encouraging activity, response rates between 20.0 and 24.7% and median overall survival between 5.7 and 7 months, have been reported in two phase II studies [11, 18]. However, survival benefit of this combination therapy was not shown in a phase III study [12], in which, 360 patients were randomized to treatment with a combination of gemcitabine 1,000 mg/m^2 followed by irinotecan 100 mg/m^2 given on days 1 and 8 of a 3-week cycle versus gemcitabine monotherapy. The response rate for the combination therapy was higher at 16.1% compared with 4.4% for gemcitabine alone, but there was no difference in median overall survival (6.3 vs. 6.6 months). However, several clinical studies have recently indicated that irinotecan-based chemotherapy seemed to be an effective treatment for advanced pancreatic cancer after gemcitabine failure: irinotecan–raltitrexed combination demonstrated overall response rate of 16% (3/19) in patients with gemcitabine-pretreated pancreatic cancer [21], and Cantore et al. [3] reported that irinotecan plus oxaliplatin showed response rate of 10% (3/30) with a clinical benefit response of 20% (6/30) for patients with advanced pancreatic cancer after gemcitabine failure.

Because biliary excretion is a major elimination pathway for irinotecan and its metabolites, we investigated the impact of biliary drainage on the pharmacokinetics for this agent. Our results suggested that patients with biliary drainage tended to have higher area under the concentration versus time curve of irinotecan and metabolites compared with patients without biliary drainage. Meyerhardt et al. [10] reported that modest elevation of bilirubin (1.0–1.5 mg/dl) is associated with increased grade 3 to 4 neutropenia in patients treated with irinotecan. The fact that the two patients with biliary drainage in the current study had slight elevation of baseline serum bilirubin level (1.4 and 1.7 mg/dl) might influence pharmacokinetics for irinotecan. Although no severe hematological or non-hematologic toxicities appeared in these two patients, careful observation may be required when treating patients with biliary drainage.

In conclusion, single-agent irinotecan showed a substantial antitumor activity for patients with metastatic pancreatic cancer, rendering a 27.0% response rate. The toxicity with this schedule appears manageable, though it must be monitored carefully.

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ORIGINAL REPORT

Pharmacokinetics of Gemcitabine in Japanese Cancer Patients: The Impact of a Cytidine Deaminase Polymorphism

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ABSTRACT

Purpose
Gemcitabine is rapidly metabolized to its inactive metabolite, 2′,2′-difluorodeoxyuridine (dFdU), by cytidine deaminase (CDA). We previously reported that a patient with homozygous 208A alleles of *CDA* showed severe adverse reactions with an increase in gemcitabine plasma level. This study extended the investigation of the effects of *CDA* genetic polymorphisms on gemcitabine pharmacokinetics and toxicities.

Patients and Methods
Genotyping of *CDA* was performed by a direct sequencing of DNA obtained from the peripheral blood of Japanese gemcitabine-naïve cancer patients (n = 256). The patients recruited to the association study received a 30-minute intravenous infusion of gemcitabine at a dose of either 800 or 1,000 mg/m^2, and eight blood samples were periodically collected (n = 250). Plasma levels of gemcitabine and dFdU were measured by high-performance liquid chromatography. Plasma CDA activities toward cytidine and gemcitabine were also measured (n = 121).

Results
Twenty-six genetic variations, including 14 novel ones and two known nonsynonymous single nucleotide polymorphisms (SNPs), were detected. Haplotypes harboring the nonsynonymous SNPs 79A>C (Lys27Gln) and 208G>A (Ala70Thr) were designated **2* and **3*, respectively. The allelic frequencies of the two SNPs were 0.207 and 0.037, respectively. Pharmacokinetic parameters of gemcitabine and plasma CDA activities significantly depended on the number of haplotype **3*. Haplotype **3* was also associated with increased incidences of grade 3 or higher neutropenia in the patients who were coadministered fluorouracil, cisplatin, or carboplatin. Haplotype **2* showed no significant effect on gemcitabine pharmacokinetics.

Conclusion
Haplotype **3* harboring a nonsynonymous SNP, 208G>A (Ala70Thr), decreased clearance of gemcitabine, and increased incidences of neutropenia when patients were coadministered platinum-containing drugs or fluorouracil.

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INTRODUCTION

Gemcitabine (2′,2′-difluorodeoxycytidine) is a nucleoside anticancer drug that has a broad spectrum of antitumor activity against various solid tumors, such as non–small-cell lung cancer and pancreatic cancer.[1] In a randomized clinical trial, gemcitabine was confirmed to provide a survival advantage over fluorouracil in addition to symptom-relieving benefits in patients with advanced pancreatic cancer.[2] On the basis of these results, gemcitabine has generally been accepted as a standard chemotherapeutic agent for advanced pancreatic cancer.

Gemcitabine is transported into cells by concentrative and equilibrative nucleoside transporters,[3-8] where it is phosphorylated to its monophosphate form by deoxycytidine kinase. Gemcitabine triphosphate, an active form of gemcitabine, is incorporated into an elongating DNA strand, and is followed by the addition of another deoxynucleotide that leads to the halt of DNA synthesis.[9,10] Another mode of action in solid tumors, associated with the inhibition of ribonucleotide reductase, has also been suggested.[11]

Gemcitabine is rapidly metabolized to an inactive metabolite, 2′,2′-difluorodeoxyuridine (dFdU)

Table 1. *CDA* Haplotypes Estimated in This Study

Region		5'-Flanking			Exon 1 (5'-UTR)			Exon 1	Intron 1	Exon 2		Intron 2		
SNP ID		CDA001	CDA002	CDA003	CDA004	CDA005	CDA007	CDA009	CDA010	CDA011	CDA012	CDA014	CDA016	CDA017
Nucleotide change		−451C>T	−205C>G	−182G>A	−116G>A	−92A>G	−33_−31 delC	79A>C	IVSI+37 G>A	208G>A	210T>C	IVS2 +87_+88 insTCAT	IVS2+242 A>G	IVS2+296 T>A
Amino acid change								Lys27Gln		Ala70Thr	Ala70Ala			
Haplotypes														
*1	*1a													
	*1b													
	*1c													
	*1d													
	*1e													
	*1f													
	*1g													
	*1h													
	*1i													
	*1j													
	*1k													
	*1l													
	*1m													
	*1n													
	Other *1													
*2	*2a													
	*2b													
	*2c													
	*2d													
	Other *2													
*3	*3a													
	*3b													

(continued on next page)

NOTE. The haplotypes were described as a number plus a small alphabetical letter. Four single nucleotide polymorphisms (SNPs) (CDA006, 008, 013, 015) were found only in the very rare ambiguous **1* haplotypes. Since these ambiguous haplotypes were grouped and described as "Other **1*" in this table, the four SNPs are not shown in the row of nuclotide change. White, major allele; gray, minor allele.

by cytidine deaminase (CDA),[9] and most of an administered dose is recovered as dFdU in the urine.[12] CDA is expressed at varying levels in the human tissues,[13] and the rapid clearance of gemcitabine can be attributed to its plentiful occurrence in the liver.[14] Two single nucleotide polymorphisms (SNPs), 79A>C (Lys27Gln) and 435T>C (Thr145Thr), have been discovered in *CDA*, the CDA-encoding gene in humans.[15,16] The 79A>C SNP reportedly reduces the deamination activity (maximum velocity/Km) toward 1-beta-D-arabinofuranosyl cytosine (cytarabine),[15] and increases Km toward gemcitabine,[17] in vitro. A recently discovered third SNP, 208G>A (Ala70Thr) displayed a decrease in deamination activity of 60% for cytidine and 68% for cytarabine when introduced into a *CDA*-null yeast strain.[18]

Toxicity of gemcitabine is generally mild,[19,20] but unpredictable severe toxicities such as myelosuppression are occasionally experienced.[21,22] Our previous case report described a patient with homozygous 208A alleles of the *CDA* gene who showed severe adverse reactions with increased plasma gemcitabine levels.[23] In addition, there has been controversy over the relationship between cellular CDA activity and the clinical effects of cytarabine.[24-27] This study examined the relationship between *CDA* polymorphisms, and the pharmacokinetics of gemcitabine, plasma CDA activity, or adverse reactions in Japanese cancer patients.

PATIENTS AND METHODS

Gemcitabine and dFdU for analytic standards were supplied by Eli Lilly Japan K.K. (Kobe, Japan). Tetrahydrouridine, 3'-deoxy-3'-fluoro-thymidine (3'-dFT), cytidine and uridine (Sigma-Aldrich Chemical Co, St Louis, MO) were purchased. All other chemicals were of highest grade available.

Patients

The participants in this study consisted of 256 Japanese patients with carcinoma, including six patients described in a previous report,[23] at the National Cancer Center Hospital (Tokyo, Japan) and National Cancer Center Hospital East (Kashiwa, Japan). Two hundred fifty-one patients received a 30-minute intravenous infusion of gemcitabine at a dose of either 800 or 1,000 mg/m^2, and five patients received a fixed dose-rate (10 mg/m^2/min) infusion at a dose between 1,000 and 1,500 mg/m^2. The eligibility criteria for the study were as previously reported.[23] The ethics committees of the National Cancer Center and the National Institutes of Health Sciences approved this study. Written informed consent was obtained from each participant.

