Table 4 Results of randomized controlled trials on neoadjuvant transarterial chemoembolization and non-transarterial chemoembolization before hepatectomy for resectable hepatocellular carcinoma (HCC)

Study	Year	Total patients (*n*)	(TACE/non-TACE) patients (*n*)	Percentage of HBV (TACE/non-TACE)	Percentage of HCV (TACE/non-TACE)	Percentage of Child–Pugh class A (TACE/non-TACE)
This study		124	81/43	12/26	73/53	88/91
Zhou et al. [18]	2009	108	52/56	98/98	0/0	84/89
Yamasaki et al. [15]	1996	97	50/47	NR	NR	NR
Wu et al. [17]	1995	52	24/28	75/68	NR	92/86

Study	Mean preoperative tumor size (cm) (TACE/non-TACE)	Cytotoxic agent	TACE sessions per patient (*n*)	Complete necrosis (%) (TACE/non-TACE)
This study	4.1/5.0	EPI	1	**21/0**
Zhou et al. [18]	9.0/9.5	5FU, CDDP	1.5	**15/0**
Yamasaki et al. [15]	3.1/3.3	DOX	1	**16/NR**
Wu et al. [17]	14.3/14.5	DOX	3	NR/NR

Study	Morbidity (%) (TACE/non-TACE)	Mortality (%) (TACE/non-TACE)	3-year disease-free survival (%)	3-year overall survival (%) (TACE/non-TACE)
This study	10/19	1/2	28/32	75/60
Zhou et al. [18]	**Adhesions and longer operating time in TACE group**	0/0	26/21	40/32
Yamasaki et al. [15]	NR	6/9	54/42	91/88
Wu et al. [17]	NR	4/7	40/50	**33/60**

Significant differences are shown in **bold**. The number of patients receiving TACE in this study was 81 (42 patients in the selective group and 39 patients in the whole-liver group)

TACE transcatheter arterial chemoembolization, *NR* not reported, *HBV* hepatitis B virus, *HCV* hepatitis C virus, *EPI* epirubicin, *5FU* 5-fluorouracil, *CDDP* cisplatin, *DOX* doxorubicin

Conflict of interest None.

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ORIGINAL ARTICLE

Neo-adjuvant Chemoradiation Therapy Using S-1 Followed by Surgical Resection in Patients with Pancreatic Cancer

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Abstract
Objective The aim of this study was to compare short-term surgical results in pancreatic cancer patients who underwent surgical resection after neo-adjuvant chemoradiation therapy (NACRT) using S-1.
Methods The study population comprised 77 patients with pancreatic cancer between 2006 and 2010. Out of 34 patients who underwent staging laparoscopy between 2008 and 2010, 31 patients without occult distant organ metastasis underwent chemoradiation and of whom 30 underwent pancreatectomy (NACRT group). Of the other 43 patients, 36 underwent surgical resection in 2006–2008, followed by adjuvant therapy (adjuvant group). The primary endpoint was frequency of pathological curative resection (R0).
Results The new regimen of NACRT was feasible and safe. Twenty-eight of 30 (93%) patients in the NACRT group had R0 resection, which was significantly higher than in the adjuvant group (21 of 36 patients, 58%, p=0.005). The number and extent of metastatic lymph nodes in the NACRT group (1 (0–25), N0/1; 18 of 38) was significantly lower than in the adjuvant group (2 (0–19), N0/1; 23 of 30), p=0.0363). The frequency of intractable ascites in the NACRT group (eight of 30) was significantly higher than in the adjuvant group (two of 36, p=0.035).
Conclusion Neo-adjuvant chemoradiation therapy using S-1 followed by pancreatectomy can improve the rate of pathologically curative resection and reduces the number and extent of lymph node metastasis.

Keywords Chemoradiation · S-1 · Adjuvant chemotherapy · Residual tumor grading · Mortality and morbidity

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Introduction

Pancreatic cancer is a lethal disease with a poor prognosis, even in patients who have undergone curative resection. The results of surgical therapy alone for ductal pancreatic adenocarcinoma are disappointing, and the 5-year actual survival rate ranges from 3% to 17%, even after surgical resection.[1–5] Bradley proposed that further improvements in the numbers of long-term survivors from this dread disease, or increases in the number of actual cures, are unlikely to result from modifications of current surgical techniques.[6] To achieve a 5-year survival rate exceeding 50% in patients with pancreatic cancer, Traverso[7] advocated appropriate patient selection for curative resection by accurate staging, balanced resection, centralized treatment in high-volume centers, and the use of an effective adjuvant or neo-adjuvant therapy.

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Neo-adjuvant chemoradiation therapy (NACRT) has several possibilities, such as improved patient selection after the re-staging evaluation, increased resectability rate with clear margins (R0 resection), and a decreased rate of metastatic lymph nodes (LN) and local relapse.[8] We previously reported that NACRT increased the resectability rate with clear margins and decreased the rate of metastatic spread to the lymph nodes, resulting in a significant improvement of the 5-year actual survival rate in curative cases with pancreatic cancer and who had not received adjuvant therapy.[9–11] However, there were three limitations and/or issues associated with our previous study that need to be addressed. Firstly, approximately 20% of patients who underwent NACRT did not undergo subsequent surgical resection because of tumor progression or newly developed distant organ metastases. Secondly, no partial or complete responses were observed after pre-operative chemoradiation using low-dose 5-fluorouracil and cisplatin or gemcitabine (400 mg/m^2, three times in 4 weeks). Finally, although the actual disease-free survival rate at 1 year was approximately 50%, this was similar to that of the surgery-alone group. To address these issues, we have introduced a new strategy of treating patients with pancreatic cancer.

The objective of this study was to investigate the short-term results in patients with pancreatic cancer after surgical resection following NACRT using S-1[12], an orally administered drug consisted of a combination of tegafur, 5-chloro-2,4-dihydroxypyridine, and oteracil potassium.

Patients and Methods

Between January 2006 and September 2010, 103 consecutive patients with a clinical diagnosis of pancreatic ductal adenocarcinoma met our resectability criteria[9,10] and were regarded as potentially or borderline resectable or unresectable pancreatic cancer patients, as defined by the National Comprehensive Cancer Network (NCCN) guideline.[13] This diagnosis was made using cine-imaging multi-detector row CT (MDCT) at Kansai Medical University Hospital. The patients who were expected to achieve pathologically curative resection by Appleby operation[14] or distal pancreatectomy with the celiac axis (CA) resection[15] were also eligible for this study. Cases involving an endocrine tumor of the pancreas, intraductal papillary mucinous cancer, acinar cell cancer, anaplastic cancer, duodenal cancer, distal common bile duct cancer, or ampullary cancer were excluded. The period during which the patient was treated determined which group they were classified under, namely adjuvant (2006–2008) or NACRT (2008–2010) groups (Fig. 1).

Adjuvant Group Between January 2006 and September 2008, among 48 consecutive patients, 43 with T3/T4 pancreatic cancer (International Union Against Cancer (UICC) classification, sixth edition[16]) who met our resectability criteria[9,10] were classified as the adjuvant group, as shown in Fig. 1. Peri-operatively, all 43 patients had pathological evidence of pancreatic ductal adenocarcinoma. It was planned that all patients in the adjuvant group who underwent pancreatectomy would then receive adjuvant chemotherapy comprising weekly gemcitabine (1,000 mg/m^2) with three times in 4 weeks and a total of 18 times of gemcitabine administration.

