in both the benign IPMT group and the malignant IPMT group and in the pancreatic body (tail) in 8 patients in the benign IPMT group and 3 in the malignant IPMT group. The dilatation of MPD measured by ERP was 7.8±6.5 mm in the benign IPMT group and 10.7±6.5 mm in the malignant IPMT group. There were no significant differences in location and dilatation of MPD between the benign and malignant IPMT groups. However, the mean tumor size of 81±18 mm in the malignant IPMT group was significantly larger than that of 31±4 mm in the benign IPMT group ($P=.002$). The mural nodules were present in 6 patients with benign IPMTs and 12 patients with malignant IPMTs. There was a significant difference between the benign IPMT group and the malignant IPMT group related to the presence of mural nodule ($P=.007$). In addition, the mean mural nodule size of 9.8±4.4 mm in the malignant IPMT group was significantly larger than that of 3.3±5.7 mm in the benign IPMT group ($P=.002$). Sensitivity, specificity, and accuracy for differentiating benign from malignant IPMTs were 75%, 50%, and 62% by US; 92%, 85%, and 89% by CT; 91%, 64%, and 78% by endoscopic US; and 91%, 57%, and 73% by ERP, respectively. The accuracy rate by CT was higher than that of US ($P=.009$).

CYTOLOGIC ANALYSIS OF PURE PANCREATIC JUICE

Brushing for cytologic analysis was performed. A total of 20 mL of pure pancreatic juice was collected in 23 patients by intravenous injection of secretin. The cyto-

Table 2. Comparison of Cytologic Analyses of Pure Pancreatic Juice Between Benign and Malignant Intraductal Papillary Mucinous Tumor (IPMT) Groups

Class	Benign IPMT Group (n = 11)	Malignant IPMT Group (n = 12)
I	0	1
II	7	7
III	3	2
IV	1	1
V	0	1

logic specimen of pure pancreatic juice after brushing could be examined in 11 patients in the benign IPMT group and 12 patients in malignant IPMT group (**Table 2**). In the remaining 4 patients, deep cannulation for brushing for cytologic examination could not be performed or a sufficient amount of sample of pure pancreatic juice could not be collected for cytologic analysis. Two (17%) of 12 patients with malignant IPMTs were diagnosed as having a malignancy (class IV or V), and 10 (91%) of 11 patients with benign IPMTs were diagnosed as having benign disease (class I, II, or III). One patient diagnosed as having a cytologic malignancy in the benign IPMT group had histologic dysplasia and premalignant lesions.

TUMOR MARKERS IN PURE PANCREATIC JUICE AND SERUM

Both CEA and CA19-9 levels in pancreatic juice could be measured in 11 patients in the benign IPMT group and 9 patients in the malignant IPMT group. In the other 7 patients, a sufficient amount of supernatant could not be collected after the centrifugation of pancreatic juice. Serum CEA and CA19-9 levels could also be measured in all patients. Carcinoembryonic antigen levels in pancreatic juice in the malignant IPMT group (3051±7556 ng/mL) were significantly higher than those (41±80 ng/mL) in the benign IPMT group ($P=.003$). However, we found no significant differences between the benign and malignant IPMT groups in serum CEA levels (1.8±1.2 ng/mL vs 3.0±2.4 ng/mL), CA19-9 levels in pancreatic juice (412±416 ng/mL vs 9630±13511 ng/mL), and serum CA19-9 levels (18±35 ng/mL vs 262±777 ng/mL).

PREDICTIVE FACTORS FOR MALIGNANT IPMT DIAGNOSIS

This study clarified that predictive factors for differentiating benign IPMTs from malignant IPMTs were tumor size, mural nodule size, and CEA levels in pure pancreatic juice. The ROC curves for tumor size, mural nodule size, and CEA levels of pure pancreatic juice are presented in **Figure 1**. With regard to the tumor size and mural nodule size by preoperative imaging findings, diagnostic cutoff levels for differentiation between benign IPMTs from malignant IPMTs were 30 and 5 mm, respectively (**Figure 2**). The sensitivities of the cutoff line in tumor size and mural nodule were 92% and 85%, respectively, and the specificity of the cutoff line was 71% for both. Therefore, the accuracies were 81% and 81%, respectively. The cutoff level of CEA in pure pancreatic juice was at 110 ng/mL for differentiation between benign and malignant IPMTs (**Figure 3**). The sensitivity of the CEA cutoff line was 78%, the specificity was 91%, and the accuracy was 80%.

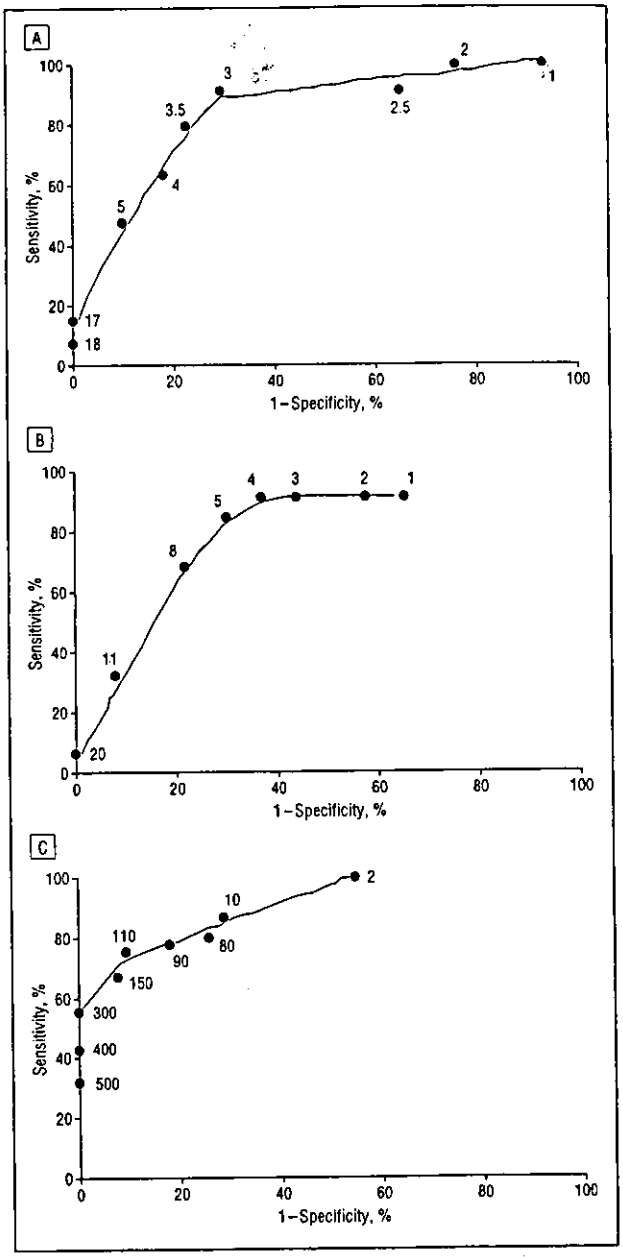

Figure 1. Receiver operating characteristic curve of tumor size (A), mural nodule size (B), and carcinoembryonic antigen level of pure pancreatic juice (C) for estimation of a cutoff level differentiating malignant from benign intraductal papillary mucinous tumors.

COMMENT

The early diagnosis of malignant IPMTs is important for improving prognosis. Invasive carcinoma derived from IPMTs has been reported to be the most important factor that influences survival in patients with IPMTs and