Distribution of ifosfamide and metabolites between plasma and erythrocytes

Summary

The distribution of ifosfamide and its metabolites 2-dechloroethylifosfamide, 3-dechloroethylifosfamide, 4-hydroxyifosfamide and ifosforamide mustard between plasma and erythrocytes was examined in vitro and in vivo. In vitro distribution was investigated by incubating whole blood with various concentrations of ifosfamide and its metabolites followed by separation of plasma and erythrocyte layers and subsequent analysis. In vivo distribution of ifosfamide, 2- and 3-dechloroethylifosfamide and 4-hydroxyifosfamide was determined in seven patients receiving a 72-hour intravenous continuous infusion of 9 g/m² ifosfamide. In vitro distribution equilibrium between erythrocytes and plasma was obtained quickly after drug addition. Mean (±sem) in vitro and in vivo erythrocyte (e)-plasma (p) partition coefficients (Pₑ/p) were 0.75±0.01 and 0.81±0.03, 0.62±0.09 and 0.73±0.05, 0.76±0.10 and 0.93±0.05 and 1.38±0.04 and 0.98±0.09 for ifosfamide, 2- and 3-dechloroethylifosfamide and 4-hydroxyifosfamide respectively. These ratios were independent of concentration and unaltered with time. The ratios of the area under the erythrocyte and plasma concentration-time curves (AUCₑ/p) obtained in patients were 0.96±0.03, 0.87±0.07, 0.98±0.06 and 1.34±0.39, respectively. A time- and concentration dependent distribution-equilibrium phenomenon was observed during incubation with ifosforamide mustard. It was hypothesized that the more hydrophilic ifosforamide mustard is less capable of penetrating the erythrocyte compared to the other analytes. Complete spontaneous in vitro conversion of 4-hydroxyifosfamide to ifosforamide mustard was observed. The equivalency in red blood cell partitioning of ifosfamide and its metabolites under in vitro and in vivo conditions was corroborated by the AUCₑ/p's, because similar ratios were observed. It is concluded that ifosfamide and metabolites rapidly reach distribution equilibrium between erythrocytes and plasma; the process is slower for ifosforamide mustard. Drug distribution to the erythrocyte fraction ranged from about 38% for 2-dechloroethylifosfamide to 58% for 4-hydroxyifosfamide.
and was stable over a wide range of clinically relevant concentrations. A strong parallelism in the erythrocyte and plasma concentration profiles was observed for all compounds. Thus, pharmacokinetic assessment using only plasma sampling yields direct and accurate insights into the whole blood kinetics of ifosfamide and metabolites and may be used for pharmacokinetic-pharmacodynamic studies.

**Introduction**

![Chemical Structures]

Figure 1. Metabolism of prodrug ifosfamide with activation through 4-hydroxyifosfamide to the alkylating ifosforamide mustard and deactivation to 2- and 3-dechloroethylifosfamide.

Ifosfamide, \( N,3\text{-bis(2-chloroethyl)}\text{tetrahydro-2H-1,3,2-oxazaphosphorin-2-amine 2-oxide} \) (Holoxan\textsuperscript{®}, Ifex\textsuperscript{®}), is commonly used for the treatment of various solid tumours, soft tissue sarcomas and haematological malignancies in adults and children.\textsuperscript{[1]} Ifosfamide is a prodrug which needs activation via the cytochrome P450 enzymatic system in the liver to 4-hydroxyifosfamide. 4-Hydroxyifosfamide exists in equilibrium with its tautomeric form aldoifosfamide, which converts into the final alkylating agent ifosforamide mustard with concomitant acrolein release. Ifosfamide is also deactivated to the non-cytotoxic compounds 2-dechloroethylifosfamide and 3dechloroethylifosfamide with simultaneous release of the potentially neurotoxic chloracetaldehyde.\textsuperscript{[1]} The metabolism of ifosfamide is depicted in figure
1. The measurement of ifosfamide and metabolites has mostly been limited to plasma.\[1\] Recently, Highley et al. reported a strong preference of ifosfamidemustard, the active antitumour metabolite for the erythrocytes.\[2\] They observed that erythrocytes from five patients treated with ifosfamide contained as much as 77% of the total whole blood concentration of ifosfamidemustard, resulting in a ratio of the areas under the concentration-time curve of erythrocytes over plasma (\(AUC_{e/p}\)) of 2.7. This indicated that the relationship between concentrations in the plasma compartment and associated with erythrocytes may not be fixed and the two compartments could behave rather differently. Consequently, drug concentration-time profiles in whole blood and plasma could differ, thereby yielding different values for the pharmacokinetic parameters. To support ongoing pharmacokinetic and pharmaco-dynamic studies of ifosfamide, the possibility of any preferential distribution of ifosfamide and its metabolites to erythrocytes must be assessed.\[3\] The present work deals with the \textit{in vitro} and \textit{in vivo} distribution of ifosfamide, 2- and 3-dechloroethylifosfamide, 4-hydroxyifosfamide and ifosfamidemustard in erythrocytes.

