Altered Pharmacokinetics of Vinblastine in Mdr1a P-glycoprotein-Deficient Mice

Judith van Asperen, Alfred H. Schinkel, Jos H. Beijnen, Willem J. Nooijen, Piet Borst, Olaf van Tellingen*

Background: P-glycoprotein (Pgp) is a membrane protein that acts as an extrusion pump for many cytotoxic drugs. Pgp is expressed in normal tissues, and its (over)expression in tumor cells contributes to their drug resistance. Human Pgp is encoded by the MDR1 gene. In mice, two Pgps (encoded by the mdr1a and mdr1b genes) appear to perform the same function as the single human protein. The simultaneous use of cytotoxic drugs and agents that block Pgp function has raised questions of safety, since a blockade of Pgp in normal tissues could alter drug pharmacokinetics and change the spectrum of toxic side effects. Analysis of the consequences of Pgp blockade has been facilitated by the generation of mice with disrupted mdr1a genes [mdr1a(-/-)]. Purpose: We studied the plasma pharmacokinetics, tissue distribution, and excretion of the cytotoxic drug vinblastine (VBL) and its metabolites in mdr1a(-/-) mice and in wild-type [mdr1a(+/+)] mice. Methods: VBL was administered to mice in bolus doses of either 1 or 6 mg/kg body weight by intravenous injection. VBL and its metabolites were quantified in tissue specimens, plasma, feces, and urine by use of high-performance liquid chromatography. Liquid scintillation counting was used to measure radioactivity in specimens from animals that had received 3H]VBL. Pharmacokinetic parameters were calculated by use of noncompartmental methods. Only two-sided P values are reported. Results: The half-life (t0.5) of VBL during its terminal phase of elimination was longer in mdr1a(-/-) mice than in wild-type mice. The t0.5 values with a 1-mg/kg dose were 3.6 hours ± 0.3 hour (mean ± standard error) and 2.1 hours ± 0.3 hour, respectively (P<0.05); with a 6-mg/kg dose, the values were 8.6 hours ± 1.8 hours, respectively (P = .058). Fecal excretion of nonmetabolized VBL was reduced from 20%-25% of the administered dose (either 1 or 6 mg/kg) in wild-type mice to 9.3% (1-mg/kg dose) or 3.4% (6-mg/kg dose) in mdr1a(-/-) mice (both P<0.05); the cumulative urinary excretion of VBL was low (<6% of the administered dose) and not substantially different in the two types of mice. The metabolism of VBL to hydrophilic compounds, a primary mechanism involved in its elimination, was not altered in mdr1a(-/-) mice. The brains of mdr1a(-/-) mice accumulated substantially more VBL than the brains of wild-type mice. In mdr1a(-/-) mice, a few other tissues, such as the heart and the liver, accumulated increased amounts of VBL, but the relative levels of accumulation were lower than those found in the brain. Conclusions: Mice lacking the Pgp encoded by the mdr1a gene exhibit reduced fecal excretion of VBL, leading to a prolonged elimination t0.5 for this drug. Intact mdr1a function appears to protect the brain against high plasma levels of VBL, but most other tissues are not similarly protected. Implications: Enhanced drug accumulation in nonmalignant tissues after Pgp blockade should be carefully considered in future clinical trials of Pgp modulation. [J Natl Cancer Inst 1996;88:994-9]

