Influence of alternate sequences of epirubicin and docetaxel on the pharmacokinetic behaviour of both drugs in advanced breast cancer

G. Lunardi¹, M. Venturini²*, M. O. Vannozzi¹, G. Tolino², L. Del Mastro², C. Bighin², G. Schettini¹ & M. Esposito¹

¹Servizio di Farmacologia e Neuroscienze, Laboratorio di Farmacologia Tossicologica and ²Dipartimento di Oncologia Medica I, Istituto Nazionale per la Ricerca sul Cancro, Genoa, Italy

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Background: Previously we observed a pharmacokinetic interference of epirubicin elimination when paclitaxel is given in combination in a sequence-dependent manner (i.e. when paclitaxel is administered as first drug). The aim of this study was to determine whether these sequence-dependent pharmacological effects were also evident when epirubicin was combined with docetaxel.

Patients and methods: Patients who received epirubicin 75 mg/m² or 90 mg/m² as an intravenous bolus followed immediately by docetaxel 70 mg/m² or 80 mg/m² over a 1-h infusion, or the opposite sequence, every 3 weeks were eligible for this study. The pharmacokinetics of docetaxel, epirubicin and its metabolites were studied at the first and second cycle of treatment. Pharmacokinetic data were normalised to the lower dose of each drug. Toxicity was recorded at nadir and graded according to National Cancer Institute Common Toxicity Criteria.

Results: Twelve consecutive patients, each acting as their own control, entered the study. The sequence of drug administration of docetaxel and epirubicin did not affect the pharmacokinetics of the parent anthracycline. Statistically significant lower glucuronidation metabolism of epirubicin was observed in patients who received docetaxel before epirubicin. The pharmacokinetics of docetaxel were not influenced by the sequence of drug administration. No difference in haematological and non-haematological toxicity was observed in the two sequences of treatment.

Conclusions: The pharmacokinetics of the parent anthracycline and of docetaxel were similar between the two schemes of treatment. The metabolic variations observed, i.e. differences in the plasma levels of epirubicin glucuronides, seem not to have clinical relevance.

Key words: combination treatment, docetaxel, epirubicin, pharmacokinetics

Introduction

Optimal combination of anthracyclines and taxanes in breast cancer is under intensive investigation. Due to the high activity of anthracyclines and taxanes as single agents, and the lack of cross-resistance, several clinical trials have been conducted using these drugs in combination.

However, anthracycline/taxane combinations may lead to complex pharmacological interactions that can have a significant impact on toxicity, and these effects seem to be either sequence- or schedule-dependent [1, 2]. Paclitaxel administration can modify the disposition of both doxorubicin and epirubicin, as well as their metabolic pathway [3–5], whereas docetaxel does not seem to affect the pharmacokinetics of these anthracyclines [5, 6]. However, the metabolic profile of epirubicin appeared to be altered when docetaxel administration immediately followed an intravenous (i.v.) bolus of this anthracycline [5]. Recently, it has been observed that the maximum tolerable dose (MTD) of docetaxel in combination with epirubicin is influenced by the schedule of drug administration [7], and that the area under the curve (AUC) of docetaxel in combination with doxorubicin was higher than that obtained by its administration as a single agent [6].

Based on these findings, we conducted a pharmacokinetic study to evaluate whether a sequence-dependent pharmacokinetic interaction exists between epirubicin and docetaxel when used in combination.
Patients and methods

The study was conducted at the Department of Medical Oncology and the Pharmacotoxicology Laboratory of the Istituto Nazionale per la Ricerca sul Cancro, Genoa, Italy. All clinical and pharmacokinetic protocols were approved by the Protocol Review Committee and by the Ethics Committee of the same institute. Informed consent was obtained from all patients before study entry.

Patient selection and drug administration

Stage III and IV breast cancer patients who had received a combination treatment of epirubicin and docetaxel as first-line chemotherapy were eligible for the pharmacokinetic investigations. Other eligibility criteria were a performance status of < 1, no other serious medical or psychiatric illness that would preclude intensive treatment, and informed consent. All patients had to have normal bone marrow, liver and kidney functions.

