Species Comparison of In Vivo P-Glycoprotein-Mediated Brain Efflux Using mdr1a-Deficient Rats and Mice

Christoffer Bundgaard, Christian Jes Nyberg Jensen, and Mats Garmer

Discovery DMPK, H. Lundbeck A/S, Valby, Denmark

Received September 30, 2011; accepted November 23, 2011

ABSTRACT:
The experiments described herein compared the extent of in vivo P-glycoprotein (P-gp)-mediated brain efflux between rats and mice for a set of known central nervous system compounds. With use of newly introduced genetically modified mdr1a-deficient rats and their gene-competent counterparts, the brain to plasma distribution was assessed and compared with the distribution pattern in mdr1a-deficient and wild-type mice. Four compounds (aripiprazole, cilazapril, risperidone, and venlafaxine) were administered using a continuous subcutaneous osmotic minipump infusion paradigm. Steady-state brain and plasma concentrations of the compounds, including selected metabolites (9-hydroxyrisperidone, O-desmethyl-venlafaxine and N-desmethyl-venlafaxine) were measured in mdr1a-deficient rats and mice and their wild-type counterparts along with their free fractions to determine total and unbound brain to plasma distribution between genotypes within and between species. The results revealed qualitative as well as quantitative similarities between P-gp functionality in vivo at the blood-brain barrier level in rats and mice. All compounds tested were shown to have a significantly higher brain to plasma distribution in both mdr1a-deficient rats and mice compared with their wild-type counterparts. Moreover, the relative enhancement in extent of brain penetration between mdr1a-deficient and wild-type rats could be directly correlated to the enhancement ratios obtained in mice. From the unbound brain to unbound plasma distributions, the impact of P-gp on the overall brain penetration capabilities showed minor differences between rats and mice for the compounds tested. In conclusion, a comparable functional role of P-gp between rats and mice with respect to brain efflux mediated by this transporter is suggested.

Introduction

The physiology of the blood-brain barrier (BBB) with its endothelial cells lining the brain capillaries connected by tight junctions limits the extent of brain penetration for many xenobiotics. In addition to the physical barrier, numerous transport proteins are present at the BBB, acting as efflux pumps to actively exclude a wide variety of chemically diverse compounds from the central nervous system (CNS). One of the most studied members of this group of transporters is P-glycoprotein (P-gp, ABCB1), a mammalian plasma membrane phosphoglycoprotein encoded by the multidrug resistance (MDR) 1 gene (mdr1 in rodents). P-gp belongs to the ATP-binding cassette (ABC) transporter superfamily and is located in the brain in the luminal membrane of the endothelial cells but is also expressed in other organs (Thiebaut et al., 1987). For compounds designed to exert their effect in the CNS, drug-P-gp interactions could influence the potential efficacy by reducing the brain concentration because of this efflux mechanism. Thus, P-gp has been shown in mice to have a profound influence on brain concentrations of many types of drug substrates including antidepressants and antipsychotics (Ejsing et al., 2007; Linnet and Ejsing, 2008). Therefore, screening for P-gp substrate recognition and assessment of the potential impact hereof are important parts of the profile evaluation of new chemical entities, particularly those targeted to the CNS (Wager et al., 2011).

During lead optimization programs and drug candidate characterization, in vivo pharmacology and pharmacokinetic studies are often performed in rats and mice. In such studies it is important to understand the potential differences in the functional activity of P-gp between these species with respect to drug distribution to the brain to make proper comparisons and extrapolations (Kim et al., 2008; Syvänen et al., 2009). In both rats and mice, P-gp is the most highly expressed ABC protein in brain endothelial capillary cells (Warren et al., 2009). Rats and mice share two genes encoding for P-gp, mdr1a and mdr1b, which are similarly expressed between the two species, and in both of these species, mdr1a is the predominant form in the brain (Croop et al., 1989; Kwan et al., 2003). In addition, there is a high degree of homology in the amino acid content of P-gp between rats and mice of approximately 93% (Hubbard et al., 2007). Studies investigating differences in P-gp functionality between rats and mice have primarily been conducted in vitro, applying efflux ratios derived from bidirectional transport studies in cell systems transfected with mdr1 from each species. These studies suggest some correlation between rat and mouse P-gp-mediated efflux and also differences, depending on the substrate (Booth-Genthe et al., 2006; Katoh et al., 2006; Takeuchi et al., 2006). However, comparison of efflux ratios among species from in vitro transport studies is circumvented with indecisive
biological challenges such as total protein expression and density of the functional transporter in each cell line.