Treatment with cytotoxic drugs is an important tool in the management of leukemias and disseminated or locally advanced inoperable cancers. However, intrinsic and/or acquired resistance of populations of tumor cells to cytotoxic agents remains a major impediment to achieving complete and lasting remissions. To date, the best characterized form of drug resistance is caused by (over)expression of the MDR1 gene, which encodes a membrane-localized, glycosylated protein called P-glycoprotein (Pgp). Pgp acts as an extrusion pump for a variety of large, structurally unrelated hydrophobic and neutral or cationic compounds, including many cytotoxic drugs (e.g., vinca alkaloids, anthracyclines, epipodophyllotoxins, and taxanes), thereby leading to their decreased intracellular accumulation (1,2). While only one gene encoding a drug-transporting Pgp (MDR1) has been identified thus far in humans (3-5), two genes, mdr1a (also called mdr3) and mdr1b (also called mdr1), have been found for drug-transporting Pgps in mice. The mdr1a gene is expressed predominantly in the intestines and in the capillaries of the brain and the testis, whereas the mdr1b gene is expressed mainly in the adrenal gland, the placenta, the ovarium, and in the (pregnant) uterus. Both the Mdr1a and the Mdr1b Pgps are expressed in the kidney and the liver (6-9). Although some differences in the substrate specificities of the two mouse Pgps have been reported (10), the data suggest that these proteins play a role primarily in protecting against xenotoxins and also that Mdr1a and Mdr1b together fulfill the same role in mice as Mdr1 does in humans.

Several studies (11-15) have shown that Pgp-mediated multidrug resistance is of clinical relevance, and the findings have stimulated a search for agents that are capable of reversing this form of drug resistance. Thus far, however, clinical results with identified reversal agents have been modest, particularly in the treatment of solid tumors (16-28). The use of reversal agents concomitantly with cytotoxic drugs raises important questions about the safety of chemotherapeutic treatment. A blockade of Pgp in normal tissues might alter the pharmacokinetics of co-administered drugs and, as a result, increase or change the spectrum of associated toxic side effects (29-32).

Mice with homozygously disrupted mdr1a genes [mdr1a(-/-) knock-out mice] have been generated at our institute.

*Affiliations of authors. J. van Asperen, W. J. Nooijen, O. van Tellingen (Department of Clinical Chemistry), A. H. Schinkel, P. Borst (Department of Molecular Biology), The Netherlands Cancer Institute, Amsterdam; J. H. Beijnen, Department of Clinical Chemistry, The Netherlands Cancer Institute, and Department of Pharmacy, Slotervaart Hospital, Amsterdam.

Correspondence to: Olaf van Tellingen, Ph.D, Department of Clinical Chemistry, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands.

See "Note" section following "References."
(9), and the generation of mdr1b and mdr1alb double-knock-out mice is in progress. The use of these knock-out mice in pharmacokinetic studies with cytotoxic drugs may provide valuable information, since they embody the genetically engineered reference standard for a complete blockade of Pgp activity. Although the phenotype of the mdr1alb double-knock-out mouse would resemble the human MDR1-inactivated phenotype most closely, relevant data can be obtained from mdr1a(-/-) mice because Mdr1a is the prevalent Pgp form in a number of important tissues (e.g., the blood–brain barrier) (9).

We report here results of the first comprehensive analysis of the plasma pharmacokinetics, the tissue distribution, and the excretion of vinblastine (VBL) and its metabolites in mdr1a(-/-) mice and in their wild-type [mdr1a(+/+)] counterparts.

Materials and Methods

Laboratory Animals

All studies were performed with male mice 9-16 weeks of age. The mdr1a(-/-) knock-out mice of mixed genetic background (F2 and F3 generations of a cross between the 129/OLA and the FVB strain) were generated as described previously (9). The control group consisted of wild-type [mdr1a(+/+)] mice of a similar mixed genetic background. The protocol for this study was approved by the animal welfare committee at our institute as required by Dutch law. Animals were housed according to institutional guidelines and were given food and water ad libitum.

Drugs and Chemicals

VBL sulfate, daunovirublastine (DVBL) sulfate, and vintriptol methan sulfonate were purchased from the Medigenix Group (Fleurus, Belgium). [3H]VBL sulfate in ethanol (specific activity, 338 MBq/mg) was obtained from Amer sham International (Little Chalfont, Buckingham shire, U.K.). Radiolabeled drug was mixed with unlabeled VBL to produce a solution with a specific activity that allowed the injection of about 250 kBq (range, 185-370 kBq) of radioactivity per animal. Briefly, unlabeled VBL was dissolved in 1 mL ethanol, radiolabeled VBL was added, and the solution was evaporated under nitrogen (37°C). The dried mixture was prepared for injection by dissolution in 5% dextrose to yield solutions with a final VBL concentration of either 0.2 or 1 mg/mL.

