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# Pharmacology of the paclitaxel–cisplatin, gemcitabine–cisplatin, and paclitaxel–gemcitabine combinations in patients with advanced non-small cell lung cancer

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**Abstract** *Purpose*: To compare the pharmacology of the paclitaxel–cisplatin, gemcitabine–cisplatin and paclitaxel–gemcitabine combinations in patients with advanced non-small cell lung cancer (NSCLC). *Patients and methods*: Twenty-four chemo-naive patients with advanced NSCLC were randomized to receive one of the three regimens. Plasma pharmacokinetics and pharmacologic parameters in mononuclear cells were compared and related to toxicity and efficacy. *Results*: Pharmacological parameters of gemcitabine and cisplatin were not influenced by the combination with one of the other agents, while the paclitaxel clearance was significantly lower for the combination with cisplatin as compared to gemcitabine ($P = 0.024$). The percentage decrease in platelets was significantly higher for the gemcitabine combinations ($P = 0.004$) and related to the dFdCTP-$C_{max}$ ($P = 0.030$). Pharmacologic parameters were not related to response or survival. *Conclusions*: Gemcitabine and cisplatin pharmacology were not influenced by the combination with one of the other agents, while paclitaxel has a lower clearance in combination with cisplatin as compared to gemcitabine.

**Keywords** Paclitaxel · Cisplatin · Gemcitabine · Non-small cell lung cancer

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## Introduction

The cisplatin–paclitaxel, cisplatin–gemcitabine and paclitaxel–gemcitabine combinations are active regimens in advanced NSCLC [1, 2]. A phase III trial conducted by the European organization for research and treatment of cancer (EORTC) Lung Cancer Study Group comparing these regimens showed no significant differences in response rate and survival compared to cisplatin–paclitaxel [2]. There was a trend toward lower progression free survival and response duration for the nonplatinum arm. The only toxicity that differed significantly among the three treatment arms was myelosuppression, being more common for the cisplatin–gemcitabine combination as compared to the cisplatin–paclitaxel combination. We investigated possible drug–drug interactions and their potential effects on toxicity and efficacy for the three regimens.

Gemcitabine (2′,2′-difluoro-2′-deoxycytidine; dFdC) a deoxycytidine analogue is attractive for combination chemotherapy, due to its mechanism of action [3] and mild toxicity profile [4]. Gemcitabine acts by incorporation of its active triphosphate (2′,2′-difluoro-2′-deoxycytidine triphosphate; dFdCTP) into DNA. In a panel of 21-tumor cell lines intracellular dFdCTP accumulation was correlated to gemcitabine's sensitivity [5]. Several self-potentiating mechanisms have been described, including inhibition of ribonucleotide reductase, dCMP-deaminase and CTP synthetase [3, 6] enhancing the incorporation of dFdCTP into nucleic acid and disturbance of (deoxy) ribonucleotide pools. In addition, gemcitabine induces a G1/S phase arrest and triggers apoptosis [7, 8]. Gemcitabine is deaminated to its inactive metabolite 2′,2′-difluoro-2′-deoxyuridine (dFdU).

Paclitaxel promotes microtubule assembly and stabilization by preventing depolymerization, resulting in G2/M arrest, inhibition of cell proliferation and cell death [9].

Cisplatin is the basis for most of the effective combination chemotherapy regimens in NSCLC [1, 10]. Cisplatin acts by formation of platinum DNA adducts (Pt-DNA) [11]. A relation between the exposure to unbound cisplatin, Pt-DNA-adduct formation in white blood cells (WBCs) and tumor response in patients has been demonstrated [12].

