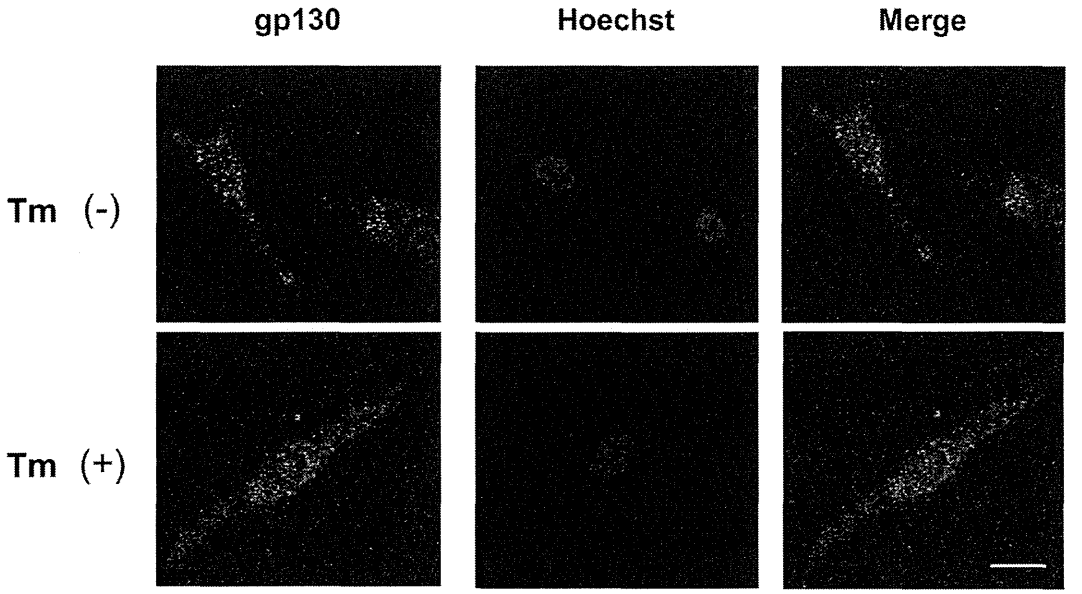

Figure 2. Tunicamycin does not influence the localization of gp130 in cardiomyocytes. Neonatal rat cardiomyocytes were treated with or without Tm (2 μg/mL) for 8 hours. Cultured cells were fixed and immunostained with anti-gp130 antibody or Hoechst for nuclei. Bar indicates 15 μm. Representative images were shown.
doi:10.1371/journal.pone.0111097.g002

Immunoblot analyses

Immunoblot analyses were performed as described previously [11]. Cell lysates were prepared by adding SDS-PAGE sample solution to cells and boiled for 5 minutes. Proteins were separated by SDS-PAGE on polyacrylamide gels and transferred to polyvinylidene difluoride membranes (Millipore). The membrane was blocked with 2% skim milk for 1 hour, followed by incubation with the first antibody overnight at 4°C. Horseradish peroxidase (HRP)-conjugated antibody (Santa Cruz Biotechnology) was used as secondary antibody. The bands were detected by ECL system (GE Healthcare). Densitometric analyses of the detected bands were performed with Image-J software.

The first antibodies used for this study are as follows; anti-phospho-STAT3 (#9131), anti-phospho-ERK1/2 (#9101), anti-ERK1/2 (#9102), anti-phospho-JAK2 (#3771), anti-JAK2 (#3230) and anti-CHOP (#2895) antibodies were purchased from Cell Signaling Technology. Anti-STAT3 (sc-7179), anti-gp130 (sc-656), anti-phospho-JAK1 (sc-16773), anti-JAK1 (sc-7228), anti-LIF receptor (LIFR) (sc-659), anti-IL-11 receptor α (IL-11Rα) (sc-993) and anti-PTP1B (sc-1718) antibodies were obtained from Santa Cruz Biotechnology. Anti-GAPDH antibody (MAB374) was purchased from Millipore, and anti-Bip/GRP78 (610978) antibody was from BD Biosciences.

Immunofluorescence microscopy

Cells were fixed with 3.7% formaldehyde in PBS and washed with PBS twice. Permeabilized with 0.2% TritonX-100 in PBS for 2 minutes, cells were stained with anti-gp130 antibody as a primary antibody for 1 hour at room temperature. After washed with PBS twice, Alexa Fluor488 anti-rabbit IgG (Molecular Probes) was applied for 1 hour at room temperature as a secondary antibody. Nuclei were also stained with Hoechst 33258 (Sigma). Cells were examined with confocal microscopes (Leica TCS SP5) and fluorescent microscopy (Olympus FSX100).

PCR

Real time PCR with cDNA was performed to quantify mRNA as described previously [12]. Briefly, total RNA was prepared from neonatal rat cardiomyocytes with QIAzol reagent (QIAGEN) according to the manufacture's protocol, and 1 μg of total RNA was subjected for first strand cDNA synthesis with Oligo (dT) primer (Invitrogen), dNTPs (Roche), RNase Inhibitor (TOYOBO) and Rever Tra Ace (TOYOBO). Using the synthesized cDNA, the expression levels of SOCS1, SOCS3, PTP1B and GAPDH were quantified by real time PCR using the SYBR Green Master Mix (Applied Biosystems) according to the manufacture's protocol. The primers used in this study were shown in Table 1.

Statistical analysis

All data are presented as means ± S.D. Multiple comparisons were performed by One-way ANOVA with post-hoc multiple comparison test using SPSS software. $P<0.05$ was considered to be statistically significant.

Results

The treatment with tunicamycin completely inhibited the glycosylation of gp130 in cardiac myocytes

Since Tm is an inhibitor of N-acetylglucosamine phosphotransferase, we examined the effects of Tm on the glycosylation of gp130 in cultured cardiac myocytes. Cultured cardiomyocytes were incubated with various concentrations of Tm for 8 hours or with 2 μg/mL of Tm for the indicated times (0, 3, 6, 8 hours). The immunoblot analyses have demonstrated that Tm treatment reduced the molecular weight of gp130 in a dose- and time-dependent manner (Figure 1A and 1B). This reduced molecular weight (slightly bigger than 100 kDa) corresponds with that of its unglycosylated form, 101 kDa as previously reported [5]. These results indicate that Tm could completely replace glycosylated gp130 with its unglycosylated form, and then cells were pretreated with 2 μg/mL of Tm for 8 hours for further experiments. The

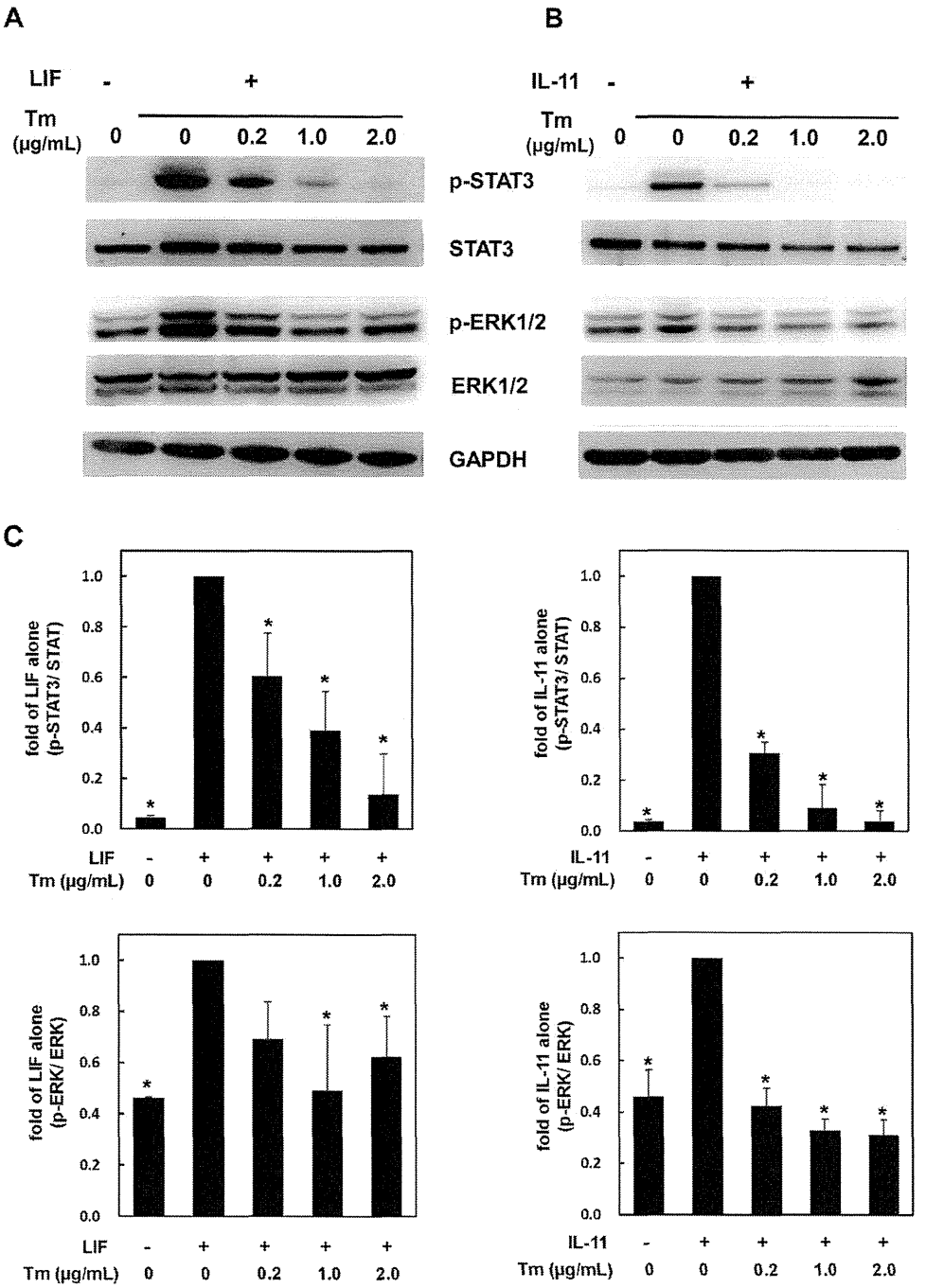

Figure 3. Tunicamycin inhibits the activation of STAT3 and ERK by LIF or IL-11, respectively. Cultured cardiomyocytes were pretreated with the indicated concentration of Tm for 8 hours, and stimulated with LIF (300 U/mL) (A) or IL-11 (20 ng/mL) (B) for 15 minutes. Activation of STAT3 and ERK1/2 was analyzed by immunoblotting with each phospho-specific antibody. Membranes were stripped and reprobed with anti-STAT3, anti-ERK1/2, or anti-GAPDH antibody, respectively. Representative images were shown (A and B). (C). For quantification, densitometric analyses for STAT3 or ERK1/2 phosphorylations were normalized with those of total STAT3 or total ERK, respectively. Values were converted based on that of each group treated with cytokine alone. Data were mean ± S.D. of three independent experiments. Dunnett test was performed for post-hoc multiple comparison test. *; $P<0.05$ versus cytokine alone.
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inhibitory effect of Tm on the N-glycosylation of gp130 continued more than 16 hours after Tm washed out from the culture media in cardiomyocytes (Figure S1).

