Cancer Chemother Pharmacol (2008) 61:875–882
DOI 10.1007/s00280-007-0567-8

ORIGINAL ARTICLE

Effects of tumor selective replication-competent herpes viruses in combination with gemcitabine on pancreatic cancer

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Received: 29 April 2007 / Accepted: 25 July 2007 / Published online: 29 August 2007

Abstract
Purpose Pancreatic cancer still has a poor prognosis, even if aggressive therapy is pursued. Currently, new modalities of oncolytic virus therapy are being tested against this cancer. The combination of one of two representative mutant herpes simplex viruses (R3616: $\gamma_1$34.5 inactivated, hrR3: UL39 inactivated) with a standard anti-pancreatic cancer chemotherapy drug (gemcitabine), was investigated in this study.
Experimental design The intracellular concentration of ribonucleotide reductase was estimated by Western blotting. The effect of gemcitabine on viral replication and the total cytotoxic effect of the combination therapy were investigated on pancreatic cancer cell lines. We compared the results of two oncolytic viruses, R3616 and hrR3. A mouse model of pancreatic cancer with peritoneal dissemination was used to evaluate the in vivo effect of the combination therapy.
Results Although the replication of both viruses was inhibited by gemcitabine, the combination caused more tumor cell cytotoxicity than did virus alone in vitro. The results with R3616 were more striking. Although the difference was not statistically significant, R3616 with gemcitabine had a greater effect than did R3616 alone, while hrR3 with gemcitabine had a weaker effect than did hrR3 alone in vivo experiments.
Conclusion The combination of oncolytic virus with gemcitabine is a promising new strategy against advanced pancreatic cancer. Each virus has different functional characteristics, and can affect the results of the combination of viruses and chemotherapy drugs. The results indicate that there is a complicated interaction among viruses, cells, and chemotherapy drugs and that the best combination of oncolytic virus and chemotherapeutic agents should be studied more extensively before embarking on a clinical trial.

Keywords Herpes oncolytic virus · Pancreatic cancer · Gemcitabine · Combination therapy

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Introduction

Pancreatic cancer is a disease with an extremely poor prognosis. Surgical therapy for pancreatic cancer is still insufficient to cure most patients [1]. Recently gemcitabine has shown a modest survival advantage over 5-fluorouracil (5-FU) in patients with this cancer [2]. Gemcitabine has become one of the standard chemotherapy drugs against pancreatic cancer but more effective therapies must be devised in order to significantly improve survival. Oncolytic virus therapy has been highly trusted as a new type of therapy for advanced incurable pancreatic cancer, and may provide some clinical benefit to those patients in the near future. Currently, clinical trials using oncolytic viruses have been started against many types of cancer in world-wide [3], such as brain cancer [4, 5], prostate cancer [6, 7], pancreatic cancer [8], breast cancer [9], and head and neck cancer [10, 11]. This study investigated the possibility of combination therapy using gemcitabine and two herpes mutant oncolytic viruses (R3616 and hrR3) against pancreatic cancer.

Gemcitabine (difluorodeoxycytidine; dFdC) is intracellularly phosphorylated to difluorodeoxycytidine diphosphate (dFdCDP) and difluorodeoxycytidine triphosphate

(dFdCTP). dFdCTP competes with deoxycytidine triphosphate (dCTP) for incorporation into DNA, and DNA synthesis is inhibited [2, 12, 13]. In addition, dFdCDP acts as an inhibitor of ribonucleotide reductase (RR) in cells, which in turn causes a major decrease in the dCTP pool. Therefore, gemcitabine reduces the activity of RR in cancer cell lines [14] (Fig. 1a). However, some cells have been known to acquire chemoresistance to gemcitabine due to over expression of RR [15–21].

R3616 and hrR3 are genetically engineered herpes simplex viruses [3]. R3616 lacks the $\gamma_1$34.5 gene that produces the ICP34.5 protein. Replication of R3616 is severely restricted in normal cells, because the expression of ICP34.5 in normal cells prevent a protein shutoff mechanism that is associated with eIF2α dephosphorylation through the protein kinase receptor (PKR). Most cancer cells lose this normal protein shutoff mechanism so that viral replication can proceed, which induces the virally infected cells to undergo apoptosis to protect the integrity of the cell's DNA and block viral replication [3, 22–24]. hrR3 lacks the UL39 gene that produces the ICP6 proteins (viral RR), a key enzyme in the biosynthesis of DNA in all prokaryotic and eukaryotic cells. The viral replication of hrR3 is severely restricted in cells that have high levels of holding proteins involved in nucleic acid synthesis such as cancer cells [25, 26] (Fig. 1b).

We investigated the effect of tumor-selective, replication-competent herpes viruses (R3616 and hrR3) against pancreatic cancer under the same conditions in which gemcitabine effects cancer cells. Our major concern was how gemcitabine may interrupt viral replication, and whether the combination of an oncolytic virus with gemcitabine can significantly improve anti-pancreatic cancer therapy.

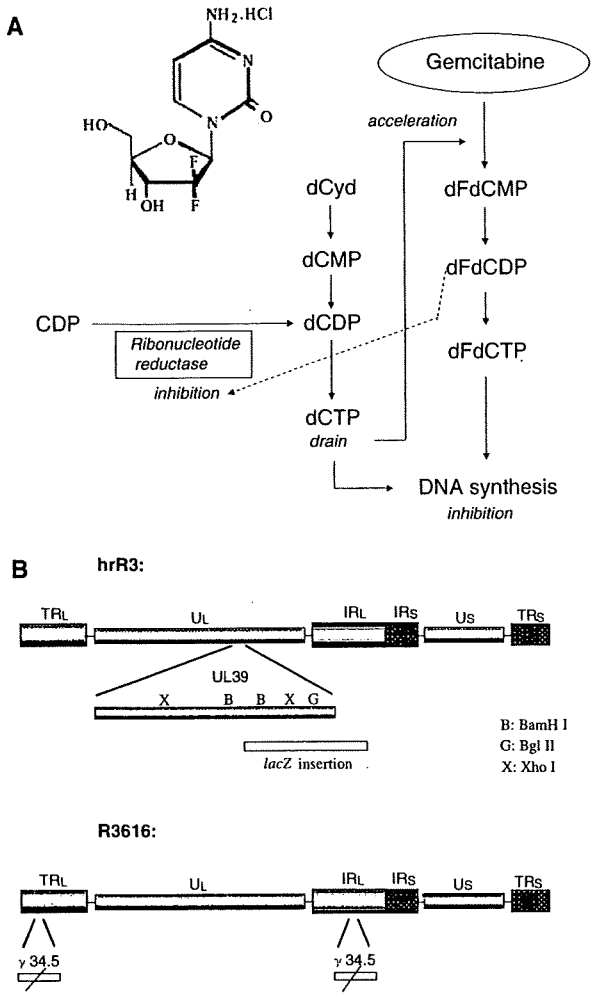

Fig. 1 **a** Gemcitabine structure and pathway. Gemcitabine HCl is a nucleoside analog that exhibits anti-tumor activity. Gemcitabine HCl is 2′-deoxy-2′, 2′-difluorocytidine monohydrochloride (β-isomer). The empirical formula for gemcitabine HCl is C9H11F2N3O4 × HCl. It has a molecular weight of 299.66. Gemcitabine is metabolized intracellularly by nucleoside kinases to the active diphosphate (dFdCDP) and triphosphate (dFdCTP) nucleosides. **b** Schematic illustration of hrR3 and R3616. hrR3 is a mutated herpes simplex virus (*HSV*) that has the LacZ gene inserted into the site of UL39 (ICP6), causing inactivation of ribonucleotide reductase activity that is associated with UL39. Ribonucleotide reductase is a key enzyme for viral DNA synthesis. R3616 is a mutated HSV that has a deletion of both $\gamma_1$34.5 genes. The $\gamma_1$34.5 gene produces ICP 34.5 that dephosphorylates eIF2α-phosphate to permit continued viral protein synthesis. Those mutated HSVs replicate and destroy only the cancer cells

Materials and methods

Viruses and cells

R3616 was kindly provided by Bernard Roizman Sc. D (University of Chicago, Chicago, IL, USA) and hrR3 was kindly provided by Sandra K. Weller Ph.D. (University of Connecticut, Storrs, CT, USA). SW1990, derived from a human pancreatic carcinoma, was kindly provided by Dr. T. Sawada (First Department of Surgery, Osaka City University, Osaka, Japan). CAPAN 1, also derived from a human pancreatic carcinoma, was obtained from the Japanese Cancer Research Resources Bank, Tokyo, Japan. PACA2, another cell line derived from a human pancreatic carcinoma, was obtained from the American Type Culture Collection, Manassas, VA, USA. Cells were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum and 1% penicillin/streptomycin at 37°C (Sigma, Tokyo, Japan).

Western blot assay

A total of 10^6 cells were harvested and rinsed twice with phosphate-buffered saline, pH 7.4. Cell extracts were prepared with lysis buffer (20 mM Tris, pH 7.5, 0.1% Triton-X, 0.5% deoxycholate, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, and 10 μg/ml leupeptin) and clarified by

centrifugation at 12,000*g*, for 15 min, at 4°C. Cell lysates containing equal amounts of protein as determined by a BCA assay kit were electrophoresed on a NuPAGE, Novex 4–12% Bis-Tris Gel (Invitrogen, Carlsbad, CA, USA), and the resolved proteins were transferred to PVDF membranes (Invitrogen).The membranes were blocked with 5% nonfat milk overnight at room temperature, and incubated with 0.2 μg/ml human anti-RRM1 antibody (CHEMICON International, Temecula, CA, USA) for 1 h. RRM1 was detected using an enhanced chemiluminescence (ECL) system following the manufacturer's instructions (Amersham Life Science, Uppsala, Sweden). β-actin also was detected on the same membrane to serve as a control for the amount of protein loaded.

Cytotoxic assay

Gemcitabine and viral-induced cytotoxicity assays were performed using the MTT assay as previously described [27, 28]. Briefly, 10^6 cells were plated in a 10-cm plate and 10 μg/ml of gemcitabine was added. After 24 h, a replication-competent virus (R3616 or hrR3) was added at multiplicity of infection (MOI) values ranging from 0.01 to 10 and incubated for an additional 48 h. The number of surviving cells was quantified by a colorimetric MTT assay. The results, expressed as mean ± SD of four samples, were compared with the results from the cytotoxicity assays of gemcitabine alone and the virus alone. Statistical significance was determined by the two-sided Student's *t*-test using SPSS (SPSS, Chicago, IL, USA).