Preclinical and clinical drug–drug interactions for the gemcitabine, paclitaxel and cisplatin doublets have been studied quite extensively. The paclitaxel–cisplatin combination has demonstrated a marked schedule dependent synergistic interaction both in vitro and in patients, showing superior antitumor effect and less toxicity when paclitaxel was administered first [13, 14]. Various gemcitabine–paclitaxel combinations did not show sequence dependent cytotoxic effects in NSCLC cells; all combinations were not more than additive [8]. However, the exposure of paclitaxel prior to gemcitabine seemed to be favorable since paclitaxel enhanced gemcitabine metabolism and apoptotic index. In addition, in patients, paclitaxel increased dFdCTP accumulation in mononuclear cells of NSCLC patients [15]. The gemcitabine–cisplatin combination clearly showed schedule dependent additive and synergistic effects in vitro and in vivo [16, 17]. Preclinical studies indicated an advantage of the gemcitabine prior to cisplatin schedule, while in a pharmacological study the schedule with cisplatin prior to gemcitabine produced the best pharmacological profile with an increased dFdCTP accumulation [18], but resulted in more severe leucopenia [19]. Although for all three combinations clear schedule dependencies at the cellular level have been observed, the underlying interactions at the pharmacological level are less clear.

Pharmacokinetic monitoring may help to identify possible drug–drug interactions, in order to optimize drug regimens, and to explain differences in toxicity and/or efficacy between these promising combinations presently used in treatment of patients with NSCLC. For this purpose plasma pharmacokinetics and dFdCTP in mononuclear cells were compared and related to toxicity and efficacy.

## Patients and methods

### Patients and study design

#### *Patient selection*

Eligible patients were entered in the EORTC study 08975 [2] and consecutively asked to participate in this study; patients had cytologic or histologic diagnosis of NSCLC stage IIIb (due to malignant pleural effusion or supraclavicular lymph node involvement) or stage IV, without prior chemotherapy; aged between 18 and 76 years; measurable or evaluable disease; World Health Organization (WHO) performance status $\leq 2$ according to WHO scale; no symptoms of brain metastasis; adequate bone marrow function (white blood cell count [WBC] $\geq 4\times10^9$/L, absolute neutrophil count [ANC] $\geq 2\times10^9$/L and platelet count $\geq 100\times10^9$/L); adequate renal function (creatinine clearance ≥60 ml/min); serum bilirubin level $\leq 1.25\times$ the upper normal limit; adequate cardiac function; and written informed consent both for randomization into one of the treatment arms and for performing the pharmacokinetic study. Results of the clinical part of this EORTC trial 08975 have been reported separately [2].

#### *Study design*

Patients were randomized to receive paclitaxel at 175 mg/m$^2$ administered as a 3 h infusion followed by cisplatin at 80 mg/m$^2$ in 1 h on day 1 ($n=8$), or gemcitabine at 1,250 mg/m$^2$ as a 30 min infusion on days 1 and 8 followed by cisplatin at 80 mg/m$^2$ in 1 h on day 1 ($n=8$), or paclitaxel at 175 mg/m$^2$ administered in 3 h on day 1 followed by gemcitabine at 1,250 mg/m$^2$ as a 30 min infusion, on days 1 and 8 ($n=8$). Treatment cycles were repeated every 3 weeks. The plasma pharmacokinetics of gemcitabine, paclitaxel and cisplatin on day 1 were compared between the regimens and with the plasma pharmacokinetics of gemcitabine on day 8.

#### *Drugs*

Gemcitabine (Gemzar®; 2′,2′-difluoro-2′-deoxycytidine; Eli Lilly & Co, Indianapolis, IN) was supplied as a lyophilized powder in sterile vials containing 200 or 1,000 mg of gemcitabine as hydrochloride salt, mannitol, and sodium acetate. Gemcitabine was administered in 500 ml 0.9% sodium chloride, as a 30 min i.v. infusion.

Paclitaxel (Taxol®) was provided by Bristol–Myers Squibb (Waterloo, Belgium). It was dissolved in 500 ml 0.9% sodium chloride and infused over 3 h by the use of a constant volume infusion pump. Premedication consisted of 20 mg dexamethasone orally 12 and 6 h before paclitaxel infusion, diphenhydramine 50 mg IV, and cimetidine 300 mg i.v. 30 min prior to paclitaxel.

