Gemcitabine, epirubicin and paclitaxel: pharmacokinetic and pharmacodynamic interactions in advanced breast cancer

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Background: The objectives of this study were to investigate the disposition of gemcitabine, epirubicin, paclitaxel, 2',2'-difluorodeoxyuridine and epirubicinol, and characterize the pharmacokinetic and pharmacodynamic profile of treatment in patients with breast cancer.

Patients and methods: The drug disposition in 15 patients who received gemcitabine 1000 mg/m², epirubicin 90 mg/m² and paclitaxel 175 mg/m² (GEP) on day 1 of a 21-day cycle, was compared with that of patients treated with epirubicin 90 mg/m² and paclitaxel 175 mg/m² (EP, n = 6) and epirubicin 90 mg/m² alone (n = 6). Drug and metabolite levels in plasma and urine were assessed by high-performance liquid chromatography and parameters of drug exposure were related to hematological toxicity by a sigmoid-maximum effect (Emax) model.

Results: Paclitaxel administration significantly increased the epirubicinol area under the concentration–time curve, from 357 ± 146 (epirubicin) to 603 ± 107 (EP) and 640 ± 81 h × ng/ml (GEP), and reduced the renal clearance of epirubicin and epirubicinol by 38 and 52.2% and 34.5 and 53% in GEP- and EP-treated patients, respectively, compared with epirubicin alone. Gemcitabine had no apparent effect on paclitaxel and epirubicin pharmacokinetics, and renal clearance of epirubicin and epirubicinol. The only pharmacokinetic/pharmacodynamic relationship observed was between neutropenia and the time spent above the threshold plasma level of 0.1 µmol/l (tC0.1) of paclitaxel, with the time required to obtain a 50% decrease in neutrophil count (Et50) of GEP being 7.8 h, similar to that of EP.

Conclusions: Paclitaxel and/or its vehicle, Cremophor EL, interferes with the disposition and renal excretion of epirubicin and epirubicinol; gemcitabine has no affect on epirubicin and paclitaxel plasma pharmacokinetics and renal excretion of epirubicin, while neutropenia is not enhanced by gemcitabine.

Key words: bone marrow, drug interactions, gemcitabine–epirubicin–paclitaxel, pharmacokinetics, toxicity

Introduction

The combination of paclitaxel and anthracyclines has been evaluated using a variety of doses and schedules of administration, and a number of studies show drug interaction with respect to disposition and toxicity [1]. In particular, the analysis of doxorubicin pharmacokinetics in regimens containing paclitaxel demonstrated that the schedule-dependent increase in Cmax and area under the concentration–time curve (AUC) and reduction in doxorubicin clearance were associated with severe neutropenia and mucositis [2]. Furthermore, the higher plasma exposure to doxorubicin and doxorubicinol in patients given doxorubicin immediately before paclitaxel compared with the two drugs administered 24 h apart was regarded as a key factor in the high incidence of cardiac toxicity [3]. Likewise, paclitaxel significantly affected epirubicin disposition and metabolism, albeit to a lower extent, and this effect was dependent on drug dose and/or schedule of administration [1, 4–6].

Early studies on paclitaxel showed that neutropenia correlates with drug exposure by a sigmoid-maximum effect (Emax) function [7]. The predictivity of this relationship was established for paclitaxel as a single agent and in combination regimens [8, 9]. Of note, a favorable pharmacodynamic interaction with less severe thrombocytopenia than expected has been demonstrated with the combination treatment of paclitaxel and carboplatin [9].

Gemcitabine (2',2'-difluorodeoxycytidine) is a cytidine analog with high efficacy in various malignant tumors and a manageable toxicity profile [10]. In view of its single-agent activity and tolerability, gemcitabine represents an attractive...
candidates to be used in combination therapy including taxanes and anthracyclines for the management of advanced breast cancer [11]. However, the peculiarities of the pharmacology of these drug classes suggest that the integration of new drugs in unexplored combination regimens with taxanes and anthracyclines should be associated with pharmacokinetic/pharmacodynamic monitoring in order to identify possible drug interactions and prevent unexpected toxicity.

On these premises, this study investigated the pharmacokinetics and pharmacodynamics of a combination regimen containing gemcitabine, epirubicin and paclitaxel administered to breast cancer patients and compared these findings with those obtained in patients given epirubicin and paclitaxel or epirubicin alone.