\section*{Materials and methods}

\subsection*{Chemicals}

Ifosfamide, 2- and 3-dechloroethylifosfamide, ifosfamidemustard and 4-hydroperoxyifosfamide were a kind gift of Dr J. Pohl, ASTA Medica AG (Frankfurt, Germany). Acetonitrile and methanol (HPLC supra-gradient) were purchased from Biosolve (Val-kenswaard, The Netherlands). 0.9 \% Sodium chloride solution (NaCl) was obtained from the Slotervaart Hospital Pharmacy (Amsterdam, The Netherlands). Ethyl acetate (p.a. grade), anhydrous potassium dihydrogen phosphate (suprapure grade) were obtained from Merck (Darmstadt, Germany). Semicarbazide hydrochloride (p.a. grade) was purchased from Acros (Geel, Belgium). A 2 M semicarbazide solution (SCZ) for stabilising 4-hydroxyifosfamide was made and adjusted to pH 7.40 with 1 M HCl and 1 M NaOH prior to use. Semicarbazide is a derivatising agent for 4-hydroxyifosfamide yielding an ultra-violet detectable chromophore. Moreover, 4-hydroxyifosfamide is trapped by this derivatization and can no longer spontaneously decompose to ifosfamidemustard.\[4\] A 2 M semicarbazide with 1 M NaCl solution (SCZ/NaCl) for stabilising ifosfamidemustard was made and adjusted to pH 7.40 with 1 M HCl and 1 M NaOH, prior to use.\[5\] Distilled water was used throughout. Blank plasma, erythrocytes and heparinized whole blood were collected from healthy volunteers in the Slotervaart Hospital (Amsterdam, The Netherlands).

\subsection*{Preparation of standards and solutions}

4-Hydroperoxyifosfamide was used as a substitute for 4-hydroxyifosfamide. In aqueous solution, 4-hydroperoxyifosfamide rapidly liberates 4hydroxyifosfamide/aldoifosfamide and hydrogen peroxide. Fresh solutions of 4-hydroxyifosfamide were prepared immediately prior to use by dissolving an equimolar amount of 4-hydroperoxyifosfamide in distilled water. Dissolution was accelerated by ultra-sonification. Ifosfamidemustard was also prepared freshly for each experiment. Four separate \textit{in vitro} experiments were performed. Experiment I
investigated the distribution of ifosfamide, 2- and 3-dechloroethylifosfamide between plasma and erythrocytes. 3 ml Venous blood at 37°C was added to 30 µl of a 50, 500 and 5000 µg/ml solution of ifosfamide, 2- and 3-dechloroethylifosfamide in a 0.9% NaCl solution. By using isotonic NaCl solutions undesired haemolysis of the erythrocytes by the stock solutions could be prevented. Next, 500 µl samples were aliquoted in six Eppendorf cups. The final drug concentrations in whole blood were 0.5, 5.0 and 50 µg/ml ifosfamide, 2- and 3-dechloroethylifosfamide. Experiment II investigated the distribution of 4-hydroxyifosfamide between plasma and erythrocytes. 12 ml Venous blood at 37°C was added to 120 µl of a 25 and 250 µg/ml solution of 4-hydroxyifosfamide in a 0.9 % NaCl solution. Next, 2000 µl samples were aliquoted in six Eppendorf cups. The final drug concentrations in whole blood were 0.25 and 2.5 µg/ml 4-hydroxyifosfamide. Experiment III investigated the distribution of ifosforamide mustard between plasma and erythrocytes. 6 ml Venous blood at 37°C was added to 60 µl of a 100 and 500 µg/ml solution of ifosforamide mustard in a 0.9 % NaCl solution. Next, 1000 µl samples were aliquoted in six Eppendorf cups. The final drug concentrations in whole blood were 1.00 and 5.00 µg/ml ifosforamide mustard. Experiment IV investigated the spontaneous conversion of 4-hydroxyifosfamide to ifosforamide mustard and subsequent distribution of ifosforamide mustard between plasma and erythrocytes. 6 ml Venous blood at 37°C was added to 60 µl of a 25 and 250 µg/ml solution of 4-hydroxyifosfamide in a 0.9 % NaCl. Next, 2000 µl samples were aliquoted in six Eppendorf cups. The final drug concentrations in whole blood were 0.25 and 2.5 µg/ml 4-hydroxyifosfamide. All venous blood samples were carefully mixed by gently tilting the tubes several times before they were aliquoted. All experiments were executed in triplicate. Since three bioanalytical methods with different stabilization and/or derivatization steps were applied, the in vitro experiments were not conducted simultaneously.

