Pharmacokinetics and Metabolism of Epidoxorubicin and Doxorubicin in Humans

By K. Mross, P. Maessen, W.J.F. van der Vijgh, H. Gall, E. Boven, and H.M. Pinedo

Pharmacokinetics of doxorubicin (DOX), epidoxorubicin (EPI), and their metabolites in plasma have been performed in eight patients receiving 40 to 56 mg/m² of both anthracyclines as a bolus injection in two sequential cycles. Terminal half-life and volume of distribution appeared to be smaller in case of EPI, whereas plasma clearance and cumulative urinary excretion was larger in comparison to DOX. The major metabolite of DOX was doxorubicinol (Aol) followed by 7-deoxy-doxorubicinol (7d-Aolon). Metabolism to glucuronides was found in case of EPI only. The area under the curves (AUC) of the metabolites of EPI decreased in the order of the glucuronides E-glu > Eol-glu > 7d-Aolon > epirubicinol (Eol). The AUC of Eol was half of the value in its counterpart Aol. In the case of EPI, the AUC of 7d-Aolon was twice the level of that of the corresponding metabolite of DOX. The terminal half-lives of the cytostatic metabolites Aol and Eol were similar, but longer than the corresponding values of their parent drugs. Half-lives of the glucuronides (E-glu, Eol-glu) were similar to the half-life of their parent drug. 7d-Aolon had a somewhat shorter half-life in comparison to both DOX and EPI. Approximately 6.2% of EPI and 5.9% of DOX were excreted by the kidney during the initial 48 hours. Aol was found in the urine of patients treated with DOX, whereas Eol, E-glu, and Eol-glu were detected in urine of patients treated with EPI. The cumulative urinary excretion appeared to be 10.5% for EPI and its metabolites, and 6.9% for DOX and its metabolite. The plasma concentration vs time curves of (7d)-aglycones showed a second peak between two and 12 hours after injection, suggesting an enterohepatic circulation for metabolites lacking the daunosamine sugar moiety. The plasma concentrations of the glucuronides were maximal at 1.2 hours for E-glu and 1.9 hours for Eol-glu. All other compounds reached their maximum plasma concentration during the first minutes after the administration of DOX and EPI. Deviating plasma kinetics were observed in one patient, probably due to prior drug administration.


Epidoxorubicin (EPI; epirubicin Pharmarubicin [Farmitalia Carlo Erba, Milan, Italy]), the 4'-epimer of doxorubicin (DOX; Adriamycin [Farmitalia Carlo Erba]) (Fig 1), is one of the analogs presently being studied in phase II and III trials. 1-3

From preclinical studies with EPI it was concluded that differences in therapeutic and toxicologic manifestations exist between EPI and DOX, reflecting apparent alterations in pharmacologic properties and possible mode of action. 4 A comprehensive review of clinical activity and adverse effects of EPI was published recently in the Journal of Clinical Oncology. 5

Search for anthracycline analogs is necessary because clinical use of DOX is hampered by unfavorable side effects. Furthermore, human tumors of major importance, such as pancreatic cancer and lung cancer, are not generally responsive to DOX. One of the major drawbacks of DOX treatment is its cardiotoxic effect. The incidence of congestive heart failure (CHF) is 5% at a cumulative dose of 550 mg/m² and 800 to 900 mg/m² of DOX and EPI, respectively. 6,7

The differences between DOX and EPI recorded for preclinical and clinical pharmacological behavior are probably attributed to differences in tissue distribution, metabolism, and pharmacokinetics. EPI and DOX do not differ substantially in their affinity to double-stranded DNA, most likely the main biological target of these two anthracyclines. 8

Human metabolism of DOX involves carbonyl reduction by aldo-keto reductase, the major enzymatic conversion, 9 as well as reductive glyco-

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sidic and hydrolytic glycosidic cleavage. Molecular structures of the metabolites are shown in Fig 2. The main metabolite doxorubicinol (Aol), maintains anticancer activity, whereas the aglycones are not active.10

The pharmacokinetics of DOX using different schedules is well documented.11,12 The 4'-glucuronides, only present as metabolites in case of EPI, were first described by Weenen et al.13 By that time, pharmacokinetic studies of EPI and its metabolites were hampered by low recoveries of the very polar glucuronides due to existing liquid-liquid extraction procedures.14 This problem was solved by the introduction of a liquid-solid extraction procedure for anthracyclines.15,16,17 However, description of pharmacokinetic data for EPI has been incomplete with respect to all known metabolites up to now.

