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Original article

Drug distribution and pharmacokinetic/pharmacodynamic relationship of paclitaxel and gemcitabine in patients with non-small-cell lung cancer

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Summary

Background Gemcitabine and paclitaxel are two of the most active agents in non-small-cell lung cancer (NSCLC), and pharmacologic investigation of the combination regimens including these drugs may offer a valuable opportunity in treatment optimization. The present study investigates the pharmacokinetics and pharmacodynamics of paclitaxel and gemcitabine in chemotherapy-naive patients with advanced NSCLC within a phase I study.

Patients and methods: Patients were given i.v. paclitaxel 100 mg/m^2 by one-hour infusion followed by gemcitabine 1500, 1750 and 2000 mg/m^2 by 30-min administration. Plasma levels of paclitaxel, gemcitabine and its metabolite 2',2'-difluorodeoxyuridine (dFdU) were determined by high-performance liquid chromatography (HPLC). Concentration-time curves were modeled by compartmental and non-compartmental methods and pharmacokinetic/pharmacodynamic (PK/PD) relationships were fitted according to a sigmoid maximum effect (E_{max}) model.

Results Paclitaxel pharmacokinetics did not change as a result of dosage escalation of gemcitabine from 1500 to 2000 mg/m^2. A nonproportional increase in gemcitabine peak plasma levels (C_{max}, from 18.56 ± 4.94 to 40.85 ± 14.85 μg/ml) and area under the plasma concentration-time curve (AUC, from 9.99 ± 2.75 to 25.01 ± 9.87 h μg/ml) at 1500 and 2000 mg/m^2, respectively, was observed, suggesting the occurrence of saturation kinetics at higher doses. A significant relationship between neutropenia and time of paclitaxel plasma levels ⩾0.05 μmol/l was observed, with a predicted time of 10.4 h to decrease cell count by 50%. A correlation was also observed between percentage reduction of platelet count and gemcitabine C_{max}, with a predicted effective concentration to induce a 50% decrease of 14.3 μg/ml.

Conclusion This study demonstrates the lack of interaction between drugs, the nonproportional pharmacokinetics of gemcitabine at higher doses and the E_{max} relationship of paclitaxel and gemcitabine with neutrophil and platelet counts, respectively. In addition, gemcitabine 1500 mg/m^2 is the recommended dosage in combination with paclitaxel 100 mg/m^2 for future phase II studies, due to its predictable kinetic behaviour and less severe thrombocytopenia than expected.

Key words bone marrow, drug combination, metabolism, pharmacologic interaction, toxicity

Abbreviations NSCLC – non-small-cell lung cancer, HPLC – high-performance liquid chromatography, dFdU – 2',2'-difluorodeoxyuridine, PK/PD – pharmacokinetic/pharmacodynamic, E_{max} – maximum effect, ECOG – Eastern Cooperative Oncology Group, ANC – absolute neutrophil count, PLTC – platelet count, AST – aspartate aminotransferase, ALT – alanine aminotransferase, QoL – Quality of Life, DLT – dose-limiting toxicity, MTD – maximum tolerated dose, UV – ultraviolet, QC – quality control, C_{max} – peak plasma concentration, T_{max} – time to C_{max}, AUC – area under the plasma concentration-time curve, $t_{½}$ – half-life, CL_{TB} – total body clearance, V_{ss} – steady-state volume of distribution, V_d – volume of distribution, MRT – mean residence time, $tC_{0.05}$ – time of paclitaxel levels ⩾0.05 μmol/l; ET_{50} – $tC_{0.05}$ of paclitaxel required for 50% decrease of ANC, EC_{50} – C_{max} of gemcitabine required for 50% decrease of PLTC.

Introduction

The therapeutic options for combined treatment of non-small-cell lung cancer (NSCLC) have been expanded through the extensive clinical development of chemotherapeutic agents for the management of advanced disease, including cisplatin, carboplatin, gemcitabine, paclitaxel, and docetaxel [1]. However, which combination is preferable in terms of drug, dosage and schedule is still an open issue that awaits further clinical investigation. Rational drug development based on the analysis of drug distribution, metabolism and application of pharmacokinetic/pharmacodynamic (PK/PD) principles allows the optimization of treatment schedules and reveals drug interactions that may occur in combination regimens.

Cumulative experience with single-agent paclitaxel in advanced NSCLC suggests that it is a highly active cytotoxic agent, the use of which is consistently associated with a one-year survival rate of 35%–40% [2]. The major toxicities include neutropenia, neuropathy, and myalgia-arthralgia syndrome [2]. Paclitaxel has been used in combination with several other nonplatinum agents for the treatment of NSCLC, and the order of administra-

tion and schedule are clearly relevant in these combinations [2]. For example, in one study, etoposide and paclitaxel given simultaneously proved to be ineffective, with substantial grade IV neutropenia [2, 3]. Another schedule with an identical dose of etoposide, given daily for three days, followed by paclitaxel, achieved a response rate of 41%, with markedly diminished neutropenia [2, 4, 5]. Additional data from phase I trials demonstrated a schedule-dependent pharmacokinetics, toxicity and efficacy of gemcitabine [6, 7] and paclitaxel [5, 8]. Therefore, it is advisable that the pharmacokinetics and pharmacodynamics of gemcitabine, and in particular paclitaxel, should be monitored in clinical studies in which these drugs are used in combination schedules to assess drug-drug interactions that may contribute to unexpected adverse effects, as previously demonstrated with the association of anthracyclines and paclitaxel [8, 9].