Table 1. *CDA* Haplotypes Estimated in This Study (continued)

Intron 3					Exon 4	Exon 4 (3'-UTR)					
CDA018	CDA019	CDA020	CDA021	CDA022	CDA023	CDA024	CDA025	CDA026			
IVS3+71 T>C	IVS3 −194_−193 insAlu	IVS3-56 G>A	IVS3-36 G>A	IVS3-23 C>T	435C>T	510 (*69) G>T	637_638 (*196_*197) insC	676 (*235) A>G			
					Thr145Thr						
									No.	Frequency	
									175	0.342	
									63	0.123	
									52	0.102	
									17	0.033	
									13	0.025	
									12	0.023	
									12	0.023	
									11	0.021	0.756
									8	0.016	
									5	0.010	
									4	0.008	
									4	0.008	
									2	0.004	
									1	0.002	
									8	0.016	
									84	0.164	
									11	0.021	
									5	0.010	0.207
									3	0.006	
									3	0.006	
									18	0.035	0.037
									1	0.002	
									512	1.000	1.000

Monitoring and Toxicities

A complete medical history and data on physical examinations were recorded before the gemcitabine therapy. CBC and platelet counts, as well as blood chemistry, were measured once a week during the first 2 months of gemcitabine treatment. Toxicities were graded according to the National Cancer Institute Common Toxicity Criteria, version 2.

DNA Sequencing

All four exons and the 5′-upstream region (approximately 800 base pairs [bp] from the translation initiation codon) of *CDA* were amplified from 100 ng of DNA extracted from peripheral blood, and sequenced along both strands. Polymerase chain reaction (PCR) primers[23] and sequencing and PCR conditions[28] were described previously. For detection of an approximately 300-bp Alu insertion (IVS3-194_-193insAlu), PCR was performed using a specific primer set (5′- TTGTCATAGCAGAAGGAGGTT-3′ and 5′- TCAG CTCTCCACACCATAAGG-3′) and 100 ng of DNA as a template. Then, sizes of the amplified fragments were determined by 1% agarose gel electrophoresis. NT_004610.17 (GenBank, National Center for Biotechnology Information, Bethesda, MD) was used as the reference sequence.

Linkage Disequilibrium and Haplotype Analyses

Hardy-Weinberg equilibrium and linkage disequilibrium (LD) analyses were performed by SNPAlyze software (Dynacom Co, Yokohama, Japan). All of the detected variations were found to be in Hardy-Weinberg equilibrium ($P \geq .05$), except for the SNP IVS1+37G>A ($P = .002$). Some of the haplotypes were unambiguously assigned from subjects with homozygous variations at all sites or a heterozygous variation at only one site. The diplotype configurations (a combination of haplotypes) were separately inferred by LDSUPPORT software,[29] which determines the posterior probability distribution of the diplotype configuration for each subject based on the estimated haplotype frequencies. The diplotype configurations of all but 11 subjects were inferred with probability of more than 0.93. All haplotypes inferred in single subjects were gathered as the groups "Other *1" and "Other *2" in Table 1.

Pharmacokinetic Study

Five patients with fixed dose-rate infusion and one patient with interruption of infusion for more than 15 minutes were excluded from the pharmacokinetic analysis described herein. Heparinized blood was collected before administration of gemcitabine and used to measure plasma CDA activity. Five milliliters of heparinized blood was also sampled for pharmacokinetic analysis before the first gemcitabine administration, and at 0, 15, 30, 60, 90, 120, and 240 minutes after the termination of the infusion. Fifty microliters of 1% tetrahydrouridine was immediately added to these samples to prevent ex vivo deamination. Plasma levels of gemcitabine and dFdU were determined using the high-performance liquid chromatography method previously reported.[23] The area under the curve (AUC) and mean residence time from 0 to infinity, peak concentration (C_{max}), clearance (CL/m^2) and distribution volume based on the terminal phase (Vz/m^2) were calculated using WINNonlin (Scientific Consultant, Apex, NC) version 4.01 (Pharsight Corporation, Mountain View,

CA). AUC and C_{max} were corrected for dose, assuming that all patients received 1,000 mg/m^2 of gemcitabine.

CDA Activities in Plasma

Determination of CDA activities was performed using the method by Richards et al[30] with slight modifications (modifications are as follows: gemcitabine was used as a substrate as well as cytidine, internal standards for analysis [3′-dFT for gemcitabine or dFdU for cytidine] were added to the mixtures at the beginning of the reaction, and high-performance liquid chromatography was used for detection of reaction products). CDA activity was expressed by unit, and one unit of enzyme activity was defined as the concentration that produces 0.1 nmol of dFdU or uridine per minute per milliliter of plasma.[30]

Statistical Analysis

Kruskal-Wallis, Mann-Whitney, and Pearson's correlation tests were performed using the JMP software (SAS Institute Inc, Cary, NC). Two ordinally scaled categoric data were subjected to χ^2 analysis for a correlation test. A significance level of .05 was applied to all two-tailed and correlation tests. Multiplicity was adjusted by the false-discovery rate,[31] if necessary.

RESULTS

Genetic Variations and Haplotype Structures of CDA

Twenty-six (14 novel) genetic variations were detected in the 256 Japanese cancer patients enrolled onto this study (Table 2). Three of the novel variations were found in the 5′-untranslated region, one in exon 2, three in the 3′-untranslated region and seven in the introns. Three known SNPs in the coding region of *CDA* were also detected. Among these, the nonsynonymous SNPs, 79A>C (Lys27Gln) and 208G>A (Ala70Thr), exhibited allelic frequencies of 0.207 and 0.037 (Table 2), respectively, and they were comparable to those reported previously.[18] One patient was found to be homozygous for the 208A polymorphism. A novel insertion of an approximately 320-bp Alu element (IVS3-194_-193insAlu) was newly found in intron 3.

The detected variations were used to analyze LD (Fig 1). Four novel variations (IVS3-56G>A, IVS3-36G>A, IVS3-23C>T and

Table 2. Variations of the *CDA* Gene Found

SNP ID				Position				
This Study	NCBI (dbSNP)	JSNP	Location	NT_004610.17	From the Translational Initiation Site or From the Nearest Exon	Nucleotide Change and Flanking Sequences (5′ to 3′)	Amino Acid Change	Allele Frequency
MPJ6_CDA001	rs532545	IMS-JST008767	5′-Flanking	3739514	−451‡	TGCCTCCTGCCT**C/T**GGGATGCCGCAG		0.199
MPJ6_CDA002	rs603412	IMS-JST008768	5′-Flanking	3739760	−205‡	CACACGTAGGCA**C/G**TGTCTTACACCA		0.266
MPJ6_CDA003	rs12726436		5′-Flanking	3739783	−182‡	CACACCTGCTGA**G/A**TCCAAACCATGG		0.061
MPJ6_CDA004*			Exon 1 (5′-UTR)	3739849	−116‡	CTGAGAGCCTGC**G/A**GTCTGGCTGCAG		0.059
MPJ6_CDA005	rs602950		Exon 1 (5′-UTR)	3739873	−92‡	GGGACACACCCA**A/G**GGGGAGGAGCTG		0.205
MPJ6_CDA006*			Exon 1 (5′-UTR)	3739884	−81‡	AAGGGGAGGAGC**T/C**GCAATCGTGTCT		0.002
MPJ6_CDA007	rs3215400	IMS-JST076939	Exon 1 (5′-UTR)	3739934	−33_−31‡	GCTCCTGTTTCC**C/-**GCTGCTCTGCTG		0.451
MPJ6_CDA008*			Exon 1 (5′-UTR)	3739957	−8‡	TGCCTGCCCGGG**G/A**TACCAACATGGC		0.002
MPJ6_CDA009†	rs2072671	IMS-JST008769	Exon 1	3740043	79‡	CAGGAGGCCAAG**A/C**AGTCAGCCTACT	Lys27Gln	0.207
MPJ6_CDA010	rs12059454		Intron 1	3740155	IVS1+37	CCCAGCCCAGCA**G/A**CCTGGGTGGTGG		0.184
MPJ6_CDA011†			Exon 2	3755816	208‡	GCTGAACGGACC**G/A**CTATCCAGAAGG	Ala70Thr	0.037
MPJ6_CDA012*			Exon 2	3755818	210‡	TGAACGGACCGC**T/C**ATCCAGAAGGCC	Ala70Ala	0.004
MPJ6_CDA013*			Intron 2	3755932	IVS2+58	GCCAACATCTTC**C/T**TTACACATATTA		0.002
MPJ6_CDA014*			Intron 2	3755961_3755962	IVS2+87_+88	TCATTCATTCAT**-/TCAT**CTGACATATGTT		0.135
MPJ6_CDA015*			Intron 2	3756043	IVS2+169	ATAAGGAGATAA**A/G**TAAGAAATGGAG		0.002
MPJ6_CDA016	rs10916825		Intron 2	3756116	IVS2+242	CATACAAGGGCC**A/G**GTATGCCCCTGT		0.289
MPJ6_CDA017	rs818194		Intron 2	3756170	IVS2+296	GTCCTACAAGAT**T/A**TAACAGAAAGGC		0.217
MPJ6_CDA018	rs3738130	IMS-JST083844	Intron 3	3764805	IVS3+71	AGCCACGCCAAG**T/C**TGCAGGCATGGC		0.053
MPJ6_CDA019*			Intron 3	3769093_3769094	IVS3-194_−193	CTGTTCAGTTTC**-/(Alu)**§ACAGCATTCTTT		0.293
MPJ6_CDA020*			Intron 3	3769231	IVS3-56	CAGACCCAGTCC**G/A**TCTCAGCCCCCT		0.293
MPJ6_CDA021*			Intron 3	3769251	IVS3-36	CCCCTCAGCCAC**G/A**CTGTGTCTCTCA		0.293
MPJ6_CDA022*			Intron 3	3769264	IVS3-23	CTGTGTCTCTCA**C/T**GCCAGCTTTGCC		0.293
MPJ6_CDA023†	rs17846527		Exon 4	3769397	435‡	CCTGCAGAAGAC**C/T**CAGTGACAGCCA	Thr145Thr	0.293
MPJ6_CDA024*			Exon 4 (3′-UTR)	3769472	510 (*69)‡	CTCACAGCCCTG**G/T**GGACACCTGCCC		0.002
MPJ6_CDA025*			Exon 4 (3′-UTR)	3769599_3769600	637_638 (*196_197)‡	ACCGCCGCCCCC**-/C**TGCCCCACCTTT		0.293
MPJ6_CDA026*			Exon 4 (3′-UTR)	3769638	676 (*235)‡	GGGCCCTCTTTC**A/G**AAGTCCAGCCTA		0.010

