Table II. Allele frequencies of the genetic polymorphisms of *CDA*, *DCK* and *SLC29A1* investigated in this study

Gene	Single nucleotide polymorphism ID	Location	Nucleotide change	Amino acid change	Subjects (n)			Allele frequency
					wild-type	heterozygous	homozygous	
CDA	MPJ6_CDA007	Exon1 (5'-UTR)	-31delC		83	110	55	0.444
	MPJ6_CDA009	Exon1	79A>C	Lys27Gln	160	73	15	0.208
	MPJ6_CDA010	Intron1	IVS1+37G>A		175	59	14	0.175
	MPJ6_CDA011	Exon2	208G>A	Ala70Thr	230	16	2	0.040
DCK	MPJ6_DCK008	5'-Flanking	-360C>G		187	58	3	0.129
	MPJ6_DCK016	Exon3	364C>T	Pro122Ser	219	28	1	0.060
SLC29A1	MPJ6_ET1005	5'-Flanking	-5851G>A		215	31	0	0.063
	MPJ6_ET1008	5'-Flanking	-3797A>G		84	128	34	0.398
	MPJ6_ET1011	5'-Flanking	-3268_-3249del AGGCTCGCGAGCGGAGGTGC		15	114	117	0.707
	MPJ6_ET1026	Intron8	IVS8+169G>A		222	23	1	0.051
	MPJ6_ET1029	Intron10	IVS10+160A>C		154	73	19	0.226
	MPJ6_ET1036	Exon12 (3'-UTR)	1840(*469)C>A		161	69	16	0.205
	MPJ6_ET1039	3'-Flanking	1984+69 (*682)A>C		139	83	24	0.266

CDA = cytidine deaminase; **DCK** = deoxycytidine kinase; **SLC** = solute carrier family; **UTR** = upper translational region.

Plasma Gemcitabine and dFdU Concentrations and Genotypes

Plasma concentrations of gemcitabine and dFdU and genotypes of *CDA*, *DCK* and solute carrier family 29A1 (*SLC29A1* [coding *hENT1*]) have been previously reported.[12,15,16] Blood samples for determining the plasma concentrations were collected before and at timepoints 0, 15, 30, 60, 90, 120 and 240 minutes after completion of the infusion. Table II summarizes the allele frequencies of *CDA*, *DCK* and *hENT1* genotypes for which effects on the pharmacokinetic parameters of gemcitabine were investigated in this study.

Population Pharmacokinetic Model Development

Population pharmacokinetics of gemcitabine and dFdU were analysed using nonlinear mixed-effects modelling software (NONMEM® version V level 1.1; ICON Plc, Dublin, Ireland). The first-order method was applied during the building of population pharmacokinetic models, and the first-order conditional estimation (FOCE) method was applied to obtain estimations in the final model. Selection of covariates was carried out by the forward stepwise inclusion and deletion method at a threshold p-value of 0.001 by a χ^2 test.

De Pas et al.[17] reported linear pharmacokinetics of gemcitabine up to 1500 mg/m^2, after which nonlinear pharmacokinetic behaviour and higher interpatient variability in the maximum plasma concentration (C_{max}) and the area under the plasma concentration-time curve (AUC) were reported. Since all patients received gemcitabine at doses ≤1000 mg/m^2, linear compartment models were selected as gemcitabine population pharmacokinetic models.

To develop a basic population pharmacokinetic model for gemcitabine (selection of a compartment model and description of interindividual and residual error variability), we fitted a one-compartment or two-compartment linear model to plasma concentrations of gemcitabine. The estimated population parameters for a one-compartment model were the volume of distribution (V_d) and clearance (CL), and those for a two-compartment model were the volume of distribution in the central compartment (V_1), clearance (CL_1), the volume of distribution in the peripheral compartment (V_2) and intercompartmental clearance (Q) [step 1]. As previously reported, two patients carrying homozygous *CDA**3 showed unexpectedly high plasma concentrations of gemcitabine;[12,14] therefore, these patients were excluded from this step. Four patients who showed bimodal concentration-time curves for gemcitabine were also excluded from the analysis because an extraordinarily large apparent V_1 for gemcitabine was estimated when they were included. Next, after the data obtained from the two *CDA**3 homozygous patients were added, the contribution of *CDA**3 to the population pharmacokinetics of

gemcitabine was determined (step 2). Similarly, additional candidate covariates, most of which had previously been shown to have univariate correlations with model-independent pharmacokinetic parameters,[12] were examined for their contributions; they included other genetic polymorphisms of *CDA*,[12] *DCK*[15] and *SLC29A1* (*hENT1*),[16] regimens of chemotherapies and patients' characteristics (step 3).

In order to consider the metabolic pathway from gemcitabine to dFdU and dFdU pharmacokinetics, we used subroutines provided by NONMEM® (ADVAN5 and MODEL); the former is prepared for general linear models and the latter defines compartment attributes and rate constants. We examined whether dFdU followed a one-compartment or two-compartment model. The estimated population parameters of dFdU for a one-compartment model were total dFdU clearance (CL_m) and the dFdU volume of distribution (V_m), and those for a two-compartment model were CL_m, the dFdU volume of distribution in the central compartment (V_{m1}), the dFdU volume of distribution in the peripheral compartment (V_{m2}) and intercompartmental dFdU clearance (Q_m). Since only the subroutine TRANS1 (in which micropharmacokinetic parameters such as intercompartmental rate constants [k_{12} or k_{21}] are used) is provided by NONMEM® for ADVAN5, we adequately transformed micropharmacokinetic parameters to macropharmacokinetic ones such as the V_1, V_2, CL and Q. The metabolic fraction of dFdU (f_m) was assumed to be 1 because more than 90% of the administered gemcitabine was recovered as dFdU in the urine.[6] Compartment and error models for dFdU were determined (step 4). Subsequently, selection of candidate covariates for dFdU, which had previously been reported to have correlations with model-independent pharmacokinetic parameters of dFdU,[12] was carried out (step 5). Finally, pharmacokinetic parameters of the model constructed in step 5 were re-estimated using the FOCE method. The final model was evaluated by plots of the observed and predicted individual plasma concentrations of gemcitabine and dFdU, and by plots of the observed plasma concentrations and population conditional weighted residuals, which were calculated using Xpose 4.0 software (Uppsala University, Uppsala, Sweden).[18]

Results

Development of a Population Pharmacokinetic Model for Gemcitabine

We used 1973 plasma concentrations of gemcitabine obtained from 248 patients (figure 1a) for the following analysis. As described in the Methods, the two patients with homozygous *CDA**3 (*CDA* 208G>A) were excluded from the first step analysis because of their extraordinarily different pharmacokinetic profiles (figure 1). Pharmacokinetics of gemcitabine in Japanese cancer patients, excluding the *CDA**3-homozygous patients, were estimated using a two-compartment model. The objective function value (OFV) was at a minimum when interindividual variabilities of pharmacokinetic parameters were assumed to distribute log-normally, and when the variation in the residual errors was assumed to have both proportional and additive components (model 1).

In order to build a model taking into account the *3/*3 patients with extremely low clearance,[12] we added a

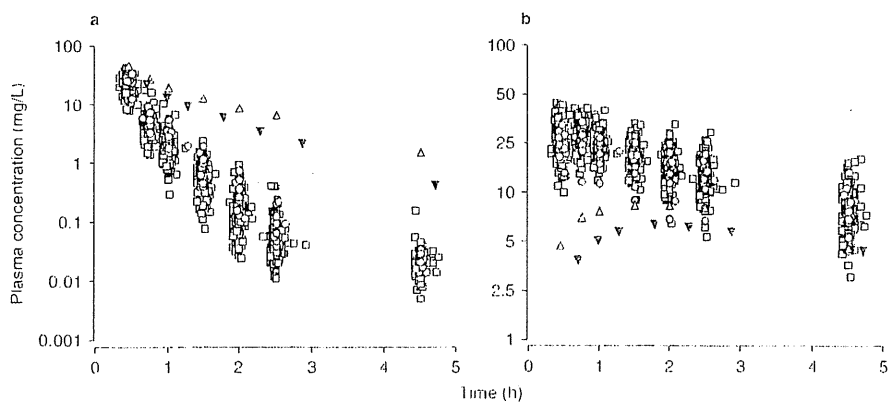

Fig. 1. Plasma concentration-time plots of (**a**) gemcitabine (dFdC) and (**b**) 2′,2′-difluorodeoxyuridine (dFdU). The light grey squares represent patients without *CDA**3 and the dark grey circles represent patients with heterozygous *CDA**3. The white triangles and black inverted triangles represent patients with homozygous *CDA**3 receiving 1000 and 454 mg/m^2 of gemcitabine, respectively.

