was delivered in 28 fractions over 5.5 weeks. Primary tumor and metastatic lymph nodes >1 cm identified on CT were contoured as gross tumor volumes (GTV). The clinical target volume (CTV) included the primary tumor with a 0.5-cm margin and metastatic lymph nodes. Regional lymph nodes were not treated electively. The definition of planning target volume (PTV) include the CTV with a 1-cm margin laterally and a 1- to 2-cm margin in the craniocaudal direction to take into account respiratory organ motion and daily set-up errors. The reference point for the radiation dose was set at the center of the PTV. The spinal cord dose was maintained at <45 Gy. The volume of liver to receive 30 Gy was required to be <40%, and the volume to receive 20 Gy was required to be <67%. At least 75% of both kidneys was required to receive less than 18 Gy.

S-1 was administered orally at a dose of 40 mg/m^2 twice daily after breakfast and dinner on the day of irradiation (Monday through Friday) during radiation therapy. The 3 initial doses were determined according to the body surface area (BSA) as follows: patients with a BSA of <1.25 m^2 received 40 mg/dose; those with BSA of 1.25 m^2-<1.5 m^2 received 50 mg/dose; and those with BSA of ≥1.5 m^2 received 60 mg/dose. The dose of S-1, which is the standard dose when S-1 is used as a single agent for systemic therapy (15, 16), had been previously determined in our phase I trial (19).

The occurrence of grade 4 hematological toxicity, grade 3 non hematological toxicity excluding nausea, anorexia, fatigue, constipation, and hyperglycemia, or a serum AST or ALT >200 IU/l resulted in the suspension of radiation therapy and S-1 administration. When the toxicities improved by at least 1 grade compared to the suspension criteria, treatment was resumed. When suspension criteria were met, dose modification was allowed as follows: patients with a BSA of <1.25 m^2 received 25 mg/dose; those with a BSA of 1.25 m^2-<1.5 m^2 received 40 mg/dose; and those with a BSA ≥1.5 m^2 received a 50 mg/dose. Chemoradiation therapy was discontinued when the patient developed grade 4 non-hematological toxicities or other unacceptable toxicities, including gastrointestinal ulcer or bleeding, interruptions in treatment of >15 days, or unequivocal tumor progression. After treatment discontinuation, patients could receive other anticancer treatments excluding S-1 with concurrent radiation therapy at their physician's discretion.

Maintenance S-1 chemotherapy

From 2-8 weeks after completion of S-1 with concurrent radiation therapy, maintenance S-1 chemotherapy was initiated at a dose of 40 mg/m^2 twice daily orally, after breakfast and dinner, for 28 consecutive days, followed by a 14-day rest period per course. Treatment cycles were repeated until the appearance of disease progression, unacceptable toxicities, or the patient's refusal to continue treatment. If a grade 3 or higher hematological toxicity or a grade 2 or higher non hematological toxicity was observed, temporary interruption or dose reduction of S-1 administration was allowed as follows: patients with a BSA of <1.25 m^2 received 25 mg/dose; those with a BSA of ≤1.25 m^2-<1.5 m^2 received a 40 mg/dose; and those with a BSA of ≥1.5 m^2 received a 50 mg/dose. When grade 4 non hematological toxicities, unacceptable toxicities, a rest period >28 days, or an unequivocal tumor progression was observed during maintenance S-1 chemotherapy, treatment was discontinued. After treatment discontinuation, patients could be given other anticancer treatment, excluding S-1 monotherapy, at their physician's discretion.

Response and toxicity assessment

Evaluations of tumor response during chemoradiation therapy and maintenance therapy were performed at the completion of chemoradiation therapy and every 6 weeks thereafter until tumor progression or 24 weeks from the start of S-1 and radiation therapy, using the Response Evaluation Criteria in Solid Tumors version 1.0 questionnaire. Responses were evaluated centrally by 3 independent reviewers. Serum carbohydrate antigen 19-9 (CA19-9) levels were measured at least every 6 weeks. In patients with a pretreatment CA19-9 level ≥100 U/ml, the CA19-9 response was assessed; a positive response was defined as a reduction of >50% from the pretreatment level (13). Overall survival was measured from the date of initial treatment to the date of death or the date of the last follow-up. Progression-free survival was defined as the time from the date of initial treatment to the first documentation of progression or death. Basic laboratory tests that included a complete blood count with differentials, serum chemistry, and urinalysis were administered at least weekly during S-1 therapy and radiation therapy and then at least once every 2 weeks during S-1 maintenance therapy. Common Terminology Criteria for Adverse Events, version 3.0, were used for the assessment of treatment-related toxicities.

Radiation therapy quality assurance

All radiation therapy treatment plans for the enrolled patients were reviewed centrally by an independent radiation committee consisting of 9 radiation oncologists. To assess radiation therapy protocol compliance, the following parameters were reviewed: fraction size, prescribed dose to the reference point, energy, relationships between GTV, CTV, PTV and radiation field, overall treatment time, isodose distributions at the transverse section of the reference points, and doses to organs at risk. The quality assurance assessment was given as per protocol (PP), deviation acceptable (DA), and violation unacceptable (VU). After parameter compliance was assessed, overall radiation therapy compliance was classified as: PPoverall, no DA or VU in any parameter; VUoverall, at least 1 VU in any parameter; or DAoverall, neither PP nor VU.

Statistical considerations

Primary endpoints of this trial were overall survival for the efficacy evaluation and frequency of adverse events for the safety evaluation; secondary endpoints were progression-free survival, response rate, and serum CA19-9 level response.

The enrollment goal was set at 60 eligible patients. The number of enrolled patients was determined using a statistical power analysis. Under the assumptions of a median survival time of 10 months for patients receiving conventional chemoradiation therapy (1-4), a 2-year registration period followed by a 2-year follow-up period and a one-sided alpha level of 5%, the statistical power of the hazard ratio test was over 70% or 90% with the expected median survival time of 14 or 16 months, respectively. Therefore, the number of planned enrolled patients, the registration period, the follow-up period, and the total research period were set at 60, 2 years, 2 years, and 4 years, respectively. The full analysis set (FAS) was defined as any patient who received at least 1 course of study medication. Overall and progression-free survival curves were calculated using the Kaplan-Meier method. This open-label, multi-institutional, single arm

phase II study was approved by the review board of each institution and was conducted in accordance with the Declaration of Helsinki and Ethical Guidelines for Clinical Research (Ministry of Health, Labour, and Welfare, Japan). The trial was registered at University Hospital Medical Information Network-Clinical Trial Registry (UMIN-CTR) (http://www.umin.ac.jp/ctr/index-j.htm), identification number (UMIN000000486).

Patient registration and data collection were managed by the Makimoto-han datacenter. The quality of the data was ensured by a careful review performed by the data center staff and the coordinating investigator of this study (MI). All data were fixed on November 13, 2009, and all analyses in this study were performed by statisticians (NY and TS).

Results

Patient characteristics

Sixty-one patients were enrolled in this trial between July 2006 and November 2007 at 20 institutions in Japan (see the Appendix in Supplementary Material). However, 1 patient was excluded before the start of protocol treatment because distant lymph node metastases were detected during a CT examination for radiation field planning; this patient received systemic chemotherapy with gemcitabine alone. Table 1 shows the characteristics of the 60 FAS patients.

Table 1 Patient characteristics (n = 60)

Characteristics	No. of patients	Value(s)	% of patients
Age (y)			
Median		64	
Range		31-80	
Sex			
Male	35		58
Female	25		42
Eastern Cooperative Oncology Group performance status			
0	34		57
1	26		43
Biliary drainage			
Present	16		27
Pathology			
Adenocarcinoma	59		98
Adenosquamous carcinoma	1		2
Tumor location			
Head	33		55
Body or tail	27		45
Maximum tumor size, cm			
Median		3.6	
Range		2.0-6.5	
Regional lymph node swelling			
N0	44		73
N1	16		27
CA19-9 (U/ml)			
Median		304	
Range		0-4400	
Planning target volume (cm^3)			
Median		240	
Range		102-442	

Abbreviation: CA19-9 = carbohydrate antigen 19-9.

Fifty-three patients (88%) completed S-1 therapy and radiation therapy but the remaining 7 patients (12%) discontinued S-1 and radiation therapy. Reasons for treatment discontinuation were disease progression (2 patients), duodenal and bile duct perforation (1 patient), acute myocardial infarction (1 patient), treatment interruption for >15 days because of cholangitis (1 patient), severe confusion (1 patient), and patient refusal to continue treatment because of grade 3 nausea and vomiting (1 patient). The treatment delay during chemoradiation therapy was observed in 20 patients (33%), and the median delay was 3 days (range, 1-17 days). Compliance with S-1 therapy was high, with a rate of 99% (1170 of 1176 doses). Of the 53 patients who completed chemoradiation therapy 47 (89%) patients received maintenance S-1 chemotherapy, but 6 patients did not for the following reasons: disease progression (3 patients); sudden death because of septic shock of unknown origin occurring 40 days after the completion of S-1 and radiation therapy (1 patient); and patient refusal to continue treatment because of grade 2 nausea and grade 2 diarrhea (1 patient) or grade 3 appetite loss and grade 2 fatigue (1 patient). The median number of S-1 maintenance chemotherapy courses was 4 (range, 1 to ≥19). At the time of the final analysis, S-1 maintenance chemotherapy had been terminated in 46 (98%) of 47 patients because of disease progression (29 patients, 63%), adverse events (12 patients, 26%), patient refusal (2 patients, 4%), or other reasons (3 patients, 7%). Treatment delay during the first and second courses of maintenance S-1 therapy was observed in 9 patients (19%) and 7 patients (18%), respectively. The rate of compliance with S-1 chemotherapy was 91% (2503 of 2744 doses) in the first course and 98% (2149 of 2184 doses) in the second course. After the completion of protocol treatment, 53 patients (88%) received subsequent therapy including gemcitabine (47 patients), S-1 (11 patients), radiation therapy for bone metastases (2 patients), and other treatments (4 patients).

Toxicity

The toxicities of S-1 and radiation therapy observed in the 60 FAS patients are listed in Table 2. Grade 3 leukocytopenia, neutropenia, and anemia occurred in 6 (10%), 3 (5%), and 2 (3%) patients, respectively; no grade 4 hematological toxicity was seen. The most common and troublesome non-hematological toxicities for patients undergoing chemoradiation therapy were usually gastrointestinal toxicities, including anorexia, nausea, and vomiting. However, grade 3 or higher cases of these toxicities were observed only in 4 (7%), 3 (5%), and 2 (3%) patients, respectively, and the toxicities were generally mild and manageable. One treatment-related death arising from perforation of the duodenum and biliary tract occurred during chemoradiation therapy.

Toxicities occurring during S-1 maintenance chemotherapy were also mild and transient (Table 3). Grade 4 leukocytopenia was the only hematological toxicity, and it was observed in only 1 patient (2%); the incidence of grade 3 or higher gastrointestinal toxicities was <6%. In addition, no serious adverse events occurred during S-1 maintenance chemotherapy. No late toxicities that could be associated with S-1 and radiation therapy were reported.

Efficacy

The response evaluation included all 60 FAS patients, but tumor response was not evaluable in 1 patient in whom contrast-enhanced CT examination could not be performed due to deterioration of her general condition following duodenal perforation.

