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Pharmacokinetics of Gemcitabine at Fixed-Dose Rate Infusion in Patients with Normal and Impaired Hepatic Function

Alessandra Felici,[1,2] *Susanna Di Segni*,[1] *Michele Milella*,[2] *Simona Colantonio*,[1] *Isabella Sperduti*,[3] *Barbara Nuvoli*,[1] *Michela Contestabile*,[1] *Andrea Sacconi*,[1] *Massimo Zaratti*,[2] *Gennaro Citro*[1] and *Francesco Cognetti*[2]

1 Laboratory of Pharmacokinetics, Regina Elena Cancer Institute, Rome, Italy
2 Division of Medical Oncology A, Regina Elena Cancer Institute, Rome, Italy
3 Division of Biostatistics, Regina Elena Cancer Institute, Rome, Italy

Abstract

Background and objectives: Gemcitabine (2,2-difluorodeoxycytidine [dFdC]) can be administered in a standard 30-minute infusion or in a fixed-dose-rate (FDR) infusion to maximize the rate of accumulation of triphosphate, its major intracellular metabolite. The standard 30-minute infusion requires dose adjustment in patients with organ dysfunction, especially in patients with elevated baseline serum bilirubin levels. On the other hand, the FDR infusion is burdened by increased haematological toxicity. The primary aim of this study was to evaluate the pharmacokinetics of dFdC and its metabolite difluorodeoxyuridine (dFdU) in patients with normal and impaired hepatic function.

Patients and methods: In this prospective study, patients with pancreatic or biliary tract carcinoma and normal or impaired hepatic function tests were considered eligible for recruitment. Patients were recruited according to the following criteria: (i) serum bilirubin <1.6 mg/dL and AST and ALT <2 times the upper the limit of normal (ULN) [cohort I]; and (ii) serum bilirubin >1.6 mg/dL and/or AST/ALT >2 times the ULN (cohort II). An FDR infusion of gemcitabine 1000 mg/m^2 was administered on days 1, 8 and 15 every 4 weeks. The pharmacokinetic analysis of gemcitabine and dFdU was performed with high-performance liquid chromatography-tandem mass spectrometry assay in cycles 1 and 2.

Results: Thirteen patients were enrolled, four in cohort I and nine in cohort II. All patients were assessable for toxicity and pharmacokinetic analysis. The grade and rate of toxicities were similar in both groups, and patients with elevation of bilirubin and/or transaminases did not require dose reduction of gemcitabine. Pharmacokinetic analysis revealed a reduction of the experimental area under the plasma concentration-time curve for gemcitabine and dFdU in patients with hepatic dysfunction when compared with patients with normal hepatic function. All other pharmacokinetic parameters were similar in the two cohorts. No statistical difference was demonstrated for all parameters evaluated between cycle 1 and cycle 2 in the two groups.

Conclusion: Gemcitabine 1000 mg/m^2 can be administered as an FDR infusion in patients with altered hepatic function without causing additional toxicity compared with patients with normal hepatic function.

Background

Gemcitabine (2,2-difluorodeoxycytidine [dFdC]) is a fluorinated analogue of deoxycytidine, which has shown a broad spectrum of activity against several solid tumours, such as non-small-cell lung cancer and pancreatic adenocarcinoma.[1,2]

dFdC is a prodrug that requires intracellular activation; after its uptake, the nucleoside analogue is converted by deoxycitidine kinase to its monophosphate form (dFdCMP), followed by subsequent phosphorilation steps to its diphosphate (dFdCDP) and triphosphate forms (dFdCTP).[3] Gemcitabine also undergoes intracellular and extracellular metabolism by cytidine deaminase, the enzyme that converts

the prodrug into its inactive metabolite difluorodeoxyuridine (dFdU). The rate-limiting step in the intracellular accumulation of dFdCDP and dFdCTP is the conversion of dFdC to dFdCMP by deoxycitidine kinase.[4] It has been demonstrated that deoxycytidine kinase has saturable kinetics, and the optimal plasma dFdC concentration to obtain maximal dFdCTP formation and accumulation by mononuclear cells is 10–20 μmol/L.[5]

Several studies have reported that a gemcitabine dose in the range from 1000 to 1500 mg/m^2 is active and well tolerated when given over 30 minutes as an intravenous infusion on a weekly schedule,[6,7] although patients receiving doses ranging from 800 to 2600 mg/m^2 as a 30-minute intravenous infusion generate plasma concentrations of dFdC >60 μmol/L. Under these conditions, the triphosphate accumulation process may be saturated[5,8] and target cells may not use a substantial portion of the drug because of metabolic clearance. A fixed-dose-rate (FDR) infusion of 10 mg/m^2/min has been proposed to circumvent this problem and thereby achieve plasma steady-state concentrations of 10–20 μmol/L,[4,8] optimizing the intracellular dFdCTP accumulation.[9-12]

A phase II trial,[13] which compared a 30-minute gemcitabine infusion (2200 mg/m^2) with an FDR infusion (1500 mg/m^2 over 150 minutes) in patients with pancreatic adenocarcinoma, revealed improvements in survival and clinical benefit with the FDR infusion, with an increased incidence of haematological toxicity and grade 3 hypertransaminaesemia in the FDR arm. Despite this initially promising result, other studies using gemcitabine alone or in combination with other drugs (cisplatin, carboplatin, paclitaxel) failed to show any clinical benefit in favour of the FDR infusion.[7]

The gemcitabine FDR infusion implies a linear increase in the dFdCTP intracellular concentration, and its intracellular area under the concentration-time curve (AUC) is higher following the prolonged 10 mg/m^2/min infusion than with the standard 30-minute infusion schedule.[14]

The pharmacokinetics of gemcitabine concerning clearance or metabolic capacity can be influenced by abnormal hepatic function due to liver metastases from pancreatic/biliary tract carcinoma or other hepatic diseases (cirrhosis, hepatitis, etc.). Venook et al.[15] explored the pharmacokinetic disposition of gemcitabine given as 30-minute standard infusion in patients with hepatic and renal impairment, suggesting a dose reduction for patients with elevated serum bilirubin levels because of an elevated risk of hepatic toxicity. Supported by their pharmacokinetic and clinical results, Venook et al.[15] suggested initially treating patients showing elevated bilirubin levels with gemcitabine 800 mg/m^2 and subsequently escalating the dose if it is well tolerated.

