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SHORT COMMUNICATION

Pharmacokinetic study of gemcitabine, given as prolonged infusion at fixed dose rate, in combination with cisplatin in patients with advanced non-small-cell lung cancer

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Abstract
Introduction Although some studies have suggested that gemcitabine delivered as a fixed dose rate (FDR) infusion of 10 mg/m^2/min could be more effective than when administered as the standard 30-min infusion, the available pharmacokinetic data are still too limited to draw definitive conclusions. This study is aimed to investigate the plasmatic and intracellular pharmacokinetics of gemcitabine given as FDR at doses of 600 and 1,200 mg/m^2 in combination with 75 mg/m^2 of cisplatin in advanced non-small-cell lung cancer (NSCLC) patients.
Patients and method The patients were divided into two groups receiving different initial doses of the drug: 4 patients received 600 mg/m^2 gemcitabine 60-min i.v. infusion and 4 patients 1,200 mg/m^2 gemcitabine 120-min i.v. infusion both as a FDR of 10 mg/m^2/min on days 1 and 8 of a 21-day cycle (at first cycle). At the second cycle, all patients were treated with gemcitabine at 1,200 mg/m^2 120-min i.v. infusion (FDR of 10 mg/m^2/min) on days 1 and 8 of a 21-day cycle. At each cycle, gemcitabine was administered alone on day one, and in combination with 75 mg/m^2 of cisplatin on day 8. Plasmatic and intracellular pharmacokinetic analyses were performed on blood samples collected at defined time points before, during and after gemcitabine infusion.
Results The plasmatic pharmacokinetic parameters were clearly different when the patients received a higher gemcitabine dose in the second cycle compared to the lower dose of the first course; in the same time, the intracellular drug levels were not modified. Comparing the pharmacokinetic parameters of different patients treated at different dose levels, the results appeared to be quite similar.
Conclusions A substantially higher accumulation of metabolites in peripheral blood mononuclear cells was observed when the longer infusion time was employed, suggesting a pharmacological advantage for this treatment schedule.

Keywords Non-small-cell lung cancer · Chemotherapy · Cisplatin · Gemcitabine · Prolonged infusion · Pharmacokinetics

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Introduction

Gemcitabine, 2′,2′-difluoro-2′-deoxycytidine (dFdC) is a pyrimidine antimetabolite, with a broad spectrum of antitumor activity [1], representing one of the reference drugs in combination chemotherapy of non-small-cell lung cancer (NSCLC) [2].

Gemcitabine is a pro-drug that enters the cell by means of nucleoside transporters and becomes active through an intracellular transformation catalyzed by deoxycytidine kinase to the final triphosphate form (dFdCTP) in a rate-limiting reaction [1, 3, 4]. Gemcitabine pharmacokinetics in humans are characterized by rapid elimination and extensive deamination to its inactive 2′,2′-difluorodeoxyuridine (dFdU) that is the main metabolite present in plasma [3, 4].

Gemcitabine is usually administered intravenously as a 30-min infusion at the dose of 1,000–1,200 mg/m^2, but previous studies suggest that under these conditions, the formation of dFdCTP in normal peripheral blood mononuclear cells (PBMC) is saturated when gemcitabine concentrations of 15–20 μM are achieved in plasma [3, 4]. Gemcitabine delivered as a fixed dose rate (FDR) infusion of 10 mg/m^2/min has been suggested to be more effective than when administered as the standard 30-min infusion [3–5]. This schedule seems adequate for maintenance of the plasma target level for a longer period of time [5, 6], but the available pharmacokinetic data are still too limited to draw definitive conclusions.

In terms of clinical outcomes, the picture is unclear. For example, in pancreatic cancer, despite the promising results from a randomized phase II study showing a survival advantage for patients treated with FDR infusion [5], a more recent report failed to confirm the same advantage for FDR infusion that led an increased toxicity [7].

We have therefore conducted a phase II study in advanced NSCLC patients, aimed to investigate the pharmacokinetics of gemcitabine and dFdU administered as a FDR of 10 mg/m^2/min at doses of 600 and 1,200 mg/m^2 in combination with 75 mg/m^2 of cisplatin.