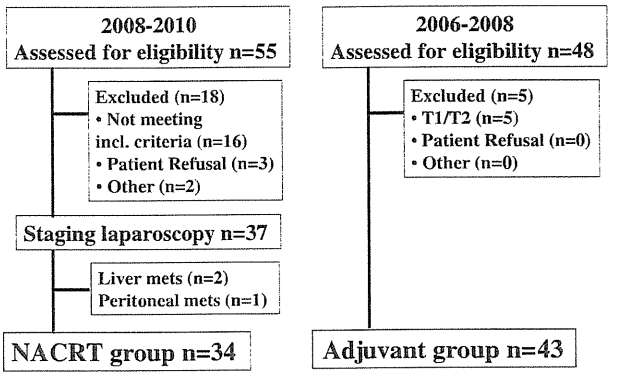

Fig. 1 Study profile

NACRT Group In October 2008, we introduced a new strategy for treating T3/T4 pancreatic cancer patients (UICC classification, sixth edition[16]) who met our resectability criteria,[9,10] and all patients since that date have been treated with this method, as described below. The main criteria for inclusion in this NACRT group were (1) T3/T4 pancreatic cancer (UICC classification, sixth edition[16]) and coincided with our resectability,[9,10] (2) confirmation of pathological evidence of pancreatic cancer, (3) no distant organ metastasis under the staging laparoscopy, and (4) introduction of adjuvant chemotherapy. There were 55 consecutive patients with clinically diagnosed pancreatic cancer between October 2008 and September 2010. Eighteen patients were excluded due to no pathological evidence (n=4), pre-operative diagnosis of lower bile duct cancer (n=4), localized tumor within pancreatic parenchyma (n=3), patients' refusal (n=3), poor performance status (n=2), and other reasons (n=2). The remaining 37 patients underwent staging laparoscopy, following which three additional patients with occult liver (n=2) and peritoneal (n=1) metastases were also excluded. Thus, eventually, 34 patients underwent the planned NACRT (described below) and were classified as the NACRT group (Fig. 1). The tumor extension in these patients was re-evaluated by cine-imaging MDCT 3 weeks after NACRT. It was planned that all patients in whom the MDCT did not show progressive disease or the development of newly distant organ metastasis would undergo

pancreatectomy at approximately 1 week after this re-evaluation. All patients who underwent pancreatectomy following NACRT were to undergo adjuvant chemotherapy with the same regimen as patients in the adjuvant group.

Regimen of NACRT Following the result of the phase I trial of S-1 with concurrent radiotherapy by Ikeda et al.,[12] radiotherapy was administered by 10 or 15 MV photons using three-dimensional treatment planning. A total dose of 50.4 Gy was delivered in 28 fractions over 5.5 weeks. The clinical target volume (CTV) included only the gross primary tumor and nodal involvement enlarged over 10 mm, as detected by computed tomography. Elective nodal irradiation was not used. The planning target volume was defined as CTV plus a 10-mm margin in the lateral direction and 10–20-mm margin in the craniocaudal direction to account for respiratory organ motion and daily setup error. The four-field technique was used. S-1 was administered orally, twice daily (80 mg/m^2/day) on the day of irradiation (Monday to Friday) during radiotherapy.

Extent of Lymph Node and Nerve Plexus Dissection LN dissection around the CA, superior mesenteric artery (SMA), middle colic artery (MCA), superior mesenteric vein (SMV), para-aortic region, and of the hepatoduodenal ligament and right-sided dissection of the nerve plexus around the CA and the SMA was carried out in patients who underwent pancreaticoduodenectomy. In patients who underwent distal pancreatectomy, LN dissection around the CA, SMA, MCA, SMV, and para-aortic region was performed in all patients, while left-sided dissection of the nerve plexus around the CA and the SMA was limited to patients with pancreatic body cancer. In a few cases, distal pancreatectomy with celiac axis resection was performed.

Post-operative morbidity and mortality, defined as in-hospital death due to any cause, were also recorded. Informed consent was obtained from all patients according to institutional regulations, and this study was approved by the local ethics committee. Patient data were obtained from the prospective database of pancreatobiliary disease at Kansai Medical University Hospital.

Endpoints and Statistical Analysis The primary endpoint was the frequency of pathological curative resection (R0) defined by residual tumor grading. The specimen was serially cut with the thickness of 5 mm. All of these were histologically examined according to "General Rules for the study of pancreatic Cancer[17]." Within the general rule, when all of surgical margin factors, such as the pancreatic and bile duct transection margins and dissected peripancreatic tissue margin, were negative, we determined no residual tumor (R0). If at least one of them was positive, pathological residual tumor (R1) was determined. Furthermore, the stumps of the nerve plexus and the retroperitoneal tissue were pathologically examined independently of the resected specimen, in order to evaluate, in detail, the extent of resection (namely R0 or R1) in the NACRT group. Secondary endpoints were feasibility of NACRT and its associated adverse effects, response rate defined by Response Evaluation Criteria in Solid Tumor,[18] pathological tumor grading defined by Evans classification,[19] and safety of pancreatectomy following NACRT. The study design to predict the number of patients necessary for statistical validity (two-sided) was based on the premise of improving the rate of pathologically curative resection from 70% to 90%, with the α set at 0.05 and the β set at 0.2, yielding a power of 80%. It was calculated that 30 patients were required in this study group. The countable data were expressed as the median and range. The countable data using Mann–Whitney U test or the category data using Fisher's exact test or chi-square test were compared between the NACRT and adjuvant groups. Results were considered significant at $p<0.05$.

Results

Clinical Courses of Patients in NACRT and Adjuvant Groups As shown in Fig. 2, among 34 patients in the NACRT group, one patient withdrew her consent to continue NACRT treatment due to grade 1 nausea and fatigue; she underwent pancreatectomy 19 days after discontinuation of NACRT. MDCT for re-evaluation showed the presence of multiple liver metastases in two patients, and one patient refused subsequent pancreatectomy due to poor performance status. Among 31 patients who underwent open laparotomy,

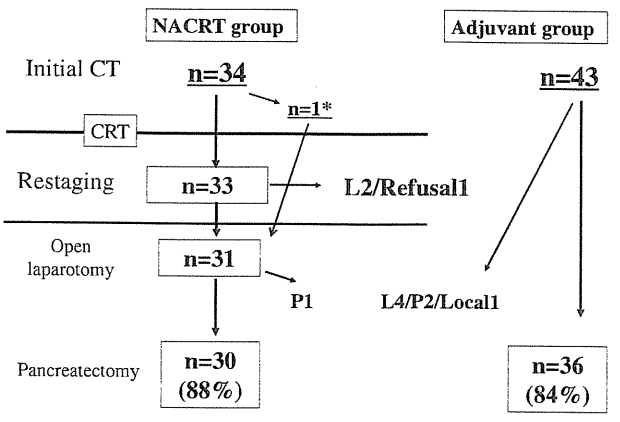

Fig. 2 Clinical course of NACRT and adjuvant groups. *Asterisk* the patient who had refused to continue this regimen underwent pancreatectomy without re-evaluation. *L* liver metastasis, *P* peritoneal metastasis, *Local* locally advanced tumor

one patient incidentally had occult peritoneal metastases. Consequently, 30 out of the 34 patients underwent pancreatectomy in NACRT group.

In the adjuvant group, seven patients did not undergo pancreatectomy because of liver metastasis (n=4), peritoneal metastasis (n=2), and progressive local disease (n=1) which became apparent during open laparotomy. Thus, eventually, 36 of 43 patients underwent surgical resection in the adjuvant group. There was no difference in the surgical resectability rate between the two groups.

Radiological Response and Adverse Effects of Chemoradiotherapy A total of 33 out of 34 patients completed the regimen of NACRT and were evaluated for efficacy in terms of radiological response at 3 weeks after the end of CRT. The patient who had refused to continue this regimen underwent pancreatectomy without re-evaluation. Complete response was not observed in any patient. Partial response and stable disease were achieved in six and 11 patients, respectively. The overall response rate and disease control rate were 18% and 88.0%, respectively. Twenty-five of 34 patients in NACRT group had pancreatic cancer with radiographic findings of portal or superior mesenteric vein (PV/SMV) invasion at pre-NACRT period. Six of nine patients with PV/SMV involvement demonstrating tumor abutment had tumor shrinkage with no radiographic evidence of PV/SMV abutment after NACRT. Three of 16 patients with PV/SMV involvement with impingement and narrowing of the lumen had tumor shrinkage with no radiographic evidence of PV/SMV abutment after NACRT. A total of 33 patients were evaluated for toxicity of NACRT as shown in Table 1. Adverse events were reported in 18 (55%) patients. No on-treatment deaths or grade 4 toxicity occurred. The most severe hematologic toxicity was leukocytosis (grade 3), reported in only one patient (3.0%). Grade 3 anorexia and fatigue were each seen in one patient (3.0%). Problems with biliary stenting were seen in seven patients (21%), who underwent procedures to replace the stenting. All toxicities were tolerable and reversible after temporarily withholding therapy.

