Comparison of different classes of CpG-ODN in augmenting the generation of human epitope peptide-specific CTLs

MASAHIRO KATSUDA, MAKOTO IWAHASHI, KENJI MATSUDA, MOTOKI MIYAZAWA, MIKIHITO NAKAMORI, MASAKI NAKAMURA, TOSHIYASU OJIMA, TAKESHI IIDA, KEIJI HAYATA and HIROKI YAMAUE

Second Department of Surgery, School of Medicine, Wakayama Medical University, Wakayama, Japan

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Abstract. Three distinct classes of CpG-oligonucleotides (ODN) (CpG-A, CpG-B and CpG-C) have been identified on the basis of differences in their structures and immune effects. To date, only CpG-B is applied for clinical treatments; however, it is still unknown which of the different CpG-ODN classes is most useful as an adjuvant for human cancer vaccine therapy. In the present study, we examined the activity of these 3 types of CpG-ODN in enhancing the induction of human peptide-specific CTLs. Our data showed that the specific cytotoxicity was augmented in the presence of CpG-A, -B and -C but not in the presence of control ODN, and the augmenting effect was most potent with CpG-A. Flow cytometric analysis showed the subpopulation of effector-memory cells in $CD8^+$ cells was most increased with CpG-A. Furthermore, depletion of PDCs from PBMCs before stimulation with peptide and CpG-ODN completely abrogated the augmenting effect of CpG-ODN. These data indicated that the stimulation of PDCs by CpG-ODN augmented the generation of peptide-specific CTLs, and CpG-A was superior to CpG-B and CpG-C in terms of augmenting the generation of human peptide-specific CTLs *in vitro*.

Introduction

The innate immune system is activated via exposure to pathogen-associated molecular patterns (PAMPs) that are expressed by a diverse group of infectious microorganisms. Subsequently, the host mounts an adaptive immune response directed against determinants that are uniquely expressed by the pathogen. The resultant antigen-specific immunity is characterized by the production of high-affinity antibodies and the generation of cytotoxic T cells that provide long-lasting protection (1). The key feature of innate immune cells that enables them to detect and categorize infection seems to be their repertoire of what have been termed pattern-recognition receptors (PRRs). The best understood family of PRRs is the toll-like receptors (TLRs), of which 10 are known in humans.

In contrast to viruses and other pathogens, vaccines containing recombinant proteins or synthetic antigenic peptides usually fail to induce significant immune responses unless they are mixed with adjuvant (3,4). Because of their high efficacy, several recently identified TLR ligands are promising vaccine adjuvants. Bacterial unmethylated CpG-rich oligodeoxy-nucleotides (ODN), which bind to TLR-9, are one of the most promising candidates for a cancer vaccine adjuvant and are currently being tested in many human clinical trials (5-8).

In numerous murine models, TLR-9 activation enhances antigen-specific cellular responses to a wide variety of antigens. The mechanism that contributes to the potent adjuvant activity of CpG-ODNs is maturation and differentiation of dendritic cells resulting in the strong induction of CTLs, even in the absence of CD4 T-cell help (9). On the other hand, the cellular patterns of TLR expression vary between different species (2,10). B cells, monocytes and all DC subsets express TLR-9 in mice; however, only plasmacytoid dendritic cells (PDC) and B cells express TLR-9 in humans (11-14). Consequently, the murine immune system produces different actions from human systems when exposed to CpG-ODN. Therefore, it is impossible to extrapolate the experimental results from murine models to humans. Furthermore, little is known about the mechanism by which CpG-ODNs augment acquired cellular immunity in humans, although systemically administered CpG-ODNs have shown substantial evidence of augmenting the activity of anti-tumor immunity in human clinical cancer vaccine trials (5-8).

Three distinct classes of CpG-ODN have been identified on the basis of differences in their structures and immune-stimulating effects (9,15-17). CpG-A induces the production of high levels of IFN-α from PDC with relatively little B-cell stimulation. In contrast, CpG-B induces the production of low levels of IFN-α along with profound B-cell activation. CpG-C has intermediate immune effects with excellent *in vivo* stability and ease of formation. To date, only CpG-B has been applied for clinical treatments; however, the class of CpG-ODN

Correspondence to: Dr Makoto Iwahashi, Second Department of Surgery, School of Medicine, Wakayama Medical University, 811-1 Kimiidera, Wakayama 641-8510, Japan
E-mail: makoto@wakayama-med.ac.jp

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that is most useful as an adjuvant for a human cancer vaccine is still unknown.

In the present study, we examined the immuno-modulatory activity of these 3 types of CpG ODN in terms of the generation of peptide-specific CTLs.

Materials and methods

Cell lines. A24+LCL cells (HLA-A24/24) were a generous gift of Takara Shuzo Co., Ltd. (Otsu, Japan). The A24LCL cells were used for peptide-mediated cytotoxicity assays. These cells were maintained in a tissue culture flask using RPMI and supplemented with antibiotics and 10% heat-inactivated fetal calf serum (Gibco BRL).

Oligodeoxynucleotides. CpG-A was synthesized by Gene Design (Osaka, Japan). CpG-B, CpG-C and GpC-ODN were synthesized by Hokkaido System Science (Sapporo, Japan); CpG-A, 5'-ggTGCAT**CG**ATGCAGGGGgg-3'; CpG-B, 5'-**tcgtc** gttttg**tcg**ttttgtcgtt-3'; CpG-C, 5'-**tcgtcg**aa**cg**ttcgagagatgat-3'; GpC-ODN, the GC control to CpG-ODN, 5'-ggTGCATG CATGCAGGGGgg-3' (lower case letters indicate phosphorothioate linkage; capital letters, phosphodiester linkage 3' of the base; bold, CpG-dinucleotides).

Peptides. Peptide derived from the squamous cell carcinoma-associated differentiation antigen LY6K-177 (RYCNLEGPPI), influenza (flu) virus-derived peptide (RFYIQMCYEL) and HIV-derived peptide (RYLRDQQLL) with the HLA-A24 binding motif were purchased from Takara Bio Inc. (Otsu, Japan). The purity (>90%) and the identity of the peptides were determined by analytical HPLC and mass spectrometry analysis, respectively. Peptides were dissolved in dimethylsulfoxide (DMSO) at 20 mg/ml and stored at -80°C.

Cytokine assays. Human PBMCs from healthy volunteers (n=15) were isolated from freshly drawn peripheral blood by Ficoll-Hypaque (Biochrom, Berlin, Germany) density gradient centrifugation. Blood donors were negative for HIV, hepatitis B virus (HBV) and HCV infection.

Freshly isolated PBMC ($1x10^6$ in 500 μl of AIM-V media, Invitrogen) were incubated at 37°C with 5% CO_2 in 48-well flat-bottom plates with each class of CpG-ODN at different concentrations. Cell supernatants collected after 48 h were stored at -80°C until assayed. IFN-α in cell supernatants was measured by ELISA according to the manufacturer's instructions (PBL Biomedical Laboratories, Piscataway, USA). All assays were performed in triplicate.

Induction of flu peptide-specific CTLs. For this study, HLA-A24-positive donors were selected. PBMCs ($2x10^6$/ml) isolated from healthy volunteers were stimulated with the flu peptide at a concentration of 10 μg/ml in the presence of CpG-ODNs (20, 5 and 5 μg/ml for CpG-A, -B and -C, respectively) in 24-well culture plates in AIM-V containing 2% heat-inactivated autologous serum (AS). In some experiments, PBMCs were depleted of PDCs using BDCA4-coupled magnetic beads (Miltenyi Biotec) according to the manufacturer's protocol (<0.01% PDCs identified as $BDCA2^+$ and $CD123^+$ after depletion) to investigate the role of PDCs on the adjuvant effect of CpG-ODNs. On day 7, the T cells were further stimulated with the peptide-pulsed adherent cells that were cultured with autologous irradiated PBMCs for 4 h. The cytotoxic activity of CTLs was tested against peptide-pulsed A24-LCL cells on day 14 as indicated.

Induction of LY6K peptide-specific CTLs. Monocyte-derived dendritic cells (DCs) were used as antigen-presenting cells to induce CTLs against peptides presented on HLA. DCs were generated *in vitro* as previously described (18). Briefly, PBMCs isolated from healthy volunteers (HLA-A*2402) were separated by adherence to a plastic tissue culture dish (Becton-Dickinson) so as to enrich them for the monocyte fraction. The monocyte-enriched population was cultured in the presence of 1,000 U/ml GM-CSF (Kirin) and 500 U/ml IL-4 (Ono) in AIM-V containing 2% heat-inactivated AS. After 5 days in the culture, TNF-α (10 ng/ml), IL-6 (1,000 U/ml), IL-1β (10 ng/ml) and PGE2 (1 μg/ml) were added to the culture to mature DCs. After 7 days, DCs were pulsed with 20 μg/ml of the synthesized peptides in the presence of 3 μg/ml β2-microglobulin (Sigma), pulsed on the cytokine-generated DCs for 4 h at 37°C in AIM-V. These peptide-pulsed DCs were then inactivated by γ irradiation (50 Gy) and used as stimulator cells. To increase the precursor frequency of peptide-specific cells, $CD8^+$ T cells were enriched by one round of positive selection using anti-CD8 antibody beads and MACS technology according to the manufacturer's protocol (Miltenyi Biotec; Bergisch-Gladbach, Germany). Then, $CD8^+$ T cells and unseparated PBMCs were mixed at a 1:2 ratio and used as the responder cells. These cultures were set up in 48-well plates (Corning); each well contained $5x10^4$ stimulator cells and $1.5x10^6$ responder cells in the presence of CpG-ODNs in 0.5 ml of AIM-V/2% AS. In some experiments, PBMCs were depleted of PDCs using BDCA4-coupled magnetic beads (Miltenyi Biotec) according to the manufacturer's protocol. Two days later, these cultures were supplemented with IL-2 to a final concentration of 20 IU/ml. On day 7, the T cells were further re-stimulated with the autologous peptide-pulsed DCs. The peptide-pulsed DCs were prepared in the same manner as described above. CTL activity was tested against peptide-pulsed A24-LCL cells on day 14 as indicated.