Paclitaxel and gemcitabine are two of the most active single agents currently available in the treatment of NSCLC [5, 6], and their combination represents a logical direction of clinical investigation for the improvement of treatment both in terms of efficacy and quality of life (QoL) For these reasons, a pharmacokinetic and pharmacodynamic analysis of paclitaxel and gemcitabine administered on a weekly basis, was performed on 15 chemotherapy-naive NSCLC patients enrolled in a phase I, dose-finding trial during their first cycle of therapy

Patients and methods

Patient characteristics and treatment plan

The study was performed in accordance with the provisions of the Helsinki Declaration and after approval by the local Ethics Committee All patients were advised of the investigational nature of this protocol and written, informed consent was obtained before enrollment Chemotherapy-naive patients with histologically or cytologically proven stage IIIb–IV NSCLC were eligible for this study Additional eligibility criteria were (a) 18–65 years of age, (b) ECOG performance status ≤ 2, (c) life expectancy of at least three months, (d) adequate hematopoiesis (white blood cell count $\geq 3500/\mu l$, absolute neutrophil count [ANC] $\geq 1500/\mu l$ and platelet count [PLTC] $\geq 100{,}000/\mu l$), hepatic function (aspartate aminotransferase [AST] and alanine aminotransferase [ALT] ≤ 2 times, and total bilirubin ≤ 1.25 times institutional upper limit of normal) and renal function (creatinine ≤ 1.25 times institutional upper limit of normal), (e) no active or uncontrolled infection, (f) no prior chemotherapy/radiation therapy within the previous 4/6 weeks, respectively, (g) radiation therapy to $\leq 30\%$ of bone marrow reserve, (h) no known central nervous system involvement, (i) no medical conditions (uncontrolled hypertension, congestive heart failure, serious arrhythmia, unstable angina, recent myocardial infarction and interstitial lung disease with moderate-severe dyspnea) or psychiatric conditions that might expose patients at risk for participation in investigational treatment, (l) previous or concurrent malignancies, (m) absolute contraindication to the administration of steroids, and (n) no pregnancy or lactation Additional details of the phase I study, including pretreatment evaluation, follow-up, assessment of Quality of Life (QoL), dose-limiting toxicity (DLT), maximum tolerated dose (MTD), response and toxicity are provided elsewhere [10, 11] Patients received paclitaxel 100 mg/m^2 by one-hour i v infusion immediately followed by gemcitabine 1500, 1750 and 2000 mg/m^2 by 30-min i v infusion, on days 1, 8, and 15 with cycles repeated every four weeks

Sample collection

Blood (5 ml) was drawn at baseline, 15 min and 1 h after the start of paclitaxel infusion, 5 and 30 min after the beginning of gemcitabine infusion and 5, 15, 30 min, 1, 2, 4, 12 and 24 h after the end of gemcitabine administration Samples were obtained by venipuncture or indwelling i v cannula from the arm, contralateral to the infusion line and collected into heparin-containing tubes If a heparin lock was used, 1 ml of blood was withdrawn and discarded before sample collection Tubes were placed into a slurry of ice water, and plasma was separated by centrifugation at 1500 g for 15 min Tetrahydrouridine (Sigma, St Louis, Missouri) was added to plasma specimens to inhibit the conversion of gemcitabine to its metabolite 2',2'-difluorodeoxyuridine (dFdU) by deoxycytidine deaminase Samples were stored at −70 °C for a maximum of two months until drug assay

Drug analysis

Plasma levels of paclitaxel and gemcitabine/dFdU were assayed by validated reverse-phase, high-performance liquid chromatographic (HPLC) methods with ultraviolet (UV) monitoring [12, 13] The HPLC instrument was an LC Module I Plus equipped with a 715 autosampler and a 486 variable wavelength UV detector (Waters, Milford, Massachusetts) Limits of quantitation of paclitaxel and gemcitabine/dFdU were 10 nmol/l and 0.08 μg/ml, respectively Human, blank plasma was used as the calibrant matrix, and methods were linear (linear regression analysis, weighting $1/X^2$) over the analytical range of 0.01–100 μmol/l for paclitaxel ($r^2 \geq 0.995$) and 0.08–100 μg/ml for gemcitabine and dFdU ($r^2 \geq 0.998$) The mean assay precision, expressed as the coefficient of variation of the estimated concentrations of quality control (QC) standards, averaged 2.5%, 3.1%, and 7.2%, respectively, for low (0.01 μmol/l), medium (2.5 μmol/l), and high (100 μmol/l) concentrations of paclitaxel, 3.8%, 2.9%, and 6.6%, respectively, for low (0.08 μg/ml), medium (5 μg/ml), and high (100 μg/ml) levels of gemcitabine, and 2.9%, 4.1%, and 7.1%, respectively, for low (0.08 μg/ml), medium (5 μg/ml), and high (100 μg/ml) concentrations of dFdU Assay accuracy, expressed as the percent ratio of the estimated vs theoretical QC standard concentrations, averaged 92.2%–98.6% for paclitaxel, 91%–99.2% for gemcitabine and 90.5%–98.9% for dFdU

