Table 3. Toxicities across first two cycles by dose level (patient number)

Toxicity	Dose level 1 (n = 3)				Dose level 2 (n = 3)				Dose level 3 (n = 6)				Dose level 4 (n = 6)			
Grade:	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Leucopenia	1	2	0	0	0	2	1	0	1	4	1	0	1	2	3	0
Neutropenia	1	1	0	0	0	1	2	0	0	5	0	1	0	3	1	2
Anemia	2	0	0	0	3	0	0	0	4	1	0	0	4	2	0	0
Thrombocytopenia	2	0	0	0	1	2	0	0	4	1	0	0	3	0	0	0
Nausea	2	0	0	0	1	0	1	0	2	2	0	0	2	0	0	0
Vomiting	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0
Anorexia	1	0	0	0	0	0	1	0	2	1	0	0	2	1	0	0
Diarrhea	1	0	0	0	1	0	0	0	0	0	0	0	2	0	0	0
Stomatitis	2	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0
Rash	0	1	0	0	1	2	0	0	2	0	0	0	3	1	1	0
ALT elevation	1	0	0	0	2	0	0	0	3	2	0	0	1	0	0	0
Creatinine elevation	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fever	0	0	0	0	0	1	0	0	0	0	0	0	3	0	0	0
Fatigue	1	0	0	0	1	0	0	0	1	1	0	0	2	1	0	0

Toxicity was graded according to the National Cancer Institute Common Toxicity Criteria version 2.0. ALT = Alanine aminotransferase.

dose of 80 mg/m^2/day) was considered the recommended dose in further studies with this schedule.

Toxicity

All 18 patients were assessable for toxicity. The major toxicities observed during the first two cycles are summarized in table 3. Hematological toxicity, particularly neutropenia, was the most pronounced toxicity of gemcitabine and S-1 with this schedule of administration. Although 3 patients experienced grade 4 neutropenia during the first two cycles of treatment, all of them recovered quickly without any severe complications. The neutrophil nadir typically occurred on day 15, and neutrophil counts recovered to baseline values by day 22. The non-hematological toxicities commonly observed with our regimen were gastrointestinal toxicities, such as nausea (≥grade 1; 55.6%) and anorexia (≥grade 1; 44.4%), although most of them were mild and transient. Although 1 patient at dose level 2 experienced grade 3 anorexia and grade 3 nausea in the first cycle, he recovered from the toxicities with the use of antiemetic agents and could continue treatment without reducing the doses of gemcitabine and S-1. Skin rash was also frequently seen in the current study (≥grade 1; 61.1%). The rash typically appeared on the arms and legs and spread to the trunk within 10 days of the initiation of chemotherapy. Most rashes were mild and resolved promptly with appropriate medical treatment such as antihistamines and steroids, although 1 patient at dose level 4 exhibited grade 3 rash that required temporary treatment discontinuation and dose reduction in the next cycle. Although 125 cycles of chemotherapy have been administered, there was no indication of cumulative toxicity.

Table 4. Objective tumor response

Dose level	Patients	Response				Response rate, %
		CR	PR	NC	PD	
1	3	0	2	1	0	66.7
2	3	0	0	1	2	0
3	6	0	3	3	0	50
4	6	0	1	4	1	16.7
Total	18	0	6	9	3	33.3

CR = Complete response; PR = partial response; NC = no change; PD = progressive disease.

Efficacy

The objective tumor responses at each dose level are shown in table 4. A partial response was seen even at the lowest dose level, and across all dose levels, 6 of the 18 patients achieved a partial response, resulting in an over-

all response rate of 33.3 (95% confidence interval, 13.3–59.0%). No change was noted in 9 patients (50%) and progressive disease in 3 patients (16.7%). The mean response duration was 4.8 months (range 2.8–15.9). The serum CA 19-9 level was reduced to less than half from baseline values in 8 (61.5%) of the 13 patients who had a pretreatment level greater than the upper limit of normal (37 U/ml). At the time of analysis, 9 patients had died because of disease progression. The median progression-free and the median overall survival times were 5.0 and 7.6 months, respectively.

Discussion

To improve the prognosis of patients with advanced pancreatic cancer, gemcitabine-based combination chemotherapy has been actively investigated, although many phase III trials have failed to demonstrate any survival benefit of combination chemotherapy in comparison with gemcitabine as a single agent. 5-FU has been selected as a candidate to be investigated in combination with gemcitabine in patients with pancreatic cancer because of its favorable toxicity profile and modest but substantial activity in this disease. Gemcitabine is considered to enhance the effect of the 5-FU metabolite 5-FdUMP by reducing the concentration of its physiological competitor via inhibition of ribonucleotide reductase [24]. Preclinical studies have demonstrated synergy between gemcitabine and 5-FU in tumor cell lines, including pancreatic cancer cells [25, 26]. Clinical studies have reported activity of gemcitabine in pancreatic cancer patients with refractoriness to 5-FU [27], suggesting the lack of cross-resistance between the two agents. Several phase I and II studies of combination therapy with gemcitabine and 5-FU for advanced pancreatic cancer have demonstrated relatively good response rates of around 20% with acceptable toxicity profiles [14–18]. A phase III study comparing gemcitabine alone with gemcitabine plus weekly bolus 5-FU showed that median progression-free survival was significantly longer in the combination arm compared with gemcitabine alone (3.4 vs. 2.2 months, $p = 0.022$); however, median overall survival was not significantly prolonged (6.7 vs. 5.4 months, $p = 0.09$) [5].

The novel oral anticancer agent S-1 was developed to improve the tumor-selective toxicity of 5-FU and has shown efficacy in a variety of solid tumors, including pancreatic cancer [9–13]. With the aim of developing a more effective chemotherapeutic regimen for pancreatic cancer, we decided to conduct a clinical study of combination therapy with gemcitabine and S-1. Since this combination has not previously been investigated, a phase I study was carried out to determine MTD and DLT.

In the present study, MTD was not reached because only 2 of the 6 patients experienced DLT at the highest dose, level 4. Although the 6 patients at level 4 have received a total of 34 cycles of treatment (average 5.7, range 2–12), there was no indication of cumulative toxicity. Therefore, dose level 4 (gemcitabine 1,000 mg/m^2/week, S-1 80 mg/m^2/day) was considered the recommended dose in further studies of this combination regimen. Because 2 of the 6 patients experienced DLT at this level, it goes without saying that more large-scale studies will be necessary to confirm the safety of our recommended dose. The overall toxicity of this regimen was mild, and neither unexpected nor life-threatening toxicities were observed during the study, indicating that S-1, like other fluoropyrimidines, can be safely combined with gemcitabine.

Neutropenia was the major DLT of this combination regimen: 1 of the 6 patients at dose level 3, and 2 of the 6 patients at dose level 4, experienced grade 4 neutropenia. Neutropenia as the DLT was to be expected because myelosuppression, especially neutropenia, is one of the most common toxicities of each individual drug. The neutrophil nadir typically occurred on day 15, but in most cases, the neutrophil count spontaneously recovered to baseline values within a week. Furthermore, no febrile neutropenia was observed during any of the 125 cycles of treatment, suggesting that the myelosuppression caused by this combination regimen is manageable on an outpatient basis.

The non-hematological toxicities commonly observed with our regimen were gastrointestinal toxicities such as nausea and anorexia. Although 1 patient at dose level 2 experienced transient grade 3 nausea and grade 3 anorexia, no DLTs associated with gastrointestinal toxicities were observed. Diarrhea was also mild and rare in the current study, similar to previous reports from Japanese studies of single-agent S-1; however, relatively severe diarrhea induced by S-1 has been reported in studies from Europe and the United States [28–30]. For example, Hoff et al. [28] reported that severe diarrhea occurred in all of the 3 patients who received S-1 at a dose of 40 mg/m^2 b.i.d. It is not clear why the toxicity profile and MTD of S-1 in Western studies differ from those in studies with Japanese populations, although a pharmacokinetic study suggested that the conversion of tegafur to 5-FU may occur more slowly in Japanese patients than in patients from other ethnic groups [31]. In any event, it may be dangerous to apply the results of our study directly to

treatment of Western patients, particularly from the viewpoint of gastrointestinal toxicity.