In addition, the effects of Tm on the glycosylation of LIFR and IL-11Rα were also proved because each of them heterodimerizes with gp130 respectively and activates downstream signals. As shown in Figure 1, Tm reduced the molecular weight of LIFR

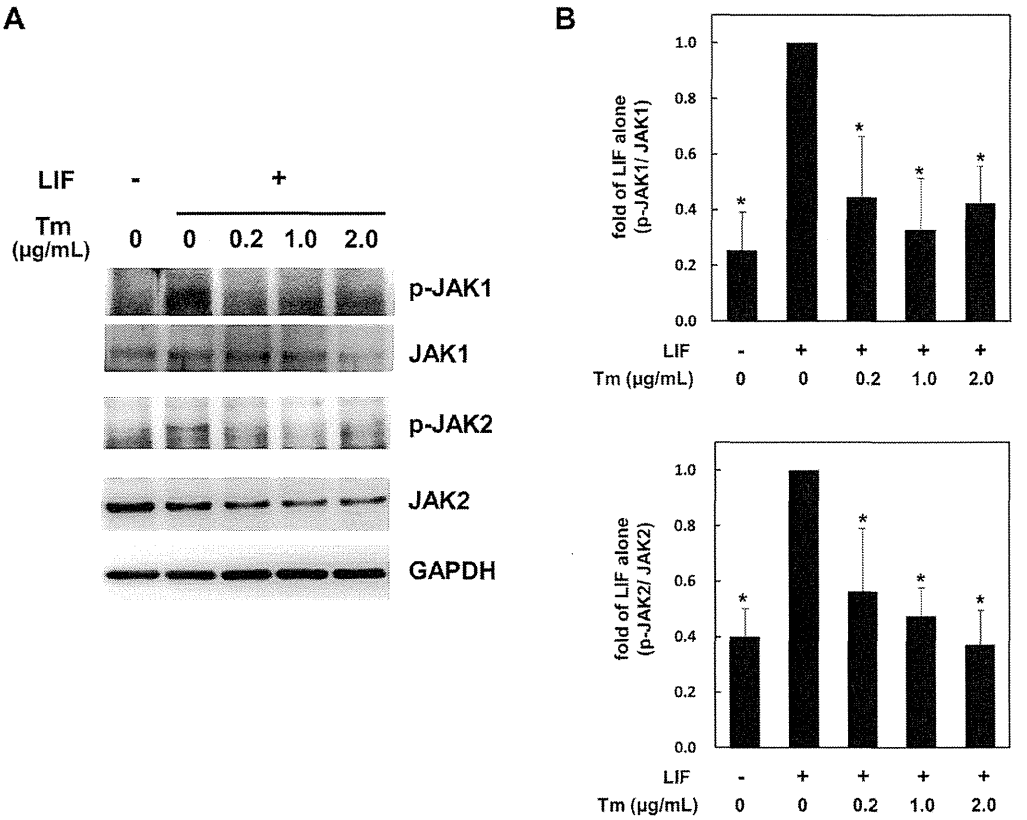

Figure 4. Tunicamycin suppresses the gp130-mediated activation of JAK1 and 2. Neonatal rat cardiac myocytes, pretreated with the indicated concentrations of Tm for 8 hours, were stimulated with LIF (300 U/ml) for 15 minutes. The activation of JAK1 and JAK2 was analyzed by immunoblotting with the phospho-JAK1 and phospho-JAK2 specific antibodies. Membranes were stripped and reprobed with anti-JAK1, anti-JAK2, or anti-GAPDH antibody, respectively. Representative images were shown (A). (B). For quantification, densitometric analyses for JAK1 and JAK2 phosphorylation were normalized with those of total JAK1 or total JAK2, respectively. Values were converted based on that of each group treated with LIF alone. Data were mean ± S.D. of three independent experiments. Dunnett test was performed for post-hoc multiple comparison test. *; $P<0.05$ versus LIF alone.
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partially, whereas Tm did not affect the glycosylation of IL-11Rα. For LIFR, the molecular weight of reduced band is consistent with that in the previous report [13], indicating that Tm incompletely inhibited the glycosylation of LIFR in cardiac myocytes.

Tunicamycin did not alter the localization of gp130

Previously, it was reported that the inhibition of gp130 glycosylation does not impair its translocation to cellular membrane [13]. Consistently, immunofluorescence microscopic analyses revealed that Tm does not alter the localization of gp130 (Figure 2).

Tunicamycin inhibits STAT3 activation by IL-6 family cytokines in cultured cardiac myocytes

In order to examine the biological significance of gp130 glycosylation, cardiac myocytes were stimulated with LIF, an IL-6 family cytokine, in the presence of various concentrations of Tm. As reported previously [14], LIF activated STAT3 and ERK1/2; however, Tm inhibited LIF-mediated activation of these signaling pathways (Figure 3A). Recently, we reported that IL-11, which also belongs to IL-6 family cytokines, activates STAT3 and ERK1/2 through IL-11R that is expressed in cultured cardiac myocytes [3]. Therefore, we examined the effects of Tm on IL-11 activation of STAT3 and ERK1/2. As is the case with LIF, IL-11-induced activation of STAT3 and ERK1/2 was inhibited by Tm in a dose-dependent manner (Figure 3B). As shown in Figure 3C, quantitative analyses exhibit Tm significantly inhibited the activation of STAT and ERK1/2 by these two IL-6 family cytokines, respectively. Next, we checked whether Tm influences JAKs activities using phospho-JAK1 and phospho-JAK2 specific antibodies. LIF enhanced the phosphorylation of both JAK1 and JAK2, which was suppressed by Tm significantly (Figure 4). These data indicate that Tm inhibits JAKs/STAT3 activation by IL-6 family cytokines in cardiac myocytes.

Tunicamycin inhibited JAK/STAT3 pathway downstream of gp130 independently of PTP1B and SOCSs

Previously, ER stress was reported to inhibit JAK/STAT3 pathway in leptin signaling pathway through PTP1B [7,8] In cardiac myocytes, Tm increased the expression of the marker genes for ER stress, such as C/EBP-homologous protein (CHOP) and glucose-regulated protein 78 (Grp78) (Figure 5A & 5B). Therefore, we examined whether Tm induced PTP1B and found that the expression of PTP1B mRNA was not influenced by Tm (Figure 5C). Consistently, Tm inhibited LIF-mediated activation even in the presence of JTT551, a PTP1B inhibitor [15] (Figure 5D). We also examined the expression of SOCS1 and 3,

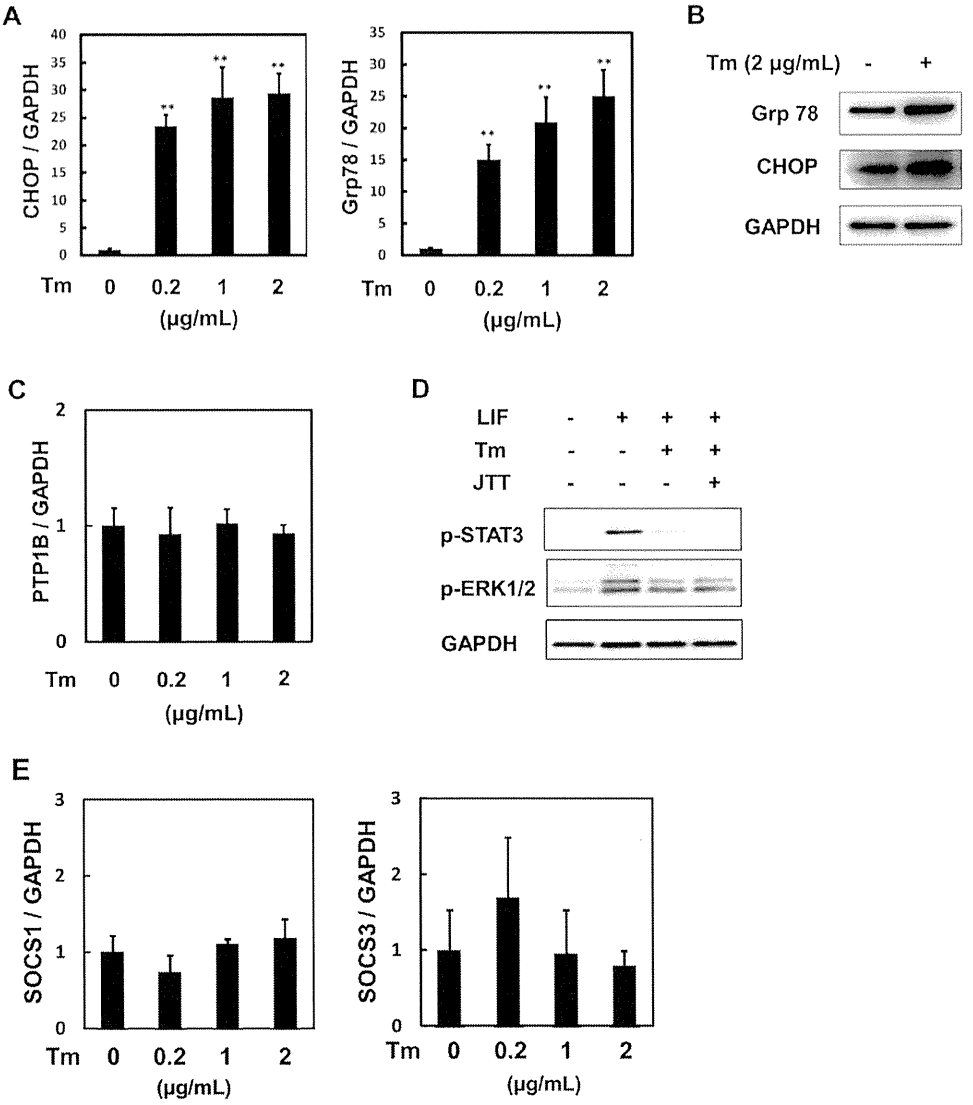

Figure 5. Tunicamycin inhibits JAK/STAT3 pathway downstream of gp130 independently of ER stress, PTP1B and SOCSs. Neonatal rat cardiac myocytes were cultured with the indicated concentrations of Tm for 8 hours. Total RNA was prepared and applied for reverse transcription. Real time PCR system was used to detect the mRNA expression of CHOP (A), Grp78 (A), PTP1B (C), SOCS1 and 3 (E) as described under 'Material and Methods'. The expression level of each gene was normalized with that of GAPDH, an internal control, and represented as value of fold induction relative to those of each non-treated group with Tm (control). Data were shown as mean ±S.D. (n = 3). **; $P<0.05$ versus control at the multiple comparison test. After cardiac myocytes were pretreated with or without Tm (2 μg/mL) for 8 hours, cells were washed with medium and incubated for more 15 hours. Afterward, cells lysates were prepared for immunoblotting analyses with anti-CHOP, anti-Grp78 and GAPDH antibody. Experiments were repeated three times with similar results and representative data are shown in (B). Cardiac myocytes were pretreated with or without Tm (2 μg/mL) for 8 hours in the presence or absence of JTT551 (JTT), PTP1B inhibitor, and stimulated with LIF (300 U/l) for 15 minutes. Activations of STAT3 and ERK1/2 were analyzed by immunoblotting with anti-phospho-STAT3, anti-phopho-ERK1/2 and anti-GAPDH antibody. Representative images were shown in (D).
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but the expression of these genes was not influenced by Tm (Figure 5E). These data indicate that Tm inhibits the gp130-mediated activation of JAK/STAT3 differently from leptin-induced STAT3 activation.