Pharmacokinetic analysis

Paclitaxel, gemcitabine and dFdU plasma levels vs time curves were modeled using the MW/PHARM software (Mediware, The Netherlands, [14]) Initial parameter estimates were determined by curve-stripping with the Kinstrip module and then fitted with the Kinfit module to obtain equations describing the profile of plasma levels vs time The non-linear least-squares, iterative regression analysis of Kinfit determines the slopes and intercepts of the logarithmically-plotted curves of polyexponential functions and provides a correlation coefficient for the fitted curve Modeling of the concentration-time curve was done with the Nelder-Mead simplex procedure to determine the parameter values that minimize a weighted least-squares criterion, and the performance of the fitting procedure was controlled by an accuracy factor defined interactively [14] While analysing the polyexponential pharmacokinetic data, the convergence was reached when the relative change in the sum of squares was less than 1×10^{-6} for non-linear curve-fitting/modeling An open, two-compartment model, with model input via constant infusion of drugs, best described the disposition kinetics of paclitaxel and gemcitabine in the present study The fitting of dFdU levels vs time curve was performed by a bi-exponential decay equation, assuming that the conversion of gemcitabine to dFdU is a first-order process The following time-concentration functions were used to describe the post-infusion profiles of paclitaxel, gemcitabine and dFdU

$$\text{Paclitaxel/gemcitabine } C_t = \sum_{i=1}^{N}\left\{C_i/(L_i \times T_{inf}) \times \left[e^{L_i(t-T_{inf})} - e^{-L_i t}\right]\right\}$$

$$\text{dFdU } C_t = \sum_{i=1}^{N}(C_i \times e^{-L_i t}) - \sum_{i=1}^{N}(C_i \times e^{-k_m t})$$

where C_t is the plasma level measured at time t, N is the number of compartments, C_i and L_i are, respectively, the x^1 coefficient and exponent of polyexponential functions, T_{inf} is the infusion time of paclitaxel and gemcitabine and k_m is the rate of dFdU input into the central compartment. Curve-fitting yielded the parameters C_i, L_i, k_m and the intercompartmental rate constants k_{xy}. Peak plasma concentration (C_{max}, μmol/l or μg/ml) and time to reach C_{max} (T_{max}, h) were graphically determined based on the plasma concentration-time data. Half lives ($t_{1/2}$, h) were calculated as $0.693/L_i$, where L_i (1/h) is the negative slope of the log-linear α (distribution) and β (elimination) phases of the plasma concentration-time profiles. The area under the plasma concentration-time curve (zero moment curve, AUC, h μmol/l, h μg/ml) was calculated on the experimental values (trapezoidal rule) with extrapolation to infinity, obtained by the terminal elimination rate constant [15]. Mean residence time (MRT, h) for paclitaxel and gemcitabine was determined by dividing the area under the first-moment curve (AUMC, h^2 μmol/l, h^2 μg/ml) by AUC, with correction for infusion time [15]. Apparent, total-body clearance was normalized to the body-surface area and calculated as CL_{TB} = dose/AUC (l/h/m^2), while the apparent volume of distribution was obtained as $V_d = CL_{TB}$/terminal L_i and expressed as l/m^2. The apparent volume of distribution at steady state (V_{ss}, l/m^2) was computed as $V_{ss} = Vl \times [1 + k_{12}/k_{21}]$, where k_{xy} are the intercompartmental rate constants and Vl is the volume of distribution of the central compartment [15]. Finally, the time interval of paclitaxel levels ≥0.05 μmol/l ($tC_{0.05}$) was measured on individually-fitted plasma concentration-time plots [16].

Pharmacodynamic analysis

The relationship between drug exposure and hematologic toxicity, and the principal and dose-limiting effect of paclitaxel and gemcitabine [5, 6] was evaluated. The percentage of decrease in hematologic count (neutrophils, leukocytes and platelets) was calculated as follows:

$$\%\text{Decrease in hematologic count} = 100 \times \frac{\text{pretreatment count-nadir count}}{\text{pretreatment count}}$$

and plotted as a function of plasma AUC, C_{max} and $tC_{0.05}$ of paclitaxel, a threshold concentration associated with hematologic toxicity [16], or gemcitabine C_{max} and AUC. Relationships were fitted according to sigmoid maximum effect (E_{max}) model [15] using non-linear least-squares regression and a weighting factor of unity, defined as follows:

$$\%\text{Change in hematologic count} = E_{max} \times \frac{PK^{\kappa}}{PK^{\kappa} + PK_{50}^{\kappa}}$$

where κ is the shape-factor that describes the sigmoidicity of the concentration-effect curve and PK_{50} is the value of the pharmacokinetic parameter (PK) that results in 50% of the E_{max}. The performance of the pharmacodynamic model was evaluated using the relative-root, mean-square error (%RMSE) value and its standard error (%SE), defined as follows:

$$\%\text{RMSE} = \left[N^{-1} \times \sum_{i=1}^{N}(pe_i)^2\right]^{1/2} \times 100$$

$$\%\text{SE} = \left[N \times (N-1)^{-1} \times \sum_{i=1}^{N}(pe_i)^2\right]^{1/2} \times 100$$

where N is the number of P pairs (i.e., true with predicted values), and the prediction error is $pe = [\ln(P_{\text{predicted value}}) - \ln(P_{\text{true value}})]$, in the best model the %RMSE approaches zero.