Table 2 Toxicity during S-1 and concurrent radiation therapy (n=60)

Toxicity	No. of patients (%)*			
	Grade 1	Grade 2	Grade 3	Grade 4
Hematological				
Leukocytes	15 (25)	28 (47)	6 (10)	0 (0)
Neutrophils	9 (15)	15 (25)	3 (5)	0 (0)
Hemoglobin	16 (27)	13 (22)	2 (3)	0 (0)
Platelets	24 (40)	3 (5)	0 (0)	0 (0)
Non hematological				
Rash	2 (3)	0 (0)	0 (0)	0 (0)
Pigmentation	6 (10)	0 (0)	0 (0)	0 (0)
Hand-foot syndrome	1 (2)	0 (0)	0 (0)	0 (0)
Gastric ulcer/gastritis	0 (0)	1 (2)	1 (2)	0 (0)
Abdominal pain	0 (0)	0 (0)	1 (2)	0 (0)
Bilirubin	4 (7)	1 (2)	1 (2)	0 (0)
Aspartate aminotransferase	11 (18)	3 (5)	0 (0)	0 (0)
Alanine aminotransferase	10 (17)	5 (8)	0 (0)	0 (0)
Alkaline phosphatase	4 (7)	0 (0)	0 (0)	0 (0)
Hypoalbuminemia	15 (25)	7 (12)	0 (0)	-
Amylase	0 (0)	1 (2)	0 (0)	-
Creatinine	0 (0)	0 (0)	0 (0)	0 (0)
Hyperglycemia	2 (3)	4 (7)	0 (0)	0 (0)
Cholangitis	0 (0)	1 (2)	0 (0)	0 (0)

* Grading followed Common Terminology Criteria for Adverse Events version 3.0.

Tumor response was evaluated based on the best response as of 24 weeks after S-1 and radiation therapy were started. Overall, a partial response was seen in 16 patients for an overall response rate of 27% (95% confidence interval [CI], 16%-40%). The median survival in patients with partial response was 19.4 months (range, 9.8-32.6 months; 95% CI, 13.9-25.1 months), with a median duration of response of 7.3 months (range, 5.5-10.1 months). Forty patients (67%) showed stable disease, and 3 patients (5%) had progressive disease. Additionally, tumor response was evaluated for all periods because tumor shrinkage was obtained in some patients after 24 weeks. Of the 40 patients who were judged to have stable disease on the response evaluation at 24 weeks, an additional 6 patients were judged to have a partial response by the central independent reviewers. The median time to partial response was 4.7 months (range, 1.4-16.8 months) after chemoradiation therapy commenced. Therefore, the response rate for all periods was 37% (95% CI, 25%-50%). Of the 42 patients with a pretreatment serum CA19-9 level ≥100 U/ml, 34 (81%) patients had a >50% decrease compared to the pretreatment level. During this protocol treatment, 2 patients underwent surgical resection because tumor shrinkage occurred and their tumors became resectable.

Fifty-four of the 60 patients had disease progression at the time of the analysis. The median progression-free survival time and the 6-month and 1-year progression-free survival proportions for all patients were 9.7 months (95% CI, 6.9-11.6 months), 68%, and 32%, respectively (Fig.). The pattern of disease progression was distant metastases in 26 patients (46%), locoregional recurrence in 16 patients (27%), distant metastases and locoregional recurrence in 3 patients (5%), and deterioration of general condition in 9 patients (15%). At the time of analysis, 49 patients had died, and the median follow-up period was 16.3 months (range, 3.0-34.0 months). The median survival time and the 1-year and 2-year survival proportions for the 60 patients were 16.2 months (95% CI, 13.5-21.3 months), 72% (95% CI, 59%-82%), and 26%, respectively (Fig.).

Table 3 Toxicity during S-1 maintenance therapy (n=47)

Toxicity	No. of patients (%)*			
	Grade 1	Grade 2	Grade 3	Grade 4
Hematological				
Leukocytes	4 (9)	27 (57)	4 (9)	1 (2)
Neutrophils	5 (11)	19 (40)	6 (13)	0 (0)
Hemoglobin	8 (17)	18 (38)	3 (6)	0 (0)
Platelets	8 (17)	2 (4)	1 (2)	0 (0)
Non hematological				
Malaise	13 (27)	8 (17)	2 (4)	0 (0)
Anorexia	15 (32)	11 (23)	3 (6)	0 (0)
Nausea	7 (15)	4 (9)	1 (2)	0 (0)
Vomiting	4 (9)	1 (2)	0 (0)	0 (0)
Diarrhea	3 (6)	3 (6)	0 (0)	0 (0)
Stomatitis	4 (9)	0 (0)	0 (0)	0 (0)
Alopecia	1 (2)	0 (0)	-	-
Rash	2 (4)	1 (2)	0 (0)	0 (0)
Pigmentation	11 (23)	1 (2)	0 (0)	0 (0)
Hand-foot syndrome	1 (2)	0 (0)	0 (0)	0 (0)
Duodenal ulcer	0 (0)	1 (2)	0 (0)	0 (0)
Taste alteration	1 (2)	2 (4)	-	-
Bilirubin	7 (15)	5 (11)	0 (0)	0 (0)
Aspartate aminotransferase	8 (17)	3 (6)	1 (2)	0 (0)
Alanine aminotransferase	5 (11)	2 (4)	0 (0)	0 (0)
Alkaline phosphatase	1 (2)	0 (0)	0 (0)	0 (0)
Hypoalbuminemia	10 (21)	5 (11)	0 (0)	-
Amylase	0 (0)	1 (2)	0 (0)	-
Creatinine	3 (6)	0 (0)	0 (0)	0 (0)
Hyperglycemia	2 (4)	4 (9)	0 (0)	0 (0)

* Grading followed Common Terminology Criteria for Adverse Events version 3.0.

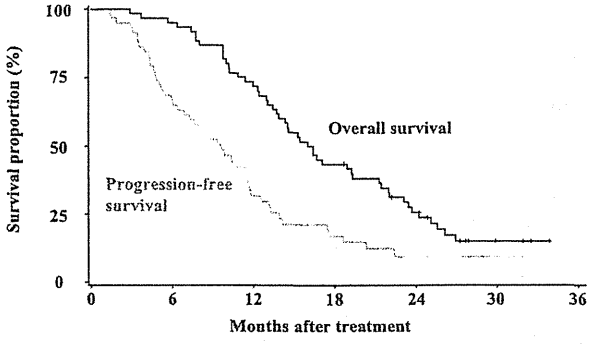

Fig. Overall survival and progression-free survival curves of the 60 locally advanced PC patients treated with S-1 with concurrent radiation therapy. Censored cases are shown by tick marks.

Radiation therapy quality assurance

Radiation therapy quality assurance was reviewed centrally by an independent radiation committee for all 60 FAS patients. DA was observed for 2 parameters in 4 patients (relationship between GTV and radiation field, 2 patients; isodose distribution, 2 patients), but no instances of VU were seen in this study. Therefore, PPoverall, DAoverall, and VUoverall were assessed in 56 (93%) patients, 4 (7%) patients, and 0 (0%) patients, respectively.

Discussion

The combination of radiation therapy and 5-FU chemotherapy has been acknowledged as a standard therapy for locally advanced PC (1-4). However, optimal chemotherapeutic regimens continue to be pursued, as the survival benefit remains modest. S-1 is the first single anticancer agent to be judged non-inferior to gemcitabine in a large-scale randomized phase III trial for advanced PC (10), and it is expected to become a first-line treatment for patients with advanced PC, at least in Asian countries. In addition, it has been shown that combined S-1 and radiation therapy has a synergistic effect against 5-FU-resistant cancer xenografts; thus, S-1 may also have a radiosensitizing effect (11). With S-1 and standard-dose radiation therapy (50.4 Gy/28 fractions), the full dose (80 mg/m^2) of S-1 can be given on the day of irradiation (12) with a reduced risk of distant metastases. Therefore, S-1 may act not only against systemic tumor spread but also a as a potent radiosensitizer to enhance local control. Furthermore, the fact that S-1 can be given orally is an additional benefit over 5-FU infusion.

In the present multicenter trial, the 24-week tumor response rate was 27%, although the overall tumor response rate for the complete period was 37%; in fact, tumor resection was possible in 2 patients after treatment. Thus, excellent tumor shrinkage appears to be an additional benefit of this treatment. Furthermore, other outcomes, including the serum CA19-9 level response (81%), progression-free survival (median, 9.7 months), and overall survival (median, 16.2 months), showed excellent results. As the subsequent therapy, most patients (78%) received gemcitabine, as it might lead to favorable overall survival. However, the outcome of S-1 and concurrent radiation therapy has been reported by other groups (14-16), which were single institutional studies with small numbers of enrolled patients and had slight differences in S-1 administration (Table 4). Similar results were obtained, although such nonrandomized data must be interpreted with caution. Given the recent reports of chemoradiation therapy (4-8, 17, 18), S-1 with concurrent radiation therapy appears to have a favorable treatment efficacy for locally advanced PC, and its survival time will approach that of resected PC patients.

During chemoradiation therapy the major troublesome adverse events were gastrointestinal toxicities (anorexia, nausea, and vomiting), which required intravenous fluid infusion and, sometimes, the termination of chemoradiation therapy (4). One approach to reducing these toxicities that has recently come to be used in chemoradiation therapy using conventional photons for the treatment of PC (4, 6), is a limited radiation field, with a PTV including gross tumor volume alone, without prophylactic nodal irradiation; this minimizes the irradiation of normal tissue and was adopted in the present study. Grade 3 or higher of the above-mentioned toxicities were observed in less than 7% of the patients, and the gastrointestinal toxicities were very mild and easily managed. Other grade 3 or higher non hematological and hematological toxicities of S-1 and concurrent radiation therapy were observed in only 10% or less of the patients and were mild, although there was one treatment-related death due to a perforated duodenum. The toxicities associated with maintenance S-1 therapy were also mild, and this regimen was considered to be well tolerated.

Regarding the results of the radiation therapy quality assurance evaluations performed in this study, 93% of the treatments were assessed as PPoverall; this result is excellent compared with that of a previous trial (5). This result was achieved thanks to the efforts made by the radiation oncologists. The radiation technique that was used in this study was thoroughly explained to all of the radiation oncologists at each institution before patient registration, and the radiation therapy records of the enrolled patients were reviewed by the radiation committee. Results of the review were returned to the radiation oncologists at each institution if any problem with the radiation technique was noted. Therefore, a high quality of radiation therapy was maintained in this study.