Viral replication assay

Viral replication assays were performed as described [28, 29]. Briefly, 10^6 cells were plated in a 10-cm plate and 10 μg/ml of gemcitabine was added. After 24 h, replication competent viruses (R3616 or hrR3) were added at MOI of 2. Forty-eight hours after infection, the supernatant and cells were harvested, exposed to three freeze- thaw cycles to release the virions, and titered. The results were compared with the assays of viral replication without gemcitabine.

Animal studies

Mice (6-week-old females BALB/c nu/nu) were obtained from the Charles River Japan, Yokohama, Japan. Animal studies were performed in accordance with the guidelines issued by the Nagoya University Animal Center. The mice, used in a peritoneal-disseminated carcinoma model, were injected with 10^6 PACA2 cells into the intraperitoneal cavity. The condition of the animals was checked once or twice a day for the duration of the study. The mice were divided randomly into six groups (A–F). Group A ($n = 10$), group D ($n = 10$), and group E ($n = 10$) were injected with 1 mg of gemcitabine into the intraperitoneal cavity on day 14 after the injection of the PACA2 cells. The mice in groups A and B ($n = 10$) each were injected with 10^6 particles of R3616 on day 15 after the injection of the PACA2 cells. Group C ($n = 10$) and group D ($n = 10$) were injected with 10^6 particles of hrR3 on day 15 after the injection of the PACA2 cells. Group F ($n = 10$) was the control group, which was injected with only PACA2 cells into the intraperitoneal cavity.

Statistical differences between groups were determined by the log-rank test with the use of JMP 5.0 software (SAS Inc., Cary, NC, USA). $P < 0.05$ was considered statistically significant.

Results

Expression of RRM1 by Western blotting

As previously reported by many researchers on their papers, overexpression of ribonucleotide reductase subunit 1 (RRM1) is associated with chemoresistance to gemcitabine [15–17]. We examined the intensity of RRM1 protein expression in Capan1, PACA2, and SW1990 cells (Fig. 2). The intensity of RRM1 expression in the PACA2 cells was greater than in the other cell lines. The results from many previous related papers regarding chemoresistance to gemcitabine, indicated that PACA2 cells might have the highest potential of chemo resistance to gemcitabine among the three cell lines.

Comparison of cytotoxic assays between hrR3 and R3616, with or without gemcitabine

We compared the cytotoxicity of R3616 ($\gamma_1$34.5 deficiency) and hrR3 (ICP6: RR gene deficiency) viruses' combination with gemcitabine by the MTT assay (Fig. 3). With both R3616 and hrR3, the cytotoxicity was increased by their

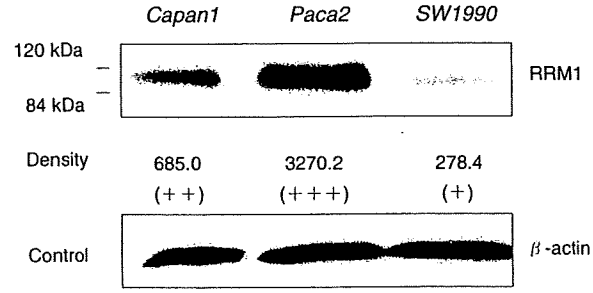

Fig. 2 Expression of ribonucleotide reductase M1 (*RRM1*) by Western blotting. PACA2 cells expressed the most ribonucleotide reductase M1 (*RRM1*) by Western blot assays among three pancreatic cancer cell lines tested. β-actin served as a control for the amount of protein loaded in each lane

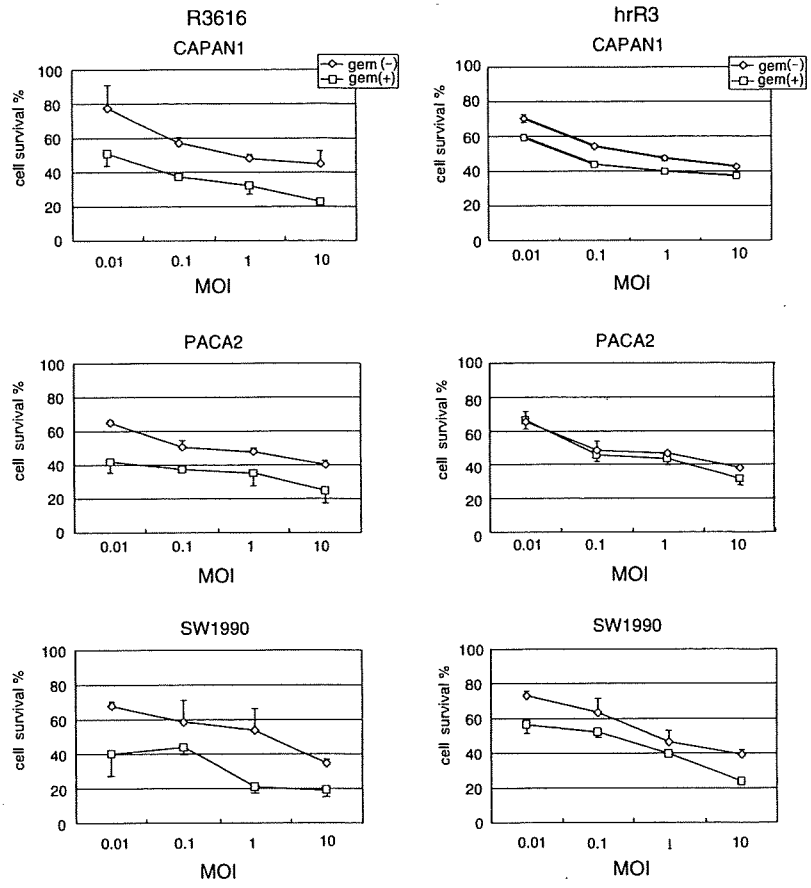

Fig. 3 Comparison of cytotoxic assays between hrR3 and R3616, with or without gemcitabine. For both R3616 ($\gamma_1$34.5 gene inactivated) and hrR3 (ICP6: ribonucleotide reductase gene inactivated), the cytotoxity was increased when combined with gemcitabine. However, there was a trend toward greater cytotoxity with R3616 than with hrR3 against all cell lines

combination with gemcitabine, but the more significant increase in cytotoxicity was observed with R3616 than hrR3. On the other hand, PACA2 cells, which expressed the most RRM1 by a Western blot assay, had the lowest increase in cytotoxicity with the combination of hrR3 and gemcitabine.

Comparison of cytotoxic assays between gemcitabine alone and gemcitabine with low titer virus

We also compared the cytotoxicity between gemcitabine alone and gemcitabine with low titer virus by the MTT assay (Fig. 4). Of all cell lines, the combination of gemcitabine and an MOI 0.01 of R3616 showed more cytotoxic tendency than did gemcitabine alone ($P = 0.04$ on PACA2 cell line), while the combination of gemcitabine with an MOI 0.01 of hrR3 tend to be less cytotoxic than gemcitabine alone. PACA2 cells.

Comparison of viral replication between hrR3 and R3616, with or without gemcitabine

We compared the viral replication between R3616 and hrR3 in the presence of gemcitabine by the plaque-forming assay (Fig. 5). The replication of both viruses was inhibited by gemcitabine. The titer of hrR3 declined more than did R3616 in combination with gemcitabine. The replication of hrR3 was inhibited by gemcitabine in all cell lines. PACA2 cells expressed the most RRM1 by Western blot assay, and hrR3 replicated more vigorously with gemcitabine in the PACA2 cells than in the other two cell lines, while R3616 was also inhibited by gemcitabine in all cell lines but with somewhat weaker inhibition comparing to hrR3.

Animal studies

Long-term survival (LTS: 100 days) was achieved in 60% of mice treated with an intraperitoneal injection of R3616 followed by gemcitabine (group A). Mice treated with an intraperitoneal injection of R3616 had only a 50% LTS (group B). Mice treated with hrR3 had a 30% LTS (group C). Mice treated with hrR3 followed by gemcitabine had a 20% LTS (group D). Mice treated with gemcitabine alone had only a 10% LTS (group E). All mice in the control group died within 60 days (group F) (Fig. 6). Statistical differences in the survival rates were determined by log-rank analyses (group A versus group F, $P = 0.0011$; group A versus group D, $P = 0.0078$; group E versus group F,

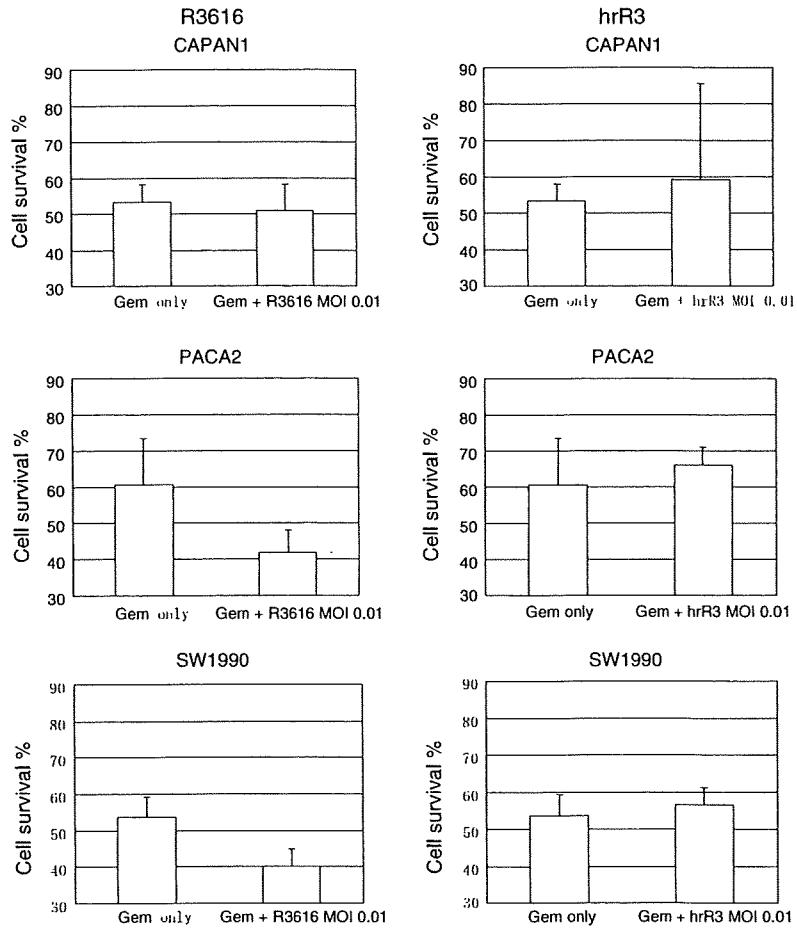

Fig. 4 Comparison of cytotoxic assays between gemcitabine alone and gemcitabine with a very low titer of virus. For all cell lines, the combination of gemcitabine and a very low titer (MOI 0.01) of R3616 tended to exhibit greater cytotoxicity than did gemcitabine alone ($P = 0.04$ on PACA2 cell line), on the other hand, the combination of gemcitabine and a very low titer (MOI 0.01) of hrR3 tended to be less cytotoxic than gemcitabine alone

$P = 0.006$; group B versus group D, $P = 0.0174$; group B versus group F, $P = 0.0049$). There were no other statistically significant differences between the other groups except for shown above. Although it was not significantly different, R3616 with gemcitabine tended to have a stronger effect than did R3616 alone, while hrR3 with gemcitabine tended to be weaker than hrR3 alone.