Statistical analysis

Data are presented as mean ± standard deviation (S.D.). Statistical analysis was performed by ANOVA followed by Student–Newman–Keuls test [17], the level of significance was $P < 0.05$.

Results

Pharmacokinetics of paclitaxel, gemcitabine and dFdU

The plasma concentration-time profiles of paclitaxel 100 mg/m^2 followed by gemcitabine are represented in Figure 1, and the major pharmacokinetic parameters are reported in Table 1. Plasma levels of paclitaxel rapidly increased during administration, reaching a C_{max} of 9.10 ± 2.27, 8.96 ± 1.28 and 8.94 ± 4.09 μmol/l at the end of infusion, in combination with gemcitabine 1500, 1750 and 2000 mg/m^2, respectively. Paclitaxel concentrations then decreased in the postinfusion period by a bi-exponential decay with a $t_{½}β$ ranging from 2.77 ± 1.47 h to

Table 1 Pharmacokinetic parameters of paclitaxel in combination with gemcitabine

	Paclitaxel 100 mg/m^2 +		
	Gemcitabine 1500 mg/m^2 (n = 5)	Gemcitabine 1750 mg/m^2 (n = 5)	Gemcitabine 2000 mg/m^2 (n = 5)
C_{max} (μmol/l)	9.10 ± 2.27	8.96 ± 1.28	8.94 ± 4.09
AUC (h μmol/l)	13.86 ± 4.07	14.45 ± 2.03	12.73 ± 4.22
$t_{½}α$ (h)	0.24 ± 0.05	0.19 ± 0.07	0.23 ± 0.11
$t_{½}β$ (h)	3.23 ± 1.71	2.77 ± 1.47	4.17 ± 2.88
CL_{TB} (l/h/m^2)	7.61 ± 1.81	8.25 ± 2.97	8.57 ± 2.62
V_{ss} (l/m^2)	13.15 ± 6.39	14.02 ± 8.53	14.63 ± 10.24
V_d (l/m^2)	34.46 ± 19.81	34.19 ± 23.11	43.89 ± 21.03
MRT (h)	1.79 ± 0.86	1.66 ± 0.82	1.70 ± 0.89

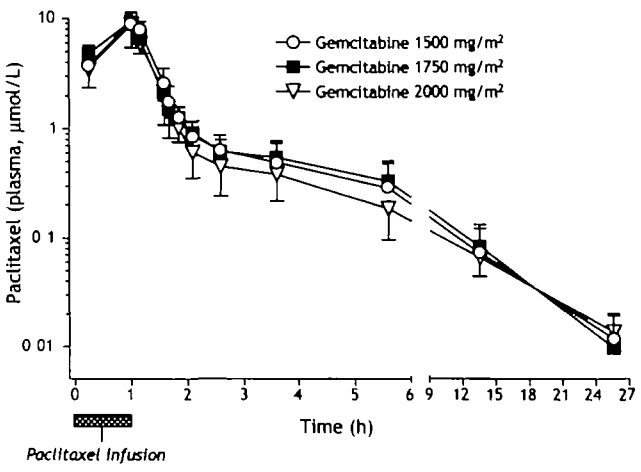

Figure 1 Plasma concentration vs time profiles of paclitaxel 100 mg/m^2 by one-hour i.v. infusion in patients given gemcitabine 1500, 1750 and 2000 mg/m^2 by 30-min i.v. infusion. Points, mean values (n = 5 patients), bars, SD of the mean.

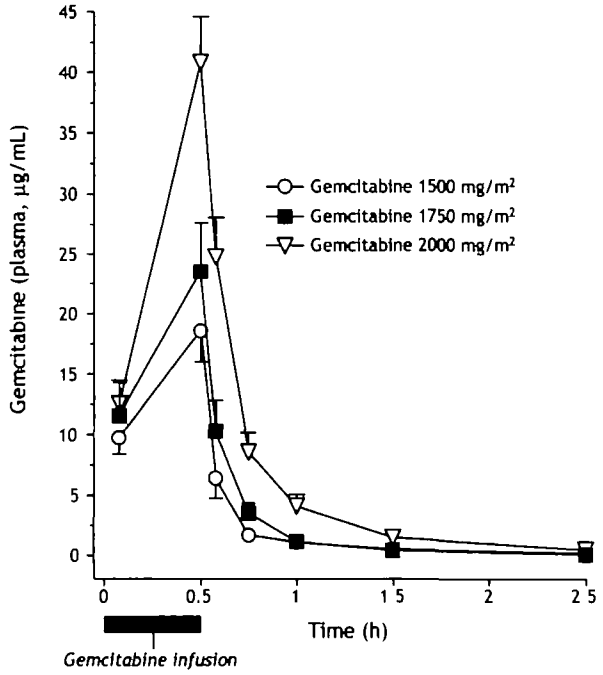

Figure 2 Plasma concentration vs time profiles of gemcitabine 1500, 1750 and 2000 mg/m^2 by 30-min i v infusion in patients given paclitaxel 100 mg/m^2 by one-hour i v infusion Points, mean values (n = 5 patients), bars, SD of the mean