Cytotoxicity assay. Target cells were labeled with 100 μCi of $Na_2{}^{51}CrO_4$ (Perkin-Elmer Life Sciences) for 1 h at 37°C in a CO_2 incubator. Peptide-pulsed targets were prepared by incubating the cells with 20 μg/ml of the peptide for 16 h at 37°C before labeling. Labeled target cells were rinsed and mixed with effector cells in a final volume of 0.2 ml in round-bottom microtiter plates. The plates were centrifuged (4 min at 800 x g) to increase cell-to-cell contact and placed in a CO_2 incubator at 37°C. After 4 h of incubation, 0.1 ml of the supernatant was collected from each well and the radioactivity was determined with a γ counter. The percentage of specific cytotoxicity was determined by calculating the percentage of ^{51}Cr release in 4 h using the following formula: [(cpm of the test sample release - cpm of the spontaneous release)/(cpm of the maximum release - cpm of the spontaneous release)] x 100. Spontaneous release was determined by incubating the target cells alone, in the absence of effector cells, and the maximum release was obtained by incubating the targets with 1 N HCl. All measurements were carried out in triplicate and the standard

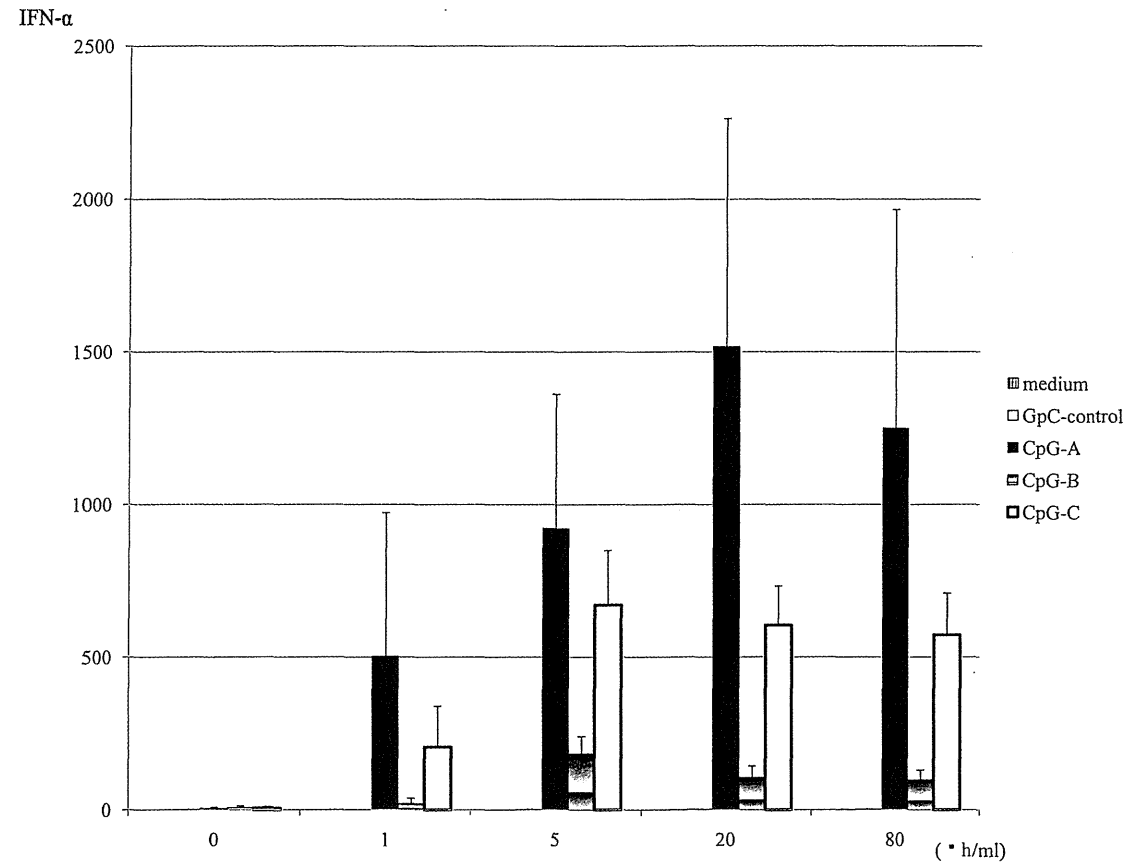

Figure 1. IFN-α secretion from PBMCs stimulated by CpG-ODNs. Levels of IFN-α secreted by PBMC from healthy donors (n=15) after 48-h culture with media, GpC control ODN, A-Class, B-Class or C-Class CpG (all ODN at 0, 1, 5, 20, 80 μg/ml). Bars show mean values and standard error of the means for each group of subjects.

errors of the means were consistently below 10% of the value of the mean.

Flow cytometric immunofluorescence analysis. Monoclonal antibodies against human CD8PerCP, CD45RAFITC and CCR7PE were purchased from BD Biosciences Pharmingen (Franklin Lakes, NJ, USA). Cells were incubated with specific antibodies in PBS for 30 min at 4˚C, then analyzed using a FACSCalibur with the Cell Quest software package (Becton-Dickinson).

Results

IFN-α secretion from PBMCs stimulated by CpG-ODNs. Distinct ODN classes were studied for their ability to stimulate human PBMCs to secrete IFN-α. Consistent with previous reports (15-17), IFN-α secretion from PBMCs was greatest with CpG-A, and it was moderate with CpG-C; it was lowest with CpG-B. The dosages of CPG-A, B and C that induced the maximum level of IFN-α were 20, 5 and 5 μg/ml, respectively (Fig. 1).

The effect of CpG-ODNs on the induction of the influenza peptide-specific CTL. The flu peptide-specific CTLs showed clear cytotoxicity against flu peptide-pulsed A24+LCL but not against HIV peptide-pulsed A24+LCL (Fig. 2A and B). The cytotoxicity against flu peptide-pulsed A24+LCL was augmented in the presence of CpG-A, -B and -C but not in the presence of control ODN, and the augmenting effect was greatest with CpG-A; it was moderate with CpG-C and low with CpG-B (Fig. 2A). The depletion of PDCs from PBMCs before stimulation with peptide and CpG-ODNs completely abrogated the augmenting effect of each class of CpG-ODN (Fig. 2C-E). These data indicated that the stimulation of PDCs by CpG-ODNs augmented the expansion and activation of flu peptide-specific CTL to increase the specific cytotoxicity.

Flow cytometric analysis showed the population of $CD8^+$ cells in flu peptide-specific CTLs. Interestingly, the subpopulation of effector-memory cells in $CD8^+$ cells was most increased with CpG-A, and moderately increased with CpG-C (Fig. 3).

CpG-A augmented the LY6K peptide-specific CTL induction in a PDC-dependent manner. The population of LY6K peptide-specific CTL precursor cells in healthy volunteers may be much smaller than that of flu peptide-specific CTLs because LY6K is a cancer-testis antigen (19). Therefore, we investigated whether CpG-ODNs could also affect the induction of LY6K-specific CTLs. Although the induction of influenza-specific CTL was augmented by all types of CpG-ODN (Fig. 2A), the LY6K peptide-specific CTLs were induced only with CpG-A from both donors 1 and 2, but not with CpG-B or CpG-C (Fig. 4A-D). Depletion of PDCs from PBMCs of donor 2 before stimulation with peptide and CpG-A completely abrogated the effect of CpG-A (Fig. 4E).