A pharmacokinetic study of DOX and EPI in patients with advanced cancer, designed as a crossover study, was implemented to describe the pharmacokinetic behavior of both drugs and their metabolites. The results of this study and their relationship to suggested modes of action are discussed.

PATIENTS, MATERIALS, AND METHODS

Treatment Schedule

EPI and DOX were obtained as a sterile lyophilized powder (50 mg/vial, Farmitalia, Carlo Erba SpA, Italy). The drug was reconstituted with 25 mL sterile water (United States Pharmacopeia). The prescribed dose of DOX or EPI was administered subsequently within one to two minutes through the line of rapid saline infusion. The pharmacokinetic study was performed in patients receiving 40 to 56 mg/m² DOX or EPI in a crossover design with an interval of 3 weeks.

Eight female patients, not previously treated with anthracyclines, were included in the study after informed consent was obtained. All patients had advanced disease requiring treatment with anthracyclines either as single agent, in combination with fluorouracil and cyclophosphamide for breast cancer, or in combination with mitomycin for adenocarcinoma of unknown primary origin. During the 48-hour sampling period no other cytostatic agents were administered. Thereafter, anticancer treatment was continued according to the treatment protocol.

Patient characteristics and baseline laboratory values are summarized in Table 1. All patients had total bilirubin levels < 15 μmol/L and a normal serum creatinine. Three patients had metastatic lesions in the liver. Only one patient (J.O.) had been
treated with chemotherapy (cyclophosphamide, methotrexate, fluourouracil) and hormonal therapy (aminogluthethimide, hydrocortisone) before.

**Blood and Urine Samples**

Blood samples of 10 mL were obtained at −5, 0, 5, 10, 15, 30, 60 minutes, and 2, 4, 6, 9, 12, 24, 36, and 48 hours after bolus injection. Blood was collected in heparinized (150 IU Li-heparine) glass tubes (Terumo, Leuven, Belgium) and immediately centrifuged at 4°C, 4,000 g for 15 minutes. The plasma was transferred to polystyrene tubes. Urine samples were obtained in portions of 6 hours over 48 hours. Plasma and urine samples were maintained at −20°C until analysis. After thawing, all samples were sonicated and centrifuged at 4°C for five minutes with 4,000 g to remove clotted material.