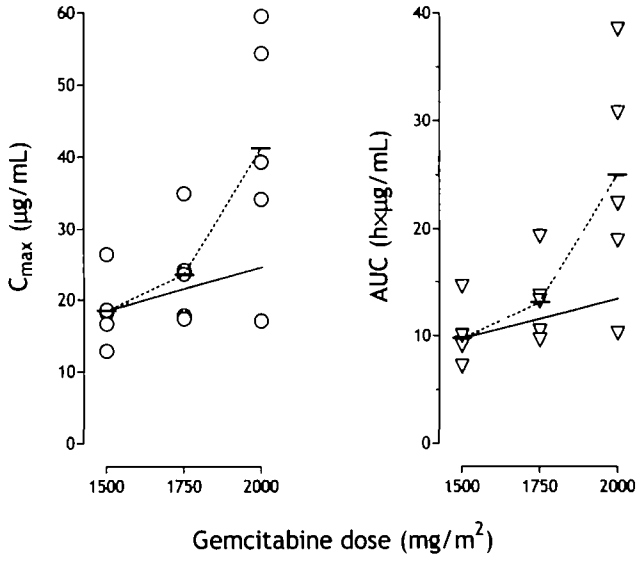

Figure 3 Scatter plots of individual values of C_{max} (left) and AUC (right) of gemcitabine vs dosage level in 15 subjects The dotted-line links the observed mean values (horizontal bars) of C_{max} and AUC for each group of patients, while the solid line intersects the theoretical mean values if C_{max} and AUC proportionally increased with the drug dosage

Table 2 Pharmacokinetic parameters of gemcitabine in combination with paclitaxel

	Paclitaxel 100 mg/m^2 +		
	Gemcitabine 1500 mg/m^2 (n = 5)	Gemcitabine 1750 mg/m^2 (n = 5)	Gemcitabine 2000 mg/m^2 (n = 5)
C_{max} (µg/ml)	18 56 ± 4 94	23 56 ± 7 06	40 85 ± 14 85[a]
AUC (h µg/ml)	9 99 ± 2 75	13 21 ± 3 80	25 01 ± 9 87[a]
$t_{1/2}\alpha$ (h)	0 05 ± 0 01	0 09 ± 0 01	0 11 ± 0 02
$t_{1/2}\beta$ (h)	0 52 ± 0 10	0 34 ± 0 08	0 64 ± 0 25
CL_{TB} (l/h/m^2)	160 4 ± 22 0	142 6 ± 20 66	92 54 ± 38 93[a]
V_{ss} (l/m^2)	36 16 ± 9 23	31 24 ± 8 32	25 26 ± 5 16
V_d (l/m^2)	127 7 ± 34 7	72 65 ± 21 53	75 90 ± 29 30
MRT (h)	0 21 ± 0 03	0 21 ± 0 03	0 33 ± 0 13

[a] $P < 0.05$, ANOVA followed by the Student–Newman–Keuls test

4.17 ± 2 88 h The disappearance profiles of the drug after the infusion of gemcitabine at three dosage levels, were almost identical to overlapping profiles, suggesting that the dosage-escalation of gemcitabine does not significantly affect the pharmacokinetics of paclitaxel, as also shown by the CL_{TB} values ranging from 7.61 ± 1.81 l/h/m^2 to 8 57 ± 2.62 l/h/m^2 (Table 1).

The administration of gemcitabine 1500, 1750 and 2000 mg/m^2 was associated with a nonproportional increment in drug levels (Figure 2) and pharmacokinetic parameters (Table 2) In particular, gemcitabine C_{max} increased from 18.56 ± 4.94 to 23 56 ± 7.06 and 40.85 ± 14.85 µg/ml at 1500, 1750 and 2000 mg/m^2, respectively, while the AUC displayed an increase from 9.99 ± 2.75 to 13.21 ± 3.80 and 25.01 ± 9.87 h µg/ml (Table 2) Thus, a dosage increment of 17% (1750 mg/m^2) and 33% (2000 mg/m^2) as compared to 1500 mg/m^2, resulted in a nonproportional change in C_{max} of 27% and 120%, and in AUC of 32% and 150% at 1750 and 2000 mg/m^2, respectively, as compared to the pharmacokinetic parameters calculated at 1500 mg/m^2, suggesting a saturation kinetics of gemcitabine above 1750 mg/m^2. Likewise, drug CL_{TB} decreased from 160.4 ± 22.0 to 142.6 ± 20.66 (−11%) and 92.54 ± 38.93 (−42.5%) at gemcitabine 1500, 1750 and 2000 mg/m^2, while no changes would have been expected if the pharmacokinetics were dose-proportional. Statistical analysis indicated a significant difference when comparing the C_{max}, AUC and CL_{TB} of gemcitabine at 2000 vs 1500 mg/m^2 (Table 2).

