The brain entry of risperidone and 9-hydroxyrisperidone is greatly limited by P-glycoprotein

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Abstract
P-glycoprotein (P-gp) in the brain capillary endothelial cell limits the entry of many drugs into the brain. Our previous in-vitro study using ATPase as a marker of P-gp activity suggested that risperidone might be effectively transported by P-gp. In the present study, we compared the concentrations of risperidone and its major pharmacologically active metabolite 9-hydroxyrisperidone (9-OH-risperidone), in plasma, brain and various other tissues between abcb1ab−/− knockout mice which are functionally devoid of P-gp in their blood–brain barrier vs. FVB wild-type mice. One hour after intraperitoneal injection of 4 mg/g risperidone, the brain concentrations and ratios of brain:plasma concentrations of risperidone (13.1-fold and 12-fold respectively, \( p < 0.05 \)) and 9-OH-risperidone (29.4-fold and 29-fold respectively, \( p < 0.01 \)) were significantly higher in the abcb1ab−/− mice than those in the FVB mice. These results indicate that P-gp in the blood–brain barrier significantly influences the brain concentrations of risperidone and 9-OH-risperidone by limiting their CNS access.

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Key words: P-glycoprotein, 9-OH-risperidone, risperidone.

Introduction
The multidrug resistance gene ABCB1 that encodes for P-glycoprotein (P-gp) exists in various normal tissues including the apical membrane of the gastrointestinal tract, the biliary canalicular membranes of hepatocytes, the luminal membranes of proximal tubular epithelial cells in the kidney, and the luminal membranes of endothelial cells of the blood–brain barrier (BBB). It is clear that P-gp acts as a cellular efflux pump playing an important role in limiting organ exposure to various endogenous and exogenous compounds (Schinkel and Jonker, 2003). P-gp in brain endothelial capillary cells limits compounds of diverse structures from entering the brain thereby minimizing or preventing their CNS effects (Kim et al., 1998; Schinkel et al., 1994, 1996, 1997; Uhr et al., 2000, 2002; Uhr and Grauer, 2003; Wang et al., 2004a,b). This defensive function of P-gp may be beneficial for peripherally acting drugs that are substrates of P-gp by blocking their brain penetration but may diminish the therapeutic efficacy of CNS-acting drugs that are substrates of P-gp. In this regard, identifying CNS-active drugs that are substrates of P-gp may have important clinical significance as the expression and activity of P-gp can vary substantially between individuals.

Among the various in-vitro and in-vivo approaches used in studying the P-gp function in CNS drug penetration, the abcb1 a/b knockout mouse model with functional deficiency in the BBB is unique for reflecting the complex in-vivo situation of P-gp in the BBB (Schinkel et al., 1994). This model has been used to test the P-gp-mediated CNS penetration of diverse classes of drugs including protease inhibitors, loperamide, ondansetron, ivermectin, vincristin, cyclosporin A, amitriptyline, morphine, olanzapine and methadone (Kim et al., 1998; Schinkel et al., 1994, 1996, 1997; Uhr et al., 2000, 2002; Uhr and Grauer, 2003, Wang et al., 2004a,b).
By using ATPase activity as a marker for P-gp activity, our previous in-vitro study suggested that several atypical antipsychotic drugs (AADs), i.e. quetiapine, risperidone and olanzapine may be effectively transported by P-gp (Boulton et al., 2002). The rank order of ATPase activity was verapamil (positive control) > quetiapine > risperidone > olanzapine. In a subsequent in-vivo study using the abcb1a gene knockout mouse model, we found that P-gp limited the brain penetration of olanzapine, as its brain concentration was 3-fold higher in the abcb1a knockout mice than that in the FVB control mice (Wang et al., 2004a). Finally, Mahar Doan et al. (2002) classified risperidone as a P-gp substrate based on the results of the in-vitro fluorescent probe calcein-AM. No previous reports have evaluated 9-hydroxyrisperidone (9-OH-risperidone), the major active metabolite of risperidone, as a P-gp substrate. Based on these previous studies, we hypothesized that brain entry of risperidone and 9-OH-risperidone would be significantly limited by P-gp in vivo.

Materials and methods

Chemicals

Risperidone, 9-OH-risperidone and methylrisperidone (internal standard) were generous gifts from Janssen Pharmaceutica (Titusville, NJ, USA). Hanks’ balanced salt solution (HBSS) was purchased from Gibco–BRL (Carlsbad, CA, USA). Other chemicals and reagents were of the purest grade available and were obtained from Fisher Scientific Co. (Fairlawn, NJ, USA).

