Comparative Pharmacokinetics of Vinblastine after a 96-Hour Continuous Infusion in Wild-Type Mice and Mice Lacking mdr1a P-Glycoprotein

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ABSTRACT

To determine the tissue-specific impact of P-glycoprotein on the accumulation of a substrate drug, we have studied the tissue distribution of vinblastine in mdr1a(−/−) and wild-type mice at approximately similar, relatively low plasma levels. Vinblastine was administered as a 96-h continuous infusion at dose rates of 1 to 10 µg/h, which were delivered by a s.c. implanted osmotic pump. Drug concentrations were determined in plasma and tissues by HPLC. In comparison to wild-type mice, 4.4- to 9.6-fold higher drug concentrations were observed in the brains of mdr1a(−/−) mice (p ≤ .014), whereas a 2-fold increase was found in the heart (p = .014) and the intestinal tissues (p ≤ .028). No or only slight differences were observed in all other tissues. These results indicate that, except for the brain and, to a lesser extent, the heart and the intestinal tissues, P-glycoprotein does not protect individual organs against vinblastine. Given its polarized cell-specific and organspecific distribution and its affinity for a broad range of compounds, it is suggested that P-glycoprotein has mainly evolved to provide a general protection of the complete organism against potentially toxic substrates by inhibiting their uptake and by mediating their transport from the internal to the external environment. For the clinical application of reversal agents, these data indicate that, in general, a blockade of endogenous P-glycoprotein will probably not result in an increased accumulation of the concomitantly administered anticancer drug in complete organs, but, possibly, only in classes of cells making up a fraction of an organ.

Multidrug resistance is a major limitation to the successful chemotherapeutic treatment of locally advanced or disseminated cancer. Various mechanisms can cause multidrug resistance, of which the (over)expression of P-glycoprotein is the most intensively studied thus far. P-Glycoprotein is a membrane-associated protein that has affinity for a variety of large, structurally unrelated, neutral or cationic amphipathic compounds, including many anticancer drugs, e.g., Vinca alkaloids, taxanes, anthracyclines, and epipodophyllotoxines. By pumping substrate drugs out of the cell, this protein decreases the intracellular drug accumulation, resulting in a diminished therapeutic efficacy (reviewed in Germann, 1996). The observation that verapamil was able to reverse vincristine resistance in murine leukemia cell lines (Tsuruo et al., 1981) initiated a search for potent and selective reversal agents, compounds that are capable of blocking or inhibiting P-glycoprotein. Theoretically, their clinical application may be complicated by the presence of P-glycoprotein in normal tissues because it is likely that reversal agents inhibit endogenous P-glycoprotein as effectively as P-glycoprotein in tumor cells. Consequently, the accumulation of the concomitantly administered anticancer drug might increase in normal tissues, thereby augmenting the potential of toxic side-effects.

Two drug-transporting P-glycoproteins have been identified in mice, mdr1a and mdr1b, which together probably fulfill the same role as the single drug-transporting MDR1 P-glycoprotein in humans. This is reflected in the tissue distribution of MDR1 P-glycoprotein, which embodies all tissues expressing mdr1a, mdr1b, or both genes (O’Brien and Cordon-Cardo, 1996). The mdr1a gene is highly expressed in the intestinal epithelium and in the capillaries of the brain and the testes, whereas the mdr1b gene is predominantly expressed in the adrenal gland, pregnant uterus, and ovaries. Significant levels of both mdr1a and mdr1b are present in many other tissues, such as liver, kidney, lung, heart, and spleen (Croop et al., 1989). The initial ideas on the physiological function of P-glycoprotein were based on its tissue-specific localization. The results of several studies suggested

ABBREVIATIONS: Css, steady state plasma concentration; LLQ, lower limit of quantitation.
that P-glycoprotein plays a role in the protection of the organism against potentially toxic substances, e.g., by limiting the absorption of orally ingested compounds, by mediating the elimination of substrates from the body, and by protecting crucial organs such as the brain and the testis against toxic substances in the circulation (Thiebaut et al., 1987; Cordon-Cardo et al., 1989).