Treatment with IL-6 plus sIL-6R failed to stimulate STAT3 and ERK1/2 in the presence of Tunicamycin

We have demonstrated that Tm inhibited activation of STAT3 and ERK1/2 by LIF and IL-11; however, we cannot completely rule out the possibility that the glycosylation of the endogenous LIFR and IL-11R might be modulated by Tm and lose the capacity of activating gp130, though the molecular weight of some fraction of LIFR and/or IL-11R was not apparently affected by Tm (Figure 1). Therefore, to exclude the effects of Tm on glycosylation of the endogenous receptor α subunits, we treated cultured cardiomyocytes with IL-6 and sIL-6R with or without Tm pretreatment. Since sIL-6R is an agonistic receptor, the combined treatment with IL-6 and sIL-6R activated STAT3 and ERK1/2 in the absence of Tm; however, this combined treatment

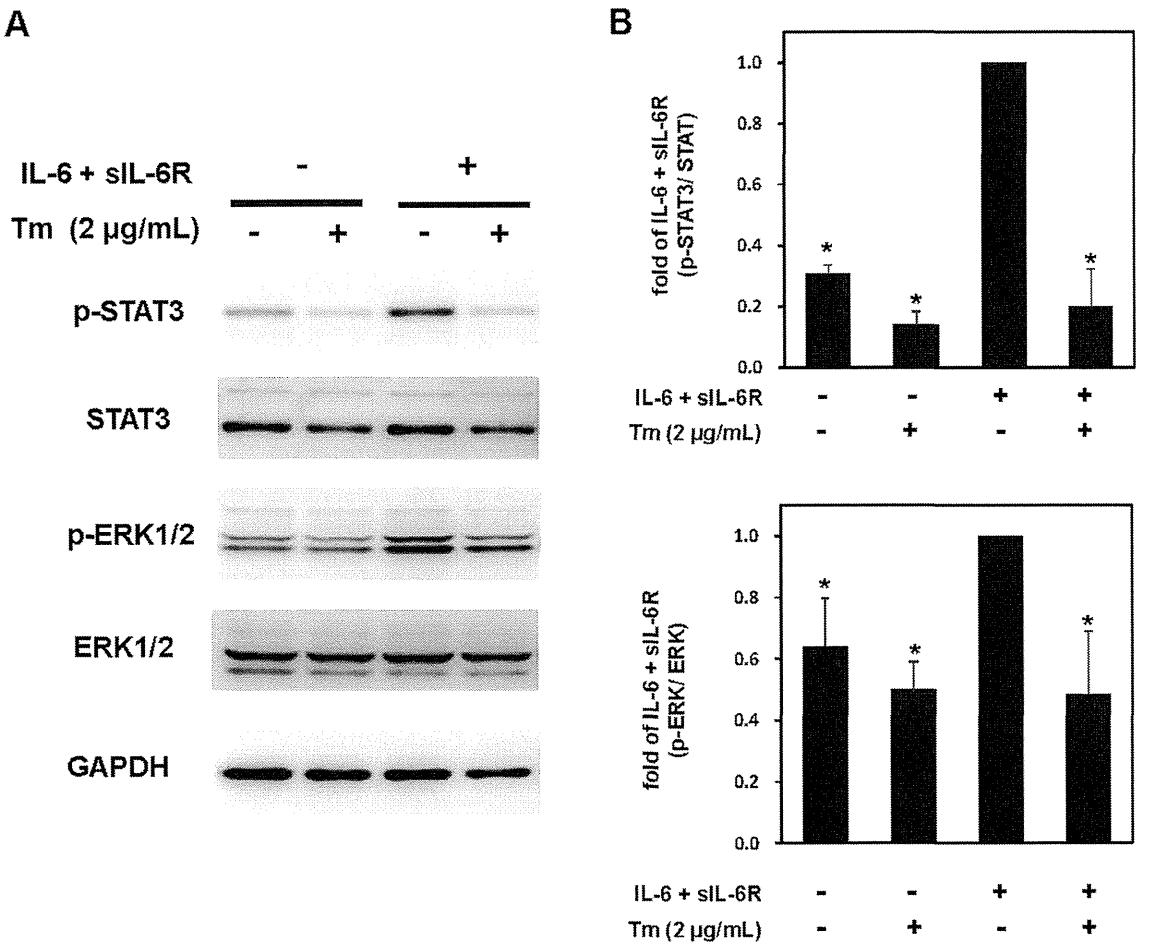

Figure 6. The combined treatment with IL-6 and sIL-6R fails to activate STAT3 in the presence of tunicamycin. Neonatal rat cardiac myocytes, pretreated with or without Tm (2 μg/mL) for 8 hours, were stimulated with IL-6 (20 ng/mL) plus sIL-6R (100 ng/mL) for 15 minutes. Activation of STAT3 and ERK1/2 were analyzed by immunoblotting with each phospho-specific antibody. Membranes were stripped and reprobed with anti-STAT3, anti-ERK1/2, or anti-GAPDH antibody, respectively. Representative images were shown (A). (B). For quantification, densitometric analyses for STAT3 or ERK1/2 phosphorylation were normalized with those of total STAT3 or total ERK, respectively. Values were converted based on that of each group treated with cytokine alone. Data were mean ± S.D. of three independent experiments. Dunnett test was performed for post-hoc multiple comparison test. *; $P<0.05$ versus the combination of IL-6 and sIL-6R alone.
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failed to transduce STAT3 and ERK1/2 signalings when the cardiomyocytes were pretreated with Tm (Figure 6).

Discussion

In this study, we examined the effects of Tm on gp130 signaling in cultured cardiomyocytes. The treatment with Tm inhibited the activation of JAK/STAT3 in response to LIF or IL-11 in cardiomyocytes. Tm induced ER stress in cardiomyocytes; however, PTP1B is not involved in the inhibition of JAK/STAT3 by Tm, suggesting that gp130 signaling was suppressed by Tm differently from leptin signal.

The treatment with Tm replaced the N-glycosylated gp130 with its unglycosylated form completely, whereas Tm partially inhibited the glycosylation of LIFR and Tm did not affect that of IL-11Rα. In addition, the inhibitory effect of Tm on unglycosylated gp130 continued more than 15 minutes even after Tm was washed out from the medium. Under these conditions, the unglycosylated form of gp130 failed to activate STAT3 and ERK1/2 in response to the stimulation with LIF, IL-11 or IL-6 plus sIL-6R. Based on these findings, it is concluded that N-glycosylation of gp130 is essential for the signal transduction of gp130 system.

Previously, Yanagisawa et al. evaluated the functional role of N-glycans of gp130 in mouse embryonic neural stem cells (NSCs) using Tm (13). In NSCs treated with Tm, some fraction of gp130 was detected as its unglycosylated form. Unglycosylated gp130 was translocated to the cell surface but did not form a heterodimer with LIFR, analyzed by immune-precipitation assays. In spite of its loss of heterodimerization ability with LIFR, LIF stimulation activated STAT3 and ERK in the presence of Tm to the same level with their activation in the absence of Tm. This discrepancy might be explained by the limitation of their experimental system; significant fraction of gp130 was still expressed as its N-glycosylated form in NSCs even in the presence of Tm. Another possibility is that LIFR was also unglycosylated by Tm and might be functionally modified, resulting in transducing LIF signal independently of gp130. In this study, when we successfully replaced N-glycosylated gp130 with the unglycosylated form

completely, LIFR was not completely replaced with the unglycosylated forms and IL-11Rα was not affected. Under this glycosylation state of each receptor, the signals of JAK/STST3 and ERK1/2 by LIF or IL-11 were inhibited. Besides these results, we demonstrated that the glycosylation is essential for gp130 signaling by using IL-6 and sIL-6R, which form ligand/receptor complex and then proceed trans-signaling.

In contrast to our results, Waetzig et al reported that N-glycosylation is not essential for the signal function of gp130 by using the gp130 mutant with amino acid substitution from Asn to Gln at N-glycosylation sites (16). The loss of N-glycosylation in gp130 molecule reduces its stability but retains the ability to activate STAT3 in response to the agonistic complex of IL-6 and sIL-6R. Since amino acid substitutions could result in the intramolecular conformational changes in mutant gp130, it might be difficult to address the necessity of N-glycosylation by amino acid substitution method. Moreover, the authors used the agonistic complex of IL-6 and sIL-6R as hyperactive IL-6 (17). These approaches might artificially potentiate the mutant gp130 signaling function, though further studies would be required.

Previously, it was demonstrated that Tm inhibits leptin-mediated JAK2/STAT3 pathway through ER stress (7, 8). Under the ER stress, activation of JAK2 is inhibited by PTP1B. In cardiac myocytes, we have also confirmed that Tm induces ER stress; however, PTP1B is unlikely to be involved in Tm-mediated inhibition of STAT3 activation by IL-6 family. Indeed, PTP1B inhibitor did not recover STAT3 activity in the presence of Tm. Moreover, PTP1B specifically inhibits JAK2 activity, while IL-6 family cytokines activate STAT3 though both JAK1 and JAK2 (14).

Recently, the activation of the hexosamine biosynthesis pathway (HBP), which converts glucose to UDP-N-acetylglucosamine (GlcNAc) for N- and O-glycosylation of proteins, has been reported to protect various biological insults, such as ER stress, ischemia/reperfusion injury in heart and Tm toxicity (18, 19). Interestingly, supplementation with GlcNAc to worms led to Tm resistance (18). It is not clear whether GlcNAc or its metabolites could affect directly N-glycosylation, which maintains effective protein folding and ER proteostasis, to regulate cellular protein homeostasis. Further research on the crosstalk between HBP and gp130/JAKs/STAT signalings might provide insights into the molecular mechanisms of the onset of heart failure.