Discussion

In this study, we compared the efficacy of hrR3 or R3616 plus gemcitabine against pancreatic cancer. An in vitro cytotoxic assay indicated that R3616 plus gemcitabine caused a significant increase in the cell-killing effect in all three pancreatic cancer cell lines than did hrR3. We postulate that this result was due to the functional differences caused by each deleted viral gene. The viral replication of hrR3 might be more interrupted by the effect of gemcitabine than that of R3616, and this reduction might have been responsible for the slight decrease in the cell-killing effect of hrR3. Cellular RR is important for viral replication especially for hrR3 that has no RR [3, 25–27, 29, 30]. Gemcitabine is well known to reduce the activity of cellular RR in cancer cell lines [14]. Therefore, it is a possible that the effect of gemcitabine was greater in combination with hrR3 than with R3616 reducing the replication and cytotoxicity of the viruses.

Interestingly, infection with hrR3 at a very low concentration (MOI 0.01) in the presence of gemcitabine caused less cytotoxic than did gemcitabine alone. This may be the result of the virus protecting the cancer cells from the apoptosis caused by gemcitabine. The virus itself has some anti-apoptotic effects on cells in order to protect the host cells from bursting too early and until the virus particles have matured. Although gemcitabine reduced the replication of hrR3, some viral anti-apoptosis genes might still have worked in the infected cells without the burst-cell effect that is caused by an abundance of mature viruses. The apoptosis mechanism might malfunction as a result of this low virus concentration, causing an anti-apoptotic effect against gemcitabine. This effect might apply not only to HSV, a critical

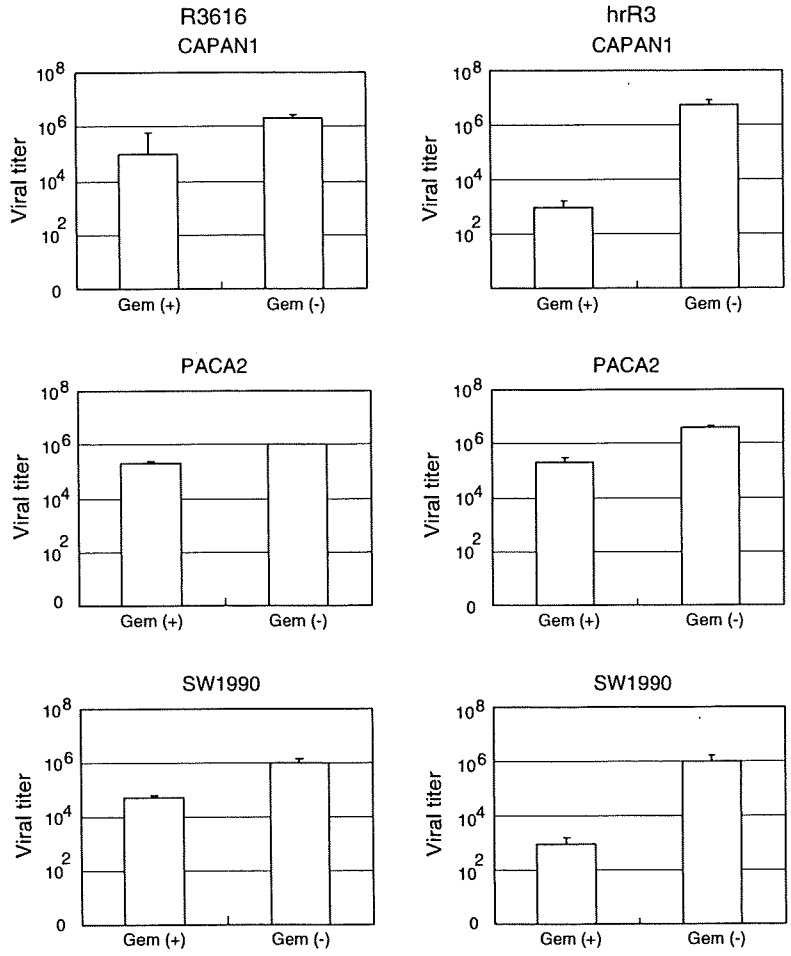

Fig. 5 Comparison of viral replication between hrR3 and R3616, with or without gemcitabine. The viral replication of both R3616 and hrR3 were inhibited by the presence of gemcitabine. This phenomenon was more prominent with hrR3 and gemcitabine than with R3616

consideration when using a viral vector or an oncolytic virus with chemotherapy drugs, because most viruses have such an anti-apoptosis gene. Examples include US3 and US5 in HSV [31, 32], and E1b 19 kDa in adenovirus [33]. Furthermore, several distinct viruses have been shown to develop mechanisms to block premature apoptosis of infected cells [34–36]. This phenomenon should be considered when using any viral vector for gene therapy or oncolytic virus therapy with chemotherapy drugs. In our opinion, the anti-apoptosis genes in a virus should be studied more intensively if future development of oncolytic virus therapy is to proceed.

PACA2 cells had the highest density of RR by Western assays and also the lowest cytotoxic effect from single agent gemcitabine among the three pancreatic cancer cell lines tested as 60% cell survival in Fig. 4, which indicates that PACA2 cells have some type of resistance against the cytotoxicity of gemcitabine comparing to other two cell lines. For the combination of R3616 with gemcitabine, increased efficacy was observed against all the pancreatic cancer cell lines even if the cells had some resistance to the chemotherapy alone. On the other hand, the combination of hrR3 with gemcitabine was of weak cytotoxity toward PACA2 cells, which expressed the most RRM1 by Western blot assay, and the effect was less pronounced than when R3616 was used. These results suggest that, the combination of R3616 and gemcitabine might be suitable for the cancer cell type that is expected to offer resistance to gemcitabine.

In the in vivo experiments, the combination of R3616 with gemcitabine yielded a 60% LTS rate (100 days) in the mice. This was higher than in mice treated with an intraperitoneal injection of R3616 alone that resulted in a 50% LTS rate, while mice treated with only hrR3 had a 30% LTS rate; however, there was no statistically significant difference in the LTS rate between R3616 and R3616 with gemcitabine. Thus, combination therapy with R3616 and gemcitabine had the same or slightly higher efficacy than the virus alone. However, mice treated with hrR3 followed by gemcitabine showed a lower LTS rate (20%) than those treated with hrR3 alone. And moreover, there was a statistically significant difference between group A (R3616 + GEM) and group D (hrR3 + GEM) ($P = 0.0078$). From the results of our in vivo and in vitro, we determined that the

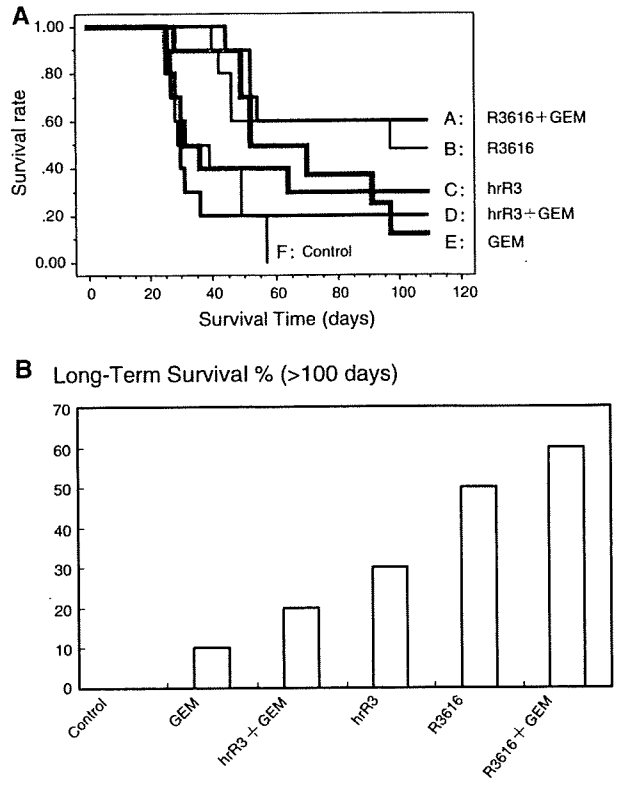

Fig. 6 **a** Cumulative survival curves of an in vivo mouse model. The PACA2 cells were injected into the peritoneal cavity. Each group was treated as shown below. Group A: R3616 + gemcitabine (GEM), group B: R3616 only, group C: hrR3 only, group D: hrR3 + GEM, group E: GEM only, and group F: no treatment. Differences in the survival rates were assessed by log-rank analysis (group A versus group F, $P = 0.0011$; group A versus group D, $P = 0.0078$; group E versus group F, $P = 0.006$; group B versus group D, $P = 0.0174$; and group B versus group F, $P = 0.0049$). There were no other statistic ally significant differences between the other groups except for shown above. There was a statistically significant difference between group A (R3616 + GEM) and group D (hrR3 + GEM) ($P = 0.0078$). **b** Long-term survival (*LTS*: over 100 days). LTS was achieved in 60% of mice treated with an intraperitoneal injection of R3616 followed by gemcitabine (group A). Mice treated with an intraperitoneal injection of R3616 only had a 50% LTS survival rate (group B). Mice treated with hrR3 only had a 30% LTS rate (group C). Mice treated with hrR3 followed by gemcitabine had a 20% LTS rate (group D). Mice treated with gemcitabine alone had only a 10% LTS rate (group E). The untreated group (group F) had a 0% LTS survival rate

combination of gemcitabine with R3616 ($\gamma_1$34.5 inactivated) might be more effective than the combination with hrR3 (RR inactivated).

