reduced activity of the variant enzyme with Thr^{70} might have resulted in the abnormal pharmacokinetics in patient 1.

The allelic frequency of the 208G>A polymorphism of the *CDA* gene in the Japanese population is 4.3% (10). Recently, genetic polymorphisms in the gemcitabine metabolic pathway, including *CDA* SNPs in Europeans and Africans, were reported by Fukunaga et al. (15). The SNP 208G>A was not detected in Europeans, whereas the allelic frequency of 208A was 0.125 in Africans (15). According to the two previous studies (10, 15), frequencies of homozygous 208G>A individuals in the Japanese and African populations were estimated to be about 0.18% and 1.56%, respectively. Therefore, severe toxicity caused by 208G>A could occur more frequently in Africans than in Japanese.

Based on the results of the analyses of the pharmacokinetic profiles and the 208G>A SNP, we can conclude that decreased CDA activity might have been responsible for the severe drug toxicity observed in this Japanese cancer patient.

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Original Paper

Prognostic Factors in Patients with Advanced Biliary Tract Cancer Receiving Chemotherapy

Toshimitsu Saisho MD, Takuji Okusaka MD, PhD, Hideki Ueno MD, PhD
Chigusa Morizane MD, Shuichi Okada MD, PhD
Hepatobiliary and Pancreatic Oncology Division, National Cancer Center Hospital, Tokyo, Japan
Corresponding Author: Takuji Okusaka MD, PhD, Hepatobiliary and Pancreatic Oncology Division
National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan
Tel: +81 3 3542 2511, Fax: +81 3 3542 3815, E-mail: tokusaka@ncc.go.jp

KEY WORDS:
Biliary tract cancer; Chemotherapy; Prognosis; Prognostic factors

ABBREVIATIONS:
Biliary Tract Cancer (BTC); World Health Organization (WHO); Eastern Cooperative Oncology Group (ECOG); Median Survival Time (MST); C-Reactive Protein (CRP); Lactate Dehydrogenase (LDH); Carcinoembryonic Antigen (CEA); Carbohydrate Antigen 19-9 (CA19-9)

ABSTRACT

Background/Aims: Prognostic factors in patients with advanced biliary tract cancer receiving chemotherapy have not been fully examined. This study investigated prognostic factors in patients with advanced biliary tract cancer receiving chemotherapy.

Methodology: Sixty-five consecutive chemo-naive patients with advanced biliary tract cancer, who received chemotherapy, were analyzed retrospectively to investigate prognostic factors.

Results: Median survival time and overall survival rates at 1 and 2 years were 180 days, 21%, and 5%, respectively. By multivariate analysis using the Cox proportional hazards model, performance status of 0, 1, serum C-reactive protein level of ≤1.0mg/dL, serum albumin level of ≥3.5g/dL, serum lactate dehydrogenase level of ≤500 U/L, and being female were independent favorable prognostic factors. A prognostic index based on the coefficients of these prognostic factors was used to classify patients into three groups with good, intermediate, and poor prognoses. The median survival times for these three groups were 246, 152, and 33 days, respectively.

Conclusions: The results may be helpful for predicting life expectancy, determining treatment strategies, and designing future clinical trials in patients with advanced biliary tract cancer.

INTRODUCTION

Biliary tract cancer (BTC) is diagnosed at an advanced stage in most patients despite the recent improvement in diagnostic techniques. Even if resection is performed, the recurrence rate is extremely high (1-5). Therefore, to improve the prognosis of BTC patients, effective non-surgical treatment is indispensable. With regard to chemotherapy for advanced BTC, numerous clinical trials have been conducted (6-10). However, at present, chemotherapy for advanced BTC has been of limited value in clinical practice, because the majority of patients do not respond well and suffer only the adverse effects of chemotherapy.

The identification of prognostic factors will be helpful for predicting life expectancy, and designing and analyzing clinical trials. However, prognostic factors in BTC patients treated with chemotherapy have not been fully examined. The current study was designed to retrospectively analyze several variables that may affect survival in patients with advanced BTC receiving chemotherapy. To our knowledge, this is the first study concerning prognostic factors and a staging system for patients with advanced BTC receiving chemotherapy.

METHODOLOGY

Patients

The study group included 65 consecutive chemo-naive patients with advanced BTC who had received chemotherapy at the National Cancer Center Hospital, Tokyo, Japan, between April, 1988 and March, 2001 **(Table 1)**. None had received any anti-cancer treatment except for surgical resection before chemotherapy. All diseases were diagnosed as advanced BTC using various imaging modalities including chest X-ray, ultrasonography, and computed tomography. Pathological confirmation of adenocarcinoma was obtained in 62 patients (95%) by a surgical procedure or by a fine-needle aspiration biopsy. Cytological examination of the peritoneal fluid was performed for patients with intraperitoneal fluid collection, and peritoneal dissemination was diagnosed by positive cytology. Patients with obstructive jaundice underwent percutaneous transhepatic or endoscopic

TABLE 1 Chemotherapeutic Regimens for Advanced Biliary Tract Cancer

Regimen	No. of patients
Fluorouracil	1
Fluorouracil + methotrexate	1
Cisplatin	8
UFT (tegafur + uracil)	2
S-1 (tegafur + gimeracil + oteracil potassium)	9
Fluorouracil + mitomycin C	1
Fluorouracil + cisplatin + epirubicin	43

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TABLE 2 Patient Characteristics

Characteristics	No. of patients (%)
Age (yrs) *	63 (28-76)
Gender	
Male	33 (51)
Female	32 (49)
Primary tumor location	
Gallbladder	53 (82)
Extrahepatic bile duct	12 (18)
Prior surgical resection (+)	16 (25)
Performance status	
0	31 (48)
1	28 (43)
2	6 (9)
Biliary drainage (+)	20 (31)
White blood cell (/mm^3) *	7,200 (3,500-25,200)
Hemoglobin (g/dL) *	11.7 (7.7-15.5)
Albumin (g/dL) *	3.6 (2.4-4.3)
Total bilirubin (mg/dL) *	0.8 (0.3-4)
LDH (IU/L) *	429 (228-5,178)
C-reactive protein (mg/dL) *	1.3 (0.0-17.1)
CEA (ng/mL) *	13.6 (1-13,680)
CA19-9 (U/mL) *	209 (1-1,480,000)

* median (range); LDH: lactic dehydrogenase; CEA: carcinoembryonic antigen; CA19-9: carbohydrate antigen 19-9.

biliary drainage before chemotherapy. The tumor response was evaluated according to the criteria of the World Health Organization (WHO) every 4 weeks after the first course of chemotherapy. Survival was measured from the first day of chemotherapy until death from cancer or the last day of follow-up.

Factors Analyzed

Pretreatment clinical variables were investigated for their relation to survival by univariate analysis and multivariate analysis. The pretreatment variables were chosen by considering the possible effects on the prognosis as indicated by previous investigations (11,12) or suggested from our own clinical experience. The variables, divided into two subgroups, were as follows: age (<60 or ≥60 years), gender (male or female), prior surgical resection for BTC (presence or absence), Eastern Cooperative Oncology Group (ECOG) performance status (13) (0, 1 or 2), biliary drainage (presence or absence), white blood cell count (<7,000 or ≥7,000/mm^3), hemoglobin level (<12 or ≥12g/dL), serum albumin level (<3.5 or ≥3.5g/dL), serum total bilirubin level (<1.0 or ≥1.0mg/dL), serum lactate dehydrogenase (LDH) level (<500 or ≥500 IU/L), and serum C-reactive protein (CRP) level (<1.0 or ≥1.0mg/dL), as host-related variables; primary tumor location (extrahepatic bile duct or gallbladder), serum carcinoembryonic antigen (CEA) level (<10 or ≥10ng/mL), and serum carbohydrate antigen 19-9 (CA 19-9) level (<1,000 or ≥1,000 U/mL), as tumor-related variables.

Statistical Methods

Actuarial survival probabilities were calculated using the Kaplan-Meier method (14), and compared with the log-rank test (15). Multivariate analysis was performed following the Cox proportional hazards model (16). A prognostic index was calculated based on the regression coefficients of the variables identified from multivariate analysis. All P values presented in this report are of the two-tailed type; $P \leq 0.05$ was considered to be statistically significant.