In order to assess whether the pharmacokinetics of gemcitabine could be dependent on paclitaxel, the findings of this work were compared with a phase I study in which gemcitabine was administered as a single agent to patients with advanced cancer [18] The C_{max} and AUC of gemcitabine 1500 mg/m^2 in combination with paclitaxel 100 mg/m^2 displayed a dose-proportional increase as compared to historical data of gemcitabine up to 1000 mg/m^2, suggesting that paclitaxel did not affect the linear relationship between the dose and disposition of the nucleoside analogue. However, at gemcitabine 1750 and 2000 mg/m^2, C_{max} and AUC showed a sharp increase (Figure 3), indicating the loss of pharmacokinetic linearity with nonproportional change in drug distribution parameters, unlikely to be dependent on paclitaxel but rather on saturation of gemcitabine metabolism and/or elimination.

Peak concentrations and plasma exposure to the

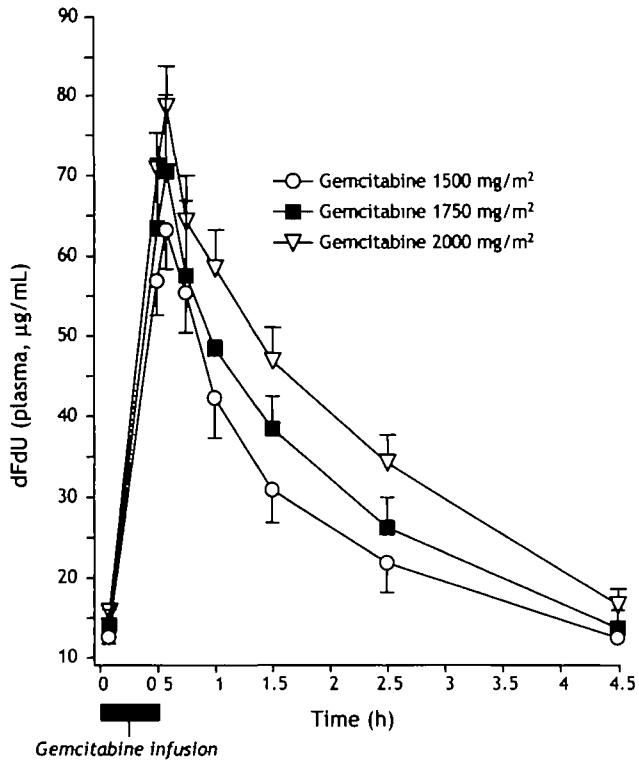

Figure 4 Plasma concentration vs time profiles of dFdU in patients given gemcitabine 1500, 1750 and 2000 mg/m^2 by 30-min i v infusion and paclitaxel 100 mg/m^2 by one-hour i v infusion Points, mean values (n = 5 patients), bars, SD of the mean

Table 3 Pharmacokinetic parameters of dFdU after administration of gemcitabine in combination with paclitaxel

	Paclitaxel 100 mg/m^2 +		
	Gemcitabine 1500 mg/m^2 (n = 5)	Gemcitabine 1750 mg/m^2 (n = 5)	Gemcitabine 2000 mg/m^2 (n = 5)
C_{max} (μg/ml)	63 2 ± 9 7	70 6 ± 19 0	79 73 ± 15 32
T_{max} (h)	0 6 ± 0 1	0 59 ± 0 02	0 50 ± 0 03
AUC (h μg/ml)	159 9 ± 46 2	182 0 ± 49 0	224 9 ± 61 5
$t_{½}\alpha$ (h)	0 22 ± 0 08	0 17 ± 0 03	0 17 ± 0 06
$t_{½}\beta$ (h)	2 1 ± 1 0	2 6 ± 0 3	2 19 ± 0 43

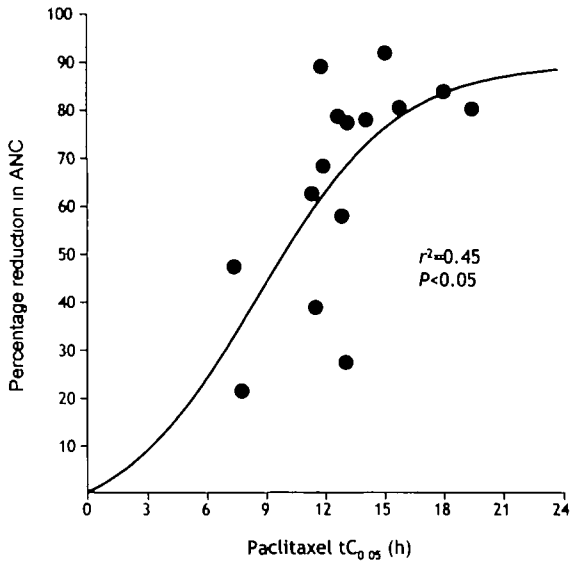

Figure 5 Relationship between percentage of reduction inANC and $tC_{0.05}$ of paclitaxel Data were modeled according to the E_{max} sigmoid (variable slope) fitting Points, individual data from 15 patients