Table III. Building of covariate population pharmacokinetic models for gemcitabine (dFdC) and 2',2'-difluorodeoxyuridine (dFdU)

Model name	Parameter	Factor[a]	OFV	Model used for comparison	ΔOFV	p-Value
Gemcitabine						
1		Basic model	−532.19			
	CL_1	*CDA**3 (linear)	−2206.79	1	−1674.60	<0.000001
2	CL_1	***CDA**3 homozygous**	−2260.86	1	−1728.68	<0.000001
3	CL_1	***CDA**3 heterozygous**	−2276.14	2	−15.28	9.28E−05
4	CL_1	BSA	−2405.26	3	−129.12	
	V_1	BSA	−2204.71	3	71.43	
	CL_1	Bodyweight	−2276.05	3	0.094	
	V_1	Bodyweight	−2176.05	3	100.09	
	CL_1	Age	−2409.77	4	−4.51	0.034
	V_1	Age	−2410.98	4	−5.72	0.017
	CL_1	Sex	−2406.40	4	−1.14	0.29
	V_1	Sex	−2406.52	4	−1.26	0.26
	CL_1	Cisplatin	−2407.248	4	−1.99	0.16
5	CL_1	**S-1**	−2427.66	4	−22.40	2.21E−06
6	CL_1	***CDA*−31delC**	−2464.89	5	−37.23	1.05E−09
	CL_1	*CDA**2	−2441.21	5	−13.55	0.00023
	CL_1	*CDA* IVS1+37G>A	−2441.84	5	−14.18	0.00017
dFdU						
7		Basic model	91.694			
8	CL_m	**BSA**	45.958	7	−45.736	
	V_{m1}	BSA	10.795	7	−80.899	
9	V_{m1}	**BSA**	−31.64	8	−77.598	
	CL_m	Bodyweight	163.251	7	71.557	
	V_{m1}	Bodyweight	2.496	7	−89.198	
10	CL_m	**Creatinine**	−166.798	9	−135.158	3.05E−31
11	CL_m	**Age**	−197.342	10	−30.544	3.26E−08
12	V_{m1}	**Age**	−212.069	11	−14.73	0.000124
13	V_{m1}	**Sex**	−243.914	12	−31.845	1.67E−08
	CL_m	Sex	−253.677	13	−9.763	0.00178

a The factors indicated in bold type were selected as covariates for the final model.

BSA = body surface area; **CL_1** = clearance of gemcitabine; **CL_m** = clearance of the metabolite dFdU; **OFV** = objective function value; **S-1** = an oral product of tegafur with gimeracil and oteracil; **V_1** = apparent volume of distribution of the central compartment of gemcitabine; **V_{m1}** = apparent volume of distribution of the central compartment of dFdU.

covariate to the basic model to account for the effect of homozygosity of *3 (θ_{*3homo}) on the clearance of gemcitabine (equation 1):

$$CL = \theta_1 \times (1 - \theta_{*3homo} \times CDA*3homo) \quad \text{(Eq. 1)}$$

where CL is total gemcitabine clearance in a patient of interest; θ_1 is gemcitabine clearance in patients without *3/*3; and $CDA*3homo$ is 1 for *3/*3 and 0 for other patients (*3/non-*3 or non-*3/non-*3). This modification significantly reduced the OFV, as shown in table III (model 2).

Next, the effect of heterozygous *3 on gemcitabine clearance was examined by comparing equations 2 and 3:

$$CL = \theta_2 \times (1 - \theta_{*3homo} \times CDA*3) \quad \text{(Eq. 2)}$$

$$CL = \theta_2 \times (1 - \theta_{*3hetero} \times CDA*3hetero) \times (1 - \theta_{*3homo} \times CDA*3homo) \quad \text{(Eq. 3)}$$

where θ_2 is gemcitabine clearance for patients without *3, *CDA**3 is 0 for non-*3/non-*3, ½ for *3/non-*3 and 1 for *3/*3; $\theta_{*3hetero}$ is a parameter related to the effect of heterozygous *3 but independent of θ_{*3homo}; and *CDA**3hetero is 1 for *3/non-*3 and 0 for *3/*3 or non-*3/non-*3. Equation 3 assumes a nonlinear gene-dose effect of *CDA**3 on CL. The OFV of equation 3 (model 3) was slightly but significantly smaller than that of equation 2, which indicates that the *CDA**3 gene-dose effect is not linear.

The effects of the body surface area (BSA), bodyweight, age and sex on the CL and V_1 of gemcitabine were investigated. As shown in table III, while consideration of an effect of size on the V_1 did not improve the OFV, examination of proportionality between the CL and BSA (model 4) considerably reduced the OFV. Age and sex did not significantly affect the CL and V_1 of gemcitabine (table III), although they were significantly correlated with these parameters in our previous univariate analyses.[12] As shown in table I, 66 patients received a gemcitabine-based combination chemotherapy with either cisplatin, carboplatin, fluorouracil, S-1 (an oral anti-cancer multicomponent drug containing tegafur, gimeracil and oteracil) or vinorelbine. Among the coadministered drugs, only S-1 significantly increased CL (model 5).

The effects of genetic polymorphisms of *CDA* other than *3 on the pharmacokinetics of gemcitabine were also examined. *CDA*–31delC (rs3215400; previously described as *CDA*–33_–31delC [precisely *CDA*–33_–31 C3>C2]), *CDA* 79A>C (Lys27Gln, *2) and *CDA* IVS1+37G>A increased gemcitabine clearance, and their effects were all statistically significant (table III). A delC factor was adopted in the final model for gemcitabine because it gave the smallest p-value and OFV (model 6 in table III).

Although we previously reported that 29 genetic variations of *DCK* were detected in our patients, they were very rare except for *DCK*–360C>G and 364C>T (Pro122Ser) [the allele frequencies were 0.131 and 0.061, respectively, as shown in table II],[15] and their functions were reported to be altered.[19,20] We analysed the effects of *DCK*–360C>G and 364C>T (Pro122Ser) on gemcitabine population pharmacokinetics, but no effects were detected. Thirty-nine genetic polymorphisms of *SLC29A1* (*hENT1*), including two nonsynonymous ones, were also previously reported.[16] Although we analysed the effects of genetic polymorphisms of *hENT1* whose allele frequencies were higher than 0.05 (table II), no effects were observed in univariate analyses (data not shown).

Development of a Combined Population Pharmacokinetic Model for Gemcitabine and dFdU

Next, we added compartments for dFdU where its central compartment was connected with the central compartment of gemcitabine with a first-order metabolic rate constant (CL/V_1) (figure 2). The f_m was assumed to be 1 because >90% of administered gemcitabine was recovered in the urine as dFdU.[6] Since an extraordinarily large V_m for dFdU was obtained if the V_1 for gemcitabine was not fixed, the V_1 was fixed to the value estimated in the previous section (12.60 L). Although the sampling duration in this study was not sufficiently long for pharmacokinetic analysis of dFdU (which has a longer half-life than that of gemcitabine, as shown in figure 1b), a two-compartment model (model 7, the combined basic model for gemcitabine and dFdU) provided a better fit for the data than a one-compartment model (the ΔOFV was –3402.44). Inclusion of covariates such as the BSA, age, serum creatinine level and sex in the model significantly reduced the OFV, as shown in table III.

All covariates selected by the inclusion steps remained after the stepwise exclusion/deletion process. The final population pharmacokinetic model (model 13) for Japanese cancer patients is shown in table IV. This model indicated that gemcitabine clearance was decreased by 64% and 17% in the *3-homozygotes and heterozygotes, respectively, compared with patients without *CDA**3. The increases in gemcitabine clearance by delC were 7.5% for heterozygotes and 15% for homozygotes. If S-1 was coadministered, gemcitabine clearance increased by 19%. CL_m was reduced by 8.6% if a patient was 10 years older than the average age (62.67 years in our patient group) and by about 7.3% if the creatinine level of a patient was 0.1 mg/dL higher than the average level (0.7 mg/dL in our patient group). The V_{m1} for dFdU was decreased by 8.1%

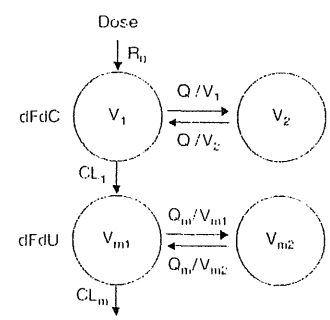

Fig. 2. Compartmental representation of gemcitabine (dFdC) and 2′,2′-difluorodeoxyuridine (dFdU) pharmacokinetics. CL_1 = clearance of gemcitabine; CL_m = clearance of the metabolite dFdU; **Q** = intercompartmental clearance between the central and peripheral compartments of gemcitabine; Q_m = intercompartmental clearance between the central and peripheral compartments of dFdU; R_0 = zero-order infusion rate constant; V_1 = apparent volume of distribution of the central compartment of gemcitabine; V_2 = apparent volume of distribution of the peripheral compartment of gemcitabine; V_{m1} = apparent volume of distribution of the central compartment of dFdU; V_{m2} = apparent volume of distribution of the peripheral compartment of dFdU.