RESULTS

Patient Characteristics

The characteristics of the patients are shown in **Table 2**. Of the 65 patients with BTC, 33 were males and 32 females. The median age was 63 years old (range, 28-76). Performance status was 0, 1 in 59 patients (91%) and 2 in 6 patients (9%). The primary tumor location was the gallbladder in 53 (82%) and the extrahepatic bile duct in 12 patients (18%). Fifty-six patients (86%) had distant metastasis. Twenty patients (31%) underwent percutaneous or endoscopic biliary drainage before chemotherapy. Of 65 patients, 6 were evaluated as showing a partial response, twenty-eight showed no change and 29 showed progressive disease. The tumor response was not evaluated in 2 patients due to early death related to chemotherapy.

Survival

The median survival time and survival rate at 1 and 2 years in 65 patients were 180 days, 21%, and 5%, respectively **(Figure 1)**. At the time of analysis, 63 patients had died; the causes of death were cancer-related in 61 patients (97%) and chemo-related in 2 (3%).

Univariate Analysis

Table 3 lists the results of univariate analyses in relation to each variable. Patients with a performance status of 0, 1 showed better survival than those with a performance status of 2 (P=0.01); one of the 6 patients with a performance status of 2 survived 13 months, but the other 5 survived less than 4 months. Moreover, survival was significantly affected by serum albumin level (P<0.01), serum CRP level (P<0.01), and serum LDH level (P=0.01).

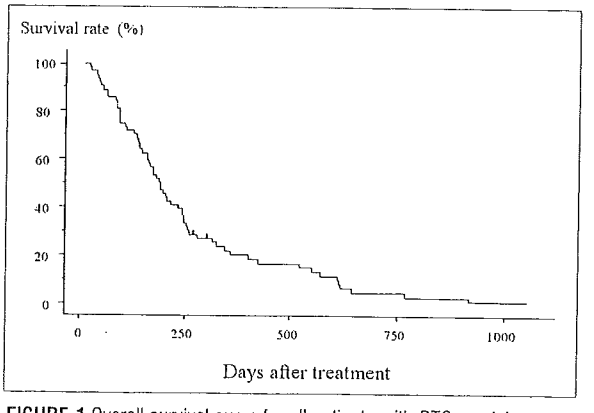

FIGURE 1 Overall survival curve for all patients with BTC receiving chemotherapy. Tick marks indicate censored cases.

Multivariate Analysis

In addition to gender and age, variables with prognostic significance in univariate analysis were subsequently included in the multivariate Cox regression model. Among them, 5 factors, performance status, serum CRP level, serum albumin level, serum LDH level, and gender were identified as independent prognostic factors **(Table 4)**.

Risk Groups Based on the Regression Model: For the clinical application of these findings, a prognostic index was calculated based on the regression coefficients derived from the five variables identified by multivariate analysis. The index equation was as follows: 1.97 (0, performance status of 0, 1; 1, performance status of 2) + 0.94 (0, CRP <1.0mg/dL; 1, CRP ≥1.0mg/dL) + 0.81 (0, albumin ≥3.5mg/dL; 1, albumin <3.5mg/dL) + 0.73 (0, LDH <500 IU/L; 1, LDH ≥500 IU/L) + 0.58 (0, female; 1, male). The individual index values for the patients ranged from 0.00 to 5.03. The patients were then classified into three groups according to the prognostic index, as follows: group A, a prognostic index <1.50 (25 patients); group B, a prognostic index from 1.50 to 2.50 (32 patients); group C, a prognostic index >2.50 (8 patients). The survival curves for these groups are shown in **Figure 2**. The median survival times in groups A, B, and C were 246, 152, 33 days, respectively. There was a significant difference among these three groups in the survival time ($P<0.01$).

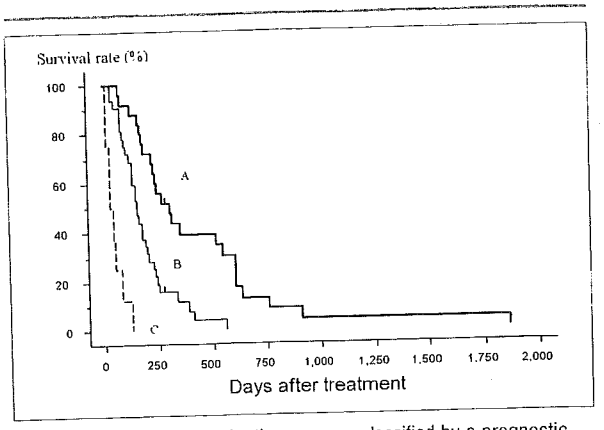

FIGURE 2 Survival curves for three groups classified by a prognostic index based on the findings of multivariate analysis.
Group A, prognostic index less than 1.5 (25 patients); Group B, prognostic index from 1.5 to 2.5 (32 patients); Group C, prognostic index greater than 2.5 (8 patients). Tick marks indicated censored cases.

TABLE 3 Univariate Analysis of Prognostic Factors Associated in Patients with Advanced Biliary Tract Cancer

Variable		No. of patients	Median survival (days)	*P* value
Age, years	<60	27	186	
	≥60	38	164	0.93
Gender	Male	33	164	
	Female	32	186	0.64
Primary tumor location	Gallbladder	53	180	
	Extrahepatic bile duct	12	138	0.25
Prior surgical resection	+	16	150	
	-	49	180	0.70
Performance status	0, 1	59	186	
	2	6	47	0.01
Biliary drainage	+	20	186	
	-	45	165	0.46
White blood cell	<7,000/mm³	35	236	
	≥7,000/mm³	30	138	0.14
Hemoglobin	<12g/dL	34	138	
	≥12g/dL	31	238	0.07
Albumin	<3.5g/dL	23	124	
	≥3.5g/dL	42	224	<0.01
Total bilirubin	<1.0mg/dL	40	181	
	≥1.0mg/dL	25	165	0.92
LDH	<500 IU/L	44	199	
	≥500 IU/L	21	152	0.01
C-reactive protein	<1.0mg/dL	28	250	
	≥1.0mg/dL	37	138	<0.01
CEA	<10ng/mL	31	206	
	≥10ng/mL	34	155	0.36
CA19-9	<1,000 U/mL	40	180	
	≥1,000 U/mL	24	172	0.82

LDH: lactic dehydrogenase; CEA: carcinoembryonic antigen; CA19-9: carbohydrate antigen 19-9.

TABLE 4 Significant Prognostic Factors Identified in 65 Patients as Determined by Multivariate Analysis with Cox Proportional Hazards Model

Variable	Hazards ratio Coefficient (β)	(95% confidence interval)	*P* value
Performance status	1.97	7.14 (2.67-19.06)	<0.01
C-reactive protein	0.94	2.57 (1.46-4.53)	<0.01
Albumin	0.81	2.24 (1.23-4.09)	<0.01
LDH	0.73	2.07 (1.12-3.84)	0.02
Gender	0.58	1.79 (1.02-3.14)	0.04

LDH: lactic dehydrogenase.

DISCUSSION

The prognosis of patients with advanced BTC is extremely poor, with a median survival of 4-12 months (1,4,5,8,9). To improve the prognosis of this disease, the development of effective chemotherapy is essential. However, chemotherapy for advanced BTC has been of limited value, because the majority of patients does not respond well and suffer only the adverse effects of chemotherapy. Therefore, in chemotherapy for advanced BTC, patient selection with reference to expected survival time may be important. In addition, identifying prognostic factors may be useful for the design of future trials of chemotherapy for BTC. In the present study, we investigated the prognostic factors in patients with advanced BTC receiving chemotherapy. This single institution study was undertaken using unified methods for staging the disease and identical procedures for supportive care throughout, thus enabling us to confirm important prognostic factors.

Among the 14 potential prognostic factors investigated, four factors, performance status, serum CRP level, serum albumin level, and serum LDH level, were identified as a significant predictor of survival by both univariate analysis and multivariate analysis. Moreover, in addition to these four factors, gender was

found to have independent prognostic value by multivariate analysis.