Cisplatin (cis-diamminedichloroplatinum(II)) was diluted in 500 ml hypertonic saline (2.9%) and administered as a 1 h i.v. infusion. Before cisplatin, patients received i.v. hydration with 1,000 ml normal saline plus 20 mmol potassium chloride and 2 g magnesium sulphate over 2 h. After cisplatin infusion 4,000 ml normal saline plus 80 mmol potassium chloride and 8 g magnesiumsulphate were given over 24 h. Prophylactic antiemetics, 8 mg ondansetron and 8 mg dexamethason, were administered twice on the day of cisplatin infusion.

### Pharmacokinetics

#### *Blood sampling*

Blood samples (9 ml) for analysis were collected at days 1 and 8 (in case of gemcitabine administration) during

the first cycle of therapy. Based on previous studies a limited sampling model was used [15, 18]. At day 1 patients were hospitalized and blood samples were taken as follows: (paclitaxel–cisplatin regimen) pretreatment, at the end of paclitaxel administration and 1, 4 and 18 h after the start of cisplatin infusion; (gemcitabine–cisplatin regimen) pretreatment, at the end of gemcitabine administration, and 1, 4 and 18 h after the start of cisplatin infusion and (paclitaxel–gemcitabine regimen) pretreatment, at the end of paclitaxel infusion and at 30 min, 2, 4 and 18 h after the start of gemcitabine infusion. On day 8 samples were drawn just before gemcitabine infusion; 30 min and 2 h post-gemcitabine infusion. Samples for gemcitabine analysis were drawn in heparinized tubes containing 0.25 mg tetrahydrouridine to prevent deamination of gemcitabine and the tubes were immediately placed on ice. Plasma was obtained by centrifugation of the samples (4,000 rpm for 5 min at 4°C) and stored at −20°C until analysis. The buffy-coat at the interface between plasma and erythrocytes was used for isolation of mononuclear blood cells, using a Ficoll-Hypaque density gradient (Pharmacia, Sweden) as described previously [20]. After purification the cell pellet was immediately frozen in liquid nitrogen and subsequently stored at −80°C until analysis.

*Gemcitabine and dFdU analysis*

Gemcitabine and dFdU were analyzed as described previously [15]. Briefly, 150 $\mu$L of plasma were extracted as described and stored at −20°C until analysis. Separation and quantification of gemcitabine and dFdU from the plasma was achieved with an isocratic reversed-phase high-performance liquid chromatography (HPLC) system using a $\mu$Bondapak C18 column (length 300 mm, internal diameter 3.9 mm and particle size 10 μm). Peak areas were quantified using the data acquisition program Chromeleon version 3.02 (Chromeleon Chromatography Data Systems, Gynkotek HPLC, Germering, Germany). Retention times of gemcitabine and dFdU were 7.1 and 13.5 min, respectively. The limit of quantification was about 25 pmol/50 μl (0.5 μM) for both gemcitabine and dFdU , with an inter-assay variation of $<8.5\%$ and $<6.2\%$, respectively.

*dFdCTP analysis*

Cellular nucleotides were extracted and analyzed by HPLC as reported previously [15]. Briefly, after extraction, separation and quantification of both the normal ribonucleotides and of dFdCTP was achieved with a gradient HPLC (Partisphere SAX anion exchange column; length: 110 mm, internal diameter: 4.7 mm, particle size: 5 μm) connected to photo-diode array detector set at 254 and 280 nm. Peak areas were calculated using the data acquisition program Chromeleon version 3.02. The detection limit for dFdCTP was 50 pmol per injection (175 μl) and the quantification limit 75 pmol, with an inter-assay variation index of $<8\%$. The cellular concentrations of dFdCTP and ribonucleotides were calculated as pmol/$10^6$ mononuclear cells.