Table IV. Population pharmacokinetic parameters for gemcitabine (dFdC) and 2′,2′-difluorodeoxyuridine (dFdU) in the final model

Pharmacokinetic parameter	Estimated value	CV%
Gemcitabine		
CL_1 (L/h/m^2)	$73.70 \times BSA \times (1-0.639 \times$ *3homo[a]$) \times (1-0.171 \times$ *3hetero[b]$) \times (1+0.0749 \times$ delC[c]$) \times (1+0.191 \times$ S-1[d]$)$	17.1
V_1 (L)	12.60 (Fixed)	58.9
Q (L/h)	37.50	Not estimated
V_2 (L)	9.54	25.3
dFdU		
CL_{m1} (L/h/m^2)	$11.00 \times BSA \times (1-0.00855 \times (AGE-62.67)) \times (1-0.732 \times (Cre-0.70))$	20.5
V_{m1} (L)	$15.00 \times BSA \times (1-0.00806 \times (AGE-62.67)) \times (1+0.239 \times$ Sex[e]$)$	27.9
Q_m (L/h)	58.0	22.7
V_{m2} (L)	31.7	26.4
Residual error	SD (ε_3); 0.0844	
	CV (ε_1) and CV (ε_2); 0.200 and 0.0412, respectively	

a *3homo: 1 for homozygous *CDA**3 and 0 for others.

b *3hetero: 1 for heterozygous *CDA**3 and 0 for others.

c delC: number of *CDA*–31delC in a patient (delC = 0, 1 or 2).

d S-1: 1 for S-1 coadministered to patients and 0 for others.

e Sex: 1 for male and 0 for female.

ε = variance; **AGE** = age (years); **BSA** = body surface area (m^2); **CL_1** = clearance of gemcitabine; **CL_m** = clearance of the metabolite dFdU; **CL_{m1}** = clearance of the metabolite dFdU from central compartment; **Cre** = serum creatinine (mg/dL); **CV** = coefficient of variation (interindividual); **Q** = intercompartmental clearance between the central and peripheral compartments of gemcitabine; **Q_m** = intercompartmental clearance between the central and peripheral compartments of dFdU; **S-1** = an oral product of tegafur with gimeracil and oteracil; **V_1** = apparent volume of distribution of the central compartment of gemcitabine; **V_2** = apparent volume of distribution of the peripheral compartment of gemcitabine; **V_{m1}** = apparent volume of distribution of the central compartment of dFdU; **V_{m2}** = apparent volume of distribution of the peripheral compartment of dFdU.

if a patient was 10 years older than the average age, and was increased by 24% in males compared with females.

Evaluation of the Goodness of Fit

The observed plasma concentrations of gemcitabine and dFdU were plotted against concentrations predicted by the final model, as shown in figure 3a and b, respectively. Most gemcitabine concentrations distributed into two peaks: one peak with scattering around 25 mg/L (collected at the end of the gemcitabine infusion [30 minutes after initiation of the infusion]) and a second peak with scattering close to the point of origin. This dual peak plot was the result of very rapid gemcitabine metabolism. One point at an extremely high concentration represented the C_{max} obtained from a *3/*3 patient, who was administered 1000 mg/m^2 of gemcitabine.[12,13] For both gemcitabine and dFdU, higher plasma concentrations gave more widely scattered plots, indicating that the variation in the residual errors was proportional to the measured concentration (a constant coefficient of variation type). The slopes of the regression lines for gemcitabine and dFdU were very close to 1.0 (1.007 and 0.9908, respectively). Conditional weighted residuals (CWRES) were recently reported as a diagnostic tool for the FOCE approximation.[18] The slopes of the regression lines of CWRES for gemcitabine and dFdU against predicted plasma concentrations were very close to 0.0 (–0.00482 and –0.00926, respectively), indicating a very good fit for the constructed model. Further validation of the model by a visual predictive check or bootstrapping was not performed, because the distribution of some covariates, such as diplotypes of *CDA**3 (non-*3/non-*3 : non-*3/*3 : *3/*3 = 230 : 16 : 2), and coadministration of S-1 (in only 10 of the 248 patients) were unevenly distributed.

Discussion

Recently, Jiang et al.[21] performed population pharmacokinetic analyses on gemcitabine and dFdU, and they adopted two-compartment models for both plasma gemcitabine and dFdU pharmacokinetics. Likewise, in our study, the

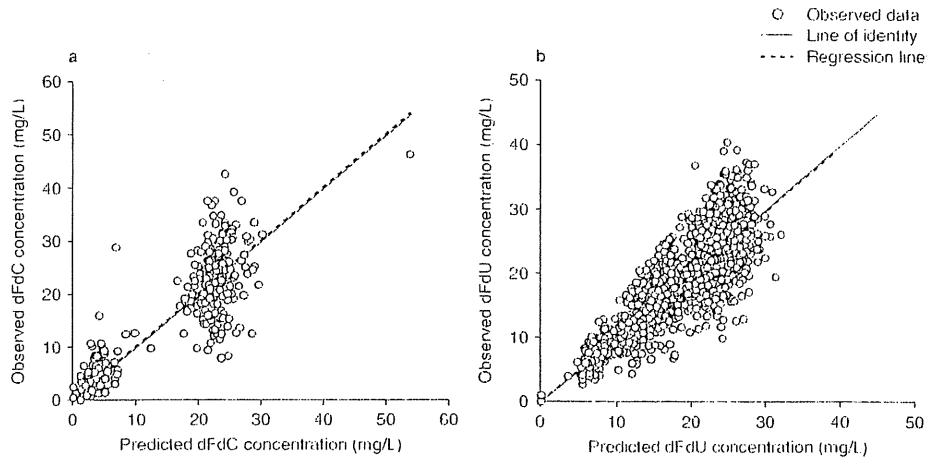

Fig. 3. Plots of observed concentrations against predicted concentrations of (a) gemcitabine (dFdC) and (b) 2′,2′-difluorodeoxyuridine (dFdU).

pharmacokinetics of gemcitabine and dFdU were effectively described by two-compartment models. The values of the estimated CL ([115.0 L/h] from a typical patient with an average BSA of 1.56 m^2), V_1 (12.60 L) and V_2 (9.54 L) were comparable to the values reported by Jiang et al.[21] (162 L/h, 15 L and 15 L, respectively). The estimated CL was slightly smaller and the V_1 was slightly larger than the values reported by Tham et al.[22] (222.8 L/h and 2.96 L, respectively). Although the reasons for these discrepancies are unknown, it should be noted that the population pharmacokinetic analyses performed by Tham et al.[22] included gemcitabine triphosphate (dFdCTP, an active form of gemcitabine) in addition to gemcitabine and dFdU, and the pharmacokinetic models applied in their study were completely different from ours.

The gemcitabine clearance in the *3/*3 patients, obtained from the model-independent analysis, was 80% less than the average clearance in patients without *3.[12,13] The effect of homozygous *3 on gemcitabine clearance, as estimated by the final population pharmacokinetic model, was a 64% decrease. This value, although slightly less than 80%, was the most significant among the covariates. Our current study also confirmed a finding from our previous report that the gene-dose effect of *CDA* was not linear. So far, we have encountered three patients with *3/*3, and all of them experienced life-threatening toxicities, including prolonged severe neutropenia.[12-14] Some of the non-*3/non-*3 and non-*3/*3 patients experienced transient grade 4 neutropenia, but only one patient required supportive treatment.[14] Thus, special attention to *3 homozygotes is advisable.

The effects of −31delC, 79A>C and IVS1+37G>A of *CDA* on gemcitabine clearance were found to be small but significant in this study (table III). All of these genotypes had slightly increased gemcitabine clearance (by <10%). The single nucleotide deletion −31delC is simultaneously present in both the haplotype *2 harbouring 79A>C and several *1 haplotypes (*1b, *1d, etc.) harbouring IVS1+37G>A in the Japanese population.[12] Thus it is reasonable that −31delC, rather than 79A>C or IVS1+37G>A, was selected as the covariate in the final model. This finding suggests that −31delC may be a functional SNP.

The haplotype analysis in our previous report[12] indicated that 208G>A, the tagging SNP of *CDA**3, is not present on a chromosome carrying −31delC, 79A>C or IVS1+37G>A. However, some patients simultaneously carried both haplotypes *2 and *3 (*2/*3). The median value of gemcitabine clearance observed in patients with *2/*3 was slightly higher than that observed in patients with *1/*3, although the difference was not statistically significant.[12]

The SNP 79A>C, a tagging SNP of the haplotype *2, results in the amino acid substitution, Lys27Gln.[12] A recent study[23] has suggested that the average enzymatic activity of CDA was significantly lower in cytoplasmic extracts of red blood cells obtained from patients with homozygous 79A (Lys27) than in those from patients with 79C (Gln27). Furthermore, it was reported that *CDA* 79A, the major allele, was a predictive marker of better response, more severe toxicity, longer time to disease progression and overall survival in Caucasian patients with advanced non-small-cell lung cancer who were treated with cisplatin and gemcitabine.[24] Haplotype *2 harbouring 79A>C also harbours −31delC, which has an incomplete association with the intron SNP IVS1+37. Our findings may explain the effects of 79A>C observed in Caucasian patients, since 79A>C is closely linked with −31delC, and the single nucleotide deletion −31delC in the 5′-untranslated region is responsible for increased clearance, a decreased AUC and less response to gemcitabine. This speculation warrants further study.