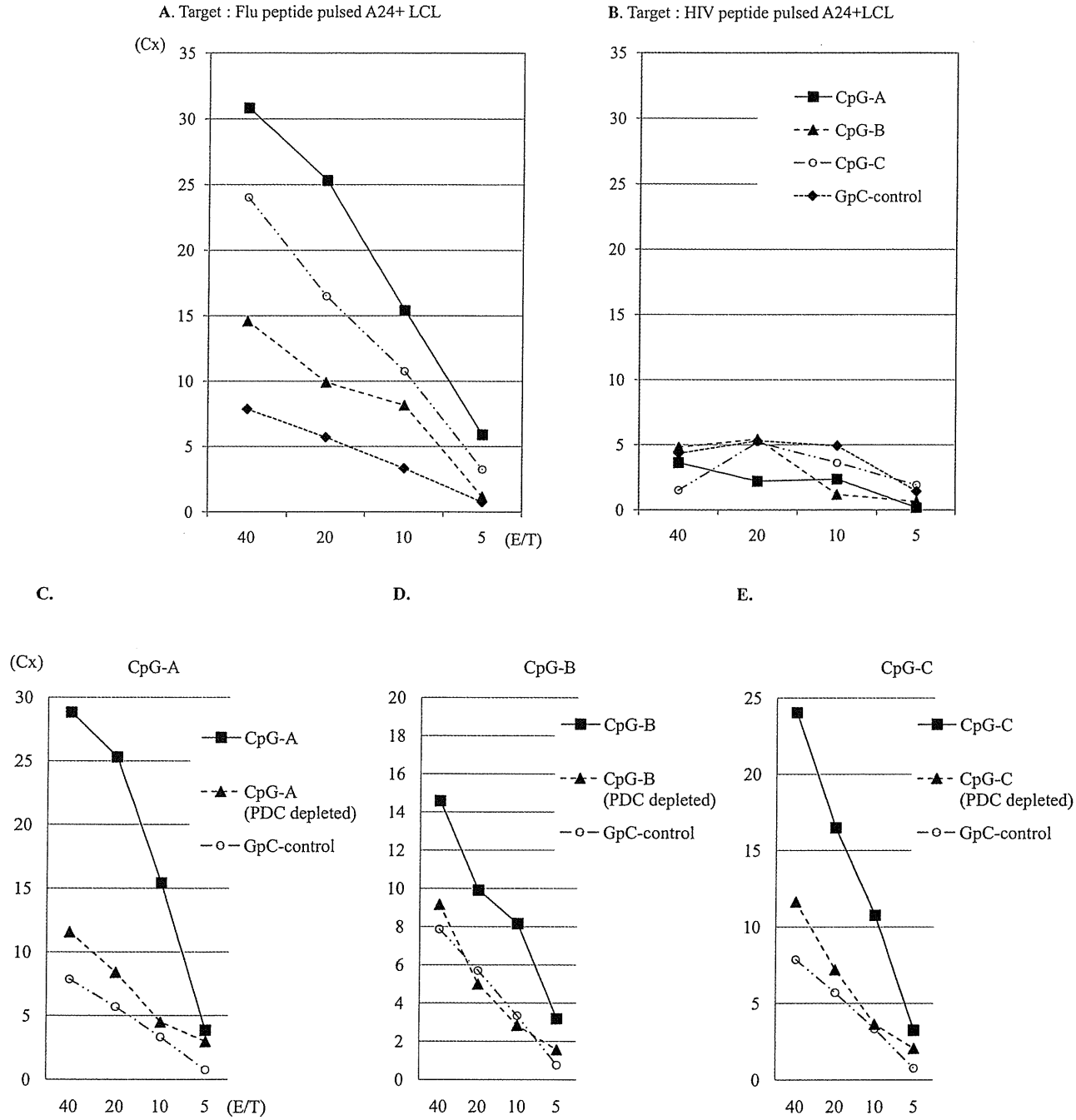

Figure 2. CpG-ODNs augments the influenza peptide-specific CTL induction in a PDC-dependent manner. PBMCs from HLA-A24-positive healthy volunteers were stimulated with the flu peptide in the presence or absence of each class of CpG-ODN (20, 5 and 5 μg/ml for CpG-A, -B and -C, respectively). After 7 days, effector cells were harvested and re-stimulated with the flu peptide pulsed on adherent cells of irradiated PBMCs. After another 7 days, the cytotoxicity of harvested cells against the HLA-A24-positive LCL-A24 cell line pulsed with the flu peptide (A) or a control HIV peptide (B) was assessed by standard ^{51}Cr release assay. Data shown are representative of three independent experiments. PDCs were depleted from PBMCs on day 0. After stimulation with peptide and CpG-ODNs, harvested cells were assessed by standard ^{51}Cr release assay in the same way (C-E).

These data indicated that the stimulation of PDCs by CpG-A augmented the expansion and activation of LY6K peptide-specific CD8$^+$ T cells.

Discussion

Recently, dozens of clinical trials of vaccine therapy for infectious diseases or cancers using CpG-ODNs as an adjuvant have been performed, and some of these trials have shown promising results (5-8). In contrast, there have been few studies that showed the augmenting effects of CpG-ODNs on acquired immunity as a vaccine adjuvant (20). Therefore, the mechanism by which CpG-ODNs augment the efficiency of a vaccine has yet to be clarified in sufficient detail.

In the present study, the efficiency of *in vitro* flu peptide-specific CTL induction by flu peptide was enhanced in the presence of each type of CpG-ODN, and CpG-A showed a more potent adjuvant effect than CpG-B and CpG-C. Moreover, the

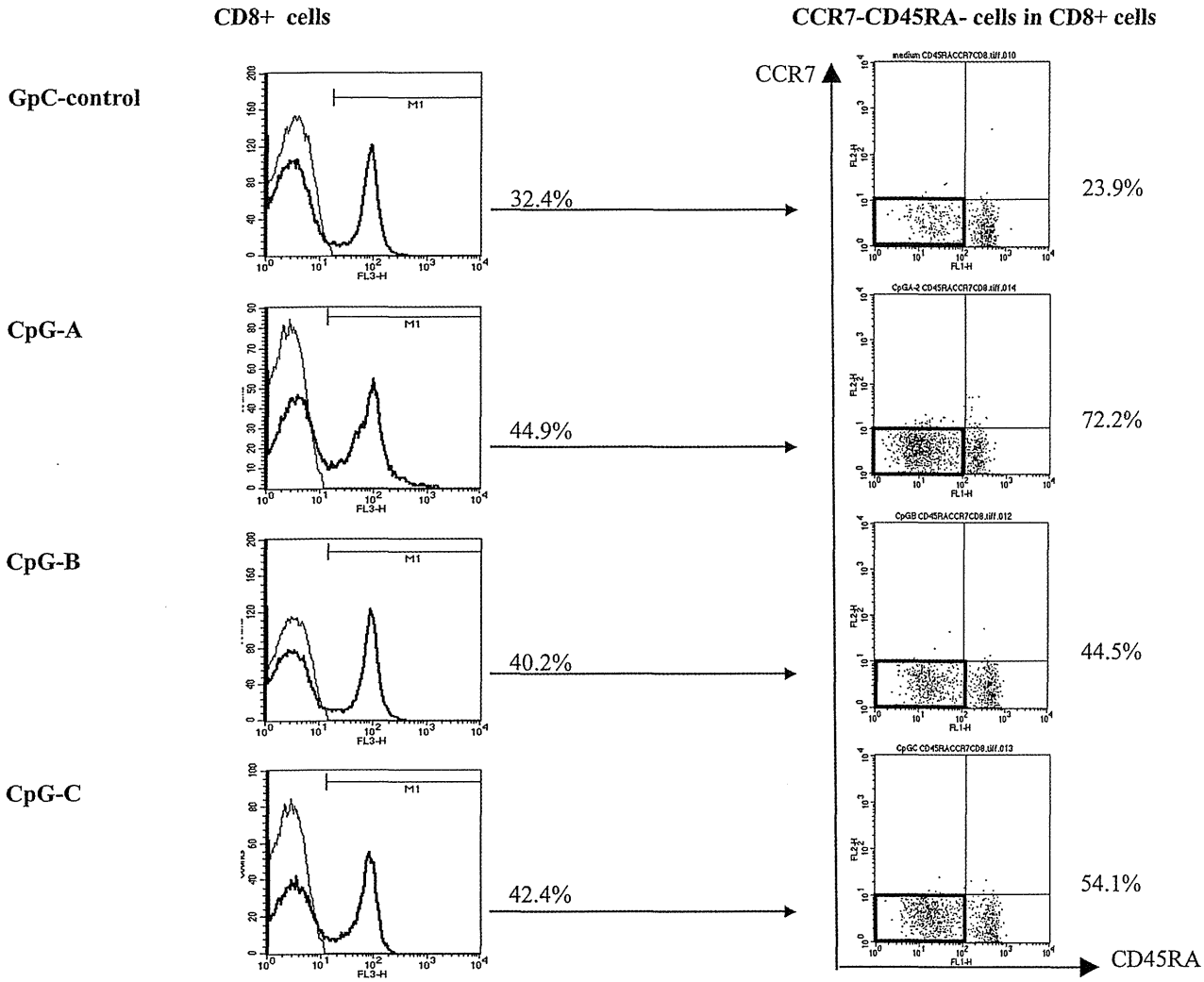

Figure 3. The subpopulation of effector-memory cells in CD8$^+$ cells was increased with CpG-ODN. Populations of CD8$^+$ cells in harvested cells and CCR7$^-$ CD45RA$^-$ effector-memory T cells among CD8$^+$ cells were assessed by flow cytometry.

Ly6K peptide-specific cytotoxicity was induced only with the coexistence of CpG-A, but not with CpG-B and CpG-C. It was suggested that the stimulation of CpG-B or CpG-C was insufficient to elicit Ly6K peptide-specific CTLs because the number of Ly6K peptide-specific precursor CTLs in healthy volunteers is much smaller than that of flu peptide-specific precursor CTLs. These data suggested that CpG-A might be more effective than CpG-B or CpG-C in terms of inducing peptide-specific CTLs *in vitro*.

Our data showed that this CpG-ODN-induced enhancement of cytotoxicity completely disappeared when PDCs were depleted from PBMCs, which means that PDCs were responsible for this enhancement effect. CpG-ODNs mature PDCs by up-regulating the expression of CD80, CD83 and CD86 (21). While most studies have indicated that MoDCs are better antigen-presenters than PDCs (22), many studies have demonstrated the ability of PDCs to function as APCs for both CD4- and CD8-positive cells (22-24). On the other hand, it is well known that CpG-B and CpG-C are more potent to mature PDCs than CpG-A (1,2). Because our data showed that CpG-A was superior to CpG-B and CpG-C in inducing peptide-specific CTLs, the maturation of PDCs by the stimulation of CpG-ODNs could not affect the results in view of our study design. Therefore, we considered that any cytokines produced from PDCs stimulated by CpG-ODNs must contribute to the enhancement of peptide-specific CTL induction. In addition to IFNs, PDCs also produced the pro-inflammatory cytokines TNF-α and IL-6 (data not shown). Type-1 IFN, TNF-α and IL-6 are known to drive the differentiation of DCs into mature antigen-presentation cells. Our data also showed that the supernatant of PBMCs stimulated by CpG-ODNs up-regulated the expression of CD80, 83 and 86 on monocyte-derived DCs, and the expression level was highest with CpG-A, less with CpG-C, and least with CpG-B (data not shown). However, the production levels of TNF-α and IL-6 from PBMCs stimulated by CpG-A were almost the same as those for CpG-B and CpG-C. Murine PDCs are known to produce IL-12, which induces Th1 differentiation by the stimulation of CpG-ODNs, but human PDCs do not induce IL-12 (25,26). In contrast, CpG-A produced a much higher level of IFN-α than CpG-B or CpG-C, and we considered that the reason why CpG-A has the most potent augmenting effect to induce peptide-specific CTLs is that CpG-A induces the highest level of type-1 IFN from PDCs.

