Cancer Chemother Pharmacol (2003) 51: 328–336
DOI 10.1007/s00280-002-0560-1

ORIGINAL ARTICLE

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Gemcitabine pharmacokinetics and interaction with paclitaxel in patients with advanced non-small-cell lung cancer

Received: 28 May 2002 / Accepted: 1 November 2002 / Published online: 13 March 2003

Abstract *Purpose*: Gemcitabine administered at a fixed dose rate of 10 mg/m^2 per min has been reported to achieve plasma steady-state concentrations ranging from 10 to 20 μ*M* in patients with acute leukemia. These concentrations have been shown to saturate the intracellular accumulation of the active triphosphate metabolite. We designed this pharmacokinetic study to assess the ability of a fixed dose rate of gemcitabine to achieve the desired steady-state concentration in the absence and presence of paclitaxel in patients with solid tumors. *Patients and methods*: A group of 14 patients with advanced non-small-cell lung cancer received paclitaxel 110 mg/m^2 over 3 h on days 1 and 8 and gemcitabine 800 mg/m^2 over 80 min on days 1 and 8 every 21 days. Patients received gemcitabine alone on cycle (C) 1, day (D) 1. Pharmacokinetic samples were collected at 0, 15, 30, 45, 60 and 80 min during infusion and 0.25, 0.5, 1, 2, 4, 6, and 8 h after infusion on C1D1, C1D8, C2D1, C4D1 and C6D1. *Results*: Of 13 patients included in the pharmacokinetic analysis, 61% achieved the desired steady-state concentration (C_{ss}) with gemcitabine alone (C1D1), whereas only 0 to 45% of patients achieved the desired C_{ss} with paclitaxel and gemcitabine, depending on the treatment cycle. Paclitaxel significantly decreased systemic clearance (Cl_T; $P = 0.012$) and volume of distribution (V_d; $P = 0.050$) and significantly increased C_{ss} ($P = 0.009$). Gemcitabine plasma pharmacokinetic parameters demonstrated great interpatient variability in the absence of paclitaxel (C_{ss} 30%, Cl_T 30%, V_d 55%). Interpatient and intrapatient variability in gemcitabine pharmacokinetics were not observed when gemcitabine was administered in combination with paclitaxel ($P > 0.05$). *Conclusions*: Gemcitabine plasma pharmacokinetic parameters are significantly altered in the presence of paclitaxel.

Keywords Gemcitabine · Paclitaxel · Pharmacokinetics · Interaction

Supported by a Clinical/Translational Research Award granted by the Lineberger Comprehensive Cancer Center and a grant (RR00046) from the General Clinical Research Centers Program of the Division of Research Resources, National Institutes of Health.

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Introduction

Gemcitabine (2′,2′-difluorodeoxycytidine, dFdC) is a fluorinated pyrimidine analog with significant single-agent activity in non-small-cell lung cancer (NSCLC) [1, 2, 3]. It is extensively metabolized in vivo to both active and inactive metabolites, with gemcitabine undergoing sequential intracellular phosphorylation by deoxycytidine kinase and other nucleotide kinases to the active diphosphate (dFdCDP) and triphosphate (dFdCTP) metabolites. Gemcitabine also undergoes intracellular and extracellular metabolism in the presence of cytidine deaminase to the inactive metabolite difluorodeoxyuridine (dFdU) in the liver, lung, intestine, kidney, plasma, and other tissues [4, 5]. The amount of dFdU excreted in the urine accounts for up to 98% of a radiolabeled gemcitabine dose, demonstrating that deamination is the major metabolic pathway for gemcitabine [6, 7].

Many in vitro and in vivo studies have shown that the gemcitabine plasma concentration affects the accumulation of the active triphosphate metabolite [6, 8, 9, 10, 11, 12, 13]. In these studies it was found that gemcitabine plasma concentrations ranging from 10 to 20 μ*M* produce maximum intracellular triphosphate

concentrations. Higher plasma concentrations fail to significantly increase intracellular triphosphate concentrations [6, 10, 11]. In fact, gemcitabine plasma concentrations $>20\ \mu M$ either maintain or decrease the intracellular triphosphate concentrations achieved compared to concentrations $<20\ \mu M$ [6, 10, 14]. In vitro evidence shows that high gemcitabine plasma concentrations may inhibit the intracellular accumulation of the triphosphate metabolite [13]. A dose rate of 10 mg/m^2 per min has been reported to provide gemcitabine plasma concentrations between 10 and 20 μM. Abbruzzese et al. [6] have reported that patients receiving dose rates >10 mg/m^2 per min experience greater hematologic toxicity (anemia and thrombocytopenia) than patients receiving lower dose rates, suggesting that gemcitabine or its inactive metabolites may contribute to the toxicity associated with gemcitabine. However, it is currently unclear whether the parent compound, dFdU, or other metabolites contribute to the toxicity associated with gemcitabine. Clinically, patients typically receive gemcitabine doses ranging from 800 to 2600 mg/m^2 as an intravenous infusion for 30 min. These doses generate gemcitabine plasma concentrations substantially higher (mean $\geq 60\ \mu M$) than the concentrations that saturate the intracellular accumulation of the triphosphate metabolite [6, 14, 15]. Although gemcitabine regimens are generally well tolerated, patients receiving current gemcitabine regimens may be experiencing avoidable toxicity [7, 16, 17, 18].