**High-Performance Liquid Chromatography Analysis**

The high-performance liquid chromatography (HPLC) assay used for detection and quantification of the two parent drugs and their metabolites was recently developed by Maessen et al.17 Briefly, the chromatographic system consisted of a WISP 710B injection system, a model 6000A solvent delivery system, and a data module with system controller 720 (Waters, Etten-Leur, The Netherlands). This system was provided with a stainless steel HPLC column (4.6 × 100 mm, 3 µm CP MicroSph, Chrompack, Middelburg, The Netherlands) including a guard column (4 × 4 mm, 5 µm LiChrosorb RP-18, Merck, Amsterdam) and a F-1000 fluorescence detector (excitation wave length, 480 nm; emission wavelength, 580 nm) from Merck-Hitachi, Amsterdam. An isocratic eluent was used consisting of 0.02 mol/L NaH₂PO₄ pH 4/acetonitrile (2.5/1 v/v) at a flow rate of 1 mL/min. DOX, EPI, and their metabolites were extracted from human plasma using C-18 Sep-Pak cartridges (Waters), pretreated with 5 mL methanol, 5 mL H₂O, and 5 mL buffer (0.02 mol/L NaH₂PO₄ pH 4/acetonitrile 9/1, v/v). One milliliter plasma was injected onto the cartridge, subsequently purged with 2 mL buffer, dried with a flow of air, and eluted with methanol/chloroform (3/1 v/v). The eluate was evaporated to dryness at 50°C under a stream of air. The residue was redissolved in 50 µL buffer, of which 30 µL was injected onto the analytical column. EPI was added as internal standard (2.5 × 10⁻⁸ mol/L) to all plasma samples containing DOX and its metabolites, whereas DOX was added (2.5 × 10⁻⁸ mol/L) to all samples containing EPI and metabolites. Samples were prepared in duplicate. Six spiked plasma samples containing the two drugs and their metabolites (see Fig 2) (5 × 10⁻¹⁰ to 5 × 10⁻⁸ mol/L) were included in each series to construct a within-run standard line. A typical chromatogram of a spiked blank plasma sample is shown in Fig 3. The detection limit of the assay ranged from 1 × 10⁻¹⁰ mol/L for Aolon to 4 × 10⁻¹⁰ mol/L for 7d-Aon and DOX. The other compounds could be detected within this range. Plasma samples were diluted with blank heparinized plasma from a blood donor. For the first two samples

![Fig 3. Typical chromatogram after extraction of a plasma sample spiked with DOX and EPI and their metabolites and separation of all compounds. The peaks in front of Eol-glu (hatched area) represent co-extracted plasma material with fluorescent properties.](image-url)
(t = 0 and 5 minutes) various dilutions were prepared in order to determine all metabolites as accurately as possible because of the large differences in drug and metabolite concentrations. The urine samples were sonicated after pH adjustment to 2.5 and subsequently centrifuged. After addition of an external standard (DOX, respectively, EPI), samples were directly injected onto the column.

**Pharmacokinetic Analysis**

Each set of concentration time [c(t)] values of DOX and EPI in plasma was fitted to the appropriate polyexponential equation using the program, NONLIN. The final decision to describe the results according to a three compartment model was performed by AUTO AN. The r² of NONLIN least-squares fit was always better than .999, with one exception (Patient J.O., r² = .97). All fittings were performed to the three exponential equation:

\[ c_p = A \times e^{-\alpha t} + B \times e^{-\beta t} + C \times e^{-\gamma t} \]

The pharmacokinetic parameters for the parent drugs were calculated using the following equations:

1. AUC = \( \frac{A}{\alpha} + \frac{B}{\beta} + \frac{C}{\gamma} \) (nmol \times L⁻¹ \times min);
2. \( \text{Cl}_p = D \times \text{AUC} \) (L \times min⁻¹) (normalized to 1.74 m²);
3. \( V_d = D \times \frac{\alpha^2 + \beta^2 + \gamma^2}{\text{AUC}^2} \) (L) (normalized to 1 kg);
4. \( t_{1/2} = \frac{0.693}{k} \times 60 \) (h) (k = \( \alpha, \beta, \gamma \)).

The abbreviations used are AUC, area under the curve; Clₚ, plasma clearance; \( V_d \), volume of distribution; D, dose; \( t_{1/2} \), half-life; and k, rate constant. The terminal half-lives of DOX and EPI were also analyzed by the curve-stripping method using all time points from four hours onward. Additionally, the AUCs of the parent drugs and their metabolites were determined by means of the trapezoidal method because of irregular c(t) curves of the metabolites, not allowing calculations according to a pharmacokinetic model. The terminal half-lives of DOX, EPI, and their metabolites were determined from their concentrations in the final plasma samples by least square fitting.

**RESULTS**

The following results represent summarized data from seven of eight patients studied. All pharmacokinetic data from one patient (J.O.) and \( T_{1/2}^\gamma \) of second patient (V.R.) were omitted from the calculation of mean values because the data differed < 2 standard deviations from the mean.