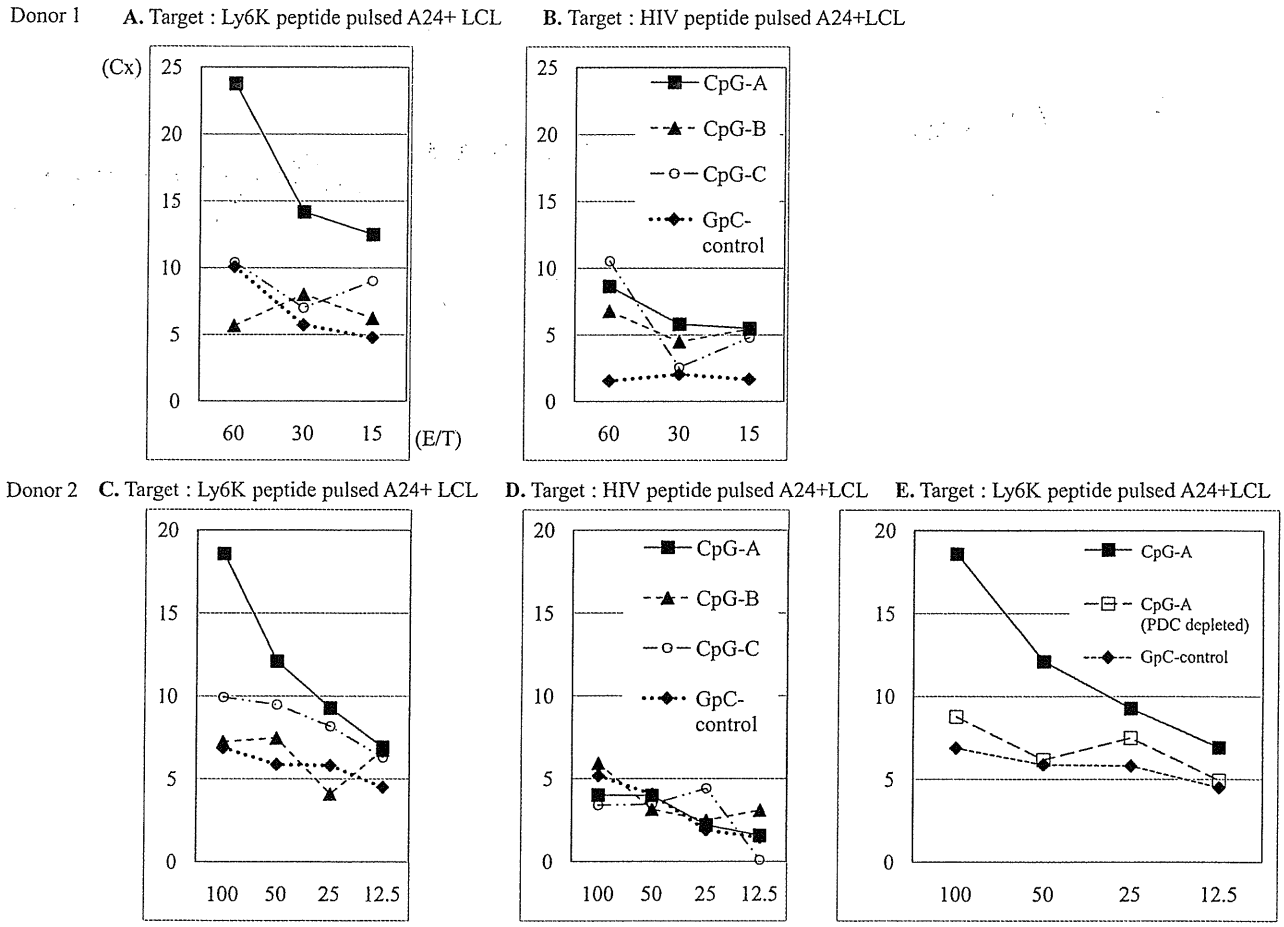

Figure 4. CpG-A augments the Ly6K peptide-specific CTL induction in a PDC-dependent manner. LY6K peptide-specific CTL was generated from 2 different donors in the presence or absence of each class of CpG-ODN (20, 5 and 5 μg/ml for CpG-A, -B and -C, respectively) as described in Materials and methods. The cytotoxicity of harvested cells against the HLA-A24-positive LCL-A24 cell line pulsed with the LY6K peptide (A and C) or a control HIV peptide (B and D) was assessed by standard ^{51}Cr release assay. PDCs were depleted from PBMCs on day 0. After stimulation with peptide and CpG-A, harvested cells were assessed by standard ^{51}Cr release assay in the same way (E).

Type-1 IFNs are known to activate NK cells (27,28) and induce activation of DCs (29-32). In murine models, it is known that type-1 IFNs promote Th1 cytokine production, effector differentiation, proliferation and contribute to the clonal expansion and formation of memory CD8$^+$ T cells (33-40).

Our data revealed that the population of CD8$^+$ cells and that of effector-memory cells in CD8$^+$ cells after induction of flu peptide-specific CTLs were increased the most with CpG-A, less with CpG-C and least with CpG-B. Memory cells persist for extended periods owing to antigen-independent homeostatic turnover and they respond rapidly upon re-encountering a pathogen (41). Two subsets of memory T cells were described on the basis of their anatomical location, expression of cell surface markers and effector functions (42). Memory T cells that express molecules such as CCR7, which allow efficient homing to lymph nodes (LN), are termed central memory cells (T_{CM}), whereas memory T cells that lack expression of these LN homing receptors and are located in no lymphoid tissues are termed effector memory cells (T_{EM}). Some studies have also shown that T_{EM} acquire effector functions, such as cytokine production and killing, more rapidly than T_{CM} (42-44). The mechanisms that contribute to the generation of memory cells are poorly understood. Previous studies suggested that infectious antigen-experienced CD8$^+$ T cells undergo programmed expansion for about 1 week after infection and then undergo programmed cell death (45-47). A majority of the daughter cells derived from antigen-experienced CD8$^+$ T cells undergo death in parallel with proliferation during the acute phase of viral infection and direct type-I IFN action rescues them from this death, thereby tilting the balance effectively toward clonal expansion (40). Therefore, the type-I IFN-mediated rescue from death during antigen-driven proliferation might be critical for the expansion of memory precursors.

In conclusion, our data showed that the stimulation of PDCs by CpG-ODN augmented the generation of effector-memory peptide-specific CTLs. Furthermore, CpG-A might be superior to CpG-B and CpG-C in augmenting the generation of human peptide-specific CTLs *in vitro*. Therefore, CpG-A could become a superior vaccine adjuvant rather than CpG-B or CpG-C in clinical application.

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ORIGINAL ARTICLE

Predictive risk factors for clinically relevant pancreatic fistula analyzed in 1,239 patients with pancreaticoduodenectomy: multicenter data collection as a project study of pancreatic surgery by the Japanese Society of Hepato-Biliary-Pancreatic Surgery

Manabu Kawai · Satoshi Kondo · Hiroki Yamaue · Keita Wada · Keiji Sano · Fuyuhiko Motoi · Michiaki Unno · Sohei Satoi · A-Hon Kwon · Takashi Hatori · Masakazu Yamamoto · Joe Matsumoto · Yoshiaki Murakami · Ryuichiro Doi · Masahiro Ito · Shuichi Miyakawa · Hiroyuki Shinchi · Shoji Natsugoe · Hisatoshi Nakagawara · Tetsuo Ohta · Tadahiro Takada

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Abstract

Background/purpose It is important to predict the development of clinically relevant pancreatic fistula (grade B/C) in the early period after pancreaticoduodenectomy (PD). This study has been carried out as a project study of the Japanese Society of Hepato-Biliary-Pancreatic Surgery (JSHPBS) to evaluate the predictive factors associated with clinically relevant pancreatic fistula (grade B/C).

Method The data of 1,239 patients from 11 medical institutions who had undergone PD between July 2005 and June 2009 were retrospectively analyzed to review patient characteristics and perioperative and postoperative parameters.

Results A drain amylase level >4,000 IU/L on postoperative day (POD) 1 was proposed as the cut-off level to predict clinical relevant pancreatic fistula by the receiver operating characteristic (ROC) curve. The sensitivity, specificity, and accuracy of this cut-off level were 62.2, 89.0, and 84.8%, respectively. A multivariate logistic regression analysis revealed that male [odds ratio (OR) 1.7,

M. Kawai · H. Yamaue
Second Department of Surgery, School of Medicine, Wakayama Medical University, Wakayama, Japan

S. Kondo (✉) · J. Matsumoto
Department of Surgical Oncology, Hokkaido University Graduate School of Medicine, N15 W7, Kita-ku, Sapporo, Hokkaido 060-8638, Japan
e-mail: info@jshbps.jp

K. Wada · K. Sano
Department of Surgery, Teikyo University School of Medicine, Tokyo, Japan

F. Motoi · M. Unno
Division of Hepato-Biliary-Pancreatic Surgery, Department of Surgery, Tohoku University Graduate School of Medicine, Sendai, Japan

S. Satoi · A.-H. Kwon
Department of Surgery, Kansai Medical University, Moriguchi, Osaka, Japan

T. Hatori · M. Yamamoto
Department of Surgery, Institute of Gastroenterology, Tokyo Women's Medical University, Tokyo, Japan

Y. Murakami
Division of Clinical Medical Science, Department of Surgery, Hiroshima University Graduate School of Biomedical Sciences, Hiroshima, Japan

R. Doi
Department of Hepato-Biliary-Pancreatic Surgery and Transplantation, Kyoto University, Kyoto, Japan

M. Ito · S. Miyakawa
Department of Biliary Pancreatic Surgery, Fujita Health University School of Medicine, Toyoake, Aichi, Japan

H. Shinchi · S. Natsugoe
Department of Surgical Oncology, Kagoshima University, Kagoshima, Kagoshima, Japan

H. Nakagawara · T. Ohta
Department of Gastroenterological Surgery, Graduate School of Medical Science, Kanazawa University, Kanazawa, Ishikawa, Japan

T. Takada
Japanese Society of Hepato-Biliary-Pancreatic Surgery, Department of Surgery, Teikyo University School of Medicine, Tokyo, Japan

$P = 0.039$], intraoperative bleeding >1,000 ml (OR 2.5, $P = 0.001$), soft pancreas (OR 2.7, $P = 0.001$), and drain amylase level on POD 1 ≥4,000 IU/L (OR 8.6, $P < 0.001$) were the significant predictive factors for clinical pancreatic fistula.

Conclusion The four predictive risk factors identified here can provide useful information useful for tailoring postoperative management of clinically relevant pancreatic fistula (grade B/C).