Since the systemic clearance and volume of distribution of gemcitabine have been reported to vary approximately fivefold in patients receiving single-agent gemcitabine at a fixed dose rate of 10 mg/m^2 [6, 11], a fixed dose rate may not reliably achieve gemcitabine plasma concentrations between 10 and 20 μM. Individualized dosing based on specific individual parameters (i.e. therapeutic drug monitoring) may be necessary to achieve this goal. Studies are needed to determine if a fixed dose rate of 10 mg/m^2 per min can reliably achieve gemcitabine plasma concentrations between 10 and 20 μM in patients with NSCLC.

Paclitaxel has been administered in combination with gemcitabine in a number of clinical studies, since these agents show significant single-agent activity in NSCLC and they have different mechanisms of action with only partially overlapping toxicities. Phase I and II studies with gemcitabine and paclitaxel in NSCLC have been reported [16, 17, 18]. This combination chemotherapy regimen is currently being evaluated in several phase III clinical trials in NSCLC, as well as in breast and ovarian cancer. The potential effects of paclitaxel administration on the plasma pharmacokinetic parameters of gemcitabine have not been investigated. Although the current understanding of the metabolism of gemcitabine and paclitaxel does not suggest a possible drug interaction between these agents, several in vitro studies have shown that the sequence and schedule of administration of this combination of gemcitabine and paclitaxel affects cytotoxicity, suggesting a possible interaction between these agents [19, 20, 21]. Therefore, clinical investigation is warranted into the effects of paclitaxel on the disposition of gemcitabine.

This pharmacokinetic study was conducted in patients with locally advanced or metastatic NSCLC receiving combination chemotherapy with paclitaxel and gemcitabine. Each patient received paclitaxel 110 mg/m^2 over 3 h on days 1 and 8 with gemcitabine 800 mg/m^2 over 80 min on days 1 and 8 every 21 days based on a phase I trial conducted in patients with NSCLC [16]. The specific aims of the present study were to investigate: (1) the ability of a fixed dose rate (10 mg/m^2 per min) of gemcitabine to achieve plasma concentrations ranging between 10 and 20 μM, (2) the effects of paclitaxel on gemcitabine pharmacokinetic parameters, and (3) the interpatient and intrapatient variability in gemcitabine plasma pharmacokinetic parameters.

Patients and methods

Patient eligibility

A group of 14 adult patients with histologically or cytologically proven stage IIIB or IV NSCLC were enrolled into this study as a companion study to a phase II trial. All patients were evaluated in the Multidisciplinary Thoracic Oncology Program at the University of North Carolina from December 1999 to December 2000. The inclusion criteria for the phase II study included: no prior chemotherapy; measurable disease; Eastern Cooperative Group (ECOG) performance status <2; >2 weeks since prior radiation therapy; and adequate hepatic, renal and bone marrow function defined as a serum creatinine <2 mg/dl; total bilirubin <1.5 times the upper limit of normal; transaminases <3 times the upper limit of normal; white blood cell count $\geq 3.5 \times 10^9$/l; platelet count $\geq 100 \times 10^9$/l; and hematocrit $\geq 30\%$. Exclusion criteria included: untreated central nervous system metastases; <5 years since prior malignancy; and seropositive for human immunodeficiency virus. The Protocol Review Committee at the Lineberger Comprehensive Cancer Center and the Institutional Review Board at the University of North Carolina School of Medicine approved both the phase II and the pharmacokinetic trial. Written informed consent to undergo pharmacokinetic studies was obtained from each patient in conjunction with the consent for the phase II trial.

Study design

All patients received paclitaxel on days 1 and 8 and gemcitabine on days 1 and 8 every 21 days. However, patients did not receive paclitaxel on cycle (C) 1, day (D) 1 to determine gemcitabine pharmacokinetic parameters in the absence of paclitaxel. Each patient initially received gemcitabine 800 mg/m^2 in 100 ml 0.9% sodium chloride solution over 80 min (10 mg/m^2 per min). Each patient also initially received paclitaxel 110 mg/m^2 in 300 ml 0.9% sodium chloride solution over 3 h before the gemcitabine infusion. Patients requiring dose reductions secondary to significant bone marrow suppression following treatment (absolute neutrophil count 0.100 to 1.5×10^9/l or platelet count 7.5 to 100×10^9/l) on days 1 or 8 received dose-reduced gemcitabine 600 mg/m^2 and paclitaxel 75 mg/m^2. All patients received ondansetron 24 mg orally, dexamethasone 20 mg intravenously, diphenhydramine 50 mg intravenously, and ranitidine 50 mg intravenously 30 min before chemotherapy except on C1D1 (gemcitabine alone) when patients received solely ondansetron 24 mg orally 30 min before chemotherapy.