In conclusion, pharmacological approach using Tm, an inhibitor of enzymes involved in N-glycosylation, has revealed that N-glycosylation of gp130 is essential for its signal functions. Since gp130 signaling pathway plays crucial roles in the maintenance of cardiac homeostasis, the disturbance of its N-glycosylation might cause cardiovascular diseases.

Supporting Information

Figure S1 The reversibility of unglycosylated gp130 by Tm in cardiomyocytes. Treated with or without Tm (2 μg/mL) for 8 hours, neonatal rat cardiac myocytes were washed twice with serum free medium and incubated again for the indicated times. Cells lysates were applied for immunoblotting analysis with anti-gp130 antibody to detect the reversibility of N-glycosylation of gp130. Representative images are shown.
(TIF)

Author Contributions

Conceived and designed the experiments: YF MM. Performed the experiments: RM HM MM SM KT. Analyzed the data: YF HN MM. Contributed reagents/materials/analysis tools: YF HN TM MM. Contributed to the writing of the manuscript: YF MM.

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ORIGINAL ARTICLE

A phase I trial of combination therapy using gemcitabine and S-1 concurrent with full-dose radiation for resectable pancreatic cancer

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Abstract
Purpose Use of the fourth-generation oral fluoropyrimidine S-1 together with gemcitabine has shown striking anticancer effects. In this single-arm phase I trial of preoperative combination therapy using gemcitabine and S-1 concurrently with radiotherapy, we verified the safety and feasibility and determined the maximum-tolerated dose of each drug in patients with resectable pancreatic cancer.
Methods A standard 3+3 dose escalation scheme was used. Patients with cytologically or histologically proven resectable pancreatic ductal adenocarcinoma were administered 30-min intravenous gemcitabine infusions on days 1, 8, 22, and 29 and S-1 orally on days 1–5, 8–12, 22–26, and 29–33. A total radiation dose of 50.4 Gy (1.8 Gy/day, 5 times per week, 28 fractions) was concurrently delivered. Surgical exploration was scheduled 4–7 weeks after the final radiation fraction.
Results Twenty-one patients were enrolled. No treatment-related deaths occurred during this study. Recommended doses were determined to be 80 mg/m^2 of S-1 daily and 1,000 mg/m^2 of gemcitabine. CA19-9 was reduced to <50 % of baseline values in 12 (75 %) of 16 measurable patients. Nineteen of 21 enrolled patients successfully underwent surgical resection.
Conclusions Preoperative chemoradiotherapy consisting of gemcitabine and S-1 concurrent with full-dose radiation is feasible and well tolerated.

Keywords Pancreatic ductal adenocarcinoma · Neoadjuvant chemotherapy · Gemcitabine · S-1 · Chemoradiotherapy

Abbreviations

PDAC	Pancreatic ductal adenocarcinoma
CTCAE	Common Terminology Criteria for Adverse Events
MTD	Maximum-tolerated dose
RD	Recommended dose
DLT	Dose-limiting toxicity

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is one of the most deadly diseases, having a 5-year survival rate of 6 % [1]. Surgical resection is the only curative treatment, but the 5-year survival rate after surgical resection is only 5.5–21 % [2, 3]. Adjuvant chemotherapy with either 5-fluorouracil or gemcitabine improves disease-free survival and overall survival compared to observation alone, and

UMIN Clinical Trials Registry Number: 000002649.

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is widely accepted as the standard of care after resection; however, the prognosis remains unsatisfactory [3–5].

Efforts to increase the postoperative survival rate have included extensive testing of various preoperative therapies followed by surgical resection. Recently, we and others reported encouraging survival rates following preoperative gemcitabine-based chemoradiotherapy in patients with potentially resectable PDAC [6–8]. While preoperative therapy seems to be a promising strategy, it may also have disadvantages. Of primary concern, preoperative therapy requires at least several months, potentially delaying surgical resection. In patients for whom the preoperative therapy is not sufficiently effective, this therapy can be a waste of time and may result in the patient missing the chance for surgery. The anticancer effect of gemcitabine is acceptable, but not satisfactory; therefore, a more effective short-term protocol has been long awaited.

The fourth-generation oral fluoropyrimidine S-1 contains tegafur, gimeracil, and oteracil potassium in a molar ratio of 1:0.4:1, and its efficacy has already been shown in a variety of solid tumors, particularly gastric cancer [9]. S-1 reportedly has high anticancer activity in patients with pancreatic cancer, with a combination of gemcitabine and S-1 showing stronger anticancer activity than gemcitabine alone in patients with unresectable pancreatic cancer [10–14]. It is thus presumable that a combination of gemcitabine and S-1 with concurrent radiotherapy is the most powerful and suitable protocol for preoperative treatment. However, such combination therapy has not yet been reported and, therefore, a recommended dose (RD) of each drug has not yet been established.

The present report describes the first phase I study of combination therapy using gemcitabine and S-1 with concurrent radiotherapy. This study aimed to verify the safety and feasibility of such treatment, and to determine the maximum-tolerated dose (MTD) of each drug in patients with resectable pancreatic cancer.

Patients and methods

Patient eligibility

This study was a single-arm phase I trial conducted at Osaka University Hospital. Patients with resectable cytologically or histologically proven ductal adenocarcinoma of the pancreas were prospectively enrolled in the clinical trial. Cases were considered resectable if the pancreatic cancer did not involve the hepatic artery, celiac trunk, or superior mesenteric artery and had no evidence of metastatic disease; if the tumor could be resected by distal pancreatectomy with celiac axis resection (DP-CAR); or if there was venous involvement of the SMV/PV, but the SMV/PV had suitable vessel proximal and distal to the area of vessel involvement to allow for safe resection and reconstruction. Eligible patients were aged ≥20 years, had an Eastern Cooperative Oncology Group performance status of <2, and had adequate organ function to tolerate surgery. Patients were excluded from the study for the following reasons: (1) concomitant or past history (within 3 years) of other malignant disease; (2) inadequate bone marrow reserves as measured by a total white blood cell count of 3,500–12,000/l, neutrophil count of ≤2,000/l, and a platelet count of ≤100,000/l; (3) laboratory tests indicating abnormal data, such as total bilirubin >2.0 mg/dL, asparagic aminotransferase >150 U/l, alanine aminotransferase >150 U/l, or creatinine >1.2 mg/dL; or (4) active inflammatory bowel disease, active gastric/duodenal ulcer, mental disorder, or other severe concurrent disease.

This study was approved by the institutional review board of the Osaka University Hospital (IRB number 09125) prior to enrolling patients and was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice. This study was registered with the University Hospital Medical Information Network Clinical Trials Registry (UMIN-CTR), officially accepted registries by International Committee of Medical Journal Editors (ICMJE), with a registration number of UMIN000002649. Written informed consent was obtained from all patients prior to undergoing any study procedure or receiving any study treatment.

Chemotherapy

The trial used a standard 3+3 dose escalation scheme with preplanned cohort expansion at the MTD. Patients were administered an intravenous infusion of gemcitabine on days 1, 8, 22, and 29; and S-1 orally on days 1–5, 8–12, 22–26, and 29–33 (Fig. 1). The starting dose (level 1) consisted of S-1 30 mg/m^2 twice daily and gemcitabine 600 mg/m^2. Patients were enrolled sequentially to receive escalating doses up to level 4 (Table 1). Gemcitabine was dissolved in saline and administered at a standard 30-min infusion. Administration at a fixed dose rate (FDR) of 10 mg/m^2/min was not used. Antiemetics were not routinely used but were allowed as the need arises. According to the observed toxicities, chemotherapy was suspended or terminated, but administration with reduced dose was not permitted. No intrapatient dose escalation was permitted.

Dose-limiting toxicity (DLT) and maximum-tolerated dose (MTD)

The patients were evaluated following the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) Version 3.0. DLT was defined as any of the following: (1) nonhematologic toxicity of grade 3 or

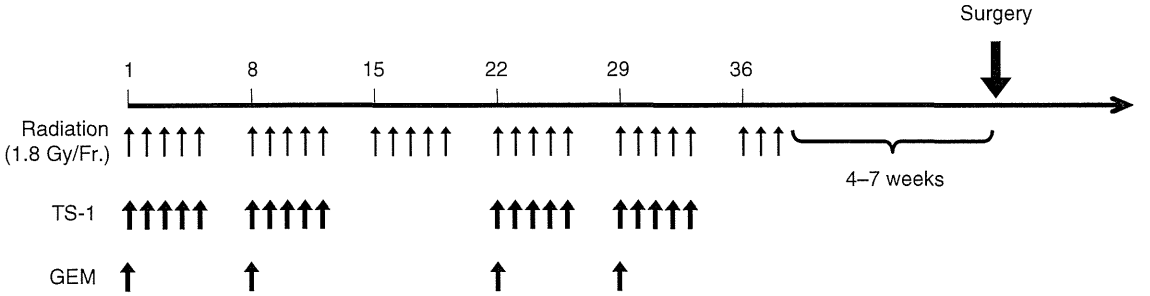

Fig. 1 The treatment schedule included 50.4 Gy (1.8 Gy/day, 5 times per week, 28 fractions total) of preoperative radiation that was administered along with concurrent 30-min intravenous infusions of gemcitabine on days 1, 8, 22, and 29 or S-1 orally on days 1–5, 8–12, 22–26, and 29–33. Surgical exploration was performed 4–7 weeks after the final fraction of radiation

Table 1 Dose escalation schedule and the number of treated patients

Dose level	Gemcitabine (mg/m^2)	S-1 (mg/m^2)	No. of patients
0	600	40	0
1	600	60	6
2	800	60	6
3	800	80	3
4	1,000	80	6

higher, except nausea and vomiting; (2) any grade 4 hematologic toxicity; (3) grade 3 neutropenia with fever; (4) any study-related toxicity that required radiation interruption for more than 14 days; (5) inability to deliver gemcitabine on two or more occasions; and (6) inability to deliver S-1 for 8 days or more. An infusion reaction was not considered a DLT. To enable full DLT evaluation, all patients were observed for 1 week following completion of the last dose of radiation. At least three patients were enrolled at each dose level. If DLT was observed in one or two patients, three additional patients were placed on that dose level. If only one or two of six patients experienced DLT, dose escalation would continue. The MTD of the combination was defined as the dose level that produced DLT in three or more of six patients, or in all of the initial three patients. The RD was defined as the dose level one level below the MTD. If the maximum dose level (level 4) will not reach the MTD, level 4 will be concluded as the RD.