Experimental animals

Male abcb1ab+/− transgenic mice and genetically matched male wild-type FVB mice aged 8 wk weighing 22–30 g were obtained from Taconic Farms Inc. (Germantown, NY, USA). The founding of this colony has been previously described (Schinkel et al., 1994). Animals were housed individually and maintained at a 12:12 h light–dark cycle. The animals were cared for in accordance with the US Public Health Service policy for the Care and Use of Laboratory Animals, and the experimental studies were approved by the Medical University of South Carolina Animal Care Committee.

Experimental procedures

Risperidone was dissolved in 0.9% saline containing 1% ethanol and 0.5% acetic acid. A dose of 4 μg risperidone/g mouse was administered intraperitoneally (i.p.). The entire volume of injection was 10 μl/g mouse. One hour after injection, the mice were anaesthetized with isoflurane and decapitated. Trunk blood was collected in EDTA-coated tubes and centrifuged at 3000 g for 20 min to determine plasma concentrations of risperidone and 9-OH-risperidone.

Brain, lung, heart, liver, kidney, spleen, small intestine, muscle and testis were dissected and weighed and then homogenized in 5 × vol of HBSS and 0.02 M Hepes buffer (pH 7.2). The homogenates were frozen at −70 °C for subsequent high-performance liquid chromatography (HPLC) analysis.

HPLC analysis of risperidone and 9-OH-risperidone concentrations in plasma and tissues

Plasma and tissues samples obtained at 1 h following i.p. administration of risperidone were extracted and determined using an HPLC method previously developed and validated in our laboratory with slight modification for tissue samples (Titier et al., 2002). Briefly, 25 μl of internal standard working solution (8 μg/ml methylrisperidone), 0.5 ml of 1 M borate buffer (pH 10), and 5 ml of pentane: dichloromethane were added to 200–1000 μl of plasma or organ samples. The mixture was shaken for 20 min and centrifuged at 3000 g for 5 min. The organic layer was transferred to another tube and evaporated under slow nitrogen flow at 45 °C. The residue was dissolved in 100 μl of mobile phase (35% acetonitrile/65% 0.01 M phosphate buffer, pH 4.5), and 50 μl was injected into the chromatographic system. Risperidone, 9-OH-risperidone and internal standard were separated by a Waters (Milford, MA, USA) Novapak C18 column (150 × 3.9 mm), and the effluent was monitored at a UV wavelength of 280 nm with a flow rate of 1.0 ml/min at ambient temperature. The detection limit for risperidone, 9-OH-risperidone and internal standard was 10 ng/ml. The intra-day and inter-day coefficients of variation were lower than 7% at relevant concentrations (n = 7). The plasma and tissues extraction recoveries ranged from 85 to 98%.

Data analysis

Drug quantitation was performed by comparing peak area ratios of risperidone and 9-OH-risperidone to the internal standard with ratios derived from calibration curves of standards containing spiked amounts of chemicals extracted from plasma or tissues as described above. Final concentrations of risperidone and 9-OH-risperidone were expressed as either ng/ml for plasma or ng/g for tissues.

An unpaired t test was used to compare the differences between the concentrations of risperidone and...
9-OH-risperidone in abcb1ab−/− mice and control FVB mice and their tissue:plasma ratios. A two-tailed p value was used and the level of statistical significance was set as p < 0.05.

Results

The plasma and organ concentrations of risperidone and 9-OH-risperidone 1 h after i.p. injection of 4 μg risperidone/g mouse are shown in Tables 1 and 2. Risperidone and 9-OH-risperidone were extensively localized in liver, kidney, lung, testis and gut, and to a lesser extent in heart, spleen and muscle in both groups of mice. The brain concentrations of risperidone and 9-OH-risperidone in the abcb1ab−/− mice were markedly higher (13.1- and 29.4-fold respectively, p < 0.0001) than those of the FVB wild-type mice (Table 1). Another tissue showing significant difference in drug concentrations was testis, although not as dramatic as brain. The testis concentrations of risperidone and 9-OH-risperidone were 1.9-fold (p < 0.05) and 5.2-fold (p < 0.01) higher respectively, in the abcb1ab−/− mice than in the FVB controls.