Potentially, chemotherapy drugs connote to inhibit oncolytic virus replication to some degree, but this effect may be influenced by the differences in the characteristics of each virus caused by gene mutation. UL 39 (ICP6)-deleted HSVs, such as G207 [3, 37], and Myb34.5 [38, 39] also have some kind of potential likely to be inhibited by gemecitabine, as is hrR3, because of the genetic characteristics of RR. In other words, UL39-intact HSVs, such as HF10 [40, 41], RH105 [42], and DFγ34.5 [43] are likely to interact differently from a UL 39-deleted HSV (e.g., hrR3), in combination therapy with gemcitabine. Additional studies must be needed for further confirmation of the efficacy depending upon the functional characteristics among the chemotherapy drugs, viruses, and the cancer cells.

In the future, oncolytic virus therapy in combination with chemotherapy drugs may become more popular for use in clinical trials. Therefore, the characteristics of each virus must be considered carefully to determine if they are suitable for use with the chemotherapy drugs chosen.

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Pancreatic Cancer With Paraaortic Lymph Node Metastasis

A Contraindication for Radical Surgery?

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Objectives: The purpose of this study was to determine the operative indications for pancreatic cancer with paraaortic lymph node metastases (No. 16 [+]).

Methods: Between July 1981 and March 2007, 335 patients with pancreatic cancer including 45 No. 16 (+) patients underwent extended radical surgery at the Department of Surgery II, Nagoya University. The overall survival rates and clinicopathological parameters were analyzed using univariate and multivariate analyses.

Results: Although there was no significant difference in survival between the No. 16 (+) patients and the unresectable cases, there were some long-term survivors among the No. 16 (+) patients. Multivariate analysis of the No. 16 (+) patients identified age (59 years or younger), tumor size (>4 cm), and pathologically confirmed portal invasion (pPV[+]) as independent prognostic factors. The survival of No. 16 (+) patients without these factors was significantly better than the unresectable cases. The survival of patients with only 1 metastatic paraaortic lymph node also was significantly better than the unresectable cases, and tended to be better than those with more than 2 metastatic nodes.

Conclusions: No. 16 (+) pancreatic cancer patients with age 60 years or older, tumor size 4 cm or less, and pPV(−) may benefit from resection.

Key Words: pancreatic cancer, paraaortic lymph node, indication

(*Pancreas* 2009;38: e13–e17)

Pancreatic cancer continues to have the worst prognosis of all the gastrointestinal malignancies, and the actual 5-year survival rate after a curative resection reportedly ranges from 6.8% to 19.8%.[1–5] Surgical resection remains the only chance for cure, although a moderate improvement in outcome has been achieved through a gradual increase in the resection rate and a decline in the surgical mortality after pancreatoduodenectomy that currently ranges from 1% to 5.4%.[1–5]

Lymph node involvement is one of the most important prognostic factors for gastrointestinal cancer, including pancreatic cancer. Paraaortic lymph nodes (No. 16 nodes) are considered to be the final nodes for periampullary and gastric cancers before the cancer enters the systemic lymphatic circulation. Metastases to the No. 16 nodes (No. 16 [+]) are observed commonly among patients with carcinoma of the head of the pancreas,[6–9] and anatomic or clinical studies detailing the patterns of lymphatic flow from the pancreas to the No. 16 nodes have been reported.[6,7] Some consider these nodes to be regional lymph nodes and dissect them as a part of a routine lymphadenectomy for pancreatic cancer. Others believe that metastases to these nodes represent systemic disease and recommend that radical surgery including extended lymphadenectomy should be abandoned for No. 16 (+) patients.[10–12] Although the optimal extent of lymphadenectomy for pancreatic cancer thus remains a matter of controversy, there is growing skepticism as to the survival benefit of extended lymphadenectomies in general, and the authors share the opinion that systematic dissection of all No. 16 nodes may not be beneficial when performed routinely for all patients with pancreatic cancer.[13] On the other hand, we have encountered some No. 16 (+) patients who have experienced long-term survival after an extended nodal resection, suggesting that this procedure may have value for a selected population of patients, the identification of which is the aim of current study.

MATERIALS AND METHODS

Patients Selection and Study Design

Between July 1981 and March 2007, 511 patients with pancreatic cancer underwent surgery at the Department of Surgery II, Nagoya University. Three hundred thirty-five patients had extended radical surgery with systematic lymph node dissection, including regional and No. 16 lymph nodes, whereas 176 patients were deemed unresectable because of macroscopic hepatic metastases, macroscopic peritoneal metastases, or extensive local invasion. The cohort of resected pancreatic cancer patients included 222 men and 113 women, with a median age of 62.2 years (range, 35–83 years). All patients were followed until death or through March 2007. Tumor location included the head of the pancreas (n = 258), the body of the pancreas (n = 68), and the entire pancreas (n = 9). One hundred sixty-one pancreatoduodenectomies, 44 pylorus-preserving pancreatoduodenectomies, 59 distal pancreatectomies, 70 total pancreatectomies, and 1 pancreatic head resection with segmental duodenectomy[14] were performed. The pathologic findings were evaluated in accordance with the second English edition of the *Classification of Pancreatic Carcinoma* proposed by the Japan Pancreatic Society.[15] This classification scheme is more detailed than the classification of the Union Internationale Contre le Cancer.[16] Lymph nodes were classified into several lymph node stations named according to the anatomic location and were numbered: 3, lesser curvature; 4, greater curvature; 5, suprapyloric; 6, infrapyloric; 7, left gastric artery; 8, common hepatic artery; 9, celiac trunk; 10, splenic hylus; 11, splenic artery; 12, hepatoduodenal ligament; 13, posterior pancreatoduodenal; 14, superior mesenteric artery; 15, middle colic artery; 16, paraaortic; 17, anterior pancreatoduodenal; and 18, inferior pancreas lymph nodes. The No. 16 lymph

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Received March 20, 2008.
Accepted for publication July 24, 2008.
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ISSN: 0885-3177
DOI: 10.1097/MPA.0b013e3181889e2d

TABLE 1. No. Positive and Total Lymph Nodes by Station

Lymph Node Station	Positive LN Number, mean (min-max)	Dissected LN Number, mean (min-max)
No. 3	0.0 (0–0)	0.8 (0–5)
No. 4	0.0 (0–0)	2.4 (0–13)
No. 5	0.0 (0–0)	0.4 (0–3)
No. 6	0.4 (0–4)	2.9 (0–11)
No. 7	0.0 (0–1)	0.7 (0–6)
No. 8	0.4 (0–4)	2.2 (0–10)
No. 9	0.3 (0–11)	1.1 (0–17)
No. 10	0.0 (0–0)	1.0 (0–12)
No. 11	0.2 (0–2)	2.6 (0–21)
No. 12	0.8 (0–4)	4.6 (0–15)
No. 13	1.7 (0–7)	3.7 (0–11)
No. 14	1.9 (0–31)	7.7 (0–38)
No. 15	0.0 (0–0)	0.3 (0–3)
No. 16	2.9 (1–10)	7.4 (1–33)
No. 17	1.5 (0–10)	4.1 (0–13)
No. 18	0.0 (0–1)	0.4 (0–5)
Total	10.1 (1–49)	42.4 (2–105)

nodes in this study refer to those that are surrounded by the celiac trunk, the inferior mesenteric artery, the right margin of the inferior vena cava, and the left margin of the abdominal aorta. Consequently, 45 No. 16 (+) patients were identified. Intraoperative radiation therapy (IORT, 30 Gy) had been administered to the retroperitoneal fields, and 5-fluorouracil (5-FU) portal injection, 5-FU–based chemotherapy, or gemcitabine had been given as postoperative adjuvant chemotherapy to some patients.

Statistical Analysis

The overall survival rates were calculated using the Kaplan-Meier method, and the difference in survival curves was analyzed using the log-rank test. The prognostic value of each clinicopathologic factor was evaluated by univariate analysis among the 45 No. 16 (+) patients. Significant independent prognostic factors were then identified by multivariate analysis using the Cox proportional hazards regression model. Data are expressed as the mean (SD). The level of statistical significance was set at $P < 0.05$.

RESULTS

The numbers of positive and dissected lymph nodes in each of the lymph node stations as defined in the second English edition of the *Classification of Pancreatic Carcinoma*[15] are given in Table 1. The average number of dissected lymph nodes was 42.4 (range, 2–105), and the average number of positive lymph nodes was 10.1 (range, 1–49). As for the No. 16 nodes (No. 16), a mean of 7.4 nodes (range, 1–33 nodes) were dissected, and a metastasis was found in a mean of 2.9 nodes (range, 1–10 nodes).

The overall survival rate of patients stratified by the extent of lymph node involvement is given in Figure 1. Lymph node metastases were observed in 230 (68.7%) of 335 patients. The survival of patients with metastases to the regional nodes (n[+], No. 16 [−]) was significantly worse than that of node-negative patients (n[−]), whereas it was significantly better than that of No. 16 (+) patients ($P = 0.0012$ and $P = 0.0029$, respectively). On the other hand, there was no significant difference in survival between the No. 16 (+) patients and the unresectable cases.

The clinicopathologic characteristics of the 45 No. 16 (+) patients are provided in Table 2. As postoperative adjuvant chemotherapy, 5-FU portal injection was given to 5 patients, and gemcitabine was injected in 9 patients. Intraoperative radiation therapy was also administered to 26 patients. The survival time of the No. 16 (+) subjects ranged from 0.1 to 45.4 months (median, 7.8 months). There were some long-term survivors even among this population.