Pharmacokinetic studies

Blood samples were drawn during C1D1, C1D8, C2D1, C4D1, and C6D1. Two intravenous lines were maintained in each patient, one for chemotherapy and the other for pharmacokinetic sampling. Blood (7 ml) was collected into heparinized tubes and immediately placed on ice. All heparinized tubes were pretreated with 5 μ*M* tetrahydrouridine to inhibit the metabolism of gemcitabine to its inactive metabolite dFdU by cytidine deaminase ex vivo [22]. Blood was collected before treatment and at 15, 30, 45, 60, 80 min during infusion and 0.25, 0.5, 1, 2, 4, 6, and 8 h after infusion. All samples were immediately centrifuged in a fixed-angle rotor centrifuge at 3000 *g* for 5 min at 25°C. The plasma was transferred in 1.5-ml aliquots to a microcentrifuge tube and immediately stored at −70°C until analysis.

High-pressure liquid chromatography determination of gemcitabine

All assays were performed at the University of North Carolina, School of Pharmacy, Chapel Hill, North Carolina. Plasma concentrations of gemcitabine were determined by reverse-phase high-pressure liquid chromatography modified from a previously reported method [23]. Authentic gemcitabine (2′2′-difluorodeoxycytidine, LY188011) was graciously supplied by Eli Lilly and Company (Indianapolis, Ind.). Briefly, 400 μl of patient plasma or pooled plasma treated with known concentrations of gemcitabine was mixed with 5 μl (180 ng/ml) of the internal standard 3′-deoxythymidine (Aldrich Chemical Company, Milwaukee, Wis.) and 400 μl of a 20% solution of trifluoroacetic acid (Sigma Chemical Company, St. Louis, Mo.). The mixture was vortexed for 5 min and centrifuged at 10,000 *g* for 5 min. The resulting supernatant was transferred to 13 × 100 borosilicate culture tubes and concentrated using a Savant Turbo-Vacuum concentrator with a refrigerated vapor trap (ThermoSavant, Holbrook, N.Y.). The dried residue was reconstituted with 200 μl mobile phase A (97.5% 15 m*M* ammonium acetate, pH 5.0, and 2.5% acetonitrile). A 100-μl aliquot of the reconstituted material was injected onto a 5-μm Symmetry 250 × 4.6 cm C_{18} column (Waters Associates, Milford, Mass.) preceded by a Symmetry 2 × 0.39 cm C_{18} guard column using a Hewlett Packard 1100 liquid chromatograph connected to a Hewlett Packard 1100 ultraviolet detector set at 275 nm. Mobile phase A and mobile phase B (40% 15 m*M* ammonium acetate, pH 5.0, and 60% acetonitrile) were pumped at 1 ml/min with a gradient elution. The gradient was linearly decreased from 100% A to 83% A from 0 to 22.5 min to resolve the gemcitabine and deoxythymidine peaks within 25 min. Mobile phase A was further decreased to 40% from 23.5 min to 31.5 min to remove materials slowly eluting from the column before the next injection. The column was allowed to equilibrate with 100% mobile phase A from 31.5 min to 37.5 min for a total run time of 37.5 min. The retention times for gemcitabine and 3′-deoxythymidine were 8 and 17 min, respectively, under these conditions (Fig. 1).

Plasma concentrations for gemcitabine were calculated from the ratio of the area of the gemcitabine peak to the area of the 3′-deoxythymidine peak using least-squares linear regression and weighting by 1/y. The lower limit of quantification for gemcitabine was 0.05 μg/ml and the assay was linear between 0.05 μg/ml (0.1 μ*M*) and 15 μg/ml (50 μ*M*). Within-day and between-day variabilities (measured by a coefficient of variation) were < 10% for gemcitabine peak area ratios. There were no endogenous materials in the plasma that interfered with the integration of the peaks of interest.

Pharmacokinetic analysis

A noncompartmental model was fitted to the concentration versus time data for gemcitabine to estimate systemic clearance (Cl_T) and volume of distribution (V_d) using the program WinNonlin Professional version 3.2 (Pharsight Corporation, Mountain View, Calif.). Steady-state plasma concentrations for gemcitabine were calculated as the arithmetic means of the plasma concentrations measured after three half-lives had elapsed from the start to the end of infusion. The steady-state concentrations for patients receiving gemcitabine 600 mg/m^2 over 80 min were dose-adjusted using the following equation: C_{ss}(measured)/600 mg/m^2 = C_{ss}(dose-adjusted)/800 mg/m^2. The elimination half-lives ($t_{1/2}$) were calculated using the following equation: $t_{1/2} = 0.693 \times (Cl_T/V_d)$.

Criteria for toxicity

Patients were assessed for toxicity based on the criteria defined in the phase II trial. Toxicity was evaluated using National Cancer Institute Common Toxicity Criteria (version 2.0) after each cycle of chemotherapy. Patients with unacceptable toxicity were withdrawn from the clinical trial and, subsequently, this pharmacokinetic study.