Patients and methods

Inclusion criteria

Eligibility criteria for this study were as follows: histologically proven breast cancer; no adjuvant chemotherapy within 6 months prior to enrollment; total cumulative dose of doxorubicin ≤240 mg/m² or epirubicin ≤360 mg/m² and no prior radiotherapy on the mediastinal fields; Eastern Cooperative Oncology Group (ECOG) performance status (PS) ≤2; life expectancy ≥3 months; age ≥70 years of age; absolute neutrophil count ≥2 × 10⁹/l, platelets ≥100 × 10⁹/l; bilirubin ≤25 µmol/l, AST and ALT ≤2.5 × normal values, creatinine ≤120 µmol/l and left ventricular ejection fraction (LVEF) ≥50% at bidimensional ultrasonography. The study was performed in accordance with the provisions of the Helsinki Declaration and after approval by the Institutional Review Board of Pisa University Hospital. All patients were informed of the investigational nature of the study and written, informed consent was obtained before enrollment.

Study treatment

Patients were categorized into one of three cohorts as follows. Cohort I: patients receiving gemcitabine 1000 mg/m² by 30 min i.v. infusion on days 1 and 4, followed 10 min later by paclitaxel 175 mg/m² by 3 h i.v. infusion on day 1, every 21 days (GEP, n = 15 patients). Cohort II: patients given paclitaxel 90 mg/m² i.v. bolus followed 10 min later by paclitaxel 175 mg/m² infused i.v. in 3 h (EP, n = 6). Cohort III: patients administered with epirubicin 90 mg/m² i.v. bolus followed by paclitaxel 175 mg/m² 24 h later, termed ‘epirubicin alone’ for pharmacokinetic purposes (n = 6). All patients were examined at the first cycle of therapy. Patients assigned to the GEP cohort were enrolled within a phase II trial designed to assess the safety and activity of the combination [12], while those included in EP and epirubicin alone cohorts participated in a phase II trial reported previously [13]. In the latter group of patients, paclitaxel administration was delayed by 24 h with respect to epirubicin administration to minimize the pharmacokinetic interaction between epirubicin and paclitaxel. In addition, these patients were evaluated with respect to epirubicin distribution only, and plasma and urine sampling was suspended before paclitaxel administration.

Blood counts/chemistry and urinalysis monitoring were performed at baseline and on days 1, 4, 7, 14 and 21 of each cycle; additional samples were obtained if required by the patient’s clinical conditions. Cardiac function was assessed by physical examination and electrocardiogram recording before each cycle; LVEF was measured by echocardiography every two cycles during treatment and at 3-month intervals during the follow-up. The clinical characteristics of patients including hematological and non-hematological toxicity data are reported elsewhere [12, 13].

Drug administration and plasma/urine sampling

Gemcitabine hydrochloride (Eli Lilly, Firenze, Italy) was reconstituted with 0.9% NaCl and administered i.v. with an infusion pump over 30 min on days 1 and 4. Epirubicin (Pharmacia & Upjohn, Milano, Italy) was purchased as a 2 mg/ml concentrated solution in a 25 ml amouple in sterile water for intravenous use; the drug was diluted with sterile NaCl 0.9% to 30 ml and administered at a fixed dose of 90 mg/m² by i.v. bolus injection on day 1. Paclitaxel (Bristol-Myers Squibb, Princeton, NJ, USA) was obtained as a concentrated solution with 6 mg/ml of drug in 5 ml ampoules in 50% polyoxyethylated castor oil (Cremophor EL; BASF, Blagden, UK) and 50% dehydrated ethanol. The drug was diluted in 5% dextrose to a final concentration of ≤0.6 mg/ml and administered i.v. over 3 h on day 1. Dexamethasone 20 mg i.v., cimetidine 300 mg i.v. and omeprazole 40 mg i.m. were given 0.5–1 h before paclitaxel as pre-medication.

Blood samples (5 ml each) for drug assays were taken from patients on day 1 of the first cycle of therapy from an antecubital vein controlateral to the site of injection. In particular, patients receiving GEP were sampled at 0 (before gemcitabine administration), 10, 15, 30 and 45 min, and 1, 2, 4, 6, 8, 12 and 24 h after the beginning of gemcitabine infusion, and collected in heparinized test tubes (Becton Dickinson, Rutherford, NJ, USA) that contained tetrahydrouridine to prevent deamination of gemcitabine to 2′,2′-difluorodeoxyuridine (dFdU). Blood samples from patients given the EP regimen were obtained at 0 (before the administration of paclitaxel) and 30 min, and 1, 2, 3 h (end of paclitaxel infusion), 4, 6, 8, 12, 18 and 24 h thereafter, while blood specimens from patients treated with epirubicin were obtained at 0 (before the administration of epirubicin) 5, 10 and 30 min, and 1, 2, 3, 4, 6, 8, 12, 18 and 24 h after drug administration; the last sample was obtained immediately before paclitaxel administration. Plasma was obtained by centrifugation at 1400 g for 10 min, split in three aliquots for the analysis of gemcitabine and dFdU, epirubicin and epirubicinol, and paclitaxel and stored at –20°C until assays were performed. The renal excretion of epirubicin and epirubicinol was evaluated by collecting urinary samples at baseline (pre-bolus of epirubicin) and at 4-h intervals up to 24 h after epirubicin administration. The volume of urine and the time of collection were recorded and samples were stored frozen at –20°C in light-protected tubes until analysis as reported below.