Comparisons of Surgical and Pathological Results Between NACRT and Adjuvant Groups There were no significant differences in the clinical backgrounds between NACRT and adjuvant groups, apart from the resectability status defined by NCCN[12] as shown in Table 2. A significantly higher frequency of borderline resectable and unresectable pancreatic cancer was seen in the NACRT group relative to the adjuvant group (p=0.022). No significant differences were seen in operative factors between the two treatment groups, as shown in Table 3. Resection of other organs, including vascular resection, was carried out in 17 of 36 patients in the adjuvant group and 19 of 30 patients in NACRT group.

Regarding the rate of pathologically curative resection (R0), which was the primary endpoint in this study, 28 of 30 (93%) patients in NACRT group had R0 resection, which was significantly higher than the rate in the adjuvant group, where 21 of 36 (58%) patients had R0 resection (p=0.005). The reason of R1 resections of two patients in the NACRT group was the SMA margin.[20] The SMA margins were positive in 12 of 13 patients with R1 resections and in all patients with R2 resections (n=2). The neck margins were positive in the residual one of 13 patients with R1 resections and in one of two patients with R2 resections.

The number of metastatic lymph nodes in the NACRT group was significantly lower than the adjuvant group (p= 0.0363). When comparing the extent of metastatic lymph nodes, the frequency of N0/1 in the NACRT group was higher than in the adjuvant group (p=0.041). The lymph node ratio in the NACRT group was significantly lower than that in the adjuvant group (p=0.032). There was a tendency for a lower rate of negative lymph nodes in the NACRT group relative to the adjuvant group, but the difference did not reach statistical significance. In the NACRT group, there were three patients with T1/2 defined by pathological findings, with evidence of down-staging. Pathological effect, as defined by Evans classification[19], was grade IIA (n=21), IIB (n=7), and III (n=2).

Comparisons of Post-operative Mortality and Morbidity Between NACRT and Adjuvant Groups With one exception, there were no significant differences in mortality and morbidity between the two groups (Table 4). The exception was rate of intractable ascites, defined as drug resistance or ascites needed paracentesis, which was significantly higher in the NACRT group (eight of 30 patients, 27%) compared with the adjuvant group (two of 36 patients, 6%) (p=0.035). Diarrhea needing oral administration of loperamide hydrochloride and tincture of opium was reported in five of 36 (14%) patients in the adjuvant group and in nine of 30 (30%) patients in the NACR group, but the difference was not significant (p=0.138). There were three in-hospital deaths in the NACRT group. They had borderline resectable pancreatic cancer that needed vascular resection and/or other organ resection such as colon, adrenal gland, or stomach. They had adverse events of grade 2 anorexia and/or fatigue during NACRT. Postoperatively, three patients had anastomotic failure of the colon followed by liver failure, massive ascites followed by aspiration pneumonia, or fungemia followed by multiple organ dysfunction syndrome.

Discussion

In the majority of patients with pancreatic cancer, the tumor is classified as unresectable at diagnosis, and only

Table 1 Toxicity of NACRT

		0	1	2	3	4	G1–G4 (%)	G3–G4 (%)
Hematologic	Leucopenia	29	1	3	1	0	15	3.0
	Neutropenia	32	0	2	0	0	6.0	0
	Anemia	33	1	0	0	0	3.0	0
	Thrombocytopenia	34	0	0	0	0	0	0
Non-hematologic	Nausea	33	1	0	0	0	3.0	0
	Vomiting	33	0	1	0	0	3.0	0
	Anorexia	21	5	7	1	0	38	3.0
	Diarrhea	33	0	1	0	0	3.0	0
	Fatigue	27	0	6	1	0	21	3.0
	Weight loss	25	7	2	0	0	26.4	0
	Gastric ulcer	33	0	1	0	0	3.0	0
	DVT	33	0	1	0	0	3.0	0
	Skin rash	33	0	1	0	0	3.0	0
	Fever	26	8	0	0	0	23.5	0
	Stent trouble	28	0	0	7	0	20.8	20.8
No adverse events $n=15$								

Toxicity was graded according to Common Terminology Criteria for Adverse Events v4.0

DVT deep vein thrombosis

approximately 20% of patients are indicated for surgical resection. Even after "curative" resection, patients with pancreatic cancer face a 50–80% local recurrence rate and a 25–50% chance of developing distant metastases in the peritoneum and liver, resulting in an actual 5-year survival rate of approximately 10%.[1–5] Recently, some randomized studies have shown favorable results in pancreatic cancer patients who underwent curative resection followed by adjuvant therapy, reporting median survival times within the range of 20.1–23.6 months.[21–23] A systematic review and meta-analysis by Gillen et al. showed that an estimated median survival time of patients with resectable pancreatic cancer who underwent surgical resection following neo-adjuvant therapy was similar to those of patients who had adjuvant therapy.[24] Recently, a few centers have reported better actual survival rate in patients with pancreatic cancer who underwent surgical resection following NACRT. For example, the M.D. Anderson Cancer Center Group showed that the actual 5-year survival rate of patients after multidisciplinary management including surgical resection was 27%,[25] and in patients with resectable pancreatic head cancer who underwent surgical resection following preoperative gemcitabine-based chemoradiation, the actual 5-year survival rate was 36%.[26] Our previous study[9–11] demonstrated that the actual 5-year survival and disease-free survival rates in the pre-CRT group, who did not receive adjuvant chemotherapy, were significantly longer than in the surgery-alone group, in a sub-group analysis of patients who underwent curative resection. In fact, the actual survival curves in these studies demonstrated that a fall of the survival curve within 3 years after surgical resection, plateaued when it passed the 3-year mark. Thus, surgical resection following NACRT can be associated with improvement of long-term survival rate through good local disease control. In our previous study,

Table 2 Clinical background between NACRT and adjuvant groups

Parameter	Adjuvant ($n=36$)	NACRT ($n=30$)	p value
Sex (male/female)	25:11	15:15	0.133
Age (years)[a]	68 (51–81)	65.5 (36–79)	0.107
CA19–9 (U/ml)[a]	127 (6–1,729)	247 (1–2,232)	0.067
Diabetes mellitus (+/−)	10:26	11:19	0.596
Obstructive jaundice (+/−)	30:6	20:10	0.153
Albumin (g/dl)[a]	3.8 (1.9–4.4)	3.6 (2–4.3)	0.286
Hemoglobin (g/dl)[a]	12.1 (7.9–15.2)	11.4 (9.1–13.9)	0.142
Platelet count ($\times 10^4$)[a]	24 (12–43)	21 (13–40)	0.908
PR vs BR/UN	19:17/0	7:21/2	0.022
Stent exchange (+/−)	3:33	7:23	0.089

PR potentially resectable pancreatic cancer, *BR* borderline resectable pancreatic cancer, *UN* unresectable pancreatic cancer

[a]Values are median (range)

Table 3 Comparisons of surgical results between NACRT and adjuvant groups

Parameter	Adjuvant (n=36)	NACRT (n=30)	p value
Extent of blood loss (ml)	999 (324–5,238)	1,376 (438–3,853)	0.151
Op time (min)	514 (210–672)	531 (380–711)	0.146
Op type (PD/PpPD/DP/TP)	22:8:5:1	22:1:6:1	0.112
PV resection (+/−)	14:22	17:13[b]	0.216
CA/CHA resection (+/−)	0:36	2:28	0.203
Blood transfusion (none/auto/allo)	4:23:9	8:13:9	0.166
Location (Ph/Pbt)	31:5	22:8	0.227
Tumor size (mm)	32.5 (23–65)	30 (10–65)	0.341
Numbers of harvested LNs	26 (7–56)	33 (6–65)	0.340
Numbers of metastatic LNs	2 (0–19)	1 (0–25)	0.0363
Lymph node ratio[a]	0.07 (0–0.62)	0.02 (0–0.38)	0.032
N (−/+)	8:28	14:16	0.065
N 0/1:2/3	18:18	23:7	0.041
T 1/2:3/4	0:36	3:27	0.089
R0:1:2	21:13:2	28:2:0	0.005
Evans classification (IIA/IIB/III)	N/E	21:7:2	

TNM classification was defined by Japanese Pancreas Society

NACRT neo-adjuvant chemoradiation therapy, *Op* operation, *PD* pancreaticoduodenectomy, *PpPD* pylorus preserving pancreaticoduodenectomy, *DP* distal pancreatectomy, *TP* total pancreatectomy, *PV* portal vein, *CA* celiac axis, *CHA* common hepatic artery, *auto* autologous blood transfusion, *allo* allogeneic blood transfusion, *Ph* pancreatic head, *Pbt* pancreatic body and tail, *LN* lymph node, *R0* negative microscopic margin, *R1* positive microscopic margin, *R2* positive gross margin

[a] Lymph node ratio is calculated as number of metastatic lymph nodes/harvested lymph nodes

[b] One patient who underwent renal vein resection was included

approximately half the patients who underwent curative resection had disease recurrence at 1 year in both the NACRT and surgery-alone groups. NACRT followed by surgical resection did not have enough power to improve the short-term survival rate and the frequency of early liver metastases, which was one of the major post-operative recurrence sites.