Pharmacokinetic parameters for the parent drugs, calculated according to the formulas 1 through 5 described in the previous section, are shown in Tables 2 and 3. Besides the calculations with the NONLIN program, the terminal half-life was also calculated by curve-stripping. This procedure allowed a consequent calculation of all final half-lives over an interval of four to 48 hours and may, therefore, provide additional information about interpatient and interdrug variation. The disappearance of both parent drugs was triphasic. The half-lives calculated for DOX were always longer than those calculated for EPI. The mean volume of distribution was larger in the case of DOX when compared with EPI, while the plasma clearance (normalized to 1.74 m² body surface area) was slower in the case of

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### Table 2. Calculated Pharmacokinetic Parameters of DOX in Eight Patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>PPC⁺ (mol/L × 10⁶)</th>
<th>AUC (nmol min⁻¹ × 10⁻⁴)</th>
<th>( Cl_p ) (L/h)</th>
<th>( V_{du} ) (L/kg)</th>
<th>( t_{1/2\alpha} ) (h)</th>
<th>( t_{1/2\beta} ) (h)</th>
<th>( t_{1/2\gamma} ) (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>J.O.</td>
<td>14.06</td>
<td>96.65</td>
<td>9.83</td>
<td>6.59</td>
<td>0.167</td>
<td>1.49</td>
<td>48.21</td>
</tr>
<tr>
<td>K.U.</td>
<td>11.5</td>
<td>19.7</td>
<td>48.38</td>
<td>31.14</td>
<td>0.062</td>
<td>3.02</td>
<td>48.4</td>
</tr>
<tr>
<td>R.O.</td>
<td>16.0</td>
<td>16.5</td>
<td>53.99</td>
<td>14.05</td>
<td>0.050</td>
<td>0.30</td>
<td>24.1</td>
</tr>
<tr>
<td>S.T.</td>
<td>12.0</td>
<td>9.8</td>
<td>87.08</td>
<td>20.37</td>
<td>0.017</td>
<td>0.07</td>
<td>17.6</td>
</tr>
<tr>
<td>L.A.</td>
<td>14.9</td>
<td>13.0</td>
<td>65.46</td>
<td>14.60</td>
<td>0.046</td>
<td>0.22</td>
<td>21.4</td>
</tr>
<tr>
<td>B.O.</td>
<td>16.5</td>
<td>14.9</td>
<td>60.38</td>
<td>12.29</td>
<td>0.029</td>
<td>0.09</td>
<td>18.7</td>
</tr>
<tr>
<td>B.A.</td>
<td>10.0</td>
<td>8.3</td>
<td>87.46</td>
<td>31.24</td>
<td>0.034</td>
<td>0.17</td>
<td>24.8</td>
</tr>
<tr>
<td>V.R.</td>
<td>24.0</td>
<td>43.0</td>
<td>20.11</td>
<td>44.63</td>
<td>0.047</td>
<td>1.68</td>
<td>157.85</td>
</tr>
<tr>
<td>Mean</td>
<td>15.0</td>
<td>17.9</td>
<td>60.40</td>
<td>24.00</td>
<td>0.041</td>
<td>0.79</td>
<td>25.8</td>
</tr>
<tr>
<td>± SD</td>
<td>4.7</td>
<td>11.7</td>
<td>23.40</td>
<td>12.00</td>
<td>0.020</td>
<td>1.13</td>
<td>11.4</td>
</tr>
</tbody>
</table>

**NOTE.** Explanation of the calculated parameters in Pharmacokinetic Analysis section.

*Peak plasma concentrations normalized to 50 mg/m².
†Plasma clearance normalized to 1.74 m².
‡Calculations with curve stripping method from four hours onward.
§Omitted for calculation of the mean.
DOX than in the case of EPI. The AUC of DOX was larger than it was in the case of EPI.