Univariate analysis among the No. 16 (+) patients revealed that age 59 years or younger, tumor size greater than 4 cm, pathological portal vein invasion (pPV[+]), and perineural invasion were the factors significantly associated with survival (Table 3). These 4 variables were included in the multivariate analysis using Cox proportional hazards regression model along with clinical portal vein invasion, a factor that was almost significant in the univariate analysis. Consequently, age 59 years or younger, tumor size greater than 4 cm, and pPV(+) were identified as independent prognostic factors among this population (Table 4). The overall survival curves stratified for these 3 independent prognostic factors are shown in Figures 2A–C.

Finally, the survival of the No. 16 (+) cases was evaluated. These cases were subclassified into 2 groups, patients with only 1 metastatic No. 16 lymph node and those with 2 or more nodes. The survival of patients with 1 positive lymph node was significantly better than that of the unresectable cases and tended to be better than that of patients with 2 or more positive nodes ($P = 0.049$ and $P = 0.14$, respectively; Fig. 2D).

DISCUSSION

Lymph nodes are the most frequent site of metastases for gastrointestinal cancer, including pancreatic cancer, and their removal in theory offers a therapeutic potential, particularly to achieve local control. Efforts to obtain improved tumor clearance through enlarging the anatomic extent of lymphadenectomy have often been found unrewarding in survival benefit not only for pancreatic cancer[17–20] but also for gastric

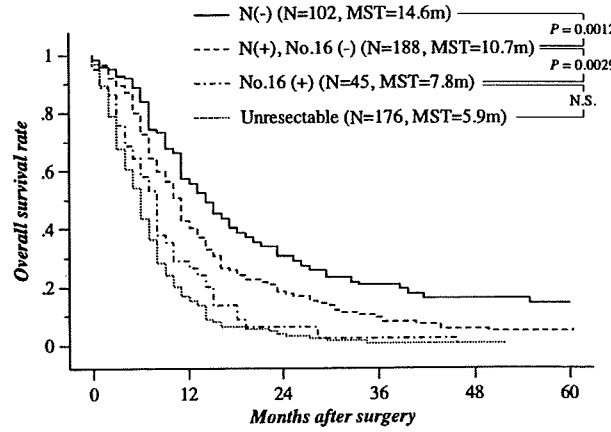

FIGURE 1. The overall survival rates of patients based on lymph node involvement (n) are shown. The survival of patients with n(+), No. 16(−) was significantly worse compared with those who were n(−) and was significantly better than those with No. 16(+) ($P = 0.0012$ and $P = 0.0029$, respectively). There was no significant difference in the survival curves of the patients with the No. 16(+) and the unresectable cases. m indicates months; MST, mean survival time; NS not statistically significant.

TABLE 2. Patient Characteristics With Paraaortic Lymph Node Metastases

Age, range (mean [SD]), y	43–79 (62.5 [7.8])
Sex	
Male	29
Female	16
Tumor location	
Head	39
Body, tail	5
Whole	1
Operative procedure	
PD	24
PpPD	2
DP	4
TP	15
Postoperative chemotherapy	
5-FU	5
Gemzal	9
Others	3
None	26
IORT	
30 Gy	26
None	19
Survival time, range (mean [SD]), mo	0.1–45.4 (9.6 [8.6])

DP indicates distal pancreatectomy; PD, pancreatoduodenectomy; PpPD, pylorus-preserving pancreatoduodenectomy; TP, total pancreatectomy.

cancer.[21–23] These findings and experience with other types of cancer suggest that the indications for extended lymphadenectomy should be seriously reconsidered.

There is a general consensus, however, that localized pancreatic cancer with regional lymph node metastases can be cured only by extended radical surgery. According to recent clinical observations, the absolute contraindications for pancreatectomy are the presence of liver metastases and peritoneal deposits, whereas the relative contraindications are the involvement of the portal venous system or major arteries. In addition, some authors suggest that radical surgery with extended lymphadenectomy should be abandoned for No. 16 (+) patients because long-term survival is extremely infrequent in this population, although extended lymphadenectomy did not adversely affect morbidity or mortality.[10–12] Consequently, radical surgery may not be recommended when a No. 16 (+) lymph node is confirmed during surgery through sampling and intraoperative examination of the frozen section. On the other hand, the inclusion of No. 16 to the routine range of lymphadenectomy, although time-consuming, is technically feasible, with acceptable morbidity and mortality reported from the authors and from others.[13,17,19] Furthermore, there were some patients with paraaortic lymph node involvement who had a chance of long-term survival.[6–9] Therefore, it is important to identify No. 16 (+) patients who may benefit from radical surgery with extended lymphadenectomy.

The purpose of this study was to determine the indications for the resection of No. 16 (+) pancreatic cancer. The survival of No. 16 (+) patients was significantly worse than those with n(+) No. 16 (−) disease, whereas no significant difference in survival was observed between the No. 16 (+) patients and the unresectable cases in this study. Given these results, No. 16 (+) was initially considered not to be a good target for radical surgery. The results in the current study indicate that age 59 years or younger, tumor size 4 cm or greater, and pPV(+) are independent factors that predict a poor outcome after radical surgery for No. 16 (+) patients, and the survival of patients without these factors was significantly better than that of patients who were deemed unresectable. Because the tumor of No. 16 (+) patients was mainly located in the head of pancreas in this study, we could not evaluate the prognostic relation in cancer location or operative procedure and could not deny biologic involvement. Along with size of the tumor, age has been reported to affect prognosis in some other cancer types. Early age of onset is often considered a poor prognostic factor for colorectal cancer, for example, which tends to be diagnosed at a more advanced stage. It tends to show more aggressive histopathologic features and to result in lower survival rates in younger patients.[24,25] On the other hand, pPV(+) may not be as useful for making a decision regarding whether or not to proceed with radical surgery, because this information is essentially unavailable before surgical resection and histopathologic examination. However, the authors have shown that intraportal endovascular ultrasonography (IPEUS) is capable of accurately detecting or excluding histologic invasion of the portal vein wall.[26,27] Correlation of IPEUS results with pathologic examination of resected specimens revealed that tumor-vessel contiguity with an intact echogenic band was indicative of tumor within 1 mm of the adventitia of the portal vein wall but without actual invasion. Although most centers might not use this IPEUS, recent intraoperative ultrasonography (IOUS), which provides a distinct image, could be used as a substitute for IPEUS. It can be assumed, therefore, that future indications for radical surgery in No. 16 (+) patients could be decided based on the age, tumor size, and the IPEUS or IOUS findings.

Recent reports indicated that the number of positive nodes at a given lymph node station is an important predictor for survival of the surgically treated patients.[28] It was also reported

TABLE 3. Univariate Analysis for Patients With Paraaortic Lymph Node Metastases

Variable	Odds Ratio	95% CI	*P*
Age (≤59 y)	2.354	1.226–4.520	0.0101
Sex (male)	0.981	0.524–1.836	0.9517
Location			
Body, tail	1.008	0.136–7.459	0.9937
Whole	1.217	0.475–3.118	0.6825
Tumor size (>4 cm)	3.589	1.789–7.200	0.0003
PV(+)	2.652	0.909–7.738	0.0742
Vascular invasion	1.177	0.577–2.401	0.6547
Invasion of anterior pancreatic capsule	1.346	0.737–2.460	0.3335
Invasion of retroperitoneal tissue	1.803	0.880–3.693	0.1073
Bile duct invasion	0.778	0.370–1.636	0.5086
Duodenal invasion	1.326	0.706–2.490	0.3804
pPV(+)	2.051	1.033–4.073	0.0401
Arterial invasion	1.716	0.851–3.461	0.1315
Perineural invasion	2.045	1.008–4.148	0.0474
pDPM	1.641	0.881–3.056	0.1184
No. 16–positive (≥2)	1.585	0.851–2.952	0.1464

CI indicates confidence interval; PV, clinical portal vein invasion; pDPM, pathological dissected peripancreatic tissue margin.

TABLE 4. Multivariate Analysis for Patients with Paraaortic Lymph Node Metastases

Variable	Odds Ratio	95% CI	*P*
Age (≤59 years)	3.438	1.621–7.290	0.0013
Tumor size (>4 cm)	2.693	1.304–5.561	0.0074
PV(+)	1.062	0.304–3.717	0.9245
pPV(+)	2.359	1.055–5.278	0.0367
Perineural invasion	1.84	0.868–3.898	0.1115

that the removal and pathologic examination of a greater number of lymph nodes can influence staging accuracy and even improve the overall survival after pancreatectomy.[29] Furthermore, some authors revealed that the ratio of the number of positive lymph nodes to the total number of dissected lymph nodes (lymph node ratio) was one of the most powerful predictors of survival.[30,31] In this study, the mean number of lymph nodes retrieved was 42.4, and the mean number of metastatic lymph nodes was 10.1. These data are comparable to what has been reported in the literature. The survival of patients with only 1 metastatic No. 16 lymph node was significantly better than the unresectable cases and also tended to be better than those with more than 2 metastatic nodes, although the difference did not reach statistical significance due to the small sample size. Regarding the relationship between No. 16 (+) and other stations, as we reported previously, statistical analysis showed that metastases to paraaortic lymph nodes had a strong correlation with metastases to Nos. 12, 13, 14, and 17 lymph nodes, and they were seldom observed among the patients who had no metastases to Nos. 13, 14, and 17 lymph nodes.[7] Before performing IPEUS or IOUS, therefore, sampling of the paraaortic lymph nodes and subsequent evaluation of the frozen sections could facilitate a decision regarding whether the patient had only 1 positive paraaortic lymph node. Our data might indicate that radical surgery should be abandoned when 2 or more examined lymph nodes harbor metastases.

Finally, the effect of adjuvant therapies need to be mentioned, because the treatment strategy of the patients analyzed in this study was rather mixed. No significant difference in survival was observed between the patients who received chemotherapy and those who did not. Likewise, IORT performed in 26 patients did not confer any survival benefit (data not shown). The difference in adjuvant therapies given to the patients can therefore be considered to have had little influence on the results obtained in this study. This lack of efficacy of adjuvant treatment indeed justifies our policy to find ways to increase candidates for radical surgery, the only treatment modality that has been shown to have a significant impact on the survival of patients.