Table 4 Comparison of morbidity and mortality between NACRT and adjuvant groups

Parameter	Adjuvant	NACRT	p value
Overall complication (+/−)	12:24	10:20	1.000
Mortality (+/−)	0:36	3:27	0.098
Re-operation/no re-operation	0:36	1:29	0.455
DGE (+/−)	3:33	2:28	1.000
POPF (+/−)	7:29	1:29	0.063
Grade A/B/C	4:3:0	1:0:0	0.245
Wound dehiscence (+/−)	4:32	6:24	0.492
Intra-abdominal abscess (+/−)	1:35	1:29	1.000
Cholangitis (+/−)	0:36	2:28	0.203
Pneumonia (+/−)	0:36	2:28	0.203
Bile leakage (+/−)	0:36	0:30	–
PPH (+/−)	0:36	0:30	–
Intractable ascites (+/−)	2:34	8:22	0.035
Diarrhea (+/−)	5:31	9:21	0.138

DGE delayed gastric emptying, *NACRT* neo-adjuvant chemoradiation therapy, *POPF* post-operative pancreatic fistula, *PPH* post-pancreatectomy hemorrhage

There were several limitations and issues with our previous study that we aimed to resolve in this present study, namely (1) approximately 20% of patients who received pre-operative CRT did not undergo surgical resection because of progressive disease, resulting in a median survival time of 5.5 months (unpublished data); (2) surgical resection followed by pre-operative CRT only did not improve the short-term results; and (3) the previous regimen of pre-CRT was not aggressive enough to achieve tumor shrinkage. Therefore, we introduced (1) staging laparoscopy before patient recruitment to the new regimen of NACRT, (2) standard adjuvant chemotherapy, and (3) full dose of S-1 (80 mg/m^2) and radiotherapy (50.4 Gy).

S-1 is an orally administered drug, which is a combination of tegafur, 5-chloro-2,4-dihydroxypyridine, and oteracil potassium. Very recently, the results of the gemcitabine and S-1 trial study (a randomized, prospective, open-label, three-arm, and phase III study) were presented to the public at the annual meeting of the American Society of Clinical Oncology 2011.[27] The results showed that oral S-1 provided

Table 5 Surgical results of neo-adjuvant chemoradiation therapy

Authors (reference number)	Year of publication	No of patients	Regimen of CRT	Resection rate; *n* (%)	Vascular resection rate; *n* (%)	R0 (%)	Negative LN mets rate (%)
White et al.[33]	2001	53	5-FU based (45 Gy)	28 (53)	2 (7)	71	19 (70)
Moutardier et al.[34]	2004	61	5-FU based (60 Gy)	40 (66)	5 (13)	95	30 (75)
Evans et al.[26]	2008	86	GEM (30 Gy)	64 (74)	13 (20)	89	40 (63)
Le Scodan et al.[35]	2009	41	5-FU based (50 Gy)	26 (63)	N/A	80.7	12 (46)
Ohigashi et al.[36]	2009	38	GEM (50.4 Gy)	31 (82)	17 (55)	97	28 (90)
Turrini et al.[37]	2010	34	Docetaxel-based (45)	17 (50)	N/A	100	13 (76)
Stokes et al.[38]	2011	40	Capecitabine (50 Gy)	16 (25)	4 (25)	88	13 (81)
Present study	–	34	S-1 (50 Gy)	30 (88)	17 (57)	94	14 (47)

CRT chemoradiation therapy, *5-FU* 5-fluorouracil, *GEM* gemcitabine, *LN* lymph node, *mets* metastasis, *N/A* not available

similar efficacy and tolerable toxicity to gemcitabine when used as first-line treatment for unresectable pancreatic cancer. The response rates of gemcitabine, S-1, and gemcitabine+S-1 were 13.3%, 21.0%, and 29.3%, respectively. In addition to the benefit of the oral drug on its own, the combination of S-1 and radiotherapy has been demonstrated to exert a synergistic effect against 5-FU-resistant cancer xenografts.[28,29] The response rate of CRT using S-1 was around 20% in phase I and II studies in patients with unresectable pancreatic cancer[12,30–32] As expected, our results showed that the response rate and disease control rate of NACRT using S-1 were 18% and 88.0%, respectively. To our knowledge, this is the first study of NACRT using S-1 and concurrent radiation for patients with resectable pancreatic cancer.

Despite the fact that we excluded patients with occult metastasis by using staging laparoscopy before study entry for NACRT, three of 34 patients had occult liver or peritoneal metastasis after NACRT. During the 9 weeks between study entry and surgical resection, approximately 10% of pancreatic cancer patients had progressive disease. In this study, the majority of patients who underwent NACRT using S-1 did not suffer from severe adverse effects, and 33 of 34 patients completed this regimen. However, seven of 34 (21%) NACRT patients required hospitalization because of cholangitis, resulting in a delay of the operation date. The primary endpoint of pathologically curative resection rate in this study showed a statistically significant difference in favor of NACRT over the adjuvant group, despite the fact that the NACRT group had a higher frequency of borderline resectable and unresectable pancreatic cancer cases. In this study, we pathologically examined the cut stump of the nerve plexus and retroperitoneal tissue independently in all cases, which was the main reason for a positive surgical margin (R1 resection). Moreover, there was a tendency for a higher rate of negative lymph node metastasis in the NACRT group than in the adjuvant group, but this did not reach statistical significance because of the small sample size. The frequency of N0 and N1 and number of metastatic lymph nodes in the NACRT group were significantly improved relative to those in adjuvant group. Most studies have reported that predictive factors for prognosis in patients with pancreatic cancer were pathologically curative resection and negative lymph node metastasis.[1–5] In addition to our results, some authors have reported the promising results of a higher rate of R0 resection (70–100%) and lower rate of metastastic lymph nodes (46–90%) in patients who underwent surgical resection following NACRT, as summarized in Table 5.[26,33–38]

We recognize that a limitation of our study was its prospective non-randomized design. Approximately 30% of patients were excluded before staging laparoscopy in the NACRT group due to no pathological evidence of pancreatic cancer, misdiagnosis of bile duct cancer, and so on. In contrast, 43 of 48 patients were included in the adjuvant group; the five excluded patients had T1/2 pancreatic cancer. Consequently, the frequency of borderline resectable pancreatic cancer in the NACRT group was significantly higher than in the adjuvant group. However, irrespective of this one important difference in the baseline characteristics between the two groups, the primary endpoint of this study was still reached.

Although there were no statistical differences in morbidity and mortality between the two groups, three in-hospital deaths were observed in the NACRT group. The common clinical features of these three patients were borderline resectable pancreatic cancer, grade 2 anorexia or fatigue during CRT, and other organ resection including vascular

resection. Gillen et al.[24] reported that in-hospital mortality after neo-adjuvant treatment and tumor resection was estimated at 2.2–6.0% in resectable patients and at 5.1–9.5% in non-resectable patients. In this present study, 23 of 30 (77%) patients in the NACRT group had borderline resectable and unresectable pancreatic cancer, and 17 of 30 (57%) patients underwent pancreatectomy with vascular resection. The patient population in the NACRT group had shifted to an advanced stage. Thus, special attention should be paid to patients with advanced pancreatic cancer who undergo this new type of surgical strategy.

In conclusion, in this study, NACRT using the orally administered drug S-1 resulted in a better response rate than was seen among the patients in the adjuvant group; it was also feasible and safe. Pancreatectomy after NACRT improved the rate of pathologically curative resection and reduced the number and extent of lymph node metastasis. A large-scale randomized controlled trial will be needed to confirm the clinical efficacy of NACRT.

