Edelfosine is the prototype molecule of a promising family of antitumor compounds collectively known as synthetic alkyl-hydroxyl phospholipids, which include also clinically relevant drugs such as miltefosine and perifosine, and which can be administered orally (1–4). This class of synthetic anticancer agents acts at the level of the cell membrane, unlike most currently available chemotherapeutic drugs that target the nuclear DNA, and induces selective apoptosis in malignant cells, sparing normal cells (5–7). Although the precise mechanism of action is not yet fully elucidated, edelfosine-induced apoptosis is mediated by Fas/CD95 death receptor (8–10), c-jun NH2-terminal kinase activation (11, 12), endoplasmic reticulum stress (13), and mitochondria (14). Edelfosine has been applied as a purging agent in autologous bone marrow transplantation (15), and recent data suggest a putative clinical relevance for this agent in cancer (7). However, there is very little information about the disposition and oral bioavailability of edelfosine in vivo.

The kinetic behavior of radioactive edelfosine in rats and mice after i.v. administration has previously been investigated. Arnold et al. (16) administered 15 daily injections of an amount of radioactive edelfosine of 1 × 10⁶ counts per minute (cpm) i.v. to methylcholantrene-induced fibrosarcoma-bearing mice. Results showed that 1 day after the last injection, an overall activity of only 2 × 10⁶ cpm was recovered. This indicated that edelfosine did not accumulate in the organism. However, measurement of total radioactivity does not reflect the pharmacokinetic behavior or the tissue distribution of the parent drug edelfosine because the radiolabel may be included in some metabolites.

Kötting et al. (17) studied biodistribution in rats after oral administration of edelfosine. After 24 hours, no significant...
levels of edelfosine were detected either in feces or urine. This information led them to conclude that ~96% of the drug was absorbed in the first 24 hours (17).

As yet no precise pharmacokinetic parameters have been determined for free edelfosine. Characterization of the pharmacokinetics of edelfosine is crucial to understand the in vivo concentration-effect and concentration-toxicity relationships and to choose dosing regimens. Furthermore, knowledge of the in vivo concentration range, oral bioavailability, and pharmacokinetics of free edelfosine is crucial to evaluate the effects of edelfosine delivery systems on the pharmacokinetic behavior of this antineoplastic agent.

To determine the levels of edelfosine in plasma, tissue, or tumor, sensitive and selective techniques must be used to detect only the compound of interest and not others, like metabolites or very similar molecules. We have recently developed a simple and highly selective and sensitive high-performance liquid chromatography-mass spectrometry technique (HPLC-MS) technique with a quantitation limit of 0.3 ng, which avoids the use of radiolabeled compounds (18). With this technique, it is possible to quantify edelfosine concentrations in plasma, tissues, or tumor accurately and sensitively to determine pharmacokinetic parameters.

The present study investigates and compares the dose-dependent pharmacokinetics and oral bioavailability of edelfosine in healthy, immunodeficient, and tumor-bearing immunosuppressed mouse model animals.

Severe combined immune deficiency (SCID) mice are routinely used as hosts for malignant cells and for in vivo testing of new antitumor agents. SCID mice are characterized by the complete inability of the adaptive immune system to mount an appropriate immune response due to absence of functional lymphocytes as a result of defects in T- and B-cell development. On these grounds, use of SCID mice permits the long-term engraftment of human tumor cells. Because most of the in vivo antitumor drug testing assays are carried out with SCID mice, accomplishment of the pharmacokinetics in these immune compromised animals is imperative, and thus we studied the tissue and tumor drug distributions in these immunodeficient mice. In addition, in vitro experiments with the Z-138 mantle cell lymphoma (MCL) cell line, used for xenograft assays, were done to determine both edelfosine uptake and apoptotic activity.

**Translational Relevance**

Edelfosine is the prototype molecule of a family of antitumor drugs collectively known as synthetic alkyllyosphospholipids. This drug holds promise as a selective antitumor agent, and a number of preclinical assays are in progress. However, information about the tissue disposition and oral bioavailability of edelfosine in vivo is absent or rather scarce. Here we have studied the pharmacokinetics, biodistribution, and oral bioavailability of edelfosine in both tumor-free and tumor-bearing mice by the use of a high-performance liquid chromatography-mass spectrometry technique. We found that edelfosine is scattered in different organs but accumulated preferentially in the tumor. Multiple oral administration of edelfosine was required to reach a clinically relevant plasma concentration. The preclinical pharmacokinetic data reported here are essential to predict the human disposition of edelfosine and provide a basis for the development of the experimental design of clinical phase I trials as well as to set up a drug dosing scheme in cancer treatment.