Methods

Patients, study design and objectives

On the basis of results from our previous dose-escalating study with cisplatin and gemcitabine administered at 7 different dose levels (from 600 to 1,200 mg/m^2) [8] as prolonged FDR in advanced NSCLC, we planned to explore both the plasmatic and intracellular pharmacokinetics of gemcitabine at the lower (600 mg/m^2) and higher (1,200 mg/m^2) levels of dose.

From July 2005 to February 2006, we enrolled 8 patients affected by advanced NSCLC and treated at the Department of Oncology of Santa Chiara Hospital, Trento, Italy. The treatment schedule was planned to evaluate both intra- and inter-patient pharmacokinetic variability of gemcitabine given as a prolonged FDR infusion in combination with cisplatin.

According to the study plan, the patients were divided into two groups receiving different initial doses of the drug: 4 patients treated with 600 mg/m^2 gemcitabine 60-min i.v. infusion (Group A), and 4 patients treated with 1,200 mg/m^2 gemcitabine 120-min i.v. infusion (Group B), both at FDR of 10 mg/m^2/min on days 1 and 8 of a 21-day cycle (at first cycle). At the second cycle, all patients received 1,200 mg/m^2 gemcitabine 120-min i.v. infusion (FDR of 10 mg/m^2/min) on days 1 and 8 of a 21-day cycle.

At each cycle, gemcitabine was administered alone on day one and in combination with 75 mg/m^2 of cisplatin on day 8. Cisplatin was infused for 30 min, immediately before the administration of gemcitabine.

All patients, in absence of progressive disease, received further 4 courses of gemcitabine 1,200 mg/m^2 on days 1 and 8 plus cisplatin 75 mg/m^2 on day 8 for a total number of 6 cycles: in these courses, no pharmacokinetic analyses were planned.

All of the patients gave their written informed consent to participate in the study, which was approved by the local Ethics Committee.

Pharmacokinetic study

Blood samples were collected at the following times: pre-infusion, 15 and 30 min during the infusion, at the end of the infusion, 30 min, 2, 4, and 24 h after the end of the infusion.

Blood was withdrawn by venipuncture, immediately placed into heparinized tubes containing 50 μL of the cytidine deaminase inhibitor tetrahydrouridine (10 mg/mL, in bi-distilled water) (Calbiochem, La Jolla, CA, USA) to prevent the spontaneous deamination of gemcitabine to dFdU and placed in an ice bath until following separation of plasma and PBMC. After centrifugation at 4°C for 10 min at 2,500 rpm, the plasma fraction was separated and stored frozen at −20°C until analysis of gemcitabine and dFdU. Then the cellular fraction, kept at 4°C, was suspended (1:1) with phosphate buffered saline (PBS) and layered over 5 ml Ficoll-Hypaque (Pharmacia, Stockholm, Sweden) (specific gravity 1.077 g/mL) (2:1 v/v) and centrifuged at 1,000 g for 25 min at 4°C. The buffy coat containing PBMC was collected, washed twice with 20 mL ice-cold PBS (4°C) and centrifuged at 400 g for 8 min at 4°C. Finally, the cell pellet was resuspended in 110 μL PBS. An aliquot of 10 μL of the suspension was used to determine the cell number by automatic coulter counter system. The rest of the suspension was immediately stored at −40° C until shipping and analysis.

The determination of the plasma concentrations of gemcitabine and dFdU was performed by using the recently published method based on HPLC coupled to mass spectrometry [9].

The concentration of dFdCTP in PBMC cells was determined by HPLC (lowest sensitivity limit 0.25 mg/L) as described by Kirstein et al. [10] and Huang et al. [11] for chromatographic conditions and final sample preparation following deproteinization, respectively.

Pharmacokinetic analysis

Gemcitabine and dFdU pharmacokinetic parameters were calculated with WinNonLin Pro Node 4.1 pharmacokinetic

software (Pharsight Co., Mountain View, CA, USA) by using a non-compartmental approach.

Statistical analysis

Two-tailed *t* test was used to determine the significance of differences between measurements of dFdCTP values.

Results

Patients' characteristics

The patients' (6 men and 2 women) median age was 61.5 years (range 34–69). All tumors were adenocarcinoma. Three patients achieved a partial response, while 5 patients experienced progressive disease. All patients relapsed with a median progression-free survival of three months (range 1–7 months); the median survival was 4.5 months (range 2–34). Grade 3–4 haematological toxicities were observed only in two patients of group A during the second course of therapy.

