INFLUENCE OF DOSE AND INFUSION DURATION ON PHARMACOKINETICS OF IFOSFAMIDE AND METABOLITES


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ABSTRACT:

The anticancer drug ifosfamide is a prodrug requiring activation through 4-hydroxyifosfamide to ifosforamide mustard, to exert cytotoxicity. Deactivation of ifosfamide leads to 2- and 3-dechloroethylifosfamide and the release of potentially neurotoxic chloracetaldehyde. The aim of this study was to quantify and to compare the pharmacokinetics of ifosfamide, 2- and 3-dechloroethylifosfamide, 4-hydroxyifosfamide, and ifosforamide mustard in short (1–4 h), medium (24–72 h), and long infusion durations (96–240 h) of ifosfamide. An integrated population pharmacokinetic model was used to describe the autoinducible pharmacokinetics of ifosfamide and its four metabolites in 56 patients. The rate by which autoinduction of the metabolism of ifosfamide developed was found to be significantly dependent on the infusion schedule. The rate was 52% lower with long infusion durations compared with short infusion durations. This difference was, however, comparable with its interindividual variability (22%) and was, therefore, considered to be of minor clinical importance. Autoinduction caused a less than proportional increase in the area under the ifosfamide plasma concentration-time curve (AUC) and more than proportional increase in metabolite exposure with increasing ifosfamide dose. During long infusion durations dose-corrected exposures (AUC/D) were significantly decreased for ifosfamide and increased for 3-dechloroethylifosfamide compared with short infusion durations. No differences in dose-normalized exposure to ifosfamide and metabolites were observed between short and medium infusion durations. This study demonstrates that the duration of ifosfamide infusion influences the exposure to the parent and its metabolite 3-dechloroethylifosfamide. The observed dose and infusion duration dependence should be taken into account when modeling ifosfamide metabolism.

Ifosfamide (Holoxan, Ifex) is an alkylating agent, which has been proven to be active against a number of solid tumors and hematological malignancies in adults and children. Currently, ifosfamide is mostly used in combination with other anticancer drugs, but also as a single agent. Many different infusion schedules are in use, e.g., continuous infusion over either 24, 72, or 240 h with doses of 5, 9, or 7.5–15 g/m² ifosfamide, respectively (Kaijser et al., 1996; Nielsen et al., 2000).

Ifosfamide is a prodrug, which needs activation by cytochrome P450 3–4A4 (CYP3A4) to 4-hydroxyifosfamide, as depicted in Fig. 1. Spontaneous decomposition of 4-hydroxyifosfamide yields the ultimate alkylating metabolite ifosforamide mustard and acrolein (Kerbusch et al., 2001). The alkylating activity of ifosforamide mustard is responsible for both the antitumor activity and hematological toxicity. Acrolein causes hemorrhagic cystitis, which can generally be prevented by mesna coadministration. Ifosfamide is deactivated to the noncytotoxic metabolites 2- and 3-dechloroethylifosfamide. Each dechloroethylation reaction yields an equimolar amount of chloracetaldehyde, which is held responsible for neurotoxicity observed in about 10% of all patients receiving conventional single-agent dosing of ifosfamide (Cerny and Küpfer, 1992). In addition, observed renal tubular abnormalities may also be correlated with the formation of chloracetaldehyde (Kerbusch et al., 2001). Chloracetaldehyde is very unstable. Therefore, assessment of 2- and 3-dechloroethylifosfamide exposure is preferred when quantifying the relationship between the pharmacokinetics and neurotoxicity after ifosfamide infusion.

Ifosfamide is subject to autoinduction, which leads to an increase in metabolism of ifosfamide over time (Kerbusch et al., 2001). Several comparative studies observed that fractionation of the ifosfamide dose leads to increased autoinduction (Lewis et al., 1990; Kerbusch et al., 2000b). Pharmacokinetic studies can provide further insight into the effect of the infusion schedule on the autoinduction of ifosfamide metabolism. Aim of this study is to quantify and to compare the pharmacokinetics of ifosfamide, 2- and 3-dechloroethylifosfamide, 4-hydroxyifosfamide, and ifosforamide mustard in short (1–4 h), medium (24–72 h), and long infusion durations (96–240 h) of ifosfamide. Assessment of pharmacokinetics will indicate whether the development of autoinduction is dependent on the dose and/or infusion duration, and may further aid in the design of ifosfamide infusion schedules.