Drug analysis

Gemcitabine and dFdU

Gemcitabine and its metabolite dFdU were determined by a validated high-performance liquid chromatography (HPLC) method with ultraviolet (UV) detection. Gemcitabine and dFdU were extracted by adding 2 ml of isopropyl alcohol and 5 ml ethyl acetate to 0.5 ml plasma samples; the suspension was mixed, centrifuged for 5 min at 1400 g and the supernatant was transferred to another tube and blown to dryness at 40°C under N₂. The dry sample was dissolved in 250 µl of mobile phase, consisting of phosphate buffer 30 mM (pH 6.8)/acetonitrile/methanol (96:2:2 v/v); a sample of 50 µl of this solution was injected into a Symmetry Shield C₁₈ 5 µm, 300 × 4.6-mm column (Waters, Milford, MA, USA), eluted at a flow rate of 1 ml/min and monitored at 270 nm by UV absorption.
Paclitaxel

Paclitaxel was measured following a previously described method [14]. Sample extraction was performed by protein precipitation with acetonitrile (0.25 ml) of 0.25 ml plasma aliquots. The samples were briefly mixed and particulate material cleared by centrifugation in a microfuge at 19 800 × g for 15 min. The supernatant (50 µl) was analysed on a Shandon Hypersil ODS 5 µm, 100 × 4.6-mm column (Alltech, Deerfield, IL, USA) protected with a pre-column (30 × 4.6 mm) of the same packing material, and eluted with acetonitrile 45% / water 55% at 1.2 ml/min. Eluents were monitored by UV absorption at 230 nm.

Epirubicin and epirubicinol

The analysis of epirubicin and epirubicinol in plasma and urine samples was performed by HPLC with fluorescence detection [15]. Extraction was performed by adding 4 ml of chloroform–1-heptanol (9:1) to 0.5 ml samples previously buffered with 0.5 ml of Na2HPO4 0.2 M (pH 8.4). After mixing for 15 min, the samples were centrifuged at 14000 g for 10 min and 3.5 ml of the organic phase was back-extracted with 0.5 ml of H2PO4 0.1 M. The acidic phase was chromatographed on a Supelcosil LC-CN 5 µm, 250 × 4.6-mm column (Supelco, Bellefonte, PA, USA) with isocratic elution of the mobile phase (acetonitrile 35% / Na2HPO4 50 mM 65%, pH 5.6) and fluorescence detection at λex 480 nm and λem 560 nm.

Method validation

Drug analyses were performed by a Waters LC Module I plus equipped with a WISP 416 autosampler, a variable wavelength UV detector and a 4.6-mm column (Supelco, Bellefonte, PA, USA) protected with a pre-column (30 × 4.6-mm column) of the same packing material, and UV detection at 230 nm.

Pharmacokinetic analysis

Gemcitabine, dFdU, paclitaxel, epirubicin and epirubicinol plasma levels versus time curves were modeled using the MW/PHARM software (Mediware, Groningen, The Netherlands; [16]). Initial parameter estimates were determined by curve stripping with Kinstrip module and then fitted by Kinfit module. The non-linear least-squares iterative regression procedure of Kinfit determines the slopes and intercepts of the logarithmically plotted curves of polyexponential functions and provides a correlation coefficient of the fitted curve. Modeling of the concentration–time curves was done with the Nelder–Mead simplex procedure to determine the parameter values that minimize a weighted least-squares criterion [16]. Epirubicin disposition kinetics was modeled as an open three-compartment linear model, whereas gemcitabine, paclitaxel, dFdU and epirubicinol dispositions were fitted according to an open two-compartment linear model, assuming that the model input occurred via constant infusion of drugs and their conversion to the respective metabolites was a first-order process. The following time–concentration polyexponential functions were obtained:

\[
C_i = \sum_{i=1}^{N} \left( \frac{C_i}{(L_i \times T_{ini})} \times \left( 1 - e^{-LT_i} \right) \right)
\]

gemcitabine/paclitaxel during infusion:

\[
C_i = \sum_{i=1}^{N} \left( \frac{C_i}{(L_i \times T_{ini})} \times \left( 1 - e^{-LT_i} \right) \right)
\]

epirubicin: \[
C_i = \sum_{i=1}^{N} \left( \frac{C_i \times e^{-LT_i}}{L_i \times T_{ini}} \right)
\]

2′,2′-difluoroxyuridine/epirubicinol:

\[
C_i = \sum_{i=1}^{N} \left( \frac{C_i \times e^{-LT_i}}{L_i \times T_{ini}} \right) - \sum_{m=1}^{N} \left( \frac{C_m \times e^{-kt_m}}{m} \right)
\]

where \(C_i\) is the plasma concentration measured at time \(t\), \(N\) is the number of compartments, \(C_i\) and \(L_i\) are the \(x\) coefficient and exponent of polyexponential functions, respectively, \(T_{ini}\) is the time of constant infusion of gemcitabine and paclitaxel, and \(k_m\) is the rate constant of metabolite input into the central compartment. Curve fitting yielded the parameters \(C_i\), \(L_i\), and \(k_m\), and the intercompartmental rate constants.