Incubation and sample pre-treatment

The samples were placed in a thermostatically controlled waterbath at 37°C. At various time-points (0, 5, 10, 20, 30 and 60 min) samples were taken and placed on ice water followed by immediate centrifugation for 2 min at 3,000 g at 4°C. After centrifugation the plasma layer was separated from the erythrocyte layer. The buffy coat with the leukocytes was removed carefully with sufficient margins and discarded, followed by transferring the erythrocytes. In order to exclude interference of haemolysis, a 50 µl plasma sample was used to determine the percentage haemolysis. In experiment I the ifosfamide, 2- and 3-dechloroethylifosfamide spiked samples were aliquoted in three 50-µl volumes of plasma and 50-µl volumes of the erythrocytes. In experiment II the 4-hydroxyifosfamide samples were aliquoted in two 250-µl volumes of plasma and two 250-µl volumes of the erythrocytes. In experiment III the ifosforamide mustard spiked samples were aliquoted in a 200-µl volume of plasma and a 200-µl volume of erythrocytes. In experiment IV the 4-hydroxyifosfamide spiked samples were aliquoted in a two 200-µl volume of plasma and a two 200-µl volume of erythrocytes for ifosforamide mustard determination. Immediately after incubation and matrix separation, samples containing 4-hydroxyifosfamide and ifosforamide mustard were stabilized by addition of 25 µl SCZ or 20 µl SCZ/NaCl, respectively, followed by vortex mixing for 15 sec. All samples were stored at -70°C pending analysis.
Determination of haemolysis

Interferences due to possible haemolysis have been investigated as follows: a volume of 2.5 ml distilled water was added to 25 µl venous blood yielding full haemolysis (100% standard sample) and 2.5 ml 0.9% NaCl was added to 25 µl venous blood with no haemolysis (0% standard sample). To check for haemolysis during the distribution experiments, a volume of 2.5 ml 0.9% NaCl was added to 25 µl plasma samples. If haemolysis of the erythrocytes would have occurred, erythrocyte cell-fragments would cause increased absorbance of the plasma matrix. The resulting mixture was mixed by gently tilting the tube several times and subsequent centrifugation for 10 min at 3,000 g. The absorption of all supernatants was measured at 540 nm. The 0 and 100% standard samples were measured in triplicate and the plasma samples were measured in duplicate. The percentage haemolysis was calculated by the ratio of the absorbance of the plasma sample ($A_{\text{plasma}}$) corrected for the background absorbance of 0.9% NaCl ($A_{\text{NaCl}}$) divided by the absorbance of 100% haemolysed venous blood ($A_{\text{water}}$) again corrected for the background absorbance of 0.9% NaCl, as described by equation 1.

$$\frac{A_{\text{plasma}} - A_{\text{NaCl}}}{A_{\text{water}} - A_{\text{NaCl}}} \times 100\%$$  \hspace{1cm} (eq. 1)