Radiation therapy

All patients were treated with 3D conformal radiotherapy. The clinical target volume was defined as the gross tumor volume with a 5-mm margin plus the neuroplexus region and the locoregional elective lymph node region, which included the celiac, superior mesenteric, peripancreatic, portal, and para-aortic regions for pancreatic head cancers and the splenic region for pancreatic body and tail cancers. The posterior margin of the target volume was set 1.0 cm behind the anterior margin of vertebral bodies. The planning target volume included the clinical target volume with a 10-mm margin for possible positioning errors, respecting anatomical boundaries, such as the stomach, duodenum, small intestine, and transverse colon. In patients with tumors located near critical organs, the margins were reduced accordingly. The radiation fields were delineated in each of the multiple CT cut-sections, thereby constructing the planning target volumes. The total radiation dose was 50.4 Gy, which was delivered 5 times per week in daily fractions of 1.8 Gy.

Surgery

In the event that neither distant metastasis nor cancer progression requiring reconstruction of the arteries was detected, surgical exploration was scheduled at 4–7 weeks after the final radiation fraction. If careful inspection revealed neither liver metastasis nor peritoneal implantation, a pancreatectomy together with lymphatic and connective tissues clearance was performed. In cases where the pancreatic tumor was fixed with the PV/SMV, it was resected together with the pancreas (en bloc resection). Regarding the GI tract, reconstruction procedures—pancreaticojejunostomy, cholangiojejunostomy, and jejunojejunostomy—were performed after pancreaticoduodenectomy, while none of the anastomotic procedure was performed after caudal pancreatectomy.

Postoperative course

If the patient's performance status was adequate, postoperative therapy comprised 6 months of gemcitabine treatment. Postoperative follow-up consisted of a routine physical examination and laboratory tests, including the serum levels of CEA and CA19-9. Both chest X-ray and CT/ultrasonography of the abdomen were performed every 3 months, and the presence or absence of cancer recurrence was carefully monitored.

Table 2 Patient characteristics at baseline

Characteristics	No. of patients
No. of patients (levels 1:2:3:4)	21 (6:6:3:6)
Age in years (median, range)	66.0, 56–79
Gender (male:female)	15:6
Performance status (0:1)	18:0
Tumor location (head:body:tail)	13:5:3
TNM stage (I:IIA:IIB:III:IV)	0:18:3:0:0
Tumor size in mm (median, range)	23.0, 13–53
CA19-9 in U/l (median, range)	197, 5–1,859
CEA in ng/ml (median, range)	4.0, 1–9

Statistical analysis

All data were reported as the mean ± SD and/or median. We used Fisher's exact test for categorical data and the Mann–Whitney U test for continuous data. Data analyses were performed on an intention-to-treat basis using the SPSS software package (SPSS Inc., Chicago, IL, USA). A P value of less than 0.05 was considered statistically significant.

Results

Patients' characteristics

From December 2009 to December 2012, 21 patients were enrolled in this study. Table 2 lists the patient characteristics. The median age was 66 years (range 56–79 years). All patients had an Eastern Cooperative Oncology Group performance status of 0. Pancreatic cancers were located in the pancreatic head in 13 patients, body in five, and tail in three. Eighteen patients were diagnosed as TMN stage IIA and three as stage IIB. The median tumor size was 23 mm (range 13–53 mm). Median CA19-9 and CEA were 197 U/ml (range 5–1,859 U/ml) and 4.0 ng/ml (range 1–9 ng/ml), respectively.

Toxicity and recommended dose

Table 3 lists the toxicities observed in the 21 enrolled patients. No treatment-related deaths occurred during this study. One of the initial three patients at level 1 experienced grade 4 leukopenia/neutropenia and AST/ALT/γ-GTP elevation (probably due to biliary stent obstruction); thus, an additional three patients were enrolled at level 1, who all completed the chemoradiotherapy without DLT. At level 2, one of the initial three patients experienced grade 4 leukopenia/neutropenia; therefore, an additional three patients were enrolled, who all completed the protocol without DLT. At level 3, no patients experienced DLT and the level was completed with only three patients enrolled. Level 4 had been defined as the maximum dose level; six patients were enrolled at level 4 to verify that it was the RD. Among the six enrolled patients, one experienced grade 4 leukopenia/neutropenia and another experienced grade 3 γ-GTP/bilirubin elevation (probably due to biliary stent obstruction). Because four of the six patients enrolled at level 4 completed their treatment without DLT, the level was considered well tolerated and set as the RD.

Tumor response and resectability

Although tumor response assessment was not a primary objective of this phase I study, patients were evaluated for tumor response and resectability. Of the 21 patients, none had complete response, 5 had partial response, 15 had stable disease, and 1 had progressive disease

Table 3 Adverse events

Dose escalation	Level 1			Level 2			Level 3			Level 4		
No. of patients	$n = 6$			$n = 6$			$n = 3$			$n = 6$		
CTCAE grade	2	3	4	2	3	4	2	3	4	2	3	4
Leukopenia	3	1	1	1	3	1	0	3	0	1	3	1
Neutropenia	2	1	1	1	3	1	2	1	0	3	1	1
Thrombocytopenia	1	0	0	0	0	0	0	0	0	1	2	0
AST elevation	0	0	1	1	0	0	1	0	0	0	0	0
ALT elevation	0	1	0	1	0	0	1	0	0	0	0	0
γ-GTP elevation	0	1	0	0	0	0	1	0	0	0	1	0
Hyperbilirubinemia	0	0	0	0	0	0	1	0	0	0	1	0
Constipation	1	0	0	3	0	0	0	0	0	1	0	0
Anorexia	2	0	0	2	0	0	1	0	0	0	0	0
Diarrhea	0	0	0	0	0	0	1	0	0	0	0	0
Rash	0	0	0	0	0	0	2	0	0	1	0	0

Table 4 Clinical outcomes

Level	No. of patients	Tumor (head/body/tail)	Stage (IIA:IIB)	RECIST (CR/PR/SD/PD)[a]	More than 50 % reduction of CA19-9 (yes/no)[b]	Surgery (PD/DP/unresected)[c]
1	6	3/2/1	6/0	0/2/4/0	2/1	3/3/0
2	6	5/0/1	5/1	0/1/5/0	3/1	5/0/1
3	3	3/0/0	1/2	0/1/2/0	2/1	2/0/1
4	6	2/3/1	6/0	0/1/4/1	5/1	2/4/0
Total	21	13/5/3	18/3	0/5/15/1	12/4	12/7/2

[a] *CR* complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease

[b] Patients who had a pretreatment value of more than the upper limit of normal were evaluated

[c] *PD* pancreaticoduodenectomy, *DP* distal pancreatectomy

(Table 4). The average tumor size slightly decreased from 26.7 ± 11.0 mm to 22.9 ± 10.4 mm. CA19-9 significantly decreased from 398 ± 489 U/ml to 128 ± 208 U/ml. CA19-9 was reduced to less than 50 % of baseline values in 12 (75 %) of the 16 patients who had a pretreatment value above the upper limit of normal. After completion of preoperative chemoradiotherapy, 19 of the 21 enrolled patients successfully underwent surgical resection, whereas two could not be resected because liver metastases were observed during the re-evaluation of resectability following chemoradiotherapy. Perioperative outcomes in resected patients were acceptable, with median operation time of 451 min (range 190–628 min) and median blood loss of 450 ml (range 120–1,900 ml). Postoperative complications included three patients of intra-abdominal abscess after caudal pancreatectomy (two patients in Clavien–Dindo grade II and one in grade IIIa) and one patient of delayed gastric emptying (ISGPS grade A) after pancreaticoduodenectomy [15, 16]. No patients died postoperatively.

Discussion

For the treatment of resectable pancreatic cancers, studies have tested a variety of preoperative combination therapies, including 5-FU and radiation, gemcitabine and radiation, or S-1 and radiation, with each of these protocols showing considerable effectiveness [6–8, 17, 18]. Preoperative chemoradiotherapy provides numerous potential therapeutic advantages [19, 20]. First, the macroscopic and microscopic levels of down-staging induced by the locoregional effects of preoperative chemoradiotherapy could lower the rates of margin-positive resections compared with those obtained without preoperative chemoradiotherapy. Second, preoperative therapy can reduce the cancer cell viability, potentially preventing the implantation and dissemination of cancer cells at laparotomy and thereby decreasing subsequent peritoneal tumor recurrence.

Previous studies have shown that the number of residual cancer cells in resected specimens after preoperative chemoradiotherapy is an important prognostic factor, regardless of the chemotherapeutic reagents used [21, 22]. The combination of gemcitabine and S-1 reportedly has higher anticancer activity (objective response rate 29.3 %) compared with either treatment alone, suggesting that combination therapy using gemcitabine and S-1 concurrently with radiation is a better choice [12–14]. Recent reports indicate similar or even higher response rates by other combinations including FOLFIRINOX (response rate 31.6 %) or nab-paclitaxel plus gemcitabine (response rate 23 %) [23, 24]. However, FOLFIRINOX was administered to patients with metastatic pancreatic cancer and showed increased toxicity. FOLFIRINOX thus seems to be inadequate as a part of chemoradiotherapy in resectable pancreatic cancer patients.

A previous study used combination therapy with gemcitabine and S-1 and concurrent radiation in patients with locally advanced pancreatic cancer [25]. The rationale behind this combination is simply applying the strongest therapy to advanced cancers and hoping that unresectable lesions will become resectable. However, only a small percentage of enrolled patients will actually become resectable; thus, the tolerability of surgical resection after the therapy remains to be clarified. Moreover, when applying this combination therapy to resectable pancreatic patients, it may be possible to increase the dose of each chemotherapeutic reagent because patients with resectable cancers usually have higher performance status and have smaller radiation fields. The objective of the present study was to evaluate the tolerance and to determine the RD of preoperative combination therapy using gemcitabine and S-1 concurrently with full-dose radiation for resectable pancreatic cancer. In this dose escalation study, dose level 4—which consisted of 40 mg/m^2 of S-1 twice daily and 1,000 mg/m^2 of gemcitabine—was determined to be the RD. Nineteen of the 21 enrolled patients successfully underwent surgical resection without any severe postoperative complications.

According to the previous data by us and others, 1,000 mg/m^2 of gemcitabine with 50 Gy of radiation was feasible and safe [7, 26]. It has also been reported that a combination of 1,000 mg/m^2 of gemcitabine, 85 mg/m^2 of oxaliplatin, and radiation was feasible and safe [27]. However, the RDs of 80 mg/m^2 daily of S-1 and 1,000 mg/m^2 of gemcitabine seem to be something of an overdose. We speculate that these high doses were tolerable for the following reasons: Gemcitabine was scheduled to be administered on days 1 and 8, and was not administered on day 15; and S-1 was administered on the same days as radiation (weekdays) with breaks on weekends. Only one patient on level 4 experienced grade 4 leukopenia, and none needed granulocyte colony-stimulating factor (G-CSF) during the protocol. Moreover, previous studies have shown that gemcitabine and S-1 work as radiation sensitizers; therefore, our protocol of simultaneous administration on weekdays seems to have advantages both in avoiding adverse events and in improving long-term prognoses [28].