Chemotherapy consisted of epirubicin as an i.v. bolus followed immediately by docetaxel given over 1 h (ED sequence), or the reverse, i.e. docetaxel over 1 h followed immediately by epirubicin i.v. bolus (DE sequence). Drug doses consisted of epirubicin 75 mg/m^2 combined with docetaxel 80 mg/m^2, or epirubicin 90 mg/m^2 combined with docetaxel 70 mg/m^2. Cycles were repeated every 3 weeks. The pharmacokinetic analyses were performed in patients who received both sequences of drug administration at the first and second cycle of treatment; therefore, each patient acted as her own control. All patients received premedication therapy and antiemetic treatment as described previously [5]. Epirubicin, purchased as a sterile lyophilised powder in 50 mg vials, was dissolved in 25 ml normal saline, and administered i.v. over 10 min. Aventis (Milan, Italy) supplied docetaxel as a sterile lyophilised powder in 50 mg vials, was dissolved in 2 ml polysorbate 80 (Tween 80). Docetaxel was diluted in a 13% ethanol solution to a concentration of 10 mg/ml. The drug was diluted in 250 ml 5% dextrose and infused over 1 h immediately after the epirubicin injection.

Specimen collection and sample analyses

For plasma analyses of docetaxel, epirubicin and its metabolites, heparinised venous blood samples were collected before treatment and at various times thereafter. Plasma samples for epirubicin were obtained at 5, 15, 30 and 60 min and at 2, 3, 5 and 24 h following i.v. bolus. Plasma samples for docetaxel were obtained at 15, 30, 45, 60, 75 and 90 min, and 2, 3, 4, 6 and 24 h from the beginning of the taxane infusion. Blood samples were centrifuged immediately at room temperature, and plasma was separated and stored in aliquots at –20°C until analysis.

Concentrations of epirubicin and its metabolites in plasma were determined as described previously [3, 5] according to Maessen et al. [8].

The method proposed by Huizing et al. [9] for the paclitaxel assay was validated for the analysis of docetaxel. Three calibration curves, each constructed daily by triplicate chromatographic analyses of eight docetaxel standard points, ranging from 2000 to 8 ng/ml, were executed on different days. The recovery of docetaxel from plasma ranged from 85% to 95% and linear regression analysis between docetaxel standard concentrations and chromatographic responses provided a good linearity (\( r^2 \geq 0.998 \)). Within- and between-day accuracy and precision evaluated as relative mean error (RME%) and coefficient of variation (CV%) [10], respectively, were always lower than 10%. The lower limit of quantification for docetaxel, evaluated according to Shah et al. [10], was 15 ng/ml. Chromatographic analyses of six different plasma samples obtained from healthy subjects showed no interfering substance near to the elution zone of docetaxel.

All pharmacokinetic data were analysed by an integrated computer system (Siphar program, Simed, Creteil, France) on an IBM/IC computer. Values obtained by non-compartmental analysis (statistical moment theory) were considered, and the maximum peak plasma concentration (\( C_{\text{max}} \)) was put on par with the mean concentration in the plasma samples following drug administration. Because it was demonstrated that the pharmacokinetics of epirubicin is at least linear for doses up to 150 mg/m^2 [11] and that the pharmacokinetics of docetaxel is linear for doses up to 100 mg/m^2 given over 1 h by infusion [12], pharmacokinetic data were normalised for lower doses of each drug, i.e. 75 mg/m^2 for epirubicin and 70 mg/m^2 for docetaxel.

Toxicity

At the beginning of therapy, all patients underwent clinical evaluation, cardiac assessment with ECG and physical examination (including measurement of blood pressure, heart rate, evaluation of oedemas and presence of dyspnea), complete blood cell count (CBC) with white blood cells differential, and blood chemistries (blood urea nitrogen, creatinine, bilirubin, alkaline phosphatase, lactate dehydrogenase, total protein, albumin, glucose, transaminases, serum electrolytes). CBC was performed twice a week, and daily whenever absolute neutrophil count (ANC) was < 500/µl. At each cycle of chemotherapy, clinical examination, biochemical profile and toxicity evaluation were performed. Toxicity was graded according to the National Cancer Institute Common Toxicity Criteria.

Statistical analysis

Pharmacokinetic data were compared using the nonparametric Wilcoxon matched-pairs signed-ranks test for paired data. A probability of \( P < 0.05 \) was considered significant. The program SPSS for Windows (Version 5.0.1) was used for the statistical analysis.