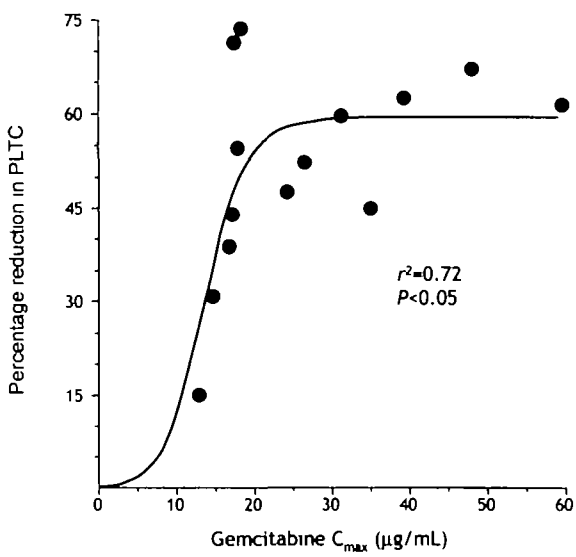

Figure 6 Relationship between percentage of reduction in PLTC and C_{max} of gemcitabine Data were modeled according to the E_{max} sigmoid (variable slope) fitting Points, individual data from 15 patients

inactive metabolite dFdU, resulting from deamination of the parent drug, differed from that of gemcitabine, since the metabolite distribution was characterized by dose-proportional changes, with C_{max} values of 63.2 ± 9.7 μg/ml, 70.6 ± 19 0 μg/ml (+11 7%) and 79 73 ± 15 32 μg/ml (+26 1%), and AUC values of 159 9 ± 46.2 h μg/ml, 182 0 ± 49 0 h μg/ml (+13.8%) and 224.9 ± 61.5 h·μg/ml (+40 6%) at gemcitabine 1500, 1750 and 2000 mg/m^2, respectively, suggesting that the non-linearity of gemcitabine disposition was largely dependent on saturation of metabolism rather than elimination. Other pharmacokinetic parameters, including distribution (α) and elimination (β) $t_{1/2}$ as well as T_{max}, did not change significantly (Figure 4 and Table 3)

Pharmacokinetic/pharmacodynamic relationship of paclitaxel and gemcitabine

The analysis of the pharmacokinetic/pharmacodynamic profile of paclitaxel showed a significant correlation (r^2 = 0.45, P < 0.05) between $tC_{0.05}$ of paclitaxel and the percentage of decrease in ANC (Figure 5). The $tC_{0.05}$ associated with 50% reduction in neutrophil count (ET_{50}), with respect to the fitted E_{max}, was 10.4 h. The plot represented in Figure 6 shows the significant relationship (r^2 = 0.72, P < 0.05) between the percentage of decrease in PLTC and the C_{max} of gemcitabine, as described by the sigmoid E_{max} pharmacodynamic model. On the basis of data modeling, the C_{max} of gemcitabine was predicted to

yield a 50% reduction in PLTC (EC_{50}), with respect to the fitted E_{max}, which was 14.3 μg/ml. In this patient population, only 2 out of 15 patients had a percentage of decrease in PLTC higher than 70% (Figure 6)

Discussion

The identification of new drug combinations for the management of NSCLC represents an important objective of current research Until recently, the role of chemotherapy for NSCLC has generally been questioned, due to marginal activity, substantial toxicity and high cost. There has, however, been increasing evidence that chemotherapy in advanced NSCLC is able to increase survival and improve QoL [1, 19]. In the past few years, a number of active drugs with established activity and favourable toxicity profiles have been introduced in the treatment of advanced disease [1, 5, 6]. In particular, the administration of the doublet paclitaxel and gemcitabine, or the triple drug combination paclitaxel, gemcitabine and cisplatin, which has been proven to be safe and effective in untreated patients with NSCLC [10, 11, 20, 21] These agents appear to hold the promise of added therapeutic benefit, provided that the development of drug combinations is supported by pharmacokinetic and pharmacodynamic analysis, in order to identify possible drug interactions that may affect the efficacy and toxicity profile of treatments The treatment schedule adopted in the present work consisted of paclitaxel followed by gemcitabine on the basis of *in vitro* data, showing that paclitaxel increased the accumulation of active metabolites of gemcitabine and their incorporation into nucleic acids as well as the apoptotic index [22] The enhanced biotransformation of gemcitabine into active compounds induced by paclitaxel was further confirmed in human mononuclear cells from peripheral blood [23], and an *in vivo* study demonstrated that the best antitumor activity was obtained with paclitaxel before gemcitabine [24]. In our work, the clinical pharmacokinetics and relationship between parameters of drug exposure and hematological toxicity of paclitaxel and gemcitabine have been examined The findings of this study demonstrated the lack of reciprocal pharmacokinetic interaction between drugs, the nonproportional pharmacokinetics of gemcitabine and the E_{max} relationship of paclitaxel and gemcitabine with neutrophil and platelet counts, respectively The administration of gemcitabine 1500 mg/m^2 was associated with a predictable kinetic behaviour and modest thrombocytopenia, and thus it is the recommended dosage in combination with paclitaxel 100 mg/m^2 for future, phase II studies.