Maximum plasma concentration (\(C_{max}\), µg/ml, µmol/l) and time to reach \(C_{max}\) (\(T_{max}\), h) of parent drugs and metabolites were determined from observed values of the plasma concentration–time curves. Half lives (\(t_{1/2}\)) were calculated as 0.693L/\(k_m\), where \(L_i\) (\(h^{-1}\)) is the negative slope of the log-linear \(\alpha\), \(\beta\) and \(\gamma\) phases of the plasma concentration–time profiles. The areas under the plasma concentration–time curve (zero moment curve, AUC, h × µg/ml, h × µmol/l) from the first to the last sampling time were calculated using the experimental values (trapezoidal rule). Mean residence time (MRT, h) was determined by dividing the area under the first moment curve (AUMC; h² × µg/ml, h² × µmol/l) by AUC with correction for infusion time, while the MRT of epirubicin was calculated as AUMC/AUC + 1/\(k_m\). Total body clearance (CLtot, l/h/m²) and volume of distribution at steady state \((Vsg, l/m²)\) were calculated as dose/ AUC and \(VI \times (1 + k_{21}/k_{31} + k_{31}/k_{21})\), respectively, where \(k_{21}\) and \(k_{31}\) are the intercompartmental rate constants and \(VI\) is the volume of distribution of the central compartment and then normalized to body surface area. Finally, paclitaxel pharmacokinetics included the measurement of the time spent above the threshold plasma level of 0.1 µmol/l on individually fitted plasma concentration–time plots (\(\tau_{C_{0.1}}\), a parameter related to neutropenia [17]. The amounts of epirubicin and epirubicinol eliminated by urinary route in patients given GEP, EP and epirubicin were calculated at each time interval to obtain the cumulative amount excreted (CAE) from time 0 to 24 h after dosing, while renal clearances (CLR) of epirubicin and epirubicinol were calculated by dividing the CAE by plasma AUC during the sampling period [18].

Pharmacokinetic–pharmacodynamic analysis

The relationship between drug exposure and hematological toxicity, and the dose-limiting effect of gemcitabine, epirubicin and paclitaxel [1, 7, 19] was evaluated. The percentage decrease in hematological count (neutrophils, leukocytes and platelets) was calculated as follows:
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and plotted as a function of time of plasma concentrations ≥ 0.1 µmol/l of paclitaxel, a threshold level associated with myelotoxicity [17] or parameters of epirubicin, epirubicinol or gemcitabine exposure (Cmax, AUC and AUMC) [20]. These relationships were fitted according to a sigmoidal maximum effect (Emax) model:

\[
\% \text{ change in ANC} = \frac{E_{\max} \times PK^x}{PK_{50}^x + PK^x}
\]

where \( \kappa \) is the shape factor that accommodates the sigmoidicity of the concentration–effect curve, \( PK \) represents the pharmacokinetic parameter of interest and \( PK_{50} \) denotes the value at 50% of the \( E_{\max} \), which in turn, is defined as the 100% reduction in hematologic count [21] (ANC = absolute neutrophil count). The performance of the pharmacodynamic model was assessed using the relative root mean square error value and its standard error [22].