*Novel variations detected in this study.
†Yue et al.[18]
‡A of the translation initiation codon ATG is numbered 1, and the number with * in parentheses indicates the position from the termination codon TGA.
§The sequence of the Alu insertion was as follows: 5′ - (T)nGAGACGGAGTCTCGCTGTCGCCCAGGCTGGAGTGCAGTGGCGCAATCTCGGCTCACTGCAGGCTCCG CCCCCTGGGGTTCACGCCATTCTCCTGCCTCAGCCTCCCGAGTAGCTGGGACTACAGGCGCCCGCCACCTCGCCCGGCTAATTTTTTGTATTTTTAGTAGAGACGGGGTT TCACCGTGTTAGCCAGGATGGTCTCGATCTCCTGACCTCGTGATCCGCCCGCCTCGGCCTCCCAAAGTGCTGGGATTACAGGCGTGAGCCACCGCGCCCGGCCCACTG TTCAGTTTC-3′ (n = approximately 25).

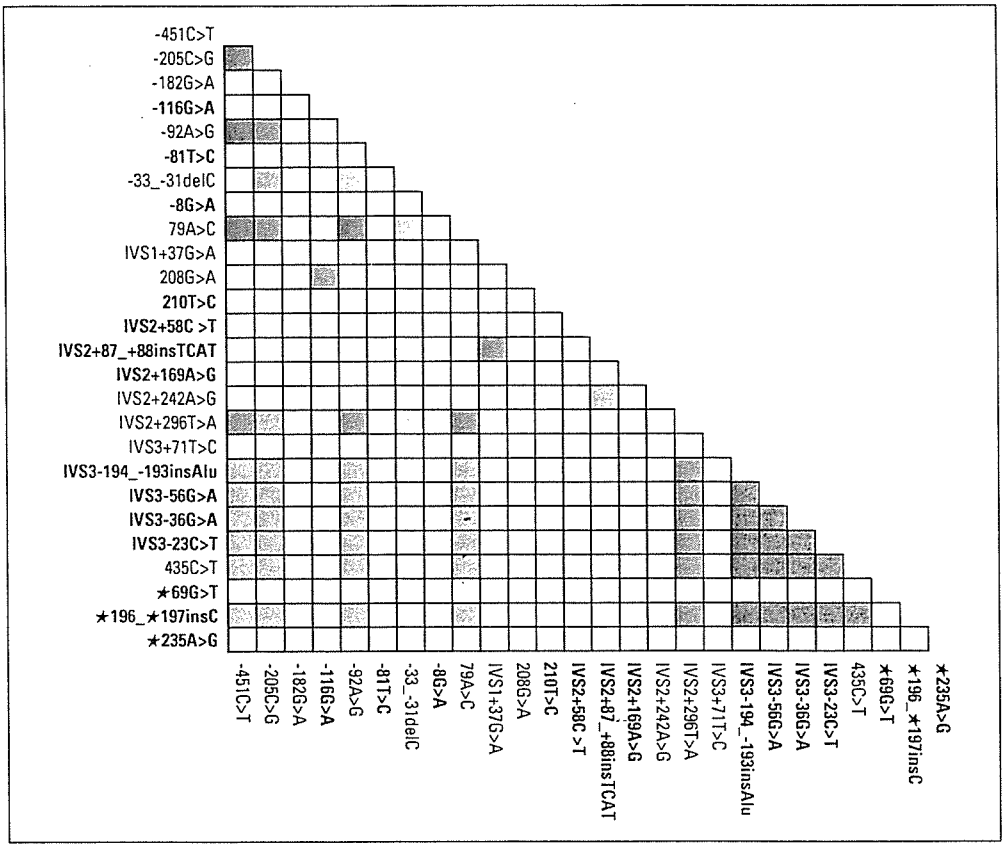

Fig 1. Linkage disequilibrium (LD) among 26 *CDA* variations. Pairwise LD as r^2 (from 0 to 1) is expressed as 10-graded blue color. The density of the blue color increases with higher linkage rates.

*196_*197insC), the Alu element insertion and a known SNP 435C>T (Thr145Thr) showed complete linkage (Fig 1) with a frequency of 0.293. Strong LD ($r^2 \geq 0.93$) was also observed among SNPs −451C>T, −92A>G, and 79A>C. Note that moderate linkages ($r^2 \geq 0.42$) were observed between the two completely and strongly linked groups (Fig 1). Because relatively close linkages were observed throughout the entire *CDA* gene spanning approximately 30 kb, the *CDA* haplotypes were analyzed as one LD block.

The haplotypes determined/inferred in this study are summarized in Table 1. Haplotypes without amino acid changes were defined as the **1* group. These harboring the nonsynonymous SNPs 79A>C and 208G>A were designated **2* and **3*, respectively. The most frequent haplotype was **1a* (frequency, 0.342), followed by **2a* (0.164), **1b* (0.123), and **1c* (0.102).

Effects of Patient Background Factors on Gemcitabine Pharmacokinetics

Characteristics of the 250 patients recruited for the pharmacokinetic study are shown in Table 3. As previously reported, the patient who was homozygous for 208A showed extraordinarily high gemcitabine and low dFdU plasma concentrations.[23] Therefore, this patient was excluded when effects of patient background factors on the pharmacokinetic parameters of gemcitabine were analyzed.

The effects of age and sex on pharmacokinetic parameters are summarized in Table 4. Vz/m^2 was significantly higher in males than in females, even after adjustments for their body surface areas (Mann-Whitney $P = .0031$). The C_{max}, AUC, CL/m^2, and Vz/m^2 of gemcitabine showed significant correlations with age ($P < .0001$ for all parameters). Values of any clinical tests, including creatinine concentration, did not correlate with pharmacokinetic parameters of gemcitabine. Although approximately 30% of patients in this study underwent combined chemotherapy, no clinically significant effects of coadministered drugs on pharmacokinetic parameter values of gemcitabine were detected.

Effects of CDA Genetic Polymorphisms on Gemcitabine Pharmacokinetics

Because age and sex were unbiasedly distributed among the patients, with the various genotypes compared in the following analysis (data not shown), the 250 patients were not further stratified.

After careful examination, the data did not identify any **1*, **2*, or **3* subtypes that showed statistically significant differences from each major subtype within the three groups (Table 5; unpublished data). Therefore, each subtype was combined into one group (the **1*, **2*, or **3* group) to investigate the association between pharmacokinetic parameters and genetic groups.

The relationships between the diplotype groups and the pharmacokinetic parameters of gemcitabine are shown in Figure 2 and summarized in Table 6. The data clearly showed a haplotype **3*–dependent decrease in clearance and increases in C_{max} and AUC values (χ^2 trend $P < .0001$ for all parameters). The values of C_{max}, AUC, and CL/m^2 observed in the patient bearing a homozygous 208G>A (**3/*3*) were two-fold, five-fold, and one-fifth of the means of the **1/*1* group, respectively (Table 6). In contrast, the pharmacokinetic parameters of gemcitabine except for mean residence time (data not shown) were not significantly influenced by the haplotype **2*.

Table 3. Characteristics of Patients Recruited to Pharmacokinetic Studies (N = 250)

Characteristic	
Sex	
Male	165
Female	85
Age, years	
Mean	62.6
Range	32-80
SD	9.2
Body surface area, m^2	
Mean	1.57
Range	1.18-1.99
SD	0.17
Weight, kg	
Mean	54.8
Range	34.4-80.3
SD	9.7
Performance status	
0	122
1	118
2	10
Primary tumor	
Pancreas	205
Lung	38
Mesothelium	7
Dose, mg/m^2	
1,000	246
800	4
Regimen	
Gemcitabine alone	180
Gemcitabine-based combination	70
Cisplatin	30
Carboplatin	16
Fluorouracil	14
Vinorelbine ditartrate	10
Previous treatment	
None	134
Surgery	66
Radiation	74
Chemotherapy	65

*Effect of Haplotypes *2 and *3 on Plasma CDA Activity*

Plasma CDA activities were measured in 121 patients of the 250 patients in this study. One patient in the **1/*2* group who showed extremely high plasma CDA activities to both gemcitabine and cytidine (43.04 and 29.04 units, respectively; far higher than the 99% upper confidence limits of plasma CDA activities for the **1/*2* group) was excluded as an outlier from the following statistical analysis, although his pharmacokinetic parameters were quite normal.