To get more information on the physiological and pharmacological role of mdr1a P-glycoprotein, mice with a homozygous disruption of the mdr1a gene (mdr1a<sup>−/−</sup>) mice have been generated in our institute (Schinkel et al., 1994). We have used these mice in a previous study with vinblastine administered as an i.v. bolus injection to gain more insight into the pharmacokinetic consequences of blocking P-glycoprotein in normal tissues, which may occur upon clinical application of reversal agents (van Asperen et al., 1996). An important finding of this study was the substantially reduced elimination of vinblastine in the absence of mdr1a P-glycoprotein, which was reflected in the prolonged presence of high drug concentrations in plasma of mdr1a<sup>−/−</sup> mice as compared with wild-type mice. This observation complicated the investigation of tissue-specific differences in drug accumulation between both types of mice because the tissue levels of a drug depend, for a considerable part, on its concentration in plasma. For all tissues except the brain, relatively small differences in vinblastine accumulation between mdr1a<sup>−/−</sup> and wild-type mice were observed. The most pronounced differences were found when the plasma concentrations had declined to 10 ng/ml. Therefore, we hypothesized that the high plasma levels of vinblastine present during the first hours after i.v. bolus administration may have saturated P-glycoprotein in wild-type animals. For these reasons, the aim of the present experiments was to study the tissue distribution of vinblastine in mdr1a<sup>−/−</sup> and wild-type mice at similar, constant plasma levels which do not exceed a concentration of 10 ng/ml. This study design has the additional advantage of a close resemblance to the clinical situation in which vinblastine is frequently administered as a continuous infusion with steady-state plasma levels around 2 ng/ml (Zeffren et al., 1984; Ratain and Vogelzang, 1986; Rahmani and Zhou, 1993).

Materials and Methods

Animals. All experiments were performed with male FVB mdr1a<sup>−/−</sup> or wild-type mice between 12 and 14 weeks of age (b.wt., 25.7–32.7 g). The animals were housed and handled according to institutional guidelines. Food (Hope Farms BV, Woerden, the Netherlands) and acidified water were given ad libitum.

Drugs and Chemicals. Vinblastine sulfate was obtained from Eli Lilly (Nieuwegein, the Netherlands). Solutions of 1, 2, 4, and 10 mg/ml vinblastine sulfate were prepared in 5% dextrose (NPBI Emmer-Compascuum, the Netherlands). Vintriptol methane sulfonate originated from the Medegenix Group (Fleureus, Belgium). The commercially available formulations of ketamine (Nimatek; 100 mg/ml ketamine HCl; A.U.V., Cuijk, the Netherlands) and medetomidine (Domitor; 1 mg/ml medetomidine HCl; SmithKline Beecham Farma, Rijswijk, the Netherlands) were freshly diluted in saline to final concentrations of 20 mg/ml and 0.01 mg/ml, respectively. All chemicals (E. Merck, Darmstadt, Germany) were of analytical quality except for acetonitrile, which was of gradient grade. Diethyl ether was distilled once before use in the analytical procedure; the other chemicals were used as supplied. Water was purified by the Milli-Q Plus system (Millipore, Milford, MA). Blank human plasma was obtained from healthy volunteers.

Study Design. Alzet osmotic pumps (model 2001; Alza Corporation, Palo Alto, CA) were filled with a vinblastine solution of 1, 2, 4, or 10 mg/ml. Four to six animals were used at each dose level. The pumps were implanted s.c. on the back of the mouse. Briefly, mice were anesthetized with medetomidine (0.064 mg/kg b.wt. s.c.) and ketamine (100 mg/kg b.wt. i.p.). The skin was shaved at the level of the implantation site and disinfected with 70% ethanol. A small midscapular incision was made through the skin. Subsequently, a pocket for the pump was created by detaching the s.c. tissue from the skin. The pump was inserted in a vertical direction and the wound was closed with surgical sutures. According to the manufacturer, the pump should reach a stable infusion rate of 1.0 µl/h at approximately 4 h after insertion, resulting in dose rates of 1.2, 4, and 10 µg/h vinblastine. Blood and tissue samples were collected approximately 96 h after implantation of the pump. Blood was obtained by orbital bleeding under diethyl ether anesthesia and collected in heparinized tubes. The plasma fraction was separated by centrifugation (10 min at 2000g; 4°C) and stored for analysis. Animals were sacrificed by cervical dislocation to collect the following tissues: eye, brain, skeletal muscle, colon, cecum, small gut, stomach, liver, kidney, lung, spleen, heart, testis, epididymis, organ fat, thymus, and peripheral and mesenteric lymph nodes. The contents of tissues from the gastrointestinal tract were carefully removed. Each tissue was homogenized in a volume of 1.0 to 3.0 ml blank human plasma (to obtain approximately 0.05–0.2 g tissue/ml) with a Polytron tissue homogenizer (Kinematica AG, Littau, Switzerland). All biological specimens were stored at −20°C until analysis.