Keywords Pancreatic fistula · Pancreaticoduodenectomy · Predictive risk factors · Drain amylase level

Introduction

In most patient series, the incidence of pancreatic fistula has been reported to vary between 5 and 20% after pancreaticoduodenectomy (PD) and to be associated with a high mortality rate [1–7]. In 2005, the International Study Group of Pancreatic Fistula (ISGPF) proposed a consensus definition and clinical grading of postoperative pancreatic fistula [8]. The most important issue currently being debated regarding pancreatic fistulas is whether it is possible to predict the development of clinically relevant pancreatic fistula (grade B/C) according to the ISGPF proposal in the early period after PD. The risk factors for developing a pancreatic fistula in previously reported studies may have been able to predict all grades of pancreatic fistulas but, unfortunately, could not predict the extent of severe clinically relevant pancreatic fistula (grade B/C) [9–13].

Molinari et al. [14] proposed that a drain amylase value on postoperative day (POD) 1 of >5,000 U/L was a significant predictive factor for the incidence of all grades of pancreatic fistula after PD. However, this amylase value could not distinguish clinically relevant pancreatic fistulas (grade B/C) from insignificant disease during the early postoperative period. Kawai et al. proposed that a combination of two predictive postoperative factors on POD 4, namely, serum albumin level ≤3.0 g/dL and leukocyte counts >9,800/mm^3, can predict the development of clinically relevant pancreatic fistula [15]. However, these two factors may also reflect serious systemic inflammation, such as that due to other intra-abdominal or respiratory complications. It therefore remains unclear which predictive risk factor(s) can be used to precisely distinguish the risk of clinically relevant pancreatic fistula (grade B/C) in the early postoperative period. In an attempt to clarify this situation, the Japanese Society of Hepato-Biliary-Pancreatic Surgery (JSHPBS) decided to perform a survey of high-volume PD centers in Japan to evaluate the predictive factors for the development of clinically relevant pancreatic fistula (grade B/C) in the early period after PD.

Methods

Patients

Data were collected by a questionnaire survey on all patients who underwent PD between July 2005 and June 2009 at one of 11 high-volume centers participating in the project study of the JSHPBS. The following patient characteristics and perioperative and postoperative parameters were reviewed: age, gender, body mass index (BMI), American Society of Anesthesiologists (ASA) class, preoperative laboratory data, such as hemoglobin, creatinine, HbA1c (glycated hemoglobin), albumin, total bilirubin, and amylase, preoperative biliary drainage, length of the surgery, intraoperative bleeding, blood transfusion, pancreatic texture (soft or hard), presence or absence of dilatation of the main pancreatic duct, histologic diagnosis (malignant or benign), and the serum C-reactive protein (CPR) and drain amylase levels on POD 1, 3, and 4. In total, data on 1,331 patients were collected from the 11 institutions. Of these 1,331 patients, 1,239 (749 men, 490 women; median age 67 years, age range 35–91 years) were enrolled in the study, and their data used for the analysis of the occurrence of pancreatic fistula using the ISGPF criteria.

Postoperative complications

The diagnosis of pancreatic fistula was made based on the ISGPF guidelines [8], namely, an amylase level in the drainage fluid on POD 3 of more than threefold the serum amylase level. Pancreatic fistulas were classified into three categories according to the ISGPF guidelines:

Grade A: Transient fistula. There is no clinical impact. The patient is fed orally and remains clinically well.
Grade B: Patients are usually supported with partial, total parenteral, or enteral nutrition. Antibiotics are usually used for signs of infections and a somatostatin analogue may also be required. Percutaneous drainage or persistent drainage for more than 3 weeks is usually required.
Grade C: A major change in clinical management or deviation from the normal clinical pathway. Total parenteral, enteral nutrition, antibiotics, or somatostatin analogue is often instituted in an intensive care unit (ICU) setting. Radiologic intervention or reoperation is required. The patients typically require an extended hospital stay with a major delay in hospital discharge and have life-threatening complications, such as intra-abdominal bleeding or sepsis. There is a real possibility of postoperative mortality[8].

Grades B + C were defined as "clinically relevant pancreatic fistula". Delayed gastric emptying (DGE) was

defined according to a consensus definition and the clinical grading of postoperative DGE according to the proposals of the International Study Group of Pancreatic Surgery (ISGPS) [16], using the web-based calculator (http://pancreasclub.com/calculator/) to improve the homogeneity of the definition [17]. DGE was then classified into three categories (grade A, B, or C) by the ISGPS clinical criteria based on the clinical course and postoperative management, such as reinsertion of a nasogastric tube, the period of inability to tolerate a solid diet, presence or absence of vomiting, and the use of prokinetics. Other postoperative complications were graded according to the Clavien classification [18], where grade I is any deviation from the normal postoperative course but without any need for pharmacologic treatment or surgical, endoscopic, or radiologic intervention; grade II is indicated by complications requiring pharmacologic treatment; grade III, by complications requiring surgical, endoscopic, or radiologic intervention; grade IV, life-threatening complications requiring intermediate or ICU management; grade V, death. Complications in this study were defined as a condition that was more than grade II according to the Clavien classification.

Statistical analysis

Continuous variables were expressed as the mean ± standard deviation (SD). Patient characteristics and perioperative and postoperative factors between the groups were compared using chi-square statistics, the Fisher exact test, and the Mann–Whitney U test. Variables with $P < 0.05$ were entered into a logistic regression model to determine independent risk factors of postoperative complications. The independent risk factors of the variables were expressed as odds ratios (OR) with their 95% confidence intervals (CI). The measurement of drain amylase levels on POD 1 has a major benefit by enabling the development of clinically relevant pancreatic fistula (grade B/C) to be predicted in the early period after PD. In fact, amylase values in drains on POD 1 of >5,000 U/L have been reported to be a significant predictive factor for the incidence of all grades of pancreatic fistula after PD [14]. Therefore, the optimal cut-off levels of the drain amylase level on POD 1 for differentiation between the no pancreatic fistula/grade A group and the grade B/C group were sought by constructing receiver operating characteristic (ROC) curves, which were generated by calculating the sensitivities and specificities of the drain amylase level on POD 1 at several predetermined cut-off points. Line graphs were used for graphical visualization (SPSS, Chicago, IL). Statistical significance was defined as $P < 0.05$.

Results

The indications for PD in the 1,239 patients were 573 pancreatic adenocarcinoma, 237 bile duct carcinoma, 124 ampullary adenocarcinoma, 127 intraductal papillary neoplasms, 38 duodenal adenocarcinoma, 37 pancreatic endocrine neoplasms, 46 tumor-forming pancreatitis, and 57 "other" diseases.

Postoperative complications

Table 1 shows the postoperative complications among the patient cohort after PD. The overall morbidity was 44.2% (548/1,239 patients). The overall rate of pancreatic fistula was 30.2% (374 patients). When the pancreatic fistula was classified into the three categories according to the ISGPF criteria, 15.8% (196/1,239) of the patients had grade A fistula; 11.8% (146 patients) had grade B; 2.6% (32 patients) had grade C. In total 139 (11.2%) patients had intra-abdominal abscess, and 58 patients (4.7%) required percutaneous drainage for the development of intra-abdominal abscess related to pancreatic fistula after PD. The reoperation rate due to pancreatic fistula was 0.72% (9/1,239 patients), and the overall incidence of DGE was 16.9% (211/1,239 patients). The DGE was categorized according to the ISGPS guidelines into grade A (107 patients, 9.6%), grade B (51 patients, 4.1%), and grade C (53 patients, 4.3%). The overall mortality rate was 0.97% (12/1,239 patients).

Comparison of patient characteristics, intraoperative status, and postoperative outcome among types of pancreatic fistula

Table 2 shows the general characteristics, intraoperative status, and postoperative outcome of the 1,239 patients classified by ISGPF. Based on the ISGPF criteria, 865 of the 1,239 patients (69.8%) did not develop a pancreatic fistula, and 196 patients (15.8%) developed only transient pancreatic fistula (grade A), while a clinically relevant pancreatic fistula (grade B/C) developed in 178 patients (14.4%). The ratio of male to female patients was significantly higher in those patients with a grade B/C fistula than in those with no pancreatic fistula or a grade A fistula. In those patients with a grade B/C fistula, the length of the surgery was significantly longer, and the intraoperative bleeding or the need for blood transfusion was significantly higher, compared to subjects with no pancreatic fistula or a grade A fistula. Patients with soft pancreatic parenchyma or a pancreatic duct <3 mm had a significantly higher incidence of pancreatic fistula (grade A and grade B/C) than those with no pancreatic fistula ($P < 0.01$). There were no

Table 1 Postoperative complications and the outcome after 1,239 pancreaticoduodenectomies

Postoperative complications/outcome	No. of patients (%)
Overall morbidity	548 (44.2)
Local complication	
Pancreatic fistula[a]	374 (30.2)
Grade A	196 (15.8)
Grade B	146 (11.8)
Grade C	32 (2.6)
Intra-abdominal abscess	139 (11.2)
Intra-abdominal bleeding	34 (2.7)
Biliary leakage	29 (2.3)
Gastrointesitinal leakage	72 (5.8)
Delayed gastric emptying[b]	211 (16.9)
Grade A	107 (9.6)
Grade B	51 (4.1)
Grade C	53 (4.3)
Wound infection	130 (10.5)
Systemic complications	
Cardiac complication	35 (2.8)
Pulmonary complications	23 (1.9)
Sepsis	35 (2.8)
Percutaneous drainage	58 (4.7)
Persisting drain (more than 3 weeks)[c]	104 (8.4)
Reoperation	9 (0.7)
Mortality	12 (1.0)

[a] Pancreatic fistula as defined by the International Study Group of Pancreatic Fistula (ISGPF)

[b] Delayed gastric emptying as defined by the International Study Group of Pancreatic Surgery (ISGPS)

[c] Used in postoperative management of intra-abdominal abscess related to pancreatic fistula

significant differences between patients with grade A and grade B/C pancreatic fistual in term of the presence of soft pancreatic parenchyma or pancreatic duct <3 mm.

With regard to the postoperative outcome, the drain amylase level on POD 1 in the grade B/C group (18,846 ± 37,287 IU/L) was significantly higher than that in the grade A group (7,951 ± 15,322 IU/L) ($P = 0.002$), whereas there was no significant difference between the grade A group and grade B/C group in terms of drain amylase level on POD 3 and 4.