With respect to in vivo studies investigating quantitative species differences in P-gp function between rats and mice, a very limited amount of data has been published. Cutler et al. (2006) used infusion of the P-gp inhibitor GF-1-129198 (elacridar) and reported that similar total plasma concentrations of the inhibitor were required in rats and mice to achieve the same relative increase in the brain/plasma distribution ratio of an undisclosed P-gp substrate. However, species differences in plasma protein binding of the inhibitor or substrate are confounding factors that may influence the total plasma concentrations required to inhibit P-gp. Thus, such chemically induced knockout studies are not readily comparable between species.

With the advent of 
mdr1a
-deficient mice either from genetic knockout modifications or mutant origin (Schinkel et al., 1996), these animals has become widely applied in drug discovery to assess the role of P-gp in vivo on drug disposition including BBB transport studies. Use of these transporter-deficient animal models has suffered because they are only available in mouse species, making quantitative interspecies comparisons difficult. Recently, however, 
mdr1a
-knockout rats became commercially available through the application of zinc finger nuclease technology, enabling targeted gene knockout (Geurts et al., 2009; Chu et al., 2011). The purpose of this study was therefore to compare the P-gp functionality between 
mdr1a
-deficient rats and mice and their gene-competent counterparts (wild types) with respect to in vivo brain distribution. A range of known CNS compounds and their selected metabolites previously recognized to be P-gp substrates were evaluated in these animal models using a similar experimental design across species.

Materials and Methods

Animals. Homozygous 
mdr1a
-knockout and wild-type rats (SD-Abc1a<sup>tm1sage</sup>, 350–500 g b.wt.) were obtained from SAGE Labs, Sigma Life Science (St. Louis, MO). 
mdr1a
-deficient mice and CF-1 (non-Swiss) outbred background mice (Crl:CF1-Abcb1a<sup>−/−</sup> mutants; 25–45 g b.wt.) were purchased from Charles River Laboratories, Inc. (Wilmington, MA). All animals arrived at the animal facility at least 5 days before being used in experiments. During acclimatization, animals were housed in pairs under controlled conditions of temperature (21 ± 2°C) and relative humidity (55 ± 5%) and a 12-h light/dark cycle (light on at 6:00 AM). Food and tap water were freely available in the home cage. Approval of all animal procedures was granted by The Danish National Committee for Ethics in Animal Experimentation.

Drugs and Dosing. The test compounds administered included citalopram, venlafaxine, risperidone, and aripiprazole, which are all extensively used CNS compounds. In addition, these compounds and their selected metabolites represent a relatively wide span in their functional extent of substrate affinity toward P-gp at the BBB level in mice (Linnet and Ejsing, 2008). ALZET osmotic minipumps (Durect Corporation, Cupertino, CA) were used to deliver the test compounds in steady-state dosing regimens. ALZET pumps model 2ML1 (delivery rate 10 μl/h) and model 2001 (delivery rate 1 μl/h) were applied for rats and mice, respectively. Citalopram (hydrobromide; H Lundbeck A/S, Valby, Denmark), venlafaxine (hydrochloride; Sigma-Aldrich, St. Louis, MO), and risperidone (free base, Sigma-Aldrich) were dissolved in 9.0% NaCl. Aripiprazole (free base; Sequoia Research, Pangbourne, UK) was dissolved in 20% hydroxypropyl-β-cyclodextrin, pH 4. Test compounds were used in the following concentrations (as free base) in the pumps with corresponding average doses calculated on the basis of body weight and pump delivery rate: citalopram, rats 15 mg/ml (~9 mg/kg per day) and mice 6 mg/ml (~5 mg/kg per day); venlafaxine, rats 15 mg/ml (~9 mg/kg per day) and mice 20 mg/ml (~12 mg/kg per day); risperidone, rats 10 mg/ml (~5 mg/kg per day) and mice 4 mg/ml (~4 mg/kg per day); and aripiprazole, rats 12 mg/ml (~6 mg/kg per day) and mice 3 mg/ml (~3 mg/kg per day). Before implantation, the pumps were equilibrated by soaking them overnight in sterile saline solution at 37°C. Before surgery, animals received carprofen (Rimadyl) subcutaneously as analgesic treatment. During brief isoflurane anesthesia, mini-pumps were implanted subcutaneously on the back of the animals under aseptic conditions. Postoperative treatment was provided by buprenorphine (Temgesic) on the day of surgery and carprofen on the following 2 days. Three to five wild-type and 
mdr1a
-deficient rats and mice were used in each treatment group. The continuous delivery was maintained for 3 days in both mice and rats to attain a steady state, after which the animals were decapitated and the brains were dissectioned out. Tum blood was collected in EDTA-coated tubes, and plasma was harvested after centrifugation (5000g for 10 min at 4°C). Brain homogenate was prepared by homogenizing the whole brain with 70% acetonitrile (1:4, v/v) followed by centrifugation and collection of the supernatant. Plasma and brain supernatant samples were frozen at −80°C until analysis.