**Materials and Methods**

**Chemicals.** Edelfosine was from INKEYSA. Platelet-activating factor and PBS (10 mmol/L phosphate, 0.9% NaCl) were obtained from Sigma-Aldrich. Formic acid 99% was purchased from Fluka, and methanol was obtained from Merck. All solvents used for the analysis were of analytic grade. RPMI 1640 cell culture medium, heat-inactivated FCS, and antibiotics were from Life Technologies, Invitrogen.

**Animal experiments.** The protocol for animal experiments was approved by the University of Navarra Animal Experimentation Ethics Committee (protocol no. 060-06). BALB/c mice (20 g) were obtained from Harlan Interfarma Ibérica S.L. Animals received a standard diet and water ad libitum, except for the animals who received the oral doses, which were fasted for 24 h before administration. Female CB17-SCID mice were purchased from Charles River Laboratories. In xenograft experiments, 8-wk-old SCID mice were s.c. inoculated into the lower dorsum with 1 × 10⁷ Z-138 cells in 100 μL of PBS and 100 μL of Matrigel basement membrane matrix (Becton Dickinson).

**Cell culture and isolation of human peripheral blood lymphocytes.** The Z-138 MCL cell line (19) was grown in RPMI 1640 culture medium supplemented with 10% FCS, 2 mmol/L L-glutamine, 100 units/mL penicillin, and 100 μg/mL streptomycin at 37°C in humidified 95% air and 5% CO₂. Lymphocytes were isolated from fresh human peripheral blood by dextran sedimentation, centrifugation on Ficoll-Paque density gradients, and monocyte depletion by culture dish adherence as described previously (20).

**Edelfosine uptake in cell culture.** Drug uptake was measured as previously described (5) after incubating cells (10⁶) with 10 nmol [³H]edelfosine for 1 h in RPMI 1640/10% FCS and subsequent exhaustive washing (six times) with PBS + 2% bovine serum albumin. [³H]Edelfosine (specific activity, 42 Ci/mmol) was synthesized by tritiation of the 9-octadecenyl derivative (Amersham Buchler).

**Apoptosis assay.** Quantitation of apoptotic cells after treatment with edelfosine was calculated by flow cytometry as the percentage of cells in the sub-G₁ region (hypodiploidy) in cell cycle analysis, as previously described (14).

**Pharmacokinetic studies after oral administration.** Three treatment lines were followed. A group of BALB/c mice and a group of SCID non–tumor-bearing mice were treated with a daily oral administration of 30 mg/kg edelfosine for 6 d. At various time points after the first oral administration (0, 1, 2, 5, 8, and 24 h), blood was collected in EDTA and 5% CO₂. Then, animals were sacrificed and spleen, liver, lungs, kidneys, heart, brain, stomach, and intestine were collected and weighed. Tissues were homogenized in 1 mL of PBS (pH 7.4) using a Mini-bead Beater (BioSpect Products, Inc.) and centrifuged at 10,000 × g for 10 min (4°C) to collect plasma (100 μL). After the administration of the sixth dose, blood samples were collected at the same time intervals (0, 1, 2, 5, 8, and 24 h). Then, animals were sacrificed and spleen, liver, lungs, kidneys, heart, brain, stomach, and intestine were collected and weighed. Tissues were homogenized in 1 mL of PBS (pH 7.4) using a Mini-bead Beater (BioSpect Products, Inc.) and centrifuged at 10,000 × g for 10 min. Both plasma and tissue supernatants were collected and stored at -80°C until HPLC-MS analysis was done. An additional group, MCL-bearing SCID mice, received daily oral administration of 30 mg/kg edelfosine for 6 d. After the administration of the sixth dose, blood samples were collected at the same time intervals (0, 1, 2, 5, 8, and 24 h), and then the animals were sacrificed; liver and kidneys were collected, weighed, and processed as previously described. Tumors were collected as the other organs.