DOX and EPI were rapidly metabolized. Immediately after bolus injection all metabolites were detectable. As shown in Tables 2 and 3, peak plasma concentrations (PPC) (normalized to 50 mg/m²) ranged from 10 × 10⁻⁶ mol/L up to 24 × 10⁻⁷ mol/L for DOX, and 5.5 × 10⁻⁶ mol/L to 32 × 10⁻⁶ mol/L for EPI-treated patients. The mean PPC (normalized to 50 mg/m²) for the glucuronides was 4.7 ± 3.2 × 10⁻⁷ mol/L E-glu occurring after 1.2 ± 0.6 hours, and 1.8 ± 2.3 × 10⁻⁷ mol/L Eol-glu occurring after 1.9 ± 1.1 hours (Table 4).

Because of the irregular plasma concentration vs time curves of the metabolites, it was not possible to fit these curves with exponential terms.

Table 4. Peak Plasma Levels of the Glucuronides E-glu and Eol-glu and Time Points of Peak

<table>
<thead>
<tr>
<th>Patient</th>
<th>E-glu (× 10⁻⁷ mol/L)</th>
<th>Time (h)</th>
<th>Eol-glu (× 10⁻⁷ mol/L)</th>
<th>Time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>J.O.</td>
<td>5.63</td>
<td>2</td>
<td>4.10</td>
<td>6</td>
</tr>
<tr>
<td>K.U.</td>
<td>11.00</td>
<td>1</td>
<td>1.35</td>
<td>2</td>
</tr>
<tr>
<td>R.O.</td>
<td>5.00</td>
<td>2</td>
<td>0.70</td>
<td>4</td>
</tr>
<tr>
<td>S.T.</td>
<td>4.40</td>
<td>1</td>
<td>0.75</td>
<td>1</td>
</tr>
<tr>
<td>L.A.</td>
<td>2.40</td>
<td>2</td>
<td>0.95</td>
<td>2</td>
</tr>
<tr>
<td>B.O.</td>
<td>7.00</td>
<td>1</td>
<td>1.10</td>
<td>1</td>
</tr>
<tr>
<td>B.A.</td>
<td>3.38</td>
<td>0.5</td>
<td>1.16</td>
<td>1</td>
</tr>
<tr>
<td>V.R.</td>
<td>5.35</td>
<td>1</td>
<td>0.63</td>
<td>2</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>4.7 ± 3.2</td>
<td>1.2 ± 0.6</td>
<td>1.8 ± 2.3</td>
<td>1.9 ± 1.1</td>
</tr>
</tbody>
</table>

However, it was feasible to calculate the terminal half-lives of the metabolites from 12 hours onward (Table 5). The longest half-life was found in case of Aol and epiurubicin (Eol). The shortest half-life was calculated for 7d-Aon. The half-lives of the two glucuronides were similar to that of their parent drug. Half-lives for Aon and Aolon could not be determined accurately.

Representative plasma decay curves for DOX, EPI, and their metabolites are illustrated in Figs 4 and 5, respectively. AUCs (0 to 48 hours) for the parent drugs and their metabolites, normalized to 50 mg/m², calculated with the trapezoidal rule are listed in Table 6. It can be deduced that Aol and 7d-Aolon represent the major metabolites in case of DOX. In case of EPI, E-Glu, the glucuronidated parent drug, is more prominent. The AUC of this metabolite reached nearly twice the value of its parent drug (EPI) and gave rise to a doubling of the total AUC due to EPI compared with DOX. The AUC for Eol was only half of

Table 5. Terminal Half-Lives for the Metabolites of DOX and EPI

<table>
<thead>
<tr>
<th>Compounds</th>
<th>DOX (h)</th>
<th>EPI (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOX/EPI</td>
<td>28.3 ± 2.8</td>
<td>19.0 ± 2.4</td>
</tr>
<tr>
<td>Aol/Eol</td>
<td>32.8 ± 1.7</td>
<td>31.5 ± 6.0</td>
</tr>
<tr>
<td>7d-Aolon</td>
<td>16.8 ± 6.3</td>
<td>17.5 ± 4.9</td>
</tr>
<tr>
<td>7d-Aon</td>
<td>—</td>
<td>13.9 ± 5.2</td>
</tr>
<tr>
<td>Eol-glu</td>
<td>—</td>
<td>18.3 ± 4.0</td>
</tr>
<tr>
<td>E-glu</td>
<td>—</td>
<td>18.6 ± 2.1</td>
</tr>
</tbody>
</table>
Fig 4. Typical concentration v time curves of DOX and its metabolites in patient S.T. receiving 50 mg/m² as an IV bolus injection.