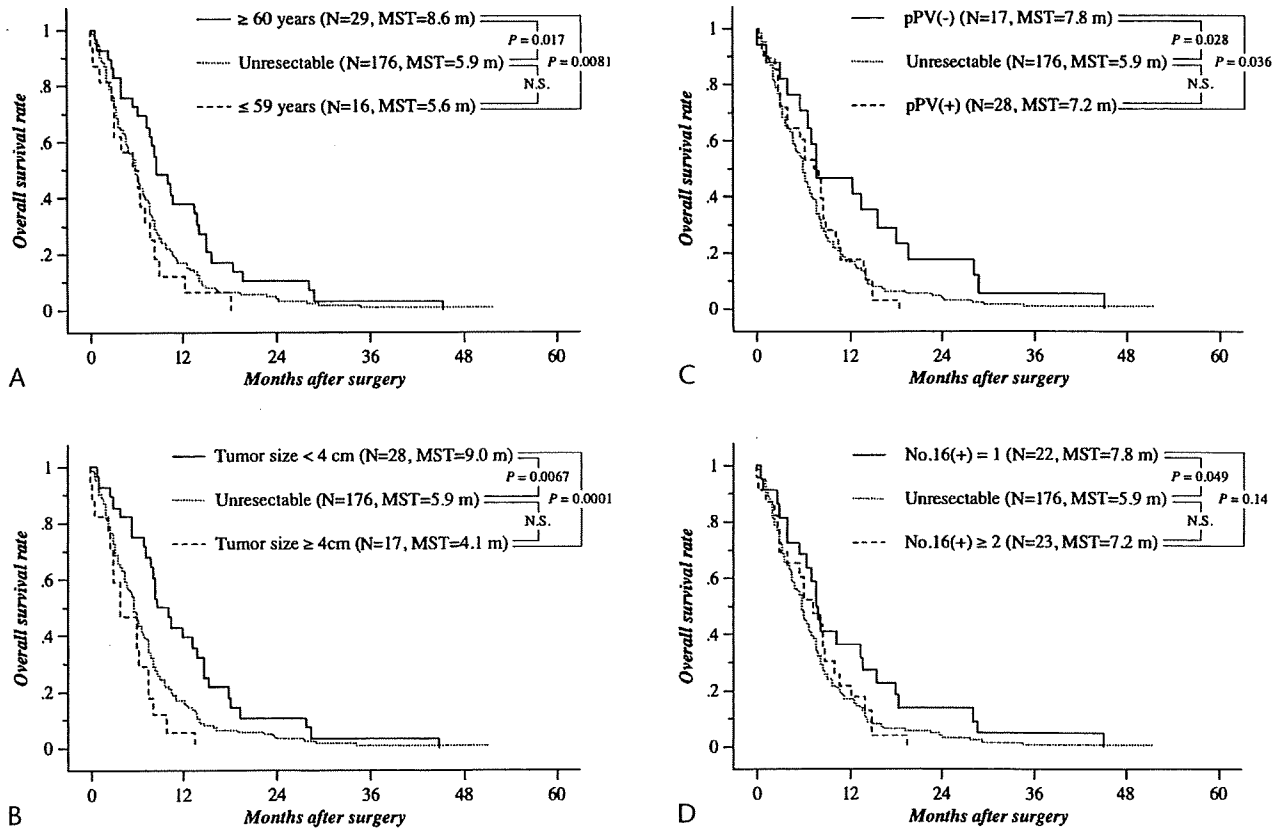

FIGURE 2. A, The overall survival curves based upon patient age. The survival of patients 60 years or older was significantly better than those 59 years or younger and those who were unresectable ($P = 0.081$ and $P = 0.017$, respectively). B, The overall survival curves were based on tumor size. The survival of patients with tumors less than 4 cm was significantly better than those with tumors 4 cm or greater and those who were unresectable ($P = 0.0001$ and $P = 0.0067$, respectively). C, The overall survival curves based upon pPV. The survival of patients with pPV(−) disease was significantly better than those who were pPV(+) or were unresectable ($P = 0.036$ and $P = 0.028$, respectively). D, The survival of patients with 1 positive lymph node was significantly better than those who were unresectable and tended to have a better survival than patients with 2 or more positive lymph nodes ($P = 0.049$ and $P = 0.14$, respectively).

In this retrospective analysis, there were 3 No. 16 (+) patients who survived for more than 2 years, all of whom were 60 years or older, had a tumor size of 4 cm or less, were pPV(−), and had only 1 metastatic paraaortic lymph node. These factors indicate that these are relatively promising targets for radical surgery among the No. 16 (+) population who usually have a dismal prognosis. Although these findings, along with the accuracy of IPEUS or IOUS in identifying pPV(−) patients, will have to be confirmed by a prospective study, radical resection with extended lymphadenectomy remains an option for selected No. 16 (+) patients at this time.

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Dig Surg 2008;25:179–184
DOI: 10.1159/000140684

Published online: June 23, 2008

A New Technique for Intraoperative Continuous Biliary Drainage during Pancreatoduodenectomy

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Key Words
Pancreaticoduodenectomy · Intraoperative biliary drainage · Preoperative biliary drainage

Abstract
Background: The common hepatic duct is divided during the early stage of pancreatoduodenectomy. Complete and prolonged closure of the proximal common duct stump can cause liver damage in the course of this long operation, resulting in associated complications. ***Methods:*** We performed intraoperative continuous external bile drainage by a new method using a novel drainage clamp in 47 consecutive patients (drainage clamp group) and compared postoperative liver enzyme levels, inflammation markers, morbidity, and outcomes with those of a conventional clamp group (n = 40). ***Results:*** The drainage clamp group had significantly lower transaminase levels within the first 14 postoperative days than the conventional clamp group. The number of patients with elevated transaminase was significantly less in drainage clamp group than conventional clamp group ($p < 0.001$). There were no significant differences between these two groups in terms of mortality rates and postoperative morbidity. ***Conclusion:*** Intraoperative complete closure of the common hepatic duct contributed to postoperative elevated transaminase levels, and the continuous decompression of the hepatic duct during pancreatoduodenectomy is beneficial to patients by avoiding liver dysfunction. The novel drainage clamp is a safe and useful tool for pancreatoduodenectomy and other operative procedure where extrahepatic bile duct is dissected.

Introduction

In most standard pancreaticoduodenectomy and pylorus-preserving pancreaticoduodenectomy procedures, the common hepatic duct is divided during the early stage of the operative procedure [1]. The proximal common duct stump is usually closed with an atraumatic bulldog vessel clamp to prevent intraoperative peritoneal bile contamination. The bile duct clamp causes abrupt and complete biliary obstruction lasting for several hours before completion of hepaticojejunal anastomosis. The clamp is released periodically, and hepatic bile juice is flushed out and sucked away, although even this maneuver involves a chance of peritoneal bile contamination. In addition, prolonged proximal clamp of the common hepatic duct can cause elevated biliary pressure and biliary reflux through hepatic sinusoid that may result in a hepatic cell damage, consequent postoperative liver dysfunction, hyperbilirubinemia, or liver abscess. Therefore, to reduce intraoperative liver damage, continuous drainage of bile after common hepatic duct closure is desirable.

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When a percutaneous transhepatic biliary drainage (PTBD) catheter is placed before surgery, it can serve as an intraoperative drainage route. In these patients, the complete biliary obstruction by vessel clamp does not increase biliary pressure, and we noted that no serious elevation of postoperative hepatic transaminase data. However, patients who are not jaundiced undergo pancreaticoduodenectomy without biliary drainage. In addition, it has been reported that many centers perform surgery without biliary drainage even in jaundiced patients [2, 3]. Therefore, we thought that intraoperative continuous bile drainage by placing a catheter immediately after dividing the common hepatic duct would better work for preventing the elevation of biliary pressure and hepatic damage.

For this purpose, we have developed a novel clamp with a shape that can easily fix a drainage catheter. To determine the effectiveness and necessity of intraoperative continuous bile drainage, we retrospectively compared the postoperative course and liver function test results of these intraoperative continuous bile drainage patients with those of an historical control group without intraoperative continuous bile drainage.

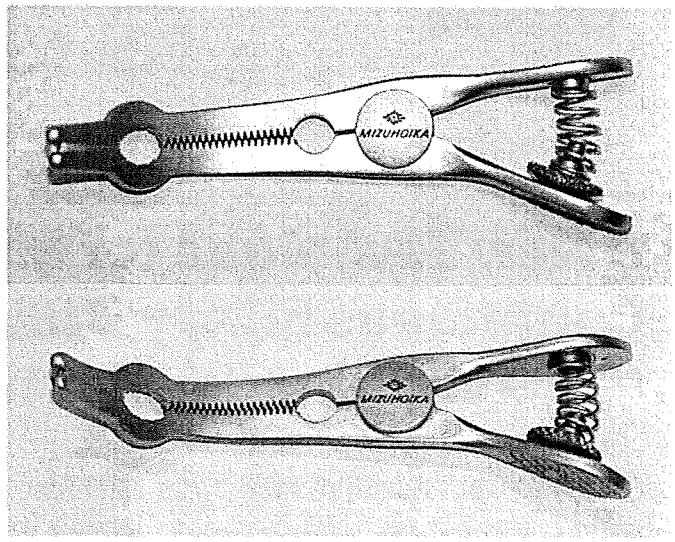


Fig. 1. The novel drainage clamp. The clamp has a hole with a diameter of 6 mm through which a 12- or 14-Fr catheter can be placed.

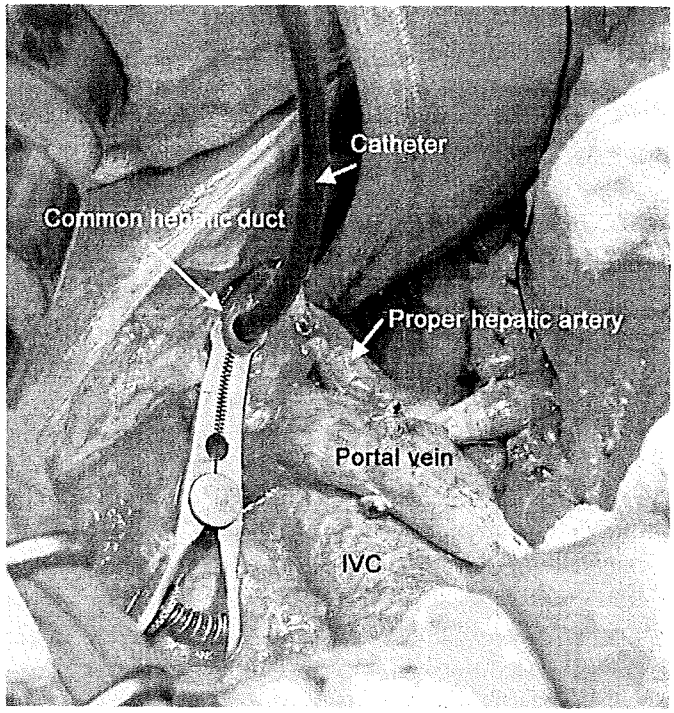


Fig. 2. Intraoperative procedure of continuous bile drainage by a drainage clamp and a nelaton catheter. A nelaton catheter was introduced into the common hepatic duct stump, and a drainage clamp was placed on it. The catheter was connected to an extracorporeal tube.