Patients and Methods

Eligibility Criteria. Thirty-three patients received ifosfamide (Holoxan, ASTA Medica, Diemen, The Netherlands) as i.v. infusion as part of the regular...
therapy with ifosfamide as single agent or as combination chemotherapy with cisplatin, carboplatin, etoposide, Adriamycin, vincristine, or methotrexate, during the period of March 1997 and April 2000. None of these anticancer agents are known to induce CYP-mediated metabolism or are known to pharmacokinetically interact with ifosfamide. Typical infusion schedules used for ifosfamide were 1.2 g/m² once daily over 1, 3, or 4 h for three consecutive days, 1.5 g/m² once daily in 22.5 h for two consecutive days, 5 g/m² in 24 h once, and 9 g/m² by means of a 72-h continuous infusion. The study protocol was approved by the Ethics Board of the Antoni van Leeuwenhoek Hospital/The Netherlands Cancer Institute. Another group of 23 patients received a 10-day continuous infusion of single-agent ifosfamide using an ambulatory pump. This group was treated at the Leiden University Medical Center, in a study that was approved by the Ethics Board of the hospital (Kaijser et al., 1996). Supportive care consisted of mesna and extensive hydration to prevent hemorrhagic cystitis and bicarbonate to prevent acidosis. Standard 5-hydroxytryptamine3 blockers as antiemetics and methylene blue (a neurotoxicity antidote) were given when indicated (Cerny and Küpfer, 1992). Pharmacokinetic sampling was performed after written informed consent was obtained from the patients.

**Pharmacokinetic Sampling.** Blood samples were drawn before the start of, during, and after the end of the infusion at selected time points, depending on the infusion schedule. Ten milliliters of whole blood was collected in a lithium heparin-coated Vacutainer (Becton Dickinson, Plymouth, UK) and placed in ice water. The plasma was immediately separated by centrifugation at 1000g for 5 min at 4°C. The plasma was aliquoted in 4 volumes of which three were precisely 1-ml volumes. To two 1-ml volumes, 100 µl of 2 M semicarbazide solution, pH 7.4, was added to stabilize 4-hydroxyifosfamide. To another 1-ml volume, 100 µl of 1 M sodium chloride, 2 M semicarbazide solution, pH 8.0, was added to prevent 4-hydroxyifosfamide degradation to ifosfamide mustard and to stabilize the latter. The remaining plasma was used for ifosfamide, 2- and 3-dechloroethylifosfamide analysis. Urine was collected from the start of the (first) infusion until 24 h after the end of the (last) ifosfamide infusion and was analyzed for ifosfamide, 2- and 3-dechloroethylifosfamide. Both plasma and urine samples were stored at −70°C, pending analysis.

**Bioanalysis.** Gas chromatography with selective nitrogen-phosphorus detection was used for the determination of ifosfamide, 2- and 3-dechloroethylifosfamide in urine and plasma (Kerbusch et al., 2000c). Sample pretreatment consisted of alkaliniﬁed liquid-liquid extraction with ethyl acetate, transfer of the organic extract to another tube, evaporation to dryness, and subsequent reconstitution in ethyl acetate. This method was validated and proved to be specific, sensitive, accurate (93.3–104.5%), and precise (within and between day, 5.5%) within the concentration range of 0.192 to 383 µM, with a lower limit of quantification (LLQ) of 0.192 µM for ifosfamide, 2- and 3-dechloroethylifosfamide.

High-performance liquid chromatography (HPLC) was used for determination of 4-hydroxyifosfamide plasma levels (Kerbusch et al., 1998). In brief, this method determined the 4-hydroxyifosfamide-semicarbazone derivative in plasma. Sample pretreatment consisted of liquid-liquid extraction with ethyl acetate. The HPLC column used was reversed phase C8 with acetonitrile-0.025 M potassium dihydrogenphosphate (32:68 v/v) as mobile phase. Detection was performed at 230 nm. This method was specific, sensitive, accurate (94.1–107.9%), and precise (within and between day <7.2%) in the concentration range of 0.361 to 361 µM, with a LLQ of 0.361 µM 4-hydroxyifosfamide (measured as semicarbazone derivative).
FIG. 2. Pharmacokinetic model for ifosfamide metabolism describing the autoinducible pharmacokinetics of ifosfamide (IFO), 2-dechloroethylifosfamide (2DCE), 3-dechloroethylifosfamide (3DCE), 4-hydroxyifosfamide (4OHIF), and ifosforamide mustard (IFM).

Autoinduction is modeled with a hypothetical enzyme compartment described by an enzyme formation rate \( K_{\text{enz,in}} \) and elimination rate \( K_{\text{enz,out}} \). The amount of enzyme increases the clearance of IFO \( (CL_{\text{IFO}}) \). The amount of IFO, described by the IFO concentration and IFO volume of distribution \( (V_{\text{IFO}}) \), inhibits \( K_{\text{enz,out}} \). Metabolite compartments are described by metabolite formation \( (F) \) and elimination rates \( (K) \).