Haplotype **2* failed to show any significant effects on the plasma CDA activities toward both gemcitabine and cytidine. On the other hand, activity decreased depending on the number of haplotype **3* (Table 6; Fig 3). The plasma CDA activities in the homozygous **3* (208A) patient were 12% (gemcitabine) and 25% (cytidine) of the median activities for the **1/*1* patients. As shown in Figure 4, a statistically significant correlation between the plasma CDA activity toward gemcitabine and the AUC values of gemcitabine was observed ($r = -0.30$; $P = .0009$). However, the correlations were not remarkable.

*Effect of Haplotype *3 on Toxicities*

Then, associations of haplotype **3* with toxicities were analyzed. Nadir grades of neutrophil counts were compared between the patient groups with and without haplotype **3* under the individual therapeutic regimens. As shown in Table 7, there were no significant differences in incidences of grade 3 or higher neutropenia between the two groups under the gemcitabine monotherapy. However, when gemcitabine was administered with carboplatin, cisplatin, or fluorouracil, grade 3 or higher neutropenia was more frequently observed in the haplotype **3*–bearing group than in the group without haplotype **3*. The increases in incidences were statistically significant. AUC values were also increased in the group with haplotype **3* under concomitant therapeutic regimen as under the monotherapy.

DISCUSSION

The pharmacokinetic parameters summarized in Table 4 showed great similarity to those obtained with adult American patients.[32] The age-dependent decrease in gemcitabine clearance in Japanese patients in this study is in agreement with the description for Gemzar injections (Eli Lilly Japan K.K.), which is based on a population pharmacokinetic study performed outside Japan. The main route of gemcitabine elimination is its metabolism into dFdU, and there was no correlation between plasma creatinine level and gemcitabine clearance. Therefore, the aging effect on gemcitabine clearance is likely to result from a decrease in distribution volume or liver function. It is

Table 4. Effects of Patient Background Factors on Pharmacokinetic Parameters of Gemcitabine

Factor	C_{max} (μg/mL)		AUC (hr · μg/mL)		CL/m^2 (L/hr/m^2)		Vz/m^2 (L/m^2)	
	Median	1/4-3/4 Quantiles	Median	1/4-3/4 Quantiles	Median	1/4-3/4 Quantiles	Median	1/4-3/4 Quantiles
Sex								
Male	23.1	18.4-26.1	9.9	8.6-11.8	100.3	83.7-115.9	42.4*	35.13-52.0
Female	24.0	19.8-28.8	10.2	9.0-11.5	97.6	86.1-111.2	38.7	32.7-43.5
Mann-Whitney *U* test	NS		NS		NS		$P < .005$	
Age								
Spearman r	0.32		0.39		−0.39		−0.39	
P value	< .0001		< .0001		< .0001		< .0001	

Abbreviations: C_{max}, peak concentration; AUC, area under the curve; CL/m^2, clearance; Vz/m^2, distribution volume based on the terminal phase.
*Significantly different from the value for female (Mann-Whitney *U* test $P = .0031$).

Table 5. Pharmacokinetic Parameters of Gemcitabine in Patients With Various *CDA* Diplotypes

Diplotype	No. of Patients	Median Gemcitabine PK Parameters				
		C_{max} (μg/mL)	AUC (hr · μg/mL)	CL/m² (L/hr/m²)	MRT (hours)	AUC Ratio (dFdU/gemcitabine)
**1a/*1a*	30	22.40	10.54	94.24	0.37	8.86
**1a/*1b*	17	22.75	10.08	97.91	0.35	9.08
**1b/*1b*	6	20.81	9.19	108.60	0.36	9.19
P value*		0.82	0.40	0.59	0.97	0.83
**1a/*1c*	23	23.23	10.87	94.31	0.35	8.73
**1c/*1c*	1	25.84	16.62	60.16	0.55	8.40
P value*		0.77	0.57	0.94	0.97	0.83
**1a/*1d*	7	22.05	9.07	108.30	0.36	9.04
**1d/*1d*	1	26.43	9.99	100.10	0.31	7.70
P value*		0.82	0.45	0.90	0.86	0.57
**2a/*2a*	8	23.94	9.34	107.20	0.33	9.70
**2a/*2b*	4	23.02	9.78	100.13	0.38	8.59
**2a/*2c*	2	21.50	9.22	111.63	0.36	10.99
P value†		0.66	0.98	0.76	0.077	0.46

Abbreviations: PK, pharmacokinetics; C_{max}, peak concentration; AUC, area under the curve; CL/m², clearance; MRT, mean residence time; dFdU, 2',2'-difluorodeoxyuridine.

*P value of a correlation test among **1a/*1a*, **1a/(*1b*, **1c*, or **1d*), and (**1b*, **1c*, or **1d*)/(**1b*, **1c*, or **1d*). Multiplicity is adjusted by false-discovery rate.

†*P* value of a Kruskal-Wallis test among **2a/*2a*, **2a/*2b*, and **2a/*2c*.

also indicated on the label that the elimination half-life of gemcitabine was longer in females than in males in a population pharmacokinetic study using 45 Japanese non–small-cell lung cancer patients. The present study did not reveal any significant sex-based difference in clearance. However, the distribution volume was significantly smaller in females than in males.

Human CDA is involved in the salvaging of pyrimidines,[33,34] and plays a key role in detoxifying gemcitabine. Although the activities of 27Gln or 70Thr variant (the products of 79A>C or 208G>A) toward cytidine and cytarabine were reported to be lower than those of the "prototype" in a yeast expression system,[18] the decreased CDA activity in patients bearing these SNPs has not been reported. Kreis et al[35] reported that the response of leukemic patients to cytarabine correlated with the phenotype of CDA deamination determined based on the ratio of plasma concentrations of a cytarabine metabolite and cytarabine.[35] They reported that 70% of subjects were slow metabolizers. However, the relationship between genetic polymorphisms and phenotypes remained to be clarified.

In our study, the haplotype **2* harboring 79C (27Gln) did not show clear effects on the AUC and CL/m² values. In contrast, the 208A (Thr70, **3*) -dependent decreases in gemcitabine clearance and plasma CDA activities were clearly demonstrated in this study. These results suggest that the *CDA* variant loses its in vivo deamination activities toward gemcitabine considerably. Moreover, the decreased plasma CDA activities toward gemcitabine and cytidine ex vivo also strongly suggest that the reduced enzymatic activity was caused by the genetic variation.

In the monotherapy group, the increased AUC in the patient with haplotype **3* did not clearly augment the incidence of toxicities including neutropenia. However, the incidences of grade 3 or higher neutropenia were higher in patients heterozygous for haplotype **3* compared with in the patients without haplotype **3* when they received concomitant chemotherapy with fluorouracil or platinum compounds. As we reported recently, one patient homozygous for haplotype **3* who received both gemcitabine and cisplatin suffered from extremely severe adverse effects including grade 3 anathema.[23] However, he experienced neither of the specific toxicities associated with cisplatin, nephrotoxicity, and neurotoxicity. Abbruzzese et al[36] reported the gemcitabine dose-dependent increase in incidence of thrombocytopenia (one of seven at 525 mg/m²/wk, three of nine at 790 mg/m²/wk, and three of six at 1,000 mg/m²/wk).[36] Therefore, we concluded that extremely high exposure to gemcitabine (AUC five times higher than the average) due to the decreased deamination activity caused the life-threatening severe toxicities in this patient. In contrast, the gemcitabine AUC of the patients with heterozygous haplotype **3* was only slightly (23% to 48%) increased from that of the patients having no haplotype **3* (Table 6). This finding coincides with the lack of life-threatening severe toxicities in the heterozygotes for **3*, although the incidences of grade 3 or higher neutropenia in the heterozygotes in combined chemotherapy groups were higher in the group without haplotype **3*.

CDA is also involved in the activation of capecitabine to its active form fluorouracil.[37] Therefore, capecitabine activation would be inefficient in patients who are homozygous for 208A. The allele frequency of the 208G>A SNP, a tagging SNP of haplotype **3*, was reported to be 0.125 in Africans, while it was not detected in Europeans.[38] The frequency of homozygous carriers of the variant could be higher in Africans than in the Japanese population. However, the frequency of 208G>A in Africans is still controversial, because it was not detected in 60 African Americans in a recent report.[17] Extra attention may be necessary for patients with the allele before treatments with gemcitabine or cytarabine are initiated, especially to **3/*3* patients, although more studies are necessary to confirm the clinical importance of this allele in the treatments using gemcitabine or cytarabine.