Patients

Venous whole blood samples of seven patients receiving 9 g/m²/72 hr intravenous ifosfamide treatment for soft tissue sarcoma were taken at 0, 3, 10, 20, 24, 34, 48, 58, 68, 72, 73, 76, 80 and 92 hours after start of the infusion. The samples were collected in 10-ml heparinized tubes and immediately cooled by placing them on ice water. The samples were then centrifuged without delay for 5 min with 1,000 g at 4°C. Plasma was transferred and the buffy coat was discarded, followed by transferring the erythrocytes. Both plasma and erythrocytes were aliquoted out for ifosfamide and metabolite analysis. Stabilization followed if required. Samples were stored at -70°C pending analysis of ifosfamide, 2- and 3-dechloroethylifosfamide and 4-hydroxyifosfamide.

Analytical methods

Ifosfamide, 2- and 3-dechloroethylifosfamide were measured using gas chromatography with flame ionization nitrogen/phosphorous detection. The samples were prepared by alkalinized liquid-liquid extraction (LLE) with ethyl acetate. Lower limit of quantification (LLQ) was 0.050 µg/ml for ifosfamide, 2 and 3-dechloroethylifosfamide. 4-hydroxyifosfamide was quantified using reversed phase high-performance liquid chromatography (RP-HPLC) with ultra-violet (UV) detection at 230 nm after LLE with ethyl acetate as reported previously. LLQ was 0.100 µg/ml 4-hydroxyifosfamide. Ifosforamide mustard was determined by using RP-HPLC with UV detection at 280 nm. This method allowed accurate determination of ifosforamide mustard in plasma, but not in erythrocytes due to interfering endogenous compounds. Samples were pre-treated by derivatization with diethyldithiocarbamate in phosphate buffer.
after stabilising with SCZ/NaCl, followed by LLE with acetonitrile. LLQ was 0.100 μg/ml ifosfamide mustard. Since separate analytical methods were used the distribution experiments were also performed in separate series. Furthermore, by combining ifosfamide, 2- and 3-dechloroethylifosfamide in one experiment and 4-hydroxyifosfamide and ifosforamide mustard in separate experiments, specific conditions could be included for optimal stabilization of the latter two compounds. Concentrations of the analytes were measured in duplicate using standard calibration curves in the same matrix. Recovery of ifosfamide and its metabolites in the \textit{in vitro} experiments was calculated according to equation 2,

\[
\text{Recovery} = \frac{C_p \times (1-H) + C_e \times (H)}{C_b \times DF} \times 100\% \quad (\text{eq. 2})
\]

in which \(C_p\), \(C_e\) and \(C_b\) are the plasma, erythrocyte (blood cells) and nominal blood concentration, respectively. \(H\) is the haematocrit (volume of blood cells) and \(DF\) is the dilution factor (1-[volume of stock solution/volume of blood]).

**Pharmacokinetic analysis**

The mean partition coefficient value (\(P_{e/p}\)) of erythrocytes over plasma was used as an indicator for any preferential distribution to erythrocytes. \(P_{e/p}\) was calculated by the ratio of the erythrocyte and plasma concentration. Ratios of the areas under the erythrocytes and plasma concentration-time curves (\(\text{AUC}_{e/p}\)) of the patients were calculated using a validated population approach.\(^{[9]}\)
Results

Analytical methods

The in vitro experiments used a clinically relevant concentration range for each compound. The haemolysis during the distribution experiments over plasma and erythrocytes was always less than 3%. The haematocrit was determined at 44%. Mean (±sem) recoveries of ifosfamide, 2 and 3-dechloroethylifosfamide were 85.5±0.7%, 93.5±0.8% and 97.0±0.8%, respectively. 4-Hydroxyifosfamide was subject to autocatalytic degradation. At the beginning of the experiment recovery was 97.9±6.4%, but after 60 minutes recovery was only 0.7±0.2%.