Results

Twelve consecutive patients were included in this study. Patient characteristics are reported in Table 1. Eight patients received the ED sequence and four received the DE sequence at the first cycle of chemotherapy. All patients received the opposite sequence at the second cycle. Three patients received haematopoietic growth factor (G-CSF) support from the first cycle of chemotherapy.

Pharmacokinetics

Plasma exposure to the anthracycline was similar in the two sequences of treatment (Figure 1) and the sequence of drug administration had no influence on the pharmacokinetic parameters of epirubicin (Table 2). Mean plasma concentration–time curves of epirubicinol (EOL) and 7-deoxydoxorubicinone (7d-Aone) in patients treated with the ED or DE sequence were superimposable and no difference was evident in the \( C_{\text{max}} \) or AUC_{0–24 h} values of these metabolites (data not shown). A clear sequence-dependent effect was evident in the metabolic pathway of glucuronidation. When patients were treated with the DE sequence a lower level of glucuronidation metabolism was found. Mean plasma concentration–time curves of epirubicinol-glucuronide (EOL–glu) and epirubicin-
glucuronide (E-glu) showed lower formation of both these metabolites when docetaxel was administered as the first drug. The \( C_{\text{max}} \) of EOL-glu and E-glu were 1.6- and 2.1-fold higher, respectively, in the ED sequence than in the DE sequence. The AUC\(_{0–24\ h}\) of EOL-glu and of E-glu were also higher in the patients treated with the ED sequence (Figure 2A and B).

No sequence dependence was observed in the peak plasma concentrations of docetaxel (Figure 3; Table 3) as well as in docetaxel total body clearance and elimination half-life. Although the AUC\(_{0–24\ h}\) of docetaxel increased by 23% on average when it was administered as the first drug, the difference was not statistically significant.

### Toxicity

Haematological toxicity was recorded at nadir. Thrombocytopenia was rarely reported. Three patients in the DE sequence and two patients in the ED sequence suffered from it. The median platelets count at nadir was \( 142 \times 10^3/\mu l \) (range 53–175), and \( 154 \times 10^3/\mu l \) (range 24–231) in the ED and DE sequences, respectively. Neutropenia was the most prominent type of haematological toxicity. Ten patients in the ED sequence and nine patients in the DE sequence had grade 4 neutropenia. ANC at nadir had a median value of 110/µl (range 0–980) and 150/µl (range 0–1100) in the ED and DE sequences, respectively. Febrile neutropenia occurred in one patient in the ED sequence.

Non-haematological toxicity was mild or moderate, and no grade III or IV toxicity was recorded. Three patients who received the ED sequence had grade II toxicity (one mucositis, one nausea and one fever) and three patients who received the DE sequence had grade II asthenia.

### Discussion

Combinations of cytotoxic drugs are used clinically for the therapeutic advantage they may provide over that of single agents. Although combination therapy with taxanes and anthracyclines has been reported to be highly effective in breast cancer [13–16], pharmacological interactions between these drugs may be clinically important. Therefore, optimal schedule administration and drug dosing remain the focus of clinical investigation. This study was performed to explore the influence of alternate sequences of epirubicin and docetaxel on the pharmacokinetic behaviour of both drugs.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sequence</th>
<th>Epirubicin → docetaxel</th>
<th>Docetaxel → epirubicin</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( C_{\text{max}} ) (µg/ml)</td>
<td>1.3 ± 0.5*</td>
<td>1.5 ± 0.4</td>
<td></td>
<td>0.57</td>
</tr>
<tr>
<td>( t_{1/2\text{,elim}} ) (h)</td>
<td>12.4 ± 2.2</td>
<td>11.7 ± 2.2</td>
<td></td>
<td>0.35</td>
</tr>
<tr>
<td>AUC(_{0–24\ h}) (h·µg/ml)</td>
<td>1.3 ± 0.4</td>
<td>1.3 ± 0.5</td>
<td></td>
<td>0.81</td>
</tr>
<tr>
<td>( CL_{TB} ) (l/h/m²)</td>
<td>60.6 ± 17.4</td>
<td>57.4 ± 17.1</td>
<td></td>
<td>0.75</td>
</tr>
</tbody>
</table>

AUC, area under the curve; \( CL_{TB} \), total body clearance; \( C_{\text{max}} \), maximum peak plasma concentration; \( t_{1/2\text{,elim}} \), elimination half-life.