*Paclitaxel analysis*

Paclitaxel was analyzed in plasma using a HPLC assay with solid phase extraction as the sample pretreatment procedure, as previously described [21]. Briefly, an APEX octyl analytical HPLC column (4.6 × 150 mm; particle size 5 μm) was used. Solid phase extraction was performed with Bond Elut Cyano Columns and UV detection was performed at 227 nm. Paclitaxel concentrations as low as 12 nM could be detected.

*Total plasma Pt analysis*

Plasma samples were diluted ten times with 0.38 M NaCl/0.5 M HCL and 0.2% triton + 0.2% antifoam before measurement of the Pt concentration, as described previously [22]. Total Pt concentration was analyzed by flameless atomic absorption spectrophotometry using a spectra AA−300 Zeeman AAS (Varian, Houten, The Netherlands). Standards of blank plasma spiked with cisplatin were treated in the same way as the samples.

Pharmacokinetic and pharmacodynamic analysis

The area under the plasma concentration versus time curve from $t=0$ (start of the infusion) to infinity was calculated using the noncompartmental linear trapezoidal analysis according to the WinNonlin computer program (version 1.5, Scientific Consulting, Inc). The half-life of the terminal log-linear phase ($t_{1/2\gamma}$) was calculated as $0.693/\lambda_z$, where $\lambda_z$ is the terminal elimination rate constant, the absolute value of the slope of the terminal log-linear phase. Total-body clearance (CL) and volume of distribution ($V_d$), were also calculated by the computer program as dose/AUC and $Cl/\lambda_z$, respectively. Peak plasma concentrations ($C_{max}$) of dFdC, dFdU, dFdCTP and paclitaxel are the mean of measured values. For dFdC no full pharmacokinetic sampling could be performed in this setting because of the large number of blood samples, which would be required; therefore the only evaluable parameter for dFdC was the $C_{max}$. The limited paclitaxel sampling based on our gemcitabine sampling model has proven to be useful before [15]. Shortly, from a historical data set of 25 concentration versus time curves, several time points were selected and the AUCs of the full data set were compared with those of several selected data points [15]. An excellent agreement was observed and therefore these points were chosen for the present evaluation. For paclitaxel the time above the threshold concentration of

0.1 μM (T≥0.1 μM), was derived graphically from the pharmacokinetic curves of each patient, as described before [21, 23]. Differences between data were evaluated using the Student's *t* , Wilcoxon signed ranks and Mann–Whitney *U* tests.

### Toxicity analysis and statistics

Toxicity was evaluated according to the NCI Common Toxicity Criteria and as percentage decrease in granulocytes, WBC, or platelets using the following equation: percentage decrease = (pretreatment value - nadir)/pretreatment value × 100%. Hematological toxicity was evaluated by weekly blood cell counts with differentials. Before each cycle, serum chemistry was repeated. Response was assessed every two cycles. All patients were evaluated for toxicity during the first cycle, details on toxicity of all cycles and response evaluation have been reported separately [2]. To investigate the determinants of inter-individual kinetic variability, patient characteristics were related with the pharmacokinetic parameters of gemcitabine, paclitaxel and cisplatin by step-wise multiple linear regression. The following patient characteristics were studied as independent variables: performance status, age, histology, plasma creatinine, and liver enzymes. Paclitaxel T≥0.1 μM was related to the percentage decrease in neutrophils, WBC and platelets. Furthermore, we tested the effects of the pharmacokinetic parameters of one agent on the parameters of the other agent used in the doublet, and vice versa, for all three doublets. The pharmacokinetic parameters of one agent used in different doublets were compared using the Mann–Whitney *U* and Student's *t* tests. A significant difference was indicated by a *P* value less than 0.05. In addition the slope of the regression line and its 95% confidence interval (95% CI) were evaluated. The computer program SPSS (version 9.0, SPSS, Inc, Chicago, IL) was used for the statistical analysis.