Based on the aforementioned hepatic toxicity[13] of the FDR gemcitabine infusion and the recommendation suggested by Venook et al.[15] in patients with high bilirubin levels, we performed a pharmacological study to evaluate the safety of gemcitabine 1000 mg/m^2 administered as a 10 mg/m^2/min infusion on days 1, 8 and 15 every 4 weeks in patients with normal and impaired hepatic function. The principal aim of this study was to define the pharmacokinetic disposition of dFdC and dFdU in the two cohorts of patients. The secondary endpoints were to evaluate the toxicity in both groups, starting with the same dose of gemcitabine, and to confirm the reproducibility of the pharmacokinetic parameters analysed within the same patients in two different cycles.

Patients and Methods

Patient Selection

Patients with a cytological or histological diagnosis of recurrent or metastatic pancreatic adenocarcinoma or biliary tract carcinoma were included in the study. The eligibility criteria included (i) age ≥18 years; (ii) WHO performance status of 0–2; (iii) life expectancy of ≥2 months; (iv) no prior systemic chemotherapy, major surgery or radiation therapy within the preceding 4 weeks; (v) granulocytes >1500/μL, platelet count >100 000/μL, plasma albumin level >2.0 g/dL, serum creatinine level <1.6 mg/dL; and (vi) compliance of the patients with testing. To limit entry to patients with hepatic dysfunction, the other eligibly criteria were (i) AST and ALT levels ≥2 times the upper limit of normal (ULN) with normal serum bilirubin levels; (ii) total serum bilirubin levels of 1.6–7.0 mg/dL with any AST/ALT level. All patients signed an informed consent form approved by the institutional ethical committee.

The exclusion criteria included (i) prior treatment with gemcitabine; (ii) known untreated brain metastases; (iii) uncontrolled or severe cardiac disease; (iv) concomitant medication that could affect hepatic function; (v) pregnant or lactating patients; (vi) patients with reproductive potential not implementing adequate contraceptives measures; and (vii) patients who could not be regularly followed up because of psychological, social, familial or geographical reasons.

Patients were enrolled in two different cohorts: (i) control patients with normal hepatic function in cohort I (serum bilirubin level <1.6 mg/dL and AST/ALT levels <2 times the ULN), and patients with impaired hepatic function in cohort II (serum bilirubin level <1.6 mg/dL and AST/ALT level ≥2 times the ULN; or serum bilirubin level from 1.6 to 7.0 mg/dL with any AST/ALT/alkaline phosphatase [AP] level).

Study Design

This single-centre study focused on gemcitabine 1000 mg/m^2 administered to patients with normal and impaired hepatic function as an FDR infusion on days 1, 8 and 15 every 4 weeks until progressive disease or unacceptable toxicity occurred. Drug toxicity and pharmacokinetics were analysed in patients with impaired hepatic function and compared with patients with normal hepatic parameters. The safety dose of gemcitabine and the required dose reduction were evaluated for a maximum of six cycles. Samplings for pharmacokinetic analysis were performed on day 1 of cycle 1 and repeated on day 1 of cycle 2, to calculate the variability of pharmacokinetic parameters in the same patient (each patient being his/her own control).

No systemic anticancer agent other than the study drug was administered, and concomitant treatment with corticosteroids was discouraged at least from day −2 to day 2 of cycles 1 and 2. Granulocyte-colony stimulating factor was not allowed in the first two cycles.

Clinical biochemistry and haematology were assessed within 7 days before starting treatment; in particular, a complete blood count and platelet count as well as hepatic function tests (serum total and fractionated bilirubin, AST/ALT level) were obtained at baseline and weekly during the treatment course. A physical examination was performed and a record of concomitant medications was obtained at baseline and before every cycle. An ECG and chest x-ray were obtained at baseline, at discontinuation from the study treatment, and at any time when clinically indicated during the trial. Patients with measurable disease were assessed for their response every three cycles with a CT scan or ultrasound of the abdomen (and of other disease sites as appropriate). Responses were documented using the Response Evaluation Criteria in Solid Tumors (RECIST).[16] After the off-treatment visit, patients were followed up monthly with clinical and instrumental evaluation.

Values of white blood cells, platelets, haemoglobin, red blood cells, neutrophils, prothrombin time (PT), partial thromboplastin time (PTT), bilirubin, AST/ALT, AP, total protein and albumin were recorded at baseline and at each cycle to evaluate a possible relationship between the blood values, drug disposition and toxicity.

Evaluation of Toxicity and Dose Modifications

The starting dose of gemcitabine was 1000 mg/m^2 infused at 10 mg/m^2/min intravenously; dose modifications were applied on the basis of toxicity. Administration of gemcitabine was delayed on day 1 until haematological recovery (absolute neutrophil count [ANC] ≥1500/μL and/or platelet count ≥100 000/μL and/or haemoglobin level ≥9 g/dL) up to a maximum of 3 weeks; on days 8 and 15, the dose was reduced as follows: (i) ANC ≥1500/μL and/or platelet count ≥100 000/μL, full dose; (ii) ANC 1500–1000/μL and/or platelet count 99 999–75 000/μL, 75% of the full dose; (iii) ANC 1000–500/μL and/or platelet count 74 999–50 000/μL, 50% of the full dose; and (iv) ANC ≤500/μL and/or platelet count ≤50 000/μL, omission. Patients who required a delay of >2 weeks but <3 weeks received a dose reduction of 25%. If the ANC was ≤500/μL, the platelet count was ≤50 000/μL or the haemoglobin level was ≤7 g/dL for a period longer than 5 days in any case of febrile neutropenia or stomatitis grade ≥3, the doses of gemcitabine was reduced by 25% in the next cycles. A 25% dose reduction was planned for gastrointestinal grade 3 and 4 toxicities. For hepatic toxicity, doses of gemcitabine were delayed when the bilirubin and AST/ALT levels were >2.5- and >5-fold higher than baseline (the starting values of each patients), respectively; doses were reduced by 50% when bilirubin and AST/ALT levels were from 1.5- to 2.5-fold and from 2.5- to 5-fold, respectively, higher than baseline; doses were reduced by 25% when the bilirubin level was from 1.5- to 2.0-fold higher than baseline and AST/ALT levels were from 2.5- to 5-fold higher than baseline. Patients who required a delay of >2 weeks but <3 weeks received a dose reduction of 25%; patients who had not recovered after 3 weeks were considered off protocol.