A number of studies have investigated the associations between cellular CDA activity and drug responses to cytarabine.[24-27,39] However, correlation between plasma CDA activity and the

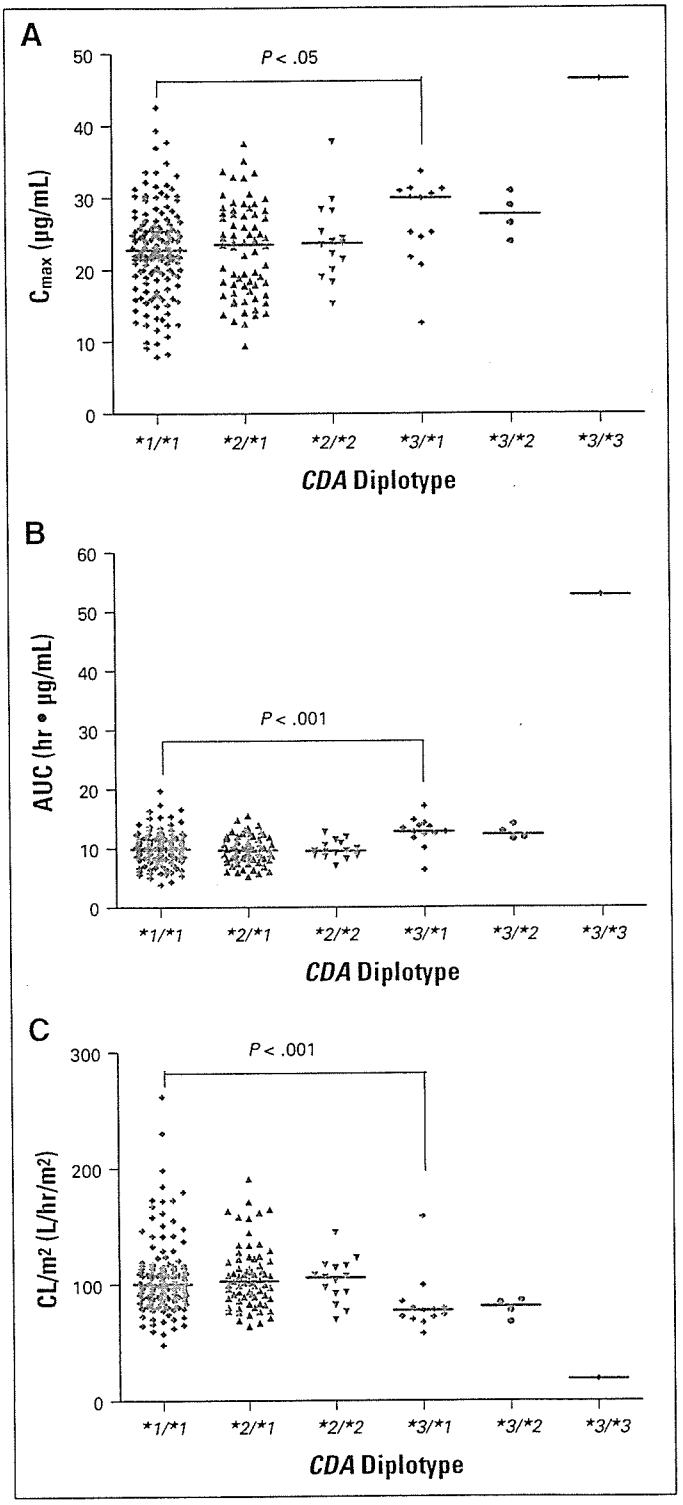

Fig 2. Effects of haplotypes **2* and **3* on the pharmacokinetic parameters of gemcitabine. (A) Peak concentration (C_{max}) and (B) area under the curve (AUC) were corrected assuming that all patients received 1,000 mg/m^2 of gemcitabine. (C) Clearance (CL/m^2). Each point corresponds to an individual patient. The bars denote the median values. *P* values are from Dunn's multiple comparison test.

pharmacokinetics of gemcitabine has not been reported. Plasma CDA activity may be a useful biomarker to screen patients with a markedly decreased metabolic CDA activity such as the patient homozygous for the **3* allele found in our study, who showed extremely low plasma CDA activity. However, a very low contribution of plasma CDA to the total clearance of gemcitabine was reported,[36] and the plasma CDA levels are increased in the inflammatory diseases.[30,40] These may account for the failure in obtaining good correlations between plasma CDA activity and the pharmacokinetic parameters of gemcitabine, as shown in Figure 4.

In conclusion, we analyzed the *CDA* genetic variations and haplotypes in Japanese cancer patients who received gemcitabine. We then investigated the associations between genetic polymorphisms and the pharmacokinetics of gemcitabine or toxicities. Depending on the haplotype **3* harboring 208A, the metabolic clearance of gemcitabine decreased, and AUC and C_{max} values were increased. Moreover, plasma CDA activities correlated well with the *CDA* genotypes. The clinical importance of the SNP 208G>A, especially of homozygotes, should be confirmed by prospective clinical studies because only one homozygous **3* patient was found in this study.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following authors or their immediate family members indicated a financial interest. No conflict exists for drugs or devices used in a study if they are not being evaluated as part of the investigation. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

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Table 6. Pharmacokinetic Parameters of Gemcitabine and Plasma CDA Activities in the Patient Groups Categorized According to Diplotypes

Diplotype	Median Gemcitabine PK Parameters: No. of Patients	C_{max} (μg/mL)	AUC (hr·μg/mL)	CL/m^2 (L/hr/m^2)	Median CDA Activity (units): No. of Patients	Gemcitabine	Cytidine
**1/*1*	148	22.81	9.96	100.30	63	6.26	5.54
**2/*1*	69	23.57	9.71	103.00	25	6.81	5.71
**2/*2*	15	23.75	9.57	106.10	14	6.53	6.24
P value*		0.52	0.46	0.99		0.47	0.19
**3/*1*	13	30.02	12.83	77.93	13	2.99	3.07
**3/*3*	1	46.42	52.86	18.92	1	0.74	1.40
P value†		5.94E-04	6.66E-13	7.77E-04		9.35E-05	2.45E-04

Abbreviations: CDA, cytidine deaminase; C_{max}, peak concentration; AUC, area under the curve; CL/m^2, clearance.
*P value of a correlation test among **1/*1*, **1/*2*, and **2/*2*. Multiplicity is adjusted by false-discovery rate.
†P value of a correlation test among **1/*1*, **1/*3*, and **3/*3*. Multiplicity is adjusted by false-discovery rate.

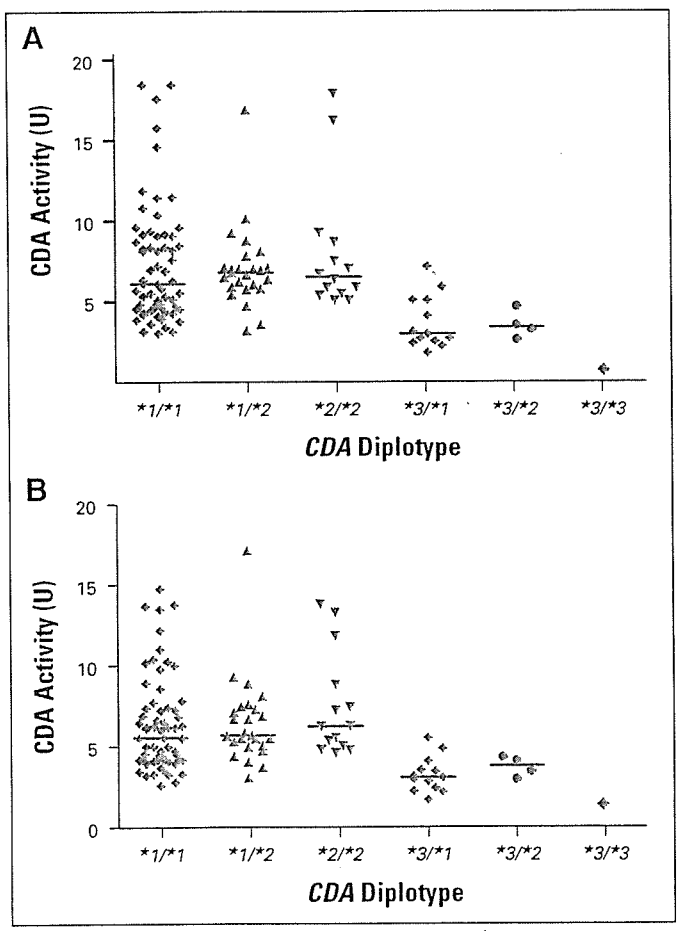

Fig 3. Effects of haplotypes **2* and **3* on plasma cytidine deaminase (CDA) activity toward gemcitabine and cytidine substrates. (A) Gemcitabine was used as a substrate, and (B) cytidine was used as a substrate. Each point corresponds to an individual patient. The bars denote the median values.

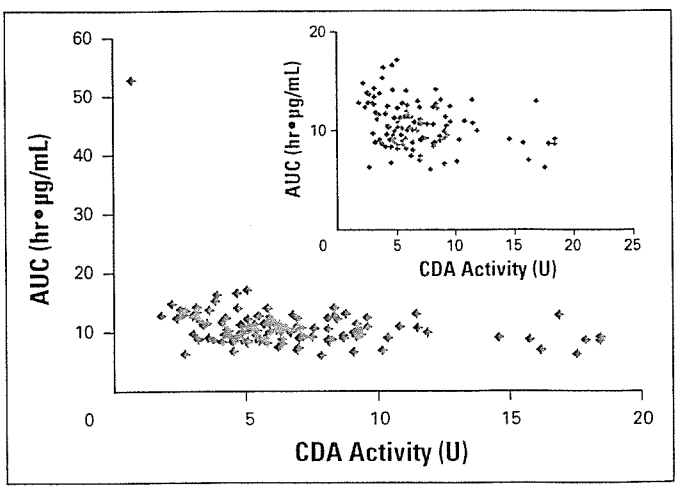

Fig 4. Correlation between plasma area under the curve (AUC) and cytidine deaminase (CDA) activity toward gemcitabine. AUC was corrected assuming that all patients received 1,000 mg/m^2 of gemcitabine. The inset excludes the data obtained from a homozygous **3* carrier. The correlation coefficient is −0.31 when the homozygous **3* carrier is included and −0.28 when the carrier is excluded.