that of the corresponding metabolite Aol. The AUCs for the 7-deoxy-agnostic were always higher in case of EPI and most pronounced in case of 7d-Aolon. The aglycones, especially 7d-Aolon, behaved irregularly in terms of their plasma concentrations v time. Highest concentrations were found immediately after injection followed by a fast decrease immediately thereafter. Invariably, a second peak could be detected at two to 12 hours after drug administration.

Fig 5. Typical concentration v time curves of EPI and its metabolites in patient B.A. receiving 40 mg/m² as an IV bolus injection.

Table 6. Areas Under the Plasma C(t) Curve (0 to 48 Hours) of Dox, EPI, and Metabolites ([nmol-min·10⁻⁴]/L) Normalized to a Dose of 50 mg/m²

<table>
<thead>
<tr>
<th>Metabolites</th>
<th>Dox</th>
<th>EPI</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOX/EPI</td>
<td>13.25 ± 2.60</td>
<td>11.34 ± 3.30</td>
</tr>
<tr>
<td>Aol/Eol</td>
<td>4.56 ± 1.56</td>
<td>2.31 ± 1.05</td>
</tr>
<tr>
<td>7d-Aolon</td>
<td>1.84 ± 0.88</td>
<td>4.35 ± 2.44</td>
</tr>
<tr>
<td>Aolon</td>
<td>0.18 ± 0.19</td>
<td>0.23 ± 0.19</td>
</tr>
<tr>
<td>7d-Aon</td>
<td>0.59 ± 0.31</td>
<td>0.93 ± 0.61</td>
</tr>
<tr>
<td>Aon</td>
<td>0.28 ± 0.26</td>
<td>0.03 ± 0.04</td>
</tr>
<tr>
<td>Eol-glu</td>
<td>—</td>
<td>4.36 ± 1.54</td>
</tr>
<tr>
<td>E-glul</td>
<td>—</td>
<td>20.04 ± 10.9</td>
</tr>
<tr>
<td>Total AUC</td>
<td>20.70</td>
<td>43.59</td>
</tr>
</tbody>
</table>

The cumulative urinary excretion (0 to 48 hours) was similar for both unchanged drugs. Aol and Eol were detectable, but not in significant amounts. Eol-glu and E-glul were excreted into the urine. Eol-glu did not contribute much to the excretion of EPI as a metabolite, whereas the urinary excretion of E-glul was remarkable (see Table 7).

One patient (J.O.) showed a deviating pharmacokinetic pattern (Tables 2 and 3). For this patient, the plasma v time curves for DOX and EPI including their metabolites are shown in Figs 6 and 7. Comparison of these figures with Fig 4 and 5, as well as the calculated pharmacokinetic parameters from Tables 2 and 3, show that most parameters were severely affected. All half-lives were longer, the AUCs of DOX and EPI were higher, and the plasma clearance was much lower. The AUCs of nearly all metabolites were higher, but not uniformly with a constant factor. Factors ranged from 3.5 (E-glul) up to 15 times (Eol-glul) higher than normal. The differences between the AUCs of the (7d-)aglycones were of minor significance. The cumulative urinary excretion was significantly lower than normal. This patient featured not only deviations in the phar-