Pharmacokinetic data

Plasma pharmacokinetics

Gemcitabine C_{max} values were in the range 10–20 μM, approximately at the end of the constant rate infusion of 60 or 120 min in all the patients, in all courses monitored. After the end of infusion, gemcitabine concentrations rapidly declined from plasma with a $T_{1/2}$ of less than 30 min. Gemcitabine was detectable up to 4 h post infusion at concentrations close to the limit of quantitation (Table 1).

Table 1 reports the means and standard deviations of the main pharmacokinetic parameters of gemcitabine and dFdU.

Cellular pharmacokinetics

A wide interpatient variability in dFdCTP peak concentrations was observed (data not shown) (Table 2).

In both the groups, the mean peak concentrations of dFdCTP in PBMC were higher on day 8 of the first cycle of treatment when compared to day 1 of the same cycle of treatment. In both cases, the difference was not statistically significant.

In group A, the mean peak concentrations of dFdCTP after the infusion of 1,200 mg/m^2 gemcitabine during a 120-min period were similar at day 1 and day 8 of the cycle 2. Within this group of patients, a slight increase in the peak levels of dFdCTP was observed at day 1 of cycle 2 when compared to day 1 of cycle 1.

In Group B, the mean dFdCTP peak concentration was similarly increased, albeit not significantly, at day 8 when compared to day 1 of the second cycle. An increase in mean peak dFdCTP concentrations was also noted at day 1 and 8 of cycle 2 when compared to the same days of cycle 1 in group B.

Intergroup variations were also observed. In group B patients, the intracellular concentrations of dFdCTP were significantly or nearly significantly higher than corresponding values in group A patients.

Peak dFdCTP concentrations occurred within a wide interval of time ranging from 15 min after the start of gemcitabine infusion and 4 h after the end of it, independently of its duration (60 or 120 min). No significant variations in T_{max} among cycles and days of treatment were observed within each group of patients. A trend to a later occurrence of the dFdCTP peak concentration was observed in patients of group B when compared to those of group A.

Planned comparisons

The comparison of pharmacokinetic parameters between different patients receiving two different dose levels in the first course showed a significant difference in terms of gemcitabine C_{max} (23.8 vs. 18.7; $P = 0.01$) and AUC_{exp} (7.7 vs. 10.5; $P = 0.005$) on day 1 and in terms of intracellular peak concentration on day 8 (173.0 vs. 316.9; $P = 0.009$). The same comparison within patients treated with two different dose levels in the first two chemotherapy courses showed that both gemcitabine AUC_{exp} and dFdU pharmacokinetic parameters were significantly higher when the patients received the higher dose in the second course (data not shown). No differences were observed assessing the differences in patients who received the same dose in the first two courses of gemcitabine.

Discussion

Gemcitabine is one of the most widely used anticancer drugs and is usually administered at the dose of 1,000–1,200 mg/m^2 over 30-min infusion. However, in recent years, several studies have tested both different infusion durations and drug doses to optimize the relationship between the drug delivery and anticancer activity. Since the intracellular metabolic conversion of gemcitabine is limited by saturation of the deoxycytidine kinase activity occurring at low-dose level, the 10 mg/m^2/min FDR infusion could enhance the drug antitumor activity [5, 12].

In our previous study we observed an apparent dose-dependent gemcitabine pharmacokinetics [8]. Between 600 and 1,200 mg/m^2, the drug plasma levels appeared to be

Table 1 Main pharmacokinetic parameters of gemcitabine and dFdU in NSCLC patients