Pharmacokinetic Sample Acquisition and Handling

Blood samples (5–10 mL) were drawn via an indwelling peripheral catheter or peripheral venipuncture into tubes containing heparin. Tetrahydrouridine (Calbiochem-Novabiochem Corp., La Jolla, CA, USA), a cytidine deaminase inhibitor, was then added (0.1 mL of a 10 mg/mL solution) to prevent *ex vivo* gemcitabine deamination. Samples were collected 30 minutes before the gemcitabine infusion, at 30, 60 and 80 minutes during the infusion, at the end of the infusion, and at 5, 30, 90, 180 and 240 minutes after completion of the infusion.

Blood samples were immediately centrifuged at room temperature for 10 minutes at 1000 rpm. The resulting plasma was frozen and stored at −20°C until analysis.

Determination of Gemcitabine and Difluorodeoxyuridine (dFdU)

All analyses were performed at the Regina Elena National Cancer Institute, Rome, Italy. Gemcitabine and dFdU plasma

Table I. Patient characteristics

Characteristic	Cohort I	Cohort II
Patients (n)	4	9
Age (y)[a]	59	67
Sex (n)		
male	1	5
female	3	4
WHO performance status		
0–1 (n)	3	7
2 (n)	1	2
Diagnosis (n)		
pancreatic adenocarcinoma	2	5
biliary tree carcinoma	2	1
gall-bladder adenocarcinoma	0	3
Locally advanced disease (n)	1	6
Metastatic disease (n)	3	3

a Median.

concentrations were determined using a hyphenated technique of high-performance liquid chromatography (HPLC)-tandem mass spectrometry (MS/MS). Gemcitabine (Ly188011) and dFdU (Ly198791) were kindly supplied by Eli Lilly and Company (Indianapolis, IN, USA), and 2′-deoxycytidine (dC) was purchased from Sigma Aldrich Inc. (St Louis, MO, USA). 10 μL of internal standard (20 μg/mL) was added to 0.2 mL of each plasma sample, and the mixture was extracted with 200 μL of isopropilic alcohol and then 400 μL of ethyl acetate. Samples were vortexed and then centrifuged for 10 minutes. The supernatant was transferred to a glass tube and the organic phase was evaporated to dryness under a nitrogen stream. 200 μL of HPLC-grade water with 0.5% acetic acid was added to each sample to reconstitute the dried residue, and the mixture was vortexed and then centrifuged for 10 minutes at $4000 \times g$. Then 20 μL of the reconstituted solution was injected into the HPLC system.

HPLC analysis was performed by using an Agilent 1100 series system (Agilent Technologies, Palo Alto, CA, USA) equipped with a binary pump, an automatic injector and a vacuum degasser. The separation was carried out on a Symmetry C18 (4.6×250 mm internal diameter, 5 μm particle size) protected by a sentry guard column Symmetry C18 (3.9×20 mm). Mobile phase consisted of: eluent A 0.5% acetic acid in water; eluent B 0.5% acetic acid in acetonitrile. Gradient elute procedure consisted of: eluent B 2% for 2 minutes, eluent B 2–50% for 10 minutes, eluent B 50–70% for 1 minute, eluent B 70% for 4 minutes, eluent B 2% for 2 minutes, eluent B 2% for 3 minutes. The flow rate was 1 mL/min, and the HPLC output was directly interfaced to the electrospray ionization (ESI) ion source, the LC/MSD ion trap mass spectrometer 1100 (Agilent Technologies). The mass spectrometer was equipped with an ESI source and operated in the positive ion mode. The ESI conditions were the following: (i) capillary voltage –3.5 kV; (ii) end-plate offset voltage 500 V; (iii) capillary exit voltage 110.9 V; (iv) nebulizer pressure 70 psi; (v) drying gas flow 12 L/min; and (vi) temperature 350°C.

The ESI-MS analyses were multiple-reaction-monitoring experiments, performed by ion fragmentation (dC: 228 → 112 m/z; gemcitabine: 264 → 112 m/z; dFdU: 265 → 113 m/z) and

Table II. Baseline laboratory values

Parameters	Cohort I		Cohort II	
	mean (SD)	range	mean (SD)	range
WBC count ($\times 10^3/\mu L$)	6.27 (2.06)	4.81–9.30	11.86 (7.08)	5.90–28.00
Platelet count ($\times 10^3/\mu L$)	159 (65.15)	96–236	369 (232.55)	146–775
Hb (g/dL)	11.7 (1.91)	10.3–14.5	11.16 (1.43)	9.3–14.0
Total bilirubin level (mg/dL)	0.80 (0.35)	0.55–1.33	5.29 (3.83)	0.66–14.24
Direct bilirubin (mg/dL)	0.39 (0.39)	0.15–0.85	2.58 (1.41)	0.22–4.52
AST (U/L)	32 (14)	16–46	70 (48)	23–168
ALT (U/L)	39 (18)	17–62	92 (32)	43–134
AP (U/L)	710 (487)	365–1055	1278 (625)	420–2258
Creatinine (mg/dL)	0.72 (0.08)	0.66–0.85	0.73 (0.35)	0.05–1.21
Total protein (g/dL)	6.50 (0.60)	6.10–7.20	6.58 (0.72)	5.10–7.50
PT (%)	99 (6.4)	95–107	97 (9.8)	86–115
PTT (sec)	31 (2.6)	29–34	32 (4.2)	28–41

AP = alkaline phosphatase; **Hb** = haemoglobin; **PT** = prothrombin time; **PTT** = partial thromboplastin time; **WBC** = white blood cell.