Bioanalysis. Rat and mice plasma and brain homogenate samples were prepared by precipitation and addition of generic internal standard. Plasma and brain concentrations of dosed test compounds and selected metabolites (O-desmethyl-venlafaxine, N-desmethyl-venlafaxine, and 9-hydroxyrisperidone) were determined using ultrasensitive performance liquid chromatography (Acquity UPLC system; Waters, Milford, MA) followed by tandem mass spectrometry detection using a Sciex API 4000 mass spectrometer (AB Sciex, Foster City, CA) (Erichsen et al., 2010). The lower limit of quantification was typically 0.5 ng/ml in plasma and 1 ng/g in brain (peak signal/noise ratio >5).

Free Fraction Determinations. Plasma protein binding and nonspecific binding in brain homogenates were determined in naive male rats (Sprague-Dawley) and mice (Crl:NMRI(Han)) by use of equilibrium dialysis. The procedure was modified from Kalvass and Maurer (2002). Dialysis membranes (96-well formats, cutoff 12–14 kDa; HTDialysis, LLC, Gales Ferry, CT) were soaked in phosphate-buffered saline-ethanol (80:20) and rinsed in deionized water before use. Brain homogenate or plasma was added to the donor side and spiked with test compound to a final concentration of 1 µM. The receiver side contained phosphate buffer (pH 7.4). Equilibrium dialysis was performed by incubating at 37°C for 5 h. Then the compound concentration was measured in the buffer phase using liquid chromatography-mass spectrometry. The free fraction in plasma (f<sub>c</sub> plasma) and intact brain tissue (f<sub>c, brain</sub>) was calculated according to Kalvass and Maurer (2002) with their dilution factor in brain taken into account. For all compounds, recovery after incubation was in the range of 91 to 109%.

Data Analysis and Statistics. Brain/plasma distribution ratios (K<sub>pu</sub>) were calculated from the measured total drug levels in the respective matrices (C<sub>brain, total</sub> and C<sub>plasma, total</sub>). To compare brain to plasma distribution in 
mdr1a
-deficient and wild-type animals between species, K<sub>pu</sub> values were normalized and expressed relative to those of wild types (100%) for each species. In addition, the unbound brain to unbound plasma partition coefficients, designated K<sub>pu, un</sub>, were calculated by associating free fractions with total brain to plasma distribution at steady state for each species as C<sub>brain, total</sub> × f<sub>c, brain</sub> plasma, total × f<sub>c</sub> plasma. (Hammarlund-Udenaes et al., 1997; Gupta et al., 2006; Student’s t test was used to compare brain/plasma distribution ratios data between 
mdr1a
-deficient and wild-type animals. Statistical and regression analyses were performed using GraphPad Prism (version 4.02; GraphPad Software Inc., San Diego, CA).