Plasma concentrations of ifosfamide mustard were determined using a validated HPLC method (Kerbusch et al., 2000a). In brief, this method determined the ifosfamide mustard-diethylthiocarbamate derivative in plasma. Sample pretreatment consisted of derivatization followed by liquid-liquid extraction with acetonitrile. Derivatization was achieved by incubating 500 μl of plasma with 350 μl of 1 M sodium chloride, 0.1 M potassium dihydrogenphosphate buffer, pH 8.0, and 50 μl of diethylthiocarbamate for 30 min at 70°C. The HPLC column used was a reversed phase C8 column with acetonitrile:0.025 M potassium dihydrogenphosphate, pH 8.0 (32:68 v/v), as mobile phase. Detection was performed at 276 nm. This method was specific, sensitive, accurate (101.4–110.0%), and precise (<15.8%) in the concentration range of 0.452 to 226 μM, with an LLQ of 0.452 μM ifosfamide mustard (measured as diethylthiocarbamate derivative).

Data Evaluation. Pharmacokinetic models were fitted to the plasma concentration data from the individuals using the population pharmacokinetic program NONMEM (Nonlinear Mixed Effects Modeling, version V 1.1, double precision, first order estimation) (Beal et al., 1992; Boeckman et al., 1994). The population pharmacokinetic models resulted in model predictions and Bayesian individual predictions for the concentrations of ifosfamide and metabolites (Sheiner and Grasela, 1991). The nonlinear pharmacokinetics of ifosfamide were described using a recently developed model, which incorporated the development of autoinduction (Kerbusch et al., 2000b). According to this recent study the time-dependent pharmacokinetics of ifosfamide can be described by a one-compartment model (Fig. 2). The change of the amount of ifosfamide \( (A_{\text{ifo}}) \) in the hypothetical enzyme compartment, as given in eq. 2:

\[
CL = \frac{C_{\text{IFO,in}} \times A_{\text{ifo}}}{V_{\text{IFO}}}. \tag{2}
\]

The change of \( A_{\text{ifo}} \) (a relative measure with no dimension) over time in the enzyme compartment is dependent on \( C_{\text{IFO,in}} \), as follows:

\[
\frac{dA_{\text{ifo}}}{dt} = K_{\text{enz,in}} - K_{\text{enz,out}} \times A_{\text{ifo}} \times \left(1 - \frac{C_{\text{IFO,in}}}{C_{\text{IFO,in}} + IC_{50}}\right) \tag{3}
\]

in which \( K_{\text{enz,in}} \) is the first order rate constant for enzyme degradation/ inactivation and \( IC_{50} \) is the ifosfamide concentration at 50% of the maximum inhibition of enzyme degradation. At \( t = 0 \) and \( A_{\text{ifo}} = 1 \), the enzyme formation rate \( (K_{\text{enz,in}}) \) is equal to \( K_{\text{enz,out}} \) (Fig. 2). The induction half-life of the enzyme \( (t\text{\_half,enzyme}) \) was calculated by the ratio of \( \ln(2) \) and \( K_{\text{enz,in}} \).

The pharmacokinetics of ifosfamide and metabolites were described sequentially: Bayes estimations (post hoc estimations) of the pharmacokinetic parameters of ifosfamide were used during the assessment of the pharmacokinetics of the metabolites. The change in the amount of a metabolite \( (A_m) \) over time could be described by eq. 4:

\[
\frac{dA_m}{dt} = \left(F_{\text{m}} \times CL \times \frac{A_{\text{ifo}}}{V_{\text{ifo}}} - (K_{\text{m}} \times A_{\text{m}})\right) \tag{4}
\]

in which \( K_m \) is the elimination rate constant of a metabolite and \( F_m \) is the fraction of ifosfamide metabolized to the metabolite. The values for \( F_m \) and \( V_{\text{m}} \) over the distribution of the metabolite \( (V_{\text{m}}, \text{liters}) \) cannot be estimated separately in this model. Therefore, the ratio of \( F_m \) over \( V_{\text{m}} \) was estimated: \( P^* \) (liters\(^{-1}\)).

The change in the amount of ifosfamide mustard \( (A_{\text{im}}) \) over time could be described by eq. 5:

\[
\frac{dA_{\text{im}}}{dt} = (F_{\text{im}} \times CL \times A_{\text{ifo}} - (K_{\text{im}} \times A_{\text{im}}) \tag{5}
\]

in which \( K_{\text{im}} \) is the elimination rate constant of ifosfamide mustard and \( F_{\text{im}} \) is the fraction of 4-hydroxyifosfamide metabolized to ifosfamide mustard. Equation 5 can be rearranged to eq. 6:

\[
\frac{dA_{\text{im}}}{dt} = (F_{\text{im}} \times V_{\text{im,short}} \times K_{\text{ifm}} \times C_{\text{ifm}} - (K_{\text{im}} \times A_{\text{im}}) \tag{6}
\]

in which \( V_{\text{im,short}} \) is the volume of distribution of 4-hydroxyifosfamide and \( C_{\text{ifm}} \) is the 4-hydroxyifosfamide concentration. The values for \( F_{\text{im}} \) and \( V_{\text{im,short}} \) cannot be estimated separately in this model and were, therefore, replaced by one parameter: \( P^{*} \times t_{\text{im,short}} \), which has no dimension.