In the present study, 11 (61.1%) of the 18 patients experienced grade 1 or greater rash. This toxicity was mild and manageable, although 1 patient at dose level 4 developed grade 3 rash, requiring temporary treatment discontinuation. The reason for the enhanced cutaneous toxicity during combination therapy with gemcitabine and S-1 is unknown, although cutaneous toxicity has already been reported in patients receiving gemcitabine and 5-FU combination regimens. Hidalgo et al. [14] reported grade 1 or greater cutaneous toxicity in 11 (42.3%) of the 26 patients in a phase I–II study with gemcitabine and 5-FU. One of these patients developed a severe cutaneous reaction, manifested as generalized exfoliative dermatitis, after the first cycle of chemotherapy.

Combination therapy with gemcitabine and S-1 was associated with promising activity in advanced pancreatic cancer. Six (33.3%) of the 18 patients achieved an objective response. Of the 13 patients who had a pretreatment serum CA 19-9 level greater than 37 U/ml, the CA 19-9 level decreased more than 50% in 8 patients (61.5%). In addition, the median progression-free survival time of 5.0 months and the median overall survival time of 7.6 months are encouraging. These efficacy data in this study, which compare favorably with those reported for single-agent gemcitabine, support further studies of this regimen.

In conclusion, our combination regimen of gemcitabine and S-1 was well tolerated up to dose level 4. The major toxicities were myelosuppression, gastrointestinal toxicity and skin rash, although most of these toxicities were mild and reversible. Six of the 18 patients showed a partial response, suggesting a promising antitumor activity of this regimen against pancreatic cancer. A multicenter phase II study of this regimen, 1,000 mg/m^2/week gemcitabine on days 1 and 8 and 80 mg/m^2/day S-1 from days 1 to 14 every 3 weeks, is under way in patients with metastatic pancreatic cancer.

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A Phase II Trial of Uracil–Tegafur (UFT) in Patients with Advanced Biliary Tract Carcinoma

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Background: Uracil–tegafur (UFT) has been reported to have broad antitumor activity in a variety of malignancies. However, its activity in biliary tract carcinoma has not been fully evaluated. The aim of this study was to evaluate the antitumor activity and toxicity of UFT in chemotherapy-naive patients with advanced biliary tract carcinoma.
Methods: Nineteen patients with advanced biliary tract carcinoma that was histologically confirmed as adenocarcinoma were enrolled in this phase II trial of UFT. A dose of 360 mg/m^2/day of UFT was administered orally if there was no evidence of tumor progression or there was unacceptable toxicity.
Results: Of the 19 patients evaluable for response, one patient (5%) achieved a partial response with a duration of 2.0 months. Six patients (32%) showed no change and the remaining 12 (63%) had progressive disease. The median survival, 6-month survival rate and 1-year survival rate for all patients were 8.8 months, 52.6 and 21.1%, respectively. The chemotherapy was well tolerated, because grades 3 or 4 toxicity were not observed.
Conclusion: UFT appears to have little activity as a single agent in treating patients with advanced biliary tract carcinoma. These findings do not support its use in practice, and further trials with this regimen in patients with biliary tract carcinoma are not recommended.

Key words: biliary tract carcinoma – chemotherapy – phase II study – uracil–tegafur

INTRODUCTION

Biliary tract carcinomas (BTCs), including carcinomas that arise from extrahepatic or intrahepatic bile duct, gallbladder or papilla of vater, are relatively rare tumors with a dismal prognosis. Surgical resection is the first choice of treatment for BTC and usually provides the only chance for a cure. However, because of the absence of early symptoms, the majority of patients are diagnosed with advanced stages of disease. Moreover, even for those who undergo surgical resection, the risk of recurrence is extremely high (1–3). To improve the prognosis of patients with this disease, effective chemotherapy is essential. However, no chemotherapeutic drug has yet shown sufficient efficacy to be acknowledged as a standard therapy, although various agents have been evaluated in clinical trials (1–3).

Uracil–tegafur (UFT) is an orally administered drug that is a combination of uracil and tegafur in a 4:1 molar concentration ratio. Uracil prevents degradation of 5-fluorouracil (5-FU) by inhibiting dihydropyrimidine dehydrogenase (DPD), which leads to an increased level of 5-FU in plasma and tumor tissues (4–6). It appears that prolonged administration of UFT results in a similar or higher maximum concentration achieved (C_{max}) as well as area under the curve (AUC) compared with those achieved with continuous infusion of 5-FU (7). In phase II trials in Japan, the antitumor activity of UFT was demonstrated in a variety of solid tumors including colorectal cancer and breast cancer (8,9). With regard to UFT for BTC, an overall response rate of 25% in eight evaluable patients was reported in a Japanese phase II trial in the early 1980s (8). However, the number of patients in that study was very small, and the results may have been unreliable because the quality of clinical trials in the early 1980s was debatable. Since then, the activity of UFT in BTC has not been re-evaluated, although UFT is approved and widely used for BTC in Japan and other countries. Therefore, we conducted a phase II trial to evaluate the antitumor activity and toxicity of UFT in patients with advanced BTC.

PATIENTS AND METHODS

ELIGIBILITY

Patients eligible for study entry had histologically or cytologically confirmed advanced BTC. The eligibility criteria were:

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20–74 years of age; an Eastern Cooperative Oncology Group performance status of 0–2; bidimensionally measurable disease; an estimated life expectancy ≥8 weeks after study entry; no prior chemotherapy; adequate hematological function (hemoglobin ≥11 g/dl, leukocytes ≥4000/mm^3, neutrophils ≥2000/mm^3 and platelets ≥100 000/mm^3); adequate hepatic function (serum total bilirubin ≤2.0 mg/dl and serum aspartate aminotransferase/alanine aminotransferase ≤2.5 times the upper limit of normal); adequate renal function (serum creatinine level within normal limits); and written informed consent. All patients with obstructive jaundice underwent percutaneous transhepatic or endoscopic retrograde biliary drainage before treatment.

The exclusion criteria were: active infection; severe heart disease; refractory pleural effusion or ascites; active gastroduodenal ulcer; severe mental disorder; active concomitant malignancy; pregnant and lactating females; females of childbearing age unless using effective contraception; and other serious medical conditions.

Pre-treatment evaluation included taking a complete history and a physical examination. The pretreatment laboratory procedures were complete differential blood count, biochemistry tests and tumor markers including serum carcinoembryonic antigen (CEA) and serum carbohydrate antigen 19-9 (CA19-9). All patients underwent electrocardiography, chest radiography and computed tomography (CT) scan within the 4 weeks before study entry.

Treatment Schedule

UFT was administered orally at a dose of 360 mg/m^2/day. The total daily dose of UFT was divided into three doses administered every 8 h. When doses could not be divided evenly, the highest dose was given in the morning and the lowest dose in the evening. The calculated UFT dose was rounded off to the nearest 100 mg.

When ≥grade 3 hematological toxicity or ≥grade 2 nonhematological toxicity was observed, treatment was delayed until the toxicity subsided to grade 1 or less. If the daily dose of UFT was considered to be intolerable, the dose was reduced by 100 mg/day (one capsule/day). UFT was administered until the appearance of disease progression or unacceptable toxicity. Patients who were refractory to this regimen were allowed to receive any other anticancer treatments at their physician's discretion.

Response and Toxicity Evaluation

We used the Japan Society for Cancer Therapy criteria, which are fundamentally similar to the World Health Organization (WHO) criteria, for evaluating the tumor responses and the adverse effects. The objective tumor response was assessed by CT every 4 weeks after the beginning of UFT therapy. During this treatment, a complete differential blood count, serum chemistry profile and urinalysis were undertaken at least biweekly. Serum CEA and CA19-9 levels were measured every 4 weeks. Progression-free survival was defined as the time from the date of initial treatment to first documentation of progression or death. Overall survival was measured from the date of initial treatment to the date of death or the date of last follow-up.