There continues to be debate about the role of chemoradiation therapy for patients with locally advanced PC. Prior to the 1990s, it was shown that concurrent external-beam radiation therapy and 5-FU chemotherapy offers a survival benefit over radiation therapy (1, 2) or chemotherapy alone (3). Since the introduction of gemcitabine, which is acknowledged as the first-line therapy for advanced PC, 2 randomized controlled trials comparing chemoradiation therapy with gemcitabine alone have been reported:

Table 4 Results of phase II trials of S-1 and radiation therapy for locally advanced pancreatic cancer

Study (ref.)	Y	Chemotherapy	Radiation therapy	No. of patients	Response rate	Median survival time (mo)	1-y survival rate (%)	Median progression-free survival time (mo)	Maintenance chemotherapy
Kim et al (20)	2008	S-1, 80 mg/m^2, days 1-14 and 22-35	50.4 Gy/28 fractions	25	24%	12.9	43%	6.5	Gemcitabine-based regimen
Sudo et al (15)	2011	S-1, 80 mg/m^2, days 1-14 and 22-35	50.4 Gy/28 fractions	34	41%	16.8	70.6%	8.7	S-1
Shinchi et al (16)	2011	S-1, 80 mg/m^2, days 1-21	50 Gy/40 fractions	50	30%	14.3	62%	6.7	S-1
Current study		S-1, 80 mg/m^2, on the day of irradiation	50.4 Gy/28 fractions	60	27%	16.2	72%	9.7	S-1

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The Hepatobiliary and Pancreatic Oncology (HBPO) Group of the Japan Clinical Oncology Group (JCOG): History and Future Direction

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The Hepatobiliary and Pancreatic Oncology Group of the Japan Clinical Oncology Group (JCOG) was constituted in April 2008 to develop new standard treatments for hepatobiliary and pancreatic cancer. In pancreatic cancer, the Hepatobiliary and Pancreatic Oncology Group focuses on establishing standard chemotherapy or chemoradiotherapy for unresectable locally advanced disease. The JCOG 0506 study was a Phase II study of gemcitabine alone to examine its efficacy and safety in patients with locally advanced disease. The results in survival significantly exceeded expectations, and gemcitabine monotherapy has come to be regarded as the provisional standard therapy by our group. Following JCOG 0506, the JCOG 1106 study, which is currently under investigation, is a randomized Phase II study to evaluate the efficacy of induction chemotherapy with gemcitabine in combination with S-1 chemoradiotherapy and select a candidate therapeutic agent in a Phase III study comparing with gemcitabine alone. The JCOG 0805 study was a randomized Phase II study comparing S-1 monotherapy with gemcitabine plus S-1 combination therapy for unresectable biliary tract cancer. As a result, gemcitabine plus S-1 combination therapy was considered the more promising candidate in comparison with the gemcitabine plus cisplatin combination therapy in a subsequent Phase III trial. The Hepatobiliary and Pancreatic Oncology Group is planning a Phase III study to compare gemcitabine plus S-1 combination therapy with gemcitabine plus cisplatin combination therapy (JCOG PC1113 study). No standard postoperative adjuvant treatment has been established. We plan to conduct a Phase III study to compare S-1 as adjuvant therapy after surgery with surgery alone in patients with biliary tract cancer (JCOG PC1202).

Key words: GI-Hepatobiliary-Med – GI-Pancreas-Med – clinical trials

INTRODUCTION

Hepatobiliary and pancreatic cancers have a high incidence and are associated with high mortality rates, not only in Japan, but also around the world. Despite the poor prognosis, no standard chemotherapeutic regimens were established for these cancers for a long time.

In Japan, based on the results of single-arm Phase I and II studies (1–4), gemcitabine was approved for the treatment of pancreatic cancer in 2001, and for the treatment of biliary tract cancer in 2006. S-1, a mixture of tegafur, gimeracil and oteracil potassium, was also approved for the treatment of pancreatic cancer in 2006, and for the treatment of biliary

tract cancer in 2007. Furthermore, in a Phase III study conducted mainly in Europe, sorafenib showed survival benefit in patients with unresectable hepatocellular carcinoma (HCC) (5), and in 2009, this drug was approved for the treatment of HCC in Japan. Thus, some agents have shown beneficial effects and have come to be established as standard or available treatments for these cancers. Nonetheless, treatment remains unsatisfactory, and in order to improve the survival in patients with these cancers, not only more effective treatments for unresectable disease, but also more effective postoperative adjuvant therapy regimens for patients who undergo surgical resection need to be developed.

The Hepatobiliary and Pancreatic Oncology Group (HBPO group) of the Japan Clinical Oncology Group (JCOG) was constituted in April 2008 to develop new standard treatments for hepatobiliary and pancreatic cancer. The HBPO group started with a membership of 16 institutes initially, and at present, 26 institutes are registered as active members. Furthermore, >30 institutes participate in regular meetings of the JCOG.

HEPATOCELLULAR CARCINOMA

Various treatment modalities, including resection, local ablation, transcatheter arterial chemoembolization and liver transplantation have been employed as local therapeutic strategies for the treatment of HCC. Intra-arterial infusion chemotherapy and systemic chemotherapy have also been used for the treatment of advanced HCC. Thus, the treatments for HCC are diverse, and appropriate strategies are selected for each patient according to the tumor stage and the grade of liver dysfunction. Although hepatic arterial infusion chemotherapy, which is applied for patients with advanced-stage HCC such as those with portal vein tumor thrombosis and/or huge tumors, has provided high response rates, the survival benefit of this treatment modality in HCC patients has never been confirmed. No standard systemic chemotherapy had ever been established until sorafenib was approved.

Sorafenib is a small-molecule multi-kinase inhibitor that inhibits several kinases such as Raf kinase, vascular endothelial growth factor receptor and platelet-derived growth factor receptor-β tyrosine kinases. A large randomized controlled trial of sorafenib versus placebo (the SHARP trial) in patients with advanced HCC and good liver function (Child-Pugh class A) demonstrated that sorafenib prolonged the survival in patients with advanced HCC (5). As a result, sorafenib has been applied as standard chemotherapy for the treatment of advanced HCC in many countries, including Japan.

New compounds have been investigated for HCC in clinical trials, including Phase III trials, conducted by pharmaceutical companies in various study settings, such as first-line therapy in comparison with sorafenib, second-line therapy (placebo-controlled trial) and in combination with local treatments. To date, however, no compound has yielded satisfactory results. Although sorafenib is the only antitumor drug that has shown survival benefit, the direct antitumor effect of the drug is not remarkable; the response rate has been reported to be only around 2–4%. Thus, there remains much room for improvement of the treatment efficacy and we think it is necessary to develop more effective treatment regimens containing sorafenib. The HBPO group is considering clinical trials using sorafenib to develop more effective treatments, e.g. combination of hepatic arterial infusion chemotherapy with sorafenib.

BILIARY TRACT CANCER

Bile duct cancer is subdivided according to the anatomic location of origin into intrahepatic and extrahepatic cholangiocarcinoma, gallbladder cancer or ampulla of Vater cancer. Each of these types of cancer has characteristic features and the treatment strategies and prognoses differ. Furthermore, most patients present with obstructive jaundice at diagnosis, and biliary drainage is generally needed before any of the aforementioned treatments. These characteristics of biliary tract cancer have made it difficult to evaluate the efficacy of chemotherapy for biliary tract cancer, resulting in a paucity of high-quality clinical trials.

In Japan, gemcitabine and S-1 were approved for the treatment of biliary tract cancer in 2006 and 2007, respectively, based on the results of single-arm Phase II studies of the two drugs. Recently, a randomized Phase III study (ABC-02) comparing gemcitabine alone with gemcitabine plus cisplatin (GC) was conducted in the UK (6), which demonstrated a statistically significant improvement in the overall survival in the GC group when compared with that in the gemcitabine-alone group. The BT22 study was conducted to confirm the efficacy and safety of GC therapy as a company-initiated trial in Japan, and similar results to those of the ABC-02 study were demonstrated in Japanese patients with biliary tract cancer (7). Thus, GC therapy has come to be recognized as the standard chemotherapy for unresectable biliary tract cancer. Based on these results, treatment with cisplatin in combination with gemcitabine was approved for the treatment of biliary tract cancer in Japan in February 2012.

On the other hand, S-1 or gemcitabine plus S-1 (GS therapy) was demonstrated to provide high response rates and good survival rates in Phase II studies (4,8), and S-1 or GS therapy was expected to yield a superior benefit to GC therapy. Therefore, the HBPO group conducted a randomized Phase II study comparing S-1 monotherapy with GS therapy (JCOG 0805 study) to examine the efficacy and safety of the two regimens and to select the more promising one for a subsequent Phase III trial of treatment for unresectable biliary tract cancer in (Fig. 1) (9,10). The main eligibility criteria of the JCOG 0805 study were the following: (i) clinically diagnosed with biliary tract cancer, which includes intrahepatic cholangiocarcinoma, extrahepatic

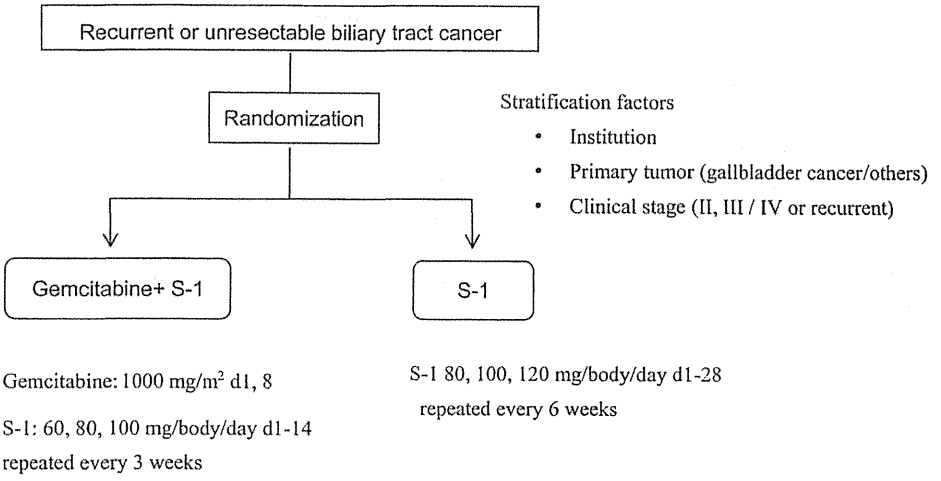


Figure 1. Study design of the JCOG 0805 study.

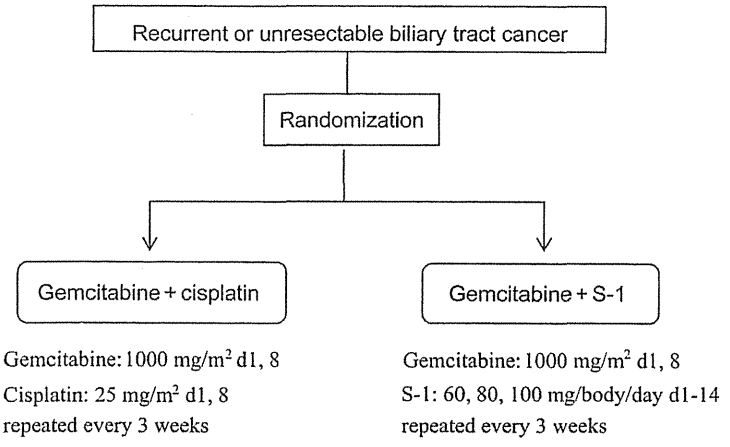


Figure 2. Study design of the JCOG PC1113 study.

cholangiocarcinoma, gallbladder cancer and ampulla of Vater cancer and histologically proven adenocarcinoma or adenosquamous carcinoma; (ii) recurrent or unresectable biliary tract cancer; (iii) no previous therapy against biliary tract cancer except surgery; (iv) no previous chemotherapy or radiotherapy for any other malignancies; (v) ECOG performance status of 0 or 1 and (vi) adequate organ function. The regimen that shows the higher point estimate in terms of the proportion of 1-year survival will be considered to be more promising. We assumed that the 1-year survival rate of one regimen is 30% and that of the other regimen is >40%. In this situation, the sample size ensuring at least 85% probability of correct selection of the more effective regimen is 98 patients, with 49 patients in each of the two arms. Considering the likelihood of some ineligible patients being enrolled, the total number of patients was set at 100 (9).