Drug Analysis. The analysis of vinblastine was performed as described in detail previously (van Tellingen et al., 1993a; van Asperen et al., 1996). In summary, vinblastine was extracted from the biological matrices with diethyl ether. The organic layers were evaporated to dryness under a gentle stream of nitrogen (37°C). The residue was reconstituted in acetonitrile and subjected to ion-pair normal-phase HPLC with fluorescence detection.

Pharmacokinetics. Assuming a steady-state plasma concentration of vinblastine after a 96-h continuous infusion and 100% bioavailability, the clearance was calculated as Clearance = dose rate/([C<sub>ss</sub> × body weight]), where [C<sub>ss</sub>] is the steady-state plasma concentration.

Statistical Analysis. For each individual mouse, the ratio of the concentration of vinblastine in tissue versus plasma was calculated. The ratios of groups of wild-type and mdr1a<sup>−/−</sup> mice with approximately similar mean plasma levels were compared in the Mann-Whitney U test to determine significant differences in the tissue concentration of vinblastine in both types of mice. The Mann-Whitney U test was also used to analyze differences in plasma levels and clearances. A p < .05 was regarded as statistically significant.

Results

In previous pharmacokinetic studies with vinblastine in mice, a terminal half-life ranging from 2.1 to 8.6 h was found (van Tellingen et al., 1993b; van Asperen et al., 1996), which indicates that steady-state plasma levels of vinblastine will be achieved within the presently used 96-h continuous infusion regimen. The pharmacokinetic parameters of vinblastine after continuous infusion in mdr1a<sup>−/−</sup> and wild-type mice are represented in Table 1. In mdr1a<sup>−/−</sup> mice, a significantly lower clearance of vinblastine was observed at the 1 µg/h dose level as compared with 4 µg/h ([p = .006). The clearances of all groups treated at dose rates higher than 1 µg/h were comparable.

The major goal of this study was to compare the tissue concentration of vinblastine between both types of mice at approximately similar, constant plasma levels. This condi-
tion was achieved with wild-type and mdr1a(−/−) mice infused at dose rates of 2 and 1 μg/h, respectively (Table 2). The accumulation of vinblastine in the brains of mdr1a(−/−) mice was 4.4-fold higher (p = .006). For all other tissues, the differences were not statistically significant. A similar comparison could be made between wild-type and mdr1a(−/−) mice, both infused with 4 μg/h vinblastine. Somewhat more pronounced differences in tissue concentrations were observed at this dose level (Table 2). The drug concentration in the brains of mdr1a(−/−) mice was increased by a factor of 9.6 (p = .014). Furthermore, mdr1a(−/−) mice accumulated approximately 2-fold higher levels of vinblastine in the heart and in all intestinal tissues (p ≤ .028). A slightly but significantly higher drug accumulation was also found in organ fat and lymph nodes of mdr1a(−/−) mice. As illustrated in Table 3, for wild-type mice, the tissue concentration of vinblastine increased almost proportionally with the plasma concentration at dose rates of 1, 2, and 4 μg/h, whereas in most tissues, a tendency toward a diminished relative drug accumulation was observed at a dose rate of 10 μg/h. These data indicate that the administered dose levels of vinblastine did not saturate P-glycoprotein in wild-type animals.