Ratios of brain:plasma concentrations for risperidone and 9-OH-risperidone were markedly higher (12- and 29-fold respectively, p < 0.05) in the abcb1ab−/− mice than in the FVB mice (Figure 1). Ratio of testis:plasma concentrations of risperidone (liver, and muscle) and 9-OH-risperidone (heart, spleen and muscle) significantly differ between the abcb1ab−/− mice and FVB mice, normalizing for plasma concentration differences of risperidone and 9-OH-risperidone between the two groups of mice eliminated the differences in the tissue:plasma ratios (Figure 1). There was a statistically significant lower heart and spleen concentration of risperidone in the abcb1ab−/− mice than in the FVB mice, and the difference for risperidone was still significant after considering the plasma differences between the two groups of mice.

Discussion

Risperidone belongs to the class of second-generation, or atypical antipsychotics. This class of drugs includes clozapine, risperidone, olanzapine, quetiapine, ziprasidone, and aripiprazole. The AADs have advantages over the traditional antipsychotics such as chlorpromazine and haloperidol. They selectively bind to the central dopamine D2 and serotonin (5-HT1c) receptor, appear more effective on the associated negative symptoms (affective flattening, avolition, cognitive impairment) of schizophrenia, and have a lower propensity for causing extrapyramidal symptoms (EPS) (Kinon and Lieberman, 1996). These advantages have resulted in the AADs quickly supplanting the traditional antipsychotics as the first choice of antipsychotic, especially in the USA (Sharma and Mockler, 1998). Despite the successful marketing of AADs, their therapeutic efficacy is frequently disappointing and dose-limited adverse events (EPS, cardiac conduction disturbances, hyperprolactinaemia, weight gain and

Table 1. Tissue (ng/g) and plasma concentrations (ng/ml) of risperidone in abcb1ab−/− (n = 6) and wild-type (abcb1ab+/+) mice (n = 6) 1 h after i.p. injection of 4 μg risperidone/g mouse

<table>
<thead>
<tr>
<th>Organ</th>
<th>abcb1ab−/−</th>
<th>abcb1ab+/+</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>352 ± 211</td>
<td>254 ± 161</td>
<td>1.4</td>
</tr>
<tr>
<td>Brain</td>
<td>1306 ± 453</td>
<td>100 ± 15</td>
<td>13.1**</td>
</tr>
<tr>
<td>Liver</td>
<td>1271 ± 96</td>
<td>574 ± 265</td>
<td>2.2*</td>
</tr>
<tr>
<td>Kidney</td>
<td>1261 ± 451</td>
<td>1727 ± 403</td>
<td>0.7</td>
</tr>
<tr>
<td>Lung</td>
<td>1538 ± 533</td>
<td>918 ± 319</td>
<td>1.7*</td>
</tr>
<tr>
<td>Heart</td>
<td>237 ± 77</td>
<td>479 ± 66</td>
<td>0.5**</td>
</tr>
<tr>
<td>Testis</td>
<td>2173 ± 81</td>
<td>1170 ± 407</td>
<td>1.9*</td>
</tr>
<tr>
<td>Small intestine</td>
<td>1603 ± 535</td>
<td>1224 ± 482</td>
<td>1.3</td>
</tr>
<tr>
<td>Spleen</td>
<td>596 ± 212</td>
<td>1367 ± 265</td>
<td>0.4**</td>
</tr>
<tr>
<td>Muscle</td>
<td>469 ± 156</td>
<td>206 ± 32</td>
<td>2.3*</td>
</tr>
</tbody>
</table>

* Statistical significance (p < 0.05) by two-tailed unpaired t test.
** Statistical significance (p < 0.01) by two-tailed unpaired t test.

Table 2. Tissue (ng/g) and plasma concentrations (ng/ml) of 9-OH-risperidone in abcb1ab−/− (n = 6) and wild type (abcb1ab+/+) mice (n = 6) 1 h after i.p. injection of 4 μg risperidone/g mouse