The residual or intr individual variability of the pharmacokinetics of ifosfamide and metabolites was described separately with a combined proportional and additive term. The interindividual variability of each pharmacokinetic parameter was estimated using a proportional error model.

The post hoc estimations of the areas under the concentration-time curves (AUCs) of ifosfamide and its metabolites were obtained by describing the cumulative concentrations of these compounds over a given time. AUCs were obtained with extrapolation of ifosfamide and metabolite concentration-time profiles up to 72 h after the end of the (last) ifosfamide infusion, where concentrations were below the LLQ. The effect of the infusion duration on the autoinduction of ifosfamide metabolism and its effect on the metabolite exposures were investigated by calculating dose-corrected AUCs. The dose-corrected AUC was obtained by dividing the AUC by the administered ifosfamide dose. A nonparametric test was conducted to determine significant differences in dose-corrected AUCs between the infusion schedules. The pharmacokinetics of each group of infusion schedules was compared with the other two groups using the Kolmogorov-Smirnov test for two independent samples.

The influence of the independent variable infusion duration was tested on the estimation of each population parameter of ifosfamide, 2- and 3-dechloroethylifosfamide according to eq. 7:

\[
P_{\text{pop}} = \theta_{\text{inf}} \times \theta_{\text{ifo,elim}} \times \theta_{\text{ifo,kin}} \tag{7}
\]
in which \( P_{\text{pop}} \) is a population parameter, \( \theta_i \) is the typical value of that parameter with short duration infusions, \( \theta_j \) is the change in \( \theta_i \) with long infusion duration. If the 95% confidence interval of the estimate of \( \theta_j \) incorporated 1, no significant difference between the short infusion durations with medium or long infusion durations was observed. The urinary excretions for ifosfamide and 2- and 3-dechloroethylifosfamide were calculated as the equimolar amount of the administered dose recovered in urine. Differences between the urinary excretions of the different infusion durations were tested for significance using the nonparametric Mann-Whitney test. Statistical analysis was performed using SPSS software (version 6.1 for Windows; SPSS, Inc., Chicago, IL). The level of significance \( (p) \) was set at 0.05. All tests for significance were two-tailed.

### Results

A total of 56 patients was divided into three subpopulations with 12 patients receiving short (1–4 h), 21 patients receiving medium (24–72 h), and 23 patients receiving long (96–240 h) infusion durations of ifosfamide (Table 1). The number of patients in which 4-hydroxyifosfamide and ifosforamide mustard were analyzed was insufficient for the separate estimation of the pharmacokinetics of these metabolites for each infusion schedule. The pharmacokinetic population parameters with their relative standard error of the mean, interindividual variability, and residual variability, including all statistically significant \((p < 0.05)\) change in the parameter estimate due to infusion duration are presented in Table 2. The corresponding estimates of 4-hydroxyifosfamide and ifosforamide mustard are listed in Table 3.

### Table 1

<table>
<thead>
<tr>
<th>Patients</th>
<th>Samples</th>
<th>Patients</th>
<th>Samples</th>
<th>Patients</th>
<th>Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFO</td>
<td>56</td>
<td>598</td>
<td>12</td>
<td>143</td>
<td>21</td>
</tr>
<tr>
<td>2DCE</td>
<td>53</td>
<td>502</td>
<td>12</td>
<td>141</td>
<td>21</td>
</tr>
<tr>
<td>3DCE</td>
<td>53</td>
<td>513</td>
<td>12</td>
<td>140</td>
<td>21</td>
</tr>
<tr>
<td>4OHIF</td>
<td>15</td>
<td>135</td>
<td>2</td>
<td>27</td>
<td>13</td>
</tr>
<tr>
<td>IFM</td>
<td>8</td>
<td>70</td>
<td>2</td>
<td>20</td>
<td>6</td>
</tr>
</tbody>
</table>

### Table 2

Estimates of pharmacokinetic population parameters of ifosfamide (IFO), 2-dechloroethylifosfamide (2DCE), and 3-dechloroethylifosfamide (3DCE) with their relative standard error of the mean, interindividual variability, and residual variability, including all statistically significant \((p < 0.05)\) change in the parameter estimate due to infusion duration.

### Table 3

Estimates of pharmacokinetic population parameters of 4-hydroxyifosfamide (4OHIF) and ifosforamide mustard (IFM) with their relative standard error of the mean, interindividual variability, and residual variability.