Bovine serum albumin (BSA) was obtained from Organon Teknika (Boxtel, The Netherlands). All other reagents were purchased from E. Merck (Darmstadt, Federal Republic of Germany) and were of analytical quality, except for acetonitrile, which was of gradient grade. Diethyl ether was distilled once before use; the remaining reagents were used as supplied. Water was purified by use of the Milli-Q system (Millipore Corp., Bedford, MA). "Blank" human plasma, used as a drug-free diluent, was obtained from healthy donors.

Study Design

Drug solutions were administered to each animal under diethyl ether anesthesia by intravenous bolus injection in the tail vein. All pharmacokinetic experiments were performed at doses of levels of 1 and 6 mg/kg body weight; three to six animals were used per time point. Animals were anesthetized with diethyl ether at 1, 4, 8, and 24 hours after drug administration, and blood samples were collected in heparinized tubes via heart puncture. Next, the following tissues were collected: brain, skeletal muscle, organ fat, colon, cecum, small gut, stomach, liver, gallbladder (bile), kidney, lung, heart, testis, spleen, thymus, and peripheral and mesenteric lymph nodes. Blank human plasma (1.0-7.0 mL) was added to the tissues (approximately 0.05-0.2 g tissue/mL), which were then homogenized at 4 °C by use of a tissue homogenizer (Biospec Products, Bartlesville, OK). Additional blood samples were collected from anesthetized animals by heart puncture at 5, 15, 30, and 45 minutes and 1, 2, 4, 8, 16, 24, and 31 hours after drug administration. Blood samples were centrifuged (10 minutes, 2000g, 4 °C), and the plasma was separated and stored for analysis. Animals were housed in Ruco-type M/l metabolic cages (Valkenswaard, The Netherlands) for the collection of urine and feces in 24-hour intervals up to 96 hours after drug administration. The fecal specimens were homogenized in 10 mL 4% (wt/wt) BSA in water. All biological specimens were stored at −20 °C until analysis.

Drug Analysis and Pharmacokinetics

VBL and DVBL were extracted from the biological matrices with diethyl ether. The organic layers were dried, reconstituted in acetonitrile, and subjected to ion-pair normal-phase high-performance liquid chromatography (33). Detection of VBL and DVBL was achieved with a Model FP920 fluorescence detector (Yasco, Hachioji City, Japan), which is more sensitive than the detector used in our previous studies (9,33); as a result, we could quantify drug levels in small volumes of plasma more accurately. As reported previously (33), two other drug-related compounds (termed M1 and M2) in addition to VBL and DVBL were detected in the feces of mice. Since these compounds are probably formed intra-intestinally and are closely related to VBL and DVBL, respectively, fecal excretions are reported here as the sums of VBL + M1 and of DVBL + M2. Radioactivity was counted in a Tri carb Series 4000 Minaxi model B4430 liquid scintillation counter (Packard Instrument Co., Meriden, CT) and corrected for quenching by means of external standardization. Specimens (100 μL) were prepared for scintillation counting by adding 5 mL Ultima Gold scintillation liquid (Packard Instrument Co.).

Pharmacokinetic parameters were calculated by noncompartmental methods using the software package Quattro Pro (Version 4.0, 1992; Borland International, Scotts Valley, CA).

The area under the plasma concentration–time curve (AUC) was calculated by the trapezoidal rule by use of the following formula:

\[
AUC = \sum_{i=2}^{n} \text{concentration}_i \cdot (\Delta t_{i-1} + \Delta t_{i}),
\]

with \(\Delta t_{i-1}\) = 0.

The standard error (SE) of the AUC was calculated with the law of propagation of errors by use of the following formula:

\[
SE_{AUC} = \sqrt{\sum_{i=2}^{n} \text{SE}_i^2 \left( (\Delta t_{i-1} + \Delta t_{i})^2 / 4 \right) / 4}
\]

The terminal elimination half-life \(t_{1/2}\) and the SE of the \(t_{1/2}\) were calculated by linear regression analysis of the log(Concentration) versus time data points of the final log-linear part of the concentration–time curve.