Predictive factors for the development of a clinically relevant pancreatic fistula (grade B/C)

With regard to the sensitivity and specificity of the drain amylase level on POD 1, an area under the ROC curve of 0.840 was obtained ($P < 0.001$; 95% confidence interval: 0.807–0.874) (Fig. 1). Drain amylase level >4,000 IU/l on POD 1 was suggested to be the best cut-off for prediction of the clinically relevant pancreatic fistula. The sensitivity, specificity, accuracy, positive predictive value and negative predictive value of drain amylase level on POD1 >4,000 IU/l were 62.2, 89.0, 84.8, 51.1 and 92.7%, respectively.

Univariate and multivariate analysis were used to reveal those factors predicting grade B/C pancreatic fistula. Table 3 presents the results of the univariate analysis of 18 parameters as potential risk factors in the 178 patients with clinically relevant pancreatic fistula (grade B/C) versus 1,061 patients with no pancreatic fistula or with transient pancreatic fistula (grade A). Nine factors were extracted and identified as being useful for discriminating between those who would develop grade B/C fistula and those who would not develop a fistula or would develop only grade A fistula: (1) preoperative factors, namely, male gender ($P < 0.001$), BMI >25 kg/m^2 ($P = 0.005$), HbA1c >7.0% ($P = 0.001$), and creatinine >1.5 mg/dL ($P = 0.039$); (2) intraoperative factors, namely, operative time >480 min ($P = 0.012$), intraoperative bleeding >1,000 mL ($P = 0.001$), soft pancreatic parenchyma ($P < 0.001$), and main pancreatic duct <3 mm ($P < 0.001$); (3) one postoperative factor, namely, drain amylase level on POD 1 >4,000 IU/L ($P < 0.001$). A multivariate logistic regression analysis revealed that male gender (OR 1.7, 95% CI 1.0–3.0, $P = 0.039$), intraoperative bleeding >1,000 mL (OR 2.5, 95% CI 1.4–4.6, $P = 0.001$), soft pancreatic parenchyma (OR 2.7, 95% CI 1.5–4.8, $P = 0.001$), and drain amylase level on POD 1 >4,000 IU/L (OR 8.6, 95% CI 5.2–14.2, $P < 0.001$) were the significant predictive factors for developing clinically relevant pancreatic fistula of grade B/C (Table 4).

Discussion

The development of pancreatic fistula has been reported to be a potentially life-threatening complication after PD [19–21]. Although lower grade fistula can still complicate patient recovery, it is very important to be able to predict whether a patient will develop clinically relevant pancreatic fistula (grade B/C) in the early period after PD, since these fistulas require changes in patient management and are associated with a higher mortality rate. This study was conducted as a project study of the JSHPBS and was designed to evaluate the predictive factors associated with the development of a clinically relevant pancreatic fistula (grade B/C) after PD. We found that male gender, intraoperative bleeding >1,000 mL, soft pancreatic parenchyma, and drain amylase level on POD 1 of >4,000 IU/L were the most significant predictive factors of the development of clinically relevant pancreatic fistula (grade B/C).

Table 2 Comparison of patient characteristics, intraoperative status, and postoperative outcome among types of pancreatic fistula

Patient characteristics, intraoperative status, and postoperative outcome	Pancreatic fistula		
	(−) (n = 865)	Grade A (n = 196)	Grade B/C (n = 178)
Patient characteristics			
Age (years)	66 ± 11	67 ± 10	67 ± 9
Gender (male/female)	507/358	110/86	132/46[c]
Body mass index (kg/m^2)	21.5 ± 3.4	21.7 ± 3.0	22.6 ± 3.3
ASA (I–II/III–IV)	740/122	172/24	144/33
Preoperative serum bilirubin level (mg/dl)	3.1 ± 4.6	2.9 ± 4.5	3.4 ± 5.9
Preoperative biliary drainage (%)	46.2	41.8	49.4
Preoperative hemoglobin (g/dL)	12.4 ± 1.6	12.3 ± 1.8	12.7 ± 1.7
Preoperative creatinine (mg/dL)	0.8 ± 10.6	0.8 ± 0.5	1.0 ± 1.0
HbA1c (%)	6.3 ± 4.5	5.8 ± 1.3	5.5 ± 0.9
Preoperative serum albumin (g/dL)	3.9 ± 0.5	3.9 ± 0.5	3.8 ± 0.5
Preoperative serum amylase (IU/L)	139 ± 244	137 ± 162	125 ± 113
Histology (benign/malignant)	132/696	43/144	35/132
Intraoperative status			
Operative time (min)	480 ± 144	456 ± 133	542 ± 403[c]
Intraoperative bleeding (mL)	1,215 ± 1,168	1,170 ± 1,056	1,596 ± 1,849[c]
Red blood cell transfusion (unit)	1.8 ± 3.9	1.7 ± 2.8	2.9 ± 6.2[c]
Pancreatic duct (>3 mm/<3 mm)	573/170	99/77[a]	76/90[b]
Soft pancreatic texture (soft/hard)	355/510	151/45[a]	142/36[b]
Postoperative outcome			
CRP on POD 1 (mg/dL)	9.1 ± 22.0	8.6 ± 3.3	9.3 ± 3.8
CRP on POD 3 (mg/dL)	12.0 ± 5.95	15.8 ± 7.4[a]	20.0 ± 7.2[c]
CRP on POD 4 (mg/dL)	8.35 ± 5.95	12.2 ± 6.1[a]	15.7 ± 6.7[c]
WBC on POD 1 (/mm^3)	10,167 ± 3,367	10,636 ± 3,757	10,380 ± 4,163
WBC on POD 3 (/mm^3)	9,127 ± 5,024	10,489 ± 3,562[a]	10,674 ± 3,840[b]
WBC on POD 4 (/mm^3)	7,677 ± 6,169	8,799 ± 3,168[a]	9,735 ± 3,529[b]
Albumin on POD 1 (g/dL)	2.8 ± 1.0	2.9 ± 0.5	2.9 ± 0.5
Albumin on POD 3 (g/dL)	2.8 ± 0.5	2.8 ± 0.5	2.7 ± 0.5
Albumin on POD 4 (g/dL)	2.9 ± 0.6	3.0 ± 0.6	2.7 ± 0.4[b]
Amylase level of drainage fluid			
POD 1 (IU/L)	1,058 ± 2,186	7,951 ± 15,322[a]	18,846 ± 37,278[c]
POD 3 (IU/L)	134 ± 181	3,638 ± 10,711[a]	6,284 ± 15,183[c]
POD 4 (IU/L)	79 ± 136	2,375 ± 9,565[a]	5,325 ± 18,452[c]
Postoperative hospital stay (days)	28 ± 18	29 ± 20	56 ± 40[c]

Data are given as the mean ± standard deviation (SD) unless otherwise indicated

ASA American Society of Anesthesiologists, *HbA1c* hemoglobin A1C (glycated hemoglobin), *CRP* C-reactive protein, *WBC* white blood cells, *POD* postoperative day

[a] No pancreatic fistula vs. grade A, $P < 0.01$

[b] No pancreatic fistula vs. grade B + C, $P < 0.01$

[c] No pancreatic fistula vs. grade B + C, $P < 0.01$ and grade A vs, grade B + C, $P < 0.01$

In particular, a drain amylase value >4,000 U/L on POD 1 correlated with 8.6-fold increased risk of developing clinically relevant pancreatic fistula.

Some authors have proposed that drain-related data, including the drain amylase level, are useful for defining the risk of developing a pancreatic fistula after PD [14, 22, 23], including Molinari et al. [14], who suggested that a drain amylase value on POD 1 >5,000 U/L was a significant predictive factor for the incidence of pancreatic fistula. However, the latter study was limited due to the small sample

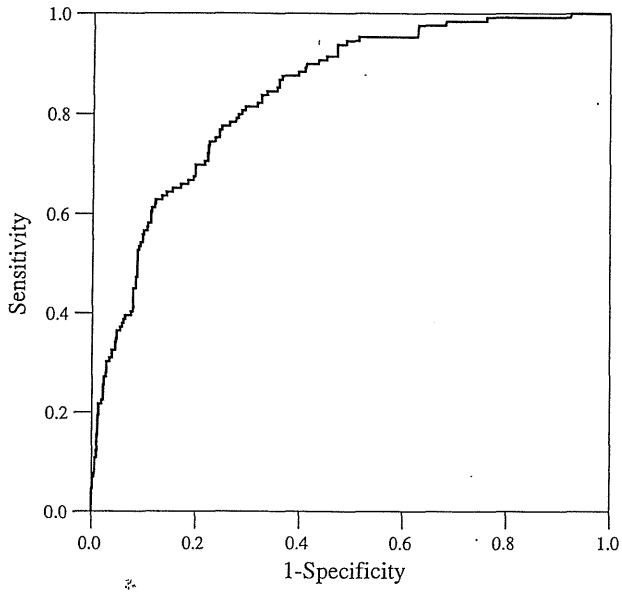

Fig. 1 Receiver operating characteristic (ROC) curves fore drain amylase level on postoperative day (POD 1) to predict clinically relevant pancreatic fistula (grade B/C). An area under the ROC curve of 0.841 was obtained taking the sensitivity and specificity of the drain amylase level on POD 1 into consideration [$P < 0.001$, 95% confidence interval (CI) 0.806–0.876]. A drain amylase level >4,000 IU/l on POD 1 was therefore suggested to be the best cut-off for predicting the clinical occurrence pancreatic fistula

size ($n = 137$). Moreover, of the 137 patients enrolled in the study, 36 patients underwent distal pancreatectomy, and 12 (44%) of the 27 patients with pancreatic fistula reported in their results underwent distal pancreatectomy, not PD [14]. In these three studies [14, 22, 23], clinically relevant pancreatic fistula (grade B/C) could not be distinguished from transient (grade A) fistula in the early postoperative period solely based on the drain amylase value. In three other studies [15, 24, 25], the authors were unable to determine whether the drain amylase value was reflective of clinically relevant pancreatic fistula, although they did propose the evaluation of predictive risk factors, such as the presence of soft pancreas, intra-abdominal bleeding, and the postoperative albumin level, as indicators for the development of clinically relevant pancreatic fistula. To the best of our knowledge, our study is the first published report demonstrating that the drain amylase value on POD 1 can predict the development of clinically relevant pancreatic fistula (grade B/C). Therefore, based on the results of this study, early removal of drains can be done when the drain amylase value is <4,000 U/L on POD 1 because these patients are unlikely to develop clinically relevant fistula. Moreover, early drain removal has previously been demonstrated to play a critical role in reducing the incidence of pancreatic fistula or intra-abdominal abscess [26, 27].