Table III. Summary of toxicities observed in the study

Toxicity	Cohort I (no. of toxicities)		Cohort II (no. of toxicities)	
	grade 2	grade 3	grade 2	grade 3
Anaemia	1		1	1
Neutropenia		2	1	
Thrombocytopenia				1
Elevated AST/ALT	1			1
Bilirubinaemia				1
Asthenia	1			1
Fever			1	
Nausea/vomiting			1	

the scan range was 100–300 m/z. In these analytical conditions, the retention times for dC, gemcitabine and dFdU were 2.3, 3.8 and 6.5 minutes, respectively.

The extraction and the analysis were based on modifications of previously published methods.[17,18]

Quant Analysis software was used to process the quantitative data. Plasma concentrations of gemcitabine and dFdU were calculated from the ratio of the gemcitabine and dFdU peaks area to the area of internal standard using least squares linear regression. The lower limit of quantification of both gemcitabine and dFdU was 0.05 μg/mL, and linearity was assessed from 0.078 μg/mL to 15 μg/mL. Within-day and between-day variability (measured as the coefficient of variation [CV]) was <12%.

Pharmacokinetic Analysis

The principal pharmacokinetic parameters were estimated for each patient using a noncompartmental method analysis; the parameters included the peak plasma concentration (C_{max} in μg/mL), determined graphically from the observed experimental values; the experimental plasma AUC (AUC_{exp} in μg • h/mL), calculated according to the trapezoidal rule, from the first to the last sampling time; the AUC extrapolated to infinity (AUC_{∞} in μg • h/mL); total plasma clearance (L/h/m^2), calculated as the ratio of the dose in μg and the AUC; the rate of elimination (k_e in h^{-1}), calculated as the negative slope estimated from the log-linear regression of the terminal part of the plasma concentration-time curve; and the terminal elimination half life ($t_{1/2}$) defined as $\ln 2/k_e$. The pharmacokinetics of gemcitabine were described by all of the above parameters; concerning dFdU, only the C_{max} and AUC_{exp} were calculated according to the sampling period performed (until 4 hours after the completion of the infusion) and the documented $t_{1/2}$ of the metabolite, reported to be >10 hours.[5]

Statistical Analysis

Summary statistics are presented as the mean, standard deviation (SD), CV, median and range or frequency for descriptive purposes. Differences between cohorts I and II were analysed with ANOVA for continuous variables at cycle 1. The normality assumptions for ANOVA were assessed using the tests available. If the normality assumption was violated, the Mann-Whitney U nonparametric test was used. Paired t tests were used to compare the C_{max}, AUC_{exp}, AUC_{∞}, $t_{1/2}$, clearance and k_e at different times for a given group; the same test was employed to compare pharmacokinetic parameters among patients experiencing different grades of toxicity. Furthermore, a Student's t-test was performed using the C_{max}, AUC and clearance of gemcitabine, and the C_{max} and AUC_{exp} of dFdU in order to compare patients experiencing at least grade 3 toxicities with patients experiencing at least grade 2 toxicities. A repeated measure ANOVA for all pharmacokinetic parameters using patient, cohort and cycle factors as variables was also performed. The Kaplan-Meier method was used to calculate overall survival and progression-free survival and reported with their 95% confidence intervals. All analyses were performed using SPSS version 13.0 software (SPSS Inc, Chicago, IL, USA).

Results

Thirteen patients were enrolled in this study; all of them were assessable for toxicity and pharmacokinetic analysis at cycle 1. The characteristics of the 13 patients are listed in table I. Seven patients were women and the median age of the whole cohort was 63 years (range 27–75 years). Seven patients had locally advanced or metastatic pancreatic adenocarcinoma, three had

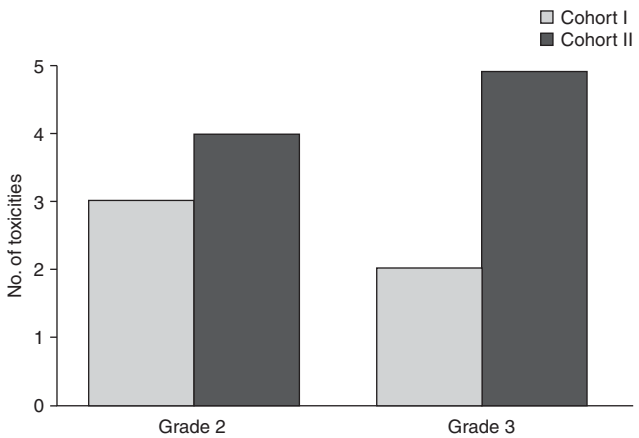

Fig. 1. Cumulative grade 2 and 3 toxicities in patients with normal hepatic function (cohort I) and in patients with impaired hepatic function (cohort II).

Table IV. Summary of pharmacokinetic parameters observed at cycle 1[a]

Parameter	Compound	Cohort I	Cohort II
C_{max} (μg/mL)	GEM	6.82 (0.73) [6.00–7.70]	7.76 (1.77) [6.50–12.20]
	dFdU	11.07 (1.58) [8.80–12.40]	8.93 (2.39) [5.40–14.0]
AUC_{exp} (μg • h/mL)	GEM	11.75 (2.61) [9.11–15.22]	8.43 (2.29) [5.06–12.54]
	dFdU	37.70 (3.74) [34.01–41.83]	25.14 (8.12) [13.80–35.14]
AUC_{∞} (μg • h/mL)	GEM	12.13 (3.12) [9.20–16.42]	8.87 (2.50) [5.15–13.17]
$t_{½}$ (h)	GEM	0.92 (1.25) [0.08–2.77]	0.18 (0.10) [0.06–0.35]
CL (L/h/m^2)	GEM	88.12 (18.65) [65.70–109.80]	127.27 (37.43) [79.76–197.59]
k_e (h^{-1})	GEM	3.35 (3.62) [0.25–8.27]	5.41 (4.11) [0.30–12.18]

a Values are expressed as mean (SD) [range].