In the JCOG 0805 study, 101 patients were enrolled between February 2009 and April 2010, with 51 allocated to the GS arm and 50 to the S-1 arm. The 1-year survival rates were 52.9 and 40.0%, the median overall survival rates were 12.5 and 9.0 months [hazard ratio 0.86; 95% confidence interval (CI): 0.54–1.36; $P = 0.52$)] and the median progression-free survival rates were 7.1 and 4.2 months (hazard ratio 0.44; 95% CI: 0.29–0.67; $P < 0.0001$), respectively, in the GS arm and S-1 arm. The most common toxicities were hematological toxicities, fatigue and rash. Grade 3 or 4 toxicities were generally more frequent in the GS arm than that in the S-1 arm, although both treatments were quite well tolerated. As a result, GS therapy was considered as the more promising candidate in comparison with the GC regimen in a subsequent Phase III trial (10). The HBOP is planning a Phase III study to compare GS therapy with GC therapy (JCOG PC1113 study), with the aim of determining whether GS therapy could be established as a new standard therapy for unresectable biliary tract cancer (Fig. 2).

Although surgery currently remains the only potentially curative treatment, most patients develop recurrence. An effective adjuvant therapy is required after surgery to increase the curability of the surgery and to prolong the survival in patients with biliary tract cancer who undergo surgery. To

date, since no large randomized controlled trials of adjuvant therapy have been conducted, no standard postoperative adjuvant treatment has been established. We consider S-1 as a potential candidate for adjuvant therapy, because a high response rate of 35% was demonstrated to S-1 in a Phase II study for unresectable biliary tract cancer (4). S-1 has also been already established as a standard adjuvant therapeutic agent for the treatment of gastric cancer. Surgical methods for the treatment of biliary tract cancer are highly diverse, including pancreaticoduodenectomy, hepatectomy, etc., when compared with those for gastric cancer. Therefore, a feasibility study of S-1 chemotherapy after surgery was conducted by a study group comprising some member institutes of the HBPO group. A treatment completion rate of 82% was achieved. The most common grade-3 toxicity was neutropenia (18%), and the rates of other grade 3 adverse events were under 5% (11). Therefore, S-1 is considered to be suitable as a postoperative adjuvant therapeutic agent for the treatment of patients with resected biliary tract cancer. Based on these results, we plan to conduct a Phase III study to compare S-1 as adjuvant therapy after surgery with surgery alone in patients with biliary tract cancer (JCOG PC1202).

PANCREATIC CANCER

Pancreatic carcinoma is a disease with one of the worst prognoses; the 5-year survival rate of patients diagnosed as having pancreatic cancer remains at 5–10%. Since it is difficult to diagnose pancreatic cancer at an early stage, 70–80% patients with pancreatic cancer have unresectable disease, including locally advanced or distant metastatic disease, at diagnosis. Since gemcitabine demonstrated a better survival benefit when compared with 5-fluorouracil (5-FU) in a Phase III study (12), it has been widely used as the standard chemotherapy for unresectable pancreatic cancer for >10 years. Despite a number of new compounds, including molecular-targeted agents, having been examined in combination with gemcitabine, no regimen, except for gemcitabine plus erlotinib, has been demonstrated to provide statistically significant improvement in the overall survival over gemcitabine alone (13,14). Thus, the prognosis of these patients with this cancer remains poor, and the development of more effective treatments for pancreatic cancer is urgently needed.

Under these situations, it is important to continue the development of new compounds in industry-initiated clinical trials and also participate in global registration trials. On the other hand, the HBPO group also considers itself as having the important role of establishing standard chemotherapy or chemoradiotherapy for unresectable locally advanced disease or postoperative adjuvant therapy.

With regard to treatments for unresectable locally advanced disease, we first conducted a Phase II study of gemcitabine alone to examine its efficacy and safety in patients with locally advanced disease of the JCOG 0506 study (15). This study was conducted to be foreseeing a Phase III trial comparing gemcitabine monotherapy with conventional chemoradiotherapy using 5-FU, which, at that time, was used as a standard therapy for locally advanced disease. The main eligibility criteria of the JCOG 0506 study were the following: (i) patients with histologically or cytologically proven pancreatic adenocarcinoma or adenosquamous carcinoma; (ii) International Union Against Cancer clinical stage III (T4N0-1 and M0); (iii) no previous chemotherapy or radiotherapy for any other malignancies; (iv) ECOG performance status of 0, 1 or 2 and (v) adequate organ function. The primary endpoint of this study was the 1-year survival rate. A sample size of 50 was required for a one-sided α of 0.20 and β of 0.10, with an expected 1-year survival rate of 40% and a threshold 1-year survival rate of 25%. Fifty patients were enrolled from January 2006 to February 2007 in this study. The results revealed a median overall survival of 15.0 months with a 1-year survival rate of 64.0% (Table 1), which significantly exceeded expectations. The toxicities were generally mild and the drug was well tolerated. Furthermore, a randomized controlled trial of gemcitabine vs. conventional chemoradiotherapy using 5-FU and cisplatin failed to show any survival benefit of chemoradiotherapy (16). Based on these results, gemcitabine monotherapy has come to be regarded as the provisional standard therapy by our group (Table 2).

Table 1. Recent randomized controlled trials using gemcitabine, cisplatin and/or S-1 for unresectable biliary tract cancer

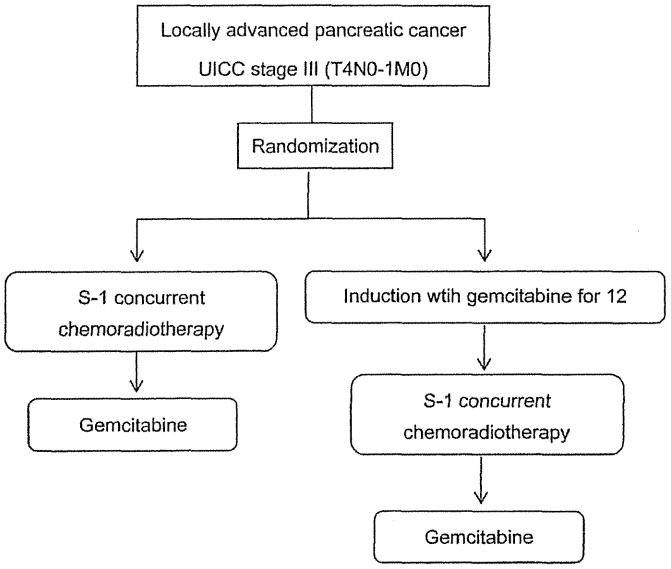
Study	Chemotherapy	*n*	Response rate (%)	Median PFS (months)	Median OS (months)	Study
ABC-02 study	Gemcitabine	206	15.5	5.0	8.1	Valle *et al.* (6)
	Gemcitabine + cisplatin	204	26.1	8.0	11.7	
BT-22 study	Gemcitabine	42	11.9	3.7	7.7	Okusaka *et al.* (7)
	Gemcitabine + cisplatin	41	19.5	5.8	11.2	
JCOG 0805 study	S-1	50	17.4	4.2	9.0	Ueno *et al.* (8)
	Gemcitabine + S-1	51	36.4	7.1	12.5	

PFS, progression-free survival; OS, overall survival.

Table 2. Recent clinical trials of chemotherapy or chemoradiotherapy for locally advanced pancreatic cancer

Study	Radiotherapy (Gy)	Chemotherapy	*n*	Median OS (month)	%1-year survival	Study
JCOG 0506 study	–	Gemcitabine	50	15.0	64	Ishii *et al.* (15)
S-1 radiation Phase II study	50.4	S-1	61	16.2	72	Ikeda *et al.* (18)
2000-01 FFCD/SFRO study	60	5-fluorouracil + cisplatin	59	8.6	32	Chauffert *et al.* (16)
	–	Gemcitabine	60	13.0	53	
ECOG 4201 study	50.4	Gemcitabine	34	11.1	50	Loehrer *et al.* (17)
	–	Gemcitabine	37	9.2	32	

%1-year survival, one-year survival rate.



Gemcitabine: 1000 mg/m^2 d1, 8, 15, repeated every 4 weeks
S-1: 80 mg/m^2/day on the day of irradiation

Figure 3. Study design of the JCOG 1106 study.

A clinical trial conducted in the USA comparing gemcitabine plus radiotherapy vs. gemcitabine alone reported that the overall survival rate was superior in the combined treatment group when compared with that in the gemcitabine-alone group in patients with locally advanced pancreatic cancer (17). Furthermore, chemoradiotherapy using S-1 demonstrated promising efficacy in a Phase II study, which was conducted as an in-house trial of some member institutes of the HBPO group; the median overall survival was 16.2 months (18). There is a possibility that new methods of chemoradiotherapy might improve the survival, especially prolonged survival of >2 years. Thus, in order to develop more promising new chemoradiotherapies, we conducted a randomized Phase II study of two chemoradiotherapeutic methods, one consisting of S-1 chemoradiotherapy and maintenance therapy with gemcitabine, and the other consisting of induction gemcitabine chemotherapy for 3 months followed by S-1 chemoradiotherapy and maintenance therapy with gemcitabine (JCOG 1106 study).

The JCOG 1106 study is a multi-institutional open-label randomized Phase II study to evaluate the efficacy of induction chemotherapy of gemcitabine in combination with S-1 chemoradiotherapy and select a candidate in a Phase III study comparing with gemcitabine alone (Fig. 3). The main eligibility criteria of the JCOG 1106 study were as follows: (i) clinically diagnosed with pancreatic cancer without distant metastasis, and histologically proven adenocarcinoma; (ii) no previous chemotherapy or radiotherapy for any other malignancies; (iii) ECOG performance status of 0 or 1 and (iv) adequate organ function. The primary endpoint is the overall survival, and we shall select the treatment method providing the better survival benefit between the two for use in a subsequent Phase III study. The 1-year survival rate of the two treatments would be expected to be >60% at least, because that of patients administered gemcitabine monotherapy was 64% in the JCOG 0506 study. The sample size is 100 patients and this study is under investigation in September 2012.

FUTURE DIRECTION

In hepatobiliary tract and pancreatic cancers, major advances have been made in relation to the establishment of standard treatments in recent years. However, the survival of patients with these cancers still remains dismal. The HBPO group considers it essential to actively conduct clinical trials to establish more effective standard treatments, including a combination of chemotherapy with local treatments including surgery or radiotherapy.