Table 7. Comparison of Adverse Reaction Incidence and Pharmacokinetic Parameters of Gemcitabine Between Two Patient Groups With and Without Haplotype **3*

		Incidence of Neturopenia (nadir)*						
		≥ Grade 3			≥ Grade 4			
Chemotherapy	Genotype	No. of Cases	Total No. of Patients	Probability	No. of Cases	Total No. of Patients	Probability	AUC† (hr·μg/mL)
Monotherapy	*non *3/non *3*	66	167	0.40	8	67	0.05	9.91
	*non *3/*3*	6	10	0.60	1	10	0.10	13.13
	P			0.205			0.514	**0.0017**
With fluorouracil	*non *3/non *3*	3	12	0.25	2	12	0.17	8.11
	*non *3/*3*	2	2	1.00	1	2	0.50	11.98
	P			**0.029**			0.327	0.055
With carboplatin	*non *3/non *3*	9	13	0.69	1	13	0.08	9.87
	*non *3/*3*	3	3	1.00	2	3	0.67	12.48
	P			0.163			**0.033**	**0.031**
With cisplatin	*non *3/non *3*	8	28	0.29	2	28	0.07	9.53
	*non *3/*3*	1	1	1.00	0	1	0.00	11.71
	**3/*3*	1	1	1.00	1	1	1.00	52.86
	P‡			**0.030**			0.128	0.061

Note. No analyses were performed in patients who received gemcitabine with vinorelbine, because only one patient bore the haplotype **3*. Boldfacing indicates a statistically significant difference ($P < .05$).
*χ^2-test.
†Kruskal-Wallis test.
‡A *P* value for comparison between *non*3/non*3* and (*non*3/*3* + **3/*3*).

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Evaluation of Acute Intestinal Toxicity in Relation to the Volume of Irradiated Small Bowel in Patients Treated with Concurrent Weekly Gemcitabine and Radiotherapy for Locally Advanced Pancreatic Cancer

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Abstract. *Background: Treatment of concurrent gemcitabine and radiotherapy for pancreatic cancer was reported to have a higher rate of severe acute intestinal toxicity. This study evaluated the acute intestinal toxicity in relation to the volume of irradiated small bowel and other factors using dosimetric analyses in pancreatic cancer patients treated with gemcitabine-based chemoradiotherapy. Materials and Methods: The patient population was derived from a phase II trial of concurrent weekly gemcitabine and radiotherapy for locally advanced pancreatic cancer. Gemcitabine was administered weekly at a dose of 250 mg/m^2. The total dose was 50.4 Gy in 28 fractions using a four-field conformal technique. A dose-volume histogram was generated for the small bowel, colon and planning target volume (PTV) and dosimetric parameters were recorded. Correlations between the acute intestinal toxicity and the volume of irradiated small bowel and other factors were evaluated. Results: Forty-two patients enrolled between July 2001 and July 2002 were analyzed. Grade 3+ acute intestinal toxicities were observed in twenty-four (62%) patients. There was no correlation between the acute intestinal toxicity and the volume of irradiated small bowel. However, the total volume of PTV was shown to be significantly correlated with the development of Grade 3+ acute intestinal toxicity ($p=0.021$). Conclusion: The volume of irradiated small bowel did not directly influence the acute intestinal toxicity, but only the volume of PTV significantly correlated with severe acute intestinal toxicity.*

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Key Words: Pancreatic cancer, chemoradiotherapy, gemcitabine, intestinal toxicity.

Pancreatic cancer is usually diagnosed as an unresectable locally advanced or metastatic disease in most patients. In patients with locally advanced pancreatic cancer, chemoradiotherapy has been commonly used as a standard treatment since it was recognized that radiotherapy with concurrent 5-fluorouracil (5-FU) prolonged survival when compared to radiotherapy or chemotherapy alone (1-3). Various novel agents and radiation schedules have been examined in clinical trials to improve the efficacy of the treatment (4).

Gemcitabine is a novel deoxycitidine analog with a broad spectrum of antitumor activity against a variety of solid tumors, including pancreatic cancer, which has demonstrated greater clinical benefit and survival compared with 5-FU in patients with advanced pancreatic cancer (5). Gemcitabine has also been shown to be a potent radiosensitizer in human pancreatic cancer (6-8). Therefore, concurrent gemcitabine and radiotherapy are currently being examined in clinical trials, suggesting that the combination of radiotherapy and gemcitabine may improve survival in patients with locally advanced pancreatic cancer (9-13).

However, significant acute intestinal toxicity (AIT) in the treatment of concurrent gemcitabine and radiotherapy was reported compared with concurrent 5-FU and radiotherapy (9, 10, 14). In rectal cancer treated with concurrent chemoradiotherapy, a significant relationship between the intestinal toxicity and the volume of irradiated small bowel is well recognized from the results of examinations using small bowel contrast and orthogonal radiographs to calculate the volume of small bowel in the high-dose volume (15-17) and more accurately three-dimensional (3D) treatment-planning tools (18). However, it has not been reported whether the volume of irradiated small intestine is related to the degree of AIT in patients treated with concurrent chemoradiotherapy for pancreatic cancer. The purpose of this study was to evaluate the AIT in relation to

the volume of irradiated small bowel and to other factors using dosimetric analyses in patients treated with concurrent weekly gemcitabine and radiotherapy for locally advanced pancreatic cancer.

Materials and Methods

Patient population. The patient population for this study was derived from a phase II trial of concurrent weekly gemcitabine and radiotherapy for unresectable locally advanced pancreatic cancer at the National Cancer Center Hospital (19). Eligibility criteria for this phase II trial included histologically or cytologically confirmed nonresectable adenocarcinoma, 20-74 years of age, an Eastern Cooperative Oncology Group (ECOG) performance status of 0-2, no evidence of distant metastasis, adequate hematological function (hemoglobin ≥10 g/dl, leukocytes ≥4000 mm^3, neutrophils >2000 mm^3, and platelets ≥100000 mm^3), adequate hepatic function (serum total bilirubin ≤2.0 mg/dl, and serum transaminase (aspartate aminotransferase (AST)/ alanine aminotransferase (ALT) < 2.5 times the upper normal limit (UNL), adequate renal function (serum creatinine within normal limit) and written informed consent.

Treatment details and dosimetric analysis. Gemcitabine was administered intravenously over 30 min starting 2 h before radiotherapy, weekly for 6 weeks, at a dose of 250 mg/m^2, which had been previously determined in a phase I trial in our hospital (20). When grade 3 hematological toxicity, serum creatinine of 1.5-2.0 times UNL, total bilirubin level of 3.0-5.0 times UNL, serum AST/APT of 5.0-10 times UNL and/or grade 2 non-hematological toxicity (excluding nausea, vomiting, anorexia, fatigue, constipation, alopecia and dehydration) were observed, gemcitabine administration was omitted and postponed to the next scheduled treatment days.

Radiotherapy was delivered *via* a racetrack microtron (MM50, Scanditronix, Uppsala, Sweden) with a 25 MV X-rays. All patients had treatment planning computed tomography (CT) scans (X-vision, Toshiba, Tokyo, Japan), 5 mm thickness with a 5 mm slice interval, with oral small bowel contrast. The clinical target volume (CTV) included the primary tumor, nodal involvement detected by CT scan, and draining and para-aortic lymph nodes. The planning target volume (PTV) was defined as CTV plus a 10 mm margin in the lateral direction and a 10-20 mm margin in the cranio-caudal direction. Four-field techniques (anterior, posterior and opposed lateral fields) were used. The spinal cord dose was maintained below 45 Gy and ≥50% of the liver was limited to ≤30 Gy, ≥50% of both kidneys were limited to ≤20 Gy. The prescription dose was 50.4 Gy, delivered in 1.8 Gy daily fractions. FOCUS (version 3.2.1, CMS, St. Louis, MO, USA) was used as a radiotherapy treatment planning system. The individual loops of small bowel and colon were delineated on each slice of the planning CT scan from the upper end level of the liver to the lower end level of the kidneys. The volumes of small bowel receiving doses between 5 and 45 Gy were recorded from DVH at 5-Gy intervals.

Toxicity assessment. Patients were evaluated at least weekly during radiotherapy, prospectively. National Cancer Institute common toxicity criteria, version 2.0, were used for toxicity assessment. AIT was defined as any toxicity that could be related to the small bowel, which included nausea, vomiting, anorexia and diarrhea, according to the previous report for rectal cancer (17) and ≥grade 3 was considered severe toxicity.