Although the effects of sex and age on model-independent pharmacokinetic parameters of gemcitabine were detected in our previous univariate analysis,[12] they were not significant in the current multivariate analysis. On the other hand, a significant effect of coadministered S-1, an oral derivative of fluorouracil, was revealed (approximately 20% higher clearance than in patients treated with gemcitabine monotherapy). In this study, nine of ten patients were coadministered S-1 in the morning a couple of hours before gemcitabine treatment. It might be noted that thymidylate synthase inhibitors such as fluorouracil can upregulate expression of hENT1, a major transporter of gemcitabine.[25] Moreover, Nakahira et al.[26] recently reported that significant increases in hENT1 expression and gemcitabine uptake were observed after S-1 treatment in mice. However, since the study duration was too short for S-1 to reveal the effects on expression of hENT1 in our study, the clinical significance of coadministration of S-1 and gemcitabine should be further investigated. In this study, four patients received fluorouracil after treatment of gemcitabine, and no effects of fluorouracil on the pharmacokinetics of gemcitabine were observed.

The metabolite dFdU is inactive and is eliminated mostly by renal excretion.[27] However, its pharmacokinetic parameters can be surrogate biomarkers of gemcitabine exposure or CDA activity because they correlate well with pharmacokinetic parameters of gemcitabine (data not shown). Serum creatinine levels and age were shown to significantly affect the clearance of dFdU. The association between dFdU clearance and renal function was also reported by Jiang et al.[21]

Conclusion

We performed population pharmacokinetic analyses of gemcitabine and dFdU in Japanese cancer patients. Clearance of gemcitabine was decreased by *CDA* 208G>A (Ala70Thr, *3) and was slightly increased by *CDA*–31delC and coadministration with S-1. Clearance of dFdU was influenced by renal function and age.

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Clinical Trial Note

Randomized Phase II Study of Gemcitabine plus S-1 Combination Therapy vs. S-1 in Advanced Biliary Tract Cancer: Japan Clinical Oncology Group Study (JCOG0805)

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A randomized Phase II selection design trial comparing gemcitabine plus S-1 combination therapy with S-1 monotherapy for chemo-naïve unresectable or recurrent biliary tract cancer patients was started in Japan. The aim of this trial is to evaluate the efficacy and safety of the two regimens and to determine which is more promising as a test arm regimen to be compared with the current standard regimen, gemcitabine plus cisplatin, in a subsequent Phase III trial. Patients with unresectable or recurrent biliary tract cancer are randomized to either gemcitabine plus S-1 combination therapy arm or S-1 monotherapy arm. A total of 100 patients will be accrued for this study from 18 institutions over 1 year. The primary endpoint is the proportion of 1-year overall survival, and the secondary endpoints are progression-free survival, response rate and adverse events.

Key words: iliary tract cancer – gemcitabine – S-1 – randomized Phase II selection design trial

INTRODUCTION

Biliary tract cancer consists of intrahepatic cholangiocarcinoma, extrahepatic cholangiocarcinoma, gallbladder cancer and ampulla of Vater cancer. In Japan, it is estimated that ~18 000 patients with biliary tract cancer die annually and it is the sixth leading cause of cancer death (about 5.6% of all deaths due to cancer) (1,2).

In biliary tract cancer, curative surgical resection offers the only chance for a cure; however, most patients are initially diagnosed with unresectable disease. Moreover, many patients who undergo curative surgery develop recurrence (3).

For unresectable or recurrent biliary tract cancer, systemic chemotherapy is recognized as a standard treatment and, globally, gemcitabine, platinum analogue and fluoropyrimidine are considered as the key drugs (3,4). Although gemcitabine alone was regarded as the standard regimen for the advanced biliary cancer until recently, gemcitabine plus cisplatin (GC) has become the new standard regimen on the basis of the results of the ABC-02 trial (5), in which the superiority of GC over gemcitabine alone was shown.

Gemcitabine plus S-1 combination therapy (GS) or S-1 monotherapy is another promising regimen for unresectable or recurrent biliary tract cancer. In a Phase II trial for biliary tract cancer, S-1 monotherapy showed a better response rate (35%) (6) than gemcitabine alone (17.5%) (7) with milder toxicity. GS also showed a better response (34%) than gemcitabine alone in a Phase II trial for biliary tract cancer (8), even though the former showed much more toxicity than the latter. Therefore, we regard both regimens as promising and

planned this randomized Phase II trial to determine which regimen is more promising as the test arm regimen in a subsequent Phase III trial, in which the test arm will be compared with the current standard regimen, gemcitabine plus cisplatin.

The Protocol Review Committee of the Japan Clinical Oncology Group (JCOG) approved this protocol in December 2008 and the study was initiated in February 2009. This trial was registered at the UMIN Clinical Trials Registry as UMIN 000001685 (http://www.umin.ac.jp/ctr/index.htm).

PROTOCOL DIGESTS OF THE JCOG0805

Objectives

The aim of this study is to evaluate the safety and efficacy of the two regimens and to determine which regimen is more promising as the test arm regimen in a subsequent Phase III trial.

Study Setting

The study was a multi-institutional open-label randomized Phase II selection design trial.

Resources

This study is supported by Grants-in-Aid for Cancer Research (20S-3, 20S-6) Health and Labour Sciences Research Grant for Clinical Cancer Research (19–22), from the Ministry of Health, Labour and Welfare of Japan.

Endpoints

The primary endpoint is the proportion of 1-year overall survival in all eligible patients. Overall survival is defined as days from randomization to death from any cause, and it is censored at the last follow-up day when the patient is alive. The secondary endpoints are progression-free survival, response rate and adverse events.

Progression-free survival is defined as days from randomization to disease progression or death from any cause, and it is censored at the latest day when the patient is alive without any evidence of progression.

Eligibility Criteria

Inclusion Criteria

For inclusion in the study, patients are required to fulfill all of the following criteria.

(i) Clinically diagnosed with biliary tract cancer, which includes intrahepatic cholangiocarcinoma, extrahepatic cholangiocarcinoma, gallbladder cancer and ampulla of Vater cancer.
(ii) Recurrent or unresectable biliary tract cancer.
(iii) Histologically proven papillary adenocarcinoma, tubular adenocarcinoma, or adenosquamous carcinoma for extrahepatic cholangiocarcinoma, gallbladder cancer and ampulla of Vater cancer patients. Histologically proven adenocarcinoma for intrahepatic cholangiocarcinoma patients.
(iv) Without central nervous system metastasis.
(v) Without moderate or more severe ascites and pleural effusion.
(vi) No previous therapy against biliary tract cancer.
(vii) No previous chemotherapy or radiotherapy against any other malignancies.
(viii) ECOG performance status of 0 or 1.
(ix) Sufficient oral intake.
(x) Aged 20–79 years old.
(xi) Adequate organ functions.
(xii) Written informed consent.

Exclusion Criteria

Patients are excluded if they meet any of the following criteria.

(i) Simultaneous or metachronous (within 5 years) double cancers, with the exception of intramucosal tumor curable with local therapy.
(ii) Pregnant or lactating women or women of childbearing potential and men who want to get their partner pregnant.
(iii) Psychosis.
(iv) Requiring systemic steroid medication.
(v) Interstitial pneumonia or lung fibrosis.
(vi) Watery diarrhea.
(vii) Active bacterial or fungous infection.
(viii) Severe complication: heart failure, renal dysfunction, liver dysfunction, hemorrhagic peptic ulcer, paresis of intestine, ileus, uncontrollable diabetes mellitus etc.
(ix) Requiring the administration of flucytosine, phenytoin or warfarin potassium.
(x) Drug allergy for iodic drugs or gadolinium.

Randomization

After confirmation of fulfillment of the eligibility criteria, registration is made by telephone or fax to the JCOG Data Center. Patients are randomized in the JCOG Data Center by a minimization method balancing the arms with institution, primary tumor (gallbladder cancer/intrahepatic cholangiocarcinoma, extrahepatic cholangiocarcinoma or ampulla of Vater cancer) and clinical stage (II, III/IV or recurrent).

Treatment Methods

For the GS arm, 1000 mg/m^2 gemcitabine is infused on days 1 and 8, and 30 mg/m^2 S-1 is orally administered twice per day from days 1 to 14, repeated every 3 weeks.

For the S-1 monotherapy arm, 40 mg/m^2 S-1 is orally administered twice per day for 4 weeks, followed by a 2-week rest, repeated every 6 weeks.