In HCC, many clinical trials using new agents are conducted as an Asian study including Japan or a global study. However, it is difficult to conduct investigator-initiated trials in HCC, because there are various differences in the etiology and treatment strategy among Asian countries, Japan and Western countries. However, it is also important for the HBPO group to discuss Asian studies on HCC and biliary tract cancer in the future, because these diseases are very common in Asia, compared with Western countries.

Establishment of standard therapies for relatively rare tumors is urgently needed. We are planning to conduct a

phase III study for the treatment of gastrointestinal neuroendocrine tumors in cooperation with other groups of the JCOG.

Although our HBPO group is growing in size, only 26 institutes are active members of the group. On the other hand, >30 institutes participate in our regular meetings as observers. It is therefore also important to increase the number of institutes as active members so as to make it possible to conduct larger clinical trials of higher quality in the future.

Funding

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Conflict of interest statement

None declared.

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ORIGINAL ARTICLE

A multicenter phase II study of S-1 for gemcitabine-refractory biliary tract cancer

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Abstract
Purpose Gemcitabine (GEM)-based chemotherapy has been used worldwide as the first-line treatment for advanced biliary tract cancer (BTC). However, no standard regimens have been established yet for patients with GEM-refractory BTC. A previous phase II trial of S-1 as a first-line treatment in patients with advanced BTC revealed promising activity of this drug. The present study was conducted to evaluate the efficacy and safety of S-1 in patients with GEM-refractory BTC.
Methods The subjects were patients with pathologically proven BTC who had shown disease progression while receiving GEM-based chemotherapy. Each treatment cycle consisted of administration of S-1 orally at the dose of 40 mg/m^2 twice daily for 28 days, followed by a rest period of 14 days. The primary endpoint of this study was objective response, and the secondary endpoints were the toxicity, progression-free survival (PFS), and overall survival (OS).
Results Forty patients were assessed for efficacy and safety from 8 hospitals in Japan between June 2007 and September 2008. There were 3 cases of confirmed partial response (7.5 %) and 22 patients (55 %) of stable disease. The median PFS and OS were 2.5 and 6.8 months, respectively. Toxicity was generally mild, and the most common grade 3 or 4 toxicities were anorexia (10.0 %), anemia (7.5 %), mucositis (7.5 %), hypoalbuminemia (5.0 %), and pneumonia (5.0 %). There were no treatment-related deaths.

This study was presented in part at the 46th Annual Meeting of the American Society of Clinical Oncology, Chicago, IL, USA, 2010.

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Conclusions Monotherapy with S-1 was well tolerated, but showed modest efficacy in patients with GEM-refractory BTC.

Keywords Biliary tract cancer · S-1 · Gemcitabine · Refractory

Introduction

Biliary tract cancer (BTC), while being relatively uncommon in Western countries, is a common cause of death in Japan, Korea, and Chile [1, 2]. Resection offers the only chance for cure of the disease. However, the resectability rate is generally low because the disease is generally diagnosed at advanced stage. Moreover, the majority of patients with resected BTC eventually develop recurrence(s) [3]. Therefore, systemic chemotherapy has been the mainstay of the treatment for most patients with BTC.

To date, various drugs have been investigated for the treatment of BTC. Among them, gemcitabine (GEM)-based regimens have exhibited moderate activity against BTC [4]. Recently, in a randomized phase III study comparing combination chemotherapy of GEM and cisplatin with GEM monotherapy (UK ABC-02 study), combination chemotherapy yielded survival benefit over GEM monotherapy, with median survival times of 11.7 months in the former arm versus 8.3 months in the latter arm ($P = 0.002$) [5]. This study was the first large-scale randomized trial conducted in patients with BTC, and the combination chemotherapy of GEM and cisplatin has been established as standard chemotherapy for patients with advanced BTC. A randomized phase II study conducted in Japan also showed similar results [6]. Despite these progresses in chemotherapy, however, the survival is still not satisfactory. In many other cancers, the second-line chemotherapy contributes to prolongation of survival. Thus, there is an urgent need to develop effective second-line chemotherapies for patients with BTC. To date, however, second-line chemotherapy for patients with BTC refractory to treatment with GEM-based regimens has not been fully examined.

S-1 (Taiho Pharmaceutical Co., Ltd., Tokyo, Japan) is a novel orally administered anticancer drug consisting of a combination of tegafur (FT), 5-chloro-2,4-dihydroxypyridine (CDHP), and oteracil potassium (Oxo) in a molar concentration ratio of 1:0.4:1 [7]. CDHP is a competitive inhibitor of dihydropyrimidine dehydrogenase, which is involved in the degradation of 5-FU, and acts to maintain efficacious concentrations of 5-FU in the plasma and tumor tissues [8]. Oxo, a competitive inhibitor of orotate phosphoribosyl transferase, inhibits the phosphorylation of 5-FU in the gastrointestinal tract, thereby serving to reduce the serious gastrointestinal toxicity associated with 5-FU treatment [9]. The antitumor effect of S-1 has already been demonstrated in a variety of solid tumors [10]. A recent late phase II study conducted to evaluate the efficacy of S-1 in chemo-naive advanced BTC patients demonstrated promising results, with a response rate of 35.0 % and a favorable toxicity profile [11]. Therefore, we conducted a phase II study to investigate the efficacy and safety of S-1 in patients with GEM-refractory BTC.

Patients and methods

Patients

The inclusion criteria for this study were as follows: (1) histologically proven BTC, (2) progressive disease (PD) during the GEM-based first-line chemotherapy, (3) 20–79 years of age, (4) Eastern Cooperative Oncology Group performance status (PS) 0–2, 5) more than 3 weeks from the last administration of the previous chemotherapy, 6) adequate bone marrow functions (white blood cell count $\geq$3,000/mm^3, neutrophil count $\geq$1,500/mm^3, platelet count $\geq$100,000/mm^3, and hemoglobin $\geq$9.0 g/dL), (7) adequate renal function (serum creatinine $\leq$1.5 mg/dL), and (8) adequate liver function (serum total bilirubin $\leq$2.0 mg/dL, serum transaminases $\leq$2.5 times the upper limit of the respective normal ranges). Patients who had obstructive jaundice or liver metastasis were considered to be eligible if their serum transaminase levels could be reduced to within 5 times the upper limit of normal by biliary drainage. The exclusion criteria were as follows: (1) under regular treatment with phenytoin, warfarin, or flucytosine (2) history of chemotherapy with fluorinated pyrimidine, (3) severe mental disorder, active infection, ileus, interstitial pneumonia or pulmonary fibrosis, uncontrolled diabetes mellitus, heart failure, renal failure, active gastric or duodenal ulcer, massive pleural or abdominal effusion, and brain metastasis, (4) active concomitant malignancy, and (5) pregnant/lactating women. Written informed consent was obtained from all of the patients. This study was conducted with the approval of the institutional review board at all the participating hospitals. The study is registered with the UMIN Clinical Trials Registry as UMIN000000919.

Treatment

S-1 was administered orally at the dose of 40 mg/m^2 twice daily, after meals. Three initial doses were set according to the body surface area (BSA), as follows: BSA $<$ 1.25 m^2, 80 mg/day; 1.25 m^2 $\leq$ BSA $<$ 1.50 m^2, 100 mg/day; 1.50 m^2 $\leq$ BSA, 120 mg/day. S-1 was administered for 28 days, followed by a 14-day rest period. The treatment cycle was repeated until the detection of disease

progression, appearance of unacceptable toxicities, or patient's refusal.

If any grade 3 or more severe hematologic, or grade 2 or more severe non-hematologic toxicity occurred, administration of S-1 was either temporarily discontinued until the toxicity recovered to grade 1 or less, and the dose of S-1 was reduced by 20 mg/day in the next treatment cycle. If no toxicity occurred, the scheduled rest period was shortened to 7 days (4-week cycle), or the dose was gradually escalated in the next course (maximum dose, 150 mg/day), or both were permitted according to the judgment of the individual physicians. In a case of the course delay more than 28 days due to toxicity, the protocol treatment was discontinued. Patients were not allowed to receive concomitant radiation therapy, chemotherapy, or hormonal therapy during the study.

Response and toxicity evaluation

The response after each course was evaluated according to the Response Evaluation Criteria in Solid Tumors. Physical examinations, complete blood cell counts, biochemistry tests, and urinalyses were assessed at least once every 2 weeks. Adverse events were evaluated according to the National Cancer Institute Common Toxicity Criteria, version 3.0.

Statistical analysis

The primary endpoint of this study was objective response rate. The secondary endpoints were toxicity, progression-free survival (PFS), and overall survival (OS). The target number of patients in this study was determined using a Southwest Oncology Group's standard [12, 13]. The null hypothesis was that the overall response rate would be $\leq$5 %, and the alternative hypothesis was that the overall response rate would be $\geq$15 %, the α level was 5 % (one tailed), and the power was 10 % (one tailed). The alternative hypothesis was established based on the data from our previous studies of first-line treatment [14, 15]. Interim analysis was planned when 20 patients were enrolled. If none of the first 20 patients showed a partial or complete response (CR), the study itself was to be discontinued. If a response was detected in the first 20 patients, 20 patients were added in the second stage if the lower limit of the 90 % confidence interval (CI) exceeded the 5 % threshold (objective response in $\geq$7 of the 40 patients), S-1 would be judged to be effective, and we would proceed to the next large-scale study. The PFS was calculated from the date of study entry to the date of documented disease progression or death due to any cause. The OS was calculated from the date of study entry to the date of death or the date of last follow-up. The median probability of the survival period and PFS were estimated using the Kaplan–Meier method. The relative dose intensity of S-1 was calculated according to the Hryniuk method [16].

Results

A total of 41 patients were enrolled in this study. Of these 41 patients, one patient was excluded on account of the rapid clinical deterioration before the first administration of S-1, and the remaining 40 patients were assessed. The patient characteristics are shown in Table 1. Of the 40 patients, 35 received GEM monotherapy and the remaining 5 received combined therapy with GEM plus cisplatin as the first-line chemotherapy. As the best response to the first-line chemotherapy, one patient showed CR, two patients showed partial response (PR), 19 patients showed

Table 1 Patient characteristics

Characteristics	Number of patients (%)
Age (years) [median (range)]	67 (35–78)
Sex	
Male	26 (65)
Female	14 (35)
Performance status	
0	18 (45)
1	20 (50)
2	2 (5)
Primary tumor site	
Intrahepatic bile duct	10 (25)
Extrahepatic bile duct	15 (38)
Gall bladder	14 (35)
Ampulla of Vater	1 (3)
Extent of disease	
Locally advanced	3 (8)
Metastatic	37 (92)
Cancer involvement	
Liver	25 (63)
Lymph node	18 (45)
Peritoneal dissemination	4 (2)
Lung	8 (20)
Biliary drainage (+)	21 (53)
Prior surgical resection (+)	20 (50)
Prior chemotherapy	
Gemcitabine	35 (88)
Gemcitabine + cisplatin	5 (13)
Carcinoembryonic antigen (ng/mL) (median [range])	5 (1–1,837)
Carbohydrate antigen 19–9 (U/mL) (median [range])	751 (3–71,900)

stable disease (SD), and the remaining 14 patients showed PD. Progress disease was observed in all patients during the first-line chemotherapy. The median time to progression during this first-line chemotherapy was 4.3 months (range 0.9–17.8).