Materials and Methods

Patients

Patients who underwent pancreaticoduodenectomy in Kyoto University Hospital between January 2004 and December 2006 had been enrolled in this study. Patients who had had PTBD were excluded, because intraoperative biliary decompression is attained by PTBD in these patients. Patients with preoperative abnormal liver function, defined by aspartate aminotransferase (AST) >200 IU/l, alanine aminotransferase (ALT) >200 IU/l, or total bilirubin >5.0 mg/dl, and patients with perioperatively diagnosed liver cirrhosis and liver metastasis were also excluded from this study because these conditions affect the evaluation of intraoperative biliary decompression.

Technique of Pancreaticoduodenectomy

All patients underwent preoperative bowel preparation with a polyelectrolyte solution, and received perioperative intravenous antibiotic prophylaxis using a second-generation cephalosporin starting 1 h before skin incision. All patients underwent pancreaticoduodenectomy or pylorus-preserving pancreaticoduodenectomy. The same two senior surgeons performed all the operations in this series. When common hepatic duct was transected, a conventional non-crushing curved bulldog vein clamp (Mizuho Co., Ltd., Tokyo, Japan) or a newly developed curved drainage clamp (Mizuho Co., Ltd.) was positioned across the hepatic duct. When a conventional clamp was used, an intermittent opening of the bile duct was repeated periodically to minimize an elevation of biliary pressure during the period between division of the common hepatic duct and subsequent biliary-enteric reconstruction. Figure 1 shows the newly developed drainage clamp that has a

Table 1. Characteristics of the patients

	Conventional clamp group (n = 40)	Drain clamp group (n = 47)	χ^2	p value
Gender (M/F)	28/12	25/22	2.56	0.11
Age, years				
Mean ± SEM	61.7 ± 1.7	65.4 ± 1.2	2.25	0.13
Range (median)	43 – 81 (64)	47 – 81 (66)		
Benign tumor/malignant tumor	6/34	6/41	0.09	0.76
Pancreatic ductal cancer	21	23		
Pancreatic cystic neoplasm	7	6		
Bile duct cancer	4	6		
Ampullary cancer	3	6		
Chronic pancreatitis	2	3		
Endocrine tumor	2	0		
Metastatic renal cancer	1	1		
Malignant lymphoma	0	1		
Duodenal cancer	0	1		
PD/PPPD	21/19	19/28	1.27	0.26

hole placed in the middle part of the clamp. When common hepatic duct was divided, a 12- or 14-Fr Safeed™ nelaton catheter (Terumo Corporation, Tokyo, Japan) was inserted into the hepatic duct stump, and the drainage clamp was placed to bite the common duct together with the nelaton catheter fixed (fig. 2). After removal of the duodenum and the pancreatic head, reconstruction was made by end-to-side pancreaticojejunostomy, end-to-side hepaticojejunostomy and end-to-side gastro- or duodenojejunostomy in this order (modified Child's method) in both groups. In all patients, biliary stent tube was not placed.

Laboratory and Clinical Data

Preoperative, perioperative, and postoperative laboratory and clinical data were retrospectively collected by chart review. As a marker for liver function and systemic inflammation, we have collected the values of total bilirubin, AST, ALT, white blood cell counts and C-reactive protein just before operation and in the first 14 postoperative days.

Major complications recorded in the postoperative period included postoperative death (death during the hospital stay for surgery or within 30 days of surgery); reoperation (during the hospital stay for surgery); postoperative intra-abdominal bleeding; intra-abdominal abscess; increased amylase in drain (drain amylase level more than 5,000 IU/l on any postoperative day without clinical sequelae); pancreaticojejunal anastomotic leak (drain amylase level more than 5,000 IU/l on any postoperative day with clinical sequelae such as fever, leukocytosis, fistula, or abscess); other anastomotic leaks (from the hepaticojejunal, gastrojejunal or duodenojejunal anastomosis); sepsis syndrome; pneumonia; gastrointestinal bleeding; and pulmonary embolism.

Other complications recorded in the postoperative period included allergic reaction, atelectasis (radiographic or clinical), cardiac arrhythmia, wound infection, cholangiitis, pancreatitis, delayed gastric emptying (gastrostomy tube output >1,000 ml on postoperative day 7 or inability to tolerate a postgastrectomy diet by postoperative day 10), ileus (absence of flatus and/or bowel sounds beyond postoperative day 7), infectious colitis (as documented by *Clostridium difficile* toxin assay), urinary tract infection (documented by positive urine culture), deep vein thrombosis, chylous ascites, pleural effusion (radiographic or clinical) and liver dysfunction (defined as either a peak AST or a peak ALT >500 IU/l).

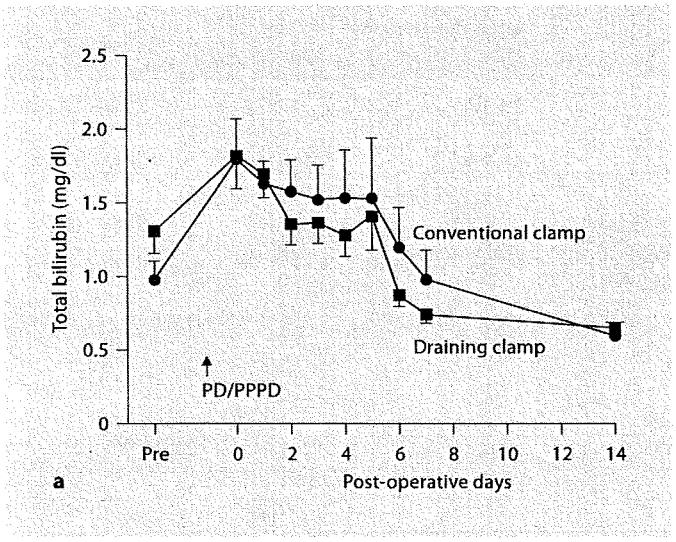
Statistical Analysis

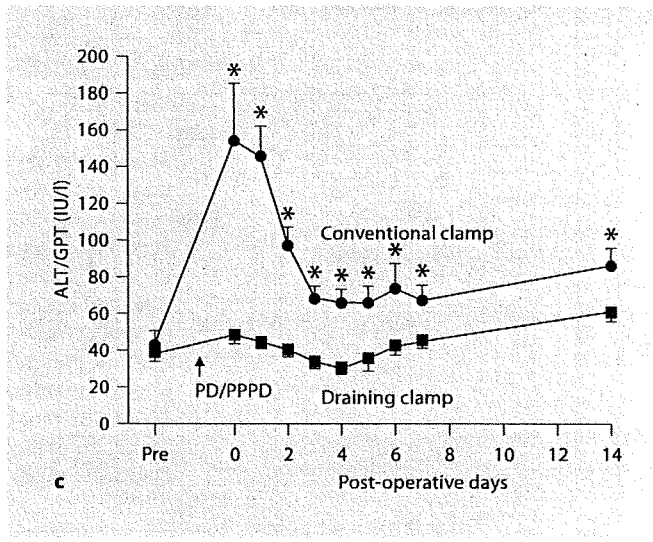
Results are expressed as the means and SEMs. Patient characteristics and perioperative and postoperative factors between 2 groups were compared by Mantel-Haenszel test. Distributions of numeric variables between groups were compared by analysis of variance, followed by a post hoc Tukey-Kramer test when appropriate. $p < 0.05$ was considered statistically significant.

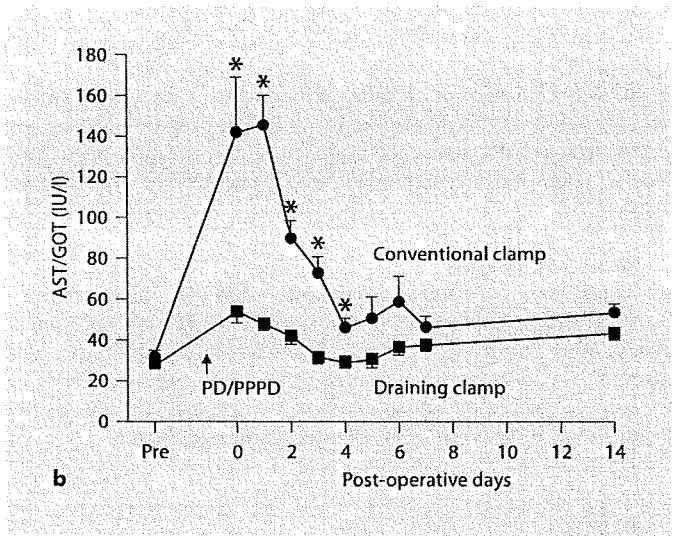
Results

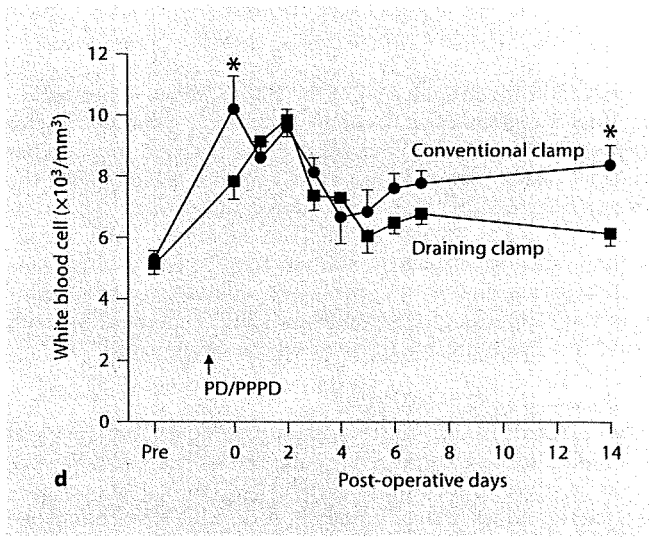
A total of 87 patients who underwent pancreaticoduodenectomy were included; the conventional clamp group consisted of consecutive 40 patients and the novel drainage clamp group consisted of consecutive 47 patients. The clinical characteristics of these 87 patients are summarized in table 1. These two patient groups are well matched for age, gender, operative time, intraoperative blood loss, transfusion requirements, pathology, and type of resection. Their preoperative liver enzyme profiles were also similar.