### Table 1. Pharmacokinetic parameters of gemcitabine, dFdU and paclitaxel in patients treated with GEP and EP

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Gemcitabine (GEP)</th>
<th>dFdU (GEP)</th>
<th>Paclitaxel (GEP)</th>
<th>Paclitaxel (EP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (µg/ml)</td>
<td>22.3 ± 3.4</td>
<td>54.7 ± 21.9</td>
<td>4.5 ± 0.4 (µmol/l)</td>
<td>4.5 ± 0.7 (µmol/l)</td>
</tr>
<tr>
<td>Tmax (h)</td>
<td>0.5</td>
<td>0.8 ± 0.1</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>AUC (h × µg/ml)</td>
<td>9.3 ± 1.8</td>
<td>325 ± 84 (h × µg/ml)</td>
<td>15.8 ± 1.1 (h × µmol/l)</td>
<td>18.5 ± 2.6 (h × µmol/l)</td>
</tr>
<tr>
<td>t1/2α (h)</td>
<td>0.06 ± 0.02</td>
<td>1.22 ± 0.90</td>
<td>0.9 ± 0.4</td>
<td>0.7 ± 0.4</td>
</tr>
<tr>
<td>t1/2β (h)</td>
<td>0.3 ± 0.1</td>
<td>19 ± 10.4</td>
<td>8.9 ± 5.7</td>
<td>13.9 ± 3.3</td>
</tr>
<tr>
<td>CLTB (l/h/m²)</td>
<td>112 ± 25</td>
<td>–</td>
<td>19.4 ± 5.9</td>
<td>12.3 ± 1.8</td>
</tr>
<tr>
<td>Vss (l/m²)</td>
<td>16.7 ± 1.4</td>
<td>–</td>
<td>82.5 ± 42</td>
<td>52.4 ± 23.5</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>0.22 ± 0.08</td>
<td>–</td>
<td>4.3 ± 1.5</td>
<td>6.6 ± 1.3</td>
</tr>
</tbody>
</table>

\( C_{\max} \): peak plasma concentration; \( T_{\max} \): time to \( C_{\max} \); AUC, area under the plasma concentration–time curve; \( t_{\alpha} \): distribution half-life; \( t_{\beta} \): elimination half-life; \( CL_{\text{TB}} \): total body clearance; \( V_{\text{ss}} \): volume of distribution at steady state; MRT, mean residence time.

% Decrease in hematologic count = 100 x (pre-treatment count – nadir count) / pre-treatment count

and plotted as a function of time of plasma concentrations ≥ 0.1 µmol/l of paclitaxel, a threshold level associated with myelotoxicity [17] or parameters of epirubicin, epirubicinol or gemcitabine exposure (\( C_{\max} \), AUC and AUMC) [20]. These relationships were fitted according to a sigmoidal maximum effect (\( E_{\max} \)) model:

\[
% \text{ change in ANC} = \frac{E_{\max} \times PK^x}{PK_{50}^x + PK^x}
\]

where \( \kappa \) is the shape factor that accommodates the sigmoidicity of the concentration–effect curve, \( PK \) represents the pharmacokinetic parameter of interest and \( PK_{50} \) denotes the value at 50% of the \( E_{\max} \); which in turn, is defined as the 100% reduction in hematologic count [21] (ANC = absolute neutrophil count). The performance of the pharmacodynamic model was assessed using the relative root mean square error value and its standard error [22].

### Statistical analysis

Data are presented as mean values ± standard deviation (SD) of the mean. Statistical comparisons among disposition parameters were performed by analysis of variance (ANOVA) followed by the Student–Newman–Keuls test [23], with \( P \) <0.05 being the limit of significance.

### Results

#### Pharmacokinetics of gemcitabine and dFdU

The \( C_{\max} \) of gemcitabine (22.3 ± 3.4 µg/ml) was reached at the end of drug administration and the concentration–time curve was characterized by biphasic decay in the post-infusion period (Figure 1). Gemcitabine rapidly disappeared from plasma, as shown by the high systemic clearance (112 ± 25 l/h/m²) and short terminal half-life (\( t_{\beta} = 0.3 ± 0.1 \) h); in addition, the small \( V_{\text{ss}} \) value (16.7 ± 1.4 l/m²; Table 1) indicates that gemcitabine is not extensively bound to tissues. Plasma levels of dFdU reached a \( C_{\max} \) of 54.7 ± 21.9 µg/ml, at ~15 min after the end of gemcitabine infusion (Figure 1; Table 1). In contrast to the parent drug, the \( t_{\beta} \) of dFdU was long (19 ± 10.4 h; Table 1) and highly variable among patients (from 6.2 to >24 h). Other relevant pharmacokinetic parameters are listed in Table 1.

#### Paclitaxel

The average plasma concentration versus time curves and pharmacokinetic parameters of paclitaxel combination regimens were similar in patients treated with EP and GEP (Figure 2; Table 1). The drug profile was characterized by increasing plasma levels during infusion to reach the \( C_{\max} \) (GEP 4.5 ± 0.4 µmol/l; EP 4.5 ± 0.7 µmol/l) at the end of pac-
Table 2. Pharmacokinetic parameters of epirubicin and epirubicinol in patients treated with GEP, EP and epirubicin alone (E)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Epirubicin</th>
<th>Epirubicinol</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_{max}</td>
<td>3.53 ± 1.93 (µg/ml)</td>
<td>3.35 ± 0.6 (µg/ml)</td>
</tr>
<tr>
<td>AUC</td>
<td>2.17 ± 0.72 (h × µg/ml)</td>
<td>1.94 ± 0.41 (h × µg/ml)</td>
</tr>
<tr>
<td>t_{1/2α} (h)</td>
<td>0.06 ± 0.04</td>
<td>0.06 ± 0.03</td>
</tr>
<tr>
<td>t_{1/2β} (h)</td>
<td>1.58 ± 0.71</td>
<td>1.58 ± 0.47</td>
</tr>
<tr>
<td>t_{1/2γ} (h)</td>
<td>22.64 ± 4.93</td>
<td>16.44 ± 5.66</td>
</tr>
<tr>
<td>CL_{TB} (l/h/m²)</td>
<td>42.46 ± 17.87</td>
<td>54.2 ± 14.4</td>
</tr>
<tr>
<td>V_{ss} (l/h/m²)</td>
<td>1000 ± 804</td>
<td>912 ± 801</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>19.52 ± 14.04</td>
<td>8.58 ± 2.99</td>
</tr>
</tbody>
</table>