**Pharmacokinetic study after i.v. administration.** An i.v. single dose of 200 μg (10 mg/kg) was administered to BALB/c mice via the tail vein.
At various time points after administration (0, 1, 2, 5, 8, and 24 h), blood was collected in EDTA surface-coated tubes and then centrifuged at 2,000 × g for 10 min (4°C) to separate the plasma (100 μL). After 24 h, animals were sacrificed by cervical dislocation and tissues were collected, weighed, and processed as explained above.

**Linearity study of the dose.** BALB/c mice were given single i.v. doses of 100, 200, 250, and 600 μg (5, 10, 12.5, and 30 mg/kg) of edelfosine dissolved in PBS via the tail vein. At various time points after administration (0, 0.5, 1, and 2 h), blood was collected as described above.

**Plasma/tissue extraction procedure for analytic process.** Ten micrograms of platelet-activating factor (0.2 mg/mL) used as internal standard were added to 100 μL of plasma or tissue supernatant. A mixture (190 μL) of 1% formic acid/methanol (5:95, v/v) was added to precipitate the proteins. Samples were vortexed for 1 min, and after centrifugation (20,000 × g, 10 min), 25 μL of the supernatant were analyzed by HPLC-MS (18).

**HPLC-MS analysis for edelfosine.** The method used for edelfosine quantitation was a slight modification of an earlier HPLC-MS method (18). Quantitation was achieved by comparing the observed peak area ratios of edelfosine and internal standard of the samples to a regression curve determined from drug-fortified plasma and tissue standards. The calibration curve of edelfosine in plasma showed good linearity over the concentration range of 0.1 to 75 μg/mL. Linear range of edelfosine concentrations in lung, kidney, liver, spleen, brain, heart, stomach, and intestine homogenates was 0.2 to 31.75 μg/mL.

**Data analysis.** The plasma concentration data were analyzed by noncompartmental and compartmental analyses using WinNonlin Professional Edition version 2.1 (Pharsight). Pharmacokinetic analysis was done for two different doses of 10 and 30 mg/kg with plasma samples obtained from experiments with all mice. The area under the plasma concentration versus time curve (AUC) is the volume of plasma completely cleared of a specific compound per unit time by the organism; it was calculated by dividing the dose by AUC. The maximum plasma concentration (C(max)) was determined directly from the plasma concentration-time curve. Oral bioavailability (F) was determined by ratio of the dose-normalized AUCs following oral and i.v. administration. Volume of distribution at steady state (V(s)) is the volume of fluid that would be required to contain the amount of drug in the body if it were uniformly distributed at a concentration equal to that in the plasma. The half-life value (t(1/2)) refers to the time taken for plasma concentration to decrease by 50%. Disposition (t(1/2)a) and elimination (t(1/2)e) half-lives were determined using the following formulas: t(1/2a) = ln(2)/α and t(1/2e) = ln(2)/β, where α and β represent the disposition and elimination constant rates, respectively, calculated from the intercompartmental mass transfer rates (k(12), k(21)) and elimination rates (k(e)).

**Statistical analysis.** Mean values of the tissue/plasma concentration ratio of more than two groups were analyzed by an ANOVA followed by Dunnett’s test for a double comparison using Social Package of Statistical Sciences (SPSS). The presence of differences in tissue/plasma ratios was measured by the Mann Whitney U test for double comparisons using the same program. A correlation analysis was done for the study of the linearity of the dose. P < 0.05 was considered statistically significant for all statistical tests.

**Results**

**Edelfosine uptake and induction of apoptosis in MCL cells.** Edelfosine has been reported to be selectively incorporated into malignant cells leading to their demise, whereas normal cells were spared (5, 8). We found that the Z-138 MCL cell line took up significant amounts of edelfosine and underwent apoptosis, whereas normal resting peripheral blood lymphocytes were not affected and drug incorporation was scarce (Fig. 1).