The performance status and serum albumin have been recognized as important prognostic factors in a variety of malignancies (17-21). The performance status is a simple but widely used method for evaluating the physical condition of cancer patients, and the serum albumin level also reflects the physical condition, especially the influence of nutritional status. The prognostic value of serum CRP and LDH have also been reported in a variety of neoplastic diseases (18,20,22-24). Serum CRP, which is known as a marker of the acute-phase protein response, is observed in different pathological states such as infection, inflammation, and malignancy. However, the elevated serum CRP in our patients with BTC was likely to be a consequence of the underlying malignancy, because no patients showed evidence of infection before treatment. It can be argued that the increasing bulk of the disease provides potential for greater tumor necrosis and associated inflammation, and, thus, serum CRP and LDH simply may reflect tumor burden. It was reported that females have a better prognosis than males in a large variety of malignant diseases (20,21,25-28). It is suggested that gender specific hormones may play a role in the regulation of tumor growth and should thus be taken into consideration as a possible reason for the survival advantage of females. However, the reasons for the better prognosis of females are still not fully clarified.

The prognosis of advanced BTC patients was poor in the present study; the median survival time was 180 days, and about 14% had died within 2 months after the beginning of chemotherapy. To predict patient survival more accurately, a prognostic index based on independent prognostic factors was proposed. The patients in the present study could be classified into three groups with good, intermediate, and poor prognosis. This prognostic index may therefore be useful in making an accurate prediction of survival in patients with advanced BTC and determining treatment strategies, although the validation of this model has to be tested using an independent data set in future studies. The poor prognosis group may be treated with different experimental approaches or may be offered only supportive care to maintain their quality of life.

In conclusion, performance status, serum CRP level, serum albumin level, serum LDH level, and gender were identified as significant independent prognostic factors in patients with advanced BTC receiving chemotherapy. The present findings may be helpful in predicting life expectancy, determining treatment strategies, and designing future clinical trials in patients with BTC.

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A Phase II Trial of Continuous Infusion of 5-Fluorouracil, Mitoxantrone, and Cisplatin for Metastatic Hepatocellular Carcinoma

Masafumi Ikeda, M.D.
Takuji Okusaka, M.D.
Hideki Ueno, M.D.
Yoriko Takezako, M.D.
Chigusa Morizane, M.D.

Division of Hepatobiliary and Pancreatic Oncology, National Cancer Center Hospital, Tokyo, Japan.

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Address for reprints: Masafumi Ikeda, M.D., Division of Hepatobiliary and Pancreatic Oncology, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan; Fax: (011) 81-3-3542-3815; E-mail: masikeda@ncc.go.jp

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BACKGROUND. The aim of the current study was to evaluate the antitumor activity and toxicity of continuous infusion of 5-fluorouracil, mitoxantrone, and cisplatin (FMP therapy) in chemotherapy-naive patients with metastatic hepatocellular carcinoma (HCC).

METHODS. Fifty-one patients with metastatic HCC who had not undergone previous systemic chemotherapy were enrolled. The therapy consisted of intravenous administration of 80 mg/m^2 cisplatin and 6 mg/m^2 mitoxantrone on Day 1 and continuous intravenous infusion of 450 mg/m^2 5-fluorouracil per day on Days 1–5. The treatment was repeated every 4 weeks for a maximum of 6 courses with dose adjustments based on the observed toxic effects if there was no evidence of tumor progression or unacceptable toxicity.

RESULTS. Of the 51 enrolled patients, 14 (27%) achieved a partial response (95% confidence interval, 16–42%) with a median duration of 7.6 months (range, 2.3–18.4 months). Twenty-seven patients (53%) showed no change and 9 (18%) had progressive disease. The median survival time, 1-year survival rate, and median progression-free survival time for all patients were 11.6 months, 44.3%, and 4.0 months, respectively. The main Grade 3 and 4 toxicities were leukocytopenia (67%), neutropenia (71%), thrombocytopenia (27%), and elevated levels of aspartate aminotransferase (37%) and alanine aminotransferase (41%). These symptoms were generally brief and reversible, with the exception of one treatment-related death due to acute hepatic failure.

CONCLUSIONS. FMP therapy had significant antitumor activity with acceptable toxicity in patients with metastatic HCC. ***Cancer* 2005;103:756–62.**

KEYWORDS: hepatocellular carcinoma, chemotherapy, metastasis, 5-fluorouracil, mitoxantrone, cisplatin.

Hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide. It is highly prevalent in Africa and Asia, and in recent years, its incidence has been increasing in Western countries. Although a range of therapeutic options are available, the efficacy of these methods remains unsatisfactory and the prognosis of patients with HCC is still poor.[1–3] Curative therapies, such as hepatic resection and liver transplantation, are applicable to only a small group of patients because of poor liver function, metastasis, or both. Local treatments, such as percutaneous ethanol injection, radiofrequency ablation, or transcatheter arterial embolization, have been reported to be useful for treating patients with unresectable disease. Unfortunately, however, in most patients with HCC, the disease progresses to an advanced stage for which effective local treatment is

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not available.[1–3] Currently, patients with HCC at this stage generally undergo chemotherapy, but this has limited value in clinical practice. The activity of single agents is limited, with only a few drugs achieving a response rate > 10%. Moreover, combination chemotherapy has proven equally disappointing because it rarely results in any meaningful clinical improvement.[1–23] Thus, despite decades of trials of various agents, no chemotherapeutic drug has shown sufficient efficacy to be acknowledged as a standard therapy. Therefore, an effective chemotherapy regimen is a much sought after goal.

Mitoxantrone is a synthetic anthraquinone, with antitumor activity against human tumor cell lines and animal models of leukemia comparable and often superior to that of doxorubicin.[4] Clinical trials of this drug have demonstrated moderate activity against HCC with a lower incidence of adverse effects, such as hematologic and cardiac toxicity, than other chemotherapeutic agents.[5–8] Cisplatin has a broad spectrum of antineoplastic activity, and there have been several reports demonstrating favorable effects of this agent on HCC.[8,9] Between the two drugs, significant therapeutic synergism has been observed against other malignancies, although the mechanism has not been elucidated fully.[4] The pyrimidine antimetabolite, 5-fluorouracil (5-FU), was the first reported chemotherapeutic agent to be used in the treatment of HCC, and there has been much interest in the possibility of increasing 5-FU activity[10–16] and therapeutic selectivity with so-called modulators such as cisplatin.[17,18] In clinical trials, combination chemotherapy including 5-FU and cisplatin has demonstrated high response rates in patients with HCC.[17,18] Therefore, we conducted a Phase II trial to evaluate the antitumor activity and toxicity of the systemic chemotherapy regimen of 5-FU, mitoxantrone, and cisplatin (FMP therapy) in patients with metastatic HCC.

MATERIALS AND METHODS

Eligibility

Patients eligible for study entry had HCC with extrahepatic metastases. The diagnosis was made by either histologic examination or typical computed tomographic scans, angiographic findings, and elevated serum α-fetoprotein levels (AFP). Eligibility criteria included the following factors: age 20–74 years; an Eastern Cooperative Oncology Group (ECOG) performance status score of 0–2; bidimensionally measurable disease; an estimated life expectancy $\geq$ 8 weeks after study entry; no previous systemic chemotherapy excluding chemoembolization; adequate hematologic function (hemoglobin level $\geq$ 10 g/dL, leukocyte count $\geq$ 3000 cells/mm^3, neutrophil count $\geq$ 1500 cells/mm^3, and platelet count $\geq$ 70,000 cells/mm^3); adequate hepatic function (serum total bilirubin level $\leq$ 2.0 mg/dL, serum albumin level $\geq$ 3.0 g/dL, and serum aspartate aminotransferase (AST)/alanine aminotransferase (ALT) levels $\leq$ 200 IU/L); adequate renal function (serum creatinine level within normal limits and creatinine clearance $\geq$ 60 mL per minute); and written informed consent. Patients with tumor thrombosis in the main portal trunk were excluded, because such patients have a reportedly poor prognosis and tumor response to systemic chemotherapy.[24–27] Previous local therapy for intrahepatic lesions before this treatment, such as hepatic resection, percutaneous local ablation, or transcatheter arterial chemoembolization, was allowed if it had not been done within the previous 4 weeks. Bone metastases were not regarded as measurable lesions.

The exclusion criteria were active infection, severe heart disease, refractory pleural effusion or ascites, known metastases to the central nervous system, severe mental disorder or encephalopathy, active gastroduodenal ulcer or esophageal bleeding within 1 month, active concomitant malignancy, pregnant and lactating females, females of childbearing age unless using effective contraception, and other serious medical conditions.