Table I. *Patient characteristics.*

Characteristic	No. of patients (N=42)
Gender	
male	19
female	23
Age, years	
range	43-73
median	59
Performance status	
0	12
1	30
Tumor size, cm	
range	2.0-10.0
median	4.0
Tumor site	
head	20
body-tail	22

Statistical analysis. For each 5-Gy dose level from 5 to 45 Gy, an association between the volume of small bowel irradiated and grade 3+ AIT was analyzed. The differences in mean small bowel volume irradiated to each 5-Gy dose level from 5 to 45 Gy were assessed using the *t*-test for the equality of means. Univariate analysis comparing the clinical and treatment factors and grade 3+ AIT was performed using the Fisher's exact test. *P*-values less than 0.05 were considered to be statistically significant.

Results

Forty-two patients were enrolled in a phase II trial between July 2001 and July 2002, and all patients were entered in this study. The patient characteristics are shown in Table I. Forty patients completed the planned radiotherapy (50.4 Gy). Two patients discontinued radiotherapy. One patient stopped at 30.6 Gy because of duodenal bleeding and another patient stopped at 45.0 Gy because of refusal due to general fatigue. The number of times gemcitabine was administered was 6 times in 17 patients, 5 times in 15 patients, 4 times in 6 patients, 3 times in 2 patients and 2 times in 2 patients. Grade 3 and grade 4 non-hematological toxicities were observed in 31% and 33% of patients, respectively. Overall, the maximum AIT encountered during radiotherapy was grade 0 in 4 patients (9.5%), grade 1 in 9 patients (21.4%), grade 2 in 3 patients (7.2%), grade 3 in 12 patients (28.6%) and grade 4 in 14 patients (33.3%). Median and range values of the dosimetric parameters of small bowel, colon and PTV are shown in Table II. The volume of irradiated small bowel ranged from 43 cm^3 to 552 cm^3, with a median value of 251 cm^3 and the volume of

Table II. *Median and range values of dosimetric parameters.*

Parameter	Median	Range
Small bowel		
total volume, cm^3	274	47-663
irradiated volume, cm^3	251	43-552
max dose, cGy	5072	3079-5229
mean dose, cGy	1485	376-2915
Colon		
total volume, cm^3	403	120-714
irradiated volume, cm^3	397	117-686
max dose, cGy	5028	1975-5221
mean dose, cGy	1516	633-2848
Planning target volume		
total volume, cm^3	555	357-1215
max dose, cGy	5120	3106-5275
mean dose, cGy	4948	3002-5045

Table III. *Volume of irradiated small intestine at each 5-Gy dose level between 5 and 45 Gy vs. the degree of acute intestinal toxicity (mean±SE, cm^3).*

RT dose level (Gy)	Grade 0-2 toxicity	Grade 3-4 toxicity	*p*-value
5	169±99	182±99	0.669
10	150±94	161±92	0.707
15	140±90	148±90	0.787
20	64±41	66±50	0.873
25	53±36	55±42	0.879
30	49±33	50±40	0.910
35	43±27	45±36	0.864
40	38±23	41±32	0.786
45	32±20	35±28	0.715

PTV ranged from 357 cm^3 to 1215 cm^3, with a median value of 555 cm^3, corresponding to a cube of 8.2 cm on a side. The average volume of small bowel irradiated at each 5-Gy dose level between 5 and 45 Gy are shown in Table III.

The average volume of small bowel irradiated at each dose level was not significantly different between the group of grade 3+ AIT and the group of grade 0-2 AIT by the *t*-test for equality of means. The relationships between grade 3+ AIT and clinical factors are shown in Table IVa. No significant correlation was seen between grade 3+ AIT and clinical factors, including age, performance status, tumor size, tumor site, and number of times gemcitabine was administered. The relationships between grade 3+ AIT and the calculated parameters are shown in Table IVb. No significant correlation was seen between grade 3+ AIT and the volume of small bowel irradiated or other parameters regarding the small bowel and the colon. However, the total volume of PTV was shown to be significantly

Table IVa. *Univariate analysis of clinical and treatment factors related to the development of ≥ grade 3 acute intestinal toxicity.*

Characteristic	n	% toxicity	*p*-value*
Gender			
male	19	63.2%	
female	23	60.9%	>0.999
Age, years			
<60	22	54.5%	
≥60	20	70.0%	0.355
PS			
0	12	41.7%	
1	30	70.0%	0.158
Tumor size, cm			
≤4	22	54.5%	
>4	20	70.0%	0.355
Tumor Site			
head	20	65.0%	
body-tail	22	59.1%	0.758
Number of times gemcitabine was administered			
<5	10	80.0%	
≥5	32	56.3%	0.270

*Fisher's exact test.

Table IVb. *Univariate analysis of calculated parameters related to the development of ≥ grade 3 acute intestinal toxicity.*

Characteristic	n	% toxicity	*p*-value*
Small bowel			
irradiated volume, cm^3			
<250	18	66.7%	
≥250	24	58.3%	0.750
max dose, cGy			
<5100	30	60.0%	
≥5100	12	66.7%	0.740
mean dose, cGy			
<1500	22	63.6%	
≥1500	20	60.0%	>0.999
Colon			
irradiated volume, cm^3			
<400	22	59.1%	
≥400	20	65.0%	0.758
max dose, cGy			
<5000	16	68.8%	
≥5000	26	57.7%	0.530
mean dose, cGy			
<1500	21	66.7%	
≥1500	21	57.1%	0.751
Planning target volume			
total volume, cm^3			
<500	16	37.5%	
≥500	26	76.9%	0.021

*Fisher's exact test.

correlated with the development of grade 3+ AIT (p=0.021). The highest incidence of grade 3+ AIT was in patients with the volume of PTV ≥500 cm^3, corresponding to a cube of 7.9 cm on a side.

Discussion

We evaluated the relationship between the AIT and the volume of irradiated small bowel in patients treated with concurrent gemcitabine and radiotherapy for pancreatic cancer and univariate analysis revealed that the volume of irradiated small bowel, which was significantly related to AIT in the treatment of rectal cancer, did not correlate to the AIT here. Minsky *et al.* reported a significant relationship between AIT and the volume of irradiated small bowel in patients with rectal cancer treated with concurrent 5-FU-based chemotherapy and pelvic radiotherapy (17). Orthogonal radiographs were used to calculate the volume of small bowel within the treated volume, using the sum of the anterior-posterior film volume and the lateral film volume. The volume of small bowel in the pelvic radiation field was greater for patients who experienced grade 3+ AIT (441±153 cm^3) compared with those who experienced grade 0-2 acute intestinal toxicity (230±43 cm^3). Baglan *et al.* reported a strong dose-relationship for the development of grade 3+ AIT in patients treated with concurrent 5-FU based chemoradiotherapy for rectal cancer using three-dimensional (3D) treatment planning tools, the same as our method (18). A highly significant association was found between the development of grade 3+ AIT and the average volume of small bowel irradiated to each 5-Gy dose level between 5 and 40 Gy (p<0.001). The volume of small bowel that received at least 15 Gy (V15) was strongly associated with the degree of AIT.

The present report represents the first analysis of AIT using dosimetric analysis in pancreatic cancer treated with chemoradiotherapy. In this study, the patient population and treatment schedule was more homogeneous compared with previous reports for rectal cancer and toxicities were evaluated prospectively, because all patients entered in this analysis were previously enrolled in a clinical trial. The reasons for the different results regarding AIT and the volume of irradiated small bowel between rectal cancer and pancreatic cancer could be several. First, the agent of chemotherapy in the combination of radiotherapy was different between the two groups. In previous reports for rectal cancer, 5-FU based chemotherapy was used, while in our study for pancreatic cancer, gemcitabine was used. An *in vivo* study showed that there was markedly increased normal tissue toxicity, such as jejunal mucosa, when gemcitabine was given more than once a week in combination with radiotherapy (21). Second, the volume of irradiated stomach and duodenum may be related to the AIT in part, since in the treatment of pancreatic cancer the upper abdomen is irradiated and the stomach and duodenum are usually included in the treated volume. However, in this study we did not evaluate the volume of irradiated stomach since it was difficult to evaluate the volume of stomach, exactly, due to the great variation in volume depending on the time of day compared with the small bowel and colon. We also did not evaluate the volume of irradiated duodenum. Because most of the duodenum was included in the radiation field with prophylactic regional lymph node area, the volume of irradiated duodenum was considered similar among the patients.

We found that the PTV was significantly associated with severe AIT. This result indicates that a larger treated volume affects a large volume of normal tissue, not just the small bowel. Recently, in an attempt to decrease the toxicity in the treatment of gemcitabine-based chemoradiotherapy, researchers at the University of Michigan and M.D. Anderson Cancer Center performed and recommended radiation treatment planning, which set only the gross tumor in the target volume without a prophylactic regional lymph node area (11, 14, 22). These authors reported that the PTV ranged from 134 cm^3 to 465 cm^3, with a median value of 255 cm^3, corresponding to a cube of only 6.3 cm on a side, which was much smaller compared with conventional radiotherapy and patients were able to tolerate the treatment (22). Our result that the smaller PTV (<500 cm^3, corresponding to a cube of 7.9 cm on a side) had less acute intestinal toxicity supports their recommendation. However, the efficacy of treatment without prophylactic regional lymph node irradiation should be evaluated in clinical trials and a longer follow-up is needed.