---

**TABLE 1**

<table>
<thead>
<tr>
<th>All Infusions</th>
<th>Short (1–4 h)</th>
<th>Medium (24–72 h)</th>
<th>Long (96–240 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>Samples</td>
<td>Patients</td>
<td>Samples</td>
</tr>
<tr>
<td>IFO</td>
<td>56</td>
<td>598</td>
<td>12</td>
</tr>
<tr>
<td>2DCE</td>
<td>53</td>
<td>502</td>
<td>12</td>
</tr>
<tr>
<td>3DCE</td>
<td>53</td>
<td>513</td>
<td>12</td>
</tr>
<tr>
<td>4OHIF</td>
<td>15</td>
<td>135</td>
<td>2</td>
</tr>
<tr>
<td>IFM</td>
<td>8</td>
<td>70</td>
<td>2</td>
</tr>
</tbody>
</table>

**TABLE 2**

Estimates of population pharmacokinetic parameters of ifosfamide (IFO) 2-dechloroethylifosfamide (2DCE), and 3-dechloroethylifosfamide (3DCE) with their relative standard error of the mean, interindividual variability, and residual variability, including all statistically significant \((p < 0.05)\) change in the parameter estimate due to infusion duration.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
<th>RE</th>
<th>IIV</th>
<th>Parameter</th>
<th>Mean</th>
<th>RE</th>
<th>IIV</th>
</tr>
</thead>
<tbody>
<tr>
<td>( CL_{\text{pop}} ) (L/h)</td>
<td>3.01</td>
<td>7%</td>
<td>26%</td>
<td>( F_{\text{DCE,short}} ) (L)</td>
<td>0.0161</td>
<td>24%</td>
<td>43%</td>
</tr>
<tr>
<td>( V_{\text{pop}} ) (liters)</td>
<td>45.9</td>
<td>10%</td>
<td>28%</td>
<td>( F_{\text{DCE,medium}} ) (L)</td>
<td>0.0109</td>
<td>20%</td>
<td>35%</td>
</tr>
<tr>
<td>( K_{\text{ext,short}} ) (h(^{-1}))</td>
<td>0.0815</td>
<td>13%</td>
<td>22%</td>
<td>( F_{\text{DCE,short}} ) (L)</td>
<td>0.00784</td>
<td>51%</td>
<td>39%</td>
</tr>
<tr>
<td>( K_{\text{ext,medium}} ) (h(^{-1}))</td>
<td>8.5</td>
<td>49%</td>
<td>39%</td>
<td>( K_{\text{DCE}} ) (h(^{-1}))</td>
<td>0.385</td>
<td>19%</td>
<td>39%</td>
</tr>
<tr>
<td>( t_{1/2\text{enz,short}} ) (h)</td>
<td>0.0391</td>
<td>52%</td>
<td>R_{\text{pop}}</td>
<td>1.73</td>
<td>66%</td>
<td>23%</td>
<td></td>
</tr>
<tr>
<td>( t_{1/2\text{enz,medium}} ) (h)</td>
<td>17.7</td>
<td>33%</td>
<td>55%</td>
<td>( P_{\text{DCE,medium}} ) (L)</td>
<td>0.666</td>
<td>23%</td>
<td>35%</td>
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<tr>
<td>( IC_{50} ) (μM)</td>
<td>2.84</td>
<td>33%</td>
<td>55%</td>
<td>( P_{\text{DCE,short}} ) (L)</td>
<td>0.00726</td>
<td>33%</td>
<td>35%</td>
</tr>
<tr>
<td>( P_{\text{DCE}} ) (μM)</td>
<td></td>
<td></td>
<td></td>
<td>( P_{\text{DCE,medium}} ) (L)</td>
<td>0.0124</td>
<td>19%</td>
<td>39%</td>
</tr>
<tr>
<td>( A_{\text{DCE}} ) (μM)</td>
<td>12.6</td>
<td>7%</td>
<td>26%</td>
<td>( P_{\text{DCE,short}} ) (L)</td>
<td>3.31</td>
<td>17.0%</td>
<td></td>
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</table>

**TABLE 3**

Estimates of pharmacokinetic population parameters of 4-hydroxyifosfamide (4OHIF) and ifosforamide mustard (IFM) with their relative standard error of the mean, interindividual variability, and residual variability.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
<th>RE</th>
<th>IIV</th>
<th>Parameter</th>
<th>Mean</th>
<th>RE</th>
<th>IIV</th>
</tr>
</thead>
<tbody>
<tr>
<td>( F_{\text{4OHIF}} ) (L)</td>
<td>0.126</td>
<td>3%</td>
<td>30%</td>
<td>( K_{\text{IPM}} ) (h(^{-1}))</td>
<td>7.00</td>
<td>27%</td>
<td>72%</td>
</tr>
<tr>
<td>( K_{\text{4OHIF}} ) (h(^{-1}))</td>
<td>7.38</td>
<td>3%</td>
<td>8%</td>
<td>( P_{\text{IFM}} ) (μM)</td>
<td>49.5%</td>
<td>49.5%</td>
<td>49.5%</td>
</tr>
<tr>
<td>( P_{\text{4OHIF}} ) (μM)</td>
<td>22.8%</td>
<td>18%</td>
<td>39%</td>
<td>( A_{\text{4OHIF}} ) (μM)</td>
<td>0.300</td>
<td>17.0%</td>
<td>49.5%</td>
</tr>
</tbody>
</table>