		Day 1				Day 8			
		C_{max} (μM)	AUC_{exp} (μg*h/mL)	AUC_{inf} (μg*h/mL)	$T_{1/2}$ (min)	C_{max} (μM)	AUC_{exp} (μg*h/mL)	AUC_{inf} (μg*h/mL)	$T_{1/2}$ (min)
Gemcitabine									
Group A ($n = 4$)									
Course 1 (600 mg/m^2)	Mean	23.8	7.7	7.7	19	22.5	6.4	–	–
	SD	2.5	1	1	6.7	4.1	1.46	–	–
	CV (%)	10.5	13	13	35.2	18.2	22.9	–	–
Course 2 (1,200 mg/m^2)	Mean	34.5	17.1	17.1	27.8	27.5	14.4	14.4	19
	SD	20	5.8	5.8	6.6	5	2.3	2.3	6.6
	CV (%)	58.8	33.9	33.9	23.7	18.2	15.7	15.8	33.9
Group B ($n = 4$)									
Course 1 (1,200 mg/m^2)	Mean	18.7	10.5	10.5	19.9	21.2	10.4	11.5	21.2
	SD	1.9	0.8	0.8	6.9	7.1	3.2	3	6.1
	CV (%)	10	7.8	7.8	34.8	33.4	31.2	26.1	28.6
Course 2 (1,200 mg/m^2)	Mean	17.8	9.2	9.3	23.7	21.6	1.1	–	–
	SD	4.1	2.7	3.4	13.3	12.5	5	–	–
	CV (%)	22.6	30.1	36.4	56.1	57.7	45.5	–	–
dFdU									
Group A ($n = 4$)									
Course 1 (600 mg/m^2)	Mean	90.7	215.2	269.1	10.7	89.2	204	281.3	13.4
	SD	10.3	31.8	57.9	2.4	17.2	33.7	75.7	5.8
	CV (%)	11.4	14.8	21.5	22.7	19.3	16.5	26.9	43.1
Course 2 (1,200 mg/m^2)	Mean	138.5	346.7	436.3	10.2	162.8	329.6	371.1	8.3
	SD	31.6	84.6	177.9	4.7	55.2	96.2	105.7	1
	CV (%)	22.9	24.4	40.8	45.8	33.9	29.2	28.5	12.2
Group B ($n = 4$)									
Course 1 (1,200 mg/m^2)	Mean	94.8	220.3	253.6	7.1	99.6	214.5	255	10.3
	SD	32.2	136.8	154.7	3.2	35.5	81	92.6	2.9
	CV (%)	34	62.1	61	45.6	35.7	37.8	36.3	28.1
Course 2 (1,200 mg/m^2)	Mean	94.1	194.8	232.9	10	88.8	215.2	271.9	11.5
	SD	19.4	43.9	58	2.3	15.5	37.5	47.1	3.3
	CV (%)	20.6	22.6	24.9	23	17.5	17.4	17.3	28.6

not related to the doses. The critical level of 15 μM, necessary to exert the pharmacological activity [3, 4], was achieved and maintained successfully in the majority of patients treated at the low or middle doses, but surprisingly, not in patients treated at the high doses. To clarify these surprising findings, we evaluated the intra- and inter-patient differences in plasmatic and intracellular gemcitabine pharmacokinetics when the drug was administered at two different doses as FDR.

As a result, the target plasma concentration of 15 μM was successfully achieved in all patients treated with 600 mg/m^2 and in 14/16 of those treated with 1,200 mg/m^2. The pharmacokinetic parameters were clearly different when the patients received a higher gemcitabine dose in the second cycle compared to the lower dose of the first course, confirming that, in this case, the intra-patient variability was mainly due to the dose level; in the same time, the intracellular drug levels were not modified, as expected by the FDR administration system. Comparing the pharmacokinetic parameters of different patients treated at different dose levels, the results appeared to be quite similar: in this case, the inter-patient variability may have a role in masking the effect of the different dose levels.

Various studies have evaluated intracellular dFdCTP concentrations in PBMC after gemcitabine treatment in patients with solid tumors [5, 13–16]. However, it is difficult to compare our results with those of the literature because the published studies were performed administering gemcitabine at variable doses and with variable infusion times. When a standard 30-min infusion was

Table 2 Mean intracellular peak concentrations and T_{max} of dFdCTP in peripheral blood mononuclear cells