C_{max}, maximum plasma concentration; AUC, area under the plasma concentration–time curve; t_{1/2α,β,γ}, initial, intermediate and terminal half-life; CL_{TB}, total body clearance; V_{ss}, volume of distribution at steady state; MRT, mean residence time; *P <0.05 versus epirubicin, ANOVA followed by the Student–Newman–Keuls test.

Figure 2. Plasma concentrations (mean ± SD) of paclitaxel in patients treated with GEP and EP.

Epirubicin and epirubicinol

The concentration–time profiles of epirubicin in the GEP, EP and epirubicin alone groups were similar up to the start of paclitaxel administration (Figure 3A), as also demonstrated by the comparison of C_{max} (3.53 ± 1.93, 3.35 ± 0.6 and 3.39 ± 0.54 µg/ml, respectively) and t_{1/2α} (0.06 ± 0.04, 0.06 ± 0.03, and 0.05 ± 0.03 h, respectively; Table 2). At the start of paclitaxel infusion, plasma levels of epirubicin in GEP and EP showed a modest increase compared with epirubicin only; however, these changes did not result in a significant difference in the AUC of epirubicin in GEP and EP versus epirubicin only (Table 2).

The plasma level versus time curves of epirubicinol in subjects given GEP, EP and epirubicin alone overlapped before paclitaxel infusion, and the first peak plasma concentrations (C_{max1}) of the three groups of patients were similar (66 ± 4, 65 ± 5 and 59 ± 3 ng/ml, respectively; Table 2). Shortly after the beginning of paclitaxel infusion, the plasma profiles of epirubicinol in patients given GEP and EP showed a rebound in metabolite plasma concentrations to generate a second peak plasma level (C_{max2}) of 41 ± 5 and 39 ± 5 ng/ml, respectively, ~4 h after the administration of epirubicin (Figure 3B). C_{max2} of epirubicinol in GEP and EP cohorts were significantly higher (P <0.05) than the metabolite concentrations (i.e. 21 ± 9 ng/ml) observed at the same time point in patients given epirubicin alone (Figure 3B).

Epirubicinol plasma levels then decreased bi-exponentially after the end of paclitaxel infusion, and the three concentration–time profiles converged to approximately the same value 24 h after epirubicin administration (Figure 3B). The infusion of paclitaxel was associated with a significant increase in epirubicinol AUC (78 and 67% in patients receiving GEP and EP, respectively) compared
with those given epirubicin alone (Table 2). The comparison of the pharmacokinetic profile and distribution parameters of epirubicinol in patients treated with GEP versus EP, demonstrated a similar behavior of the metabolite, suggesting that gemcitabine did not affect the well-known pharmacokinetic interaction between paclitaxel and epirubicin (Figure 3B; Table 2).

The data obtained from the analysis of urinary samples demonstrated that the CAEs of epirubicin and epirubicinol were in the range of 10–15% of total epirubicin dose in patients given GEP, EP and epirubicin. The amount of epirubicin excreted during the 24-h period showed a significant decrease from 21.72 ± 4.11 mg (epirubicin alone) to 14.61 ± 1.64 mg (EP) and 14.04 ± 2.72 mg (GEP), while the respective CAEs of epirubicinol were 1.82 ± 0.45, 1.04 ± 0.34 and 1.10 ± 0.24 mg (Table 3). Most notably, the CL\textsubscript{R} of epirubicin and epirubicinol were impaired by the administration of paclitaxel and a significant reduction of epirubicinol CL\textsubscript{R} was observed in the EP and GEP regimens (–53 and –52.2%, respectively) compared with epirubicin alone. Gemcitabine had no significant effect on renal clearance of anthracyclines, as shown in the present study by the similarity of the CL\textsubscript{R} values of patients treated with EP versus those treated with GEP (Table 3).