RE, relative error of the mean; IIV, interindividual variability; \( CL_{\text{pop}} \), initial ifosfamide clearance; \( V_{\text{pop}} \), volume of distribution of ifosfamide; \( K_{\text{ext,short}} \), autoinduction formation rate; N.S., not statistically significantly different; \( t_{1/2\text{enz,short}} \), half-life of enzyme; \( IC_{50} \), ifosfamide concentration at 50% of the maximum inhibition of enzyme degradation; P.E., proportional intra-individual error; A.E., additive intra-individual error; \( F^* \), ratio of fraction metabolized and volume of distribution of metabolite; K, first order rate constant for metabolite elimination.
and individual Bayesian estimated pharmacokinetic profiles of ifosfamide of all patients are presented in Fig. 3.

The urinary excretion of unchanged ifosfamide and its dechloroethylated metabolites are presented in Table 4. Increase of infusion duration resulted in a significant decrease of urinary recovery of unchanged ifosfamide and the sum of ifosfamide and metabolites. For unchanged ifosfamide the urinary recovery after short and long infusion durations was 21.2 ± 6.6 and 8.6 ± 2.6%, respectively. No clear trend was observed for 2- and 3-dechloroethylifosfamide, although urine recovery of 2-dechloroethylifosfamide during long infusions was slightly lower than during medium infusion duration.

The individual profiles of the ifosfamide clearances over time of each infusion schedule are depicted in Fig. 4. Modest interindividual variability was observed. The clearance of ifosfamide increased during the infusion and decreased after cessation. Notably, during the first 24 h for all patients the model predicted an average doubling of the clearance.

Figure 5 depicts the relationships between the AUCs of ifosfamide and its metabolites and the ifosfamide dose for the total population. The AUC of ifosfamide increased less than proportional with increase in absolute ifosfamide dose. In contrast, the AUCs of the metabolites increased more than proportional with increase in absolute ifosfamide dose.

The dose-corrected AUCs are depicted in Fig. 6. The dose-corrected exposure to ifosfamide during long infusions was decreased 26% (p < 0.001) and 25% (p < 0.05) compared with short and medium infusion duration, respectively. In comparison with medium infusion durations dose-corrected exposure to 3-dechloroethylifosfamide during long infusion durations was increased with 45% (p < 0.01).

**Discussion**

This study investigated the effect of the duration of the ifosfamide infusion on the pharmacokinetics of ifosfamide and its metabolites. In a review on the clinical pharmacokinetics of ifosfamide in 1994 it was proposed that fractionation of the ifosfamide dose increased the metabolism of ifosfamide (Wagner, 1994). However, more recent studies have indicated that the ifosfamide infusion schedule does not influence the degree of metabolism. In a comparative study in pediatric patients receiving 3 g/m² ifosfamide administered as a 1-h infusion for three consecutive days or 9 g/m² ifosfamide administered as a 72-h continuous infusion, no major differences in exposure were observed, except a minor decrease in exposure to deactivated metabolites with the bolus infusion (Bodziy et al., 1995). In addition, no differences in metabolic exposures were found in a similar study in adults (Singer et al., 1998). Singer and coworkers concluded that there is no identifiable pharmacokinetic basis for insistence on either bolus or infusional methods of ifosfamide administration.

In our study a group of 56 patients was divided into three subpopulations receiving either short (1–4 h), medium (24–72 h), or long (96–240 h) infusion durations with ifosfamide. All patients studied received ifosfamide as part of standard therapy for the treatment of various malignancies. A previously developed autoinduction model was applied to assess the population pharmacokinetics of ifosfamide and metabolites (Kerbusch et al., 2000b). This model describes the development of autoinduction with a relationship between the ifosfamide plasma concentration and a hypothetical, dynamic enzyme compartment, which is responsible for ifosfamide metabolism. Thus, clearance of ifosfamide over time changes with ifosfamide concentrations. From some of the patients included in the study only a limited number of blood samples were available for pharmacokinetic analysis. In such cases conventional noncompartmental analysis of the concentration-time profiles does not allow accurate estimation of the pharmacokinetic parameters, due to insufficient data. The applied popu-
estimates based on the total population.

action term on the synthesis of the enzyme and an identical fit to the amount of enzyme (Hassan et al. 1999). We also included the inter-

(Hassan et al. 1999) and ifosfamide (Chang et al., 1997). Whatever the through stabilization of mRNA was suggested for cyclophosphamide (Watkins et al., 1986). However, increase of enzyme synthesis but inhibited catalytic enzymes responsible for CYP degradation

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ation warranted robust and uncorrelated estimates for these metabolite parameters. In contrast, 4-hydroxyifosfamide still exhibited for-

Fig. 4. Individual time profiles of the clearance of ifosfamide (IFO) for all patients.