AUC_{exp} = experimental area under the plasma concentration-time curve; **AUC_{∞}** = AUC from time zero to infinity; **CL** = total plasma clearance; **C_{max}** = peak plasma concentration; **dFdU** = difluorodeoxyuridine; **GEM** = gemcitabine; **k_e** = elimination rate constant; **$t_{½}$** = terminal elimination half-life.

biliary tree carcinoma and the remaining three presented with advanced gall-bladder adenocarcinoma. None of the patients received prior chemo- or radiotherapy, and the liver was the major site of metastatic disease. The median WHO performance status was 1 (range 0–2). Four patients had normal hepatic function with serum bilirubin <1.6 mg/dL and AST, ALT <2 times the ULN (cohort I); nine patients had hepatic dysfunction with serum bilirubin >1.6 mg/dL and/or AST, ALT >2 times the ULN (cohort II). All patients received a gemcitabine 1000 mg/m^2 FDR infusion on days 1, 8 and 15 every 4 weeks. One patient in the control arm had a 25% dose reduction in cycle 2 because of haematological toxicity, while two patients in the experimental arm never started cycle 2, one because of disseminated intravascular coagulation (DIC) after cycle 1 and the other because of deterioration in his general condition (rapid worsening of his performance status). The baseline laboratory parameters of the patients are listed in table II. The only statistically significant difference in the baseline laboratory values between the two cohorts was in two hepatic function parameters, the total bilirubin level ($p = 0.04$) and AST level ($p = 0.01$), whereas no significant difference was observed for the ALT level ($p = 0.16$) and all other blood parameters reported in table II. Only the total bilirubin level had a statistically significant decrease from cycle 1 to cycle 2 in cohort II (5.29 mg/dL vs 1.90 mg/dL; $p = 0.03$), while all other laboratory values had comparable means between the first two cycles in both groups of patients.

The main toxicities reported after cycle 1 are detailed in table III. Although more patients in cohort II experienced grade 3 toxicities, this difference was not statistically significant (figure 1). Moreover, patients with bilirubin and/or transaminases elevation did not require a dose reduction of gemcitabine. Even though no patient experienced grade 4 toxicity, haematological toxicity represented the major adverse effect. Two patients experienced grade 3 neutropenia in the control arm, one patient experienced grade 3 thrombocytopenia and one patient experienced grade 3 anaemia in cohort II. Laboratory toxicities were low in both groups, although two episodes of transient grade 3 elevation in serum bilirubin and transaminases from baseline values were seen in one patient in cohort II. Other toxicities that occurred were asthenia and fever. The mean (SD) decrease of neutrophils from baseline to the value at the nadir during cycle 1 was 24.6% (58.1) in cohort I and 53% (26.6) in cohort II.

All patients met criteria for measurable disease; one complete response was observed in the control group, three patients

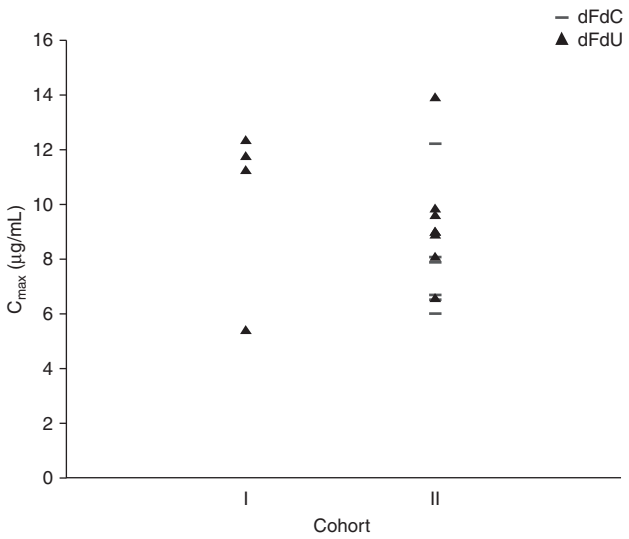

Fig. 2. Peak plasma concentration (C_{max}) of gemcitabine (2,2-difluorodeoxycytidine [dFdC]) and its metabolite (difluorodeoxyuridine [dFdU]) in patients with normal hepatic function (cohort I) and in patients with impaired hepatic function (cohort II).

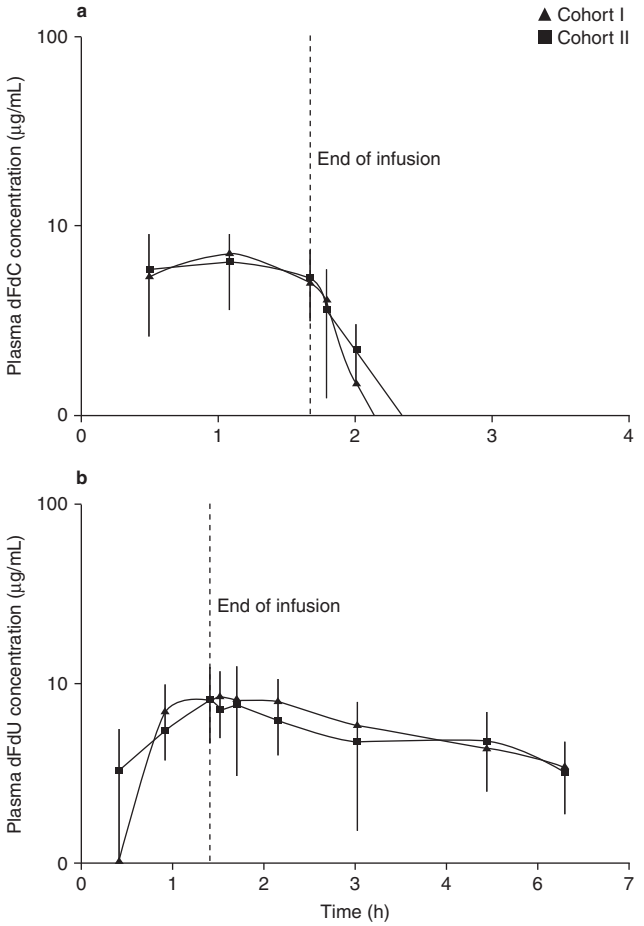

Fig. 3. Plasma concentration-time profiles of (**a**) gemcitabine (2,2-difluorodeoxycytidine [dFdC]) and (**b**) difluorodeoxyuridine [dFdU] in cycle 1 in patients with normal hepatic function (cohort I) and in patients with impaired hepatic function (cohort II).

had a partial response, two had stable disease and six patients experienced progressive disease. One patient was not evaluable for a response because of a serious adverse event (DIC) after cycle 1. At a median follow-up of 19 weeks (range 1–167 weeks), the median progression-free survival was 15 weeks (95% CI 9, 22) and the median overall survival was 20 weeks (95% CI 12, 50).