According to the RECIST criteria, none of the patients in the present study showed complete response and only five patients exhibited partial response. However, 75 % (12 of the 16 measurable patients) showed a more than 50 % reduction in CA19-9 level (Table 4). In assessing the preoperative therapy, evaluation must be performed within just a few weeks after the end of therapy, which may not be long enough to shrink the fibrous pancreatic tumor. Regardless of the modest radiologically assessed response rates, our preliminary data regarding overall survival are encouraging. The phase II study should include more patients and a longer follow-up period.

In conclusion, the present study indicates that preoperative chemoradiotherapy comprising gemcitabine and S-1 administration concurrent with full-dose radiation is feasible and well tolerated. The RD was determined to be 80 mg/m^2 of S-1 daily and 1,000 mg/m^2 of gemcitabine. Although the number of patients enrolled was too small, it is reasonable to expect that this combination therapy will lead to a better survival rate. As a follow-up to the present phase I study, a phase II study is currently underway, which should include clarification of the pathological effects and long-term survival rates following this combined therapy.

Conflict of interest None.

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Feasibility of single-site laparoscopic colectomy with complete mesocolic excision for colon cancer: a prospective case–control comparison

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Abstract
Background Single-site laparoscopic colectomy (SLC) is an emerging concept that, compared with conventional multiport laparoscopic colectomy (MLC), yields reduced postoperative pain and improved cosmesis. Complete mesocolic excision (CME) is a novel concept for colon cancer surgery that provides improved oncologic outcomes; however, there are no reports of SLC with CME. We conducted a prospective case–control study to evaluate the feasibility and safety of SLC with CME for colon cancer.
Methods Prospectively collected data of patients with stage I-III colon cancer who underwent SLC ($n = 150$) or MLC ($n = 150$) between June 2008 and March 2012 were analyzed. Patients who underwent SLC were, in terms of clinical characteristics and tumor location, matched as closely as possible with those undergoing MLC. Within each group, patients were classified as having right-sided ($n = 69$ in each group) or left-sided ($n = 81$ in each group) colon cancer, and short-term outcomes were compared between the two procedures overall and per side.
Results Overall perioperative outcomes, including operation time, blood loss, number of lymph nodes harvested, length of the resected specimen, and complications, were similar between the two procedures, whereas postoperative pain was significantly lower with SLC. Operation time for right-sided SLC was significantly shortened. SLC with CME was completed successfully in 94 % (65/69) of right-sided cases and in 88 % (71/81) of left-sided cases. Conversion rates were 1.4 % (1/69) and 1.1 % (1/81), respectively. The umbilical scars were nearly invisible 3 months after the procedure, and most patients reported being quite satisfied with the cosmetic outcomes.
Conclusions SLC with CME for colon cancer is feasible when performed by experienced surgeons in selected patients. Excellent cosmesis and reduced postoperative pain as well as oncologic clearance can be expected. A large-scale, prospective, randomized, controlled trial should be conducted to confirm the superiority of this procedure over MLC with CME.

Keywords Single-site laparoscopy colectomy · Complete mesocolic excision · Short-term outcome · Oncologic clearance · Colon cancer

Laparoscopic surgery plays a central role as a meaningful option in the management of colon cancer [1]. Laparoscopic colectomy has been compared to open colectomy in several multicenter, prospective, randomized, controlled trials (RCTs), and the short-term advantages and similar long-term survival achieved with laparoscopic colectomy have been well established by [2–5].

Complete mesocolic excision (CME) with central vascular ligation (CVL), according to the sound principles of total mesorectal excision (TME) [6, 7] for rectal cancer, has been translated to colon cancer under the concept of radical oncologic resection and following embryologic tissue planes along with the entire regional mesocolon in an intact fascial coverage of the tumor and its lymphatic

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drainage, including a high arterial tie [8, 9]. Data suggest that CME with CVL maximizes lymph node harvest, which may lead to improved oncologic outcomes [9, 10]. The technical feasibility and safety of laparoscopic CME for colon cancer also has been reported [11, 12].

Single-site laparoscopic colectomy (SLC) is performed entirely through one extraction site, theoretically reducing postoperative pain and the risk of abdominal wall morbidities, including bleeding, hernia, and internal organ damage, whereas conventional multiport laparoscopic colectomy (MLC) requires several ports and abdominal incisions [13]. Current efforts in minimally invasive treatment have shifted toward decreasing trauma by reducing the number of ports and/or size of the trocars [14]. Several groups have reported the feasibility and benefits of SLC, including improved cosmesis, reduced postoperative pain, and shortened recovery time, but there are some limitations including technical problems, such as instrument crowding, in-line viewing, insufficient countertraction, somewhat narrow patient applicability, and increased costs [15–22]. In addition, concerns over oncologic clearance in SLC remain unsettled. The less invasive procedure may bring patients some happiness or satisfaction, but oncologic clearance and technical safety are of utmost importance in the surgical treatment of colon cancer. We believe that CME also is effective and important in this minimally invasive procedure for colon cancer, especially for a locally advanced lesion; however, there is no report of SLC with CME for colon cancer at present. Therefore, we conducted a study to evaluate the feasibility and safety of SLC with CME for colon cancer in a prospective case–control analysis that examined short-term surgical results.

Patients and methods

Patients and data collection

We identified all patients scheduled to undergo SLC between 2008 and March 2012. The SLCs included right hemicolectomy for cancer of the cecum or ascending colon (right-sided colon cancer), and left hemicolectomy, sigmoidectomy, and anterior resection for cancer of the descending, sigmoid, or rectosigmoid colon (left-sided colon cancer).

In total, 150 patients undergoing SLC and 150 patients undergoing MLC during the same period and matched as closely as possible to the SLC patients were included in the study. Age, sex, body mass index (BMI), American Society of Anesthesiologists (ASA) class, tumor location, tumor size, preoperative disease stage, personal history of prior surgery, operation time, estimated blood loss, length of the incision (initial length and length required for extraction), number of lymph nodes harvested, length of the resected specimen, conversion to open surgery, insertion of an additional port, perioperative complications, morbidity, pain on postoperative day (POD) 1 (as indicated by the patient on a visual analog scale (VAS), and length of hospital stay were recorded. Patient characteristics are shown in total, per treatment group, and per right- versus left-sided procedure in Table 1.

The criteria for SLC were as follows: stage I-III colon cancer, tumor diameter <4 cm, body mass index (BMI) <35 kg/m^2, and ASA physical status <2. Each SLC patient was matched for clinical characteristics (age, sex, BMI, preoperative disease stage, prior surgery) and location of the tumor (right side of the colon or left side of the colon) to a patient undergoing MLC. No patient with rectal cancer, an advanced T4 tumor, a huge or bulky tumor ≥4 cm, severe obesity, perforated tumor, stenosis with bowel distention, prior abdominal polysurgery, or any severe comorbidity was included in the study. Patients in both groups were subclassified as those with right-sided colon cancer ($n = 69$ in each group) and those with left-sided colon cancer ($n = 81$ in each group).

Surgical techniques

All SLCs with CME were performed by one of two well-experienced laparoscopic colorectal surgeons who followed similar techniques. The conventional MLCs with CME were performed by one of five laparoscopic colorectal surgeons including the two well-experienced surgeons.

The entire SLC procedure was performed with standard laparoscopic instruments through an initial 2- to 3-cm extraction incision in the umbilicus [13]. A multichannel access device, such as a SILS Port (Covidien, Mansfield, MA, USA) or EZ Access (Hakko, Nagano, Japan), was fitted into the incision and rotated to achieve the ideal operative view and triangulation and to avoid or resolve collision of the instruments. An additional incision or trocar port was placed without hesitation if necessary to complete the procedure, and conversion to open laparotomy was maintained as an option. The indication and timing of trocar insertion or conversion to open surgery depended on the surgeon's judgment.

The abdominal cavity was explored with a 30-degree, 10-mm rigid laparoscope in all patients, with CO_2 pneumoperitoneum established and maintained at 10 mmHg. Conventional MLC required five ports, with the first 12-mm trocar in the umbilicus as a camera port, another 12-mm trocar, and three 5-mm trocars. The trocars were inserted at the right and left, upper and lower abdominal quadrant under laparoscopic guidance. The camera port was expanded to extract the specimen through an incision of 2–5 cm, as previously described [2–5].

Table 1 Patient characteristics

	SLC-total ($n = 150$)	MLC-total ($n = 150$)	p value	SLC-R ($n = 69$)	MLC-R ($n = 69$)	p value	SLC-L ($n = 81$)	MLC-L ($n = 81$)	p value
Age (year)	64.3 ± 11.7	65.6 ± 12.5	0.353	65.0 ± 11.8	66.6 ± 11.9	0.425	64.3 ± 11.7	64.8 ± 13.0	0.797
Sex (male/female)	75/75	71/79	0.644	31/38	36/33	0.394	37/44	35/46	0.752
BMI (kg/m^2)	21.7 ± 3.3	22.4 ± 4.7	0.137	21.5 ± 3.5	22.2 ± 3.7	0.257	21.9 ± 3.3	22.7 ± 5.4	0.257
ASA physical status									
1	40	33	0.572	18	15	0.807	22	18	0.704
2	83	85		38	39		45	46	
3	27	32		13	15		14	17	
Tumor location									
Cecum	34	29	0.440	34	29	0.393			
Ascending colon	35	40		35	40				
Descending colon	6	9					6	9	0.414
Sigmoid colon	53	45					53	45	
Rectosigmoid colon	22	32					22	27	
Preoperative disease stage									
I	76	65	0.290	32	31	0.82	44	34	0.220
II	48	49		23	21		25	28	
III	26	36		14	17		12	19	
Prior surgery (%)	31 (21)	39 (26)	0.275	16 (23)	19 (27)	0.557	15 (19)	20 (25)	0.340

Number (and percentage) of cases are shown unless otherwise indicated

SLC single site laparoscopic colectomy, *MLC* multiport laparoscopic colectomy, *BMI* body mass index, *ASA* American Society of Anesthesiologists, *L* left, *R* right

Right hemicolectomy for right-sided colon cancer in both groups was performed via an inferior approach, with initial peritoneal dissection between the mesoileum and the retroperitoneum performed with the patient in the Trendelenburg position (Fig. 1A). After intact mesocolic plane resection by CME, the duodenum and pancreas were sufficiently exposed (Fig. 1B), and the ileocolic vessels were ligated and dissected between clips at their origin to allow dissection of the entire right mesocolon (Fig. 1C). Laparoscopic CME with CVL was completed by dissecting the lymph nodes and lymphatic tissues at the origin of the ileocolic, right colic, and middle colic vessels (Fig. 1D). After dissection of the greater omentum, the hepatic flexure was mobilized. The specimen was extracted through the minilaparotomy incision in the umbilicus, after which extracorporeal functional end-to-end anastomosis was performed.