Table 7. Cumulative Urinary Excretion of EPI/DOX and Their Metabolites in Percent of the Dose (0 to 48 Hours)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dox</th>
<th>EPI</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOX/EPI</td>
<td>5.9 ± 1.6</td>
<td>6.2 ± 1.5</td>
</tr>
<tr>
<td>Aol/Eol</td>
<td>1.0 ± 0.3</td>
<td>0.6 ± 0.3</td>
</tr>
<tr>
<td>Eol-Glu</td>
<td>—</td>
<td>0.4 ± 0.2</td>
</tr>
<tr>
<td>E-glul</td>
<td>—</td>
<td>3.3 ± 1.2</td>
</tr>
<tr>
<td>Total</td>
<td>6.9</td>
<td>10.5</td>
</tr>
</tbody>
</table>
PHARMACOKINETICS OF DOXORUBICIN/EPIDOXORUBICIN

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**Pharmacokinetics of the Parent Drugs, but also Significant Changes in Metabolism.**

**DISCUSSION**

Although EPI and DOX differ only in the orientation of the 4'-hydroxy group in the sugar moiety, this structural modification appears to be critical as pharmacokinetics and metabolism of the two drugs differ greatly. Our present study concerned a comparison of the pharmacokinetics of DOX and EPI in eight patients with advanced cancer.

The plasma elimination of DOX and EPI appears to be triphasic after intravenous (IV) bolus injection, which is in agreement with previously reported data. However, contrary to earlier reports, we failed to detect exceptionally large interindividual variations.

Our group was the first to report on pharmacokinetics and metabolism of EPI, showing the ability of EPI and Eol to conjugate with glucuronic acid. However, the analytical procedure was not totally satisfactory at that time with regard to a relatively low extraction efficiency and the assumption that fluorescence properties of parent drug and all metabolites were identical. Our present technique has a high recovery for all metabolites, while each compound has been quantitated with a highly sensitive fluorescence detector using individual calibration lines for each of the compounds. In a study investigating the property of EPI to undergo glucuronidation, our group proposed a causal relationship between this characteristic of the analog and the differences with the parent drug with respect to pharmacokinetics and toxicity.

The half-life of distribution was shown to be extremely short for both EPI and DOX, while in all patients half-lives for DOX were longer than for EPI, being consistent with the results reported by Camaggi et al.

EPI and DOX appeared to be distributed into a deep tissue compartment from which they were slowly released. Elimination of DOX was slower than that of EPI, which may be explained by the unique glucuronidation of EPI.

The terminal half-lives of Aol and Eol are longer, whereas half-lives of the other metabolites were similar or shorter than those of the respective parent drugs. It is known that Aol and probably Eol are cytotoxic and may greatly con-
tribute to myelotoxicity and cardiotoxicity. While the degree of cytotoxicity of the glucuronides is unknown, the aglycones were reported to lack antitumor activity. Robert et al[26] reported terminal half-lives for DOX, EPI, Aol, Eol, Eol-glu, and E-glu which generally corresponded with our results, although slightly shorter, which may be related to the delayed administration of 5-fluorouracil and cyclophosphamide at two hours after the anthracyclines.

In contrast to previous reports, the major metabolite E-glu appeared to have an AUC almost twice as high as that of EPI. In addition, two prominent metabolites emerging in the case of EPI were Eol-glu and 7d-Aolon. In the case of DOX, Aol was most prominent, whereas all other metabolites were of minor importance. A remarkable difference between the metabolism of the two drugs was observed by the formation of 7d-Aolon. In the case of EPI, the AUC of the latter metabolite was twice as high as that of the corresponding metabolite of DOX.

Except for the glucuronides, all metabolites achieved their maximum plasma concentration within the first minutes after bolus injection, indicating a rapid metabolism of DOX and EPI. Glucuronides reached their peak concentrations on average at 1.2 hours in the case of E-glu, and at 1.9 hours in the case of Eol-glu. Deviant distribution and disposition may be expected of the polar glucuronides compared with the more apolar compounds, as glucuronides tend to be confined to the extracellular space. Assuming that biliary excretion is the major elimination pathway of such polar metabolites, EPI and Eol can be reabsorbed from the intestines due to the relatively high activity of β-glucuronidase in the intestines. Furthermore, DOX and EPI can undergo deglycosidation and reduction in the intestines, thereby promoting reabsorption and enterohepatic cycling of aglycones. This may explain the fact that the 7d-aglycones showed a second peak in the plasma c(t) curve after two to 12 hours, a phenomenon which has not been previously described in pharmacokinetics studies of EPI or DOX. However, it was found in rats and rabbits that DOX and its metabolites are not extensively reabsorbed from the small intestines.