The profiles were obtained on the basis of the individual Bayesian parameter estimates based on the total population.

lation approach with Bayesian estimation, on the other hand, does not have these drawbacks.

The influence of the ifosfamide concentration on the enzyme amount was modeled as an inhibiting influence on the degradation of the enzyme. Ifosfamide has been shown to reduce protein synthesis and could have a similar effect on the enzymes responsible for CYP3A4 degradation/inactivation, thus increasing ifosfamide clearance (Lewis et al., 1990). A similar mechanism of inhibition was proposed for troleandomycin, which did not increase protein synthesis but inhibited catalytic enzymes responsible for CYP degradation (Watkins et al., 1986). However, increase of enzyme synthesis through stabilization of mRNA was suggested for cyclophosphamide (Hassan et al. 1999) and ifosfamide (Chang et al., 1997). Whatever the mechanism, all modeling approaches will result in an increased amount of enzyme (Hassan et al. 1999). We also included the interaction term on the synthesis of the enzyme and an identical fit to the concentration-time data of ifosfamide was achieved. Thus, modeling autoinduction as increased enzyme translation/transcription or as decreased enzyme degradation will yield similar conclusions.

Statistically significant differences were found for $K_{enz\text{, out}}$ between long and short infusion durations and for $F^*_{2\text{dce}}$ and $F^*_{3\text{dce}}$ between medium and short infusion durations. These differences in parameter estimates were considered to be minor (33–52%) and not clinically relevant, since they were in the same order of magnitude as the interindividual variability of the respective parameters (22–43%). The observed differences may be explained by different amounts of data (information) within the concentration-time profiles of the various infusion schedules, which are necessary for the estimation of these pharmacokinetic parameters. Estimation of $K_{enz\text{, out}}$ in long infusion durations is more feasible than in short infusion durations, because the effect of autoinduction can be estimated over a longer period of time (Fig. 4). Nevertheless, the derived half-life of the enzyme ($t_{1/2\text{enzi}}$) increased from 8.5 h with short duration infusions to 17.7 with long duration infusions. Thus, in the latter group maximum induction would be reached after approximately 88 h (5 × $t_{1/2\text{enzi,long}}$), whereas maximum clearance in the short duration infusions would already be reached after 42 h. This difference in enzymatic behavior can also be observed in Fig. 4. The relatively lower number of samples (on average three per patient) drawn during the first 24 h of medium infusion durations, compared with short infusion durations (on average seven per patient), may have resulted in different estimations of the formation rates of the deactivated metabolites. Sampling frequency during the first 24 h with long infusions was more similar to that of short infusion durations (on average five samples were collected per patient). Hence, no difference was observed in metabolite formation with long infusion durations compared with short.

All other estimates of the population parameters were not significantly influenced by infusion duration. This indicates that no clinically relevant differences were observed in the pharmacokinetics between the different infusion durations.

The population pharmacokinetic parameters were in accordance with a recent study on the pharmacokinetics of ifosfamide after administration of 9 to 12 g/m² ifosfamide in a 72-h continuous intravenous infusion (Kerbusch et al., 2000b). In another study in 13 cancer patients receiving 1.5 g/m² for 5 days ifosfamide clearance and autoinduction were described using a population pharmacokinetic model for the enantiomers of ifosfamide and its metabolites (Di Marco et al., 2000). In that study clearance increased from 4 to 7 and 5 to 10 l/h from day 1 to 5 for $R$-ifosfamide and $S$-ifosfamide, respectively (Di Marco et al., 2000). This is in accordance with our findings as presented in Fig. 4. Individual ifosfamide clearances increase from 4 to 7 l/h (range 5–15).

Initially, $K$ values of the metabolites ($K_{2\text{dce}} = 0.385$, $K_{3\text{dce}} = 0.124$, $K_{\text{diohf}} = 73.8$) were greater than the value calculated for ifosfamide ($CL_{\text{lini, ifo}}/V_{\text{ifo}} = 0.0656$ h⁻¹). This indicates that the observed decay in plasma of the metabolites was formation rate limited. Maximum autoinduction was reached after 42, 42, and 88 h in short, medium, and long duration infusions, respectively. The $K$ value of ifosfamide ranged then approximately between 0.109 and 0.327 h⁻¹. In other words, after autoinduction ifosfamide elimination half-life decreased from 10.5 to 2.1 to 6.4 h and is now slower than the elimination half-life of 2- and 3-dechloroethylifosfamide (2 and 6 h, respectively). Thus, no formation rate limited elimination was observed with the dechloroethylated metabolites after development of autoinduction. Visual inspection of the terminal slopes confirmed this (data not shown). The observed lack of formation rate-limited elimination warranted robust and uncorrelated estimates for these metabolite parameters. In contrast, 4-hydroxyifosfamide still exhibited for-
ation rate-limited elimination. Unfortunately, no long infusion duration data were available for this metabolite, preventing robust parameter estimates.