Statistical Design

Analysis was to be performed when 19 patients were enrolled. In this study, the threshold response rate was defined as 5% and the expected response rate was set as 25%. If the lower limit of the 90% confidence interval (CI) exceeded the 5% threshold (objective response in four or more of the 19 patients), UFT was judged to be effective. If the upper limit of the 90% CI did not exceed the expected rate of 25% (zero or one objective response in the 19 patients), UFT was judged to be ineffective. If response was confirmed in two or three of the 19 patients, the decision whether or not to proceed to the next study was taken on the basis of the safety and survival data from the present study. In BTC, no chemotherapeutic drug has yet shown sufficient efficacy to be acknowledged as a standard therapy. Considering that this treatment also may be ineffective, the sample size in this study had to be set as a minimally required number of patients. Therefore, 90% was adopted as the CI, because the treatment could have been judged as ineffective due to the small sample size. This phase II trial was approved by the Institutional Review Board of the National Cancer Center.

RESULTS

Patients and Treatments

Nineteen patients were enrolled in this study at the two hospitals of the National Cancer Center between July 2002 and February 2004. The characteristics of the patients are listed in Table 1. A total of 33 courses were given, with an average of 1.7 courses (range 1–5) per patient. All patients discontinued this treatment because of disease progression. After abandoning UFT treatment, two patients received second-line chemotherapy with epirubicin, 5-FU and cisplatin (11); both patients showed stable disease with durations of 4.0 and 2.5 months, respectively. The remaining 17 patients received only best supportive care after the treatment.

Response

All 19 patients were evaluable for response. No patient achieved a complete response. One patient with gallbladder carcinoma achieved a partial response with a duration of 2.0 months, giving an overall response rate of 5% (95% CI 0–26). Six patients (32%) showed no change and the remaining 12 (63%) had progressive disease. During treatment, the serum CEA level was reduced by >50% of the pre-treatment level in only one patient, who achieved a partial response, and there was no patient whose serum CA19-9 level decreased from the pre-treatment level.

Table 1. Patient characteristics

Characteristic	No. of patients (%)
Age, years	
Median (range)	65 (50–74)
Gender	
Male	10 (53)
Female	9 (47)
ECOG performance status	
0	14 (74)
1	5 (26)
Prior surgery	
Positive	10 (53)
Primary site	
Gallbladder	8 (42)
Extrahepatic bile duct	2 (11)
Intrahepatic bile duct	8 (42)
Papilla of vater	1 (5)
Organs affected by metastases	
Liver	13 (68)
Lymph node	7 (37)
Lung	5 (26)
CEA (ng/ml)	
Median (range)	6.8 (2.9–133.5)
CA19-9 (U/ml)	
Median (range)	207 (4–56 000)

CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9.

Table 2. Toxicity

	Grade			
	1	2	3	4
Hematological toxicity				
Leukocytes	4 (21)	0 (0)	0 (0)	0 (0)
Neutrophils	2 (11)	1 (5)	0 (0)	0 (0)
Hemoglobin	1 (5)	1 (5)	0 (0)	0 (0)
Platelets	0 (0)	0 (0)	0 (0)	0 (0)
Non-hematological toxicity				
Nausea/vomiting	8 (42)	0 (0)	0 (0)	0 (0)
Stomatitis	2 (11)	0 (0)	0 (0)	0 (0)
Diarrhea	6 (32)	0 (0)	0 (0)	0 (0)
Fatigue	4 (21)	0 (0)	0 (0)	0 (0)
Alopecia	0 (0)	0 (0)	0 (0)	0 (0)
Skin rash	0 (0)	0 (0)	0 (0)	0 (0)
Hand-foot syndrome	0 (0)	0 (0)	0 (0)	0 (0)
Total bilirubin	–	6 (32)	0 (0)	0 (0)
Aspartate aminotransferase	8 (42)	1 (5)	0 (0)	0 (0)
Alanine aminotransferase	2 (11)	1 (5)	0 (0)	0 (0)
Alkaline phosphatase	4 (21)	0 (0)	0 (0)	0 (0)
Creatinine	2 (11)	0 (0)	0 (0)	0 (0)

Values in parentheses are percentages.

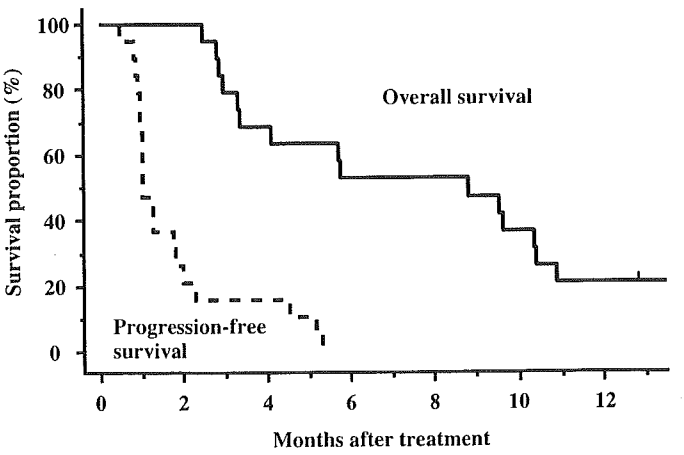

Figure 1. Overall survival and progression-free survival curves of 19 patients who received UFT therapy for advanced biliary tract carcinoma. Tick marks indicate censored cases.

TOXICITY

The toxicities observed in the 19 enrolled patients are listed in Table 2. The toxicity represents the maximum grade per patient for the entire course of therapy. Therapy with UFT was well tolerated, and all adverse events were manageable. Six patients (32%) showed grade 2 elevation of total bilirubin. However, the elevation in total bilirubin, which ranged from 1.1 to 2.0 times the upper limit of normal, was defined as grade 2 in the Japan Society for Cancer Therapy criteria, which is equivalent to grade 1 in the WHO criteria. No grade 3 or greater toxicities were observed in this study.

SURVIVAL

All enrolled patients were included in the survival assessment. At the time of the analysis, 18 patients had died because of tumor progression. The median survival, 6-month survival rate, 1-year survival rate and median progression-free survival for all patients were 8.8 months, 52.6%, 21.1% and 1.0 months, respectively (Fig. 1). The median survivals in patients with intrahepatic bile duct carcinoma and in those with other tumors, including carcinoma of the gallbladder, extrahepatic bile duct and papilla of vater, were 9.5 and 5.7 months, respectively.

DISCUSSION

The outcome of chemotherapy for BTC has not improved significantly in the last two decades, and the prognosis for patients with this disease still remains dismal. Because of

the rarity of this cancer, there have been few well-designed chemotherapeutic trials conducted with a sufficient number of patients. The most commonly used single agent has been 5-FU, with response rates of ≤10% and median survival times of ≤6 months (1–3). Mitomycin C, which was considered by some investigators to be one of the active agents for the treatment of this disease, resulted in an objective response rate of 10% in an EORTC study (12). Recently, gemcitabine has shown promising antitumor activity for BTC in several studies, with reported response rates of 8–60% and median durations of survival ranging from 6.5 to 11.5 months, but it has not yet been accepted as a standard therapy for BTC (13). Moreover, combination chemotherapy has also proven equally disappointing because it rarely results in any meaningful clinical improvement. Thus, various agents have been evaluated in clinical trials, but no chemotherapeutic drug has yet shown sufficient efficacy to be acknowledged as a standard therapy (1–3).

In Japan, only three anticancer agents, UFT, adriamycin and cytarabine, have been approved for BTC by the Ministry of Health, Labor and Welfare of Japan. UFT (tegafur combined with uracil in a molar ratio of 1:4) represents a second-generation oral 5-FU prodrug that is converted to 5-FU in tissue (4–6). Compared with 5-FU, UFT has been reported to be less toxic and to have a higher therapeutic index in a variety of solid tumors (8–9). In patients with BTC, a Japanese phase II study in the early 1980s demonstrated that UFT at a daily dose of 300–600 mg shows a relatively high response rate (two out of eight, 25%) (8). However, since then, the activity of UFT in BTC has not been re-evaluated. A re-appraisal of UFT for advanced BTC is essential, because the number of patients in the previous study was very small and the evaluation of tumor response may have been unreliable because in the early 1980s imaging modalities had not been developed sufficiently. To elucidate the true efficacy of UFT, therefore, we conducted a phase II trial of UFT in patients with advanced BTC.