Treatment

A total of 92 courses were administered to the 40 patients, with a median of two courses per patient (range 1–12). The relative dose intensity of S-1 was 97.3 %. The reasons for discontinuation of treatment were radiologically confirmed PD (31 patients), clinically confirmed PD without radiological confirmation (5 patients), unacceptable toxicities (two patients; one patient the course delay more than 28 days due to continuing grade 2 nausea, and the other patient grade 4 leukoencephalopathy), patient's request to withdraw from the study (one patient), or surgical resection because of PR (one patient).

Toxicity

Forty patients were assessable for adverse events. The treatment-related adverse events are shown in Table 2. Toxicity was generally mild, and the major grade 3 or 4 toxicities were anorexia (10.0 %), anemia (7.5 %), mucositis (7.5 %), hypoalbuminemia (5.0 %), and pneumonia (5.0 %). One patient developed grade 4 leukoencephalopathy, but recovered with just observation. Although two patients died due to rapid disease progression within 4 weeks of discontinuation of the treatment, no treatment-related deaths were observed.

Efficacy

Forty patients were assessed for response. The responses are shown in Table 3. There was no case of CR; however, 3 patients [2 patients with intrahepatic cholangiocarcinoma (IHC) and one patient with gall bladder carcinoma (GBC)] showed PR. Twenty-two patients showed SD, and 15 patients showed PD. The overall response rate was 7.5 % (95 % CI 1.6–20.4 %; 90 % CI 2.1–18.3 %), and the disease control rate was 62.5 % (95 % CI 45.8–77.2 %).

Table 3 also shows the tumor responses according to the primary tumor site. The overall response rate and disease control rate in the GBC group ($n = 14$) were 7.1 and 42.9 %, respectively. Those with the primary tumors at other sites (IHC $n = 10$, extrahepatic cholangiocarcinoma (EHC) $n = 15$, and ampulla of Vater cancer (AVC) $n = 1$) were 7.7 and 70.8 %, respectively. The median PFS and OS of the 40 patients were 2.5 and 6.8 months, respectively (Fig. 1). The median PFS and OS of the patients with GBC were 1.4 and 4.7 months, respectively, and those of the patients with the primary tumor at other sites (IHC, EHC, and AVC) were 2.5 and 7.5 months, respectively.

Table 2 Treatment-related adverse events ($n = 40$): worst grade reported during the treatment period

Toxicity grade	1	2	3	4	1–4 (%)	3/4 (%)
Hematological toxicity						
Leukopenia	7	0	0	1	8 (20)	1 (3)
Neutropenia	2	1	0	1	4 (10)	1 (3)
Anemia	4	8	3	0	15 (38)	3 (8)
Thrombocytopenia	9	2	1	0	12 (30)	1 (3)
Non-hematological toxicity						
Nausea	6	4	0	0	10 (25)	0 (0)
Vomiting	3	1	0	0	4 (10)	0 (0)
Anorexia	10	5	5	0	20 (50)	5 (13)
Fatigue	9	6	1	0	16 (40)	1 (3)
Diarrhea	2	3	2	0	7 (18)	2 (5)
Rash	2	1	0	0	3 (8)	0 (0)
Decreased serum albumin level	6	2	2	0	10 (8)	2 (5)
Elevated serum AST	5	1	0	0	6 (15)	0 (0)
Elevated serum ALT	2	0	0	0	2 (5)	0 (0)
Elevated serum total bilirubin	3	1	1	0	5 (13)	1 (3)
Elevated serum creatinine	1	0	0	0	1 (3)	0 (0)
Encephalopathy	0	0	0	1	1 (3)	1 (3)
Mucositis	6	0	3	0	9 (23)	3 (8)
Biliary tract infection	0	1	1	0	2 (5)	1 (3)
Colitis	0	1	1	0	2 (5)	1 (3)
Taste disturbance	1	1	0	0	2 (5)	0 (0)
Pigmentation	4	1	0	0	5 (13)	0 (0)
Abdominal pain	6	2	0	0	8 (20)	0 (0)

AST aspartate aminotransferase, *ALT* alanine aminotransferase

Discussion

The primary endpoint of this study was response rate. S-1 yielded a response rate of 7.5 % in the patients with GEM-refractory BTC. The lower 90 % confidence limit of the response rate, 2.1 %, was not above the null hypothesis (5 %), and hence, we did not consider that S-1 was effective.

However, since the disease control rate was 65.2 %, we concluded that the treatment showed modest efficacy. At present, several reports of clinical trials of second-line treatment are available (Table 4) [17–23]. The current study results were comparable to those of previous studies, except for another phase II trial of S-1 conducted on a small number of patients [21], in which the response rate ranged from 0 to 12.9 %.

In the current study, the median PFS and OS were 2.3 and 6.8 months, respectively. As indicated by several

Table 3 Response rate and tumor control rate in patients with gall bladder carcinoma, intrahepatic and extrahepatic cholangiocarcinoma, and ampulla of Vater cancer

Outcome	Total ($n = 40$)	GBC ($n = 14$)	IHC ($n = 10$)	EHC ($n = 15$)	AVC ($n = 1$)
CR	0	0	0	0	0
PR	3	1	2	0	0
SD	22	5	7	9	1
PD	15	8	1	6	0
Response rate (%)	7.5	7.1		7.7	
Disease control rate (%) (CR + PR +SD)	57.5	42.9		70.8	

GBC gall bladder carcinoma, *EHC* extrahepatic cholangiocarcinoma, *IHC* intrahepatic cholangiocarcinoma, *AVC* ampulla of Vater cancer

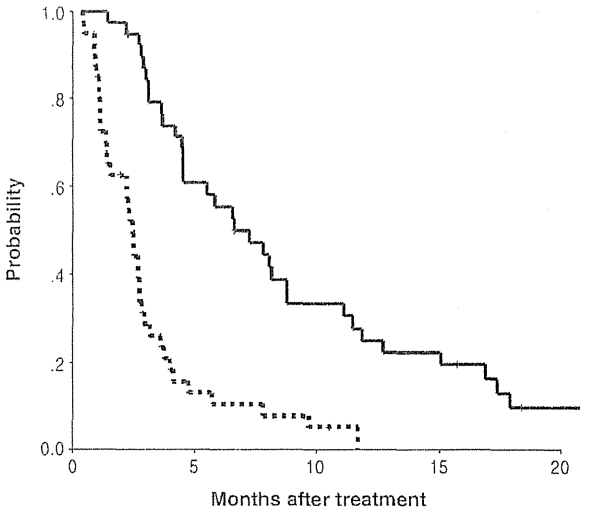

Fig. 1 Progression-free survival (*dash line*) and overall survival (*solid line*) *curves* of patients with gemcitabine-refractory biliary tract cancer receiving systemic chemotherapy with S-1 ($n = 40$). The median progression-free survival and overall survival were 2.5 and 6.8 months, respectively. *Tick marks* indicate censored cases

previous reports, BTC is a heterogeneous group, and the prognosis of cholangiocarcinoma and AVC is generally better than that of GBC [3, 24]. In this study, the disease control rate (PR + SD), PFS, and OS in the GBC group were worse than those with other primary sites.

With regard to toxicity, the results were similar to those observed during the previous first-line treatment with S-1 in chemo-naive patients with BTC [11, 25]. In addition, comparing this study with other clinical trials [17–23], we conclude second-line treatment with S-1 was well tolerated. Considering its safety and convenience, the drug can be used for treatment in the outpatient setting.

Based on the results of a randomized phase III trial of GEM + cisplatin versus GEM, the GEM + cisplatin regimen came to be recognized as standard first-line therapy for BTC. In regard to the second-line treatment, discrepant results were obtained between the randomized trials performed in the UK and those performed in Japan [26]. In the UK, the treatment for the majority of cases after disease progression in the first line was best supportive care, with only 17 % of the patients receiving further chemotherapy, mostly 5-FU-based chemotherapy. On the other hand, a much higher proportion of Japanese patients received second-line treatment, mainly with S-1 (75 % of patients). Despite this difference in the proportion of patients receiving second-line treatment, which might be expected to improve the survival after failure the first-line chemotherapy in Japanese trial patients as compared with that in the UK trial patients, the OS appeared to be very similar between the two trials. Thus, survival benefit of the second-line chemotherapy has not been confirmed. There is an urgent need to establish an effective second-line treatment(s) to improve the survival. The results of this study can serve as a reliable database for further studies on second-line treatment for BTC. The efficacy of second-line

Table 4 Clinical trials of second-line treatments for patients with advanced biliary tract cancer

Reference	Regimen	Number of patients	Response rate (%)	Median PFS/TTP (months)	Median OS (months)
Lee et al. [17]	5FU + ADR + MMC	31 (16)[a]	12.9	2.3	6.7
Oh et al. [18]	Gemcitabine	32	6.9	1.6	4.1
Pino et al. [19]	Capecitabine + celecoxib	35 (5)[a]	9	4.2	4.8
Sasaki et al. [20]	Gemcitabine + cisplatin	20	0	3.6	5.9
Sasaki et al. [21]	S-1	22	22.7	5.5	8.0
Yi et al. [22]	Sunitinib	56	8.9	1.7	12.9
Chiorean et al. [23]	Erlotinib + ADR	11	0	4.7	5.7
Current study	S-1	40	7.5	2.5	6.8

PFS progression-free survival, *TTP* time to progression, *OS* overall survival, *ADR* adriamycin, *MMC* mitomycin

[a] The number of patients includes both patients with pancreatic cancer and biliary tract cancer. The number in parentheses indicates the number of biliary tract cancer patients

treatment should be assessed prospectively within randomized controlled trials.

In conclusion, S-1, administered as single-agent chemotherapy, was well tolerated, but showed modest efficacy in patients with GEM-refractory BTC.

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Conflict of interest Takuji Okusaka is an advisory board member for Taiho Pharmaceutical Co. and receives research funding and honoraria from Taiho Pharmaceutical Co. Junji Furuse is an advisory board member for Taiho Pharmaceutical Co. and Eli Lilly.