### Pharmacokinetic–pharmacodynamic analysis

The relationship between pharmacokinetics and drug effect was examined in patients who received GEP. Only a significant correlation between paclitaxel t\textsubscript{C\textsubscript{0.1}} and reduction in absolute neutrophil count was observed; the E\textsubscript{max} pharmacodynamic model provided a good correlation (r\textsuperscript{2} = 0.96) with dose-limiting neutropenia and a Hill coefficient of 0.12, indicating a steep pharmacokinetic–pharmacodynamic curve (Figure 4). The time of drug exposure required to yield a 50% reduction of absolute neutrophil count (ET\textsubscript{0.5}) was 7.8 h; this finding compares well with historical data obtained in patients given EP [1] and provides evidence that the presence of gemcitabine does not enhance the neutropenic effect of paclitaxel in combination with epirubicin.

### Discussion

The integration of gemcitabine into combination chemotherapy including taxanes and anthracyclines represents a possible strategy for achieving higher percentages of complete responses and to increase the proportion of long-term survivors of advanced breast cancer. Because of the potential for drug interaction, as previously demonstrated for anthracyclines and paclitaxel, which may lead to unexpected drug toxicity [1, 4–6], the pharmacokinetics and pharmacodynamics of combination chemotherapy of gemcitabine, epirubicin and paclitaxel were investigated in this study in order to ascertain whether gemcitabine provided an additional factor of drug interaction.

The findings of this study suggest that the administration of epirubicin and paclitaxel does not interfere with gemcitabine disposition. Indeed, although a direct comparison to patients given gemcitabine alone has not been performed, the pharmacokinetics of gemcitabine and its metabolite dFdU are comparable to those reported previously [19]. The present data also demonstrate that gemcitabine, in turn, apparently did not affect the plasma pharmacokinetics of epirubicin, epirubicinol and paclitaxel, or the urinary excretion of epirubicin and epirubicinol. Indeed, the interaction between paclitaxel and anthracyclines, as shown in the present study by the rebound in epirubicinol plasma levels and reduction in CL\textsubscript{R} of epirubicin and epirubicinol, was not further enhanced by the administration of gemcitabine, because no significant differences between patients treated with GEP versus EP were observed.

The possible mechanisms of the pharmacokinetic interaction between paclitaxel and anthracyclines have been recently reviewed and most likely involves a pharmacological competition of paclitaxel/Cremophor EL on anthracycline excretion mediated by P-glycoprotein (P-gp) with saturation of biliary clearance, rather than modulation of aldo-keto reductase metabolism of anthracyclines [1]. With respect to this, it has been demonstrated that in vitro metabolism of epirubicin to epirubicinol was not significantly affected by

### Table 3. Urinary excretion of epirubicin and epirubicinol during a 24-h period in patients treated with GEP, EP and epirubicin alone (E)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment</th>
<th>CAE (mg)</th>
<th>Percentage change (mean values)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GEP</td>
<td>EP</td>
<td>E</td>
</tr>
<tr>
<td>CL\textsubscript{R} (l/h/m\textsuperscript{2})</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EPI</td>
<td>14.04 ± 2.72*</td>
<td>14.61 ± 1.64*</td>
<td>21.72 ± 4.11</td>
</tr>
<tr>
<td>EPIol</td>
<td>1.10 ± 0.24*</td>
<td>1.04 ± 0.34*</td>
<td>1.82 ± 0.45</td>
</tr>
<tr>
<td>CL\textsubscript{R} (l/h/m\textsuperscript{2})</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EPI</td>
<td>4.58 ± 1.2*</td>
<td>4.84 ± 1.03*</td>
<td>7.39 ± 1.87</td>
</tr>
<tr>
<td>EPIol</td>
<td>1.11 ± 0.26*</td>
<td>1.09 ± 0.2*</td>
<td>2.32 ± 0.45</td>
</tr>
</tbody>
</table>

CAE, cumulative amount excreted; CL\textsubscript{R}, renal clearance; EPI, epirubicin; EPIol, epirubicinol; *P <0.05, ANOVA followed by the Student–Newman–Keuls test.

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paclitaxel in whole blood [24]. The P-gp hypothesis is further supported by the evidence of a similar drug interaction when the anthracycline is administered in combination with P-gp competitors, including cyclosporin A and Cremophor EL, with a resulting marked change in the disposition of doxorubicin and doxorubicinol [3, 18, 25]. Cremophor effects on P-glycoprotein reach maximal inhibition at a concentration of 1 µl/ml, which was observed at the end of a 3-h infusion of paclitaxel [26, 27]. Of interest in the present work, Cmax2, corresponding to the maximal rebound of epirubicinol concentrations in GEP and EP treatments, was observed at the end of paclitaxel infusion. Furthermore, the reduction in CLR of epirubicin and epirubicinol suggested the presence of an interaction of paclitaxel and/or Cremophor EL on anthracycline urinary excretion, possibly by the competition with P-gp function in the kidney [28]. In agreement with previously published data [4, 6], the pharmacokinetic parameters of epirubicin were significantly changed, while those of epirubicin were unaffected. Of note, the lack of mutual inhibition between doxorubicin and the organic cation rocuronium on biliary excretion by the rat liver [29] indicates that major differences in the affinity of substrates for the excretory system may represent a possible explanation of the differences in pharmacokinetic behavior of the metabolites doxorubicinol and epirubicinol with respect to the parent drugs.