Total excretion of drugs via urine, including metabolites, was higher for EPI than for DOX. These results are in agreement with our findings reported earlier.

In the case of liver function impairment with an elevated serum bilirubin level (a late sign), the dose of DOX should be reduced. One patient (not included in this series) with severe liver function impairment, receiving 15 mg/m^2 DOX, appeared unable to metabolize the drug. On the other hand, as shown in Tables 2 and 3 and in Figs 6 and 7, one heavily pretreated patient (J.O.) and two patients with liver metastases (K.U., V.R.) had pharmacokinetic parameters with longer half-lives and larger AUCs than the remaining patients. Consequently, no clear relationship between the degree of liver damage and impairment of elimination of EPI and DOX could be established.

In the case of J.O., the plasma elimination of DOX was delayed in each phase, while the concentration of Aol was even higher than that of DOX at four hours after drug administration. A similar observation was made for Eol at six hours after EPI administration. Glucuronide concentrations (Eol-glu and E-glu) were much higher than in the other patients, a finding that might be related to glucuronyl transferase induction. Induction of aldoketo-reductase may explain the higher concentrations of Eol and Aol. A third enzyme system, the cytochrome P-450 reductase, involved in the formation of (7d)-aglycones, seems to be reduced in this patient, because pharmacokinetic parameters indicate a delayed excretion of the drugs. This patient had been pretreated with tamoxifen, aminoglutethimide (AG) plus hydrocortisone, and later treated with chemotherapy consisting of cyclophosphamide, methotrexate, and 5-fluorouracil. Among these drugs AG has been shown to be a potential inducer of liver enzymes. Cyclophosphamide which is activated by liver cytosomal mixed function oxydases, is known to cause a decrease of the activity of several liver enzymes, i.e., the cytochrome P-450 content. In this respect it is of interest that an interaction between the metabolism of cyclophosphamide and DOX has recently been described. Therefore, drug-DOX/EPI interactions may have been responsible for the observed alterations in metabolism and pharmacokinetics in this patient. Considering the fact that biliary excretion of anthracyclines is an active, capacity-limited process, which may be
subjected to competitive inhibition, enhanced irregular pharmacokinetics may be observed in patients with apparent normal liver functions receiving concomitantly administered drugs.

There has been a growing tendency to associate anthracycline redox cycling and radical production with chronic cardiotoxicity. Redox chemistry includes the reductive cleavage of the glycoside bond from the hydroquinone which leads to the quinone-methide. Electrophilic and nucleophile substitution leads to the aglycones and 7d-aglycones, respectively. Pharmacokinetics of the (7d)-aglycones seems to be highly interesting in this respect. In contrast to Robert who stated that (7d)-aglycones are artifactual and if present only in negligible amounts, it is evident from our results and a recently published study, that in vivo formation of this metabolite can occur. Interestingly, the absolute concentration of 7d-Aolon is at least twice as high after EPI than after DOX administration, suggesting an inverse relationship between the generation of free radicals and the formation of 7d-aglycones.

The unique glucuronidation pathway and rapid elimination may partly explain the reduced cardiotoxicity caused by EPI. However, this cannot be the sole basis, because recently differences in cardiotoxicity have been found between the two anthracyclines in animals which, in contrast to human beings, did not generate glucuronides of EPI. Also, levels of 7d-aglycones and ratios of metabolite concentrations in plasma differed considerably between animals and humans. Because differences in cardiac damage between DOX and EPI are clearly quantitative and not qualitative in nature, it would be interesting to establish a relationship between the 7d-aglycone metabolism with the cardiotoxic effects.

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