Protocol treatments in both arms are continued until progression, unacceptable toxicity or patient refusal.

Follow-up

Enhanced abdominal computed tomography (CT)/magnetic resonance imaging, chest CT/X-rays and tumor markers (CEA and CA19-9) are evaluated at least every 6 weeks during the protocol treatment. Adverse events are evaluated at least every 2 weeks during the protocol treatment using CTCAE ver. 3.0.

Study Design and Statistical Analysis

This study is a randomized Phase II selection design trial (9) to evaluate which regimen, GS or S-1, is more promising for the test arm regimen for a subsequent Phase III trial. The regimen that shows the higher point estimate in terms of the proportion of 1-year survival will be considered to be more promising.

The frequency of toxicity is expected to be higher in GS than in S-1 monotherapy, but we expect that the frequency of severe toxicity will be almost equivalent. Therefore, we will select the more promising regimen on the basis of efficacy, namely, 1-year overall survival, as long as the levels of severe toxicity do not differ markedly between the two arms.

Sample size was determined as follows by Simon's selection design. We assumed that 1-year survival of one regimen is 30% and that of the other regimen is more than 40%. In this situation, the sample size ensuring at least 85% probability of correct selection of the more effective regimen is 98 patients, with 49 patients per arm. Considering the likelihood of some ineligible patients being enrolled, the total number of patients was set at 100.

Interim Analysis and Monitoring

We do not plan the interim analysis in this study. In-house monitoring will be performed every 6 months by the JCOG Data Center to evaluate the study progress and to improve the study quality.

Participating Institutions

The participating institutions (from north to south) are as follows: Sapporo-Kosei General Hospital, Tochigi Cancer Center, Jichi Medical University, Saitama Cancer Center, National Cancer Center Hospital East, Chiba Cancer Center Hospital, National Cancer Center Hospital, Kyorin University School of Medicine, Cancer Institute Hospital, Kanagawa Cancer Center, Yokohama City University Medical Center, Shizuoka Cancer Center, Aichi Cancer Center Hospital, Osaka Prefectural Hospital Organization Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka National Hospital, National Hospital Organization Shikoku Cancer Center, National Kyushu Cancer Center and Kyushu University Hospital.

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Conflict of interest statement

None declared.

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Long-Term Administration of Wilms Tumor-1 Peptide Vaccine in Combination with Gemcitabine Causes Severe Local Skin Inflammation at Injection Sites

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The skin toxicity of vaccine therapy at injection sites is generally limited to Grades 1–2 due to the nature of their function. We experienced two cases of severe and prolonged local adverse effects in 25 patients following a Phase I study of gemcitabine and Wilms tumor-1 peptide vaccine mixed with incomplete Freund's adjuvant for inoperable pancreatic or biliary tract cancer. These patients requested to continue the treatment after the study period; however, in the course of compassionate use, they developed unacceptable local skin reactions and terminated their vaccine treatment. One patient (human leukocyte antigen, A0201, 3 mg) developed Grade 3 ulceration at the 10th vaccination and another (human leukocyte antigen, A2402, 1 mg) developed Grade 2 indulation and fibrosis at the 16th vaccination. Skin toxicity occurred at 6.4–8.4 months and continued for several months after the final vaccination during gemcitabine treatment. In these cases, activation or induction of Wilms tumor-1-specific T lymphocytes was not apparent in the peripheral blood despite their severe local reactions. Therefore, we need to monitor patients for late-onset, severe and long-lasting skin reactions at injection sites in Wilms tumor-1 cancer vaccine therapy, particularly for combination treatment with gemcitabine.

Key words: gemcitabine – WT-1 peptide vaccine – incomplete Freund's adjuvant – inflammation – ulcer

INTRODUCTION

The recent development of cancer vaccines has provided insight into anticancer immunity and assisted in the identification of numerous tumor-associated antigens; thus, numerous clinical trials are underway. Therapeutic cancer vaccines are believed to rarely have severe adverse effects. The adverse effects of immunization include systemic reactions and local reactions, such as pain, swelling and erythema. However, these effects are thought to be related to the nature of vaccine function and severe inflammation is very rare. Nevertheless, we experienced two cases showing late-onset and prolonged local inflammation during compassionate use following a Phase I study of gemcitabine (GEM) combined with Wilms tumor-1 (WT-1) peptide vaccine for patients with advanced pancreatic or biliary duct cancer. They stopped the vaccine and subsequently received only GEM; however, skin inflammation continued even during GEM treatment. In this report, we also examined the relationship between local inflammation and immunological status in the peripheral blood of these patients.

CASE REPORTS

CASE 1

A 73-year-old woman was diagnosed as having intrahepatic cholangiocellular carcinoma (mass-forming type, moderately differentiated adenocarcinoma, T2N1M0, stage

IIIC) and a tumor mass of 48 × 45 × 45 mm in the left robe of her liver was resected in May 2007. In March 2008, small lung metastatic lesions and pelvic bone metastasis were detected using CT examination. After palliative radiation therapy for bone metastasis, she consented to enter a Phase I study of the combined GEM and WT-1 peptide vaccine. The schedule and vaccine injection sites are shown in Fig. 1A and B. She had human leukocyte antigen (HLA)-A0201 genotype and received 3-mg HLA-A0201 restricted WT-1 peptide vaccine (aa126–134 RMFPNAPYL mixed with Montanide ISA 51 VG). She had Grade 1 hematological toxicity, fatigue, anorexia, nausea and vomiting caused by GEM. Local adverse effects of the vaccine (Grades 1–2: erythema, itching and nodules) appeared after the second vaccination. The maximum redness diameter was 40 mm forming a nodule after the fourth vaccination during the study period. Local inflammation was stable up to the eighth vaccination. However, redness exacerbated after the ninth vaccination to 30–40 mm, and ulcers developed at the vaccination sites on both arms after the 10th vaccination. Vaccine was discontinued as a result of ulcer development (Grade 3) (Fig. 2), although GEM was continued because disease status was stable. Despite vaccination discontinuation, she developed ulceration at the abdominal and femoral sites during continuous GEM treatment. After 2 further months, she developed obstructive jaundice. A biliary stent was inserted and GEM treatment was stopped for 1 month. Nonetheless, ulceration remained in the femoral areas, and although other sites of ulceration were covered with granulation, redness and effusion remained. The maximal response was stable disease according to RECIST criteria, although the size of small lung metastases decreased, CEA decreased from 10.7 to 3.0 ng/ml (normal, <5 ng/ml), and CA19-9 decreased from 425 to 108 U/ml (normal, <37 U/ml).

Case 2

A 73-year-old woman was diagnosed with gall bladder carcinoma [nodular type, well-differentiated tubular adenocarcinoma, T3N0M1 (peritoneum), stage IV] in January 2008. The tumor mass was 30 mm with direct invasion of the liver. She consented to enter this study. She had HLA-A2402 genotype, and received 1-mg modified HLA-A2402-restricted WT-1 peptide vaccine (modified 9-mer peptide aa235–243 CYTWNQMNL mixed with Montanide ISA 51 VG). Her treatment schedule was the same as in Case 1 (Fig. 1A and B). She had Grade 1 hypoalbuminemia, fatigue, anorexia, nausea and Grade 2 anemia caused by GEM. Local adverse effects of the vaccine (red, itching and nodule) appeared after the first injection and the maximum redness diameter was 20 mm with a nodule after the fourth vaccination. The inflammation sizes were almost no change however she complained of itching at the injection sites after the eighth vaccination and non-steroidal anti-inflammatory ointment was administered. After the 16th vaccination, she was unable to continue immunization due to strong itching and felt limitation of motility in her arms and thighs at the injection sites because of tightened skin. GEM treatment was continued because the disease status remained stable. Local reactions (redness and nodules) and itching remained for 3 months after the final vaccination (Fig. 2). The maximal response was stable disease.