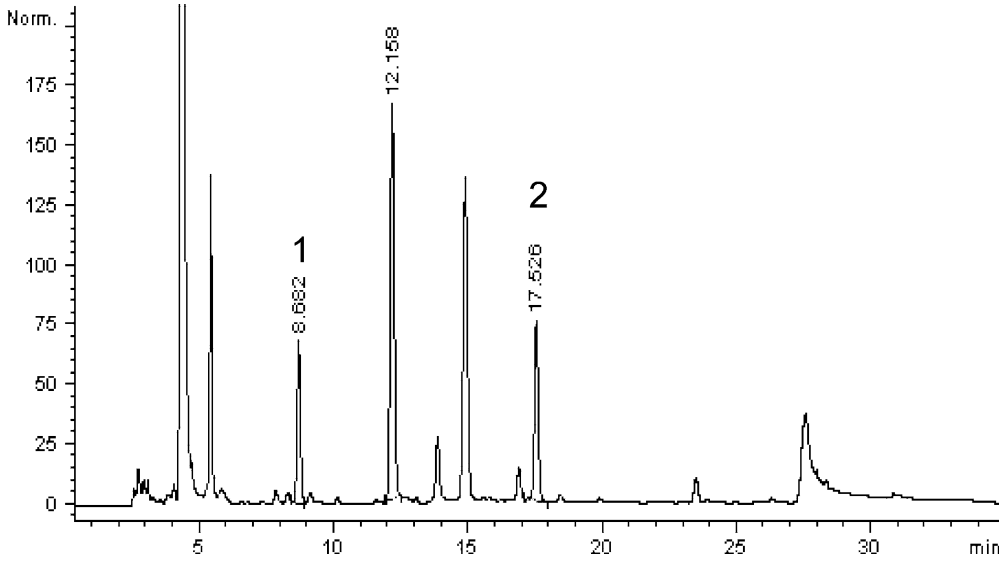

Fig. 1 High-pressure liquid chromatogram of a human plasma sample collected 15 min after the start of gemcitabine infusion on C1D1. *Peaks: 1* gemcitabine, *2* internal standard 3′-deoxythymidine

Statistical analysis

Summary statistics are presented as means and standard deviations for the gemcitabine pharmacokinetic parameters. The difference in the pharmacokinetic parameters for gemcitabine (systemic clearance, volume of distribution, and steady-state concentration) in the absence (C1D1) and presence (C1D8) of paclitaxel was assessed using the Wilcoxon sign ranks' test using the program SAS version 8.0 (SAS Institute, Cary, N.C.). The number of patients needed to detect a 25% change in the gemcitabine plasma concentrations in the absence (C1D1) and presence (C1D8) of paclitaxel with a type 1 error set at 5% and the power set at 80% was 14 using the previously published gemcitabine plasma concentrations measured following a fixed dose rate of 10 mg/m^2 [6, 10, 11]. The interpatient variability in gemcitabine and dFdU pharmacokinetic parameters for each treatment day (C1D1, C1D8, C2D1, C4D1 and C6D1) and the intrapatient variability in the presence of paclitaxel (C1D8, C2D1, C4D1 and C6D1) were described using a coefficient of variation [(standard deviation/mean) × 100]. Interpatient and intrapatient variability for gemcitabine total clearance and volume of distribution were assessed in the presence of paclitaxel (C1D8, C2D1, C4D1 and C6D1) using a two-way analysis of variance (ANOVA) with patient and cycle identified as the independent variables for all patients completing all six treatment cycles ($n = 9$).

Results

Patient characteristics

The characteristics of the 14 patients enrolled into this pharmacokinetic companion study are outlined in Table 1. Of the 14 patients, 13 were included in the pharmacokinetic analysis for C1D1 and C1D8, and 11 were included in the pharmacokinetic analysis for C2D1.

Table 1 Patient characteristics

Total number of patients	14
Gender (number of patients)	
Male	10
Female	4
Race (number of patients)	
Caucasian	12
African American	2
ECOG performance status (number of patients)	
0	4
1	10
Age (years)	
Median	56
Range	40–71
Histology (number of patients)	
Adenocarcinoma	5
Squamous cell	7
NSCLC, not specified	2
Number of patients receiving each cycle	
Cycle 1	14
Cycle 2	11
Cycle 4	9
Cycle 6	9
Number of patients receiving dose modifications	
Cycle 1, Day 1	0
Cycle 1, Day 8	3
Cycle 2	3
Cycle 4	4
Cycle 6	5

Only nine patients received all six planned chemotherapy cycles (C4D1 and C6D1). One patient was omitted from the data analysis for C1D1 due to inadequate sample collection and another patient did not receive treatment on C1D8 due to significant bone marrow suppression. Therefore, only 12 patients were included in the data analysis comparing gemcitabine pharmacokinetic parameters in the absence (C1D1) and presence (C1D8) of paclitaxel. All patients showed normal renal function and hepatic function as defined by normal laboratory values for serum creatinine, total bilirubin, alanine and aspartate transaminases, and alkaline phosphatase.