		Day 1		Day 8 (with cisplatin)	
		Peak concentrations (pmol × 10^6 cells)	T_{max} (min)	Peak concentrations (pmol × 10^6 cells)	T_{max} (min)
Group A ($n = 4$)					
Course 1 (600 mg/m^2)	Mean	92.6*	150	173.0**	105
	SD	85.9	117.5	29.5	130.8
	CV (%)	92.7	78.3	17	124.5
Course 2 (1,200 mg/m^2)	Mean	158.1	101.3	140.1***	150
	SD	134.7	172.5	98.5	60
	CV (%)	85.2	170.4	70.3	40
Group B ($n = 4$)					
Course 1 (1,200 mg/m^2)	Mean	273.5*	191.3	316.9**	142.5
	SD	139.5	145.6	69.7	155.6
	CV (%)	51	76.1	22	109.2
Course 2 (1,200 mg/m^2)	Mean	335.5	243.8	445.3***	195
	SD	117.5	162.7	210.5	139.6
	CV (%)	35	66.7	47.3	71.6

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

employed, gemcitabine doses ranged from 800 to 2,200 mg/m^2 [5, 13–16]. When gemcitabine was administered as a FDR infusion of 10 mg/m^2/min, infusion times ranged from 75 to 150 min [5, 16]. Although in our study FDR was the same, the infusion times were different, i.e. 60 and 120 min.

The increase in the accumulation of dFdCTP in PBMC that occurred at day 8 when compared to day 1 in almost all treatment conditions may have been due to enzyme induction, particularly of deoxycytidine kinase necessary for gemcitabine phosphorylation, that is an activation reaction allowing the drug to be incorporated into DNA and exert its therapeutic action in cells [17]. The administration of cisplatin before that of gemcitabine at day 8 of each cycle might have also played a relevant role in the induction of deoxycytidine kinase. The ability of cisplatin to induce nucleotide excision repair processes may up-regulate several enzymes, including deoxycytidine kinase, needed to increase deoxyribonucleotides levels (e.g. dFdCTP) [13]. They also reported a similar trend to increased dFdCTP concentrations in white blood cells in patients with solid tumors treated with a gemcitabine–cisplatin combination although with a longer interval (24 h) between platinum and gemcitabine.

In our study, only a slight increase in PBMC dFdCTP concentrations occurred at cycle 2 when compared to cycle 1 in both patient groups and both days of treatment with the exception of day 8 in group A patients. These variations were, however, not statistically significant. No evidence of dFdCTP accumulation was observed by De Lange et al. [15] as a function of courses and days of treatment.

In group B patients undergoing a longer time of FDR drug infusion (120 min), the intracellular concentrations of dFdCTP were substantially higher when compared to group A patients receiving the same fixed dose rate for a shorter infusion time (60 min).

Similar results were also reported by Tempero et al. [5] for an identical FDR infusion of gemcitabine lasting 150 min versus a standard 30-min infusion of a higher gemcitabine dose (2,200 mg/m^2). In a cohort of patients receiving gemcitabine at different dose rates (1,000 mg/m^2 over a standard 30-min infusion on week 1 and over 150-min infusion on week 2) Patel et al. [14] reported a C_{max} of dFdCTP at the end of infusion (150 min), which was higher than the plateau levels achieved after the 30-min infusion. An investigation by Soo et al. [16] comparing a combination of carboplatin and gemcitabine at standard rate or fixed dose rate infusion in patients with advanced stage NSCLC reported similar AUC of intracellular dFdCTP in both treatment arms even though the dose of gemcitabine was higher in the standard rate arm (1,000 mg/m^2 over 30 min vs. 750 mg/m^2 over 75 min).

Although the activation mechanisms of gemcitabine are well-known in terms of metabolic pathways, some aspects are relevant for future developments. Several studies have underlined that gemcitabine activity could be related to specific polymorphisms in the deoxycytidine kinase, cytidine deaminase, and/or gemcitabine transporter genes [18]. Moreover, gemcitabine may have anticancer activity also when it is administered at very low doses and with an infusion rate much lower than the well-known level of 10 mg/m^2/min: the drug delivered at 250 mg/m^2 in

360-min infusion has in fact been demonstrated to be active in NSCLC patients [19, 20] and in bladder cancer patients [21].

In conclusion, several pharmacokinetic and clinical studies suggested that gemcitabine has a complex pharmacological profile, where the transporter proteins play a central role able to influence the drug activity. In this view, data from the present study, although obtained from a very limited sample of patients, may be of value for future researches covering a wider range of clinically useful dose rates of gemcitabine with a better exploitation of its activating pharmacokinetic and metabolic mechanisms.

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