**Tissue distribution of edelfosine in tumor-bearing SCID mice.** Because Z-138 MCL cells incorporated edelfosine, we next analyzed the in vivo tissue distribution of the drug in tumor-bearing mice. Immunodeficient SCID mice were injected s.c. with Z-138 MCL cells, and xenografts were allowed to establish to an average size of 300 mm³ before drug oral treatment. The tissue distribution expressed as tissue/plasma concentration ratios of edelfosine concentrations after multiple oral dose administration of an edelfosine dose of 30 mg/kg (6-day treatment) to MCL-bearing SCID mice is shown in Fig. 2A. This oral 30 mg/kg dose was perfectly well tolerated by the mice, and we observed no side effects or body weight loss (data not shown). Mean concentration of edelfosine in plasma 24 hours after the sixth dose was 10.69 μg/mL. Kidney and liver were selected for drug accumulation because they are the major drug clearance tissues. These organs showed a 2-fold higher drug concentration compared with that in plasma (Fig. 2A).

Interestingly, the tumor showed a high accumulation of edelfosine 24 hours after the dose on day 6 (Fig. 2A). The mean concentration of edelfosine in the tumor at this stage was 138.64 μg/g, which is ~13 times higher than the plasma drug concentration, and 5 to 6 times higher than the drug concentration found in kidney and liver. Moreover, the tumor/plasma concentration ratio of edelfosine was significantly higher (P < 0.01) than the corresponding ratios observed in both kidney and liver. Thus, an in vivo and in vitro comparison indicates that edelfosine is selectively taken up by tumor cells.

**Tissue distribution of edelfosine in non—tumor-bearing SCID mice.** The tissue distribution of edelfosine was also analyzed in control non—tumor-carrying SCID mice following the same protocol as with tumor-bearing SCID mice. The mean plasma concentration of edelfosine at 24 hours after the sixth dose was 12.12 μg/mL. Figure 2B shows a higher distribution of edelfosine to lung, spleen, intestine, liver, and kidney. In contrast, low drug concentrations were found in heart, brain, and stomach (Fig. 2B). Compared with tumor-bearing SCID mice, no statistical differences in liver/plasma concentration ratios (P > 0.05) were found in non—tumor-bearing SCID mice, whereas the kidney was found to have a statistically highly significant increase (P < 0.01; Fig. 2A).

**Tissue distribution in healthy BALB/c mice after oral administration.** A multiple oral dose administration of 30 mg/kg edelfosine (6-day treatment) was done to healthy BALB/c mice. Twenty-four hours after the last dose (day 6), the mean plasma concentration of edelfosine was 13.22 μg/mL. As is shown in Fig. 2B, the highest tissue/plasma concentration ratios were found for kidney and intestine. Lower ratios were observed for stomach, spleen, lung, and liver. A low amount of edelfosine was detected in the heart and brain. Figure 2B also shows that no statistically significant differences were appreciated among the tissue/plasma concentration ratios of lung, heart, brain, intestine, and liver of BALB/c and non—tumor-bearing SCID mice. The stomach of BALB/c mice after the multiple oral administration of 30 mg/kg presented a higher ratio compared with SCID mice after the administration of same dose (P < 0.05), and the spleen/plasma concentration ratio showed a significant decrease (P < 0.05) with regard to SCID non—tumor-bearing mice.

**Tissue distribution in healthy BALB/c mice after i.v. administration.** A single i.v. administration of 200 μg of edelfosine (10 mg/kg) was administered to healthy BALB/c mice.
After 24 hours, edelfosine concentration in plasma was ~2.5 μg/mL. The tissue distribution of edelfosine expressed as tissue/plasma ratio can be observed in Fig. 2B. No drug was detectable in heart and very little was found in brain. Kidney and intestine presented the highest tissue/plasma concentration ratios, followed by lung, liver, stomach, and spleen.

**Pharmacokinetic characterization after i.v. administration.** Figure 3 depicts the concentration of edelfosine in mouse plasma plotted against time after a single-dose i.v. administration of 200 μg of edelfosine (10 mg/kg) to BALB/c mice. Pharmacokinetic analysis of edelfosine in blood plasma showed a $C_{\text{max}}$ of 50.7 ± 28.1 μg/mL and a $C_{\min}$ of 2.5 ± 1.3 μg/mL, 24 hours after i.v. administration. The obtained pharmacokinetic parameters are listed in Table 1.