Table 3 A univariate analysis of the predictive factors for clinical relevant pancreatic fistula (ISGPF grade B/C)

Predictive factors	Pancreatic fistula grade B/C		
	(−) ($n = 1,061$)	(+) ($n = 178$)	P value
Age (>75/≤75 years)	202/859	31/147	0.608
Gender (male/female)	617/444	132/46	<0.001
BMI (>25/≤25 kg/m^2)	142/919	38/140	0.005
ASA (I–II/III–IV)	146/912	33/144	0.090
COPD (yes/no)	59/1,001	11/167	0.871
HbA1c (>7.0/≤7.0%)	181/743	15/129	0.008
Hemoglobin (>12/≤12 g/dL)	647/410	120/58	0.114
Creatinine (>1.2/≤1.25 mg/dL)	32/975	11/162	0.039
Albumin (>3.5/≤3.5 g/dL)	857/198	139/37	0.539
Total bilirubin (>5/≤5 mg/dL)	212/846	36/142	0.776
Amylase (>180/≤180 IU/L)	186/865	26/152	0.257
Preoperative biliary drainage (yes/no)	482/579	88/90	0.321
Operation time (>480/≤480 min)	470/591	97/81	0.012
Intraoperative bleeding (>1,000/≤1,000 mL)	478/583	105/73	0.001
Blood transfusion (yes/no)	344/717	69/109	0.097
Pancreatic texture (soft/hard)	506/555	142/36	<0.001
Main pancreatic duct (<3/≥3 mm)	247/672	90/76	<0.001
Amylase level of drainage fluid on POD 1 (IU/L) (≤4,000/>4,000)	712/88	56/92	<0.001

BMI body mass index, *COPD* chronic obstructive pulmonary disease

Table 4 Multivariate analysis of the predictive factors for clinical relevant pancreatic fistula (ISGPF grade B/C) and predictive score

Risk factor	*P* value	Odds ratio	95% Confidence interval
Male	0.039	1.7	1.0–3.0
Intraoperative bleeding >1,000 ml	0.001	2.5	1.5–4.2
Soft pancreas	0.001	2.7	1.5–4.8
Amylase level of drainage fluid on POD 1 (IU/l) >4,000 IU/L	<0.0001	8.6	5.2–14.2

In contrast, the authors of a number of other studies have found that the amylase level in drainage fluid after PD has no clinical significance [28, 29]. The ISGPF has proposed that the ability to detect pancreatic fistula is imperfect when only drain data are used [28]. Two factors may underlie this low predictive ability: first, there have been patients who ultimately demonstrated no clinically relevant symptoms of pancreatic fistula despite having amylase-rich fluid on POD 1; second, there was the pattern of pancreatic fistula defined by Pratt et al. [30] as latent pancreatic fistula. Latent fistulas have been defined as initially lacking amylase-rich fluid but ultimately becoming clinically relevant pancreatic fistula (grade B/C). Pratt et al. [30] proposed that advanced age and small pancreatic duct size were significantly associated with latent fistulas.

There are several limitations to our study because of the multicenter and retrospective nature of the data collection. First, the surgical procedures, such as pancreaticoenterostomy (pancreaticogastrostomy or pancreaticojejunostomy) or use of pancreatic duct stent (internal, external, or no stent), were not standardized across institutions. Second, drain management, such as the number of drains, location of placed drains, or drain type, varied widely according to institutional experience. There were cases which ultimately demonstrated no clinically relevant symptoms of pancreatic fistula despite having amylase-rich fluid on POD 1. In fact, 48.9% of patients with a drain amylase value >4,000 U/L on POD 1 did not develop a clinically relevant pancreatic fistula. Therefore, further studies are necessary to prospectively validate these predictive risk factors to confirm the possible relationship between these factors and the development of clinically relevant pancreatic fistula (grade B/C).

In conclusion, the results of this study, which was an initiative of the JSHPBS, indicated that male gender, soft pancreas, intraoperative bleeding >1,000 ml, and amylase value >4,000 U/L in drains on POD 1 were significant predictive risk factors for developing clinically relevant pancreatic fistula (grade B/C). Management of pancreatic fistula in the early period after PD is not sufficiently standardized. Therefore, the identification of these predictive risk factors can provide useful information to tailor the postoperative management for patients who are at an increased risk of developing pancreatic fistula, including drain management, and the administration of antibiotics, a protease inhibitor, octreotide, or enteral nutrition.

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TOPICS

Current status of the randomized controlled trial in pancreatic surgery

Pancreaticoduodenectomy versus pylorus-preserving pancreaticoduodenectomy: the clinical impact of a new surgical procedure; pylorus-resecting pancreaticoduodenectomy

Manabu Kawai · Hiroki Yamaue

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Abstract Pylorus-preserving pancreaticoduodenectomy (PpPD) has been performed increasingly for periampullary tumors as a modification of conventional pancreaticoduodenectomy (PD) with antrectomy. Five randomized controlled trials (RCTs) and two meta-analyses have been performed to compare PD with PpPD. The results of these trials have shown that the two procedures were equally effective concerning morbidity, mortality, quality of life (QOL), and survival, although the length of surgery and blood loss were significantly lower for PpPD than for PD in one RCT and in the two meta-analyses. Delayed gastric emptying (DGE) is the major postoperative complication after PpPD. One of the pathogeneses of DGE after PpPD is thought to be denervation or devascularization around the pyloric ring. Therefore, one RCT was performed to compare PpPD with pylorus-resecting pancreaticoduodenectomy (PrPD; a new PD surgical procedure that resects only the pyloric ring and preserves nearly all of the stomach), concerning the incidence of DGE. The results clarified that the incidence of DGE was 4.5% after PrPD and 17.2% after PpPD, which was a significant difference. Several RCTs of surgical or postoperative management techniques have been performed to reduce the incidence of DGE. One RCT for surgical techniques clarified that the antecolic route for duodenojejunostomy significantly reduced the incidence of DGE compared with the retrocolic route. Two RCTs examining postoperative management showed that the administration of erythromycin after PpPD reduced the incidence of DGE.

M. Kawai · H. Yamaue (✉)
Second Department of Surgery, Wakayama Medical University, School of Medicine, 811-1 Kimiidera, Wakayama 641-8510, Japan
e-mail: yamaue-h@wakayama-med.ac.jp

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Introduction

Pancreaticoduodenectomy (PD) was first performed by Kausch [1] and was later developed by Whipple and colleagues for the treatment of carcinoma of the ampulla of Vater [2]. Conversely, pylorus-preserving pancreaticoduodenectomy (PpPD) with preservation of the entire stomach was described by Watson [3], and was popularized for the treatment of chronic pancreatitis as a modification of conventional PD with antrectomy reported by Traverso and Longmire in the late 1970s [4]. PpPD has been thought to reduce dumping syndrome, diarrhea, and bile reflux gastritis after gastrectomy and to afford patients an improved nutritional status compared to PD with antrectomy. Several reports have discussed whether preservation of the pylorus can provide a better nutritional status and more favorable quality of life (QOL) compared with PD [5–12]. However, the difference between PD and PpPD concerning long-term nutrition and QOL remains controversial.

The incidence of morbidities after PpPD remains high, in the range of 30–65%, although the mortality rate has dropped to below 5% as a result of advances in surgical techniques and perioperative management [13–17]. Delayed gastric emptying (DGE) after PpPD may occur with an incidence varying from 12 to 42%, as reported in previous series [12, 18–22]. One retrospective study reported that PpPD led to an increased incidence of DGE compared with PD [21]. At the same time, other studies have observed no difference in the incidence of DGE between PD and PpPD [6, 7, 22]. The impact of pylorus

preservation in patients undergoing PD for periampullary tumors has remained controversial concerning morbidity, mortality, QOL, and long-term survival.

Several randomized controlled trials (RCTs) or meta-analyses comparing PpPD and PD have been performed [23–29]. This review summarizes the findings of the RCTs comparing PD with PpPD and describes a new PD surgical procedure with resection of only the pyloric ring with preservation of nearly all of the stomach–pylorus-resecting pancreaticoduodenectomy (PrPD) [30].

Pylorus-preserving pancreaticoduodenectomy versus pancreaticoduodenectomy

Morbidity and mortality

Five RCTs comparing PD with PpPD have been performed at three institutions (Table 1) [23–27]. Concerning intra-operative factors, Seiler et al. [26] reported that the length of surgery, blood loss, and the volume of blood transfusions were all significantly lower for PpPD ($n = 64$) than PD ($n = 66$).

Concerning the incidence of postoperative complications, the five RCTs revealed no significant differences in the incidence of postoperative complications such as pancreatic fistula, intra-abdominal abscess, or intra-abdominal bleeding between patients who underwent PD and those who underwent PpPD. However, the development of DGE after PpPD is a persistent and frustrating complication. Lin et al. [27] reported that DGE occurred more frequently with PpPD (6 of 14 patients; 42.8%) than with PD (0 of 19 patients; 0%) ($P < 0.05$). However, the sample size of this RCT was small ($n = 33$) and the RCT was limited to patients with pancreatic head cancer. On the other hand, Tran et al. reported in their RCT that there was no significant difference between PpPD (19 of 85 patients; 22%) and PD (18 of 80 patients; 23%) concerning the incidence of DGE [25]. Seiler et al. [26] also reported that there was no significant difference between PpPD (30 of 66 patients; 45%) and PD (20 of 64 patients; 31%) with regard to the incidence of DGE. Two meta-analyses suggested that there were no significant differences in postoperative complications, including DGE, between PD and PpPD [28, 29]. PD had a mortality range of 0–7%, while the mortality due to PpPD ranged from 3 to 11% in the five RCTs. There were no significant differences between the two procedures with regard to mortality. Therefore, the two procedures were equally effective for periampullary tumors in terms of morbidity and mortality.