Postoperative liver function was assessed by the total bilirubin, AST, and ALT levels for 14 PODs (fig. 3). The total bilirubin levels of the conventional clamp group and drainage clamp group were 1.6 ± 0.2 and 1.7 ± 0.2 mg/dl, respectively, at 6 h after operation (POD 0) which was the peak value for both groups during the tested period,









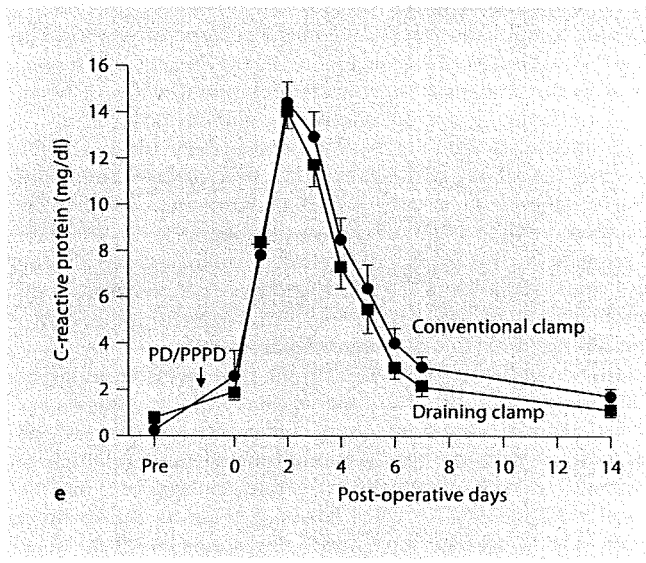


Fig. 3. Changes of total bilirubin levels (**a**), AST levels (**b**), ALT levels (**c**), WBC counts (**d**), and CRP levels (**e**) after pancreatoduodenectomy. * Significant difference between groups.

Table 2. Postoperative complications

	Conventional clamp group (n = 40)	Drain clamp group (n = 47)	p value
Death	0 (0)	0 (0)	n.a.
Reoperation	0 (0)	0 (0)	n.a.
Intra-abdominal bleeding	0 (0)	0 (0)	n.a.
Abdominal abscess	1 (3)	0 (0)	0.28
Increase of amylase in drain fluid	2 (5)	2 (4)	0.87
Pancreaticojejunal anastomotic leak	1 (3)	0 (0)	0.28
Bile leakage from the hepaticojejunostomy	0 (0)	0 (0)	n.a.
Leakage from the gastro(duodeno)-jejunostomy	0 (0)	0 (0)	n.a.
Gastrointestinal bleeding	1 (3)	0 (0)	0.28
Wound infection	2 (5)	2 (4)	0.87
Acute pancreatitis	0 (0)	0 (0)	n.a.
Delayed gastric emptying	2 (5)	1 (2)	0.47
Increase of transaminases	32 (80)	9 (19)	<0.001

n.a. = Not applicable. Data are the number (%) of patients.

and the bilirubin levels decreased gradually (fig. 3a). The postoperative total bilirubin levels did not differ between the two groups.

The AST level of the conventional clamp group hit the peak at approximately 12 h after surgery on the POD 1, and decreased gradually; however, the levels were significantly higher than those of drainage clamp group until the POD 4 (fig. 3b). After the POD 4, the AST level was not statistically different, but that of conventional clamp group was slightly higher than that of drainage clamp group.

The ALT level of the conventional clamp group hit the peak at 6 h after surgery (on the POD 0), and decreased gradually; however, the levels were significantly higher than those of drainage clamp group until the POD 14 (fig. 3c).

Postoperative inflammatory response was assessed by the peripheral white blood cell (WBC) count and serum C-reactive protein (CRP) levels for 14 PODs (fig. 3d, e). The WBC count was not much different between the two groups though, at 6 h after operation and on POD 14, the WBC count of the conventional clamp group was significantly higher than that of the drainage clamp group (fig. 3d). The CRP levels increased postoperatively and hit the peak on the POD 2 in the two groups (fig. 3e). The postoperative CRP levels did not differ between the two groups.

There was no operative death in both groups; further, as listed in table 2, there was no major leakage of the pancreaticojejunostomy or intra-abdominal bleeding. The rate of other postoperative complications was comparable between the two groups except for liver dysfunction defined by the increased AST/ALT. Increased AST/ALT was observed 32 of the 40 patients with conventional clamp (80%) and 9 of the 47 patients with drainage clamp (19%) ($p < 0.001$).

Discussion

During the postoperative period, many patients who undergo pancreatoduodenectomy have elevated serum liver enzymes of varying degrees. In most patients, this biochemical abnormality is temporary and the serum levels gradually return to normal; however, minimizing intraoperative liver damage is important after such a major operation because the liver plays a key role in recovery from the surgical trauma. First, the liver forms and secretes albumin, procoagulant factors, and acute phase reactant proteins; second, it metabolizes waste, drugs, and toxins; and third, it plays a key role in immunological response. Therefore, there is a strong possibility that postoperative liver dysfunction increases the incidence of, and compromises recovery from, other possible complications.

Intraoperative biliary decompression after dissection of the common hepatic duct by a retrograde transhepatic biliary catheter has been shown to reduce the postoperative transaminase levels within the first 7 PODs [4]. In addition, the number of patients with postoperative in-

creases of transaminase level higher than 500 IU/l was significantly less in the biliary decompression group than in the group without decompression. In agreement with this study, the results of the current study showed that intraoperative closure of the common hepatic duct resulted in elevated postoperative transaminase levels, and intraoperative drainage by our novel method significantly reduced the transaminase levels to almost normal range. The number of patients with postoperative increase of transaminase levels was significantly less in the intraoperative drainage group than in the group without drainage. These results suggest that the postoperative liver dysfunction observed after pancreatoduodenectomy is, at least, partially due to intraoperative prolonged closure of the common hepatic duct in most cases. Furthermore, intraoperative drainage by our novel drainage clamp can reduce intraoperative liver damage and prevent postoperative liver dysfunction.

When a PTBD catheter is placed in patients with jaundice preoperatively, the catheter is left, and can be used for the purpose of decompression in the hepatic duct during the postoperative period. However, it has been reported that many centers perform surgery without biliary drainage even in jaundiced patients [2, 3]. Therefore, if a PTBD catheter is not placed preoperatively, which may be the common status in patients who are scheduled to undergo pancreatoduodenectomy, the intrahepatic biliary pressure will be elevated when the common hepatic duct is dissected and closed with a conventional clamp during operation. In addition, frequent and intermittent bile drainage by opening the conventional clamp during operation would be time-consuming and might increase the possibility of postoperative infective complications by the contaminated bile.

Intraoperative insertion of a retrograde transhepatic biliary drainage catheter has been proposed as a solution to decrease biliary pressure [4]; however, placement of this catheter has been reported to accompany several complications such as local peritonitis [5], biliary stricture [6] and intrahepatic arterial bleeding. In contrast, the present method of intraoperative biliary drainage is simple and safe; the operator just needs to insert a soft silicon tube into the hepatic duct and place the novel clamp on the hepatic duct. It requires only 1 min without contamination and danger. We have not experienced any adjacent tissue injury or organ injury by this new clamp system, and intra-abdominal abscess formation postoperatively.

In conclusion, we demonstrated that continuous decompression of the hepatic duct during pancreatoduodenectomy is beneficial to patients by avoiding liver dysfunction. The novel drainage clamp, which facilitates intraoperative hepatic duct drainage, is a safe and useful tool for pancreatoduodenectomy and other operative procedure where extrahepatic bile duct is dissected.

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Prognostic value of metastin expression in human pancreatic cancer

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Published: 21 January 2009

Journal of Experimental & Clinical Cancer Research 2009, **28**:9 doi:10.1186/1756-9966-28-9

Received: 4 December 2008
Accepted: 21 January 2009

This article is available from: http://www.jeccr.com/content/28/1/9

Abstract

Background: *KiSS-1* was identified as a metastasis-suppressing gene in melanoma cells. The *KiSS-1* gene product (metastin) was isolated from human placenta as the ligand of GPR54, a G-protein-coupled receptor. The role of metastin and GPR54 in tumor progression is not fully understood.

Methods: We investigated the clinical significance of metastin and GPR54 expression in pancreatic cancer. We evaluated immunohistochemical expression of metastin and GPR54 in pancreatic ductal adenocarcinoma tissues obtained from 53 consecutive patients who underwent resection between July 2003 and May 2007 at Kyoto University Hospital. In 23 consecutive patients, the plasma metastin level was measured before surgery by enzyme immunoassay.

Results: Strong immunohistochemical expression of metastin was detected in 13 tumors (24.5%), while strong expression of GPR54 was detected in 30 tumors (56.6%). Tumors that were negative for both metastin and GPR54 expression were significantly larger than tumors that were positive for either metastin or GPR54 ($p = 0.047$). Recurrence was less frequent in patients who had metastin-positive tumors compared with those who had metastin-negative tumors (38.5% versus 70.0%, $p = 0.04$). Strong expression of metastin and GPR54 was significantly correlated with longer survival ($p = 0.02$). Metastin expression by pancreatic cancer was an independent prognostic factor for longer survival (hazard ratio, 2.1; 95% confidence interval, 1.1–4.7; $p = 0.03$), and the patients with a high plasma metastin level ($n = 6$) did not die after surgical resection.

Conclusion: Strong expression of metastin and GPR54 by pancreatic cancer is associated with longer survival. Metastin expression is an independent prognostic factor for the survival of pancreatic cancer patients. The plasma metastin level could become a noninvasive prognostic factor for the assessment of pancreatic cancer.