Plasma concentration-time data of edelfosine were well described by a biexponential function (model selection criterion, -24.12) following i.v. administration. The half-lives of distribution ($t_{1/2a}$) and elimination ($t_{1/2h}$) phases were 0.286 ± 0.076 and 22.288 ± 14.016 hours, respectively. The systemic clearance (CL) and steady-state volume of distribution ($V_{ss}$) were 0.056 ± 0.030 L/h/kg and 1.285 ± 0.556 L/kg, respectively. There was little variability in most of the values of
the parameters, indicating a well-controlled and reproducible study, except for the elimination-phase half-life value.

**Linearity of dose.** Both AUC-dose and C<sub>max</sub>-dose profiles of edelfosine showed a linear correlation (r<sup>2</sup> = 0.999), as can be seen in Fig. 4, suggesting linear pharmacokinetics with similar elimination and distribution half-lives after i.v. administration of doses between 5 and 30 mg/kg.

**Pharmacokinetic characterization after oral administration.** A very low concentration of edelfosine could be found in plasma along the time interval of 0, 1, 2, 5, 8, and 24 hours after oral administration of 30 mg/kg edelfosine in BALB/c mice, as can be observed in Fig. 3. These concentrations were close to the detectable limits of the technique and not sufficient for the calculation of pharmacokinetic parameters. Oral bioavailability for edelfosine was calculated from the ratios of the average values for AUC<sub>0→24 h</sub> for the oral and i.v. doses. Oral bioavailability (F) for edelfosine was found to be <10%.

Pharmacokinetic parameters obtained after compartmental analysis of experimental data are shown in Table 1. Oral bioavailability increased to 64% when a steady state was reached after the 6th day. At this point, a C<sub>max</sub> of 14.46 ± 2.97 µg/mL at a t<sub>max</sub> of 3 hours and a C<sub>min</sub> of 7.65 ± 2.45 µg/mL were measured 24 hours after the last administration. Due to the fast elimination during the disposition phase, only the β elimination phase could be characterized. AUC presented a value of 58.16 ± 26.76 hours, with a volume of distribution at steady state of 1.563 ± 0.163 L/kg and a systemic clearance of 0.057 ± 0.039 L/h/kg, similar values to those obtained after i.v. administration of edelfosine, with no statistically significant differences.

To determine possible interstrain pharmacokinetic differences, these results were compared with the results obtained after edelfosine was administered to non-tumor-bearing SCID mice. After reaching the steady state, pharmacokinetic parameters were estimated (Table 1). Whatever the parameters studied (half-life of elimination, clearance value, steady-state volume of distribution, and AUC), no statistically significant differences were observed between SCID and BALB/c mice.

![Time-concentration curve data of edelfosine 24 h after single-dose i.v. administration of 10 mg/kg (●) and oral single-dose administration of 30 mg/kg to BALB/c mice (●). Points, mean (n = 6); bars, SD.](image)

**Discussion**

Several attempts have been made over the years to accurately detect the accumulation of edelfosine in tumors, tissues, and plasma, as well as to estimate its pharmacokinetic parameters. This pharmacokinetic analysis is mandatory to accomplish all the preclinical assays before a rational clinical use of the drug. Because edelfosine shows a variety of medical applications, a pharmacokinetic study in different murine species, each one appropriate for distinct in vivo assays, is required. We carried out a complete pharmacokinetic analysis in SCID mice, widely used for antitumor activity testing, and in BALB/c, useful for additional putative edelfosine applications (21). In the present study, we set up a MCL-bearing animal model in immunodeficient SCID mice to analyze the distribution of edelfosine in tissues and tumors. We found that the Z-138 MCL cell line took up significant amounts of edelfosine, subsequently undergoing apoptosis, whereas normal cells hardly incorporated the drug and were spared. The in vitro and in vivo data reported here indicate a remarkably selective accumulation of edelfosine in tumor cells.

In the MCL-bearing animal model, kidney and liver were the only organs extracted as they are considered to be the main drug distribution and clearance tissues. Comparing the tissue distribution of edelfosine between these tumor-bearing SCID mice and the non–tumor-bearing SCID mice (Fig. 2A), no statistically significant differences were observed in liver (P < 0.05), whereas a major decrease (P < 0.01) was found in the kidneys of the tumor-bearing mice. The low renal uptake in the tumor model might be a result of increased drug uptake in the tumor with less drug available for renal excretion. In addition, the lower tissue/plasma ratio in kidneys of the MCL-bearing mice might be also due to a renal failure usually expected at advanced stages of the disease (22).