In the current study, only one of 19 patients obtained a partial response (response rate, 5%) with a duration of 2.0 months. Moreover, a rate of progressive disease of 63% and a median progression-free survival of only 1 month were particularly disappointing. The results of this study indicate that UFT has negligible activity in BTC and, even though it was well tolerated, cannot be recommended as routine treatment for advanced BTC. In this study, there was a large difference between overall survival (median: 8.8 months) and progression-free survival (median: 1.1 months). The difference was assumed to be due to the natural history of this disease, because only two patients received second-line chemotherapy and the remaining 17 patients received only best supportive care after the treatment. In studies by Mani et al. (14) and Chen et al. (15), combination therapy with UFT and leucovorin resulted in 0% response rates and median survivals of 7.0 and 5.2 months, respectively. These results are very similar to ours, and this regimen was also considered ineffective. However, the novel oral fluoropyrimidine derivatives S-1 (16) and capecitabine (17) have generated particular interest for the treatment of advanced BTC, since the response rates with these agents are reported to be higher than that with UFT. Further trials of these agents are currently being conducted in patients with advanced BTC.

In conclusion, UFT appears to show little activity as a single agent in treating patients with advanced BTC, although oral UFT therapy is convenient and well tolerated. These findings do not support the use of this regimen in clinical practice, and further trials in patients with BTC are not recommended. Therefore, we will continue to investigate other agents and regimens in an effort to increase response, survival and quality of life for patients with this disease.

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Chemoradiotherapy for Locally Advanced Pancreatic Carcinoma in Elderly Patients

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Key Words
Chemoradiotherapy · Pancreatic carcinoma · Elderly

Abstract
Objectives: Chemoradiotherapy, which is one of the standard treatments for locally advanced pancreatic carcinoma, is considered a high-risk procedure in elderly patients. This study investigated the outcome and tolerability of this treatment in elderly patients. ***Methods:*** We reviewed our database from November 1993 to March 2003 and retrospectively examined the clinical data of patients with histologically confirmed exocrine pancreatic carcinomas that were nonresectable but confined to the pancreatic region, who were treated with protracted 5-fluorouracil infusion (200 mg/m^2/day) and concurrent radiotherapy (50.4 Gy in 28 fractions over 5.5 weeks). We evaluated the outcome of patients ≥70 years and those <70 years. ***Results:*** There were 19 patients ≥70 and 39 patients <70. On pretreatment evaluation, the elderly patients showed lower serum albumin levels, lower transaminase levels, better ECOG performance status, more frequent body weight loss and less frequent abdominal and/or back pain with the administration of morphine than the younger patients. There were no significant differences in the frequency of severe toxicity. Neither the response rate nor the incidence of treatment discontinuation differed significantly between the two groups. The median survival time was longer in the elderly patients than in the younger patients (11.3 vs. 9.5 months, p = 0.04). ***Conclusions:*** With careful patient selection, chemoradiotherapy can be one of the treatment options for locally advanced pancreatic carcinoma in elderly patients.

Introduction

The prognosis of patients with pancreatic carcinoma is extremely poor because of difficulty in the early detection of this disease and the ineffectiveness of nonsurgical treatments. For patients with locally nonresectable disease, the results of previous randomized trials indicated that concurrent external beam radiation therapy (EBRT) and 5-fluorouracil (5-FU) therapy resulted in significantly better survival compared with EBRT alone [1, 2] or chemotherapy alone [3]. However, this combination treatment sometimes induces intolerable toxic effects, and approximately 10–20% of patients cannot complete the scheduled course of treatment [4, 5]. Consequently, this treatment is considered to be frequently contraindicated in elderly patients, who are thought to be less likely to tolerate its potential toxicity than younger patients.

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Furthermore, many physicians believe that pancreatic carcinoma is less treatable in the elderly because of the presence of comorbid illnesses. On the other hand, it was reported that elderly patients often tolerate aggressive chemotherapy or radiotherapy for other carcinomas as well as their younger counterparts [6–16].

Some studies have shown that for resectable pancreatic carcinoma, pancreatic resections can be performed for the elderly with acceptable morbidity and mortality rates and possible long-term outcome [17–25]. However, in locally advanced pancreatic carcinoma treated with chemoradiotherapy, the tolerability, efficacy of treatment and long-term outcome have not been discussed extensively.

The current retrospective analysis examines the outcome and tolerability of elderly patients (i.e. those aged ≥70 years) within our database. The main purposes of this examination were to determine if the outcome for elderly patients was different from that for younger patients and to characterize the toxicity experienced by the elderly patients.

Methods

We reviewed the database of the Hepatobiliary and Pancreatic Oncology Division of the National Cancer Center Hospital from November 1993 to March 2003. In this retrospective analysis, we examined the clinical data of all patients who met the following requirements: (1) histological diagnosis of exocrine pancreatic carcinoma, (2) nonresectable disease confined to the pancreatic region, (3) treatment with protracted 5-FU infusion and concurrent radiotherapy, and (4) absence of prior treatment for pancreatic carcinoma. We divided the patients into two groups according to age, those ≥70 years and those <70 years. We evaluated the patient characteristics, toxicities, efficacies and survival in both groups.

Treatment was performed according to the treatment protocol of our division; radiotherapy was delivered via a microtron (MM22, Scanditronix, Upsala, Sweden) with 10- or 14-MV X-rays or a racetrack microtron (MM50, Scanditronix) with 25-MV X-rays. A total dose of 50.4 Gy was delivered in 28 fractions over 5.5 weeks. All patients had treatment planning computed tomography (CT) scans (X-vision, Toshiba, Tokyo, Japan), and FOCUS (Computerized Medical Systems, St. Louis, Mo., USA) was used as a radiotherapy treatment planning system. The clinical target volume included the primary tumor, nodal involvement detected by CT scan, and regional draining and para-aortic lymph nodes, which included the peripancreatic nodes, celiac and superior mesenteric axes. The planning target volume was defined as the clinical target volume plus a 10-mm margin. Four field techniques (anterior, posterior and opposed lateral fields) were used. The spinal cord dose was maintained below 45 Gy, ≥50% of the liver was limited to ≤30 Gy, and ≥50% of both kidneys was limited to ≤20 Gy. 5-FU was given from the first day of radiation and continued through the entire course of radiation at a dose of 200 mg/m^2/day through a central venous catheter. Patients were admitted to the hospital during chemoradiotherapy. Within 8 weeks after the completion of chemoradiotherapy, maintenance chemotherapy was delivered on an outpatient basis and continued until disease progression. For the maintenance chemotherapy, we used a weekly administration of 5-FU (500 mg/m^2, 30-min infusion) before the approval of gemcitabine for pancreatic carcinoma in Japan (April 2001), and thereafter, we used weekly administration of gemcitabine (1,000 mg/m^2, 30-min infusion) 3 times every 4 weeks.

During chemoradiotherapy, the toxicity of the treatment was scored weekly according to the World Health Organization criteria [26]. Both radiotherapy and chemotherapy were suspended when ≥grade 3 toxicities other than anorexia, fatigue, nausea/vomiting, constipation and hyperglycemia occurred and were resumed when recovery to grade 2 toxicity levels was achieved. If there was a total delay of 2 weeks due to toxicity for any reason, the combined treatment was discontinued. In this retrospective analysis, we obtained the information regarding adverse events about the subjective symptoms from the doctor's record in as much detail as possible. As a rule, follow-up CT was performed within 1 week after the completion of chemoradiotherapy and every 2 months thereafter to evaluate the objective tumor response with reference to the World Health Organization criteria.

Statistics

Frequencies in 2 × 2 and larger contingency tables of the patient characteristics, response rates and toxicities were compared with the χ^2 or Fisher's exact test. Distributions of continuous variables were compared with the Mann-Whitney test. Overall survival was measured from the first day of treatment, and the survival curves were calculated according to the Kaplan-Meier method. The log rank test was used to detect differences between the curves. All p values in this study were of the two-tailed type. Significance was defined as a p value of 0.05 or less. Statistical analyses were performed with Stat View version 5.0.