In conclusion, the volume of irradiated small bowel did not directly influence the AIT in patients treated with concurrent weekly gemcitabine and radiotherapy for locally advanced pancreatic cancer. However, only the PTV significantly correlated with severe AIT. Reducing the treated volume, *e.g.*, by omitting prophylactic regional lymph node irradiation, seemed to result in decreased AIT when patients were treated concurrently with gemcitabine-based chemoradiotherapy.

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A Phase I/II Study of Combination Chemotherapy with Gemcitabine and 5-Fluorouracil for Advanced Pancreatic Cancer

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Background: In an effort to improve efficacy of single-agent gemcitabine in pancreatic cancer, several studies have examined the effects of 5-FU combined with gemcitabine. However, no studies to date have been performed in Japanese patients. We thus conducted a phase I/II study of gemcitabine and infusional 5-FU in Japanese patients to determine a recommended dosage for this combination and clarify efficacy and toxicity.
Methods: Phase I evaluated the frequency of dose limiting toxicity of two 5-FU dosages (400 and 500 mg/m^2/day) infused continuously over 5 days combined with gemcitabine 1000 mg/m^2 × 3 every 4 weeks. Results from phase I determined the recommended dosage to be examined in phase II for effect on survival period, clinical benefit response (CBR), tumor response and safety.
Results: A total of 34 chemo-naive patients were entered into the study. All had a Karnofsky performance of ≥50 points and distant metastases. Dose limiting toxicities in phase I determined the recommended 5-FU dosage at 400 mg/m^2/day. Grade 3–4 hematological toxicities (neutropenia, leukopenia and thrombocytopenia) were the most common severe toxicities. For the 28 patients administered the recommended dosage, 1-year survival rate was 14.3%, median survival time 7.1 months and progression free survival 3.2 months. Seven patients achieved a 25% overall response rate and three showed 27.3% improvement in CBR.
Conclusion: Although a meaningful survival benefit over single-agent gemcitabine was not demonstrated, 5-FU 400 mg/m^2/day infused continuously over 5 days in combination with gemcitabine 1000 mg/m^2 × 3 every 4 weeks appeared to be a moderately effective palliative treatment with low toxicity in Japanese patients with metastatic pancreatic cancer.

Key words: pancreatic cancer – phase I/II study – chemotherapy – gemcitabine – 5-FU

INTRODUCTION

Pancreatic cancer is a virulent disease with an extremely poor prognosis. Of all the treatment modalities for pancreatic cancer, only surgical resection offers the opportunity for a cure. However, because of local extension and/or metastatic disease, only a small minority of pancreatic cancer patients are candidates for curative resection. Moreover, even for these selected patients, prognosis remains unsatisfactory because of the postoperative recurrence, indicating that surgery alone has only limited value in the treatment of pancreatic cancer. Accordingly, to improve the overall survival of patients with pancreatic cancer, there is an urgent need to develop an effective non-surgical treatment for this disease.

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Previously a randomized controlled study demonstrated that gemcitabine, a nucleoside analogue, was effective in palliating symptoms and prolonging survival in patients with advanced pancreatic cancer (1). In the present study, gemcitabine showed a statistically significant advantage in both clinical benefit response (CBR) (23.8% versus 4.8%, $P = 0.0022$) and median survival (5.65 versus 4.41 months, $P = 0.0025$) compared with weekly bolus 5-fluorouracil (5-FU). Although single-agent gemcitabine has been accepted worldwide as the first-line therapy for advanced pancreatic cancer, there is substantial room for improvement in chemotherapy for pancreatic cancer because single-agent gemcitabine provides only limited benefit with a median survival of 4–6 months (1–3).

One approach has been to look for possible agents to use in combination with gemcitabine. A promising candidate has been the fluoropyrimidine, 5-FU, a key chemotherapeutic agent for pancreatic cancer before introduction of gemcitabine. Initially two *in vitro* studies in HT-29 colon cancer cells

using fluoropyrimidines in combination with gemcitabine suggested at least additive activity (4,5). Several phase II trials of gemcitabine combined with bolus 5-FU were then conducted, all of which showed promising results (6–9). Based on these findings the Eastern Cooperative Oncology Group (ECOG) conducted a phase III trial to compare gemcitabine plus bolus 5-FU with gemcitabine alone in patients with advanced pancreatic cancer (10). Although the overall survival in the combination arm tended to be superior to that in the gemcitabine alone arm, it was not possible to show a statistically significant difference. Since bolus 5-FU was adopted in this trial, we considered that administering infusional 5-FU might increase the efficacy of the regimen because (i) infusional 5-FU had previously demonstrated a superior antitumor effect to bolus 5-FU in colon cancer (11) and (ii) the effectiveness of infusional 5-FU in the combination with gemcitabine had not been elucidated in pancreatic cancer. Furthermore, since little information is available on the combination of gemcitabine and infusional 5-FU in Japanese patients, we decided to conduct a phase I/II study to determine the recommended dosage of this combination and to clarify its efficacy and toxicity in patients with metastatic pancreatic cancer.

PATIENTS AND METHODS

Eligibility Criteria

The present study included patients with a histological or cytological diagnosis of distant metastatic pancreatic adenocarcinoma not amenable to curative surgical resection or radiation therapy. Patients were required to have no history of chemotherapy, radiation therapy, curative resection or any other therapy for cancer; be between 20 and 74 years of age with a Karnofsky Performance Status (KPS) of 50 or higher; and have an estimated life expectancy of at least 3 months. Indicators of major organ functions were also required to be at normal levels: hemoglobin $\geq$ 9.5 g/dL, WBC $\geq$ 4000/mm^3, neutrophils $\geq$ 2000/mm^3, platelets $\geq$ 100 000/mm^3, alanine transaminase and aspartate transaminase levels [ALT (GPT), AST (GOT)] $\leq$ 2.5 times upper normal limit (UNL) (or $\leq$5 times UNL in patients with obstructive jaundice or liver metastasis), total bilirubin $\leq$ 2 times UNL, serum creatinine $\leq$ UNL and $PaO_2 \geq 70$ torr. Written informed consent was obtained from all patients in the study.

Patients were excluded from the study if they had pulmonary fibrosis, interstitial pneumonia, heart failure or difficult to control arrhythmia, refractory diabetes mellitus, hypercalcemia (serum Ca $\geq$ 11.5 mg/dL) or active infection. Other exclusion criteria included pregnant or lactating females, or females of childbearing age not using effective contraception, severe drug hypersensitivity, brain metastases, obvious neuropathy or mental disorders, active concomitant malignancy, other serious medical conditions or patients who received any investigational drug within 30 days before enrollment.

Study Design

This was a phase I/II study performed in two steps. The objective of Step 1 (phase I) was to evaluate the frequency of dose limiting toxicity (DLT) and then use this to determine which of the three possible 5-FU dosages (400, 500, 600 mg/m^2/day) would be recommended for continuous 24 h infusion over 5 days in combination with gemcitabine at its approved dosage (1000 mg/m^2/day). In Step 2 (phase II), this recommended 5-FU dosage was then administered in combination with gemcitabine at its approved dosage to evaluate the effect of this combination therapy on survival period. Effects on CBR, objective tumor response and the frequency and severity of adverse events were investigated as secondary objectives in Step 2. CBR, objective tumor response and survival period were also examined in those patients from Step 1 who were administered the recommended dosage.

Study Treatment

Step 1 (Phase I)

Gemcitabine (Eli Lilly and Company; Indianapolis, IN; USA) at a dose of 1000 mg/m^2 was administered as an intravenous 30-min infusion on days 1, 8 and 15 every 28 days. Continuous 24-h infusion of 5-FU (Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan) began immediately after completion of gemcitabine administration on Day 1 and continued for 5 days (Days 1–5). This 28 day period constituted one administration course.

Three possible dosage levels of 5-FU (Level 0: 400 mg/m^2/day, Level 1: 500 mg/m^2/day, Level 2: 600 mg/m^2/day) were assigned for Step 1. The first patient to enter the study began at Level 1. At least three patients were treated at this level and observed for DLT (see below for definition). If three or more patients experienced DLT at Level 1, the 5-FU dosage was reduced to 400 mg/m^2/day (Level 0) in the next three to six patients. Otherwise, patients were assigned to either Level 1 or 2 until at least three, but not more than six, patients had been assigned to two sequential levels. The dosage of 5-FU was considered tolerable according to the general method used for phase I trials of anticancer agents, i.e. DLT frequency not higher than 50%.

Treatment was discontinued if there was clear evidence of disease progression or unacceptable toxicity. Another administration course could be initiated if laboratory values met specifically defined criteria (WBC $\geq$ 4000/mm^3, neutrophils $\geq$ 2000/mm^3, platelets $\geq$ 100 000/mm^3, total bilirubin $\leq$2 times UNL, serum creatinine $\leq$ UNL, diarrhea $\leq$ Grade 1, mucosal disorders $\leq$ Grade 1). The next administration course could be delayed up to 8 weeks. Patients who experienced possible DLT received 800 mg/m^2 of gemcitabine in subsequent courses, although no dose adjustment was allowed during the same course. When patients experienced adverse effects such as Grade 3 diarrhea, Grade 3 mucosal disorders, Grade 3 hand-foot syndrome, serum transaminase of 10 times UNL, Grade 3 hepatic toxicity, or a total bilirubin level of 5.0 times UNL in patients with