Results

After 3 days of continuous treatment using minipumps, the four test compounds and three selected formed metabolites were measured in plasma and brains of wild-type and 
mdr1a
-deficient rats and mice. The observed total plasma and brain concentrations and corresponding K<sub>pu</sub> values are summarized in Table 1 for rats and Table 2 for mice. For all compounds except 9-OH-risperidone, there were no statistically significant differences between the systemic plasma exposure in 
mdr1a
-deficient animals and their wild-type counterparts. For 9-OH-risperidone, plasma concentrations were significantly higher in 
mdr1a
-deficient mice compared with those in wild-type mice (p < 0.05). This trend was also observed for 9-OH-risperidone in 
mdr1a
-deficient rats versus wild-type rats although not to a statistically significant degree. From the derived K<sub>pu</sub> values, all the compounds tested tended to display higher brain distribution in mice compared with that in rats when the species within in each genotype were
Thus, from the as being substrates for P-gp at the BBB level in both rats and mice.

exhibited significant enhancement in their normalized wild-type controls (set as 100%) for each species. All the compounds evaluated, citalopram exhibited the highest $K_p$ value in both species.

All the compounds in the data set that were tested were identified as being substrates for P-gp at the BBB level in both rats and mice. Among wild-type mice, 9-OH-risperidone and aripiprazole had the lowest $K_p$ values of 0.28 and 0.49. Of the compounds evaluated, citalopram exhibited the highest $K_p$ value in both species.

To assess the impact of P-gp deficiency on the brain to plasma distribution of the compounds independent of binding to plasma proteins and brain tissue, the plasma and brain free fractions determined in vitro were combined with measured total brain and plasma concentrations at steady state to calculate $K_{pu}$ for each compound in wild-type and mdr1a-deficient animals (Table 3). In wild-type rats, all compounds had $K_{pu}$ values well below unity, consistent with active efflux dominating the BBB distribution of these compounds. The $K_{pu}$ Values in mdr1a-deficient rats were increased to approximately unity for the majority of the compounds. In mice, all the compounds except for citalopram exhibited $K_{pu}$ values below unity. Citalopram was shown to have a $K_{pu}$ value of 2.4 in wild-type mice, which was increased to 4.8 in mdr1a-deficient mice, whereas the other compounds were increased to values closer to unity compared with the respective values in wild-type animals.

### Discussion

The experiments described herein compared the prevalence and magnitude of in vivo P-gp-mediated brain efflux between rats and mice for a set of known CNS compounds. To compare the extent of brain distribution between rats and mice and the impact of P-gp, all experiments were performed at steady state. This design allowed for calculations of $K_p$ and $K_{pu}$ between brain and plasma with species-dependent pharmacokinetics taken into account.

### Table 1

<table>
<thead>
<tr>
<th>Compound</th>
<th>Plasma</th>
<th>Brain</th>
<th>$K_p$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ng/ml</td>
<td>ng/g</td>
<td></td>
</tr>
<tr>
<td>Citalopram</td>
<td>27 ± 2.4</td>
<td>265 ± 16</td>
<td>10 ± 0.78</td>
</tr>
<tr>
<td>Venlafaxine</td>
<td>50 ± 3.7</td>
<td>158 ± 4.6</td>
<td>3.3 ± 0.29</td>
</tr>
<tr>
<td>O-Desmethyl-venlafaxine</td>
<td>4.5 ± 1.4</td>
<td>4.4 ± 0.89</td>
<td>1.0 ± 0.10</td>
</tr>
<tr>
<td>N-Desmethyl-venlafaxine</td>
<td>6.8 ± 1.5</td>
<td>9.3 ± 1.1</td>
<td>1.4 ± 0.14</td>
</tr>
<tr>
<td>Risperidone</td>
<td>194 ± 3.4</td>
<td>41 ± 0.88</td>
<td>0.22 ± 0.04</td>
</tr>
<tr>
<td>9-OH-risperidone</td>
<td>153 ± 24</td>
<td>22 ± 1.6</td>
<td>0.15 ± 0.03</td>
</tr>
<tr>
<td>Aripiprazole</td>
<td>18 ± 5.3</td>
<td>3.0 ± 0.60</td>
<td>0.19 ± 0.04</td>
</tr>
</tbody>
</table>