Figure 2B shows that the distribution of edelfosine in mice is mainly predominant in spleen, intestine, and kidney. The significant drug uptake in the gut might underlie the major toxicity of edelfosine in the gastrointestinal tract (1). The high presence of edelfosine in spleen can be explained by the incorporation of the drug into the membrane of erythrocytes inducing a hemolytic effect (17). These RBC end up being cleared from the blood in the spleen. We found a higher concentration of edelfosine in the spleen of SCID mice as compared with BALB/c mice, and this could be due to putative differences in the spleen of immunodeficient mice.

No statistically significant differences were observed between the tissue/plasma concentration ratios of edelfosine in the lung, heart, brain, intestine live, and kidney of immunosuppressed mice (SCID mice) and BALB/c mice (Fig. 2B). Moreover, no significant differences were observed in the mean plasma concentrations of edelfosine 24 hours after the sixth dose of the multiple oral administration of 30 mg/kg to tumor-bearing SCID mice, non–tumor-bearing SCID mice, and healthy BALB/c mice (10.69, 12.12, and 13.22 µg/mL, respectively). As a result, a similar pharmacokinetic profile would be expected in both strains. Therefore, we performed the characterization of edelfosine pharmacokinetic profile in healthy BALB/c mice.

Knowledge of the pharmacokinetic parameters of a drug is a must to ensure that exposure is sufficient to evaluate its pharmacodynamic properties, to predict the pharmacokinetics in other species, and to develop an appropriate dosage form. 

---

**Fig. 3.** Time-concentration curve data of edelfosine 24 h after single-dose i.v. administration of 10 mg/kg (●) and oral single-dose administration of 30 mg/kg to BALB/c mice (●). Points, mean (n = 6); bars, SD.
Following i.v. administration of a dose of 10 mg/kg edelfosine to BALB/c mice, mean blood concentrations declined biphasically, with an initial half life of 0.3 hour (Table 1). After 2 hours, plasma concentrations seemed to decline more slowly. Given that blood concentrations had declined 6-fold during the initial phase, the contribution of the secondary phase to the total drug exposure is of little relevance. According to these half-life values, edelfosine presented a rapid distribution to central compartment tissues, whereas a slower distribution to deep compartments was appreciated. The rapid distribution is mostly observed to organs that are highly irrigated, such as the kidney, liver, intestine, and lung.

The steady-state volume of distribution was 1.26 L/kg, much greater than that of the vascular volume in mice and approximately twice its total body water (23) normalized to body weight, suggesting that edelfosine is highly distributed extravascularly. In fact, $k_{12}$ presented a value five to six times higher than the corresponding $k_{21}$ value.

Edelfosine showed very low clearance values in mice compared with its respective liver and kidney blood flow. It is interesting to note that this value of 0.056 L/h/kg is translated to barely 1% of the liver blood flow (23), suggesting a much lower intrinsic hepatic clearance value.

We verified that as the drug dose increases the AUC increases equivalently, meaning that edelfosine exhibits linear pharmacokinetics. In this study, we show that edelfosine shows a linear correlation not only between the administered dose and the resulting AUC but also between the administered dose and the corresponding $C_{\text{max}}$. This fact suggests that there is no saturation of the elimination process of edelfosine at the concentration range between 5 and 30 mg/kg.

Although the mechanism for its clearance is still unknown, renal elimination of the drug might well happen because edelfosine is present in kidney. Previous studies have established that amphiphilic cationic drugs are efficiently removed by the liver from the blood (24). Additionally, amphiphilic cationic drugs were detected in other secretion organs of cationic amphiphilic drugs, such as the intestinal mucosa (25) and kidney. These data are also in good agreement with our results and the ones obtained by Marschner et al. (26).

The first determinations of oral absorption of 15 A$_\text{mol}$ edelfosine (38 mg/kg) in 1992 suggested that $f_{\text{abs}}$ of edelfosine was absorbed by rats within 24 hours (17). Conversely, our study shows very low plasma concentrations of edelfosine 24 hours after the oral administration of a single-dose of 30 mg/kg, revealing very low absorption of edelfosine by the gastrointestinal tract in mice. However, oral bioavailability showed a significant increase (64%) after daily administration of 30 mg/kg edelfosine for 6 days.