Gemcitabine plasma pharmacokinetic parameters

The mean plasma pharmacokinetic parameters for gemcitabine for all sampling events are summarized in Table 2. Following treatment on C1D1, the plasma concentration generally reached a plateau (about 90% steady-state plasma concentrations) within 45 min after the start of the infusion. The mean elimination half-life was 16 min. The maximal plasma concentration during the infusion was used in place of the steady-state plasma concentration on two occasions when the elimination half-life exceeded 26.5 min (maximal half-life capable of achieving about 90% steady-state concentrations during the 80-min infusion). Steady-state plasma concentrations ranged from 11 to 28 μM. Of 13 patients, 8 (61%) achieved steady-state concentrations (C_{ss}) within the desired range from 10 to 20 μM. The remaining patients (5/13, 39%) achieved plasma gemcitabine concentrations > 20 μM following treatment on C1D1. Table 3 summarizes the number of patients achieving the desired steady-state concentration (10 to 20 μM) for each sampling event. The volume of distribution ranged from 24 to 106 l/m^2 and the systemic clearance ranged from 80 to 215 l/h per m^2.

The remaining sampling events measured gemcitabine plasma pharmacokinetic parameters in the presence of paclitaxel. On C1D8, the steady-state plasma concentrations ranged from 15 to 31 μM. Only one patient achieved plasma concentrations within the desired concentration range of 10 to 20 μM (8%), whereas the remaining 12 patients (92%) achieved concentrations slightly higher than the desired range. The volume of distribution ranged from 16 to 70 l/m^2 and the systemic clearance ranged from 73 to 152 l/h per m^2. The pharmacokinetic parameters for the remaining sampling events were similar to the parameters calculated following C1D8. The mean steady-state plasma concentrations were 22, 18 and 24 μM following C2D1, C4D1 and C6D1, respectively. Only three and four patients on C2D1 and C4D1, respectively, and none of the patients on C6D1, had achieved the desired steady-state concentration (10 to 20 μM). The systemic clearance ranged from 81 to 169, 96 to 152 and 78 to 105 l/h per m^2, and the volume of distribution ranged from 15 to 61, 19 to 57 and 22 to 42 l/m^2 on C2D1, C4D1 and C6D1, respectively.

Table 2 Gemcitabine plasma pharmacokinetic parameters. The values presented are means (standard deviation) or number (percent) of patients (C_{ss} steady-state plasma concentration, Cl_T systemic clearance, V_d volume of distribution, $t_{1/2}$ elimination half-life, C cycle, D day)

[a]Gemcitabine alone

Parameter	Day of chemotherapy				
	C1D1 ($n=13$)[a]	C1D8 ($n=13$)	C2D1 ($n=11$)	C4D1 ($n=9$)	C6D1 ($n=9$)
C_{ss} (μM)	18.0 (5.5)	24.6 (3.8)	22.5 (3.8)	20.3 (3.9)	24.1 (2.3)
10–20 μM	8 (61%)	1 (8%)	3 (27%)	4 (45%)	0 (0%)
> 20 μM	5 (39%)	12 (92%)	8 (73%)	5 (55%)	9 (100%)
Cl_T (l/h/m^2)	136.3 (40.8)	95.5 (19.2)	105.1 (23.5)	116.5 (23.1)	95.7 (8.5)
V_d (l/m^2)	52.2 (28.6)	35.6 (15.5)	35.7 (15.1)	38.2 (13.2)	32.0 (6.0)
$t_{1/2}$ (min)	17.0 (11.6)	15.4 (6.6)	13.4 (5.1)	13.5 (3.7)	13.9 (2.3)

Table 3 Variation in gemcitabine pharmacokinetic parameters (C_{ss} steady-state plasma concentration, V_d volume of distribution, C cycle, D day)

Parameter	Intrapatient variability (%)[a]	Interpatient variability (%)[b]				
		C1D1 ($n=13$)[b]	C1D8 ($n=13$)	C2D1 ($n=11$)	C4D1 ($n=9$)	C6D1 ($n=9$)
C_{ss} (μM)	19 (8–30)	30	16	17	19	10
Cl (l/h/m^2)	22 (7–30	30	20	22	20	9
V_d (l/m^2)	32 (22–53)	55	44	42	34	19

[a]Only patients receiving all six cycles wereconsidered to determine intrapatient variability (reported as median andrange). This variability was determinedby considering only cycles administered in the presence of paclitaxel due tothe statistically significant difference reported for gemcitabine pharmacokinetic parameters in the absence and presence of paclitaxel

[b]Gemcitabine alone

Comparison of the pharmacokinetic parameters between C1D1 and C1D8

The pharmacokinetic parameters for the 12 patients who received treatment on both C1D1 and C1D8 were compared to determine the gemcitabine pharmacokinetic parameters in the absence (C1D1) and presence (C1D8) of paclitaxel. The mean systemic clearance and volume of distribution decreased 30% from C1D1 to C1D8 (Fig. 2a, b). Following treatment with paclitaxel, the mean systemic clearance decreased from 136 to 96 l/h per m^2 ($P=0.012$) and the mean volume of distribution decreased from 52 to 36 l/m^2 ($P=0.050$). The mean elimination half-life did not significantly change from C1D1 to C1D8. The observed decreases in systemic clearance and volume of distribution caused the mean steady-state plasma concentration to increase 25% from C1D1 to C1D8 (Fig. 2c) with the mean steady-state plasma concentrations increasing from 18 to 24 μM ($P=0.009$).