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Phase II trial of preoperative S-1 plus cisplatin followed by surgery for initially unresectable locally advanced gastric cancer

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Abstract

Background: The aim of this study was to evaluate the efficacy and feasibility of preoperative chemotherapy with S-1 plus cisplatin in patients with initially unresectable locally advanced gastric cancer.
Methods: We enrolled patients with initially unresectable locally advanced gastric cancer because of severe lymph node metastases or invasion of adjacent structures. Preoperative chemotherapy consisted of S-1 at 80 mg/m^2 divided in two daily doses for 21 days and cisplatin at 60 mg/m^2 intravenously on day 8, repeated every 35 days. If a tumor decreased in size, patients received 1 or 2 more courses. Surgery involved radical resection with D2 lymphadenectomy.
Results: Between December 2000 and December 2007, 27 patients were enrolled on the study. No CR was obtained, but PR was seen in 17 cases, and the response rate was 63.0%. Thirteen patients (48.1%) had R0 resections. There were no treatment related deaths. The median overall survival time (MST) and the 3-year overall survival (OS) of all patients were 31.4 months and 31.0%, respectively. Among the 13 patients who underwent curative resection, the median disease-free survival (DFS) and the 3-year DFS were 17.4 months and 23.1%, respectively. The MST and the 3-year OS were 50.1 months and 53.8%, respectively. The most common site of initial recurrence after the R0 resection was the para-aortic lymph nodes.
Conclusions: Preoperative S-1 plus cisplatin can be safely delivered to patients undergoing radical gastrectomy. This regimen is promising as neoadjuvant chemotherapy for resectable gastric cancer. For initially unresectable locally advanced gastric cancer, new trials using more effective regimens along with extended lymph node dissection are necessary.

Keywords: Neoadjuvant chemotherapy; Lymph node dissection; Bulky lymph node; TS-1; Cisplatin; Para-aortic lymph node

Introduction

Gastric cancer is still one of the most common cancers in the world; 876,000 new cases were anticipated worldwide in the year 2000.[1] In Japan, 110,323 new cases were anticipated in the year 2003 and the 5-year survival rate of gastric cancer diagnosed from 1993 to 1996 was 54.4%.[2,3]

Currently, surgery remains the mainstay of curative treatment. However, only an R0 resection is associated with significant cure rates. Patients having microscopic (R1) or macroscopic (R2) residual tumor have an extremely poor prognosis.[4]

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Preoperative and neoadjuvant chemotherapy represent investigational options. The rationale of preoperative chemotherapy is based on the difficulty of performing an R0 resection in patients with initially unresectable locally advanced tumors and the high risk of micrometastatic disease in these patients. Neoadjuvant chemotherapy has potential for resectable gastric cancer for the purpose of treating micrometastases.

Intensive chemotherapy is necessary for the improvement of the R0 resection rate and complete elimination of the micrometastases. However, it is difficult for patients who undergo gastrectomy to tolerate intensive chemotherapy. Because weight decreases by gastrectomy, it is necessary to reduce the dose of chemotherapy. The tolerance to chemotherapeutic agents with digestive organ toxicity was often reduced by gastrectomy-related gastrointestinal effects.

S-1 (TS-1, Taiho Pharmaceutical, Tokyo, Japan) is an orally active combination of tegafur (a prodrug that is converted by cells to fluorouracil), gimeracil (an inhibitor of dihydropyrimidine dehydrogenase, which degrades fluorouracil), and oteracil (which inhibits the phosphorylation of fluorouracil in the gastrointestinal tract, thereby reducing the gastrointestinal toxic effects of fluorouracil) at a molar ratio of 1:0.4:1. The response rate of S-1 alone exceeded 40% in two phase 2 trials involving patients with metastatic gastric cancer.[5,6] The combination chemotherapy of S-1 plus cisplatin (CDDP) achieved a high response rate (74%, 95%CI: 54.9–90.6) in a previous phase I/II study of patients with metastatic gastric cancer.[7]

These factors led us to perform the current phase II trial to investigate the use of an active preoperative chemotherapy regimen. The primary objectives of the trial were to investigate tolerance to the preoperative regimen, its effects on operative morbidity and mortality, and the response rate. Secondary objectives included evaluation of the R0 resection rate, disease-free and overall survival, and failure pattern.

Patients and methods

Patients

The study was conducted as a prospective multi-institutional phase II trial by the Osaka Gastrointestinal Cancer Chemotherapy Study Group (OGSG) in Japan. All patients had histologically confirmed adenocarcinoma of the stomach. They also had to have initially unresectable locally advanced tumors because of invasion to adjacent structures or severe lymph node metastases, staged by contrast-enhanced CT as T2-3N2-3M0 or T4NanyM0, according to the Japanese Classification of Gastric Carcinoma (2nd English Edition).[8] They also had to have lymph node metastases that were measurable according to the RECIST[1.0] guidelines.[9] We did not require laparoscopic staging as an entry criterion for this study. Any sites of suspected M1 disease had to be ruled out prior to entrance into the study. No prior chemotherapy or radiation was allowed. The age range was 20–75 years. The performance status (ECOG) was 0 from 1.

Because of the worse prognosis of type IV gastric cancer, also known as scirrhous or linitis plastica, we excluded such cases.[10] Acceptable hematologic profile (WBC $\geqq$ 4000 cells/mm^3, hemoglobin $\geqq$ 8.0 g/dl, platelets $\geqq$ 100,000 cells/mm^3), and renal (BUN $\leqq$ 25 mg/dl, creatinine $\leqq$ 1.2 mg/dl and/or creatinine clearance > 60 ml/min) and hepatic function (total serum bilirubin < 1.5 mg/dl) were required. In addition, certain respiratory function test results (ratio of the forced expiratory volume in one second $\geqq$ 50%, PaO2 in room air $\geqq$ 70 mmHg) were required criteria. No clinically significant auditory impairment was allowed. Patients with prior cancer diagnosed during the previous 5-year period (except for colon carcinoma *in situ*) were excluded. Other exclusion criteria included significant cardiac disease, pregnancy or serious infections. The protocol was reviewed and approved by the Institutional Review Board of each institution. All patients gave written informed consent.

Preoperative chemotherapy

Patients found to have locally advanced gastric cancer as defined above, received two cycles of S-1 plus cisplatin every 35 days. Preoperative chemotherapy consisted of S-1 at 80 mg/m^2 divided in two daily doses for 21 days and cisplatin at 60 mg/m^2 intravenously on day 8. Physical examination, abdominal CT scan and assessment of toxicity were performed prior to each cycle. The response measurement of the preoperative chemotherapy was carried out according to the RECIST[1.0] guidelines. Because it was preoperative chemotherapy, response was not confirmed at least 4 weeks apart. Toxicity was recorded and graded according to the National Cancer Institution Common Toxicity Criteria (NCI-CTC) version 2.0 scale. Operative complication was graded according to the Common Terminology Criteria for Adverse Events v4.0 (CTCAE v4.0). If a tumor decreased in size, according to protocol criteria, we added 1 or 2 more courses. If curative resection was considered possible after planned chemotherapy, the patient had surgery. If curative resection was considered difficult, a further course of chemotherapy was added. The doses of both agents were attenuated for grade $\geqq$3 toxicities, using standard reduction criteria.

Surgery

The surgery was planned for 3–6 weeks from the day of last administration of chemotherapy. Surgery involved a radical resection, the extent of which (total or distal gastrectomy) depended on the site of the primary tumor, with a D2 lymphadenectomy. We performed D2 or more dissection in patients with metastasis to N3 lymph nodes before chemotherapy. Spleen preservation in total gastrectomy procedure was entrusted to the decision of each clinician.

Patients in whom curative resection was impossible underwent palliative operation. The postoperative treatment was left to the decision of each physician.

Biostatistical considerations

The 3 primary end points of the study were as follows; 1) tolerance to preoperative chemotherapy, 2) operative morbidity and mortality, and 3) objective response rate (ORR). Secondary end points were R0 resection rate, failure pattern, and disease-free and overall survival. One of the primary end points was ORR. The number of patients to be enrolled was calculated at 24, which was required given the assumption that the 95% confidence interval (CI) would be ±20%, assuming an expected response rate of 60%. Finally, we set the number as 30 patients in consideration of disqualified patients. The early stopping criterion of the trial was 3 treatment related deaths. Analogous samples were used to estimate the response rate, R0 resection rate, operative morbidity and mortality, and incidence of treatment related grade 3–4 toxicity. Overall survival (OS) of all patients was calculated from the day of registration in the trial. OS and disease-free survival (DFS) of the patients who underwent R0 resections were calculated from the day of surgery. Survival distributions were estimated using the Kaplan–Meier method.