The clearance (Cl) was calculated by use of the following formula:

\[
Cl = \text{dose} / \text{AUC}.
\]

The unpaired Student's \(t\) test was used for statistical analyses. All reported \(P\) values are based on two-sided tests of significance.

Results

A marked increase in terminal elimination \(t_{1/2}\) and a diminished clearance of VBL were found with a 1-mg/kg drug dose in mdr1a(-/-) mice compared with wild-type mice (Fig. 1, A). A prolonged terminal elimination \(t_{1/2}\) was also observed in both mdr1a(-/-) and wild-type mice when the dose level was increased to 6 mg/kg (Fig. 1, B).

The majority of VBL and DVBL fecal excretion occurred within 24 hours after drug administration (Fig. 2). The cumulative fecal excretion of unchanged drug in wild-type mice averaged 20%-25% of the administered dose for both 1 mg/kg and 6 mg/kg VBL. In mdr1a(-/-) mice, fecal excretion was reduced to 9.3% and 3.4% of the administered dose with 1 mg/kg VBL and 6 mg/kg VBL, respectively (both \(P<0.05\)). However, clear symptoms of toxicity, including a marked 75% reduction in feces production, were observed with 6 mg/kg VBL but not with 1 mg/kg VBL in mdr1a(-/-) mice. After the administration of \(^{[3]}\)H]VBL, most of the radioactivity was excreted as polar metabolites in both wild-type and knock-out mice and was found in the aqueous phase following diethyl ether extraction of the biological matrix (Fig. 2). Urinary excretion of VBL was low and variable. With a dose of 1 mg/kg, the cumulative urinary excretion of VBL was 2.9% ± 0.7%
At all time points, the increased concentration of VBL in the brains of mdrla(-/-) mice was most striking (Table 1, A and B). After administration of a 6-mg/kg dose to mdrla(-/-) mice, the concentration of VBL in this organ remained high, even at \( t \) (time) = 24 hours (i.e., 1000 ng/g tissue), whereas a steady decline in concentration (to 22 ng/g) was observed in the brains of wild-type mice (Table 1, B). In other organs, the differences between the wild-type and mdrla(-/-) animals were less pronounced. In tissues sampled 1 hour after administration of a 6-mg/kg dose, the plasma concentration of VBL and the concentration in most tissues, except for the brain, the testis, and the small gut, of both types of mice were similar. At 8 hours, higher relative VBL concentrations were observed in the hearts and the livers of mdrla(-/-) mice. At 24 hours, the drug-level ratios [mdrla(-/-) versus wild-type] in the brain (ratio = 46.0) and, to a lesser extent, in the liver (ratio = 11.9) and the heart (ratio >10) were higher than the plasma ratio (ratio = 7.0), whereas the ratios for the stomach, the testis, and the thymus were 3.5 or less between the two mouse types. With a 1-mg/kg dose, the VBL concentration ratios in most tissues were similar to the ratio observed in plasma (Table 1, A). However, relative to the plasma ratio observed 4 hours after drug administration (i.e., 1.7), mdrla(-/-) mice tended to accumulate more drug in the heart (ratio = 3.4), the skeletal muscle (ratio = 6.7), and the small gut (ratio = 2.9). A higher concentration of the metabolite DVBL was also observed in the plasma and tissues of mdrla(-/-) mice (Table 2).

**Discussion**

We have reported previously that the approximate LD\(_{50}\) values (drug doses causing 50% lethality) of VBL for mdrla(+/-) and mdrla(-/-) mice are 22 and 6 mg/kg, respectively (9). At equitoxic dose levels, the toxic effects of this drug were similar in both types of animals. A general physical deterioration manifested itself through a reduction in food and water intake and a diminished excretion of urine and feces.