## Results

### Patient characteristics

Twenty-four patients were entered into the pharmacokinetic part of the phase III study between March 1999 and September 2000. Patient characteristics are outlined in Table 1. For two patients no blood samples were obtained at day 8, due to development of pneumonia and general malaise, respectively.

### Plasma pharmacokinetics

For gemcitabine pharmacokinetics (gemcitabine-$C_{max}$ and dFdU-AUC) and pharmacodynamics (dFdCTP-AUC) no significant difference was found between the combination with cisplatin and with paclitaxel or between days 1 and 8 of each combination (Table 2). Because of the limited sampling model we only give $C_{max}$ values for gemcitabine. The mean peak level of gemcitabine (gemcitabine-$C_{max}$) was measured at 30 min and fell below quantifiable levels within 2 h (Fig. 1). In contrast, the deamination product, dFdU, had a terminal elimination phase with a mean terminal half-life ($t_{1/2\gamma}$) of 8.6 h. Gemcitabine-$C_{max}$ and dFdU-AUC were not influenced by previous paclitaxel or successive cisplatin administrations; no differences were seen between day 1, between the gemcitabine–cisplatin and paclitaxel–gemcitabine combinations (Fig. 1), and between days 1 and 8. Neither did we find evidence that the large volume used for hydration of the patients did influence the pharmacokinetics.

The paclitaxel plasma pharmacokinetics is summarized in Table 2. Plasma paclitaxel concentrations decreased rapidly after the end of the infusion. Although the paclitaxel-AUCs were not statistically different for the studied regimens ($P = 0.058$), the paclitaxel-AUC tended to be higher for the combination with cisplatin as

**Table 1** Patient characteristics

| | Paclitaxel > Cisplatin | Gemcitabine > Cisplatin | Paclitaxel > Gemcitabine |
|---|---|---|---|
| No. of patients entered | 8 | 8 | 8 |
| Male | 6 | 3 | 7 |
| Female | 2 | 5 | 1 |
| Extent of disease | | | |
| Locoregional | 2 | 2 | 1 |
| Metastatic | 6 | 6 | 7 |
| ECOG performance status | | | |
| 0 | 2 | – | 3 |
| 1 | 5 | 7 | 5 |
| 2 | 1 | 1 | – |
| Median age (year) | 68 | 55 | 57 |
| Range | 44–73 | 43–68 | 39–75 |
| Histology | | | |
| Squamous cell | 2 | – | 2 |
| Adenocarcinoma | 2 | 5 | 2 |
| Large cell (undifferentiated) | 4 | 3 | 4 |

**Table 2** Plasma pharmacokinetics of Gemcitabine, Cisplatin and Paclitaxel (mean ± SEM)

| Drug | Day | *n* | Gem | dFdU | | | | Cis | | | | Tax | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | $C_{max}$ (μM) | $C_{max}$ (μM) | AUC (mMxmin) | $t_{1/2\gamma}$ (min) | Cl (L/min) | $C_{max}$ (μM) | AUC (mM×min) | $t_{1/2\gamma}$ (min) | Cl (L/min) | $C_{max}$ (μM) | AUC (mM×min) | $t_{1/2\gamma}$ (min) | Cl (L/min) |
| Tax > Cis | 1 | 8 | – | | | | | 20.5 ± 1.4 | 21.8 ± 1.7 | 941 ± 65 | 0.02 | 4.3 ± 0.3 | 1.06 ± 0.06 | 355 ± 15 | 0.35 |
| Gem > Cis | 1 | 8 | 46.1 ± 7.1 | 121 ± 7.1 | 51.5 ± 4.6 | 585 ± 41 | 0.16 | 19.6 ± 1.0 | 22.3 ± 1.6 | 1,061 ± 46 | 0.02 | – | – | – | – |
| Gem | 8 | 7 | 54.7 ± 7.1 | 107 ± 16 | [a] | [a] | | – | – | – | – | – | – | – | – |
| Tax > Gem | 1 | 8 | 59.6 ± 7.8 | 94 ± 7.4 | 51.0 ± 3.8 | 451 ± 29 | 0.17 | – | – | – | – | 3.9 ± 0.5 | 0.82 ± 0.1 | 318 ± 31 | 0.53 |
| Gem | 8 | 7 | 59.6 ± 7.2 | 95 ± 9.7 | [a] | [a] | | – | – | – | – | [b] | [b] | [b] | [b] |
| Statistics between schedules | 1 | | $P = 0.22$ | $P = 0.02$ | $P = 0.93$ | $P = 0.02$ | $P = 0.75$ | $P = 0.64$ | $P = 0.82$ | $P = 0.16$ | $P = 0.78$ | $P = 0.58$ | $P = 0.06$ | $P = 0.30$ | $P = 0.02$ |