Results

One hundred and ninety-nine patients with locally advanced pancreatic carcinoma admitted to the Hepatobiliary and Pancreatic Oncology Division of the National Cancer Center Hospital from November 1993 to March 2003. Thirty-nine patients were ≥70 years and 160 were <70 years. Nineteen (49%) of the 39 patients ≥70 and 39 (24%) of the 160 of those <70 met the above-mentioned conditions. The remaining 141 patients were excluded from this analysis. One hundred and thirty-eight received other anticancer treatments including chemoradiotherapy using other regimens (130), systemic chemotherapy (7) and radiotherapy alone (1). Three patients underwent only the best supportive care. The patient characteristics are shown in table 1 and the pretreatment laboratory data are shown in table 2. The male-to-female ratio was 1.7:1 in the elderly patients and 1.4:1 in the younger patients.

Table 1. Patient characteristics

	≥70 years	<70 years	p
Patients	19	39	
Age			
Median	75	60	
Range	70–86	35–69	
Sex			0.78
Male	12 (63)	23 (59)	
Female	7 (37)	16 (41)	
ECOG PS			0.004
0	6 (32)	1 (3)	
1	11 (58)	36 (92)	
2	2 (11)	2 (5)	
Diabetes mellitus	9 (47)	10 (26)	0.14
Abdominal and/or back pain[a]	3 (16)	19 (49)	0.02
Biliary drainage	4 (21)	8 (21)	>0.99
Regional lymph node	11 (58)	22 (56)	>0.99
Body weight loss[b]	14 (74)	24 (62)	0.20
Tumor location			0.42
Uncus	1 (5)	5 (13)	
Head	12 (63)	25 (64)	
Body	5 (26)	9 (23)	
Tail	1 (5)	0 (0)	
Treatment start			>0.99
Before April 2001[c]	10 (53)	21 (54)	
After April 2001[c]	9 (47)	18 (46)	

Figures in parentheses are percentages. ECOG = Eastern Cooperative Oncology Group.

[a] Abdominal and/or back pain: with consumption of morphine.

[b] Body weight loss: more than 7% of previous body weight within 6 months.

[c] April 2001: approval of gemcitabine.

Table 2. Pretreatment laboratory data

	≥70 years	<70 years	p
Albumin, g/dl	3.6 (3.0–4.3)	3.8 (3.1–4.5)	0.002
AST, IU/l	19 (11–66)	23 (10–274)	0.04
ALT, IU/l	17 (9–136)	32 (6–332)	0.01
Total bilirubin, mg/dl	0.7 (0.3–1.3)	0.6 (0.2–3.7)	0.20
CA19-9, U/ml	769.5 (3–27,000)	624.0 (4–6,310)	0.06
CEA, ng/ml	6.9 (2.1–76.4)	4.9 (0.7–1,620)	0.11

AST = Aspartate aminotransferase; ALT = alanine aminotransferase; CA19-9 = carbohydrate antigen 19-9; CEA = carcinoembryonic antigen.

Table 3. Response to chemoradiotherapy

	≥70 years	<70 years	p
Complete response	0 (0)	0 (0)	
Partial response	2 (11)	2 (5)	
No change	14 (74)	28 (72)	
Progressive disease	3 (16)	7 (18)	
Not evaluable	0 (0)	2 (5)	0.60

Figures in parentheses are percentages.

In the elderly patients, there were 6 patients (32%) who had an ECOG performance status (PS) of 0, but there was only 1 such patient (3%) among the younger patients ($p = 0.004$). The incidence of patients who had abdominal or back pain with consumption of morphine was smaller in the elderly patients ($p = 0.02$). There was no significant difference between the younger and elderly patients with regard to the period prior to treatment initiation (before or after the gemcitabine approval) ($p > 0.99$). The serum albumin level and transaminase levels were lower in the elderly patients. The other patient characteristics of those ≥70 years were generally similar to those of the younger patients.

The results of the treatment outcome are shown in table 3. Even though this study was conducted retrospectively, the antitumor response in CT was obtained in all but 2 younger patients who were transferred to another hospital before the completion of treatment. The laboratory data were also maintained for all patients, whose blood examinations were performed at least weekly. Four subjects among the elderly patients (21%) suspended the chemoradiotherapy during the schedule, as did 11 (28%) among the younger patients. One elderly patient (5%) discontinued chemoradiotherapy, as did 5 (13%) of the younger patients. Chemoradiotherapy was discontinued because of patient request due to unacceptable toxicities such as fatigue (1 younger patient), nausea/vomiting (3 younger patients and 1 elderly patient) and patient refusal (1 younger patient). A partial response was obtained in 2 (11%) elderly and 2 (5%) younger patients. Fourteen (74%) elderly patients and 28 (72%) younger patients showed no change. The survival curves are shown in figure 1. The median survival time was longer for the elderly patients than for younger patients (11.3 months in the elderly patients, 9.5 months in the younger patients, $p = 0.04$). The longest survivor in both groups was a 71-year-old male who survived 60.1 months (5.0 years) after the initiation of treatment.

Table 4. Toxicity in patients receiving chemoradiotherapy

	Grades 1–4		p	Grades 3 and 4		p
	≥70 years	<70 years		≥70 years	<70 years	
Leukocytes	9 (47)	20 (51)	>0.99	1 (5)	2 (5)	>0.99
Hemoglobin	8 (42)	16 (41)	>0.99	0 (0)	0 (0)	–
Neutrophils	3 (16)	12 (31)	0.37	0 (0)	0 (0)	–
Platelets	4 (21)	4 (10)	0.48	0 (0)	0 (0)	–
Albumin	10 (53)	16 (41)	0.58	0 (0)	0 (0)	–
AST	4 (21)	8 (21)	>0.99	0 (0)	2 (5)	0.81
ALT	3 (16)	15 (38)	0.15	0 (0)	3 (8)	0.54
Total bilirubin	2 (11)	3 (8)	>0.99	0 (0)	1 (3)	>0.99
Creatinine	2 (11)	0 (0)	0.2	0 (0)	0 (0)	–
Nausea	11 (58)	34 (87)	0.03	2 (11)	13 (33)	0.12
Vomiting	4 (21)	19 (49)	0.07	0 (0)	1 (3)	>0.99
Anorexia	16 (84)	35 (90)	0.9	6 (32)	22 (56)	0.13
Stomatitis	3 (16)	2 (5)	0.85	1 (5)	0 (0)	0.71
Diarrhea	4 (21)	13 (33)	0.47	0 (0)	2 (5)	0.81
Fatigue	3 (16)	13 (33)	0.28	0 (0)	1 (3)	>0.99

Figures in parentheses are percentages. AST = Aspartate aminotransferase; ALT = alanine aminotransferase.

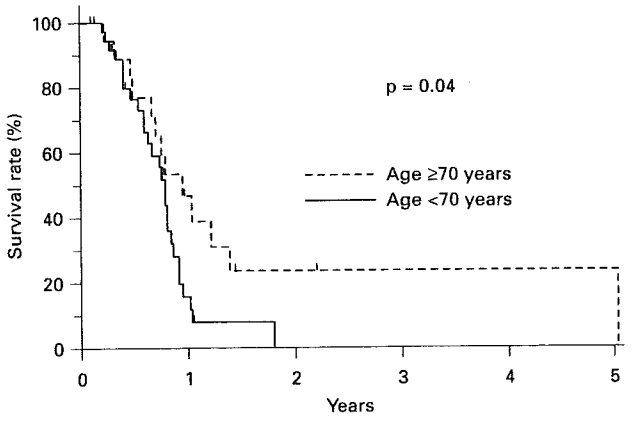

Fig. 1. Overall survival curves for patients ≥70 years (n = 19) and those for patients <70 years (n = 38).

The percentages of overall toxicities (grades 1–4) and severe toxicities (grades 3 and 4) are listed in table 4. Although the incidence of nausea (grades 1–4) was significantly higher in the younger patients, there were no significant differences in the incidence of other overall toxicities or all severe toxicities. The toxicities of both groups were generally mild and reversible. One younger patient died from a fungal infection of the lung due to pneumothorax which occurred as a complication of the insertion of a central venous catheter. There was no conspicuous late toxicity in either group.