In vitro distribution

In vitro equilibrium was obtained within 1 minute after drug addition (approximate time between spiking of the blood and sample collection at t=0). The mean (±sem) in vitro $P_{e/p}$s of ifosfamide, 2- and 3-dechloroethylifosfamide and 4-hydroxyifosfamide were 0.75±0.01, 0.62±0.09, 0.76±0.10 and 1.38±0.04, respectively (table 1).

**Table 1.** Partition coefficients ($P_{e/p}$) and ratios of the area under the erythrocyte and plasma concentration-time curves ($AUC_{e/p}$) of ifosfamide (IFO), 2-dechloroethylifosfamide (2DCE) and 3-dechloroethylifosfamide (3DCE) and 4-hydroxyifosfamide (4OHIF).

<table>
<thead>
<tr>
<th></th>
<th>in vitro $P_{e/p}$ Mean (±sem)</th>
<th>in vivo $P_{e/p}$ Mean (±sem)</th>
<th>in vivo $AUC_{e/p}$ Mean (±sem)</th>
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<tbody>
<tr>
<td>IFO</td>
<td>0.75±0.01</td>
<td>0.81±0.03</td>
<td>0.96±0.03</td>
</tr>
<tr>
<td>2DCE</td>
<td>0.62±0.09</td>
<td>0.73±0.03</td>
<td>0.87±0.07</td>
</tr>
<tr>
<td>3DCE</td>
<td>0.76±0.10</td>
<td>0.93±0.05</td>
<td>0.98±0.06</td>
</tr>
<tr>
<td>4OHIF</td>
<td>1.38±0.04</td>
<td>0.98±0.09</td>
<td>1.34±0.39</td>
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No trends with time or in concentration were observed, as presented in figure 2. $P_{e/p}$s of ifosfamide, 2- and 3-dechloroethylifosfamide were always less than one. In contrast, $P_{e/p}$ of 4-hydroxyifosfamide was always higher than one. Not all 4-hydroxyifosfamide concentrations could be determined, because they were below the LLQ. In figure 3 the autocatalytic degradation of 4-hydroxyifosfamide in plasma and erythrocytes is depicted. No difference in degradation rate was observed between plasma or erythrocytes as the profiles decayed in parallel. Mean (±sem) elimination half-lives in plasma and erythrocytes were 9.0±0.3 and 9.1±0.4 min, respectively. The plasma concentrations of ifosforamide mustard are depicted in figure 4. During incubation with 1.0 µg/ml ifosforamide mustard, plasma concentrations did not exceed 1.0 µg/ml. During incubation with 5.0 µg/ml ifosforamide mustard, plasma concentrations were higher than 5.0 µg/ml but declined towards 5.0 µg/ml after 20 minutes. No degradation of ifosforamide mustard was observed during the incubation. The formation of ifosforamide mustard from 4-hydroxyifosfamide is also depicted in figure 4.
Figure 2. Mean (±sem) in vitro partition coefficients of ifosfamide, 2- and 3-dechloroethylifosfamide and 4-hydroxyifosfamide during incubation at 37°C.

Figure 3. Plasma and erythrocyte concentrations of 4-hydroxyifosfamide (4OHIF) during incubation at 37°C.
Incubation with 0.25 µg/ml 4-hydroxyifosfamide did not yield ifosfamide mustard plasma concentrations above the LLQ. Incubation with 2.5 µg/ml 4-hydroxyifosfamide yielded an equimolar ifosfamide mustard plasma concentration of 2.0 µg/ml. Thus, complete conversion of 4-hydroxyifosfamide to ifosfamide mustard without further degradation of ifosfamide mustard was observed after incubating for 60 minutes at 37°C. The formation half-life of ifosfamide mustard was 28.1 min$^{-1}$.