In this study, we observed a nonlinear increase in plasma AUC with increasing VBL dose, a result consistent with our previous work (34). The AUCs for knock-out and wild-type animals differed by a factor of only 1.4-1.6, whereas the LD\(_{50}\) values differed by approximately fourfold, indicating that the increased toxicity of VBL in mdrla(-/-) mice is not directly related to differences in clearance. This finding is in accord with our previous proposal that the time during which the VBL concentration exceeds a threshold level (5-20 ng/mL), rather than the AUC, correlates with the drug's toxicity (34). The plasma concentration-time curves for both groups of animals overlapped during the first hours after VBL administration; however, when plasma VBL levels declined to about 40 ng/mL, the curves diverged. The longer terminal elimination \( t_{\text{d}} \) in mdrla(-/-) mice versus wild-type mice at equal VBL dose levels indicates a reduction in the rate of elimination, which results in the prolonged presence of potentially toxic plasma levels of drug.

At dose levels of both 1 mg/kg and 6 mg/kg, approximately 20%-25% of the administered VBL was recovered as unchanged drug in the feces of wild-type animals. In knock-out mice receiving VBL at a subtoxic dose level of 1 mg/kg,
Fig. 2. Cumulative fecal excretion of total $^3$H label (○), vinblastine (VBL) (●), and deacetylvinblastine (Δ) after the intravenous bolus administration of 1 mg/kg body weight (A and B) and 6 mg/kg body weight (C and D) $[^3]$HVBL to wild-type (A and C) and mdrla knock-out (B and D) mice. Values represent means ± standard errors. Dashed line with (O) represents the cumulative fecal excretion of unknown $^3$H-labeled breakdown products.

this value was diminished to 9.3%. The further reduction in fecal excretion in mdrla(-/-) mice given 6 mg/kg VBL (the approximate LD50) is likely a consequence of a more deteriorated physical condition.

The amount of unchanged VBL excreted in the urine was low. There were no significant differences in renal excretion between the wild-type and the mdrla(-/-) animals. This finding might be explained by the fivefold to 10-fold increased levels of mdrlb RNA (9) in the kidneys of mdrla(-/-) mice. Confirmatory evidence for this hypothesis must await studies with mdrlab double-knock-out mice.

With only 25%-30% of a VBL dose being recovered as unchanged drug in the feces and urine, it is evident that drug metabolism is the most important pathway of VBL elimination. VBL is metabolized to DVBL, a minor but structurally closely related metabolite (33,35). It is likely that DVBL is a Pgp substrate. Despite the higher levels of DVBL found in the plasma and the tissues of knock-out mice, the excretion of this metabolite was not significantly altered (Fig. 2). Interpretation of the effects of mdrla gene disruption, however, is difficult because the excretion of DVBL depends not only on the rate and extent of its formation and distribution but also on the rate and extent of its further breakdown. VBL and probably DVBL are extensively metabolized to one (or several) unknown hydrophilic products (33). In both mdrla(-/-) and wild-type mice receiving radiolabeled VBL, most of the radioactivity was excreted in feces, and this radioactivity remained in the aqueous phase after di-ethyl ether extraction. Metabolic breakdown to polar products thus provides an important pathway for detoxification, which is still effective in mdrla(-/-) mice.

The tissue levels of VBL are driven by many factors, such as the drug concentration in the central (plasma) compartment, drug affinity for intracellular constituents, tissue vascularization, and influx and efflux rates through cellular membranes. The consequences of lacking the Mdr1a Pgp in tissues were explored by systematic tissue distribution studies at two dose levels. A striking 22-fold difference between wild-type and knock-out mice was observed in the brain 4 hours after drug administration at a dose of 1 mg/kg, thus indicating that the Mdr1a Pgp is an important element of the blood–brain barrier for VBL (and other Pgp substrates) (9). However, despite the fact that the brain is a very well perfused organ and is relatively rich in tubulin (the target protein of VBL), the maximum level of VBL even in the brains of mdrla(-/-) mice was lower than that found in most other organs. This observation suggests that the accumulation of VBL in the brain is not restricted by Pgp alone but by other factors as well. It is likely that the physical blood–brain barrier lowers drug influx, which enables Pgp to maintain low drug levels in this tissue compartment. This hypothesis would explain why the brain is the most sensitive organ to inactivation of the mdrla gene.