The operations for left-sided colon cancer in both groups were performed via a traditional medial-to-lateral approach with the patient in the Trendelenburg position, as described previously [13] (Fig. 2A). After precise mesocolic resection with CME and partial mesorectal dissection in the TME plane (Fig. 2B), the inferior mesenteric artery was ligated and dissected between clips 0.5 cm from its aortic origin (Fig. 2C). The fat surrounding the rectum at least 5-cm distal to the lesion was removed, and the superior rectal vessels were dissected. The rectum was clamped for irrigation with saline from the anus and then transected intracorporeally by one firing of an articulating linear stapler (Fig. 2D). The specimen was extracted through the minilaparotomy incision in the umbilicus, and the double-stapling technique was applied for anastomosis.

The final incision was extended to a length comparable to the size of the specimen or the tumor. The wound was closed in layers, and the incision was remeasured. All patients were put under a similar enhanced postoperative care protocol. Intravenous narcotics were given as needed for postoperative pain control.

Statistics

Data were collected and analyzed with the use of Microsoft Excel (Microsoft Corp., Redmond, WA, USA), and statistical calculations were performed with Prism 5.0 for Mac OS X (GraphPad Software, Inc., La Jolla, CA, USA). Between-group differences in variables were analyzed by

means of the Chi square test or Student t test. A p value <0.05 was considered statistically significant.

Results

Patient characteristics did not differ significantly between the SLC group and the MLC group (age, 64.3 ± 11.7 years vs. 65.6 ± 12.5 years, respectively, $p = 0.353$; male:female ratio (1.00 vs. 1.11, respectively, $p = 0.644$; BMI, 21.7 ± 3.3 vs. 22.4 ± 4.7 kg/m^2, respectively, $p = 0.137$). No other clinical variables, i.e., ASA status, preoperative disease stage, and history of prior surgery, differed significantly between these two groups. In comparing these variables between the two groups on the basis of the tumor locations (left vs. right colon), no differences were found (Table 1).

Short-term outcomes (Table 2), including operation time, blood loss, number of lymph nodes harvested, and length of the resected specimen, were similar between the SLC group and the MLC group. The postoperative VAS pain score was significantly lower in the SLC group than in the MLC group (4.2 vs. 5.1; $p = 0.01$), but the pain scores did not differ significantly in relation to the side of the surgery. The postoperative complications are shown in Table 2. The overall complication rates were nearly equivalent in the two groups: (SLC, 12 % and MLC, 16.7 %; $p = 0.249$). There was no mortality or readmission within 30 days after the procedure in either group. Despite the lesser pain and similar short-term outcomes achieved with LCS, length of hospital stay did not differ significantly between the two groups (SLC, 8.2 days vs. MLC, 8.7 days; $p = 0.152$). The umbilical scars were almost invisible 3 months after the procedure, and almost all patients reported being very satisfied with the cosmetic outcomes.

Operation time was significantly shorter in the group treated by right-sided SLC than in the group treated by right-sided MLC (168 ± 32 vs. 179 ± 32 min, respectively; $p = 0.046$), whereas estimated blood loss was similar between the two groups (41 ± 32 vs. 46 ± 34 mL, respectively; $p = 0.381$; Table 2). There was no difference in the number of lymph nodes harvested (23.9 vs. 23.7, respectively; $p = 0.868$) or the length of the resected specimen (22.3 vs. 22.3 cm; $p = 0.991$; Table 3). The right-sided SLC procedures were completed successfully except in four cases. Three patients required an additional port in the right lower quadrant due to visceral obesity or severe adhesion and the fourth required a small laparotomy for control of bleeding. The SLC procedure was completed without additional trocars in 94 % (65/69) of the right-sided cases; conversion to laparotomy was necessary in 1.4 % (1/69) of right-sided cases. Prolonged postoperative ileus developed in three patients, and anastomotic bleeding developed in two; no anastomotic leakage occurred (Table 2). The mean length of the final incision for a right-sided SLC was 3.2 cm; 27 patients (29 %) required extension of the original incision

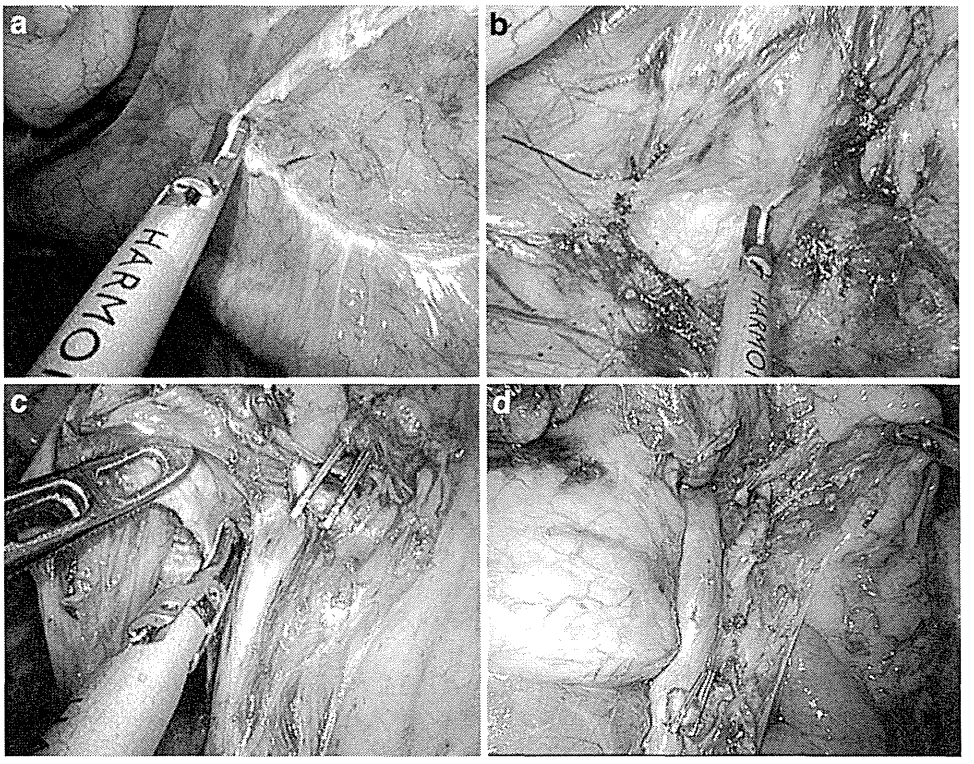

Fig. 1 Operative techniques for single-site laparoscopic right hemicolectomy with complete mesocolic excision for ascending colon cancer. **A** Inferior approach with initial peritoneal dissection between the mesoileum and the retroperitoneum. **B** Exposure of the head of the pancreas and mobilization of the duodenum by complete mesocolic excision. **C** Ligation at the origin of the ileocolic artery and vein with dissection of the entire the right-side mesocolon. **D** Completion of the lymphadenectomy in complete mesocolic excision with central vascular ligation for ascending colon cancer

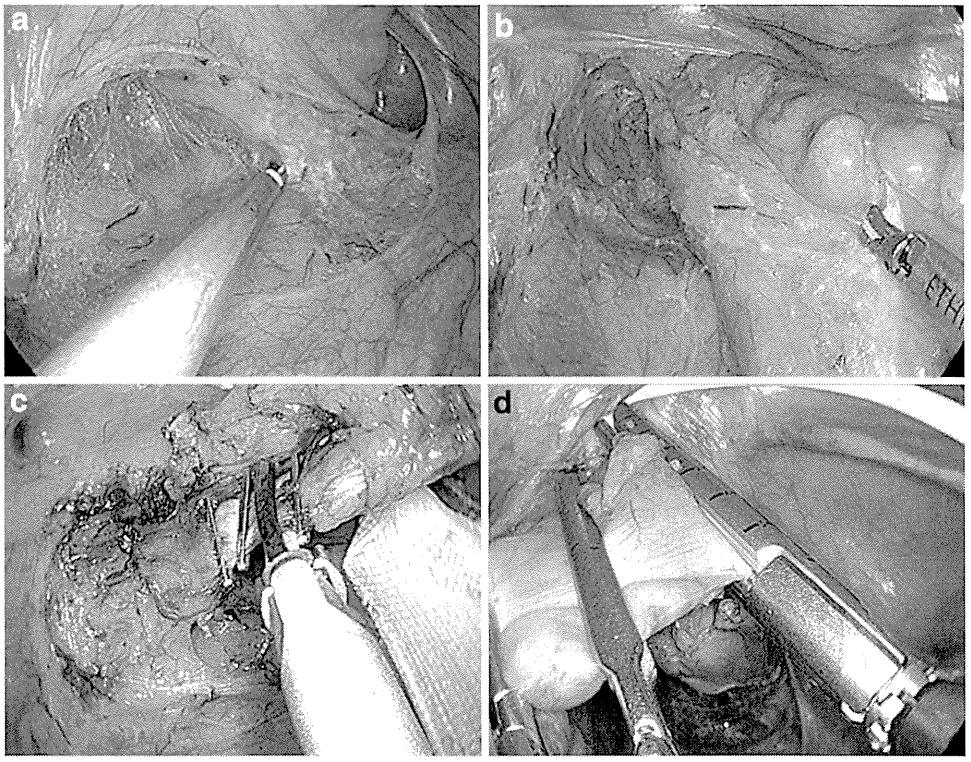

Fig. 2 Operative techniques for single-site laparoscopic sigmoidectomy with complete mesocolic excision for sigmoid colon cancer. **A** Medial-to-lateral approach with initial peritoneal dissection near the promontorium. **B** Precise plane resection of the mesosigmoid by complete mesocolic excision. **C** Ligation at the origin of the inferior mesenteric artery with dissection of the entire mesosigmoid without injury to the nerves. **D** Intracorporeal transection of the rectum with an articulating linear stapler

for gentle extraction of the tumor. Although the postoperative VAS pain score was slightly but not significantly lower for patients who underwent right-sided SCL than for those who underwent right-sided MLC (4.3 vs. 5.3; $p = 0.074$), length of hospital stay was similar between the two groups (8.0 vs. 8.5 days, respectively; $p = 0.254$; Table 2).