Clinical pharmacokinetics

Typical plasma and erythrocyte concentration-time profiles of ifosfamide, 2- and 3-dechloroethylifosfamide and 4-hydroxyifosfamide after a 72-hour continuous infusion of 9 g/m² ifosfamide are depicted in figure 5. The pharmacokinetics assessment in seven patients receiving this infusion resulted in 85, 86, 83 and 70 samples for ifosfamide, 2 and 3-dechloroethylifosfamide and 4-hydroxyifosfamide. Their mean (±sem) in vivo $P_{e/p}$s of ifosfamide, 2- and 3-dechloroethylifosfamide and 4-hydroxyifosfamide were 0.81±0.03, 0.73±0.03, 0.93±0.05 and 0.98±0.09, respectively (table 1). The plots of $P_{e/p}$s with time are depicted in figure 6. $P_{e/p}$s did not vary during the 72-hour ifosfamide infusion. However, during a brief period after cessation of the infusion a preference of ifosfamide and metabolites for the erythrocytes was observed. $P_{e/p}$s of ifosfamide, 2- and 3-dechloroethylifosfamide were always less than one, but the coefficient for 4-hydroxyifosfamide was always higher than one during the infusion. The mean (±sem) $AUC_{e/p}$s of ifosfamide, 2- and 3-dechloroethylifosfamide and 4-hydroxyifosfamide were 0.96±0.03, 0.87±0.07, 0.98±0.06, 1.34±0.39, respectively (table 1).
Figure 5. Plasma (solid lines) and erythrocyte (dashed lines) concentrations of ifosfamide (circles), 2-dechloroethylifosfamide (squares), 3-dechloroethylifosfamide (triangles) and 4-hydroxyifosfamide (diamonds) in a typical patient receiving 9 g/m² ifosfamide in a 72-hour continuous i.v. infusion.

Figure 6. Mean (±sem) in vivo partition coefficients of ifosfamide, 2- and 3-dechloroethylifosfamide and 4-hydroxyifosfamide in seven patients receiving 9 g/m² ifosfamide in a 72-hour continuous i.v. infusion.
Plasma-Erythrocyte Distribution of Ifosfamide Metabolism

Discussion

So far, the pharmacokinetics of ifosfamide and metabolites have mostly been defined with reference to drug in plasma. Considering that erythrocytes comprise about 40-50% of the whole blood volume, it is necessary to investigate possible differences in kinetics in plasma and erythrocytes. We performed in vitro and in vivo experiments to investigate possible kinetic differences of ifosfamide, 2- and 3-dechloroethylifosfamide, 4-hydroxy-ifosfamide and ifosforamide mustard in plasma and erythrocytes.

Drug measurement studies in erythrocytes must safeguard against possible lower reproducibility and accuracy in handling this matrix. The method used in the current study involved separation of erythrocyte sediments by centrifugation at 3,000 g. Recently, a novel device (MESED) was introduced for erythrocyte separation.[7] The investigators pointed out that with regular centrifugation plasma is possibly still trapped between erythrocytes which could interfere with the determination of possible accumulation of a compound in erythrocytes. However, the reduction in trapped plasma in the erythrocyte layer using MESED was only 1.9% with 10,000 g (from 3.9 to 2.0%).[7] Furthermore, this labour-intensive and costly method required three centrifugation steps, impeding routine use in clinical studies and possibly allowing degradation of unstable metabolites. Since the gain in erythrocyte purity was not considered significant, normal centrifugation was assumed to be sufficient in matrix separation.

The haemolysis during the in vitro experiments was always less than 3%. Thus, the erythrocytes remained intact during the experiment. This implies that $P_{e/p} < 1$ of a compound could not be caused by a transfer from the erythrocyte to the plasma compartment as a consequence of haemolysis. It was in vitro confirmed that total drug recovery of ifosfamide, 2- and 3-dechloroethylifosfamide and 4-hydroxyifosfamide (t=0) from erythrocyte and plasma fraction were within the accuracy of the analytical methods.[4-6] Lack of degradation of ifosforamide mustard could be assumed because equimolar conversion of 4-hydroxyifosfamide to ifosforamide mustard was observed. The nominal blood concentration of 2.5 µg/ml 4-hydroxyifosfamide could maximally be converted to 2.0 µg/ml ifosforamide mustard. This concentration was reached after 60 minutes, thus complete conversion without degradation was observed.