Variation in gemcitabine pharmacokinetic parameters

Gemcitabine plasma pharmacokinetic parameters demonstrated great interpatient variability following treatment with single-agent gemcitabine on C1D1 (Table 3, Fig. 3). The steady-state concentration and systemic clearance varied 30% between individuals and the volume of distribution varied 55% between individuals on C1D1. However, interpatient variability profoundly decreased when gemcitabine was administered following paclitaxel infusion on C1D8 to C6D1 (Fig. 3). This relationship remained valid when the interpatient variability was determined for only the nine patients who completed all six cycles of chemotherapy. A two-way ANOVA failed to identify significant interpatient or intrapatient variability ($P=0.47$ systemic clearance, and $P=0.18$ volume of distribution) when the pharmacokinetic parameters measured in the presence of paclitaxel were compared from C1D8 to C6D1 for the nine patients who completed all six cycles of chemotherapy. The intrapatient variability was determined for all nine patients who completed all six cycles of chemotherapy using the data analyzed for C1D8 to C6D1, since gemcitabine pharmacokinetic parameters are significantly changed in the presence of paclitaxel. The median (range) intrapatient variabilities were 19% (8 to 30%), 22% (7 to 30%) and 32% (22 to 53%) for the steady-state concentrations, systemic clearance and volume of distribution, respectively. In summary, the systemic clearance, volume of distribution and interpatient variability appeared to significantly decrease and the steady-state plasma concentrations appeared to significantly increase in the presence of paclitaxel.

Pharmacokinetic relationship to toxicity

The response and toxicity data for all the patients ($n=39$) enrolled in the phase II trial will be presented in detail in a subsequent report. The patients enrolled into this study received a total of 61 cycles with a median of 6 cycles per patient (range 1 to 6 cycles). Bone marrow suppression was the most commonly reported grade 3 or 4 toxicity in these patients. Grade 3 neutropenia occurred during 15 cycles

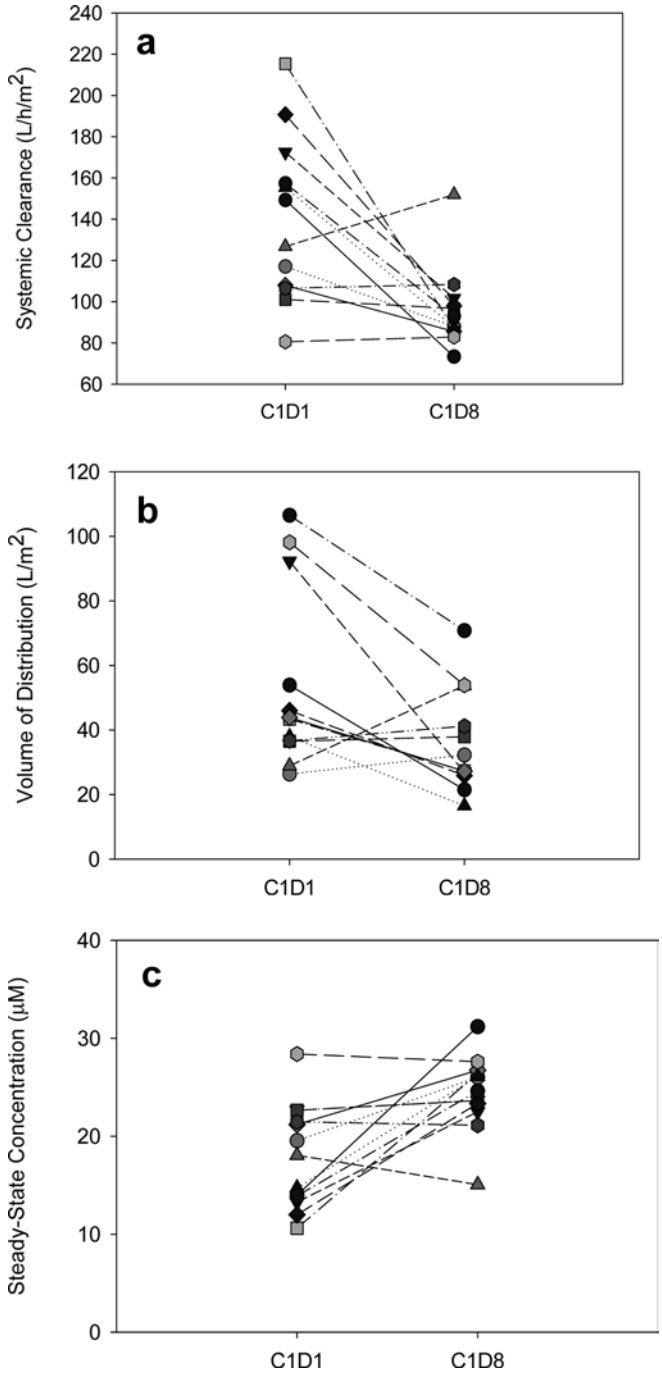

Fig. 2a–c Systemic clearance (**a**), volume of distribution (**b**) and steady-state plasma concentration (**c**) of gemcitabine in the absence (C1D1) and presence (C1D8) of paclitaxel