### Table 2

<table>
<thead>
<tr>
<th>Compound</th>
<th>Plasma</th>
<th>Brain</th>
<th>$K_p$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ng/ml</td>
<td>ng/g</td>
<td></td>
</tr>
<tr>
<td>Citalopram</td>
<td>8.8 ± 0.40</td>
<td>191 ± 19</td>
<td>22 ± 2.2</td>
</tr>
<tr>
<td>Venlafaxine</td>
<td>85 ± 9.8</td>
<td>403 ± 63</td>
<td>4.7 ± 0.40</td>
</tr>
<tr>
<td>O-Desmethyl-venlafaxine</td>
<td>5.7 ± 0.35</td>
<td>10 ± 0.87</td>
<td>1.8 ± 1.13</td>
</tr>
<tr>
<td>N-Desmethyl-venlafaxine</td>
<td>17 ± 2.8</td>
<td>25 ± 5.1</td>
<td>1.5 ± 0.12</td>
</tr>
<tr>
<td>Risperidone</td>
<td>43 ± 1.0</td>
<td>21 ± 2.3</td>
<td>0.52 ± 0.08</td>
</tr>
<tr>
<td>9-OH-risperidone</td>
<td>45 ± 8.4</td>
<td>13 ± 1.7</td>
<td>0.28 ± 0.01</td>
</tr>
<tr>
<td>Aripiprazole</td>
<td>51 ± 8.7</td>
<td>25 ± 4.8</td>
<td>0.49 ± 0.04</td>
</tr>
</tbody>
</table>
From the $K_p$ values obtained in mdr1a-deficient rats and mice and their wild-type counterparts, the experiments revealed qualitative as well as quantitative species similarities in P-gp functionality at the BBB level. Thus, all compounds evaluated were shown to be substrates in both species. Moreover, as shown in Fig. 2, the extent of the increase in the normalized $K_p$ values between mdr1a-deficient animals and their wild-type counterparts could be directly correlated between rats and mice. The indication of a comparable quantitative role of in vivo P-gp functionality at the BBB between rats and mice observed in this study correlates with expression levels and protein homology of mdr1 between these species (Kim et al., 2008). In addition, in vitro studies in mdr1a-transfected cells indicate a comparable functional role of P-gp between these species although some substrate dependence has been reported (Booth-Genthe et al., 2006; Katoh et al., 2006; Takeuchi et al., 2006).

The compounds tested have previously been reported to be substrates for P-gp in vivo at the BBB in mice as shown by increased brain to plasma distribution in P-gp knockout versus that in wild-type mice. Citalopram has been shown to have a 2- to 3-fold higher $K_p$ value in P-gp knockout mice (Uhr and Grauer, 2003; Doran et al., 2005; Uhr et al., 2008), in line with the present data in mice (201). In mdr1a-deficient rats, citalopram showed an enhancement ratio of 330/27%, which could indicate that this compound has a slightly higher propensity for P-gp-mediated brain efflux in rats compared with that in mice. Assessment of bidirectional transport properties across the BBB including the impact of P-gp can be based on the ratio between unbound concentrations in brain and plasma at equilibrium conditions, expressed as $K_{pu}$. If $K_{pu}$ is close to unity, the BBB transport is predominantly mediated by passive diffusion or the impact of influx and efflux transport is equal. If $K_{pu}$ above unity is an indication that active uptake processes are involved (Ham-

![Fig. 2](image-url)
In both mice and rats. In both mechanisms (Rochat et al., 1999). Thus, a possible species-dependent has been reported to be transported by means of a carrier-mediated transport studies across bovine brain endothelial cells, where citalopram putative involvement of active uptake is supported by in vitro trans-

was also observed for citalopram in rats although to a lesser extent. A contribution to the overall BBB distribution of citalopram. This pattern efflux, some involvement of uptake transporters is also suggested to contribute to the overall BBB distribution of citalopram. This pattern was also observed for citalopram in rats although to a lesser extent. A putative involvement of active uptake is supported by in vitro transport studies across bovine brain endothelial cells, where citalopram has been reported to be transported by means of a carrier-mediated mechanism (Rochat et al., 1999). Thus, a possible species-dependent affinity to uptake transporters could in theory influence the functional transport activity at the BBB differently between mdr1a-deficient rats and mice although this possibility remains to be explored.