<table>
<thead>
<tr>
<th>Organ</th>
<th>abcb1ab−/−</th>
<th>abcb1ab+/+</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>149 ± 1.1</td>
<td>329 ± 149</td>
<td>1.1</td>
</tr>
<tr>
<td>Brain</td>
<td>706 ± 248</td>
<td>24 ± 15</td>
<td>29.4**</td>
</tr>
<tr>
<td>Liver</td>
<td>1318 ± 80</td>
<td>1063 ± 167</td>
<td>1.2*</td>
</tr>
<tr>
<td>Kidney</td>
<td>1591 ± 268</td>
<td>1374 ± 238</td>
<td>1.1</td>
</tr>
<tr>
<td>Lung</td>
<td>1502 ± 442</td>
<td>1477 ± 188</td>
<td>1.0</td>
</tr>
<tr>
<td>Heart</td>
<td>302 ± 70</td>
<td>488 ± 47</td>
<td>0.6*</td>
</tr>
<tr>
<td>Testis</td>
<td>418 ± 90</td>
<td>81 ± 21</td>
<td>5.2**</td>
</tr>
<tr>
<td>Small intestine</td>
<td>1294 ± 139</td>
<td>1766 ± 340</td>
<td>0.7*</td>
</tr>
<tr>
<td>Spleen</td>
<td>830 ± 164</td>
<td>1167 ± 164</td>
<td>0.7**</td>
</tr>
<tr>
<td>Muscle</td>
<td>399 ± 119</td>
<td>209 ± 50</td>
<td>1.9**</td>
</tr>
</tbody>
</table>

* Statistical significance (p < 0.05) by two-tailed unpaired t test.
** Statistical significance (p < 0.01) by two-tailed unpaired t test.
metabolic disturbances) may complicate their treatment (Ereshefsky, 1996). There is emerging evidence showing that the P-gp in the BBB limits the CNS entry of some AADs, and the accumulation of AADs in peripheral tissues results in non-CNS adverse events (Boulton et al., 2002; Wang et al., 2004a). Thus, for understanding the variables that influence the disposition of AADs, it is important to evaluate their transport by P-gp.

The present results provide evidence that risperidone and its active moiety, 9-OH-risperidone, were effectively transported by P-gp in the BBB. In addition, the present data showed that the ratio of brain to plasma of 9-OH-risperidone in abcb1ab −/−/ FVB is 2.4-fold higher than that of risperidone, indicating that 9-OH-risperidone may be more effectively transported by P-gp than risperidone. Because the transport efficiency of the two moieties by the brain efflux transporter is different, a change of the plasma concentrations of the two moieties could result in differences in therapeutic efficacy or adverse reactions. The present findings verify the significance of the activity of a brain drug efflux pump in counterbalancing the active and passive brain uptake of drugs in determining their overall CNS concentration. Furthermore the present results suggest the possibility for significant drug interactions to occur by concomitant use of agents known to inhibit or induce P-gp.

Although a relatively high dose of risperidone (4 μg/g mouse) was used in the study, the very low concentrations of risperidone and 9-OH-risperidone observed in the brain tissue of FVB control mice do not support saturation of P-gp transport in the BBB causing bias in the experimental results. Consistent with the higher efficiency of transport of 9-OH-risperidone to P-gp than risperidone, the difference of 9-OH-risperidone in testis between the two groups of mice was also prominent compared to that of risperidone. Although concentrations of risperidone and 9-OH-risperidone in several other tissues showed differences between the two groups, these differences were apparently caused by the plasma fluctuations between animals since normalization of tissue concentrations to plasma eliminated the statistical significance. The explanation for the lower heart and spleen distribution of risperidone in the P-gp knockout mice is unknown and has not been previously reported. Similar, albeit weaker, differences were also noted for 9-OH-risperidone. These findings do not contradict the weak expression of P-gp in heart and spleen in wild-type mice (Schinkel et al., 1996, 1997). We postulate that if the total volume of distribution of risperidone were comparable between the mutant and FVB strains, the greater amount of risperidone distributed in the brain in the mutants than FVB mice may have resulted in lesser distribution to other tissues, including heart, spleen, and perhaps other unmeasured sites. Another possible explanation might be different metabolism and elimination characteristics of risperidone between the mutant and FVB strains. While the use of a larger number of animals in each group might clarify these differences, the dramatic differences in brain concentrations would be unlikely to change.

In conclusion, the present results showed that the expression of P-gp in the BBB significantly limits the penetration of risperidone and 9-hydroxyrisperidone into the CNS. The transport efficacy of P-gp for risperidone and 9-OH-risperidone is apparently different.
Recognition of risperidone and 9-OH-risperidone along with other AADs (Wang et al., 2004a) as P-gp substrates offers intriguing possibilities for enhancing their therapeutic utility through the use of adjunctive P-gp modulators.

Acknowledgements

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Statement of Interest

None.

References


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