These findings are in accordance with previous findings. Di Marco et al. (2000) observed the same “flip-flop” phenomenon with formation rate-limited dechloroethylated metabolite elimination at day 1, and elimination rate-limited elimination after the development of autoinduction on day 5.

Previously, Kaijser et al. (1996) calculated metabolite half-lives of 47 and 19 h for 2- and 3-dechloroethylifosfamide, respectively. This corresponds with lower K values of 0.015 and 0.036 h\(^{-1}\), respectively. However, these values were based on the assumption that these metabolites are not metabolized further. This assumption may not be valid because a didechloroethylifosfamide metabolite has been detected in human plasma (Gilard et al., 1993).

Table 4 indicates that considerable amounts of unchanged ifosfamide are excreted in the urine. Separate estimation of renal clearance, however, did not improve the goodness-of-fit of the model. The urinary excretion data found in this study were similar to previous observations. Gilard et al. (1993) reported urinary excretion of 18, 16, and 7% for ifosfamide, 2- and 3-dechloroethylifosfamide, respectively, in patients receiving 3 g/m\(^2\)/3 h ifosfamide for 3 days. The decrease in excretion of ifosfamide with increasing infusion duration can be explained by the development of the autoinduction. The decrease in excretion of ifosfamide with increasing infusion durations can be explained by decreasing AUCs due to autoinduction. For 2-
and 3-dechloroethylifosfamide increased AUCs would theoretically produce increased urine recovery. This was however not observed. The mechanism of this discrepancy remains to be elucidated. Nevertheless, the latter observation is in accordance with the previously reported finding that the urinary excretions of 2- and 3-dechloroethylifosfamide were superimposable for the different infusion schedules in pediatric patients (Boos et al. 1995).

The AUC of ifosfamide increased less than proportional with the absolute dose and the AUCs of the metabolites increased more than proportional with the absolute ifosfamide dose. This can be explained by a greater extent of autoinduction due to higher ifosfamide plasma concentrations (eqs. 2–4). These findings are in accordance with a previous report in which the pharmacokinetic model for ifosfamide was developed (Kerbusch et al., 2000b). The dose-corrected exposures to ifosfamide and its metabolites changed with increasing infusion duration. In comparison with short infusions, a decrease of 25%...
in dose-corrected ifosfamide exposure and an increase of 45% in 3-dechloroethylifosfamide exposure were observed with long infusion durations. The decrease in ifosfamide exposure can be explained by an increased average clearance during the long infusions (Fig. 4).

As a result of the increased average clearance of ifosfamide during long infusions dose-corrected exposure to the dechloroethylated metabolites was expected to increase. Both isoenzymes CYP2B6 and 3A4 have been indicated in the dechloroethylation of ifosfamide (Roy et al., 1999). Equal changes for both deactivated metabolites were expected. However, the increased average clearance observed with 3-dechloroethylifosfamide was not observed with 2-dechloroethylifosfamide. This contrasting finding may be explained by the observed large interindividual variability that could obscure the expected effect of long infusion durations on the dose-corrected exposure of 2-dechloroethylifosfamide (Fig. 6). Unfortunately, the effect of infusion duration on the dose-corrected exposure to 4-hydroxyifosfamide and ifosfamide mustards could not be evaluated since no samples were available for the long infusions. Nevertheless, the observed increased exposure to the dechloroethylated metabolites during long infusion durations demonstrates the importance of the assessment of the pharmacokinetics when comparing the pharmacodynamics during different infusion durations.

In conclusion, a population pharmacokinetic model for ifosfamide and its metabolites was used to investigate any differences in ifosfamide metabolism due to the duration of the ifosfamide infusion. Estimates of $K_{\text{enz.out}}$, $F_{\text{2dec}}$, and $F_{\text{3dec}}$ were found to depend on the infusion schedule. The clinical relevancy of the observed differences was, however, considered to be of minor importance. Concentration-dependent behavior of autoinduction produced a less than proportional increase in ifosfamide exposure and more than proportional increase in metabolite exposure with increasing ifosfamide dose. Schedule dependence could be demonstrated for the dose-corrected exposures to ifosfamide and 3-dechloroethylifosfamide between short and long infusion durations, which could be explained by the increased average clearance during infusion. No change in dose-corrected exposure to ifosfamide and metabolites was observed between short and medium infusion durations in this population. The observed dependence on dose and infusion duration should be taken into account when the pharmacodynamics of different infusion schedules of ifosfamide is evaluated.

References