Aripiprazole has previously been shown to be a substrate for P-gp with a 2.7-fold increase in Kp in knockout mice after sub-

chronic minipump treatment (Kirschbaum et al., 2010). In the present study, a similar increase in Kp was observed in mdr1a-deficient mice (269 ± 39% increase). As for citalopram, a slightly higher increase in Kp for aripiprazole was attained in mdr1a-deficient rats compared with wild-type rats (338 ± 38% increase), which cannot rule out some species difference of P-gp functionality for this compound. The Kp, uu values of aripiprazole in mdr1a-deficient rats and mice were increased to reach similar values and did not reach unity. However, some uncertainty in these derived values is to be expected because the free fractions of aripiprazole were extremely low in plasma as well as in brain.

Risperidone and its metabolite 9-OH-risperidone are well studied P-gp substrates in mice with profound P-gp effects on extent of brain penetration shown by at least 10 times higher Kp values in knockout mice in a range of different studies (Wang et al., 2004; Doran et al., 2005; Ejsing et al., 2005). These reports aligned well with the present results showing that these compounds were the most pronounced substrates in the test set with increases in Kp values in similar ranges in both mice and rats. In both mdr1a-deficient rats and mice, Kp, uu values for risperidone and 9-OH-risperidone were increased to values very close to unity, suggesting that P-gp was the main gatekeeper for brain penetration of these substances in both species.

Brain entry of venlafaxine and its demethylated metabolites O-desmethyl-venlafaxine and N-desmethyl-venlafaxine has previously been shown to be limited by P-gp using knockout mice with brain penetration enhancement ratios between 2 and 4 in mice lacking P-gp after subchronic minipump treatment (Uhr et al., 2008; Karlsson et al., 2011). In the current experiments, similar enhancement ratios were observed in mice. Venlafaxine and O-desmethyl-venlafaxine displayed equal relative enhancement ratios between mice and rats lacking P-gp. N-Desmethyl-venlafaxine had a slightly higher enhance-

ment ratio in rats (634 ± 117%) compared with that in mice (461 ± 13%). However, relatively high variability in the Kp data of formed N-desmethyl-venlafaxine was observed in rats. In regard to this, dosing of this metabolite could be considered as a means to a more accurate determination of potential species differences in the P-gp-mediated effects of this compound. Kp, uu estimates of venlafaxine and its metabolites in mdr1a-deficient and wild-type animals indicated that passive diffusion was the dominant factor for BBB distribution without the presence of P-gp across the parent-metabolite system. Kp, uu values of N-desmethyl-venlafaxine in rats and O-desmethyl-venlafaxine in mice increased to values greater than 2 in the mdr1a-deficient species, but the aforementioned variability in N-desmethyl-venlafaxine exposure and the very high free fraction of O-desmethyl-venlafaxine in mice plasma suggest that these values should be interpreted with caution.

Development of the genetically mdr1-deficient rat model provides a simple tool for evaluating the functional consequence of P-gp efflux at the BBB by the measurement of drug concentrations in brain and plasma at steady state. However, some drug- and/or species-dependent limitations in this model and methodology probably exist that may affect interpretation of the obtained Kp, uu estimates. First, it has been shown that knockout of P-gp in mice can result in alterations in the mRNA expression levels of other transporter proteins (Cisternino et al., 2004). Thus, if the compound under investigation has substrate affinity for other efflux or uptake transporters besides P-gp, this could potentially affect the brain to plasma distribution obtained in mdr1-deficient models. A recent characterization of the mdr1a-deficient rat model suggests that only minor compensatory changes in expression of other transporter-related genes occur at the BBB level in male rats lacking P-gp (Chu et al., 2011). Second, continuous drug administration may increase P-gp functionality at the BBB as a result of mdr1 induction. This has been suggested for venlafaxine in vitro in human cell systems (Ehret et al., 2007; Bachmeier et al., 2011) and in vivo in rats after 3 weeks of continuous treatment (de Klerk et al., 2010). Thus, if species differences exist in terms of liability and extent of mdr1 induction after subchronic treatment, this could theoretically hamper a quantitative comparison of P-gp-mediated efflux between rats and mice. Third, intrabrain drug distribution governed by processes other than nonspecific binding may potentially affect the estimates of Kp, uu in the present study. Hence, determining brain free fractions using the homogenate method-