### TABLE 2

<table>
<thead>
<tr>
<th></th>
<th>Dose Rate</th>
<th>Clearance</th>
<th>C_{ss, vinblastine}</th>
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<tr>
<td></td>
<td>μg/h</td>
<td>l/h/kg</td>
<td>ng/ml</td>
<td></td>
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<tr>
<td>Wild-type</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>2 μg/h</td>
<td>1.0</td>
<td>17.2 ± 3.6</td>
<td>2.2 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>1 μg/h</td>
<td>2.0</td>
<td>23.7 ± 7.5</td>
<td>3.7 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>4 μg/h</td>
<td>4.0</td>
<td>23.1 ± 3.9</td>
<td>6.0 ± 0.9</td>
<td></td>
</tr>
<tr>
<td>10 μg/h</td>
<td>10</td>
<td>24.3 ± 4.3</td>
<td>15.8 ± 3.2</td>
<td></td>
</tr>
<tr>
<td>mdr1a(−/−)</td>
<td>1.0</td>
<td>12.0 ± 1.6</td>
<td>3.3 ± 0.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.0</td>
<td>28.9 ± 3.1</td>
<td>5.1 ± 0.7</td>
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</table>

Vinblastine was administered as a 96-h continuous infusion, which was delivered by a s.c. implanted osmotic pump. The results are expressed as mean ± S.E. Four to six animals were analyzed in each group.

### Table 3

<table>
<thead>
<tr>
<th></th>
<th>Dose (μg/h)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Brain</td>
<td>&lt;3.6 a</td>
<td>4.6 ± 1.6</td>
<td>3.0 ± 0.6</td>
</tr>
<tr>
<td>Small intestine</td>
<td>32.7 ± 6.7</td>
<td>38.4 ± 9.1</td>
<td>29.5 ± 4.0</td>
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<tr>
<td>Stomach</td>
<td>33.6 ± 6.3</td>
<td>46.8 ± 9.0</td>
<td>41.5 ± 6.3</td>
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<tr>
<td>Liver</td>
<td>24.5 ± 5.3</td>
<td>35.7 ± 7.6</td>
<td>30.5 ± 5.2</td>
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<tr>
<td>Kidney</td>
<td>74.5 ± 19.6</td>
<td>73.0 ± 15.6</td>
<td>54.0 ± 6.6</td>
</tr>
<tr>
<td>Lung</td>
<td>91.4 ± 18.6</td>
<td>79.2 ± 14.0</td>
<td>71.8 ± 10.5</td>
</tr>
<tr>
<td>Spleen</td>
<td>124.5 ± 29.8</td>
<td>124.9 ± 25.1</td>
<td>100.2 ± 13.3</td>
</tr>
<tr>
<td>Heart</td>
<td>12.7 ± 2.6</td>
<td>13.8 ± 2.8</td>
<td>11.3 ± 1.8</td>
</tr>
</tbody>
</table>

Vinblastine was administered as a 96-h continuous infusion, which was delivered by a s.c. implanted osmotic pump with an infusion rate of 1 μl/h. Results are expressed as the ratio of the vinblastine concentration in tissue versus plasma (mean ± S.E.). Four to six animals were analyzed in each group.

a The observed concentration was below the LLQ of the analytical method, which was 8 ng/g brain tissue.

### Discussion

Our results show that except for the brain and, to a lesser extent, the heart and the intestinal tissues, mdr1a P-glycoprotein has only a minor tissue-specific impact on the distribution of vinblastine. Similar and constant plasma levels of vinblastine were realized in wild-type and mdr1a(−/−) mice, permitting an accurate analysis of tissue-specific differences in drug accumulation. The plasma levels were unlikely to saturate P-glycoprotein because they were below 10 ng/ml. Furthermore, they were in the same range as the plasma concentrations observed in patients treated with a continuous infusion of vinblastine (Zeffren et al., 1984; Ratain and Vogelzang, 1986; Rahmani and Zhou, 1993).