Discussion

Based on previous randomized trials [1–3], concurrent EBRT and 5-FU result in significantly better survival compared with EBRT alone or chemotherapy alone and are generally accepted as the standard treatment for locally advanced pancreatic carcinoma. However, this treatment restrains patients for more than 1.5 months during treatment. Furthermore, the life expectancy for the majority of these patients is still short, with a median survival of approximately 10–11 months. The poor prognosis and long duration of treatment makes us hesitant to indicate chemoradiotherapy for patients with locally advanced pancreatic carcinoma, especially for patients at high risk for complications. Elderly patients have been generally considered a high-risk population for chemoradiotherapy due to a number of physiological and pharmacological reasons. For example, diminished bone marrow cellularity can potentially result in decreased tolerance to myelosuppressive therapies. In addition, a decrease in hepatic and renal function may reduce the efficiency of drug metabolism and excretion, resulting in greater toxic potential.

However, in this study, no differences were found in the response rate, incidence of treatment discontinuation and toxicity profile, except for nausea, between the two groups. The median survival time was significantly longer in the elderly patients than in the younger patients. The most important reason for the favorable results of the elderly patients may be the careful selection of patients. Ikeda et al. [27] reported that a good PS was one of the independent favorable prognostic factors in patients with locally advanced pancreatic carcinoma receiving chemoradiotherapy. In our study, 32% of the patients ≥ 70 had an ECOG PS of 0, as opposed to 3% of those <70. Since this was a retrospective analysis, indication according to a physician's decision might have been different for younger and for elderly patients, only allowing the elderly patients in very good condition to receive chemoradiotherapy. As a result, this may be a comparison of elderly patients with a very good PS and younger patients with a less good or average PS.

An imbalance in the incidence of patients with abdominal pain between the two groups might also have affected the treatment outcome in our study. According to the report of Kelsen et al. [28], unresectable pancreatic carcinoma patients with abdominal pain had a median survival of 4.7 months, whereas the median survival among patients without such pain was 8.3 months.

In this study, there was no significant difference between the younger patients and the elderly patients with regard to the ratio of the patients who received maintenance chemotherapy using gemcitabine. Although it is possible that maintenance therapy had some effect on survival, the survival time did not differ significantly between the gemcitabine maintenance chemotherapy group and the 5-FU maintenance chemotherapy group in this study (data not shown).

The mild toxicity of this treatment may be another favorable factor for elderly patients [4]. This study showed that severe toxicities except anorexia were observed infrequently in both groups and that discontinuation of the treatment was required in only 1 elderly patient. Protracted 5-FU infusion with concurrent radiotherapy, which is considered a less toxic treatment than radiotherapy and bolus 5-FU [29, 30], is feasible even in elderly patients.

Krzyzanowska et al. [31] reported an attractive retrospective cohort study in 1,696 patients diagnosed with locally advanced pancreatic carcinoma. According to the report, older age was associated with a lower likelihood of receiving carcinoma-directed therapy, much less of a combined therapy such as chemoradiotherapy. However, Cox proportional hazard models showed that carcinoma-directed therapy, including chemoradiotherapy, has the potential to prolong the survival of elderly patients with locally advanced pancreatic carcinoma. These findings, which suggest that chemoradiotherapy can be an optimal treatment option for locally advanced pancreatic carcinoma in elderly patients, are supported by the results of our study.

Since this study was conducted retrospectively, the results do nothing more than suggest possibilities of the efficacy of the 5-FU-based chemoradiotherapy for selected elderly patients with locally advanced pancreatic carcinoma. To identify the benefit of the treatment in elderly patients, we must design a large prospective study. In summary, this study demonstrates that chemoradiotherapy for locally advanced pancreatic carcinoma is well tolerated and does not lead to an increase in treatment interruption or discontinuation in elderly patients. We conclude that, with careful patient selection, chemoradiotherapy can be considered an appropriate treatment for elderly patients.

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Severe Drug Toxicity Associated with a Single-Nucleotide Polymorphism of the *Cytidine Deaminase* Gene in a Japanese Cancer Patient Treated with Gemcitabine plus Cisplatin

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Abstract

Purpose: We investigated single-nucleotide polymorphisms of the cytidine deaminase gene (*CDA*), which encodes an enzyme that metabolizes gemcitabine, to clarify the relationship between the single-nucleotide polymorphism 208G>A and the pharmacokinetics and toxicity of gemcitabine in cancer patients treated with gemcitabine plus cisplatin.

Experimental Design: Six Japanese cancer patients treated with gemcitabine plus cisplatin were examined. Plasma gemcitabine and its metabolite 2′,2′-difluorodeoxyuridine were measured using an high-performance liquid chromatography method, and the *CDA* genotypes were determined with DNA sequencing.

Results: One patient, a 45-year-old man with pancreatic carcinoma, showed severe hematologic and nonhematologic toxicities during the first course of chemotherapy with gemcitabine and cisplatin. The area under the concentration-time curve value of gemcitabine in this patient (54.54 μg hour/mL) was five times higher than the average value for five other patients (10.88 μg hour/mL) treated with gemcitabine plus cisplatin. The area under the concentration-time curve of 2′,2′-difluorodeoxyuridine in this patient (41.58 μg hour/mL) was less than the half of the average value of the five patients (106.13 μg hour/mL). This patient was found to be homozygous for 208A (Thr^{70}) in the *CDA* gene, whereas the other patients were homozygous for 208G (Ala^{70}).

Conclusion: Homozygous 208G>A alteration in *CDA* might have caused the severe drug toxicity experienced by a Japanese cancer patient treated with gemcitabine plus cisplatin.

Gemcitabine (2′,2′-difluorodeoxycytidine) is a deoxycytidine analogue that is efficacious against non-small cell lung cancer and pancreatic carcinoma, as a single agent or in platinum combination therapy (1, 2). Its major adverse effects are hematologic toxicity, weakness, and emesis, and its dose-limiting toxicity is hematologic toxicity, including leukocytopenia, anemia, and thrombocytopenia (1). Single-agent and platinum combination gemcitabine therapy is relatively well tolerated, but hospitalization is occasionally required due to significant hematologic toxicity (1, 2), and it has been difficult to predict the toxicity.

Gemcitabine is activated by intracellular phosphorylation to gemcitabine monophosphate by deoxycytidine kinase, which is subsequently phosphorylated to the higher-order phosphates, gemcitabine diphosphate followed by gemcitabine triphosphate. Gemcitabine triphosphate can be incorporated into DNA followed by one more deoxynucleotide, after which DNA polymerization stops. This process is referred to as "masked chain termination" (3, 4).

Gemcitabine and gemcitabine monophosphate are deaminated to the inactive metabolite 2′,2′-difluorodeoxyuridine (dFdU) and 2′,2′-difluorodeoxyuridine monophosphate by cytidine deaminase (CDA) and dCMP deaminase, respectively. Multiple mechanisms potentiate the activity of gemcitabine both by increased formation of active gemcitabine diphosphate and gemcitabine triphosphate and decreased elimination of gemcitabine, as follows: (a) gemcitabine diphosphate, through its inhibition of ribonucleotide reductase, depletes the deoxyribonucleotide pool available for DNA synthesis and repair; (b) the decreased concentration of dCTP activates deoxycytidine kinase, which accelerates phosphorylation of gemcitabine;

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and (c) an inactivating enzyme, dCMP deaminase, is inhibited by the decreased concentration of intracellular dCTP and increased concentration of gemcitabine triphosphate (5–7). Polymorphisms of the DNAs encoding the above enzymes may influence the pharmacokinetics and pharmacodynamics of gemcitabine.

To establish the medical guidelines for treatment based on individual genetic polymorphisms, we have launched multicenter, prospective, pharmacogenomic trials (as the Millenium Genome Project) of antineoplastic agents, such as gemcitabine, paclitaxel, irinotecan, and other commonly used drugs.

At the time point when 97 gemcitabine-treated patients had been recruited, we experienced extremely severe toxicities in one patient. Because this patient was coadministered cisplatin in addition to gemcitabine, we compared the clinical data, pharmacokinetics and *CDA* genotype between this patient and the other five control patients, who were also coadministered the two drugs.