ology only measures nonspecific binding and therefore does not account for other intrabrain distributional aspects such as sequestra-

tion into lysosomes that might affect cellular retention for basic compounds. In addition, the homogenization process may alter the binding properties by unmasking binding sites that are not accessible to a drug in intact brain tissue. However, recent studies suggest that a

<table>
<thead>
<tr>
<th>Compound</th>
<th>Free Fraction, Rats</th>
<th>Free Fraction, Mice</th>
<th>Kp, uu, Rats</th>
<th>Kp, uu, Mice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( f_u, p \text{ plasma} )</td>
<td>( f_u, p\text{ brain} )</td>
<td>( K_p, uu )</td>
<td>( f_u, p \text{ plasma} )</td>
</tr>
<tr>
<td>Citalopram</td>
<td>50</td>
<td>3.1</td>
<td>0.61</td>
<td>2.1</td>
</tr>
<tr>
<td>Venlafaxine</td>
<td>64</td>
<td>9.2</td>
<td>0.45</td>
<td>0.89</td>
</tr>
<tr>
<td>O-Desmethyl-venlafaxine</td>
<td>89</td>
<td>35</td>
<td>0.38</td>
<td>1.2</td>
</tr>
<tr>
<td>N-Desmethyl-venlafaxine</td>
<td>32</td>
<td>8.0</td>
<td>0.34</td>
<td>2.0</td>
</tr>
<tr>
<td>Risperidone</td>
<td>11</td>
<td>9.0</td>
<td>0.17</td>
<td>1.4</td>
</tr>
<tr>
<td>9-OH-risperidone</td>
<td>20</td>
<td>15</td>
<td>0.11</td>
<td>1.1</td>
</tr>
<tr>
<td>Aripiprazole</td>
<td>0.10</td>
<td>0.10</td>
<td>0.19</td>
<td>0.64</td>
</tr>
</tbody>
</table>

\( f_u, p \) values are shown as averages of two independent determinations.

WT, wild-type; P-gp Def, mdr1a-deficient.

\( f_u, p \text{ plasma} \) values of 99% was used to estimate \( K_p, uu \) for venlafaxine and O-desmethyl-venlafaxine.

\( K_p, uu \) values for aripiprazole were extremely low in plasma as well as in brain.

TABLE 3

BRAIN EFFLUX COMPARISON BETWEEN P-gp KNOCKOUT RATS AND MICE
reasonable correlation can be established between nonspecific binding from homogenates and binding obtained using brain slices with a preserved cellular structure (Fridén et al., 2011).

By use of the same experimental methodology in rats as in mice, the validity of the mdr1a-deficient rat model was confirmed in the present study in which all selected P-gp substrates identified in mice were also recognized in rats. This model thus allows for a direct means to assess the potential influence of P-gp on pharmacokinetic and pharmacodynamic parameters in rats without the need for chemical P-gp inhibitors. Besides qualitative similarities in P-gp functionality between rats and mice, the data obtained here also indicate substantial quantitative similarities in the extent of P-gp-mediated efflux at the BBB between these species. This information might prove useful when existing knowledge on P-gp-mediated efflux obtained in mice is extrapolated to the rat species during pharmacokinetic and in vivo pharmacological evaluations.

Acknowledgments

Mona Elster is acknowledged for technical assistance.

Authorship Contributions

Participated in research design: Bundgaard and Jensen.

Conducted experiments: Jensen and Garmer.

Performed data analysis: Bundgaard and Garmer.

Wrote or contributed to the writing of the manuscript: Bundgaard.

References


Address correspondence to: Dr. Christoffer Bundgaard, H. Lundbeck A/S, Discovery DMPK, 9 Ottillavej, DK-2500 Valby, Denmark. E-mail: bur@lundbeck.com