Pretreatment evaluation included a complete history and physical examination. The laboratory procedures were a complete differential blood count, biochemistry tests, viral markers including serum hepatitis B surface antigen (HBsAg) and serum hepatitis C virus (HCV) antibody, urinalysis, and tumor markers including serum levels of AFP and protein induced by vitamin K absence or antagonist-II (PIVKA II). All patients underwent electrocardiography, chest radiography, gastroscopy, and computed tomography (CT) scans within 4 weeks before chemotherapy. HCC was diagnosed by histologic examination or distinctive findings of CT scans and/or angiography.

Treatment Schedule

All eligible patients were treated with the FMP regimen. 5-FU was administered as a continuous intravenous infusion at a dose of 450 mg/m^2 on Days 1–5. Mitoxantrone was administered as an intravenous infusion at a dose of 6 mg/m^2 on Day 1. Cisplatin was administered as an intravenous infusion at a dose of 80 mg/m^2 over a 2-hour period on Day 1 with standard hydration. In subsequent courses, the dose of each drug was adjusted to the toxicities observed. For example, patients who had experienced Grade 4 hematologic toxicities or Grade 3 neutropenia and/or leukocytopenia with high fever ($\geq$ 38 °C) received 4 mg/m^2 mitoxantrone, patients who had experienced

Grade 3 or 4 stomatitis, diarrhea, and/or hand–foot syndrome received 400 mg/m^2 5-FU, patients who had experienced Grade 3 or 4 elevated levels of serum creatinine and/or creatinine clearance < 40 mL per minute did not receive cisplatin. Antiemetics including 5-HT4 receptor antagonist and dexamethasone were administered prophylactically. Granulocyte colony-stimulating factor was given when neutropenia and/or leukocytopenia of Grade 3/4 with high fever (≥ 38 °C) were observed. If there was no evidence of tumor progression or unacceptable toxicity, the treatment was repeated every 4 weeks until a maximum of 6 courses were achieved. Patients who were refractory to this regimen were allowed to receive other anticancer treatment at their physician's discretion.

Response and Toxicity Evaluation

The objective tumor response was assessed by CT scan every 4 weeks after the beginning of FMP therapy. Response and toxicity were evaluated according to World Health Organization guidelines.[28] The best overall response was recorded for each patient. During this treatment, a complete differential blood count, serum chemistry profile, and urinalysis were undertaken at least weekly. Serum AFP and PIVKA II levels were measured every 4 weeks. Disease progression-free survival (PFS) was defined as the time from the date of initial treatment to first documentation of disease progression or death. The duration of response was defined as the interval from the onset of a partial response (PR) to the first evidence of disease 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

The primary end point of the current study was the efficacy and toxicity of this regimen, and the secondary end point was survival and disease PFS. The number of patients to be enrolled was planned using a 2-step design[29] based on the assumptions that the expected response rate was 30%, the response rate judged as no activity was 15%, the α error was 10%, and the β error was 10%. An interim analysis was planned after 25 patients had been enrolled. If 1 or 2 of the first 25 patients had a PR or complete response (CR), the study was to be ended. If a response was detected in > 2 of the first 25 patients studied, an additional 25 patients were to be studied in a second stage of accrual to estimate more precisely the actual response rate. This population was defined as including any patients who received at least one course of study medication. The survival time and the disease PFS were calculated by the Kaplan–Meier method.[30]

TABLE 1
Patient Characteristics

Characteristics	No. of patients (%)
Median age (range)	61 (34–74 yrs)
Gender	
Male	47 (92)
Female	4 (8)
ECOG performance status	
0	43 (84)
1	8 (16)
History of blood transfusion	
Positive	10 (20)
Alcohol abuse[a]	
Positive	12 (24)
Hepatitis B surface antigen	
Positive	20 (39)
Hepatitis C virus antibody	
Positive	27 (53)
Previous treatment	
Hepatic resection	35 (69)
Percutaneous local ablation	10 (20)
Transcatheter arterial chemoembolization	30 (59)
None	7 (14)
Child–Pugh stage	
A	*45 (88)*
B	*6 (12)*
Organs affected by metastases	
Lung	36 (71)
Lymph nodes	24 (47)
Bone	7 (14)
Adrenal gland	4 (8)
Peritoneum	2 (4)
Median CLIP score (range)	2 (0–5)
Median α-fetoprotein level (ng/dL) (range)	190 (3–509,500)
Median PIVKA II level (mAU/mL) (range)	1420 (10–185,200)

ECOG: Eastern Cooperative Oncology Group; CLIP: Cancer of the Liver Italian Program; PIVKA II: protein induced by vitamin K absence or antagonist-II.
[a] Ethanol intake ≥ 80 g per day for ≥ 5 years.

This Phase II trial was approved by the institutional review board of the National Cancer Center (Tokyo, Japan).

RESULTS

Patient Characteristics

Fifty-one patients were enrolled between September 1993 and January 2003 at the National Cancer Center Hospital. The diagnosis of HCC was confirmed by histologic examination in 45 patients (88%). In the remaining 6 patients (12%), diagnosis was based on typical CT scan findings, angiographic findings, and elevated serum AFP levels. The characteristics of the patients are listed in Table 1. There were 47 males and 4 females with a median age of 61 years (range, 34–74 years). HBsAg and HCV antibody were positive in 20 patients (39%) and 27 patients (53%), respectively. All

TABLE 2
Toxicity

Characteristics	Grade (WHO criteria) 1 (%)	2 (%)	3 (%)	4 (%)
Hematologic toxicity				
Leukocytopenia	3 (6)	14 (27)	28 (55)	6 (12)
Neutropenia	2 (4)	12 (24)	15 (29)	21 (41)
Anemia	15 (29)	13 (25)	2 (4)	0 (0)
Thrombocytopenia	9 (18)	16 (31)	12 (24)	2 (4)
Nonhematologic toxicity				
Nausea/emesis	27 (53)	10 (20)	6 (12)	0 (0)
Stomatitis	17 (33)	2 (4)	1 (2)	0 (0)
Diarrhea	6 (12)	2 (4)	0 (0)	0 (0)
Hiccup	24 (47)	2 (4)	0 (0)	0 (0)
Fatigue	27 (53)	5 (10)	5 (10)	0 (0)
Sensory neuropathy	5 (10)	0 (0)	0 (0)	0 (0)
Alopecia	13 (25)	17 (33)	0 (0)	0 (0)
Skin rash	3 (6)	0 (0)	0 (0)	0 (0)
Hand-foot syndrome	0 (0)	0 (0)	0 (0)	0 (0)
Elevated total bilirubin level	24 (47)	3 (6)	1 (2)	1 (2)
Elevated Aspartate aminotransferase level	12 (24)	12 (24)	10 (20)	9 (18)
Elevated Alanine aminotransferase level	15 (29)	11 (22)	11 (22)	10 (20)
Elevated alkaline phosphatase level	13 (25)	2 (4)	1 (2)	0 (0)
Elevated creatinine level	8 (16)	0 (0)	0 (0)	0 (0)
Elevated blood urea nitrogen level	12 (24)	1 (2)	0 (0)	0 (0)

WHO: World Health Organization.

patients showed had a good ECOG performance status score of 0–1. There were 45 (88%) and 6 (12%) patients with Child–Pugh Stage A and B,[31] respectively. The major sites of extrahepatic metastases were the lungs (n = 36 [71%]) and the lymph nodes (n = 24 [41%]). The median Cancer of the Liver Italian Program (CLIP) score was 2 (range, 0–5).[32] The median serum AFP and PIVKA II levels were 190 ng/dL (range, 3–509,500 ng/dL) and 1420 mAU/mL (range, 10–185,200 mAU/mL), respectively.

A total of 150 courses were given, with a median of 2 courses (range, 1–6 courses) per patient. The reasons for treatment discontinuation were completion of treatment (6 courses) in 9 patients (18%), disease progression in 36 patients (71%), refusal of treatment in 5 patients (10%), and treatment-related death in 1 patient (2%).

Response

Fifty patients were evaluable for response. The remaining one patient could not be evaluated because of treatment-related death. No patient achieved a CR. Fourteen patients achieved a PR, giving an overall response rate of 27% (95% confidence interval, 16–42%), and the median duration of response was 7.6 months (range, 2.3–18.4 months). Twenty-seven patients (53%) showed no change and the remaining 9 (18%) had progressive disease. Two patients with a PR underwent surgical resection for residual HCC lesions after six courses of this chemotherapy regimen. These resections were successful and both patients achieved complete clinical remission of disease after surgery.