Patients and Methods

Selection of patients and treatment schedule. Patients being treated with gemcitabine plus cisplatin were eligible for the trial if they met all of the following inclusion criteria: histologically or cytologically proven carcinoma, no prior treatment with gemcitabine, age above 20 years, Eastern Cooperative Oncology Group performance status between 0 and 2, absence of severe infectious or neurologic disease, and no evidence of heart or interstitial lung disease. Other requirements included adequate bone marrow function (WBC $\geq$3,000/μL, neutrophils $\geq$1,500/μL, and platelets $\geq$75,000/μL), hepatic function (serum total bilirubin $\leq$3 mg/d, aspartate aminotransferase and alanine aminotransferase less than five times the upper limit of normal), and renal function (serum creatinine within the upper limit of normal). The trial was approved by the Ethics Review Committees of the National Cancer Center Hospital and NIH Sciences, and oral and written informed consent was obtained from all patients before entering.

Gemcitabine was given to all patients at a dose of 1,000 mg/m^2 (30-minute infusion) on days 1, 8, and 15 and followed by 1 week of rest. If adequate bone marrow function (WBC $\geq$2,000/μL, neutrophils $\geq$1,000/μL, and platelets $\geq$70,000/μL) was confirmed, gemcitabine was given on days 8 and 15.

Cisplatin was given at a dose of 80 mg/m^2 (150-minute infusion) on day 1, immediately after gemcitabine. All patients received antiemetic prophylaxis with granisetron plus dexamethasone. Granulocyte-colony stimulating factor was not given routinely. The treatment schedule was repeated every 28 days until disease progression or unacceptable side effects occurred.

Toxicity was scored according to the National Cancer Institute Common Toxicity Criteria ver 2.0. A complete blood cell count and serum chemistry were repeated weekly. At the start of every new course, the dose was reevaluated according to toxicity. If the WBC count was <2,000/μL and the platelet count was <70,000/μL, then treatment was delayed until the recovery of bone marrow function. If grade 4 leukocytopenia, neutrocytopenia, or thrombocytopenia was observed in the previous course, the gemcitabine dose was reduced to 800 mg/m^2 in subsequent courses.

Blood sampling. Before the start of the treatment, a 5-mL heparinized blood sample was collected to measure CDA activity, and a 14-mL blood sample, to which EDTA was added, was collected to extract leukocyte DNA for genetic analysis. On day 1 of the first course, a 5-mL heparinized blood sample for gemcitabine and metabolite analysis in plasma was collected from the opposite arm before the infusion, at 3 minutes before the end of the infusion, and 15, 30, 60, 90, 120, and 240 minutes after the end of the infusion, and 50 μL of 10 mg/mL tetrahydrouridine (Wako Junyaku, Co., Ltd., Osaka, Japan) was immediately added to each of the samples. The samples were centrifuged at 3,000 × *g* for 5 minutes at 4°C, and the plasma was collected and stored at −70°C until analyzed.

Analysis of gemcitabine and its metabolite, 2′,2′-difluorodeoxyuridine. The concentrations of gemcitabine and dFdU in the plasma were determined by the method of Venook et al. with slight modifications (8). A 25 μL volume of 25 mg/mL 3′-deoxy-3′-fluoro-thymidine (Aldrich Chem. Co., St. Louis, MO) was added to an 0.25-mL aliquot of plasma sample containing 0.1 mg/mL tetrahydrouridine as an internal standard. After adding 1 mL of acetonitrile, the mixtures were centrifuged at 12,000 × *g* for 5 minutes, and the supernatant was evaporated to dryness under a nitrogen stream. The residue was dissolved in 0.25 mL of 15 mmol/L ammonium acetate buffer (pH 5.0), and the solution was filtered twice through Ultrafree-MC (0.45 μm; Millipore Corp., Billerica, MA) and Microcon YM-10 (10,000 MW; Amicon). Twenty microliters of sample were loaded into a high-performance liquid chromatography system (HP 1100 model) with diode array detection and electrospray-mass spectrometry detection. The chromatographic conditions were as follows: column, CAPCELL PACK C18 MG column (5 μm, 2.0 × 150 mm; Shiseido Co., Ltd., Tokyo, Japan) with a CAPCELL C18 MG S-5 guard cartridge (4.6 mm i.d. × 10 mm; Shiseido); column temperature, 40°C; mobile phase, 15 mmol/L ammonium acetate (pH 5.0)/methanol; running program of the mobile phase: 95:5 (0 minute), −75:25 (10-15 minutes), −60:40 (20-25 minutes), − 95:5 (30-40 minutes); flow rate: 0.3 mL/min; diode array detection: 268 nm for gemcitabine, 258 nm for dFdU, and 266 nm for 3′-deoxy-3′-fluoro-thymidine; electrospray-mass spectrometry: *m/z* 264 for gemcitabine, *m/z* 265 for dFdU, and *m/z* 245 for 3′-deoxy-3′-fluoro-thymidine. Detection and integration of chromatographic peaks were done by the HP Chemistation data analysis system (Hewlett-Packard, Les Ulis, France).

Pharmacokinetic analysis. Compartment model independent pharmacokinetic variables were calculated using WinNonlin software, ver. 4.1 (Pharsight Co., Mountain View, CA). The values are expressed as means ± SD, except for those of the patient with severe toxicity.

DNA sequencing. DNA used for sequencing was extracted from peripheral blood. All of the four exons of *CDA* were amplified from 100 ng of genomic DNA using multiplex primers listed in Table 1 (PCR). The PCR conditions have been described previously (9). After the second amplification for each exon, the PCR products were purified and directly sequenced on both strands with the sequencing primers listed in Table 1 (sequencing), as described previously (9). All variations were confirmed by repeating the sequence analysis from the first-round PCR with DNA. National Center for Biotechnology Information accession no. NT_004610.16 was used for the reference sequence.

Table 1. Primer sequences used for the analysis of the human *CDA* gene

Region	Forward primer (5′-3′)	Reverse primer (5′-3′)
PCR		
Exon 1	TCCACCCTCCAATTGAGATA	AGTCGGCAGGGTAGGAACATTC
Exon 2	TTGATGGGACACATTCAGACCA	CCGCTTTATGTTTCAATGCTGC
Exon 3	CTCTTTGACCTTTGTATTCCC	TTGACTCAGAAACGCCACTGTT
Exon 4	GCACTATGATCCAGGTACAA	TCAGCTCTCCACACCATAAGG
Sequencing		
Exon 1	TGAGACAGGGTCTGGCTCTCTGT	GTGCTTCACACTCTCCCTTA
	CAGTAGCGTGGCACCACCTTCT	CGCCTCTTCCTGTACATCTT
	ATGGCCCAGAAGCGTCCT	GGCCCCAGACACGATTGC
Exon 2	CCACCTTGTTTGGAGTAACC	CTGGCACATAGGAAGTCCAC
	TGGGATGAGTGCTGAGGATA	TGTGTAAGGAAGATGTTGGC
Exon 3	CTTCAGGACACAGTGGATCT	TTCCAGTGACTCATGCAAGC
Exon 4	ATGGTCATTCCCCTTTTACA	GTCCCTCCTAAGAGCTGCAA
		AGGCTGGAGTGTAATCTGGA

Results

We encountered a patient treated with gemcitabine and cisplatin who developed extremely severe toxicities (grade 4 neutropenia, thrombocytopenia, and stomatitis and grade 3 rash, fatigue, and febrile neutropenia). To clarify the cause of these life-threatening toxicities, we determined the plasma levels of gemcitabine and its metabolite, dFdU, and the genotypes of *CDA* encoding a major gemcitabine-metabolizing enzyme, cytidine deaminase, of this patient (patient 1) and the other five gemcitabine/cisplatin-administered patients (patients 2-6).

Pharmacokinetics. Plasma concentration-time profiles of gemcitabine and dFdU are shown in Figs. 1 and 2, and pharmacokinetic variables are summarized in Table 2. The maximum plasma gemcitabine concentration (C_{max}) and area under the concentration-time curve of patient 1 were about twice and five times higher, respectively, than the average values of patients 2 to 6. In patient 1, gemcitabine clearance was decreased to one fifth of the average value of the other five cases, and the terminal phase half-life ($T_{1/2}$) of gemcitabine was four times longer than the average value in patients 2 to 6. The C_{max} and area under the concentration-time curve of dFdU in patient 1 were one third and one half, respectively, of the average values of patients 2 to 6. The area under the concentration-time curve ratio (dFdU/gemcitabine) of patient 1 was about one tenth of the average value in patients 2 to 6.