Immunological Status in the Peripheral Blood

We frequently monitored immunological status with peripheral blood samples (Fig. 1A) from the patients according to the study protocol. We performed surface maker staining, WT1 multimer staining (HLA-A0201 WT-1 Dextramer [RMFPNAPYL], HLA-2402 WT-1 Dextramer [CMTWNQMNL], modified WT-1 Dextramer

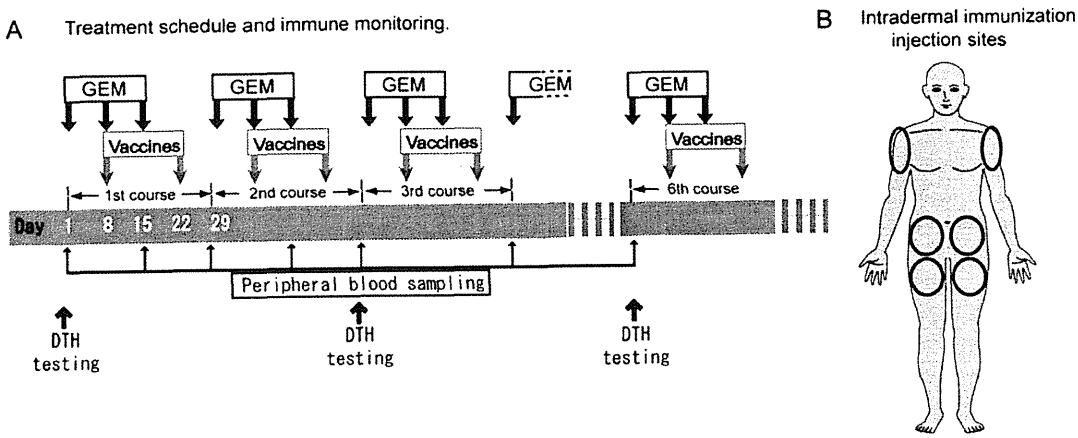

Figure 1. Study schedule and sites of immunization injection. Gemcitabine (GEM) was injected once weekly for 3 weeks with a 1-week rest period (black arrows) and WT-1 peptide vaccine was initiated on day 8 and injected biweekly (gray arrow). Treatment continued until disease progression (A). Peptide vaccine was injected intradermally at each of the six sites (100 μl) (B).

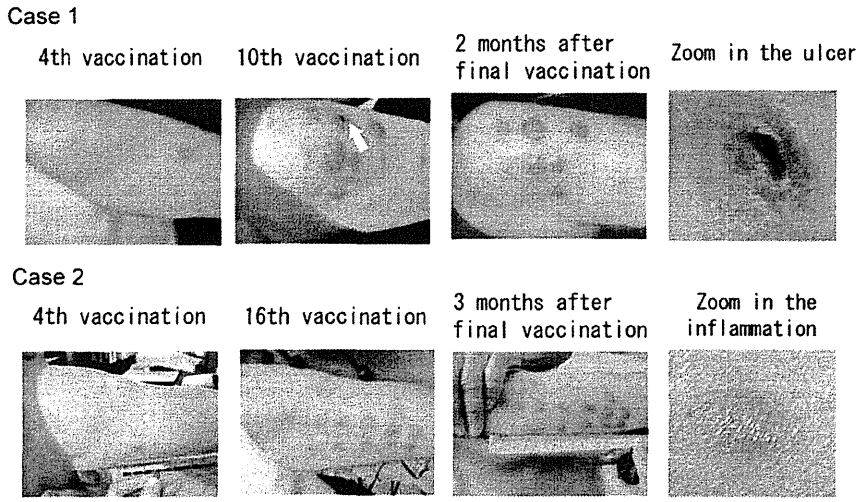

Figure 2. Injection sites in Cases 1 and 2. White arrows indicate ulcers. Inflammation continued for months even after immunization was terminated due to local adverse effects.

[CYTWNQMNL] were obtained from Immudex [Copenhagen, Denmark]. HLA-A0201 WT-1 Pentamer [RMFPNAPYL] was purchased from Proimmune [Oxford, UK]), intracellular cytokine staining by WT1 peptide stimulation and WT1-specific tetramer staining (HLA-A0201 WT-1 Tetramer [RMFPNAPYL], HLA-2402 modified WT-1 Tetramer [CYTWNQMNL]) after mixed lymphocyte and peptide culture (MLPC). The methods are shown in our previous study (1–2). In the surface maker staining, the absolute number of $CD69^+$ $CD8^+$ T cells, $CD14^+$ monocytes, and $CD11c^+$ and $CD123^+$ dendritic cells increased throughout the trial (Fig. 3A). We did not detect consistent evidence of WT1-specific lymphocyte induction in either case by multimer staining and intracellular cytokine staining; however, after MLPC a small number of WT1 tetramer binding cells were detected in Case 1 (Fig. 3B).

DISCUSSION

Clinical trials of cancer vaccines, particularly peptide vaccines, are typically carried out with no significant toxicities, although Grade 1–2 local inflammation is common. While most participants experience discomfort at local injection sites, these effects are not regarded as serious. However, some studies published over the past 10 years have reported Grade 3 local adverse effects (3–8). All of these trials targeted melanoma patients, and although the peptides were different in each study, they were all emulsified with incomplete Freund's adjuvant (IFA). Some studies added cytokines, such as interleukin-12, granulocyte-macrophage colony-stimulating factor, interferon-α2b and interleukin-2 (3,5–6, 8). The volume of IFA was over 1 ml and doses were administrated subcutaneously (3–4,8) or injected both intradermally and subcutaneously (5–7). The schedules for vaccination ranged from weekly to monthly intervals, and the total number of immunizations was 6–26 in limbs or at primary sites. The frequency of Grade 3 events at local injection sites ranged from 2.6 to 24%, and included 'local pain' (3), 'ulceration' (4) or 'injection site reactions' (5–8).

In our study, we used intradermal injections of 600 μl of peptide/IFA emulsion at six injection sites (100 μl at each site), as this delivers the antigen directly to dermal dendritic cells. Recent studies on the influenza vaccine have compared intradermal and intramuscular routes, and have confirmed the superiority of intradermal vaccination for immunological response; however, this also leads to an increase in local adverse effects (9–12). Some studies on prophylactic vaccines have also reported comparisons between percutaneous and intradermal immunization (13–15). Most of these have concluded that the response to intradermal immunization is better than that to subcutaneous immunization, although one study showed an increase in local inflammation after intradermal injection (15). Intradermal immunization may cause stronger local inflammation than other methods of vaccination.

We also used GEM in this study. Our preliminary study on the immunological effects of GEM treatment showed an increase in dendritic cells and monocytes (1). The increase in dendritic cells may have had an effect on local inflammation at the injection sites in the present chemoimmunotherapy regimen. We could not elucidate the mechanisms of severe local reactions in this trial, and planning a skin biopsy and immunohistochemical examination in future trials.

We frequently monitored immunological status with peripheral blood samples. However, we did not detect consistent evidence of antigen-specific lymphocyte induction by immunization in circulating blood without cell expansion. Furthermore, we did not detect any evidence of

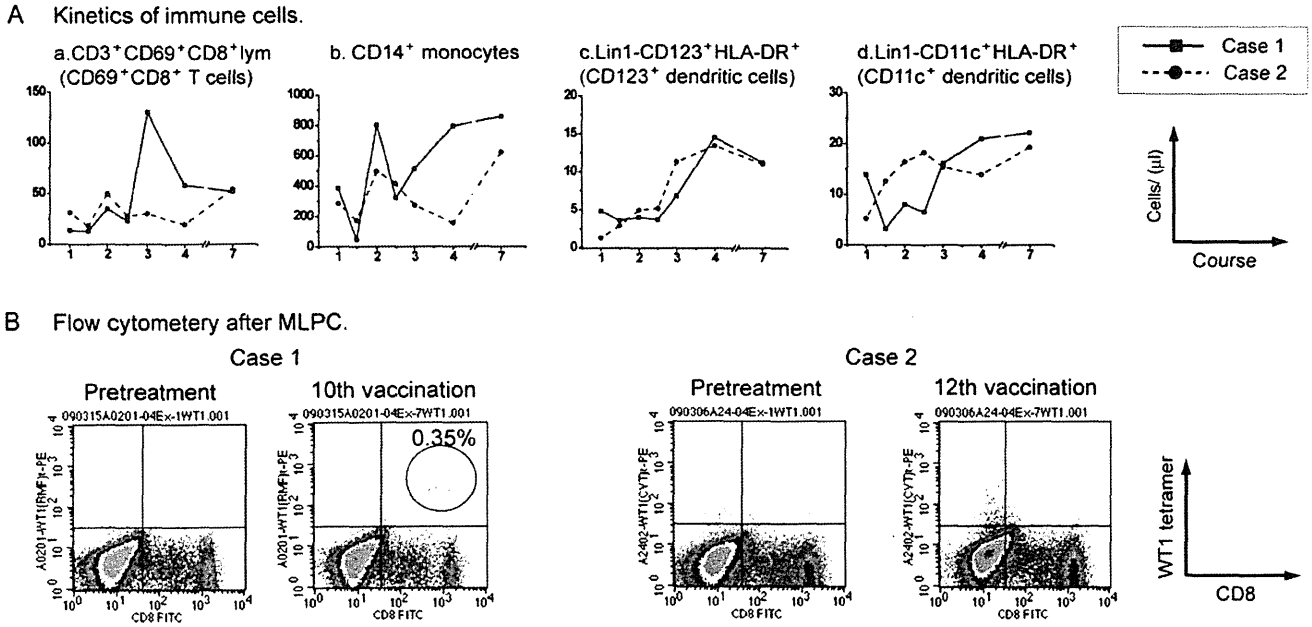

Figure 3. Kinetics of immune cells and mixed lymphocyte and peptide culture (MLPC). $CD69^+$ $CD8^+$ T cells, $CD14^+$ monocytes, $CD123^+$ and $CD11c^+$ dendritic cells increased in both patients (A). Induction of WT-1-specific CTL was detected in Case 1 by MLPC; however, the percentage was very low (B).