During the treatments, the serum AFP level was reduced by > 50% in 6 of 28 (21%) patients who had shown a pretreatment level of ≥ 100 U/mL, and the serum PIVKA II level was reduced by > 50% in 21 of 36 (58%) patients who had a pretreatment level of ≥ 100 mAU/mL.

Toxicity

The toxicities observed in the 51 enrolled patients are listed in Table 2. The toxicity represents the maximum grade per patient for the entire course of therapy. One patient died of acute hepatic failure due to neutropenic sepsis on Day 22 of the first course of treatment. Grade 4 leukocytopenia and neutropenia occurred in 6 (12%) and 21 (41%) patients, respectively, but both were generally brief and reversible. Thrombocytopenia and anemia were infrequent and mild. Except for one patient whose death was treatment related, even those who had pancytopenia before treatment tolerated this treatment hematologically.

Elevated AST and ALT levels were frequent nonhematologic adverse effects. Grade 3–4 toxicities were

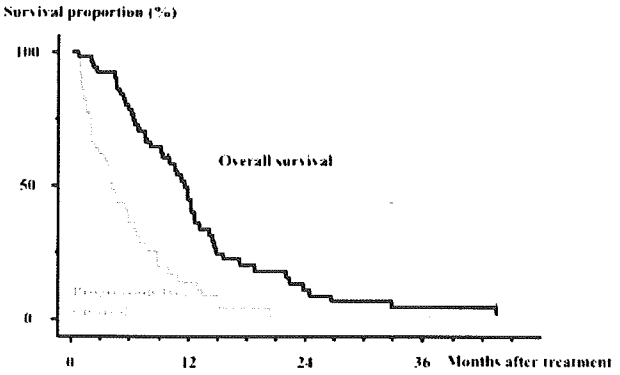

FIGURE 1. Overall survival and disease progression-free survival curves of 51 patients who received 5-fluorouracil, mitoxantrone, and cisplatin therapy for metastatic hepatocellular carcinoma. Tick marks indicate censored patients.

observed as elevated AST levels in 19 patients (37%) and elevated ALT levels in 21 patients (41%), although it was difficult to differentiate between hepatic toxicity and exacerbation of viral hepatitis. These toxicities returned to baseline levels within 1 month, and the patients were able to continue chemotherapy without dose reduction. Grade 3–4 total bilirubin elevation occurred in 2 patients (4%), 1 of whom died of acute hepatic failure due to neutropenic sepsis. However, all patients, except the 1 patient whose death was treatment related, recovered to the initial levels within 1 month without any additional treatment. There were no other serious nonhematologic toxicities.

Dose reductions according to the protocol were required in 22 patients (43%): mitoxantrone dose, 22 patients; 5-FU dose, 2 patients; and cisplatin dose, no patients.

Survival

All enrolled patients were included in the survival assessment. At the time of the analysis, 47 patients had died. The causes of death were tumor progression ($n = 40$), hepatic failure ($n = 3$), rupture of esophageal varices ($n = 1$), cerebral bleeding from brain metastasis ($n = 2$), and treatment-related death ($n = 1$). The median survival time, 1-year survival rate, and median disease PFS time for all patients were 11.6 months, 44.3%, and 4.0 months, respectively (Fig. 1). The median survival times of patients with Child–Pugh Stage A and Stage B disease were 13.2 and 6.4 months, respectively. The median survival times of patients with CLIP scores of 0–2 and 3–5 were 13.6 months and 8.1 months, respectively.

DISCUSSION

Systemic chemotherapy for unresectable HCC remains an important modality of treatment, because not all patients are suitable for effective local treatments such as surgical resection, intraarterial treatment, or local ablative therapy.[1–3] However, it has only limited value in clinical practice, because only a few patients who undergo systemic chemotherapy obtain meaningful palliation and the toxicity of chemotherapy often outweighs its benefits. Furthermore, there has been no convincing evidence so far from prospective randomized trials to suggest that systemic chemotherapy prolongs survival in comparison to no treatment.[1–3] Therefore, it remains mandatory to explore novel therapeutic strategies to improve the response and survival of patients with advanced HCC.

The possible explanations for the lack of response of HCC to anticancer agents are tumor heterogeneity, inducible overexpression of the multidrug resistance gene, and/or inherent resistance by an unexplained mechanism.[1–3] Therefore, combination therapy is considered to be more effective than monotherapy. In the current study, we chose three anticancer agents with synergic effects (i.e., 5-FU, mitoxantrone, and cisplatin). In the past, 5-FU has been administered broadly to patients with HCC, with a large variation in dosages and schedules,[10–16] although as a single agent it has shown a low response rate and no influence on overall survival.[2,3] Mitoxantrone showed a similar tumor response and fewer myelotoxic and cardiotoxic effects than epirubicin or doxorubicin,[5–8] which is considered to be one of the most active chemotherapeutic agents against advanced HCC, with response rates ranging from 3% to 26%. Cisplatin has a broad spectrum of antineoplastic activity, and there have been several reports demonstrating its favorable effects against HCC.[7,8] Furthermore, among these three drugs, significant therapeutic synergism was observed against HCC[8,17,18] or other malignancies.[6] Therefore, we conducted a Phase II trial to evaluate the antitumor activity and toxicity of this combination systemic chemotherapy of 5-FU, mitoxantrone, and cisplatin in patients with metastatic HCC. The study subjects were patients with HCC with extrahepatic metastases because such patients, for whom standard treatments are not indicated, are the most appropriate candidates for clinical trials of systemic chemotherapy.[1–3] To our knowledge, this is the first clinical trial of systemic chemotherapy only for patients with metastatic HCC.

In the current study, 14 of 51 patients achieved a PR (i.e., a response rate of 27%), and adequate tumor shrinkage was induced to allow surgical resection in 2 patients. These results were comparable with, or better than, those of the other reported chemotherapeutic regimens (response rate range, 0–26%).[1–6,8–23] This regimen yielded relatively longer overall survival outcomes (median, 11.6 months) than the other reported

chemotherapeutic regimens (median overall survival range, 4–15.5 months),[1–6,8–23] although the outcome of the patients with metastatic HCC, who were enrolled in our study, was extremely poor.[1–3,24–26] Moreover, using this therapy, the serum AFP level was reduced > 50% in 6 of 28 patients (21%) who had shown a pretreatment level of ≥ 100 U/mL, and the serum PIVKA II level was reduced > 50% in 21 of 36 (58%) patients who had a pretreatment level of ≥ 100 mAU/mL. The response rates of tumor markers were also favorable.[9,12–15,19,20,23] Therefore, FMP therapy has significant antitumor activity against metastatic HCC.

Patients with HCC tend to experience more severe myelosuppression and hepatic toxicity than those with other malignant diseases. Most of the patients with HCC have cirrhosis, which is usually associated with compromised hepatic function, leukocytopenia, and thrombocytopenia.[1–3,24,26] In the current trial, the most common toxicities were neutropenia and leukocytopenia, but these toxicities were generally brief and reversible with the exception of one treatment-related death. Hepatic toxicity also was observed frequently, but it was difficult to differentiate between hepatic toxicity induced by FMP therapy and exacerbation of viral hepatitis because all patients presented with impaired baseline liver function. The serum transaminase levels of all patients who showed a ≥ Grade 3 elevation returned to baseline levels within 1 month, and these patients were able to continue chemotherapy without dose reduction. There was only one death attributable to chemotherapy toxicity and this regimen was generally tolerated in patients with advanced HCC.

HCC is considered primarily a chemoresistant disease. However, FMP therapy resulted in a relatively higher response rate and longer survival for patients with metastatic HCC. One of the reasons is that patients with poor hepatic reserve, poor performance status, refractory pleural effusion or ascites, or tumor thrombosis in the main portal trunk—reported to be unfavorable factors for tumor response to or prognosis after systemic chemotherapy24–27—were excluded from our study. Patt et al.[12] reported that patients who had lower levels of serum AFP before treatment (i.e., patients with a response rate of 31%) responded better to combination therapy with 5-FU and interferon than those with higher levels (i.e., patients with a response rate of 0%). Leung et al.[27] also reported that patients who have normal total bilirubin levels and noncirrhotic livers might have a ≤ 50% chance of response and prolonged survival after combination chemotherapy with cisplatin, doxorubicin, alpha-interferon, and 5-FU. There are some chemosensitive subgroups of patients with advanced HCC, and it is also important to identify the appropriate candidates for systemic chemotherapy as well as to explore novel therapeutic strategies.