Pharmacokinetic Results

Pharmacokinetic analysis was performed on 13 patients in cycle 1 and on 9 of 11 patients receiving treatment in cycle 2. Two of the patients never started cycle 2 because of adverse events. All patients were studied on a dose of gemcitabine 1000 mg/m^2 at the FDR infusion of 10 mg/m^2/min. Patients in cohort I had normal hepatic function (serum bilirubin <1.6 mg/dL, and AST, ALT <2 times the ULN), and patients in cohort II had impaired hepatic function (serum bilirubin >1.6 mg/dL and/or AST, ALT >2 times the ULN). A descriptive analysis of the pharmacokinetic results is listed in table IV. With normal or impaired hepatic function, the gemcitabine C_{max} was similar in the two groups: 6.82 μg/mL (±0.73) and 7.76 μg/mL (±1.77), respectively (figure 2). The variability in the C_{max} was not very high, with concentrations ranging from 6.0 to 7.7 μl/mL for gemcitabine and from 6.5 to 12.2 μl/mL for dFdU (figure 3). The C_{max} of dFdU was observed in all patients at the end of gemcitabine infusion (after 100 minutes from the start of infusion) or after 5 minutes from the end of the infusion. After the end of the FDR infusion, the plasma concentration of gemcitabine declined rapidly in all patients (figure 3). The overall mean (±SD) clearance of gemcitabine was 88.12 (±18.65) and 127.27 (±37.43) L/h/m^2 in cohort I and II, respectively, with no significant difference between the two groups (figure 4). No relationship was found between the serum bilirubin concentration and gemcitabine clearance (figure 5). A regression analysis performed for other variables of hepatic function (transaminases, AP, PT, PTT) did not show any statistically significant result. The mean of AUC_{exp} for gemcitabine was higher in patients with normal hepatic function (11.75 μg • h/mL) than in patients with impaired hepatic function (8.43 μg • h/mL), and the difference was statistically significant ($p = 0.04$). The mean AUC_{∞} values were 12.13 μg • h/mL and 8.87 μg • h/mL in cohorts I and II, respectively ($p = 0.07$). The mean dFdU AUC_{exp} values were 37.70 μg • h/mL in cohort I and 25.14 μg • h/mL in cohort II; the difference between the two AUC_{exp} values was statistically

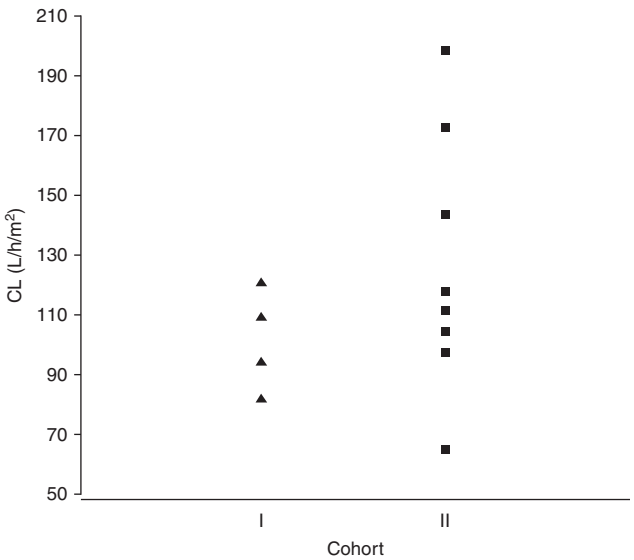

Fig. 4. Gemcitabine clearance (CL) in patients with normal hepatic function (cohort I) and in patients with impaired hepatic function (cohort II).

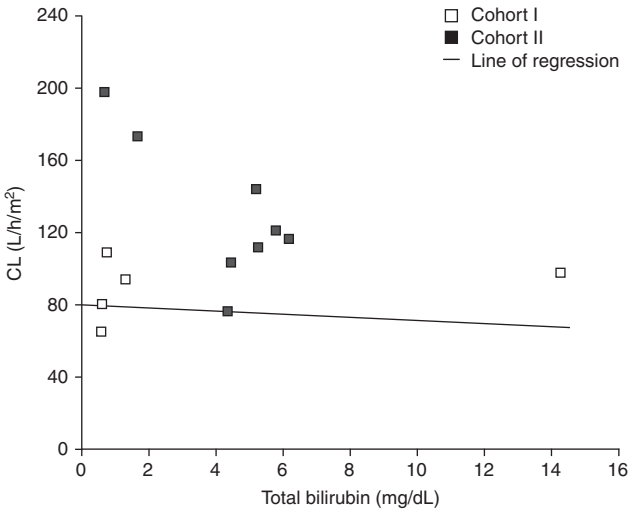

Fig. 5. Regression analysis of gemcitabine clearance (CL) as a function of total bilirubin in patients with normal hepatic function (cohort I) and in patients with impaired hepatic function (cohort II).

significant ($p = 0.01$). There were no significant differences in the $t_{1/2}$ of gemcitabine in both cohorts. Similarly, there were no significant differences between the cohorts in the k_e for gemcitabine ($3.35\ h^{-1}$ in the control group and $5.41\ h^{-1}$ in patients with impaired hepatic function).