As can be seen from our studies, edelfosine showed a moderate volume of distribution and a rapid and varied distribution through the organism when it is administered by an i.v. route. This information, along with the amphiphilic properties,

**Table 1. Comparison of pharmacokinetic parameters of edelfosine after administration of different doses to BALB/c and SCID mice by different routes ($n=6$, mean ± SD)**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>BALB/c, 200 μg i.v. single dose</th>
<th>BALB/c, 600 μg oral multiple oral dose</th>
<th>SCID, 600 μg oral multiple dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>$t_{1/2a}$ (h)</td>
<td>0.286 ± 0.076</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>$t_{1/2b}$ (h)</td>
<td>22.288 ± 14.016</td>
<td>30.404 ± 26.761</td>
<td>19.263 ± 8.262</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (μg/mL)</td>
<td>50.681 ± 28.096</td>
<td>14.460 ± 2.970</td>
<td>18.176 ± 1.023</td>
</tr>
<tr>
<td>$k_{21}$ (h$^{-1}$)</td>
<td>0.346 ± 0.137</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>$k_{10}$ (h$^{-1}$)</td>
<td>0.312 ± 0.158</td>
<td>1.943 ± 0.494</td>
<td>—</td>
</tr>
<tr>
<td>$k_{12}$ (h$^{-1}$)</td>
<td>1.943 ± 0.494</td>
<td>1.943 ± 0.494</td>
<td>—</td>
</tr>
<tr>
<td>CL (L/h/kg)</td>
<td>0.056 ± 0.030</td>
<td>0.057 ± 0.039</td>
<td>0.067 ± 0.021</td>
</tr>
<tr>
<td>$V_{ss}$ (L/kg)</td>
<td>1.285 ± 0.556</td>
<td>1.563 ± 0.163</td>
<td>1.256 ± 0.284</td>
</tr>
<tr>
<td>AUC$_{0-24}$ (μg × h/mL)</td>
<td>125.440 ± 52.511</td>
<td>258.241 ± 58.163</td>
<td>265.235 ± 51.823</td>
</tr>
</tbody>
</table>

**NOTE:** No statistical differences were found among the parameters ($P > 0.05$).
may lead us to consider the possibility of passive diffusion through the intestinal epithelium as a mechanism of absorption for edelfosine. However, this suggestion does not correlate with the low absorption rate of the drug found using the oral route. Moreover, it has been shown that the absorption of certain cationic drugs, such as quaternary ammonium compounds, shows a reduced absorption rate due to the presence of efflux transport systems on the apical membrane (e.g., P-glycoprotein). Indeed, the alkylphosphocholine miteflosine has been reported to interact with P-glycoprotein (27), and it has also been reported that edelfosine is a substrate for P-glycoprotein (28). This issue would only explain the lack of absorption at a single oral dose and the high oral bioavailability achieved after a multiple oral dose administration.

In summary, edelfosine showed a remarkable apoptotic effect in Z-138 MCL cells in vitro and a high and rather selective uptake in MCL tumor cells in vitro and in vivo. This fact, along with the knowledge of the biodistribution and pharmacokinetic behavior of edelfosine, permits the establishment of treatment regimens as well as the development of drug delivery systems to treat MCL and other tumors. In addition, because no statistically significant differences were noticed among the pharmacokinetic parameters of all three treatment lines, it can be concluded that there are no differences between the BALB/c and SCID pharmacokinetic profiles. Thus, further studies with edelfosine delivery systems will be able to be done in either one animal model or the other.

Our results show that edelfosine is widely scattered across different organs but that it is preferentially internalized by the tumor both in vitro and in vivo. All these data, together with the apoptotic action of the drug on cancer cells, support a rather selective action of edelfosine in cancer treatment, and that multiple oral administration is required to reach a clinically important plasma concentration, therefore increasing its oral bioavailability.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

Antitumor Alkyl Ether Lipid Edelfosine: Tissue Distribution and Pharmacokinetic Behavior in Healthy and Tumor-Bearing Immunosuppressed Mice

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