FMP therapy has significant antitumor activity with acceptable toxicity in patients with metastatic HCC. However, such therapy has not been shown to confer any clinically meaningful survival advantage in comparison to other palliative therapies or best supportive care. Therefore, to support our findings, we emphasize the need for larger multicenter studies of FMP therapy including prospective randomized trials in patients with metastatic HCC.

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Phase I Study of Fixed Dose Rate Infusion of Gemcitabine in Patients with Unresectable Pancreatic Cancer

Junji Furuse[1], Hiroshi Ishii[1], Takuji Okusaka[2], Michitaka Nagase[1], Kohei Nakachi[1], Hideki Ueno[2], Masafumi Ikeda[2], Chigusa Morizane[2] and Masahiro Yoshino[1]

[1]Division of Hepatobiliary and Pancreatic Medical Oncology, National Cancer Center Hospital East, Kashiwa, Chiba and [2]Division of Hepatobiliary and Pancreatic Oncology, National Cancer Center Hospital, Tokyo, Japan

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[1]Division of Hepatobiliary and Pancreatic Medical Oncology, National Cancer Center Hospital East, Kashiwa, Chiba and [2]Division of Hepatobiliary and Pancreatic Oncology, National Cancer Center Hospital, Tokyo, Japan

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Objective: The purpose of this study was to determine the feasible dose of gemcitabine when administered as a fixed dose rate infusion (10 mg/m^2/min) on a weekly schedule to Japanese patients with unresectable advanced pancreatic cancer.
Methods: Patients were required to have histologically or cytologically proven locally advanced or metastatic pancreatic cancer for which they had received no previous chemotherapy. Gemcitabine was administered intravenously weekly for three consecutive weeks every 4 weeks. Patients at three dose levels were scheduled to receive escalating doses of gemcitabine: 1000 mg/m^2 over 100 min (Level 1), 1200 mg/m^2 over 120 min (Level 2) and 1500 mg/m^2 over 150 min (Level 3).
Results: A total of 16 patients were enrolled in this study between December 2003 and September 2004. Maximum-tolerated dose was not reached during the first course. Dose-limiting toxicity was Grade 4 neutropenia. Grade 3 or 4 neutropenia was observed at Level 3 in all six patients in the first course, and administration of gemcitabine on Day 8 or 15 was skipped in all six patients. Non-hematologic toxicity was mild and the most common symptoms were anorexia, nausea and vomiting. Partial response was achieved in 1 of the 17 patients (7%). Median overall survival was 7.3 months.
Conclusions: Gemcitabine administered at a rate of 10 mg/m^2/min was tolerated up to 1500 mg/m^2, but 1200 mg/m^2 represented a more appropriate recommended dose in further studies owing to neutropenia in Japanese patients with advanced pancreatic cancer.

Key words: advanced pancreatic cancer – systemic chemotherapy – gemcitabine – fixed dose rate infusion

INTRODUCTION

Pancreatic cancer is the fifth most common cause of cancer death in Japan, with an estimated 19 000 deaths annually (1). Early-stage diagnosis of pancreatic cancer is difficult because of the lack of specific early symptoms, and surgery with curative intent can be performed in only 5–20% of patients (2). The prognosis for unresectable pancreatic cancer remains extremely poor.

Gemcitabine (2′,2′-difluorodeoxycytidine) is a novel pyrimidine antimetabolite with a broad spectrum of antitumor activity against various solid tumors, such as pancreatic and lung cancer (3). This prodrug is initially phosphorylated by deoxycytidine kinase to gemcitabine monophosphate, with subsequent phosphorylation steps yielding gemcitabine di- and triphosphate (4). Gemcitabine triphosphate inhibits DNA synthesis by competing with deoxycytidine triphosphate for incorporation into DNA by DNA polymerase (5). A dose of 790 mg/m^2 gemcitabine weekly for 3 weeks every 28 days was recommended for Phase II studies on the basis of a Phase I study in which gemcitabine was administered as a once-weekly 30 min bolus infusion (6). This dosing schedule was used in subsequent Phases II and III studies, and once-weekly 30 min infusion of the 1000 mg/m^2 dose was subsequently selected as the standard schedule (7,8). In a randomized clinical trial, gemcitabine was confirmed to provide a survival advantage over 5-FU in addition to symptom-relieving benefits in patients with advanced pancreatic cancer (8). Based on these results, gemcitabine has generally been accepted as the standard chemotherapeutic agent for advanced pancreatic cancer. However, the advantages in terms of survival rate are inadequate, and various chemotherapeutic regimens have been investigated in clinical studies in efforts to prolong survival.

For reprints and all correspondence: Junji Furuse, National Cancer Center Hospital East, 6-5-1, Kashiwanoha, Kashiwa, Chiba 277-8577, Japan.
E-mail: jfuruse@east.ncc.go.jp

The cellular pharmacokinetics of the active metabolite, gemcitabine triphosphate, in mononuclear cells have been examined in previous studies, and the rate of gemcitabine triphosphate accumulation and peak intracellular concentration were highest at a dose rate of 350 mg/m^2 over 30 min, during which steady-state gemcitabine levels of 15–20 μmol/l were achieved in plasma (6,9). A dose ~10 mg/m^2/min that achieves plasma gemcitabine concentrations of 15–20 μmol/l would thus maximize the intracellular rate of accumulation for gemcitabine triphosphate. This schedule of gemcitabine administration, with fixed dose rate (FDR) infusion of 10 mg/m^2/min, would enable exposure to higher concentrations of gemcitabine, and should improve clinical efficacy.

Phase I studies of FDR infusion of gemcitabine in the United States recommended a Phase II dose of 1500 mg/m^2 (10,11). A subsequent randomized Phase II trial comparing this FDR gemcitabine infusion schedule and high-dose gemcitabine (2200 mg/m^2) using a standard 30 min infusion showed improved median survival time for the FDR arm (12). The FDR infusion schedule is expected to become the optimal method of gemcitabine administration, but has not previously been assessed in Japan. We, therefore, conducted a Phase I study to determine whether FDR infusion of gemcitabine would be tolerated in Japanese patients with unresectable advanced pancreatic cancer. The primary objectives of this study were to confirm whether the recommended dose in the United States, 1500 mg/m^2 over 150 min, would be feasible in Japanese patients and to determine the relationship between dose and toxicity for gemcitabine administered using the FDR infusion schedule. The secondary objective was to evaluate antitumor activity of the schedule.

PATIENTS AND METHODS

Patients Eligibility

Eligibility criteria for enrollment in the study were as follows: (i) histologically confirmed pancreatic ductal adenocarcinoma; (ii) unresectable locally advanced or metastatic disease; (iii) no previous treatment for pancreatic cancer except surgery; (iv) age ≥20 and ≤74 years old; (v) Eastern Cooperative Oncology Group (ECOG) performance status of 0–2; (vi) adequate bone marrow (leukocyte count ≥4000 cells/mm^3, platelet count ≥100 000 cells/mm^3 and hemoglobin ≥9.0 g/dl), renal function (serum creatinine concentration ≤upper limit of normal) and hepatic function (serum bilirubin level ≤2.0 mg/dl, serum albumin level ≥3.0 g/dl, serum aspartate and alanine transaminase (AST and ALT) levels ≤2.5 times upper limit of normal); (vii) life expectancy ≥8 weeks; and (viii) written informed consent from the patient. Percutaneous biliary drainage was performed in patients with obstructive jaundice, and these patients were required to have serum bilirubin levels of ≤2.0 mg/dl and serum AST and ALT levels ≤5 times the upper limit of normal before enrollment. Exclusion criteria comprised serious complications such as active infection, active gastrointestinal ulcer, cardiac disease or renal disease; central nervous system metastasis; marked pleural effusion or ascites; symptomatic interstitial pneumonitis; and pregnancy or lactation for women. This protocol was approved by the National Cancer Center's institutional review board for clinical investigation.

Treatment Methods

Gemcitabine (Eli Lilly Japan K.K., Kobe, Japan) was administrated intravenously at 10 mg/m^2/min, weekly, for three consecutive weeks, followed by a week of rest. This cycle was continued until disease progression or serious adverse effects developed or until the patient requested discontinuation. When patients developed leukopenia of <2000/mm^3, neutropenia of <1000/mm^3, thrombocytopenia of <70 000/mm^3, total bilirubin >2.0 mg/dl or AST and ALT levels >5 times the upper limit of normal, gemcitabine administration was suspended until the patient recovered. If a rest period of >4 weeks was required owing to toxicity, the patient was withdrawn from the study.