All variables were similar between patients who underwent left-sided SLC and those who underwent left-sided MLC-L. Operation time (174 ± 33 vs. 167 ± 37 min, respectively; $p = 0.21$) and estimated blood loss (25 ± 16 vs. 29 ± 16 mL, respectively; $p = 0.058$) were similar (Table 2). There was no difference in the number of lymph nodes harvested (20.7 vs. 21.4, respectively; $p = 0.291$) or length of the resected specimen (20.4 vs. 21.1 cm, respectively; $p = 0.31$; Table 3). A distal tumor-free margin <5 cm was confirmed in all cases. The left-sided SLC procedure was completed in all but ten cases. Nine required an additional 12-mm trocar for insertion of a linear stapler for appropriate intracorporeal transection of the rectum or because of visceral obesity. There was only one conversion to open surgery, and this was due to severe adhesion. Successful completion and conversion rates were 88 % (71/81) and 1.1 % (1/81), respectively. Two patients developed a minor anastomotic leak, but the leaks were successfully managed conservatively without reoperation (Table 2). The mean final incision length in cases of left-sided SLC was 2.8 cm, and 18 (22 %) patients required further incision. The postoperative VAS pain score was slightly lower in the left-sided SCL group than in the left-sided MCL group (4.1 vs. 4.9; $p = 0.068$,), with similar hospital stays between groups (8.2 vs. 8.9 days; $p = 0.201$; Table 2).

In comparing right-sided SLC with left-sided SLC, the final skin incision was significantly longer ($p = 0.008$) and expansion of the initial incision was significantly more prevalent in the right-sided group than in the left-sided group (39 vs. 22 %, respectively; $p = 0.024$). In contrast, insertion of an additional port was slightly less prevalent in the right-sided group (4.3 vs. 11.1 %, respectively; $p = 0.128$), and operation time was slightly shorter in the right-sided group (168 vs. 174 min, respectively; $p = 0.254$). However, estimated blood loss was significantly greater in the right-sided group than in the left-sided group (41 vs. 25 mL, respectively; $p < 0.001$). Conversion to laparotomy and overall complication rates were nearly equivalent. No significant differences in any short-term outcomes were observed between the two surgeons who performed SLC.

Discussion

Conventional laparoscopic surgery has achieved widespread acceptance as minimally invasive abdominal surgery, and its application to colorectal cancer has increased remarkably during the past decade [2–5]. However, each surgical wound required for conventional MLC may be a cause of postoperative pain and represent potential risk. Thus, even more minimally invasive techniques have been in recent demand. Surgeons experienced in conventional

Table 2 Short-term outcomes

	SLC-total ($n = 150$)	MLC-total ($n = 150$)	p value	SLC-R ($n = 69$)	MLC-R ($n = 69$)	p value	SLC-L ($n = 81$)	MLC-L ($n = 81$)	p value
Operation time (min)	172 ± 33	173 ± 35	0.720	168 ± 32	179 ± 32	0.046	174 ± 33	168 ± 37	0.21
Estimated blood loss (mL)	32 ± 26	37 ± 27	0.114	41 ± 32	46 ± 33	0.381	25 ± 16	29 ± 16	0.058
Length of initial skin incision (cm)	2.6 ± 0.5			2.7 ± 0.6			2.5 ± 0.4		
Length of final skin incision (cm)	3.0 ± 0.7	3.1 ± 1.0	0.317	3.2 ± 0.9	3.2 ± 1.2	0.912	2.8 ± 0.5	3.0 ± 0.8	0.058
Need for an enlarged incision	45 (30)			27 (39)	–		18 (22)		
Conversion to laparotomy	2 (1.3)	5 (3.3)	0.251	1 (1.4)	2 (2.9)		1 (1.1)	3 (3.7)	
Insertion of additional port(s)	12 (8.0)			3 (4.3)			9 (11.1)	–	
Postoperative VAS pain score	4.2 ± 2.7	5.1 ± 3.3	0.01	4.3 ± 3.0	5.3 ± 3.5	0.074	4.1 ± 2.4	4.9 ± 3.1	0.068
Length of hospital stay (days)	8.2 ± 2.7	8.7 ± 3.3	0.152	8.0 ± 2.3	8.5 ± 2.8	0.254	8.2 ± 3.1	8.9 ± 3.8	0.201
Complications	18 (12.0)	25 (16.7)	0.249	9 (13.0)	13 (18.8)	0.352	9 (11.1)	12 (14.8)	0.483
Wound infection	5	4		3	2		2	2	
Anastomotic leakage	2	2		0	0		2	2	
Anastomotic bleeding	2	4		2	3		0	1	
Ileus	6	8		3	5		3	3	
Thrombosis	0	1		0	0		0	1	
Urinary	1	2		0	1		1	1	
Cardiovascular	0	1		0	0		0	1	
Pneumonia	1	1		0	1		1	0	
Wound dehiscence	1	0		1	0		0	0	
Hernia	0	2		0	1		0	1	
Re-admission within 30 days after procedure	0	0	–	0	0	–	0	0	–
Mortality	0	0	–	0	0	–	0	0	–

Number (and percentage) of cases are shown unless otherwise indicated

SLC single site laparoscopic colectomy, *MLC* multiport laparoscopic colectomy, *L* left, *R* right

Table 3 Oncologic clearance

	SLC-total ($n = 150$)	MLC-total ($n = 150$)	p value	SLC-R ($n = 69$)	MLC-R ($n = 69$)	p value	SLC-L ($n = 81$)	MLC-L ($n = 81$)	p value
Number of lymph nodes harvested	22.2 ± 5.6	22.4 ± 6.0	0.767	23.9 ± 6.7	23.7 ± 7.4	0.868	20.7 ± 4.0	21.4 ± 4.4	0.291
Length of resected specimen (cm)	22.3 ± 5.1	21.6 ± 4.4	0.502	22.3 ± 5.4	22.3 ± 4.7	0.991	20.4 ± 4.7	21.1 ± 4.1	0.31
Tumor size (cm)	3.2 ± 1.4	3.3 ± 1.4	0.537	3.3 ± 1.3	3.4 ± 1.2	0.64	3.1 ± 1.5	3.2 ± 1.6	0.682

SLC single site laparoscopic colectomy, *MLC* multiport laparoscopic colectomy, *L* left, *R* right

MLC are challenged to further decrease trauma and improve outcomes by reducing the number of ports and/or size of the trocars [23].

After SLC for colon cancer was introduced by Remizi et al. [24] and Bucher et al. [25] in 2008, the feasibility of the procedure was examined in two RCTs [21, 22] and in several case–control studies [14–20], which compared short-term outcomes between SLC and MLC. Although many authors have reported that SLC provides a better cosmetic result with similar perioperative results, the

procedure remains somewhat controversial. Until now, with the exception of one report by Champagne et al. [20], most reports were based on limited data and a small number of selected cases. In addition, several studies of SLC were designed to include both cancerous and non-cancerous lesions, such as adenoma, diverticulitis, or inflammatory disease [16–18, 20]. In the management of malignant lesions, certain oncologic clearance is the most important task. The manner by which to best dissect the regional lymph nodes or remove the mesocolon in SLC remains to be more carefully evaluated. To our knowledge, the present case–control study of SLC for colon cancer is the largest and also the first to examine SLC with CME.

Four case–control studies have been conducted to assess short-term outcomes of SLC [14, 15, 18, 20], but the results were controversial. Poon et al. conducted an RCT of SLC versus conventional laparoscopic colectomy in which postoperative pain was measured as the primary outcome variable; they reported reduced postoperative pain associated with a shorter hospital stay for patients treated by SLC [21]. Our finding that postoperative pain was greater in patients treated by MLC than in those treated by SLC corresponded to the findings that came out of the largest case–control study conducted [20] and one RCT [21]. This suggests that the lateral port sites in the abdominal wall contribute substantially to postoperative discomfort. However, reduced postoperative pain with similar perioperative outcomes (including complications) resulting from SLC was not enough to affect hospital stay in our patient series. This was largely due to our hospital's discharge policy. It also might have been due to the fact that postoperative pain was evaluated only on POD 1. It remains unclear whether the reduced postoperative pain leads to faster postoperative recovery. The minimal invasiveness of SLC should be assessed and verified by detailed analysis of postoperative pain at all port sites in a future RCT.

The significantly longer final SLC incisions and the more frequent need for extending the length of the SLC incisions in our patients with cancers on the right versus the left were considered to be due to the volume of the extraction specimens. The extraction specimens tended to be greater volume in the right-sided group because of the loop formation with the double tract. In the left-sided group, there was a single tract with the transected stump of the distal colon.

Despite the technical difficulty of SLC, all but two studies, including two RCTs, reported similar operative times [18, 19]. The reported median SLC operation time ranges from 83 to 225 min [26], and the times are quite acceptable compared with the times for MLC [2–5]. Although the more careful and precise procedure that includes CME may necessitate a longer operation, our 168 min for right-sided colon cancer and 174 min for left-sided colon cancer are reasonable. Standardization of both MLC and SLC, whether on the right or the left, will make laparoscopic CME a reliable and safe procedure. Blood loss in our SLC cohort (25 mL in right-sided SLC and 41 mL in left-sided SLC) was slightly less than the losses previously reported. Although the level of difficulty may be increased for SLC with CME, it is possible to complete this precise procedure safely.

Interestingly, operation time was shorter in our right-sided SLC group than in our left-sided SLC group, and operation time was longer in our left-sided SLC group than in our left-sided MLC group. Conversion to open surgery occurred in only two SLC cases, and this number was remarkably lower than the five MLC cases requiring conversion. This could have been due to selection bias despite our every effort to match the cases. It also is possible that the performance of SLCs by well-experienced laparoscopic surgeons in carefully selected patients influenced the outcomes. The number of patients requiring an additional port was notably high when left-sided SLC was performed. This was due mainly to appropriate transection of the rectum. Even for standard laparoscopic surgery for rectal cancer, evaluation of technical and oncologic feasibility has just begun [27]. Thus, application of single-site laparoscopic surgery to rectal cancer should perhaps be selectively applied at present. It is reassuring that the surgeon can insert one or more additional trocars according to his own judgment at any time during the procedure. We also are reassured that our data showed the overall postoperative complication rate in SLS was nearly equivalent to that in MLC regardless of the side of the procedure, and there was no mortality.

With regard to oncologic clearance, in our SLC series with CME, the mean numbers of lymph nodes harvested (24 in right-sided cases and 21 in left-sided cases) were acceptable and comparable to previously reported numbers [9–12]. More than 12 lymph nodes were dissected in all cases except 3. The mean length of the resected specimen was also acceptable, with adequate tumor-free distal and proximal surgical margins. Oncologic resection with meticulous mesocolic dissection and optimal lymph node clearance may improve oncologic outcomes [9, 10]. The embryologic tissue planes must be respected to minimize the likelihood of cancer recurrence, and true central ligation of the lymphatic drainage maximizes regional lymph node harvest [11]. Standardization of CME has improved oncologic outcomes without increasing the postoperative complication or mortality rates [28]. During a median follow-up period of 24 months, 146 patients (97 %) who underwent SLC were free of recurrence (of the remaining 4 patients, 3 suffered liver metastasis and 1 suffered lung metastasis), and no local or lymph node recurrence was found.