Quality of life and survival

Table 2 shows the comparison in long-term outcome between patients treated by PD and those treated by PpPD concerning to changes in body weight and QOL [6–10, 20–22, 25, 26]. Two retrospective studies have reported that PpPD led to improved postoperative body weight changes, and provided a more favorable QOL compared with PD [7, 10]. On the other hand, several retrospective studies have reported that the postoperative body weight change and QOL did not significantly differ between PD and PpPD [6, 8, 9, 20–22]. Moreover, two RCTs also reported that body weight change and QOL did not exhibit significant differences between the two procedures [25, 26]. Seiler et al. [26] used the sickness impact profile (SIP), a standard questionnaire that assesses various physical, psychological, and social functions, to compare long-term QOL between PD and PpPD. They reported that the capacity to work at 6 months after surgery was better after PpPD (77%) than after PD (56%), although the postoperative QOL did not significantly differ between the two procedures [26]. Further studies should be performed to clarify the comparison

Table 1 Findings of randomized controlled trials comparing PD and PpPD

Authors	Years of study	Variable	Sample size	Pancreatic fistula (%)	DGE (%)	Mortality (%)	Median survival (months)
Lin et al. [23]	1994–1997	PD	15	0	38	7	NA
		PpPD	16	13	7	0	NA
Seiler et al. [24]	1996–1999	PD	49	2	45	5	NA
		PpPD	37	3	37	3	NA
Tran et al. [25]	1992–2000	PD	83	14	23	7	11.0
		PpPD	87	13	22	3	12.0
Seiler et al. [26]	1996–2001	PD	66	3	45	3	27.0
		PpPD	64	2	31	2	34.0
Lin et al. [27]	1994–2002	PD	19	5	0[a]	11	12
		PpPD	14	7	43	7	33

PD pancreaticoduodenectomy, *PpPD* pylorus-preserving pancreaticoduodenectomy, *DGE* delayed gastric emptying, *NA* not available

[a] $P < 0.05$

Table 2 Differences in the long-term outcome between PD and PpPD concerning body weight changes and quality of life

Authors	Study design	Years of study	Variable	Sample size	Body weight (BW) change	Quality of life (QOL)
Klinkenbijl et al. [7]	Retrospective study	1984–1990	PD	44	PpPD led to a significant BW gain	NA
			PpPD	47		
van Berge Henegouwen et al. [22]	Retrospective study	1991–1995	PD	56	No significant difference between PD and PpPD	NA
			PpPD	69		
Di Carlo et al. [8]	Retrospective study	1990–1997	PD	39	No significant difference between PD and PpPD	NA
			PpPD	74		
Huang et al. [9]	Retrospective study	1981–1997	PD	39	NA	No significant difference by City of Hope QOL Survey
			PpPD	153		
Jimenez et al. [20]	Retrospective study	1991–1997	PD	33	No significant difference between PD and PpPD	No difference in nutrition status between PD and PpPD
			PpPD	39		
Yamaguchi et al. [21]	Retrospective study	1993–2000	PD	27	No significant difference between PD and PpPD	NA
			PpPD	23		
Ohtsuka et al. [10]	Retrospective study	1998–2000	PD	5	PD led to a significant BW loss	PpPD had a more favorable QOL, based on Kurihara's questionnaire [53]
			PpPD	31		
Schniewind et al. [6]	Prospective study	1993–2004	PD	34	NA	No significant difference by EORTC QLQ-C30[a]
			PpPD	57		
Tran et al. [25]	RCT	1992–2000	PD	83	No significant difference between PD and PpPD	NA
			PpPD	87		
Seiler et al. [26]	RCT	1996–2001	PD	66	No significant difference between PD and PpPD	No significant difference by SIP
			PpPD	64		

PD pancreaticoduodenectomy, *PpPD* pylorus-preserving pancreaticoduodenectomy, *RCT* randomized controlled trial, *NA* not available, *SIP* sickness impact profile

[a] EORTC QLQ-C30; European Organization for Research and Treatment of Cancer Quality of Life Questionnaire-core 30

in long-term QOL and/or nutritional status between PD and PpPD.

One of the arguments against the preservation of the pylorus in PD is the potential risk of local recurrence. Only one retrospective study reported that preservation of the pylorus significantly reduced the survival rate compared to PD for the treatment of International Union Against Cancer (UICC) stage III pancreatic cancer [31], and most other studies have reported that there was no significant difference concerning the survival rate between patients treated by PD and those treated by PpPD [6, 21, 32]. Seiler et al. [26] and Tran et al. [25], in their RCTs, reported that the long-term survival and disease-free survival were not significantly different between the two procedures. Therefore, PpPD is oncologically as effective as PD.

Two meta-analyses comparing PD with PpPD reported that the length of surgery and blood loss were significantly lower for PpPD than for PD [28, 29]. The two meta-analyses suggested that there were also no significant differences in long-term survival between the two procedures. Therefore, the two procedures were equally effective for periampullary tumors with regard to survival.

Delayed gastric emptying

Various differences in the definition of delayed gastric emptying (DGE) in previous studies may explain the wide range of reported DGE values. Therefore, in clinical studies international consensus definitions should be used. In 2007, DGE was defined according to a consensus definition and the clinical grading of postoperative DGE proposed by the ISGPS [33]. DGE is classified into three categories (grade A, B, or C) by the ISGPS clinical criteria, based on the clinical course and postoperative management required, such as reinsertion of a nasogastric tube, the period of inability to tolerate a solid diet, presence or absence of vomiting, and the use of prokinetics [33].

The pathogenesis of DGE after PpPD has been thought to include several factors, such as: (1) antroduodenal ischemia [34, 35], (2) gastric atony caused by vagotomy [36], (3) pylorospasm [37–39], (4) the absence of gastrointestinal hormones [40], (5) gastric dysrhythmia secondary to other complications such as pancreatic fistula [11, 12, 41–43], and (6) antroduodenal congestion [44]. However, its true pathogenesis remains unclear, and several

RCTs have been conducted to evaluate the effectiveness of different surgical and postoperative management techniques to reduce the incidence of DGE (Table 3). Various innovations, including new surgical techniques, intensive care medicine, and pharmacological agents have been designed to prevent DGE after PpPD.

Pylorus-resecting pancreaticoduodenectomy

DGE after PpPD is a persistent and frustrating complication. DGE after PpPD has been attributed to denervation and devascularization of the pyloric ring due to pylorospasms caused by surgical injuries of the vagus nerves innervating the pyloric ring [41–43]. To address this problem, we have proposed PrPD, in which the stomach is divided just adjacent to the pyloric ring and more than 95% of the stomach is preserved. Our study suggested that PrPD (4.5%) can lead to a significant reduction in the incidence of DGE compared with PpPD (17.2%) [30]. The definition of DGE in our study was the definition proposed by the International Study Group of Pancreatic Surgery (ISGPS) [33]. To compare gastric emptying between the PpPD and PrPD groups, the times to peak $^{13}CO_2$ content in the ^{13}C-acetate breath test at 1, 3, and 6 months postoperatively were examined and gastric emptying in PpPD group were significantly delayed compared with the PrPD group. However, examination of QOL, weight loss, and nutritional status during a 6-month follow-up period did not demonstrate any significant differences between PrPD and PpPD [30].

Surgical techniques for preventing DGE

There have been a few reports suggesting that DGE rates may vary based on how the gastrointestinal tract is routed out of the stomach [11, 45, 46]. Previous retrospective studies found that the antecolic route of duodenojejunostomy after PD or PpPD reduced the incidence of DGE more effectively than the retrocolic route [11, 45, 46]. Park et al. [45] reported that the incidence of DGE in patients treated using the retrocolic route was 31.7%, compared with 6.5% in those treated using the antecolic route ($P < 0.05$). Sugiyama et al. [46] have also supported the superiority of the antecolic route (8%) compared with the retrocolic route (72%) ($P < 0.001$) concerning the reduction of DGE. However, no randomized prospective study has been performed to compare reconstruction routes for

Table 3 Methods and results of clinical trials evaluating surgical techniques and postoperative management methods designed to prevent delayed gastric emptying

Authors	Study design	Years of study	Variable	Sample size	DGE (%)	*P* value
Surgical technique						
Tani et al. [47]	RCT	2002–2004	Antecolic duodenojejunostomy	20[a]	5	0.0014
			Retrocolic duodenojejunostomy	20	50	
Chijiiwa et al. [48]	RCT	2005–2007	Antecolic duodenojejunostomy	17	6	NS
			Retrocolic duodenojejunostomy	18	22	
Kawai et al. [30]	RCT	2005–2009	PpPD	64	17.2	0.0244
			PrPD	66	4.5	
Yeo et al. [51]	RCT	1993–1995	Pancreaticogastrostomy	73	22	NS
			Pancreaticojejunostomy	72	22	
Duffas et al. [52]	RCT	1995–1999	Pancreaticogastrostomy	81	NA	NA
			Pancreaticojejunostomy	68	NA	
Bassi et al. [49]	RCT	2002–2004	Pancreaticogastrostomy	69	3	0.03
			Pancreaticojejunostomy	82	12	
Fernàndez et al. [50]	RCT	2002–2004	Pancreaticogastrostomy	53	4	0.05
			Pancreaticojejunostomy	53	14	
Postoperative management						
Yeo et al. [43]	RCT	1990–1993	Erythromycin control	58	19	NS
				60	30	
Ohwada et al. [34]	RCT	1997–2000	Erythromycin control	14	14	0.04
				14	57	

DGE delayed gastric emptying, *RCT* randomized controlled trial, *NS* not significant, *NA* not available, *PpPD* pylorus-preserving pancreaticoduodenectomy, *PrPD* pylorus-resecting pancreaticoduodenectomy

[a] An interim analysis using Bonferroni's method was planned with 20 patients per arm, although the sample size of this RCT was calculated to require a total of 116 patients (58 per arm). This interim analysis clearly indicated a significant benefit of antecolic duodenojejunostomy over retrocolic duodenojejunostomy; therefore, the RCT was terminated based on statistical and ethical factors