Study Designs

Patients at three dose levels were scheduled to receive escalating dose of gemcitabine. At the first dose level (Level 1), gemcitabine was administered at a dose of 1000 mg/m^2. The dose level was increased to 1200 mg/m^2 for Level 2 and 1500 mg/m^2 for Level 3. Patient cohorts had a minimum of three patients at each level. If no dose-limiting toxicity (DLT) was observed in the initial three patients during the first cycle of treatment, the dose was advanced to the next level. If DLT occurred in the initial three patients, three additional patients were studied at the same dose level. If two or more of these six patients experienced DLT at that level, the dose was escalated to the next level. The maximum-tolerated dose (MTD) was defined as the highest dose level at which more than two of the six patients experienced DLT during the first cycle of treatment. If DLT occurred in three patients at Level 1, the dose was reduced to 800 mg/m^2 (Level 0). DLT was defined as follows: (i) Grade 4 leukopenia or neutropenia; (ii) febrile neutropenia; (iii) Grade 4 thrombocytopenia or Grade 3 thrombocytopenia requiring transfusion; (iv) ≥Grade e 3 non-hematological toxicity with the exception of nausea, vomiting, anorexia, fatigue and constipation; and (v) any toxicity requiring two consecutive skips of administration or a >4 week delay in treatment. Toxicity was graded according to the National Cancer Institute common toxicity criteria version 2.0.

Clinical Assessments

Physical examination, complete blood cell counts, serum chemistries and urinalysis were performed at baseline and at least once weekly after initiating treatment. Patients underwent dynamic computed tomography (CT) to evaluate response at 4–8 week intervals after start of treatment. CT was performed by obtaining contiguous transverse sections using the helical scanning method at a section thickness of 5 mm. Tumor response was assessed according to the World Health Organization criteria (13). Serum carbohydrate antigen (CA)19-9

levels were measured monthly by immunoradiometric assay. Progression-free survival was calculated from the first day of treatment until evidence of tumor progression, clinical progression or death owing to any cause. Overall survival was calculated from the first day of treatment until death owing to any cause. Survival data were analysed using the Kaplan–Meier method.

RESULTS

PATIENT CHARACTERISTICS

Between December 2003 and September 2004, a total of 16 patients were enrolled in this study. Dose escalation schedule and the number of patients at each level are shown in Table 1. The first administration of 1200 mg/m^2 of gemcitabine in one patient receiving Level 2 was later found to have been infused over 90 min, departing from the FDR of 10 mg/m^2/min. As a result, an additional patient was added to Level 2 and ultimately seven patients were treated at Level 2. Patient characteristics are shown in Table 2. The 16 patients received 60 courses of gemcitabine. Median number of courses administered per patient was 3 (range 1–9 courses). All 16 patients were evaluable for toxicity, but the Level 2 patient not infused with gemcitabine at a rate of 10 mg/m^2/min was excluded from the evaluation of DLT.

TOXICITY

Toxicities of the 15 patients evaluated for DLT during the first course are shown in Table 3. The first three patients enrolled on Levels 1 and 2 did not experience any DLT, but one of the six patients at Level 3 experienced DLT. MTD was not reached in this study. However, since all six patients at Level 3 (1500 mg/m^2 over 150 min) experienced Grade 3 or 4 neutropenia after Day 1 or 8 of the first course and did not receive the second or third dose of gemcitabine, an additional three patients were entered at Level 2 to accurately determine the recommended FDR for gemcitabine. Finally, no Grade 4 hematological toxicity was observed in any of the six patients at Level 2, and Grade 3 neutropenia developed in three of these patients. While five of the six patients received the full three doses of gemcitabine in the first course, the remaining patient did not receive the third dose owing to Grade 3 neutropenia. Level 2 (1200 mg/m^2) was therefore selected as the recommended dose for further studies of this FDR gemcitabine regimen in Japan.

Table 1. Dose escalation scheme

Dose levels	Gemcitabine (mg/m^2/wk)	Infusion time (min)	*n*
1	1000	100	3
2	1200	120	7
3	1500	150	6

Table 2. Patient characteristics

Variable	No. of patients (n = 16)
Gender	
Male	7
Female	9
Median age (range)	62 (47–74) years
ECOG performance status	
0	11
1	4
2	1
Disease stage	
Locally advanced	3
Metastatic	13
Site of metastatic disease	
Liver	10
Lung	3
Distant lymph nodes	2
CA19-9 before treatment (U/ml)	
≤37	4
>37, ≤1000	6
>1000	6

ECOG, Eastern Cooperative Oncology Group; CA19-9, carbohydrate antigen 19–9.

Table 3. Toxicities across first course by patient

	Dose levels											
	Level 1 (n = 3)				Level 2 (n = 6)				Level 3 (n = 6)			
	Grades				Grades				Grades			
	1	2	3	4	1	2	3	4	1	2	3	4
Leukopenia	0	0	2	0	3	1	2	0	0	2	4	0
Neutropenia	0	0	2	0	1	2	3	0	0	0	5	1
Anemia	1	1	2	0	2	3	0	0	4	2	0	0
Thrombocytopenia	1	2	0	0	2	0	1	0	0	2	1	0
Anorexia	1	1	0	0	2	0	1	0	2	2	0	0
Nausea	1	1	0	0	1	0	1	0	4	1	0	0
Vomiting	0	1	0	0	0	0	1	0	1	1	0	0
Rash	0	0	0	0	2	2	0	0	1	3	0	0
Fatigue	2	0	0	0	2	0	0	0	0	1	0	0
Fever	0	1	0	0	0	0	0	0	1	0	0	0
Mucositis	0	1	0	0	0	0	0	0	0	0	0	0
Alopecia	0	0	0	0	0	0	0	0	1	0	0	0
AST, ALT elevation	0	1	0	0	1	1	0	0	0	1	0	0

AST, serum aspartate transaminase; ALT, serum alanine transaminase.

Toxicities throughout the entire period of this protocol were assessed in all 16 patients enrolled in this study (Table 4). The most common toxicity was leukopenia, particularly neutropenia, with 13 of the 16 patients (81%) developing Grade 3 or 4

Table 4. Toxicities during entire course by patient

	Dose levels											
	Level 1 (n = 3)				Level 2 (n = 7)				Level 3 (n = 6)			
	Grades				Grades				Grades			
	1	2	3	4	1	2	3	4	1	2	3	4
Leukopenia	0	0	2	0	2	2	3	0	0	1	4	1
Neutropenia	0	0	2	0	1	1	5	0	0	0	3	3
Anemia	1	0	2	0	1	5	1	0	3	2	1	0
Thrombocytopenia	1	2	0	0	2	1	1	0	0	2	2	0
Anorexia	1	0	1	0	4	0	1	0	4	2	0	0
Nausea	1	0	1	0	4	0	1	0	5	1	0	0
Vomiting	0	0	1	0	2	0	1	0	1	1	0	0
Constipation	0	0	0	0	0	1	0	0	1	0	0	0
Diarrhea	0	0	0	0	1	0	0	0	0	0	0	0
Rash	0	0	0	0	3	2	0	0	1	2	0	0
Fatigue	1	1	0	0	2	0	0	0	0	1	0	0
Fever	0	1	0	0	0	0	0	0	2	0	0	0
Mucositis	0	1	0	0	0	0	0	0	0	0	0	0
Alopecia	0	0	0	0	1	1	0	0	3	0	0	0
AST, ALT elevation	0	1	0	0	1	1	0	0	0	1	0	0

AST, serum aspartate transaminase; ALT, serum alanine transaminase.

neutropenia during treatment. Non-hematological toxicities were generally mild at all levels, and one patient developed Grade 3 nausea, vomiting, and anorexia at Level 1 and Level 2, respectively. Skin rashes were mild, but tended to occur in a larger number of patients as the dose was escalated.

Tumor Response and Survival

Partial response was achieved in 1 of the 16 patients (6.3%), but no complete responses were observed. Overall response rate was thus 6.3% (95% confidence interval = 0.2–30.2%). No change was noted in 12 patients (75.0%), and progressive disease was in two patients (12.5%). The patient with DLT was not evaluated for tumor response because she received standard gemcitabine chemotherapy as second-line chemotherapy before the evaluation. Serum CA19-9 levels were reduced to >50% in 2 of the 12 patients (16.7%) in whom pretreatment level was elevated to above the upper limit of normal.