Looking at the primary results individually, the comparison on pharmacokinetic data of paclitaxel administered as a single agent [25, 26] with those of the present study, demonstrated that the drug distribution parameters were unaffected by gemcitabine 1500–2000 mg/m^2. On the contrary, the pharmacodynamic analysis of paclitaxel showed that the severity of neutropenia, as predicted by the time interval of plasma paclitaxel concentrations ≥ 0.05 μmol/l, was enhanced by gemcitabine, since the ET_{50} obtained in the present study by fitting the $tC_{0.05}$ vs. percentage of decrease in ANC was 10.4 h, a value lower than that obtained with paclitaxel alone (ET_{50} = 17.4 h, 16). The shift to the left of the sigmoid curve indicated that patients who received paclitaxel in combination with gemcitabine experienced more neutropenia than would be expected with paclitaxel alone The predictability of the pharmacodynamic/pharmacokinetic model was confirmed by the finding that grade 3–4 neutropenia was seen in 26% of patients given paclitaxel 100 mg/m^2 in combination with gemcitabine 1500, 1750 and 2000 mg/m^2 [10, 11], while 14% of patients that administered paclitaxel alone at 100 mg/m^2 as a one-hour infusion experienced this toxicity [25].

Gemcitabine displayed a nonlinear pharmacokinetics within the 1500–2000 mg/m^2 dosage range, and a remarkable inter-individual variability in drug distribution parameters was observed among patients given the highest dosage, while the inactive metabolite dFdU showed a dose-proportional disposition. The disproportional changes in gemcitabine pharmacokinetics may be due to saturable metabolic clearance via cytidine deaminase, while the presence of poor and fast metabolizers may explain the high variability in C_{max}, AUC and CL_{TB} among patients at the higher dosage level of gemcitabine. If compared with previously published data, the findings of the present work indicate that paclitaxel did not affect the linear relationship between the gemcitabine dosage and pharmacokinetics, which is in agreement with data obtained from a limited-sampling approach in 18 patients given gemcitabine 1000 mg/m^2 in combination with paclitaxel 150 and 200 mg/m^2 [23], and in patients given gemcitabine as a single agent up to 1000 mg/m^2 [18].

The analysis of the relationship between the decrease in platelet count and C_{max} of gemcitabine showed that the fitted E_{max} of the sigmoid curve corresponded to a 58 8% reduction in PLTC, with respect to baseline. Consistent with this, mild thrombocytopenia of grade 2 was observed in only one patient treated with paclitaxel 100 mg/m^2 and gemcitabine 1500 mg/m^2, while no such toxicity was observed at the higher doses of 1750 and 2000 mg/m^2 [10, 11]. On the contrary, thrombocytopenia was the DLT observed in a phase I study, with 50% of patients experiencing nadir platelet values of less than 50,000 cells/mm^3 with gemcitabine 1000 mg/m^2/week [18] Since clinically relevant PLTC reduction was not observed in patients treated with single agent paclitaxel 100 mg/m^2 by one-hour infusion [25], the data of the present study suggest a pharmacodynamic interaction between paclitaxel and gemcitabine A similar finding has been reported on the combination of paclitaxel and carboplatin, with the observation that more carboplatin is required to produce the same degree of thrombocytopenia as compared to carboplatin alone [27]. In agreement with the present data, this effect is limited to platelets, while neutrophils are not spared [27], suggest-

ing that the protective effect on platelets appears to be a common mechanism of paclitaxel. The basis of this platelet-sparing effect lies in a pharmacodynamic interaction, although the precise mechanism remains to be determined. The ability of megakaryocytes to become polyploid by allowing many rounds of DNA replication without completion of intervening mitoses, at variance with most eukaryotic cells in which the progression to the next cell-cycle phase is prevented unless the preceding phase has been completed [28], might explain, at least partly, the relative resistance of megakaryocytopoiesis to the inhibitory effect of mitotic poisons.

Finally, the findings of the present study suggest that the administration of gemcitabine at dosage levels higher than 1500 mg/m^2 in combination with paclitaxel 100 mg/m^2, may not be associated with substantial therapeutic benefit, since (i) a higher number of patients given 1750 and 2000 mg/m^2 experienced clinically significant, cumulative toxicities, as compared to 1500 mg/m^2, (ii) a dose-dependent tumor response was also lacking [11], and (iii) a high pharmacokinetic variability was observed in this scenario.

In conclusion, the administration of gemcitabine and paclitaxel does not result in pharmacokinetic interaction, while a less than expected thrombocytopenia is observed. Moreover, gemcitabine pharmacokinetics is predictable and dose-proportional at 1500 mg/m^2 and thus, it is the recommended dosage for future phase II studies with paclitaxel 100 mg/m^2.

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