*Gem* gemcitabine 1,250 mg/m$^2$, *Cis* cisplatin 80 mg/m$^2$, *Tax* paclitaxel 175 mg/m$^2$, *dFdU* the inactive metabolite of gemcitabine, 2′,2′-difluoro-2′-deoxyuridine, *n* number of patients, $C_{max}$ maximum plasma concentration, *AUC* area under the plasma concentration-time curve, $t_{1/2\gamma}$ terminal half-life, *Cl* clearance

[a] On day 8 no 4 and 18 h samples were obtained

[b] No paclitaxel infusion at day 8




**Fig. 1** Mean concentration-time curves for gemcitabine (dFdC) when combined with paclitaxel (*filled inverted triangle*) and cisplatin (*inverted triangle*) ($P = 0.22$) as well as for its inactive metabolite dFdU when combined with paclitaxel (*filled circle*) and cisplatin (*empty circle*) ($P = 0.02$). Symbols represent mean plasma levels on day 1

compared to gemcitabine. The clearance and volume of distribution were significantly lower for the paclitaxel–cisplatin combination with $P = 0.024$ and $P = 0.037$, respectively. The paclitaxel $C_{max}$ of both regimens were comparable.

Table 2 summarizes the plasma pharmacokinetics of cisplatin. The mean total plasma Pt $C_{max}$ and total plasma Pt AUC of both the paclitaxel–cisplatin and gemcitabine–cisplatin schedule were comparable.

## Cellular pharmacology

The mean cellular pharmacokinetics of dFdCTP is summarized in Table 3. In the same patients the dFdCTP accumulation on day 8, when no paclitaxel was given, was similar to dFdCTP levels on day 1 with previous paclitaxel or successive cisplatin administration. Although the $t_{1/2\gamma}$ of dFdCTP in the Paclitaxel–gemcitabine combination was more than two-fold than in the gemcitabine–cisplatin combination, the mean dFdCTP-$C_{max}$ levels and dFdCTP AUC were not clearly different.

## Pharmacokinetics–toxicity relationships

One of the objectives of this study was to relate the pharmacokinetic parameters with toxicity and response. Detailed toxicity profiles for all patients have been described elsewhere [2]. In this group of 24 patients, seven patients responded, while ten patients had stable disease. No relation between the studied parameters and the antitumor effect could be observed in this relatively small patient group.