Concerning the clinical relevance of the aforementioned pharmacokinetic interaction, the significant increase in plasma AUC of epirubicinol after paclitaxel infusion may have relevance to the risk of cardiac toxicity of the drug combination, since the reduced metabolites of anthracyclines display an enhanced potential for cardiac damage compared with the parent drugs [30, 31]. Indeed, the enhanced accumulation of doxorubicin and doxorubicinol in cardiac tissue of mice lacking the mdr1a gene [32] suggests that a blockade of endogenous P-gp in patients may increase the risk of cardiotoxicity upon administration of anthracyclines. As a matter of fact, the

Figure 3. Plasma concentrations (mean ± SD) of (A) epirubicin and (B) epirubicinol in patients treated with GEP, EP and epirubicin alone (E).

Figure 4. Percentage decrease in absolute neutrophil count as a function of the time of plasma concentrations of paclitaxel above the threshold level of 0.1 µmol/l (tC0.1) in patients treated with GEP.
pharmacokinetic interaction between doxorubicin and paclitaxel, leading to the increase in both doxorubicin and doxorubicinol plasma levels, has been related to the clinical cardiotoxicity of this regimen [3, 8]. However, epirubicin, a less cardiotoxic drug that is extensively metabolized to inactive glucuronides, was associated with mild cardiotoxicity in combination with paclitaxel [13], and the cumulative incidence of chronic heart failure was moderately higher than epirubicin alone if a cumulative dose of 1000 mg/m² was exceeded [33].

The clinical relevance of higher levels of epirubicinol on antitumor efficacy is unknown. Previous studies have demonstrated that the reduced metabolites of anthracyclines have lower cytotoxicity than the parent drugs [34], while the objective response rates reported for the association of paclitaxel and epirubicin were similar to those observed for the docetaxel–epirubicin doublet, in which the anthracycline disposition was unaffected by the taxane [35, 36]. Based on these premises, no relevant changes on the efficacy of the combination regimen are expected by increasing body exposure to epirubicinol. It should be pointed out, however, that the impaired elimination of anthracyclines and metabolites by drugs interacting with their disposition should be carefully monitored in relation to cardiotoxicity.

Pharmacodynamic analysis was performed in the present study in order to investigate the possible relationship between drug pharmacokinetics and pharmacodynamics at the first cycle of chemotherapy, and in particular the role played by gemcitabine in the hematological toxicity profile of the EP combination. In this study, neutropenia was found to be related to the \( t_{\text{Cmax}} \) of paclitaxel, while no significant correlation was observed between other hematological and drug pharmacokinetic parameters. No significant correlation between the percentage decrease in neutrophil count and the time of paclitaxel concentrations \( \geq 0.05 \mu\text{mol/l} \) was observed, although this relationship has been established previously [5]. The reason of this discrepancy may be dependent on the higher limit of quantitation of paclitaxel of the HPLC method used in the present study (0.05 \( \mu\text{mol/l} \)), compared with previous HPLC analysis characterized by higher sensitivity, which allowed a more accurate definition of duration of drug plasma concentrations \( \geq 0.05 \mu\text{mol/l} \) [5].

Of note, in the present work, the time required to obtain a 50% decrease in neutrophil count (\( E_{50} \)) of paclitaxel was 7.8 h in patients given GEP, a value similar to EP treatment (7.7 h) [24]; this finding provides evidence that gemcitabine does not interact with paclitaxel–epirubicin from a pharmacodynamic point of view. Although the underlying mechanism is as yet unknown, a similar effect has been recently reported with respect to platelet counts with the combination of paclitaxel and carboplatin in patients with non-small-cell lung cancer [9], and may be attributed to specific effects of drugs on cell cycle of stem cells in the bone marrow.

In conclusion, the data from the present study demonstrate the absence of pharmacokinetic interference between gemcitabine and epirubicin, and between gemcitabine and paclitaxel, whereas there is a drug interaction of paclitaxel affecting P-gp-mediated anthracycline excretion. Furthermore, the data here provide evidence of a favorable pharmacodynamic profile of gemcitabine, epirubicin and paclitaxel treatment, resulting in less severe neutropenia than expected.

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References

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