Disease progression was finally observed in all patients and 12 of the 16 patients died of disease progression. Median progression-free survival was 3.2 months, and overall median survival time (MST) was 7.3 months (Figs 1 and 2).

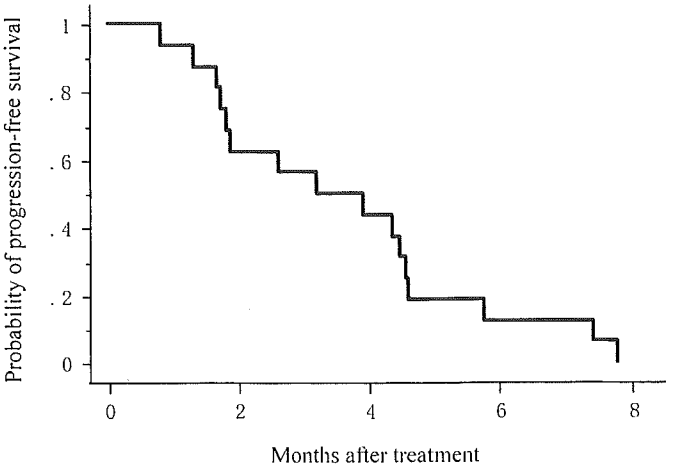

Figure 1. Progression-free survival of all 16 patients.

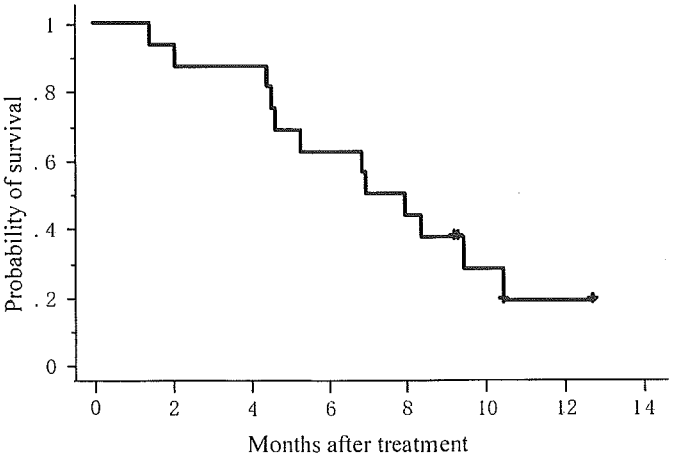

Figure 2. Overall survival of all 16 patients.

DISCUSSION

Gemcitabine is a prodrug that requires initial intracellular phosphorylation by deoxycytidine kinase, ultimately undergoing phosphorylation to the active gemcitabine triphosphate, a cytotoxic agent that inhibits DNA synthesis. Tempero et al. (12) reported on intracellular concentrations of gemcitabine triphosphate in peripheral blood mononuclear cells in a randomized trial comparing FDR infusion over 150 min and high-dose gemcitabine (2200 mg/m^2) using a standard 30 min infusion. The rate of gemcitabine triphosphate accumulation in patients who received conventional infusion decreased markedly after the end of infusion (30 min), whereas patients who received gemcitabine as FDR infusion exhibited linear accumulation of the triphosphate throughout the infusion. Intracellular gemcitabine triphosphate concentration in the FDR arm was 2-fold higher than that in the conventional infusion arm.

In the United States, two Phase I studies have been performed to determine the recommended dose for FDR infusion of gemcitabine (10,11). Brand et al. (11) conducted a Phase I study at dose levels of 1200 mg/m^2, 1500 mg/m^2 and 1800 mg/m^2, administered on Days 1, 8 and 15 of a 28 day cycle. MTD was defined as 1500 mg/m^2, with granulocytopenia and thrombocytopenia representing the DLTs. Brand et al. concluded that myelosuppression was more severe than

anticipated based on previous reports regarding standard gemcitabine administration. Touroutoglou et al. (10) conducted the other Phase I study of FDR infusion of gemcitabine in which the weekly dose was escalated from 1200 to 2800 mg/m^2 for 3 weeks every 4 weeks. They reported that MTD was 1800 mg/m^2, and recommended a Phase II starting dose of 1500 mg/m^2 owing to myelosuppressive effects.

The present study evaluated the safety of FDR infusion of gemcitabine and identified the feasible dose for Japanese patients with unresectable advanced pancreatic cancer. This Phase I study was conducted using dose levels of 1000, 1200 and 1500 mg/m^2, administered on Days 1, 8 and 15 of the 28 day cycle. DLT was observed in only one patient at Level 3, and MTD was not reached in this study. However, all six patients displayed Grade 3 or 4 neutropenia during the first course at Level 3, and no patient received all three doses of gemcitabine during the first course. In contrast, three patients at Level 2 experienced Grade 3 neutropenia, and only one patient had to skip the dose of gemcitabine on Day 15. Based on these results, the recommended dose should be 1200 mg/m^2 in further studies of FDR infusion of gemcitabine in Japan from the perspective of dose intensity for gemcitabine.

Preclinical data, using primary human tumor cell lines including pancreatic carcinoma, have suggested a possible dose–response relationship, and exposure to high concentrations of gemcitabine, independent of infusion duration, might correlate with improved cytoxicity and enhanced clinical effectiveness (12). Thus, a randomized trial of gemcitabine comparing high-dose gemcitabine (2200 mg/m^2) administered using a standard 30 min infusion to FDR infusion of 1500 mg/m^2 over 150 min was conducted in patients with locally advanced or metastatic pancreatic cancer according to the results of two Phase I studies in the United States (10–12). Although no difference in tumor response was noted between the 30 min infusion and FDR arms, MST was reported as 5.0 months in the 30 min infusion arm and 8.0 months in the FDR arm (P = 0.013), and 1 and 2 year survival rates were 9.0 and 2.2%, respectively, in the 30 min infusion arm, and 28.8 and 18.3%, respectively, in the FDR arm. In the study conducted by Burris et al. (8), MST for gemcitabine using the standard 30 min infusion of 1000 mg/m^2 was 5.7 months, and 1 and 2 year survival rates were 18 and 0%, respectively. A retrospective analysis reported that the MST of patients in Japan treated with gemcitabine by standard infusion of 1000 mg/m^2 was 5.7 months (14). In comparison, survival outcomes of patients treated using the standard 30 min infusion are similar, and MST is <6 months. In contrast, in a study with a limited number of patients using FDR infusion, MST was 7.3 months and similar to MST in the FDR arm of the randomized trial in the United States (12).

The most common toxicity for FDR infusion was myelosuppression, particularly neutropenia, as noted in a randomized trial by Tempero et al. (12). In our study, Grade 3 or 4 neutropenia developed in 81.3% of patients, and Grade 3 or 4 leukopenia and thrombocytopenia were observed in 62.5 and 18.8%, respectively. By contrast, a Phase I study for the standard infusion of gemcitabine in Japan reported rates of Grade 3 or 4 neutropenia, leukopenia and thrombocytopenia of 36.4, 27.3 and 0%, respectively (15). The FDR infusion schedule thus seems more hematologically toxic. Conversely, the non-hematological toxicity of FDR infusion was relatively mild. Grade 3 nausea and vomiting that occurred in 12.5% of patients on FDR infusion resembled the results obtained with standard infusion in the Japanese Phase I study, in which 9.1% of patients developed Grade 3 nausea and vomiting. Skin rashes were more frequent with FDR infusion, with 50% of patients developing Grade 1 or 2 skin rashes, than with standard infusion, in which 27.3% of patients developed Grade 1 or 2 skin rashes.

Various regimens of gemcitabine in combination with potentially synergistic drugs have been trialed to improve prognosis in patients with unresectable pancreatic cancer (16–22), and FDR infusion of gemcitabine has also been applied to combination chemotherapy with other anticancer drugs (20–22). A Phase III study comparing standard infusion of gemcitabine, FDR infusion of gemcitabine and combined FDR infusion of gemcitabine and oxaliplatin is under way as an ECOG study in the United States. The results of that study should be awaited before deciding whether FDR infusion of gemcitabine alone can be used as the standard treatment for unresectable pancreatic cancer. However, data from the present study confirm that FDR infusion of gemcitabine is tolerated by Japanese patients, and continued evaluation of FDR infusion, alone or in combination with other agents, is warranted in Japan.

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