*Values are expressed as mean ± SD.
No change in the pharmacokinetic parameters of epirubicin was found when the anthracycline was administered immediately before or after docetaxel. This suggests that the sequence of drug administration had no influence on the systemic exposure to parent epirubicin. In addition, the observed pharmacokinetic parameters of its metabolites EOL and 7d-Aone were similar for both sequences. However, evidence of a sequence-dependent effect on the epirubicin glucuronidation pathway was observed. The administration of docetaxel as the first drug resulted in a clear decrease in both EOL-glu and E-glu plasma concentrations compared with the opposite sequence. At present, the significance of this observation is unclear and the clinical relevance of epirubicin glucuronidation remains an open question. In a phase II study using epirubicin alone or in

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<tr>
<td>$C_{\text{max}}$ (µg/ml)</td>
<td>1.8 ± 0.8*</td>
<td>2.1 ± 0.7</td>
<td>0.29</td>
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<tr>
<td>$t_{1/2 \text{elim}}$ (h)</td>
<td>10.7 ± 2.6</td>
<td>11.2 ± 2.8</td>
<td>0.58</td>
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<tr>
<td>AUC$_{0–24\text{h}}$ (h·µg/ml)</td>
<td>2.3 ± 0.6</td>
<td>2.8 ± 1.2</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>$CL_{\text{TB}}$ (l/h/m²)</td>
<td>32.6 ± 9.8</td>
<td>28.6 ± 11.4</td>
<td>0.35</td>
<td></td>
</tr>
</tbody>
</table>

AUC, area under the curve; $CL_{\text{TB}}$, total body clearance; $C_{\text{max}}$, maximum peak plasma concentration; $t_{1/2 \text{elim}}$, elimination half-life. *Values are expressed as mean ± SD.
combination with verapamil, Mross et al. [17] reported differences in the glucuronidation metabolic pathway. However, the enhanced glucuronidation observed following combination therapy of epirubicin and verapamil was not associated with changes in the pharmacokinetics of epirubicin, and the clinical response to the two treatments was similar both in terms of objective response rate and toxicity. In a retrospective study on 48 patients with various malignancies, Robert et al. [18] did not find any correlation between low plasma levels of epirubicin glucuronides and high AUC values for epirubicin. However, the same authors observed that a low rate of epirubicin glucuronidation was correlated with a lower percentage change in granulocytes and with a better tumour response, but they had no explanation for these findings [18]. Because the administration of either paclitaxel or docetaxel as first drug resulted in a lower metabolic conversion of epirubicin to its glucuronide metabolites, but the plasma concentration of the parent drug was influenced only by paclitaxel administration [5], a relationship would be questionable between this metabolic pathway and the plasma exposure to the parent anthracycline. Some differences between docetaxel and paclitaxel in their interactions with epirubicin exist, and these could be due to the different vehicles used (cremophor or polysorbate) to dissolve, respectively, paclitaxel or docetaxel.

No sequence-dependent effect was observed in the pharmacokinetics of paclitaxel after combined treatment with epirubicin [3]. In addition, no sequence-dependent effect was observed in the pharmacokinetics of docetaxel after combined treatment with epirubicin. This result is similar to that reported by Itoh et al. [19], who studied the sequence-dependent effects of doxorubicin administration on docetaxel pharmacokinetics. The authors did not find any significant change in the pharmacokinetics of the taxane when administered either before or after the anthracycline [19]. Noteworthy, the same authors had also reported that MTD in the sequence doxorubicin after docetaxel were 40 mg/m² and 50 mg/m², respectively, while in the switched sequence MTD were 50 mg/m² and 70 mg/m², respectively. Because of the lack of pharmacokinetic interaction between the two drugs, the different pharmacodynamic effects due to the sequence of drug administration, for instance modifications of the cell cycle and of apoptotic cell death [20, 21], may explain the differences in toxicity reported. In our study, the reliability of clinical data is limited by the low number of patients. None the less, we did not find any significant difference in the haematological and non-haematological toxicity between the two sequences.

In summary, we studied the influence of the sequence of drug administration on the pharmacokinetics of docetaxel and of epirubicin and its metabolites. Parent drugs were not affected by the different modes of administration, while lower plasma concentrations of epirubicin glucuronides were observed when docetaxel was administered as the first drug. Because the haematological and non-haematological toxicities were similar in the two sequences of drug administration, this difference in epirubicin metabolism seems not to have any clinical relevance.

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References