**Table 3** Cellular pharmacokinetics of dFdCTP (mean ± SEM)

| Drug | Day | *n* | $C_{max}$ (pmol/$10^6$ cells) | AUC (nmol/$10^6$ cells × min) | $t_{1/2\gamma}$ (min) |
|---|---|---|---|---|---|
| Gem > Cis | 1 | 8 | 88.7 ± 13 | 136.3 ± 25 | 1,038 ± 177 |
| Gem | 8 | 7 | 94.8 ± 27 | –[a] | –[a] |
| Tax > Gem | 1 | 8 | 66.7 ± 9.1 | 163.6 ± 41 | 2,169 ± 480 |
| Gem | 8 | 7 | 94.5 ± 25 | –[a] | –[a] |
| Statistics between schedules | 1 | | $P = 0.23$ | $P = 0.63$ | $P = 0.10$ |

*Gem* gemcitabine 1,250 mg/m$^2$, *Cis* cisplatin 80 mg/m$^2$, *Tax* paclitaxel 175 mg/m$^2$, *n* number of patients, $C_{max}$ maximum cellular concentration, *AUC* area under the concentration-time curve, $t_{1/2\gamma}$ terminal half-life
[a]On day 8 no 4 and 18 h samples were obtained

Toxicity observed in the first treatment cycle mainly consisted of myelotoxicity; three patients developed grade 3 WBC toxicity, five patients grade 3–4 neutropenia and two patients grade 3–4 thrombocytopenia. Pharmacokinetic parameters were related with toxicity grading as described above, with percentage decrease in blood cells counts, and with patient characteristics such as pretreatment creatinine clearance and liver function. The duration of paclitaxel concentration above 0.1 μM was not related to toxicity. The percentage decrease in platelets was schedule dependent and was significantly higher for the gemcitabine–cisplatin and gemcitabine–paclitaxel combinations as compared to the paclitaxel–cisplatin combination ($P = 0.003$, $P = 0.008$, respectively, Fig. 2). Moreover, the percentage decrease in platelets was significantly related to the dFdCTP-$C_{max}$ ($P = 0.03$).

## Discussion

Plasma pharmacokinetics of gemcitabine and cisplatin were not significantly different for the three combinations or between day 1 and 8 for the gemcitabine based doublets. Interestingly, the paclitaxel-AUC tended to be higher for the paclitaxel–cisplatin schedule as compared to the paclitaxel–gemcitabine combination possibly due to the significantly lower paclitaxel clearance for the pacitaxel–cisplatin combination. The precise mechanistic explanation for cisplatin's effect on paclitaxel clearance is not clear. A possible explanation is the previously described decreased clearance of paclitaxel in combination with cisplatin, which might be attributed to cisplatin induced inhibition of cytochrome P-450 dependent paclitaxel-metabolizing enzymes [13, 30].

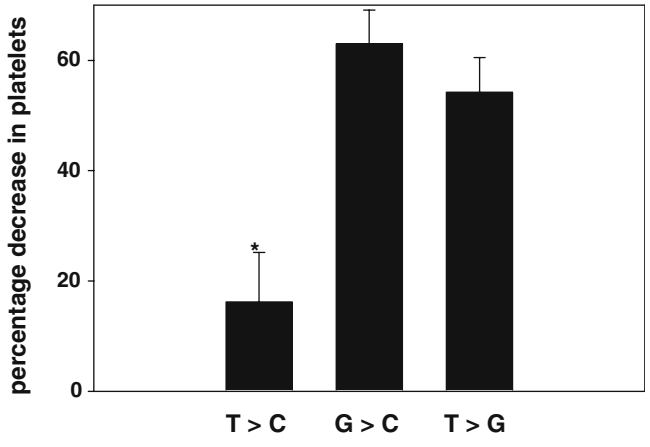


**Fig. 2** Percentage decrease in platelets (mean ± SEM) for the paclitaxel–cisplatin, gemcitabine–cisplatin and paclitaxel–gemcitabine schedules. The percentage decrease was significantly less for the paclitaxel–cisplatin schedule as compared to both the gemcitabine–cisplatin ($P = 0.003$) and paclitaxel–gemcitabine combination ($P = 0.008$)