In agreement with the presence of a blood–testis barrier, drug accumulation in the testis was also very low. Pgp appears to be less important in this tissue, since VBL levels were only about twofold higher in mdrla(-/-) mice. Furthermore, drug elimination from this organ was slow in both types of mice, suggesting avid tissue binding. Drug influx into all other organs is apparently much higher, as illustrated by the high levels observed in most tissues 1 hour after drug administration. The ratios of drug concentration between wild-type and knock-out mice in these tissues were in the same range as the ratio observed in plasma. This finding may reflect saturation of the Pgp in wild-type animals caused by relatively high drug levels during the first hours after intravenous drug administration. When the plasma level of VBL had declined to low levels (<10 ng/mL), the heart (and the
It could be hypothesized that Pgp has evolved mainly to provide protection against low levels of xenotoxins.

In conclusion, our results show that the increased toxicity of VBL in mdrla knock-out mice is related to reduced drug elimination, resulting in the prolonged presence of potentially toxic drug levels in the systemic circulation. Blocking Pgp function by use of reversal agents in patients has also led to diminished drug clearance, requiring dose adjustments in chemotherapy (29,31,32). Although many physicians are reluctant to implement dose reductions in currently utilized treatment regimens, such steps seem inevitable. The drug concentration in most normal tissues is driven mainly by the plasma level, and any advantageous effect from the use of reversal agents should come from a selective increase in drug levels in tumor cells. The effects observed here in the brain and the heart, however, warrant extreme caution, especially when reversal agents are combined with neurotoxic or cardiotoxic agents.

References

2. Riordan JR, Ling V. Purification of P-glycoprotein from plasma membrane vesicles of Chinese hamster ovary cell mutants with

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**Table 1, A. Tissue levels of vinblastine in wild-type and mdrla(-/-) mice 4 and 8 hours after intravenous injection of a 1-mg/kg dose**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>4 h</th>
<th>8 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td>mdr1a(-/-)</td>
<td>Ratio</td>
</tr>
<tr>
<td>Brain</td>
<td>5 ± 4</td>
<td>112 ± 10</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>22 ± 5</td>
<td>150 ± 8</td>
</tr>
<tr>
<td>Organ fat</td>
<td>88 ± 10</td>
<td>73 ± 1</td>
</tr>
<tr>
<td>Colon</td>
<td>210 ± 25</td>
<td>440 ± 29</td>
</tr>
<tr>
<td>Cecum</td>
<td>470 ± 17</td>
<td>470 ± 54</td>
</tr>
<tr>
<td>Small gut</td>
<td>200 ± 14</td>
<td>580 ± 59</td>
</tr>
<tr>
<td>Stomach</td>
<td>320 ± 55</td>
<td>560 ± 47</td>
</tr>
<tr>
<td>Liver</td>
<td>115 ± 35</td>
<td>280 ± 43</td>
</tr>
<tr>
<td>Gallbladder ( bile)</td>
<td>340 ± 81</td>
<td>470 ± 40</td>
</tr>
<tr>
<td>Kidney</td>
<td>400 ± 69</td>
<td>930 ± 43</td>
</tr>
<tr>
<td>Lung</td>
<td>520 ± 27</td>
<td>1060 ± 110</td>
</tr>
<tr>
<td>Heart</td>
<td>64 ± 9</td>
<td>220 ± 23</td>
</tr>
<tr>
<td>Testis</td>
<td>26 ± 3</td>
<td>62 ± 2</td>
</tr>
<tr>
<td>Spleen</td>
<td>880 ± 64</td>
<td>1440 ± 51</td>
</tr>
<tr>
<td>Thymus</td>
<td>320 ± 29</td>
<td>430 ± 15</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>390 ± 39</td>
<td>500 ± 55</td>
</tr>
<tr>
<td>Plasma</td>
<td>5.9 ± 0.5</td>
<td>10.3 ± 0.9</td>
</tr>
</tbody>
</table>