In nine patients, the pharmacokinetic analysis was also performed at the second cycle in order to evaluate the variability of the pharmacokinetic parameters in two consecutive cycles. No statistically significant difference was verified for any of the parameters analysed between cycle 1 and cycle 2 in the two groups (table V). The data for gemcitabine clearance in cycles 1 and 2 are reported in figure 6.

Finally, we investigated whether pharmacokinetic alterations could be associated with any altered toxicity profile. We identified four patients with grade ≥3 myelotoxicity, two in the control arm and two in the experimental arm; only one patient in cohort II had grade ≥3 hyperbilirubinaemia. The C_{max} of dFdU was significantly higher ($p = 0.02$) in patients with grade 3 haematological toxicities. The patient with hepatic adverse effects presented with a higher dFdU AUC_{exp} value than all other patients in the same cohort (34.63 μg • h/mL vs 28.5 μg • h/mL; $p = 0.046$), a lower gemcitabine AUC_{exp} (5.06 μg • h/mL vs 9.82 μg • h/mL; $p < 0.0001$) and a lower AUC_{∞} (5.15 μg • h/mL vs 10.27 μg • h/mL; $p < 0.0001$); furthermore, the gemcitabine clearance was $197.59\ L/h/m^2$ in this patient compared with $108.36\ L/h/m^2$ in other patients in cohort II ($p < 0.0001$). A graphic distribution of the ratio of gemcitabine and dFdU AUC_{exp} values in patients who experienced grade 3 toxicities is illustrated in figure 7.

Discussion

The pharmacokinetics and the toxicity profile of chemotherapeutic agents are usually evaluated in phase I studies and in patients with normal organ function. This methodology precludes the possibility of evaluating specific dose recommendations in patients with organ dysfunction. Gemcitabine is a drug with a broad spectrum of activity and a favourable toxicity profile. Literature data have reported an increased incidence of transient hepatic toxicity in patients with liver metastases,[19] and a phase I dose-escalation study[15] of gemcitabine over 30 minutes recommended reducing the dose of gemcitabine to $800\ mg/m^2$ in patients with elevated bilirubin levels.

Gemcitabine infusion at an FDR of $10\ mg/m^2/min$ has been shown to maximize the rate of accumulation of triphosphate, its major intracellular metabolite.[8,9] Despite these robust pharmacological data, several phase II and III studies comparing different doses of gemcitabine in standard 30-minute and FDR infusions have failed to demonstrate a substantial clinical benefit for the main outcome.

Based on these data, we were interested in evaluating the hepatic toxicity of a gemcitabine FDR infusion in patients already affected by impaired hepatic function. This is a frequent condition in patients with pancreatic and biliary tree carcinoma: hepatic function is compromised directly by cancer. We decided to use a dose of gemcitabine $1000\ mg/m^2$ in patients with pancreatic and biliary tree carcinoma, based on a current lack of evidence that gemcitabine activity is improved by increasing its dose.[20]

Although there were a limited number of patients included in this series, based on our clinical and pharmacokinetic results, we do not recommend starting gemcitabine at a reduced dose in patients with impaired hepatic function. In fact, we observed grade 3 myelosuppression in patients with both normal and altered hepatic function as well as a slightly increased incidence

Table V. Overall pharmacokinetic results at cycle 1 and cycle 2

Cycle	C_{max} (μg/mL)		AUC_{exp} (μg • h/mL)		AUC_{∞} (μg • h/mL)	$t_{1/2}$ (h)	CL ($L/h/m^2$)	k_e (h^{-1})
	GEM	dFdU	GEM	dFdU	GEM	GEM	GEM	GEM
1	7.47	9.59	9.45	29.00	9.88	0.41	115.22	4.77
2	7.26	8.73	8.14	27.01	8.73	0.25	128.45	5.36

AUC_{exp} = experimental area under the plasma concentration-time curve; **AUC_{∞}** = AUC from time zero to infinity; **CL** = total plasma clearance; **C_{max}** = peak plasma concentration; **dFdU** = difluorodeoxyuridine; **GEM** = gemcitabine; **k_e** = elimination rate constant; **$t_{1/2}$** = terminal elimination half-life.

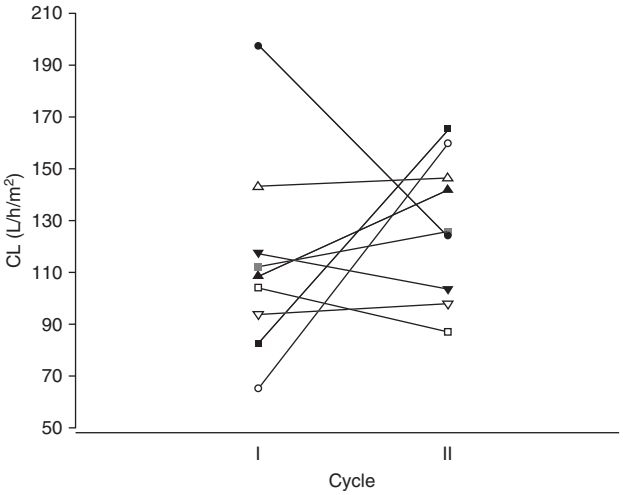

Fig. 6. Intrapatient variability: gemcitabine clearance (CL) in patients in cycles 1 and 2.

of nonhaematological toxicity in patients with hepatic dysfunction. All toxicities that occurred were manageable, and patients experiencing grade 3 toxicities in cohort II did not require dose adjustment. The only dose reduction was performed in a patient with normal hepatic function.

The pharmacokinetic analysis showed no significant differences in the C_{max}, clearance and $t_{1/2}$ values in the two cohorts, but revealed significantly lower AUC_{exp} values for gemcitabine and dFdU in patients with impaired hepatic function compared with the control group. Although the sample size was too small to confirm or refute a meaningful difference in this parameter, the overall drug exposition was lower in patients with hepatic dysfunction.

Another aim of this study was to verify and confirm the reproducibility of the pharmacokinetic parameters analysed within the same patient in two different cycles. Of all the variables analysed, none appeared to differ significantly from cycle 1 to cycle 2.