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In Vivo SPECT Imaging with ^{111}In-DOTA-c(RGDfK) to Detect Early Pancreatic Cancer in a Hamster Pancreatic Carcinogenesis Model

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Early detection of pancreatic cancer is key to overcoming its poor prognosis. $\alpha_v\beta_3$-integrin is often overexpressed in pancreatic tumor cells, whereas it is scarcely expressed in normal pancreatic cells. In this study, we investigated the usefulness of SPECT imaging with ^{111}In-1,4,7,10-tetraazacylododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid-cyclo-(Arg-Gly-Asp-D-Phe-Lys) [^{111}In-DOTA-c(RGDfK)], an imaging probe of $\alpha_v\beta_3$-integrin, for the early detection of pancreatic cancer in a hamster pancreatic carcinogenesis model. **Methods:** Hamsters were subcutaneously injected with the pancreatic duct carcinogen *N*-nitrosobis(2-oxopropyl)amine to induce pancreatic cancer. *N*-nitrosobis(2-oxopropyl)amine–treated hamsters underwent in vivo SPECT with ^{111}In-DOTA-c(RGDfK). After imaging, the tumor-to-normal pancreatic tissue radioactivity ratios in excised pancreatic samples were measured with autoradiography (ARG) and compared with the immunopathologic findings for $\alpha_v\beta_3$-integrin. In a mouse model in which inflammation was induced with turpentine, the uptake of ^{111}In-DOTA-c(RGDfK) in inflammatory regions was evaluated with ARG and compared with that of ^{18}F-FDG. **Results:** ^{111}In-DOTA-c(RGDfK) was clearly visualized in pancreatic cancer lesions as small as 3 mm in diameter. ARG analysis revealed high tumor-to-normal pancreatic tissue radioactivity ratios (4.6 ± 1.0 [mean ± SD] in adenocarcinoma and 3.3 ± 1.4 in atypical hyperplasia). The uptake of ^{111}In-DOTA-c(RGDfK) strongly correlated with $\alpha_v\beta_3$-integrin expression. In the inflammatory model, inflammation-to-muscle ratios for ^{18}F-FDG and ^{111}In-DOTA-c(RGDfK) were 8.37 ± 4.37 and 1.98 ± 0.60, respectively. These results imply that ^{111}In-DOTA-c(RGDfK) has a lower rate of false-positive tumor detection than ^{18}F-FDG. **Conclusion:** Our findings suggest that SPECT with ^{111}In-DOTA-c(RGDfK) has great potential for the early and accurate detection of pancreatic cancer.

Key Words: ^{111}In-DOTA-c(RGDfK); SPECT; $\alpha_v\beta_3$-integrin; pancreatic cancer; early detection

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Pancreatic cancer is a leading cause of cancer-related mortality in developed countries, with an increasing incidence (*1*). The 5-y survival rate is poor (*2,3*). Surgical resection remains the only curative option. The postoperative 5-y survival rate has been recorded to be high as 40%–50%, whereas only 15%–20% of tumors are found to be resectable at the time of diagnosis (*4*). Tumor size is an important prognostic factor for pancreatic cancer because better prognosis and postsurgical survival have been reported for small pancreatic cancers (≤2 cm) than for large ones (>2 cm) (*5,6*). Given the incidence and high mortality rate of pancreatic cancer, the development of novel diagnostic technologies is essential for overcoming this type of cancer.

Currently, ^{18}F-FDG PET is widely used in the diagnosis of malignant tumors. ^{18}F-FDG PET is more accurate in detecting relatively large pancreatic adenocarcinomas than conventional imaging techniques (*7–9*). However, it has some limitations in detecting pancreatic cancer (*10*). ^{18}F-FDG can accumulate in chronic and acute pancreatitis, and this fact often yields false-positive interpretations for PET (*11,12*). It is also well known that the sensitivity of ^{18}F-FDG PET in hyperglycemic patients tends to be lower than that in euglycemic patients because elevated serum glucose levels suppress ^{18}F-FDG uptake in tumors by up to 50% as a result of competitive inhibition (*13,14*). New imaging agents that are not influenced by these factors are essential for the detection of small pancreatic cancers.

Integrins are cell adhesion molecules that mediate cell–cell and cell–matrix interactions and contribute to angiogenesis, tumor invasion, and metastasis. $\alpha_v\beta_3$-integrin is a well-characterized integrin that is overexpressed in endothelial cells and various tumor cells (*15–17*). Immunohistochemical analysis demonstrated that $\alpha_v\beta_3$-integrin was expressed in 60% of invasive pancreatic ductal carcinomas of stages I–IV, and patients with $\alpha_v\beta_3$-integrin–positive carcinomas showed shorter survival times than those with $\alpha_v\beta_3$-integrin–negative carcinomas (mean survival times, 12.3 vs. 21.4 mo) (*18*). Thus, $\alpha_v\beta_3$-integrin would be an excellent target for the early detection of malignant pancreatic cancer.

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For investigating the mechanisms of the development of pancreatic cancer, an experimental pancreatic ductal carcinogenesis model has been established with the carcinogen *N*-nitrosobis(2-oxopropyl)amine (BOP) in hamsters (*19–22*). This model provides unique characteristics that are similar to a sequence of well-characterized morphologic changes in the human pancreatic duct and frequently shows point mutations in codon 12 of the K-*ras* gene, in accordance with human findings (*23,24*). We found that $\alpha_v\beta_3$-integrin was overexpressed not only in adenocarcinomas but also in atypical hyperplasia in this hamster model (*25*). Therefore, this model is useful in the development of imaging probes for the early detection of pancreatic carcinogenesis.

Radiolabeled Arg-Gly-Asp (RGD) peptides are widely used as $\alpha_v\beta_3$-integrin imaging agents (*26–28*). In a previous study, ^{111}In-1,4,7,10-tetraazacylododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid-cyclo-(Arg-Gly-Asp-D-Phe-Lys) [^{111}In-DOTA-c(RGDfK)] showed high uptake in tumors with strong expression of $\alpha_v\beta_3$-integrin, low uptake in normal pancreas, and extremely rapid clearance from the blood (*29*). These characteristics are favorable for pancreatic cancer imaging. In the present study, we investigated the usefulness of SPECT imaging with ^{111}In-DOTA-c(RGDfK) for the early and accurate detection of pancreatic cancer in a chemically induced hamster pancreatic cancer model.

MATERIALS AND METHODS

Experimental Animal Models

Ten 5-wk-old female Syrian golden hamsters were obtained from Japan SLC. For the induction of pancreatic cancer, hamsters were subcutaneously injected with BOP (Nacalai Tesque) in saline at 10 mg/kg of body weight 4 times every other day. Palpation and laparotomy were occasionally performed after BOP treatment to confirm the induction of pancreatic cancer.

Eight 6-wk-old male ddY mice (Japan SLC) were intramuscularly injected with 50 μL of turpentine oil (Kanto Chemical) in the right thigh to induce inflammation (*30,31*).

Animal studies were performed in compliance with the guidelines set for animal experiments by the Committee for Ethics of Animal Experimentation at the National Cancer Center.

SPECT with ^{111}In-DOTA-c(RGDfK) in Hamster Pancreatic Cancer Model

DOTA-c(RGDfK) was labeled with ^{111}In as described previously (*29*). Hamsters were injected via the subclavian vein with 17.5–37.0 MBq of ^{111}In-DOTA-c(RGDfK) 16 wk after treatment with BOP. They were maintained under anesthesia with isoflurane (Dainippon Sumitomo Pharmaceutical) throughout the experiment. Just before the acquisition of CT images, the hamsters were injected with 500 μL of iopamidol (Iopamiron 370; Bayer Schering Pharma).

SPECT/CT was performed with a 4-head, multiplexing, multipinhole NanoSPECT/CT scanner (Bioscan, Inc.) 1 h after the injection of ^{111}In-DOTA-c(RGDfK). First, CT scans were obtained with a tube voltage of 60 kV and a tube current of 0.12 mA. Next, SPECT scanning was performed at 300 s/projection, and 24 projection views were obtained. After imaging, the SPECT data were reconstructed with an ordered-subset expectation maximization algorithm, dedicated software (Invivoscope; Bioscan, Inc.), and Mediso InterViewXP (Mediso). SPECT and CT images were automatically superimposed with Invivoscope. The accuracy of the superimposition was regularly calibrated with phantoms. A researcher experienced in the evaluation of small-animal SPECT/CT images visually evaluated pancreatic uptake.

Autoradiography (ARG) with ^{111}In-DOTA-c(RGDfK) in Hamster Pancreatic Cancer Model

After SPECT/CT, the pancreas from each hamster was excised and macroscopically surveyed to detect pancreatic lesions. Samples were then embedded in Cryo Mount II (Muto Pure Chemicals Co., Ltd.) and frozen in liquid nitrogen. Frozen sections were cut with a cryostat to thicknesses of 20 μm for ARG and 10 μm for histologic analysis and mounted on glass slides. For ARG, glass slides were placed on an imaging plate (BAS-MS 2040; Fujifilm Co. Ltd.), and then the exposed plate was scanned with a bioimaging analyzer (FLA-7000; Fujifilm Co. Ltd.) to detect radioactivity. On the basis

TABLE 1
SPECT Detection Ratios and Tumor-to-Normal Pancreas (T/N) Ratios Calculated by ARG Analysis

Condition	Hamster	Size (mm)	Detection by SPECT	T/N ratio
Adenocarcinoma	4	2.0	ND	5.1
	5	3.0	Detected	4.0
		4.4	Detected	5.2
	6	3.0	Detected	4.5
		5.0	Detected	6.7
	7	2.0	ND	4.5
	9	3.5	ND	3.5
		5.0	Detected	4.2
	10	8.0	Detected	3.7
Atypical hyperplasia	3	1.5	ND	4.9
	7	0.7	ND	5.4
	9	0.8	ND	2.6
		1.3	ND	2.7
	10	0.9	ND	2.4

For adenocarcinoma and atypical hyperplasia, respective sizes (mean ± SD) were 4.0 ± 1.9 and 1.0 ± 0.3 mm; respective percentages detected by SPECT were 66.7% and 0%; and respective T/N ratios (mean ± SD) were 4.6 ± 1.0 and 3.9 ± 1.5. ND = not detected.

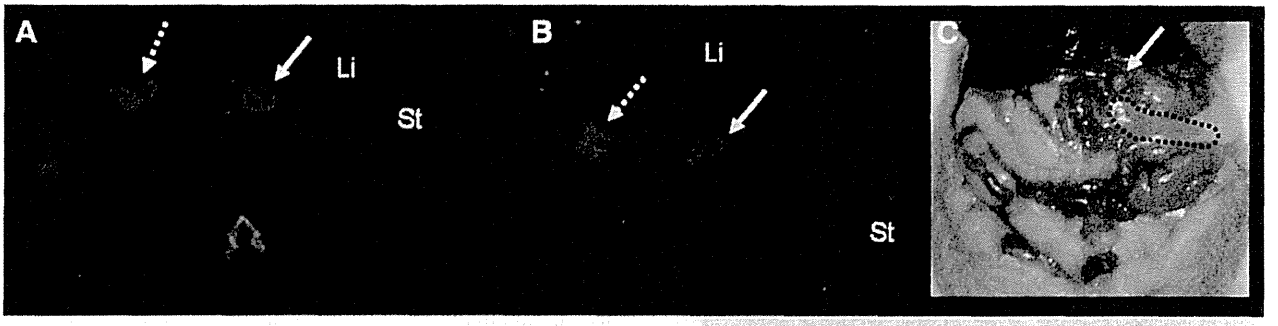

FIGURE 1. (A and B) SPECT images of pancreatic tumor in hamster 6 (A, axial; B, coronal). SPECT was performed 1 h after injection of ^{111}In-DOTA-c(RGDfK). Intense uptake was found in tumor (solid arrow). Slight uptake of ^{111}In-DOTA-c(RGDfK) was observed in intestine (dotted arrow). (C) Anatomic image of hamster abdomen. Tumor (5 mm) in pancreatic head is indicated by arrow; its position was identical to that of region of high uptake of ^{111}In-DOTA-c(RGDfK). Pancreatic gastric lobe is indicated by dotted line. Li = liver; St = stomach.

of microscopic observation of sections stained with hematoxylin and eosin, regions of interest were placed on both tumor and normal pancreatic samples. ImageQuant software (Fujifilm Co. Ltd.) was used to quantify the intensity of radioactivity.