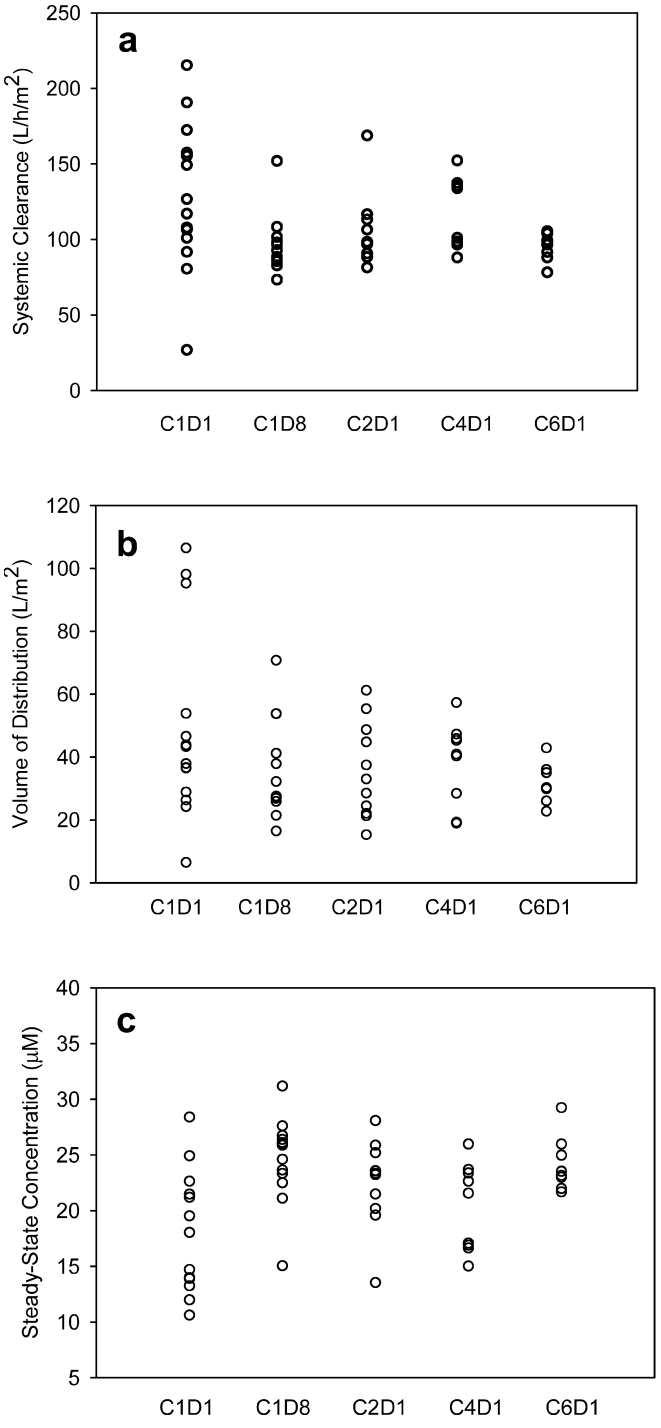

Fig. 3a–c Systemic clearance (**a**), volume of distribution (**b**) and steady-state plasma concentration (**c**) of gemcitabine for all pharmacokinetic sampling events

and grade 4 neutropenia occurred during 6 cycles. Grade 3 thrombocytopenia occurred during four cycles and grade 3 anemia occurred during two cycles. Other grade 3 and 4 toxicities observed in these patients included: dyspnea, deep venous thrombosis, dehydration, nausea and vomiting, pleural effusion, pneumonia, pulmonary embolus, syncope, and weakness; these toxicities occurred during one or two treatment cycles. Six patients required a dose reduction to 600 mg/m^2 secondary to hematologic toxicity. Only three of these patients had reached gemcitabine plasma concentrations $>20\ \mu M$ during the previous treatment cycle. No apparent relationship between the pharmacokinetic parameters and toxicity could be found in this relatively small patient population.

Discussion

In this study, the systemic clearance and volume of distribution of gemcitabine decreased 30% in the presence of paclitaxel. The decreased systemic clearance suggests that less gemcitabine underwent metabolism by cytidine deaminase with ≥98% of gemcitabine undergoing metabolism by cytidine deaminase to dFdU [6, 7]. Although gemcitabine undergoes both intracellular and extracellular metabolism, Abbruzzese et al. [6] have shown that extracellular (plasma) metabolism of gemcitabine accounts for approximately 1% of its metabolism. This study demonstrated that a 2000 mg gemcitabine dose can be metabolized within 1 h. However, the plasma cytidine deaminase can only deaminate 5.4 to 6.3 mg of gemcitabine in 1 h, suggesting that a relatively small amount of gemcitabine undergoes extracellular deamination. These data suggest that gemcitabine predominantly undergoes intracellular metabolism. Thus, the decreased gemcitabine systemic clearance most likely reflects decreased intracellular accumulation of gemcitabine in the liver, lung, kidney and other tissues rich in cytidine deaminase. The volume of distribution decreased from 0.66 l/kg (total body water) in the absence of paclitaxel to 0.5 l/kg (mostly extracellular water) in the presence of paclitaxel supporting the theory that less gemcitabine distributed into intracellular water in the presence of paclitaxel. Based on these data, paclitaxel appears to affect the intracellular transport and/or efflux of gemcitabine, and therefore prevents extensive intracellular metabolism of gemcitabine.