Follow-up

Following completion of chemotherapy and surgery, patients were followed at 3- monthly intervals until year 3. Thereafter, 6-month follow-up visits were performed. CT scans and appropriate blood studies were performed on the occasion of each evaluation.

Results

Patient population

Between December 2000 and December 2007, 27 patients with initially unresectable local advanced gastric cancer were enrolled into the study from 9 institutions. As shown in Table 1, the male to female ratio was 20:7. The median age was 63 years. As for the histologic type, 15 cases were undifferentiated (including signet ring cell carcinoma) and 11 cases were differentiated type. One case was classified as mucinous carcinoma. There were 3 cStage IIIa (11.1%) preoperatively, 8 cStage IIIb (29.6%), and 16 cStage IV (59.3%).

Preoperative chemotherapy

The median number of preoperative chemotherapy regimens was 3 courses. Grade 3–4 toxicities associated with preoperative S-1/CDDP are described in Table 2. Hematologic toxicity (Grade 3/4) was 7.4% and non-hematologic toxicity (Grade 3/4) was 3.7%. Treatment was generally well tolerated and no chemotherapy-related deaths were observed. While there was no CR, there were 17 cases of PR and the response rate was 63.0% [95%CI: 42.4–80.6] (Table 2).

Table 1
Patient characteristics ($n = 27$).

		Number	%
Age, years	Median (range)	63	(48–75)
Gender	Male	20	74.1
	Female	7	25.9
Histology	Differentiated	11	40.7
	Undifferentiated	15	55.6
	Other	1	3.7
Pretreatment cStage	T2N2M0 (IIIA)	3	11.1
	T3N2M0 (IIIB)	7	25.9
	T4N1M0 (IIIB)	1	3.7
	T2N3M0 (IV)	5	18.5
	T3N3M0 (IV)	6	22.2
	T4N2M0 (IV)	3	11.1
	T4N3M0 (IV)	2	7.4

Operative outcome

All patients who were entered into this trial had initially unresectable tumors. Nine patients were diagnosed as being unresectable when chemotherapy was completed and did not undergo surgery. Eighteen patients (66.7%) underwent laparotomy (Table 3). Thirteen patients (48.1%) had R0 resections. Three patients (11.1%) underwent R1 surgery, because of positive results of peritoneal washing cytology. Two patients underwent simple laparotomy because of peritoneal metastases or unresectable local extension of metastatic lymph nodes. Postoperative complications are described in Table 3. The incidence of complications was 22.2%. One patient underwent operative intervention because of pancreatic leakage; however, there were no surgery-related deaths.

Table 2
Courses, responses and toxicities of preoperative chemotherapy.

				n	%
Courses	Median (range)			3	(1–9)
Response	CR			0	0.0
	PR			17	63.0
	SD			6	22.2
	PD			4	14.8
Toxicities		Grade1/2		Grade3/4	
		n	%	*n*	%
	Neutropenia	10	37.0	2	7.4
	Thrombocytopenia	3	11.1	1	3.7
	Hemoglobin	21	77.8	1	3.7
	Vomiting	7	25.9	1	3.7
	Nausea	13	48.1	1	3.7
	Diarrhea	4	14.8	1	3.7
	Anorexia	17	63.0	1	3.7
	Cerebral infarction	0	0	1	3.7
Treatment related death				0	0.0

Table 3
Operative outcome ($n = 27$).

		Number	%
No operation		9	33.3
Operation		18	66.7
	R0 resection	13	48.1
	R1 resection	3	11.1
	R2 resection	0	0
	Simple Laparotomy	2	22.2
Complications			
	None	14	77.8
	Pancreatic leak	3 (Grade 1: 2, Grade 4: 1)	16.7
	Lymphorrhea	1 (Grade 1)	5.6
	Anastomotic leak	0	0.0
Re-operation		1	5.6
Mortality		0	0.0

Seven of 9 patients who did not undergo surgery received 2nd-line chemotherapy (S-1: 3 patients, S-1/CPT-11: 2 patients, CPT-11/CDDP: 1 patient, Paclitaxel: 1 patient). Four of 5 patients who underwent R1-2 surgery received further chemotherapy (S-1/Paclitaxel: 2 patients, S-1: 1 patient, CPT-11/CDDP: 1 patient).

Overall survival of all patients

Only one patient was lost to follow-up at 8 months from the first day of preoperative chemotherapy, but all other patients were followed more than three years. The median overall survival time and the 3-year overall survival rate of all patients were 31.4 months and 31.0% [95%CI: 17.5–55.1], respectively.

DFS, OS, and first relapse site of patients who underwent R0 resection

Thirteen patients underwent R0 resection. The details of these patients are shown in Table 4. Twelve of these 13 patients (92.3%) achieved PR after preoperative chemotherapy. The median number of course of chemotherapy of these patients was 3 (2–5). Of these patients, only 2 patients (15.4%) underwent D2 plus para-aortic lymph node dissection (D3). Downstaging was observed in 11 patients (84.6%). Seven of 13 patients received postoperative adjuvant chemotherapy (S-1: 4 patients, S-1 plus CDDP: 1 patient, CPT-11: 1 patient, CPT-11/CDDP: 1 patient). To date, recurrence has been diagnosed in 10 patients. First relapse site of five of ten patients was para-aortic lymph nodes. The median disease-free survival time and the 3-year disease-free survival rate of the 13 patients were 17.4 months and 23.1% [95%CI: 8.6–62.3], respectively (Fig. 1A). The median overall survival time and the 3-year overall survival rate of the 13 patients were 50.1 months and 53.8% [95% CI: 32.6–89.1], respectively (Fig. 1B).

Discussion

The combination chemotherapy of S-1 plus cisplatin was chosen because it had achieved a high response rate of 74% (95%CI: 54.9–90.6) in previous phase I/II study of patients with metastatic gastric cancer. The incidences of severe (Grade 3/4) hematological and non-hematological toxicities were 15.8 and 26.3%, respectively.[7] A randomized controlled trial in Japan showed the superiority of S-1/cisplatin compared with S-1 monotherapy according to the response rate and survival for metastatic gastric cancer.[11] Therefore, S-1/cisplatin therapy is now the standard treatment for metastatic gastric cancer in Japan.

This multi-institutional phase II prospective trial of preoperative chemotherapy in initially unresectable locally advanced gastric cancer showed that preoperative chemotherapy using S-1/cisplatin was not only feasible but also achieved a high response rate. The overall response rate was 63.0% [95%CI: 42.4–80.6]. The incidence of grade 3/4 toxicities was less than 10% and treatment related

Table 4
Patients who underwent R0 resection.

No.	cStage	Course	Response	Gastrectomy	D	Combined resection	fStage	Nodes	First relapse
1	T3N2M0 (IIIB)	2	PR	Distal	D3	Liver, Gallbladder	T2N2M0 (IIIA)	4	None
2	T3N3M0 (IV)	3	PR	Total	D2	Spleen, Panc. (tail) Gallbladder	T2N2M0 (IIIA)	6	Brain
3	T3N2M0 (IIIB)	2	PR	Total	D2	Spleen	T2N2M0 (IIIA)	10	Lymph (para AO)
4	T3N2M0 (IIIB)	2	PR	Distal	D3	None	T2N2M0 (IIIA)	3	None
5	T3N2M0 (IIIB)	3	PR	Total	D1*	Liver	T2N0M0 (IB)	0	None
6	T2N2M0 (IIIA)	2	SD	Distal	D2	Panc. (head)	T4N3M0 (IV)	7	Peritoneum
7	T4N2M0 (IV)	3	PR	Total	D2	Spleen, Panc. (tail)	T3N2M0 (IIIB)	10	Lymph (para AO)
8	T2N3M0 (IV)	4	PR	Distal	D2	Gallbladder	T2N2M0 (IIIA)	1	Bone
9	T4N3M0 (IV)	3	PR	Distal	D2	None	T1N0M0 (IA)	0	Lung
10	T4N1M0 (IIIB)	3	PR	Total	D2	Spleen	T2N2M0 (IIIA)	4	Lymph (hepatic)
11	T2N3M0 (IV)	5	PR	Total	D1*	None	T2N3M0 (IV)	2	Lymph (para AO)
12	T2N2M0 (IIIA)	3	PR	Total	D1*	None	T2N0M0 (IB)	0	Lymph (para AO)
13	T3N2M0 (IIIB)	3	PR	Total	D1*	None	T2N2M0 (IIIA)	13	Lymph (para AO)

D1*: we performed almost D2 dissection, but it classified D1 dissection according to the Japanese classification of gastric carcinoma (2nd English edition), because of preserving spleen.