Preclinically, gemcitabine has shown synergistic effects when combined with cisplatin, probably due to both increased formation of Pt-DNA adducts and a decreased Pt-DNA repair [3]. In addition, cisplatin increased gemcitabine incorporation into DNA and RNA and increased DNA strand break formation. In the present pharmacokinetic study, with gemcitabine 1,250 mg/m$^2$ (days 1 and 8) followed by cisplatin 80 mg/m$^2$ (day 1), no pharmacokinetic or pharmacodynamic interactions between both agents was observed. This is in accordance with a previous study combining gemcitabine 800 mg/m$^2$ (days 1, 8 and 15) and cisplatin 50 mg/m$^2$ (days 1 and 8), in which for the 4 h time interval no major difference in pharmacokinetics was observed [18]. In a two-week administration schedule with higher gemcitabine doses, a decrease in CG and AG-DNA intra-strand was found in WBCs [24]. In future studies, it would be of interest to further elucidate the genes involved in Pt-DNA repair and their role in drug–drug interaction with gemcitabine and paclitaxel.

For the gemcitabine–paclitaxel combination in vitro a no more than additive effect was observed [8, 25]. However, paclitaxel increased dFdCTP accumulation in NSCLC cells, increased gemcitabine incorporation into RNA and apoptosis was more pronounced when paclitaxel preceded gemcitabine as compared to the reversed schedule. In patients we previously found that paclitaxel dose dependently increased dFdCTP accumulation in peripheral blood mononuclear cells, possibly enhancing the gemcitabine metabolism [15]. In the current study, with paclitaxel at 175 mg/m$^2$ prior to gemcitabine at 1,250 mg/m$^2$ no effect of previously paclitaxel administration on dFdCTP accumulation was observed. In this study we used a higher dose of gemcitabine (1,250 mg/m$^2$ vs. 1,000 mg/m$^2$) and a fixed dose of paclitaxel. This higher dose of gemcitabine might alter the dFdCTP accumulation to such an extent that paclitaxel does not

affect dFdCTP anymore. In accordance with previous studies no plasma pharmacokinetic interactions were observed [15, 26].

For gemcitabine, plasma concentrations generally reach a plateau after 15–30 min during the standard 30 min infusion protocol and linear pharmacokinetics have been described over the range 40–4,500 and non-linear pharmacokinetics at higher doses mg/m$^2$ [27, 28]. In our pharmacokinetic studies mean gemcitabine peak plasma concentrations ranged from 24 μM at 800 mg/m$^2$ [18] to 32 μM at 1,000 mg/m$^2$ [15] and both to 70 μM and 53 μM (means of all values) at a dose of 1,250 mg/m$^2$ in both previously published [29] and the present study, respectively. At gemcitabine 1,250 mg/m$^2$, deamination was linear with mean plasma dFdU concentrations being 1.25 times higher as compared to dFdU levels using gemcitabine 1,000 mg/m$^2$. The dFdCTP-AUC (mean ± SEM; nmol/10$^6$ cells/min) for the gemcitabine–cisplatin and the paclitaxel–gemcitabine combinations were not significantly different, being 136 ± 25 and 163 ± 41, respectively. In the present study mean dFdCTP-$C_{max}$ levels were 80 pmol/10$^6$ cells.

The percentage decrease in platelets was significantly higher in the gemcitabine combinations as compared to the paclitaxel–cisplatin schedule. This decrease was also related to the dFdCTP-$C_{max}$ in the gemcitabine schedules, emphasizing the role of gemcitabine in platelet toxicity. In contrast to previous studies, pretreatment hepatic function was not related to paclitaxel pharmacokinetics, possibly because pretreatment hepatic function varied little between patients.

In conclusion, dFdCTP accumulation was related to the percentage decrease in platelets. The study revealed that the pharmacokinetics and pharmacodynamics of gemcitabine and cisplatin were not influenced by the combination with one of the other agents, while the paclitaxel clearance was influenced by concurrent administration of cisplatin. The study underlines the importance of inclusion of a pharmacokinetic evaluation when several chemotherapeutic agents are being combined, since pharmacokinetics can change as compared with single agents.

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