ARG with ^{111}In-DOTA-c(RGDfK) and ^{18}F-FDG in Mouse Inflammatory Model

Three days after turpentine oil injection, ARG analysis of inflammatory regions was performed. Eight mice were divided into 2 groups. Each group was injected via the tail vein with 740 kBq of ^{111}In-DOTA-c(RGDfK) and 925 kBq of ^{18}F-FDG. Inflammatory tissue, including the surrounding tissue, was excised 1 h after injection. ARG analysis was performed as described earlier. Regions of interest were placed on both inflammatory and muscle regions.

Immunohistochemical Analysis of $\alpha_v\beta_3$-Integrin

Frozen sections (10 μm) were fixed in methanol at −20°C. After 2 washes with phosphate-buffered saline containing 0.05% polysorbate 20 (PBS-T), endogenous peroxide was blocked with 3% H_2O_2 in methanol for 10 min. After 2 washes with PBS-T, sections were masked with 2% normal goat serum in PBS-T for 1 h at room temperature and then incubated overnight with anti-$\alpha_v\beta_3$-integrin (clone LM609; Millipore) at 4°C. Sections were incubated with biotinylated anti-mouse IgG (Dako Cytomation); this step was followed by reaction with streptavidin–biotin–horseradish peroxidase complex (StreptABComplex/HRP; Dako Cytomation). Horseradish peroxidase was detected with diaminobenzidine (Phoenix Biotechnologies) substrate. All sections were counterstained with hematoxylin.

Statistical Analysis

Data analysis was performed with GraphPad Prism (GraphPad Software). Unpaired *t* testing was used for ARG analysis in the mouse inflammatory model. The results were considered statistically significant at $P < 0.05$.

RESULTS

SPECT with ^{111}In-DOTA-c(RGDfK) in BOP-Treated Hamsters

Adenocarcinomas or atypical pancreatic hyperplasia was macroscopically or microscopically found in 7 of 10 BOP-treated hamsters (Supplemental Table 1) (supplemental materials are available online only at http://jnm.snmjournals.org). There were 9 adenocarcinoma lesions in 6 BOP-treated hamsters and 5 atypical hyperplasia lesions in 4 BOP-treated hamsters. Both adenocarcinomas and atypical hyperplasia were observed in 3 BOP-treated hamsters. The average size (mean ± SD) of the adenocarcinomas was 4.0 ± 1.9 mm, and SPECT with ^{111}In-DOTA-c(RGDfK) detected 6 of the 9 lesions (66.7%) (Table 1). The average size of the atypical hyperplasia lesions was 1.0 ± 0.3 mm, and SPECT with ^{111}In-DOTA-c(RGDfK) could not detect any such lesion.

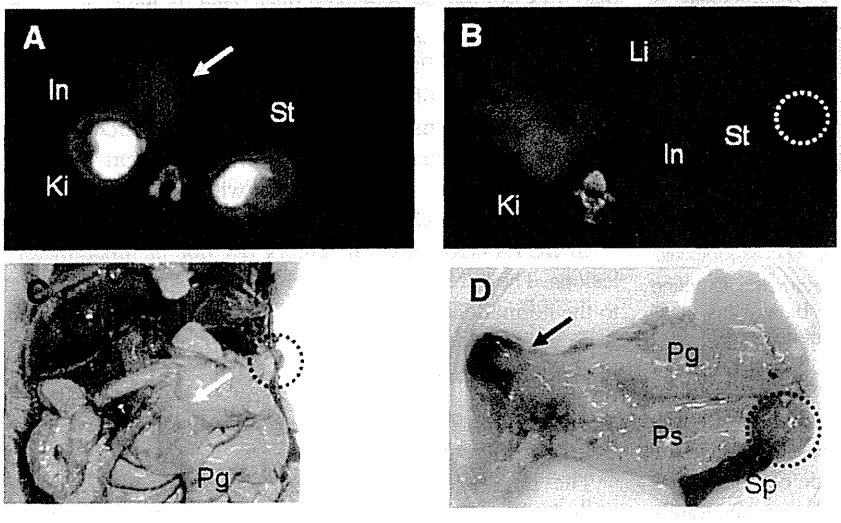

FIGURE 2. (A and B) SPECT axial images of pancreatic tumor (A) and purulent inflammatory lesion (B) in hamster 10. (C and D) Anatomic images of abdomen (C) and excised pancreas (D). Tumor (8 mm) in pancreatic head is indicated by arrow. Inflammatory lesion is indicated by dotted circle. In = intestine; Ki = kidney; Li = liver; Pg = pancreatic gastric lobe; Ps = pancreatic splenic lobe; Sp = spleen; St = stomach.

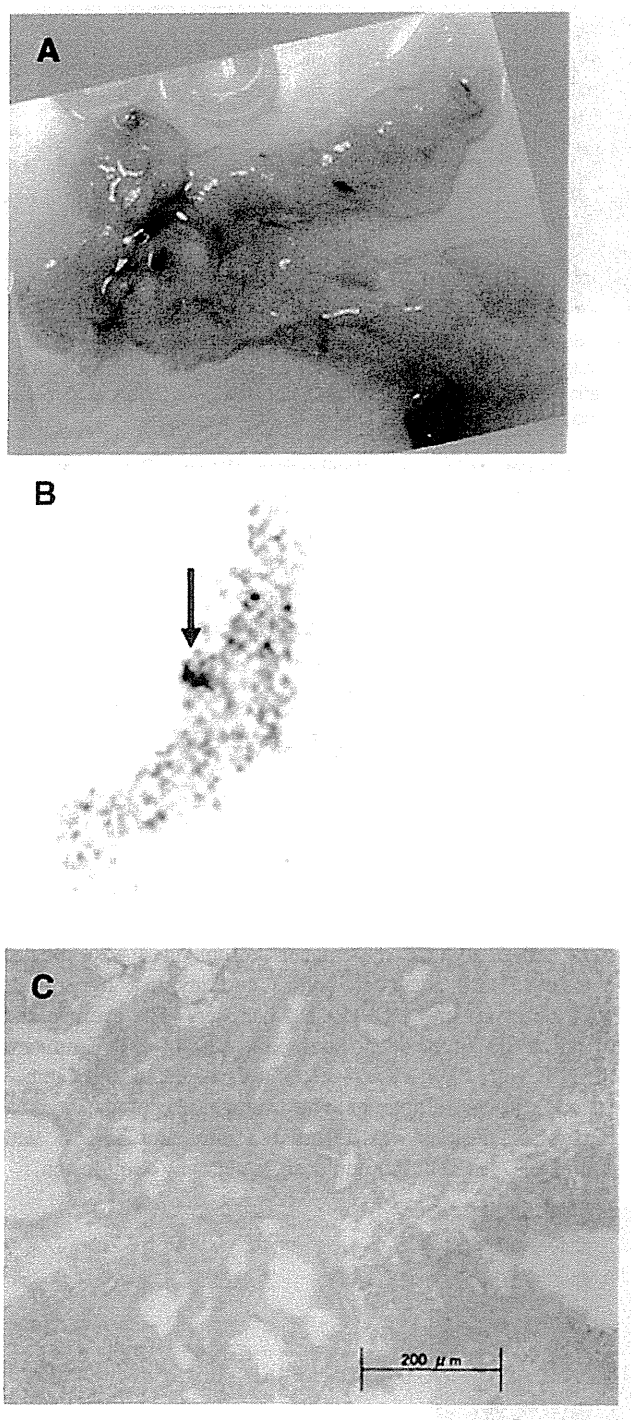

FIGURE 3. Ex vivo autoradiography and histopathologic analysis of atypical hyperplastic region in hamster 3. (A) Macroscopically, there was no lesion in pancreas. (B) One hot spot (arrow) was found in gastric lobe by ARG, but SPECT could not detect this small lesion. (C) Hematoxylin–eosin staining in region of hot spot.

Abdominal CT images of hamsters successfully depicted the liver, stomach, intestine, and kidneys. The anatomic relationships among these organs successfully indicated the location of the pancreas, although the actual pancreatic contours were not delineated. Because SPECT images were accurately superimposed on CT images, pathologic accumulation in the pancreas could be judged from the SPECT/CT fusion images. Representative SPECT/CT fusion images are shown in Figure 1 and Figure 2. Figures 1A and 1B show SPECT images of the pancreatic tumor in hamster 6. A tumor that was 5 mm in diameter and that was located near the pyloric region was clearly visualized with ^{111}In-DOTA-c(RGDfK). Although there was slight uptake in the intestine, this kind of uptake never interfered with the detection of pancreatic tumors because superimposed CT images clearly indicated that the uptake was not located in the pancreas. All tumors depicted by ^{111}In-DOTA-c(RGDfK) SPECT were verified by laparotomy findings (Figs. 1B and 1C).

In hamster 10, 1 pancreatic tumor (8 mm in diameter) in the pancreatic head and an artificially induced purulent inflammatory/foreign-body granulomatous nodule that was located in the splenic lobe of the pancreas and that was adherent to abdominal muscle were found (Figs. 2C and 2D, Ps). SPECT with ^{111}In-DOTA-c(RGDfK) accurately depicted the tumor in the pancreatic head (Fig. 2A),but the inflammatory lesion was not detected (Figs. 2B and 2D). There was intense uptake in the kidneys because of urinary excretion.

Ex Vivo ARG and Histopathologic Analysis of Excised Pancreas

ARG successfully depicted all adenocarcinoma and atypical hyperplasia lesions, but SPECT failed to detect atypical hyperplasia. The T/N ratios for adenocarcinomas and atypical hyperplasia were 4.6 ± 1.0 and 3.9 ± 1.5, respectively (Table 1). There was strong $\alpha_v\beta_3$-integrin expression in all adenocarcinoma lesions.

The contrast in ^{111}In-DOTA-c(RGDfK) accumulation on ARG images between tumors and the normal pancreas was quite good (Supplemental Figs. 1A and 1B). Strong positive results for $\alpha_v\beta_3$-integrin in tumor tissues on immunohistochemical analysis validated these results satisfactorily (Supplemental Fig. 1C).

Although SPECT failed to detect atypical hyperplasia lesions, ARG successfully depicted all of them, even when they were not macroscopically visualized. In hamster 3 (Fig. 3), the T/N ratio was 4.9—similar to that for adenocarcinoma (4.6). However, SPECT could not detect this lesion, likely because of its small size.

In hamster 10, the uptake of ^{111}In-DOTA-c(RGDfK) in the inflammatory lesion was not demonstrated even by ARG (Supplemental Fig. 2). In agreement with the in vivo SPECT findings (Fig. 2), ARG images revealed significant uptake of ^{111}In-DOTA-c(RGDfK) in tumors but not in inflammatory lesions. The T/N ratio was 3.7, and the ratio of inflammation to the normal pancreas was 0.9. These accumulation patterns were verified by the absence of $\alpha_v\beta_3$-integrin expression in inflammatory lesions.

Accumulation of ^{111}In-DOTA-c(RGDfK) and ^{18}F-FDG in Inflammatory Lesions

The uptake of ^{111}In-DOTA-c(RGDfK) was compared with that of ^{18}F-FDG in inflammatory lesions in the mouse