Nucleosides enter cells by either a facilitated diffusion or an active transport process by nucleoside-specific membrane transport carriers, because nucleosides are generally hydrophilic and do not readily permeate the plasma membrane [24, 25, 26]. Mackey et al. [27, 28, 29] have shown that the nucleoside membrane transporter hENT1 carries gemcitabine into cells, where it undergoes metabolism to its metabolites. Therefore, paclitaxel could be inhibiting this transporter and limiting the movement of gemcitabine inside cells. Alternatively, paclitaxel may be limiting the intracellular accumulation of gemcitabine by increasing the export of gemcitabine from the cells. It has recently been reported that cyclic nucleotides are substrates for the multidrug resistance associated proteins MRP5 and MRP4, two transmembrane drug efflux pump proteins. These findings suggest that paclitaxel or its formulation could limit the volume of distribution of gemcitabine by inducing these MRP proteins and increasing the export of gemcitabine from inside the cells [30, 31]. There are currently no reports of the effects of paclitaxel or its formulation on either hENT1 or MRP5 and MRP4.

Although the changes in the pharmacokinetic parameters for gemcitabine most likely affect the deamination pathway, it is unclear whether the phosphorylation pathway is affected in the presence of paclitaxel. There is one report that paclitaxel increases the intracellular accumulation of the triphosphate metabolite. However, the gemcitabine pharmacokinetic parameters did not change in the presence of paclitaxel. In that study, patients with NSCLC received gemcitabine 1000 mg/m^2 over 30 min (days 1 and 8) and paclitaxel 150 mg/m^2 over 3 h or 200 mg/m^2 over 3 h (day 1) every 21 days [32]. Interestingly, paclitaxel did not significantly affect the accumulation of the active metabolite at the lower dose (150 mg/m^2). In another study (published as an abstract only), it was also found that paclitaxel does not affect the maximal concentration or the area under the concentration-time curve of gemcitabine [33]. However, in these studies gemcitabine was administered at a higher dose rate (gemcitabine 1000 mg/m^2 over 30 min) consistent with current treatment recommendations. Additional studies are warranted to determine the affects of paclitaxel on the metabolism of gemcitabine to its active triphosphate metabolite. Although there is a plausible explanation for the apparent interaction of paclitaxel with gemcitabine, the premedications required for paclitaxel administration may also affect gemcitabine pharmacokinetic parameters.

A slight increase or no change in volume of distribution or systemic clearance from C1D1 (no paclitaxel) to C1D8 (after paclitaxel) was noted in 3 of 13 patients. A review of each individual's laboratory parameters, medical history and medications did not reveal any potential factor to explain why these patients responded differently on C1D8. It is currently unclear whether these changes coincided with any relevant clinical effects.

In this study, paclitaxel was administered as a 3-h infusion before gemcitabine was administered as an 80-min infusion. In most studies, paclitaxel has similarly been administered before gemcitabine, including the studies previously discussed [32, 33]. In several in vitro studies, the sequence of administration has been shown to affect the interaction between gemcitabine and paclitaxel. The results of these studies are disparate, suggesting that these interactions are highly time- and schedule-dependent [19, 20, 21]. Additional studies are warranted to determine the effects of the sequence and time of administration on this interaction and, more importantly, if paclitaxel significantly alters the accumulation of the active triphosphate metabolite.

Only 61% of patients achieved the desired plasma concentration following gemcitabine alone, but paclitaxel resulted in significantly increased steady-state gemcitabine concentrations causing more patients to achieve plasma concentrations exceeding the desired concentration (55% to 100%). The clinical significance of plasma concentrations >20 μM is currently unclear, because patients receiving the current dosage of 1000 mg/m^2 over 30 min achieve gemcitabine plasma concentrations >20 μM with acceptable toxicity. Interestingly, the intersubject variability was lower in the presence of paclitaxel (C1D8 to C6D1) compared to the variability observed in the absence of paclitaxel (C1D1). Since intersubject variability ranged from 10% to 19%,

therapeutic drug monitoring does not appear necessary to target the desired gemcitabine plasma concentration ranging from 10 to 20 μM. However, additional studies are warranted to determine whether paclitaxel affects the metabolism of gemcitabine to the triphosphate metabolite before this fixed dose rate can be routinely prescribed in combination with paclitaxel. If the accumulation of the triphosphate metabolite significantly decreases in the presence of paclitaxel, patients may experience lower response to this combination therapy. How other chemotherapy agents may affect the disposition and metabolism of gemcitabine is uncertain until the mechanism of this interaction can be clarified.

In conclusion, a dose rate equal to 10 mg/m^2 per min appeared to produce the desired gemcitabine steady-state plasma concentration in most patients (61%) following treatment with gemcitabine alone. Paclitaxel significantly decreased both the volume of distribution and systemic clearance of gemcitabine. The clinical significance of this interaction is uncertain, as this study did not explore how paclitaxel affects the metabolism of gemcitabine to the active triphosphate metabolite by deoxycytidine and other nucleoside kinases.

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