Genotypes. The results of *CDA* genotyping analysis are shown in Table 3. We only found three known single nucleotide polymorphisms (SNP) in the coding regions in these patients. Patient 1 was homozygous for 208G>A ($Ala^{70}Thr$) in exon 2 (10), but had homozygous wild-type alleles for the other SNPs in exons 1 and 4. All of the other patients carried the homozygous wild-type alleles in exon 2. Thus, it was assumed that the increased plasma gemcitabine levels in patient 1 might have been caused by the $Ala^{70}Thr$ substitution in cytidine deaminase.

Discussion

There was no nephrotoxicity or neurotoxicity in patient 1, which is specifically associated with cisplatin (11). In addition,

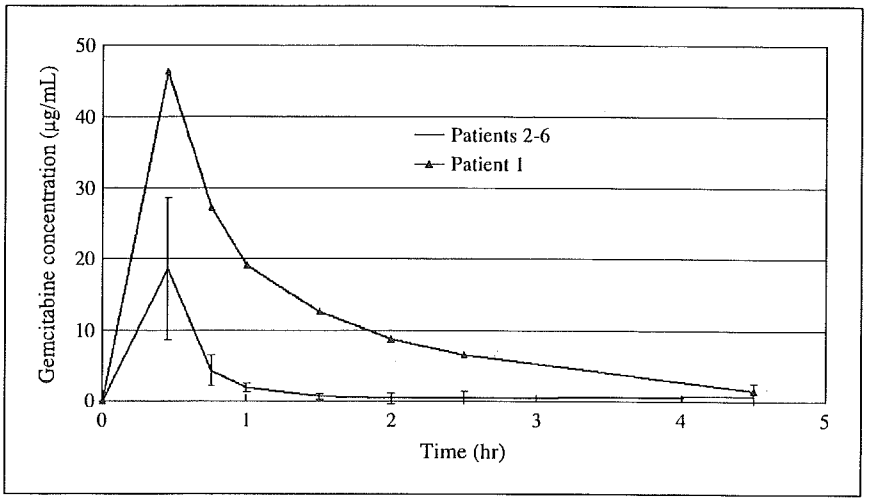

Fig. 1. Plasma disposition curve for gemcitabine in patient 1 (▲) and mean curve for patients 2 to 6 (*solid line*). Bars, SD.

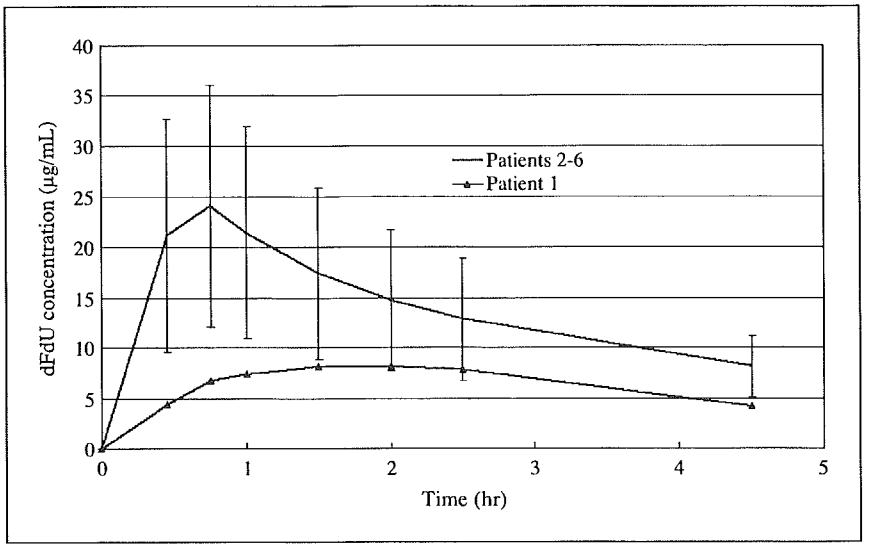

Fig. 2. Plasma disposition curve for dFdU in patient 1 (▲) and mean curve for patients 2 to 6 (*solid line*). Bars, SD.

the skin rash observed in patient 1 is common (with a reported 30.1% occurrence) in patients treated with gemcitabine in single-agent therapy (1). Therefore, we considered that the severe toxicity profile observed in patient 1 was mainly caused by the administration of gemcitabine.

Because the average pharmacokinetic profiles of gemcitabine and dFdU in patients 2 to 6 were almost the same as the population pharmacokinetic profiles in phase I and late phase II trials in Japan (12–14), the pharmacokinetic profiles of patients 2 to 6 can be regarded as standard for a Japanese population. Therefore, the plasma gemcitabine levels of patient 1 were remarkably high. Because the DLST of gemcitabine and cisplatin were negative in patient 1, the toxicities, especially a severe systemic rash including stomatitis and purpura, were unlikely to have been caused by drug allergies, such as Stevens-Johnson syndrome. Thus, the exposure to increased levels of gemcitabine is most likely responsible for the severe toxicities experienced in patient 1.

The patient backgrounds showed no major difference in age, body surface area, and performance status among patients with and without severe toxicities; age ranged from 45 to 69 years, the bovine serum albumin ranged from 1.42 to 1.78 m^2, and Eastern Cooperative Oncology Group performance status ranged from 0 to 1 (patient 1: 45 years, 1.78 m^2, performance status 0). None of the patients had received any prior chemotherapy or radiotherapy. It was unlikely that the patient backgrounds other than the *CDA* genotype caused the abnormal pharmacokinetics observed in patient 1.

Patient 1 was homozygous for the SNP 208G>A ($Ala^{70}Thr$), and all of the other patients carried the homozygous wild-type allele. Patient 1 carried no other known nonsynonymous and synonymous *CDA* polymorphisms (79A>C and 435C>T, respectively). The variant CDA enzyme with Thr^{70} was reported to show 40% and 32% of the activity of the wild-type for cytidine and 1-β-D-arabinofuranosylcytosine substrates in an *in vitro* experiment, respectively (10). Thus, the

Table 2. Compartment-independent pharmacokinetic variables of gemcitabine and its metabolite, dFdU

	Patient 1	Patients 2-6 (mean ± SD)
Gemcitabine		
C_{max} (μg/mL)	46.42	22.28 ± 5.08
AUC_{∞} (μg hour/mL)	54.54	10.88 ± 1.64
Cl (L per h per m^2)	18.34	93.17 ± 15.61
$T_{1/2}$ (h)	0.97	0.26 ± 0.03
V_z (L/m^2)	25.62	35.2 ± 7.47
dFdU		
C_{max} (μg/mL)	8.19	28.75 ± 4.09
AUC_{∞} (μg h/mL)	41.58	106.13 ± 31.44
Cl/F (L per h per m^2)	24.05	10.04 ± 2.98
$T_{1/2}$ (h)	2.17	2.46 ± 0.52
V_z (L/m^2)	75.4	34.29 ± 6.6
AUC ratio (dFdU/gemcitabine)	0.76	9.68 ± 2.05

Abbreviations: C_{max}, maximum plasma concentration; AUC_{∞}, area under the concentration-time curve; Cl, clearance; $T_{1/2}$, terminal-phase half-life; V_z = Dose / (λ_z × AUC); λ_z, elimination rate constant at terminal phase; *F*, metabolite fraction (*F* can be assumed to lie between 0.90 and 0.95).

Table 3. Genotypes of the three known polymorphic loci in exons of the *CDA* gene

Patient	Exon 1, 79A>C, K27Q	Exon 2, 208G>A, A70T	Exon 4, 435C>T, T145T (silent)
1	A/A	A/A	C/C
2	A/C	G/G	C/T
3	A/A	G/G	C/C
4	A/A	G/G	C/T
5	A/A	G/G	C/C
6	C/C	G/G	C/T