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**Table 1, B. Tissue levels of vinblastine in wild-type and mdrla(-/-) mice 1, 8, and 24 hours after intravenous injection of a 6-mg/kg dose**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>1 h</th>
<th>8 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td>mdr1a(-/-)</td>
<td>Ratio</td>
<td>Wild-type</td>
</tr>
<tr>
<td>Brain</td>
<td>120 ± 12</td>
<td>860 ± 100</td>
<td>7.2</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>3900 ± 390</td>
<td>3400 ± 290</td>
<td>0.9</td>
</tr>
<tr>
<td>Colon</td>
<td>5200 ± 470</td>
<td>7200 ± 720</td>
<td>1.4</td>
</tr>
<tr>
<td>Cecum</td>
<td>3600 ± 180</td>
<td>4900 ± 500</td>
<td>1.3</td>
</tr>
<tr>
<td>Small gut</td>
<td>6300 ± 270</td>
<td>9800 ± 340</td>
<td>1.6</td>
</tr>
<tr>
<td>Stomach</td>
<td>10300 ± 640</td>
<td>12900 ± 580</td>
<td>1.3</td>
</tr>
<tr>
<td>Liver</td>
<td>8500 ± 1200</td>
<td>11300 ± 2000</td>
<td>1.3</td>
</tr>
<tr>
<td>Kidney</td>
<td>22500 ± 1400</td>
<td>22600 ± 1800</td>
<td>1.0</td>
</tr>
<tr>
<td>Lung</td>
<td>23800 ± 1000</td>
<td>26800 ± 3400</td>
<td>1.1</td>
</tr>
<tr>
<td>Heart</td>
<td>6700 ± 1000</td>
<td>7660 ± 940</td>
<td>1.1</td>
</tr>
<tr>
<td>Testis</td>
<td>310 ± 33</td>
<td>580 ± 51</td>
<td>1.9</td>
</tr>
<tr>
<td>Spleen</td>
<td>12100 ± 630</td>
<td>14400 ± 1300</td>
<td>1.2</td>
</tr>
<tr>
<td>Thymus</td>
<td>3800 ± 170</td>
<td>3400 ± 230</td>
<td>0.9</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>4000 ± 320</td>
<td>3900 ± 360</td>
<td>1.0</td>
</tr>
<tr>
<td>Plasma</td>
<td>200 ± 35</td>
<td>240 ± 47</td>
<td>1.2</td>
</tr>
</tbody>
</table>

*Results are expressed as means ± standard error in ng/g tissue (for plasma, in ng/mL).
†Some of the data for this time point were reported previously (9); previously reported values are reprinted here with permission of the copyright holder, Cell Press, Cambridge, MA.
‡Ratio represents the drug concentration in mdr1a(-/-) mice versus wild-type mice. Three mice were analyzed in each group.
Table 2. Tissue levels of deacetylvinblastine in wild-type and mdr-la(-/-) mice 4 hours after intravenous injection of 1 mg/kg vinblastine

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Wild-type</th>
<th>mdr-la(-/-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Organ fat</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Colon</td>
<td>80 ± 7</td>
<td></td>
</tr>
<tr>
<td>Cecum</td>
<td>43 ± 19</td>
<td></td>
</tr>
<tr>
<td>Small gut</td>
<td>115 ± 17</td>
<td></td>
</tr>
<tr>
<td>Stomach</td>
<td>75 ± 8</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>621 ± 101</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>198 ± 16</td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>138 ± 16</td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>60 ± 6</td>
<td></td>
</tr>
<tr>
<td>Testis</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Spleen</td>
<td>247 ± 22</td>
<td></td>
</tr>
<tr>
<td>Thymus</td>
<td>n.d.</td>
<td></td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>56 ± 8</td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td>2.0 ± 0.9</td>
<td></td>
</tr>
</tbody>
</table>

*Results are expressed as means ± standard errors in ng/g tissue (for plasma, in ng/mL). Three mice were analyzed in each group (n.d. = not detectable).