The present results seem to contrast with the moderate to high levels of mdr1a RNA reported for many tissues (Croop et al., 1989). Although in some tissues mdr1b P-glycoprotein, which can also transport vinblastine (Tang-Wai et al., 1995), may have compensated for the loss of mdr1a P-glycoprotein,
even in the small gut of mdr1a(-/-) mice, which completely lacks both mdr1a and mdr1b P-glycoproteins (Schinkel et al., 1994), only a relatively small increase in drug accumulation was observed. In normal tissues, MDR1 P-glycoprotein is mainly present in four cell types: simple columnar epithelial cells, a subset of capillary endothelial cells, placentae trophoblasts, and certain hematopoietic and lymphoid tissues (reviewed in O'Brien and Cordon-Cardo, 1996). In detail, MDR1 P-glycoprotein was shown to be present in a highly polarized fashion on the apical membrane of epithelial cells lining luminal spaces such as the epithelium of the intestines, the brush border of renal proximal tubules, the biliary ductal epithelial cells, the epithelial cells of the trachea and the major bronchi, and the luminal membrane of capillary endothelial cells in the brain and the testis (Cordon-Cardo et al., 1989; O'Brien and Cordon-Cardo, 1996). These data clearly indicate that only a small subset of the cells in a specific organ contains P-glycoprotein; consequently, the overall substrate concentration in an organ will be mainly determined by the concentration in the large majority of cells lacking P-glycoprotein. Furthermore, in many organs, the drug influx from blood to tissue is probably not altered in mdr1a(-/-) mice because P-glycoprotein is not found in the cells lining the capillary lumen in most organs. Potential differences in the cell-specific distribution of vinblastine between mdr1a(-/-) and wild-type mice may have disappeared by analyzing homogenates of complete organs. However, a sanctuary is formed if P-glycoprotein is present in the endothelial cells of all capillaries supplying a specific organ, e.g., the brain and the testis, which may explain the substantially increased accumulation of vinblastine in brain homogenates of mdr1a(-/-) mice. Although the amounts of mdr1a and mdr1b P-glycoprotein in heart tissue are comparable, a 1.7-fold increase in accumulation of vinblastine could already be observed in heart homogenates of mdr1a(-/-) mice, which indicates that P-glycoproteins play an important role in the protection of this organ. It would be interesting to see whether this is also supported by a cell-specific distribution of P-glycoprotein in the heart.

Previous studies have demonstrated the important role of mdr1a P-glycoprotein in the elimination and limited intestinal absorption of several substrate drugs (van Asperen et al., 1996; Mayer et al., 1996; Sparreboom et al., 1997). In the present experiments, no significant difference in vinblastine elimination was observed between mdr1a(-/-) and wild-type mice at similar infusion rates (p > .2). It is possible that at these low dose levels, other elimination pathways compensate for the absence of mdr1a P-glycoprotein. The significantly higher clearance at the 4 μg/h dose level as compared with the 1 μg/h dose level in mdr1a(-/-) mice may be explained by a stronger induction of vinblastine metabolizing enzymes at the higher dose level. It has been shown previously that induction of cytochrome P450 3A by rifampicin occurs at a lower dose level in mdr1a(-/-) mice compared with wild-type mice (Schuetz et al., 1996). Furthermore, experiments with human liver microsomes have demonstrated that vinblastine metabolism is mediated by the cytochrome P450 3A subfamily (Zhou-Pan et al., 1993). For the clinical application of reversal agents, these data indicate that, in general, a blockade of endogenous P-glycoprotein will not result in an increased accumulation of the coadministered anticancer drug in complete organs, but possibly only in classes of cells making up a fraction of an organ. However, the higher concentration of vinblastine in homogenates of intestinal tissues of mdr1a(-/-) mice suggests that a blockade of endogenous P-glycoprotein may enhance the risk of intestinal toxicity. Furthermore, for potential central neurotoxic or cardiotoxic anticancer drugs, extreme caution is warranted because the absence of functional P-glycoprotein in the brain and a diminished P-glycoprotein activity in the heart increased the overall accumulation of the substrate drug vinblastine.

In conclusion, the results of the present experiments give important information on the pharmacological role of P-glycoprotein. The minor differences in drug accumulation between tissues of mdr1a(-/-) and wild-type mice indicate that, except for the brain and, to a lesser extent, the heart and the intestinal tissues, P-glycoprotein does not protect complete, individual organs against potentially toxic substrates. Because of the polarized cell-specific and organ-specific distribution of P-glycoprotein, its affinity for a broad range of compounds and its importance in limiting the absorption of orally ingested substrates and in the elimination of substrates from the body, it is suggested that P-glycoprotein has mainly evolved to provide a general protection of the complete organism against potentially toxic substrates. Its principal mechanism of action seems to be mediating the transport of substrates from milieu intérieur to milieu extérieur and inhibiting their transport in the opposite direction, finally resulting in the elimination of substrates from the body and a diminished uptake of substrates into the body.

References


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Vinblastine Infusion in Wild-Type and mdr1a(−/−) Mice