WT-1-specific lymphocytes after vaccination in Case 2, despite severe local inflammation. There may be several immunological discrepancies between local inflammation and peripheral blood samples. Thus, injection site reactions may not always induce comparable objective immunological reactions. We are preparing a report including the immunomonitoring data in our trial in another manuscript. It is also difficult to manage local adverse effects, as there are no standardized terms in common terminology criteria for adverse events to express local injection site reactions in cancer vaccine trials. We experienced conflicts with regard to the terms and definitions for the grades to apply to local reactions. Agreement is therefore needed in order to accurately characterize local reactions before vaccine trials.

Although vaccine therapies are believed to have essentially no severe adverse effects and are considered to be a safe therapeutic strategy, we observed rather serious local injection site reactions, even after treatment was discontinued. Thus, patients must be adequately informed about these unpleasant local reactions, and close observation should be extended until the later phase of study.

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Conflict of interest statement

None declared.

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Treatment Efficacy/Safety and Prognostic Factors in Patients with Advanced Biliary Tract Cancer Receiving Gemcitabine Monotherapy: An Analysis of 100 Cases

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Key Words
Biliary tract cancer · Gemcitabine · Monotherapy · Prognostic factors · Treatment efficacy · Treatment safety

Abstract
Aim: The purpose of this study was to elucidate the treatment efficacy and safety of gemcitabine monotherapy, and to identify prognostic factors in patients with advanced biliary tract cancer receiving this therapy. ***Method:*** The data of 100 patients with advanced biliary tract cancer who were treated with gemcitabine as first-line chemotherapy were reviewed retrospectively. ***Results:*** One patient showed complete response (1.0%) and 6 patients showed partial response (6.0%), yielding an overall response rate of 7.0%. The main grade 3/4 toxicities were neutropenia and leukopenia. The median survival, 1-year survival rate and progression-free survival were 7.3 months, 21.6% and 3.1 months, respectively. Multivariate analysis identified a performance status of 0–1, serum C-reactive protein level of <3.0 mg/dl, serum carcinoembryonic antigen level of <10 ng/ml and serum albumin level of ≥3.5 g/dl as factors independently associated with a favorable prognosis. ***Conclusions:*** Gemcitabine monotherapy showed modest efficacy with manageable toxicity in patients with biliary tract cancer. These results could be useful as reference data for optimizing treatment strategies and planning future clinical trials in patients with advanced biliary tract cancer.

Introduction

Biliary tract cancer (BTC) is uncommon in western countries, but it is a common cancer-related death in Japan, with an estimated 16,000 deaths occurring annually [1]. Surgery currently remains the only potentially curative treatment, but the majority of patients are diagnosed at an advanced stage of the disease because of the lack of early symptoms. Moreover, even in patients treated with surgical resection, the risk of recurrence is extremely high [2]. Although systemic chemotherapy is indicated

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for patients with unresectable disease, standard chemotherapeutic regimens have not been established in the last decades [2]. To improve survival, various agents were evaluated in clinical trials. Among these agents, gemcitabine was found to yield relatively favorable results [3, 4], and it has been administered worldwide either as a single agent or in combination with other agents for the treatment of BTC [2–5]. More recently, in a randomized phase III study of combined chemotherapy with gemcitabine and cisplatin versus gemcitabine monotherapy (UK ABC-02 study), median survival times were 11.7 and 8.3 months, respectively (p = 0.002) [6]. Therefore, the gemcitabine-cisplatin combination will become standard chemotherapeutic treatment for advanced BTC.

Numerous clinical trials investigating gemcitabine-based regimens have been conducted to date, but the numbers of patients have been small and only selected patients have been treated with gemcitabine. In addition, prognostic factors in BTC patients treated with gemcitabine have not yet been fully clarified. The objectives of this current study were to retrospectively review the treatment efficacy and safety of gemcitabine monotherapy, as well as to identify prognostic factors in patients with advanced BTC receiving this therapy.

Patients and Methods

Patients

One hundred sixteen patients with advanced or recurrent BTC received gemcitabine monotherapy from December 2001 to August 2007 at the National Cancer Center Hospital and National Cancer Center Hospital East. The diagnosis of BTC was confirmed histologically and/or cytologically as adenocarcinoma. Among these patients, the data of 16 patients were excluded from this analysis (a history of prior treatment in 10 patients; voluntary move to another hospital before the first tumor assessment in 3 patients and no evaluable tumor in 3 patients). A total of 100 patients had measureable lesion(s) and data were thus analyzed to elucidate the treatment efficacy and safety of gemcitabine monotherapy. The following criteria had to be met to be eligible for systemic chemotherapy, including gemcitabine monotherapy, at our institutions: Eastern Cooperative Oncology Group performance status (PS) of 0–2, adequate bone marrow function (white blood cell (WBC) count $\geq$3,000/mm^2, absolute neutrophil count $\geq$1,000/mm^3 and platelet count $\geq$70,000/mm^3) and availability of written informed consent from each patient. Patients were excluded if they had severe complications. Gemcitabine was administered at a dose of 1,000 mg/m^2 by intravenous injection for 30 min on days 1, 8 and 15 of each 28-day cycle until disease progression, appearance of unacceptable toxicity or patient's refusal for treatment continuation. All patients underwent physical examination and assessment of toxicity at least once every 1 or 2 weeks until the completion of gemcitabine treatment. All patients with obstructive jaundice underwent percutaneous transhepatic or endoscopic retrograde biliary drainage before treatment. These patients were required to have serum bilirubin levels of <3.0 mg/dl and serum AST and ALT levels <5 times the upper limit of normal.

Response and Toxicity Evaluation

The antitumor effect of gemcitabine was evaluated by CT/MRI conducted every 4–8 weeks after the start of treatment. Tumor response was determined according to the Response Evaluation Criteria in Solid Tumors [7]. The size of measurable lesions was determined using enhanced CT or MRI. For this analysis, tumor response was reviewed, and the best overall response was recorded for each patient. Toxicities were graded according to the Common Terminology Criteria for Adverse Events, version 3.0.

Analysis of Prognostic Factors

Eighteen variables were selected in this study based on previous investigations [8–11] and our own clinical experience. All data were obtained just before the start of the systemic chemotherapy. The variables, which were divided into two clinically meaningful subgroups, were as follows: age (<65/$\geq$65 years), sex (male/female), PS (0–1 or 2), WBC count (<8,500/$\geq$8,500/μl), hemoglobin level (<12.0/$\geq$12.0 g/dl), platelet count (<220,000/$\geq$220,000/μl), serum albumin level (<3.5/$\geq$3.5 g/dl), serum total bilirubin level (<2.0/$\geq$2.0 mg/dl), serum lactate dehydrogenase (LDH) level (<230/$\geq$230 IU/l), serum C-reactive protein (CRP) level (<3.0/$\geq$3.0 mg/dl), biliary drainage (presence/absence) and prior surgical resection (presence/absence) as the host-related variables, primary tumor location (intrahepatic, extrahepatic, bile duct and ampulla of Vater/gallbladder), extent of disease (localized/metastatic), peritoneal dissemination (presence/absence), liver metastasis (presence/absence), serum carcinoembryonic antigen (CEA) level (<10/$\geq$10 ng/ml) and serum carbohydrate antigen 19-9 (CA 19-9) level (<1,000/$\geq$1,000 U/ml) as the tumor-related variables. Peritoneal dissemination was defined as recognition of peritoneal nodules on CT/MRI or positive cytology of group V ascites.

Statistical Analysis

Progression-free survival was calculated as the time interval from the 1st day of treatment to the date of detection of disease progression, last day of follow-up, or the date of death. Overall survival was calculated as the time interval from the 1st day of treatment to the date of death or the last day of follow-up. In univariate analysis, cumulative survival proportions were calculated by the Kaplan-Meier method and differences were evaluated by the log-rank test. Only variables that were identified as showing statistical significance in univariate analysis were included into Cox's proportional hazard regression model for multivariate analysis. $p < 0.05$ was considered to be statistically significant and all the tests were two-sided. All statistical analyses were performed using the SPSS statistical software package (SPSS version 11.0 for Windows).