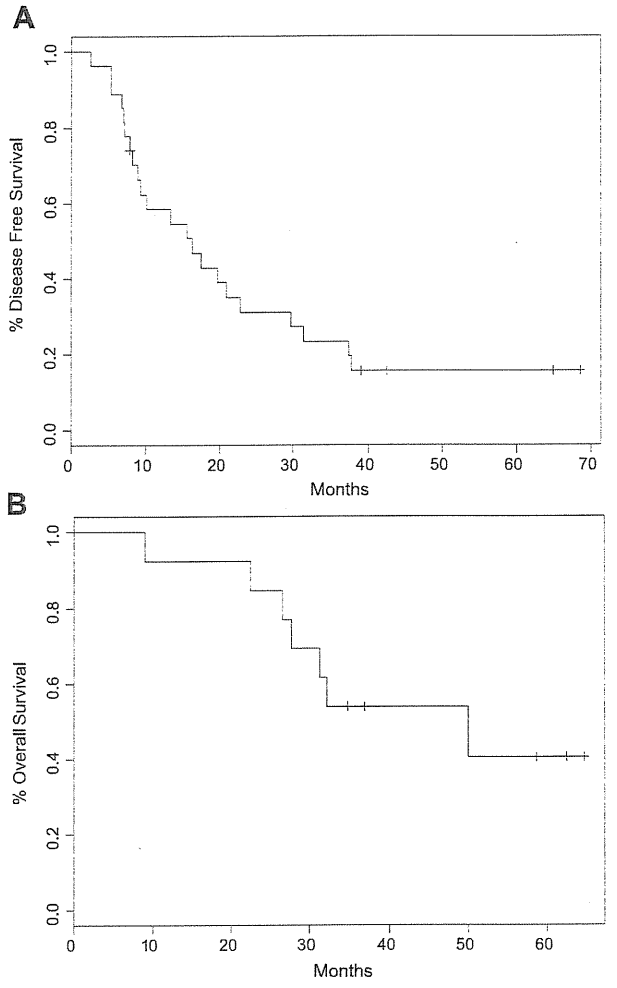

Figure 1. Disease-free and overall survival of the patients who underwent R0 surgery ($n = 13$).

mortality was 0.0%. Similar results were reported in other studies.[12,13] These results encourage the use of S-1/cisplatin combination chemotherapy as neoadjuvant treatment for patients who have resectable gastric cancer. Such trials are currently under way in Japan.[14,15]

The recently completed MAGIC trial constitutes a larger study regarding neoadjuvant chemotherapy in gastric cancer. In this study, 503 patients were randomized to three cycles of pre- and three cycles of postoperative epirubicin/cisplatin/5-FU (ECF) chemotherapy or surgery alone. Neoadjuvant chemotherapy was tolerable and was completed in 88% of patients. Significant downsizing (5.0 versus 3.1 cm median tumor size, $P < 0.001$), downstaging (54% versus 36% T1—T2 tumors, $P = 0.01$) and enhanced resectability (79% versus 69%, $P = 0.02$) were noted. Improved progression-free survival and survival were demonstrated, with an overall 5-year survival of 36% versus 23% for those undergoing surgery alone.[16] We should conduct phase III clinical trials of the neoadjuvant chemotherapy of S-1/cisplatin therapy for resectable gastric cancer.

In Japan, the ACTS-GC trail demonstrated a survival advantage of postoperative adjuvant chemotherapy after R0 resection. R0 patients were randomized to adjuvant chemotherapy using S-1 (529 patients) versus surgery alone (530 patients); improved survival (3-year overall survival rates of 80.1% versus 70.1%, $P = 0.003$) was noted.[17] Adjuvant chemotherapy, as reported by the ACTS-GC Group, is now considered a standard treatment for R0 patients. However, of the 283 patients who had stage III disease and received S-1 adjuvant chemotherapy, 73 patients died. The hazard ratio of the adjuvant chemotherapy group worsened with an increasingly advanced stage. These results suggest that S-1 monotherapy is insufficient for patients who have stage III or more. However, for patients who have initially unresectable gastric cancer like the patients enrolled in this trial, S-1/cisplatin chemotherapy is insufficient because of the high relapse rate of patients who underwent R0 resection.

For the patients immediately after gastrectomy, highly toxic chemotherapy is difficult because of overlaps between chemotherapy-induced gastrointestinal toxicity and digestive symptoms due to gastrectomy.[18] Therefore, further improvements in preoperative therapy will require development of more effective chemotherapeutic regimens. During the last decade, several new agents with promising activity against gastric cancer were identified. These include paclitaxel, docetaxel, irinotecan and trastuzumab. These agents are now undergoing phase II and III trials, as part of combination regimens.[19–22] If improved outcome is seen in metastatic disease, these agents will undergo extensive testing in the preoperative setting.

The absence of laparoscopic staging might have allowed inclusion of patients with positive peritoneal cytology or small peritoneal implants that could have disappeared with the chemotherapy; these patients have a worse prognosis, which could have impacted on the final results. Actually, there were 3 cases of positive cytology at exploration after chemotherapy. Laparoscopic staging should be mandatorily included in future similar projects.

An interesting point is that there were many para-aortic lymph node recurrences in the patients who underwent R0 resection. Among 13 patients who underwent curative resection, initial recurrence in 5 patients was in a para-aortic lymph node. These patients had not undergone para-aortic lymph node dissection. The prognostic improvement effect of the para-aortic lymph node dissection was refuted by two clinical trials.[23,24] In the JCOG 9501 trial, 523 patients with resectable gastric cancer were enrolled, and 263 were assigned to D2 group and 260 were assigned to D2 plus para-aortic nodal dissection. The 5-year overall survival rate was 69.2% for D2 lymphadenectomy group and 70.3% for the D2 lymphadenectomy plus para-aortic nodal dissection group; the hazard ratio for death was 1.03 (95%CI, 0.77 to 1.37; $P = 0.85$). There were also no significant differences in recurrence-free

survival and the pattern of recurrence between the two groups.[23] In the East Asian Surgical Oncology Group trial, 269 patients with resectable gastric cancer were enrolled, and 135 were assigned to the D2 group and 134 were assigned to the D2 plus para-aortic nodal dissection. The 5-year overall survival rates were 52.6% for the D2 lymphadenectomy group and 55.0% for the D2 lymphadenectomy plus para-aortic nodal dissection group. There was no significant difference in survival between the two groups ($P = 0.801$).[24] It was concluded that the D2 lymphadenectomy plus para-aortic nodal dissection did not improve prognosis regarding D2 lymph node dissection in the resectable gastric cancer.

However, in these trials, patients who had gross metastases to the para-aortic nodes were excluded. The incidence of metastases in the para-aortic nodes was lower than expected in 8.5% and 9.7%, respectively. The median number of metastatic nodes was only 2 nodes among the patients who underwent D2 plus para-aortic nodal dissection in the JCOG 9501. In the East Asian Surgical Oncology Group trial, the mean number of metastatic nodes was 5.9 in the para-aortic lymph node dissection group.

Recently, 15-year follow-up results of a randomized nationwide Dutch D1D2 trial were published. 711 patients underwent randomly assigned treatment with curative intent (380 in the D1 group and 331 in the D2 group). Overall 15-year survival was 21% for the D1 group and 29% for the D2 group. Gastric cancer-related death rate was significantly higher in the D1 group (48%, 182 patients) than that in the D2 group (37%, 123 patients). Local recurrence was 22% (82 patients) in the D1 group versus 12% (40 patients) in D2, and regional recurrence was 19% (73 patients) in D1 versus 13% (43 patients) in D2. After a median follow-up of 15 years, D2 lymphadenectomy was associated with lower locoregional recurrence and gastric cancer-related death rates than D1 surgery.[25] This difference was greater in the patients with lymph node metastases from 7 to 15.[26]

The observation period was shorter in the clinical trials of JCOG and East Asian Surgical Oncology Group than in the Dutch trail, and fewer mortality events occurred and also fewer metastases to lymph nodes. Therefore, para-aortic lymph node dissection might have better prognosis in patients with severe lymph node metastases like the patients enrolled in our trial.