Highley and co-workers reported differences in ifosforamide mustard distribution between plasma and erythrocytes in patients receiving ifosfamide.[2,8] An integrated bioanalytical method using gas chromatography with mass detection was applied in their studies.[9] They determined ifosfamide, 2- and 3-dechloroethylifosfamide, ifosforamide mustard, carboxyifosfamide, ketoifosfamide and two metabolites of ifosforamide mustard but not 4-hydroxyifosfamide. The ifosforamide mustard as determined by their assay represented the sum of free ifosforamide mustard and 4-hydroxyifosfamide because no stabilization of 4-hydroxyifosfamide was performed. They observed higher concentrations in the erythrocytes of ifosforamide mustard and carboxyifosfamide (a metabolite of 4-hydroxyifosfamide), but not with ifosfamide, 2- and 3-dechloroethyl-ifosfamide.

In accordance with the findings of Highley and co-workers the present study shows that to a large extent ifosfamide and metabolites are located in plasma: drug distribution to the erythrocyte compartment ranged from about 38% for 2-dechloroethylifosfamide to 43% for
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*Hydroxyifosfamide showed affinity for the erythrocytes with 58% of the total drug concentration associated with this fraction. Distribution to erythrocytes was very rapid because no change in $P_{e/p}$ was observed between the start and the end of the incubation, indicating that the compounds can enter the erythrocyte compartment freely. This was confirmed by the parallelism in *in vitro* erythrocyte and plasma concentration profiles. The pharmacokinetics of 4-hydroxyifosfamide are expected to be related with efficacy of ifosfamide therapy.\(^1\) The autocatalytic degradation of 4-hydroxyifosfamide was equal in plasma and erythrocytes, indicating that no differences between pharmacokinetic parameters of 4-hydroxyifosfamide are expected when using plasma or erythrocytes. A concentration dependent difference in distribution of ifosforamide mustard was observed. Incubation with 1.0 µg/ml ifosforamide mustard did not demonstrate a particular affinity for the erythrocytes. However, incubation with 5.0 µg/ml ifosforamide mustard demonstrated increased plasma concentrations of ifosforamide mustard during the first 20 minutes. It can be hypothesized that the more hydrophilic ifosforamide mustard in comparison to the other analytes is less capable of penetrating the erythrocyte. Furthermore, ifosforamide mustard is highly ionized and hence does not readily cross membranes.\(^10\) To reach a distribution-equilibrium of ifosforamide mustard will require time due to this concentration dependent phenomenon. This phenomenon was not observed during the conversion of 4-hydroxyifosfamide to ifosforamide mustard, possibly due to the lower incubation concentration. Furthermore, a relatively lower amount of ifosforamide mustard will be exposed to the erythrocytes during conversion. The *in vivo* experiment was evidently done with all compounds simultaneously, thus incorporating possible interactions between the metabolites that could influence their distribution between plasma and erythrocytes. The *in vivo* $P_{e/p}$ and $AUC_{e/p}$ were in accordance with the *in vitro* findings (table 1). The apparent shift in affinity towards the erythrocytes of ifosfamide and its metabolites during the elimination phase could be explained by a lack of equilibrium between plasma and erythrocytes. However, this transient shift was only minor and therefore probably not clinically relevant. The lack of *in vitro* affinity for the erythrocyte of ifosforamide mustard is apparently in disagreement with the findings in patients by Highley et al.\(^2\) However, 4-hydroxyifosfamide exhibited *in vitro* and *in vivo* affinity for erythrocytes. Moreover, the pharmacokinetics of 4-hydroxyifosfamide in the two compartments did not differ. It can be hypothesized that relatively more 4-hydroxyifosfamide will reside in the erythrocyte compartment. Consequently, relatively more ifosforamide mustard will be formed in this compartment, thus corroborating the findings of Highley and co-workers.\(^2\)

It is concluded that ifosfamide and metabolites rapidly reach distribution equilibrium between erythrocytes and plasma, with ifosforamide mustard being the slowest. Drug distribution to the erythrocyte fraction ranged from about 38% for 2-dechloroethyl-ifosfamide to 58% for 4-hydroxyifosfamide and was stable over a wide range of clinically relevant concentrations. A strong parallelism in the erythrocyte and plasma concentration profiles was observed for all compounds, indicating that no differences will arise in the assessment of pharmacokinetic parameters using either matrix. Thus, pharmacokinetic assessment using only plasma sampling can yield direct, accurate and relevant relationships with efficacy and toxicity in patients treated with ifosfamide.
References