Results

Patient Characteristics

The characteristics of the patients are shown in table 1. PS was 0 in 66 patients (66.0%), 1 in 27 patients (27.0%)

Table 1. Patient characteristics

Characteristics	Patients
Age, years, median [range]	67.5 [44–82]
Sex, n (%)	
Male	60 (60.0)
Female	40 (40.0)
PS, n (%)	
0	66 (66.0)
1	27 (27.0)
2	7 (7.0)
WBC, n/μl, median [range]	6,400 [3,200–17,200]
Hemoglobin, g/dl, median [range]	12.2 [6.2–15.3]
Platelets, n × 10^4/μl, median [range]	23.2 [7.9–56.8]
Albumin, g/dl, median [range]	3.6 [1.9–4.6]
Total bilirubin, mg/dl, median [range]	0.8 [0.2–4.1]
Lactic dehydrogenase, IU/l median [range]	203.0 [70.0–733.0]
CRP, mg/dl, median [range]	0.9 [0.0–26.3]
Primary tumor site, n (%)	
Intrahepatic bile duct	23 (23.0)
Extrahepatic bile duct	25 (25.0)
Gallbladder	45 (45.0)
Ampulla of Vater	7 (7.0)
Extent of disease, n (%)	
Locally advanced	20 (20.0)
Metastatic	80 (80.0)
Metastatic site, n (%)	
Liver	36 (36.0)
Lymph node	28 (28.0)
Peritoneal dissemination	25 (25.0)
Lung	16 (16.0)
Biliary drainage (+)	30 (30.0)
Prior surgical resection (+)	28 (28.0)
CEA, ng/ml, median [range]	6.5 [0.5–3,110.0]
CA 19-9, U/ml, median [range]	258.1 [0.0–827,000]

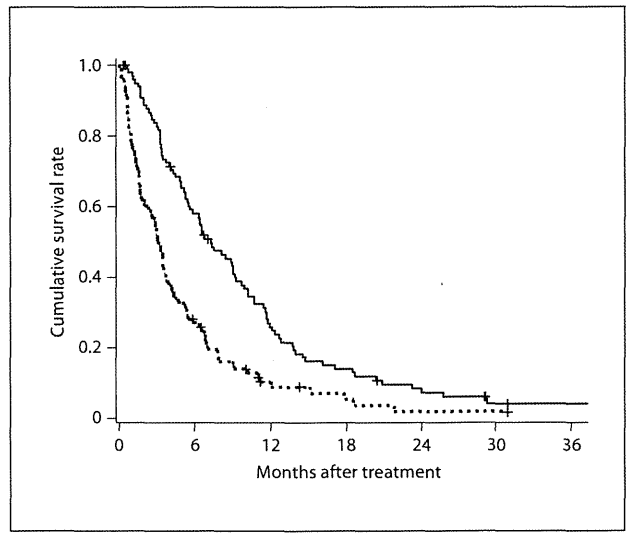

Fig. 1. Overall survival (solid line) and progression-free survival (broken line) in patients with BTC treated with gemcitabine monotherapy.

and 2 in 7 patients (7.0%). Twenty-three (23.0%) patients had intrahepatic bile duct cancer, 25 (25.0%) had extrahepatic bile duct cancer, 45 (45.0%) had gallbladder cancer, and 7 (7.0%) had cancer in the ampulla of Vater. The median number of cycles of gemcitabine monotherapy administered was 2.9 (range: 1–34). Eighteen patients (18.0%) received second-line treatment as follows: S-1 monotherapy, 7 patients; uracil/tegafur, 3 patients; uracil/tegafur + doxorubicin, 2 patients; immunotherapy, 3 patients, and other treatments, 3 patients.

Tumor Response

All the 100 patients had measureable primary or metastatic lesion(s). Of the 100 patients, complete response (CR) was achieved in 1 patient, partial response (PR) in 6 patients, stable disease (SD) was noted in 56 patients, and progressive disease (PD) in 35 patients. The remaining 2 patients could not be assessed radiologically, but both were judged as showing clinical evidence of tumor progression. The overall response rate (RR) was 7.0% [95% confidence interval (CI), 2.9–13.9]. The data were also analyzed according to the tumor type. The overall RR in patients with cancer of the intrahepatic bile duct, extrahepatic biliary duct, gallbladder and ampulla of Vater were 4.2% (1/23), 8.0% (2/25), 8.8% (4/45) and 0.0% (0/7), respectively. The overall disease control rates (CR + PR + SD) in patients with cancer of the intrahepatic bile duct, extrahepatic bile duct, gallbladder and ampulla of Vater were 69.5% (16/23), 60.0% (15/25), 57.8% (26/45) and 85.7% (6/7), respectively.

Survival

By the time of the analysis, 91 of the 100 patients had died as a result of PD. The median follow-up of censored 9 patients was 7.0 months (range, 0.4–30.9).

The overall and progression-free survival curves are shown in figure 1. The median survival, 1-year survival rate and median progression-free survival were 7.3 months (95% CI, 5.4–9.2 months), 21.6% and 3.1 months (95% CI, 2.6–3.6 months), respectively. The median progression-free survival times in PR, SD and PD patients was 12.0 (95% CI 9.5–14.5), 4.3 (95% CI 2.6–6.0) and 0.8 (95% CI 0.6–1.0) months, respectively, and the overall

Table 2. Treatment-related adverse events (worst grade reported during the treatment period)

Adverse events	Toxicity grade				
	1	2	3	4	3/4 (%)
Hematological toxicity					
Leukopenia	24	18	9	0	9 (9.0)
Neutropenia	6	9	11	5	16 (16.0)
Thrombocytopenia	9	9	2	0	2 (2.0)
Anemia	18	20	3	0	3 (3.0)
Non-hematological toxicity					
Nausea/vomiting	13	0	1	0	1 (1.0)
Anorexia	22	2	2	0	2 (2.0)
Fatigue	25	2	0	0	0
Diarrhea	3	0	0	0	0
Rash	8	1	0	0	0
Decreased serum albumin level	13	6	2	0	2 (2.0)
Elevated serum AST	16	7	2	0	2 (2.0)
Elevated serum ALT	10	5	2	0	2 (2.0)
Elevated serum ALP	3	1	5	0	5 (5.0)
Hyponatremia	9	0	0	0	0
Cognitive disturbance	0	0	1	0	1 (1.0)
Biliary tract infection	1	0	2	0	2 (2.0)

AST = Aspartate aminotransferase; ALT = alanine aminotransferase; ALP = alkaline phosphatase.

survival times were 17.1 (95% CI 14.6–19.7), 9.7 (95% CI 8.4–10.9) and 3.2 (95% CI 2.6–3.9) months, respectively.

Toxicity

The most severe hematological and non-hematological toxicities during the entire treatment period are summarized in table 2. With regard to grade 3/4 hematological toxicities, neutropenia was observed in 16 patients (16.0%), leukopenia in 9 patients (9.0%), anemia in 3 patients (3.0%) and thrombocytopenia in 2 patients (2.0%). In regard to the main grade 3/4 non-hematological toxicities, an elevated alkaline phosphatase level was observed in 5 patients (5.0%), and other adverse events occurred in <3%. Cognitive disturbance was observed in 1 patient (1.0%); however, recovery occurred in the absence of any treatment. There were no other life-threatening toxicities and no treatment-related deaths.

Univariate Analysis

Of the 18 pretreatment variables, 12 variables (female, PS 0–1, WBC count <8,500/μl, hemoglobin >12.0 g/dl, serum albumin ≥3.5 g/dl, serum total bilirubin <2.0 mg/dl, serum LDH <230 IU/l and serum CRP <3.0 mg/dl, intrahepatic, extrahepatic, bile duct and ampulla of Vater cancer, absence of peritoneal dissemination, absence of liver metastasis and serum CEA <10 ng/ml) were identified as being significantly associated with a longer survival time (table 3).

Multivariable Analysis

The 12 variables identified by univariate analysis as being of prognostic significance were subsequently incorporated in Cox's proportional hazard model for multivariate analysis, and a PS of 0–1, serum CEA <10 ng/ml, serum albumin ≥3.5 g/dl, and serum CRP <3.0 mg/dl were identified as being independently associated with a favorable prognosis (table 4).

Discussion

Gemcitabine has been used as a key drug for advanced BTC, and at present gemcitabine-based regimens are widely used as first-line treatment for advanced BTC. However, to date, reliable data of gemcitabine monotherapy based on large-scale studies are still lacking. This study shows not only efficacy and safety but also prognostic factors in a large study cohort.

Studies on gemcitabine monotherapy at doses of 800–2,200 mg/m^2 as first-line therapy for advanced BTC have reported response rates of 0–36.0%, and median survival times from 4.6 to 14.0 months [12–20]. Our overall response rate of 7.0% in BTC patients administered gemcitabine monotherapy as first-line therapy in our study was comparable to those reported from previous trials of gemcitabine monotherapy. The median survival of 7.3 months and the incidence of adverse events were also in accord with previous reports [12–20]. These findings clearly demonstrate that gemcitabine monotherapy is well tolerated in patients with advanced BTC in the clinical setting.

The study was also designed to determine prognostic factors in patients with advanced BTC administered gemcitabine monotherapy. The identification of prognostic factors can help to predict life expectancy and to select the appropriate treatment. In the current study, among the variables investigated, PS, serum CRP, serum albumin and serum CEA were found to be independently associated with patient prognosis.

PS was the strongest prognostic factor, although most of our patients (93%) had a good PS (0–1) and only 7 patients were PS 2. PS is a simple, but widely used index re-