In summary, preoperative S-1/cisplatin can be safely delivered to patients undergoing radical gastrectomy. The response rate was high, with no increase in operative morbidity and mortality compared with those upon surgery without preoperative chemotherapy.[27] Controlled trials of neoadjuvant chemotherapy using this regimen with the postoperative S-1 monotherapy for resectable gastric cancer are necessary. For initially unresectable locally advanced gastric cancer, the rate of recurrence was high, and the most common initial recurrent site was para-aortic lymph node. New trials, using a more effective regimen along with extended lymph node dissection are necessary.

Conflict of interest statement

The authors declare no conflict of interest.

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Active Hexose Correlated Compound Inhibits the Expression of Proinflammatory Biomarker iNOS in Hepatocytes

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Key Words

Active hexose correlated compound · Interleukin-1β · iNOS · Nuclear factor-κB · Type I interleukin-1 receptor · iNOS gene antisense transcript

Abstract

Background/Aims: Excess production of nitric oxide (NO) by inducible nitric oxide synthase (iNOS) has been implicated as proinflammatory biomarker in liver injury. The application of active hexose correlated compound (AHCC) as a functional food in complementary and alternative medicine has increased. The possibility that AHCC might inhibit iNOS induction was investigated as a potential liver-protective effect. ***Methods:*** Hepatocytes were isolated from rats by collagenase perfusion and cultured. Primary cultured hepatocytes were treated with interleukin-1β in the presence or absence of AHCC-sugar fraction (AHCC-SF). ***Results and Conclusion:*** AHCC-SF inhibited the production of NO and reduced expressions of iNOS mRNA and its protein. AHCC-SF had no effects on either IκB degradation or nuclear factor-κB (NF-κB) activation. In contrast, AHCC-SF inhibited the upregulation of type I interleukin-1 receptor (IL-1RI) through the inhibition of Akt phosphorylation. Transfection experiments with iNOS promoter-luciferase constructs revealed that AHCC-SF reduced the levels of iNOS mRNA at both promoter transactivation and mRNA stabilization steps. AHCC-SF inhibited the expression of iNOS gene antisense transcript, which is involved in iNOS mRNA stabilization. These findings demonstrate that AHCC-SF suppresses iNOS gene expression through a IκB/NF-κB-independent but Akt/IL-1RI-dependent pathway, resulting in the reduction of NO production. AHCC-SF may have therapeutic potential for various liver injuries.

Introduction

In the liver, nitric oxide (NO) is produced by constitutively expressed endothelial nitric oxide synthase (eNOS) or inducible NOS (iNOS). eNOS is located in vascular sinusoidal endothelial cells, and NO produced by eNOS maintains hepatic circulation and endothelial integrity. iNOS is negligible under physiological conditions, but is expressed in hepatic cells including hepatocytes and Kupffer cells under pathological conditions such as sepsis, hemorrhagic shock, ischemia-reperfusion, hepatitis, and cirrhosis. During infection and inflammation in the liver, excess production of NO by iNOS is thought to be

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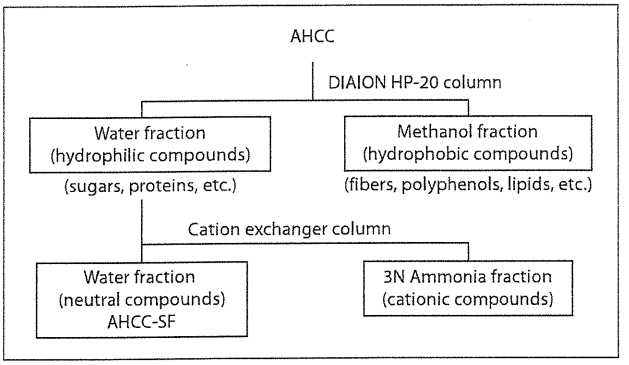

Fig. 1. Preparation of AHCC-SF. Separation flow of AHCC.

involved in liver injury. The expression of iNOS is a biomarker in proinflammation, although NO has been reported to exert either detrimental or beneficial effects depending on the insults and tissues involved. In our previous reports, clinical drugs, which showed liver-protective effects in various animal models of liver injury [1–5], prevented iNOS induction in the liver as well as decreased production of various inflammatory mediators. These drugs also inhibited iNOS induction and NO production in proinflammatory cytokine-stimulated cultured hepatocytes of rats [3, 6, 7], which is used as a simple in vitro injury model.

Proinflammatory cytokine interleukin (IL)-1β, or a mixture of IL-1β, tumor necrosis factor (TNF)-α and interferon-γ, induces the expression of iNOS gene in primary cultures of human and rat hepatocytes [8, 9]. The induction of iNOS is regulated by transactivation of the iNOS promoter with transcription factors including nuclear factor (NF)-κB, and by post-transcriptional modifications including mRNA stabilization [10]. There are two essential pathways involved in iNOS induction, IκB kinase/IκB/NF-κB activation and phosphatidylinositol-3 kinase (PI3K)/Akt/type I IL-1 receptor (IL-1RI) upregulation [11]. IL-1β stimulates the degradation of IκB after its phosphorylation by IκB kinase, which is followed by the translocation of NF-κB from cytoplasm to the nucleus and DNA binding (NF-κB activation). IL-1β also stimulates the upregulation of IL-1RI through activation of PI3K/Akt, which is essential for both transcriptional activation and mRNA stabilization in iNOS induction [7, 11–13]. In the case of mRNA stabilization, we have reported that natural iNOS gene antisense transcript interacts with 3′-untranslated region (UTR) containing AU-rich elements (ARE) of iNOS mRNA, leading to iNOS mRNA stabilization in IL-1β-stimulated hepatocytes [14].

The functional food active hexose correlated compound (AHCC) is an extract prepared from cultured mycelium of *Basidiomycetes* mushrooms. In recent reports [15–20], supplementation with AHCC has shown a generalized positive effect on the immune systems, as well as anti-inflammatory and anti-oxidant effects. AHCC is a mixture of polysaccharides, amino acids, lipids, and minerals, in which oligosaccharides are the major components constituting about 74% of the mixture. These oligosaccharides are believed to account for the biological activities of AHCC [21, 22]. In the liver, we reported that AHCC improved the prognosis of postoperative hepatocellular carcinoma patients [23]. However, the molecular mechanism by which AHCC protects the liver is not fully understood. In the current study, the possibility that AHCC might inhibit NO production was pursued as a possible liver-protecting mechanism. We intended to examine whether AHCC influences the induction of iNOS gene expression in primary cultures of rat hepatocytes, and if so, study the mechanism involved in this process.

Materials and Methods

Materials

Recombinant human IL-1β (2×10^7 U/mg protein) was provided by Otsuka Pharmaceutical Co. Ltd. (Tokushima, Japan). [γ-^{32}P]adenosine-5′-triphosphate (ATP; –222 TBq/mmol) and [α-^{32}P]deoxycytidine-5′-triphosphate (dCTP; –111 TBq/mmol) were obtained from DuPont-New England Nuclear Japan (Tokyo, Japan). Rats were kept at 22°C under a 12-h light/12-h dark cycle, and received food and water at libitum. All animal experiments were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals of the National Institutes of Health, and approved by the Animal Care Committee of Kansai Medical University.

Preparation of Sugar Fraction of AHCC

As shown in figure 1, the extract of AHCC (20 g), which was supplied by Amino Up Chemical Co. Ltd (Sapporo, Japan), was dissolved in H_2O (80 ml), applied on the column of DIAION HP-20 (5 × 25 cm; Mitsubishi Chemical Co., Japan) and eluated with H_2O (1 liter) and methanol. The first eluate (water fraction containing hydrophilic compounds) was concentrated under vacuum, followed by lyophilization (18.1 g of yellowish powder). The water fraction was dissolved in H_2O (35 ml), mixed with methanol (180 ml) and centrifuged (1,600 *g* for 15 min), which was repeated twice. Then the precipitate was dissolved in H_2O (100 ml), applied on the DOWEX 50 WX8 (4.4 × 17 cm; The Dow Chemical Company, USA), and eluted with H_2O (0.5 liter) and ammonia (3 N). The final water fraction containing AHCC-sugar fraction (AHCC-SF) was lyophilized (4.93 g) and stored at –20°C.