Finally, we investigated whether there were differences in the pharmacokinetic disposition of gemcitabine and/or dFdU between patients with increased toxicity and those with better tolerability. Our analysis failed to reveal a statistically significant difference in the pharmacokinetics of these agents between patients with an altered toxicity profile (at least grade 3) and those with grade 1–2 toxicities. Moreover, we did not identify any pharmacokinetic parameter that correlated with a pharmacodynamic outcome, such as myelosuppression. A more accurate study of the relationship between toxicity and gemcitabine disposition, based on the pharmacokinetics of the triphosphate metabolite, dFdCTP, should be performed. In fact, gemcitabine is not the active drug but a pro-drug, which requires a series of activations to be transformed into its triphosphate form. In addition, a correlation between plasma gemcitabine/dFdCTP concentrations and toxicity seems to be unlikely, as evidenced by other studies.[21] It has been demonstrated that high doses of gemcitabine (2800 mg/m^2) are not related to a high grade of toxicity. This is possibly explained by the saturable mechanism of accumulation of dFdCTP in the cells. A weak relationship between plasma gemcitabine concentrations and dFdCTP has been recently reported by Grimison et al.,[14] who reported autoinduction of dFdCTP accumulation during week 2 of gemcitabine treatment, underlying the importance of conducting a pharmacological study beyond week 1.

Only one patient in the present study had transient elevations in bilirubin and transaminases in cohort II. This singular evidence is not enough to confirm the transient hepatic toxicity observed in the FDR arm of the study by Tempero et al.,[13] and the reason for the temporary hepatic dysfunction remains unclear. The analysis of gemcitabine and dFdU disposition in this specific patient revealed a significantly lower gemcitabine AUC and higher gemcitabine clearance than in all other patients. We are unable to extrapolate this information from the only case observed, but we are currently analysing other patients with transient hepatic toxicity in order to confirm these data.

It is difficult to compare our pharmacokinetic data with pharmacokinetic data on gemcitabine reported in the literature because of the different doses and times of infusion employed and the high variability of the pharmacokinetic parameters observed. Soo et al.[22] reported the results of gemcitabine

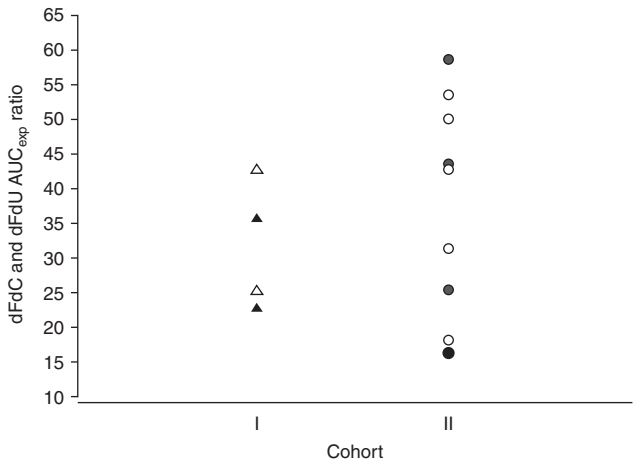

Fig. 7. Ratio of gemcitabine (2,2-difluorodeoxycytidine [dFdC]) and difluorodeoxyuridine (dFdU) experimental area under the plasma concentration-time curve (AUC_{exp}) values in patients with normal hepatic function (cohort I) and in patients with impaired hepatic function (cohort II). The black spots represent patients experiencing grade ≥3 toxicities in cohorts I and II.

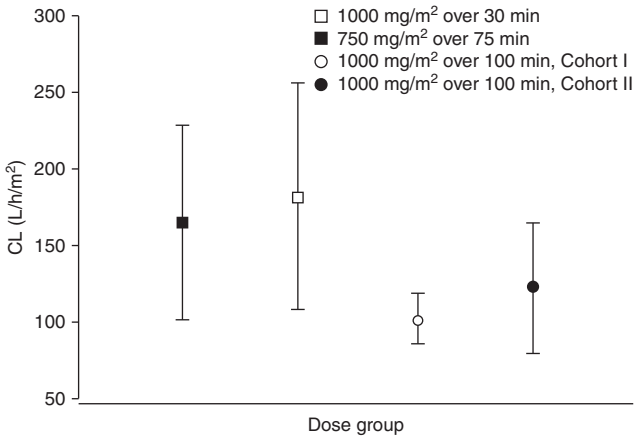

Fig. 8. Clearance (CL) of gemcitabine infused intravenously at 1000 mg/m^2 over 30 minutes, 750 mg/m^2 over 75 minutes[22] and 1000 mg/m^2 over 100 minutes.

administered at 1000 mg/m^2 in a 30-minute infusion and 750 mg/m^2 in a 75-minute infusion combined with carboplatin. We have combined our data concerning the clearance of gemcitabine with the data presented by Soo et al.[22] (figure 8).

Our results seem to exclude the possible increased toxicity of gemcitabine when administered as an FDR infusion in patients with impaired hepatic function. Nevertheless, we believe that patients with organ dysfunction do require specific studies to verify the correct drug dose and tolerability. These data cannot be extrapolated from conventional phase I trials and warrant further specifically designed investigations.

Conclusion

Our study demonstrated that gemcitabine can be safety administered at 1000 mg/m^2 in an FDR infusion in patients with impaired hepatic function. The toxicity analysis revealed similar drug tolerance, without additional adverse effects, when compared with patients with normal hepatic function. The plasma disposition of gemcitabine, when compared in patients with hepatic dysfunction and those with normal bilirubin and transaminases levels, was similar except for a reduction in the AUC_{exp}. Specific pharmacokinetic and pharmacodynamic studies should be performed to evaluate the tolerability and the disposition of chemotherapy agents in patients with potentially altered metabolic function.

Acknowledgements

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Correspondence: *Alessandra Felici MD*, Division of Medical Oncology A, Regina Elena Cancer Institute, Via Elio Chianesi, 53, 00144, Rome, Italy.
E-mail: alefelici@yahoo.it