Short Communication

The Role of P-Glycoprotein in Limiting Brain Penetration of the Peripherally Acting Anticholinergic Overactive Bladder Drug Trospium Chloride

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ABSTRACT:
The aim of the present study was to characterize the role of the drug-efflux transporter P-glycoprotein (P-gp) for the disposition of trospium chloride, a widely used anticholinergic drug for the treatment of overactive bladder. P-gp-deficient mdr1a,b(−/−) knockout mice were given either 1 mg/kg trospium chloride orally or 1 mg/kg intravenously to analyze brain penetration, intestinal secretion, and hepatobiliary excretion of the drug. The concentrations of trospium chloride in the brain were up to 7 times higher in the mdr1a,b(−/−) knockout mice compared with wild-type mice (p < 0.05), making P-gp a limiting factor for the blood-brain barrier penetration of this drug. Moreover, the residence time of the drug in the central nervous system was significantly prolonged in mdr1a,b(−/−) knockout mice. Apart from the blood-brain barrier, P-gp also had significant effects on the overall pharmacokinetics of trospium chloride. In the mdr1a,b(−/−) knockout mice, hepatobiliary excretion and intestinal secretion were significantly reduced compared with the wild-type mice. Our study indicates that the multidrug resistance transporter P-gp is a major determinant for the distribution of trospium chloride in the body and highly restricts its entry into the brain.

Antagonists of the acetylcholine muscarinic receptors, such as trospium chloride, oxybutynin, tolterodine, fesoterodine, darifenacin, and solifenacin are the cornerstone of pharmacotherapy for the symptoms of overactive bladder (OAB) (Andersson, 2005). A potential problem in OAB therapy with such drugs is the undesirable side effects involving the central nervous system (CNS) including dizziness, nervousness, sleep disorders, cognitive impairment, memory impairment, hallucination, and confusion (Scheife and Takeda, 2005; Kay and Ebinger, 2008). The occurrence of these CNS side effects is greatly dependent on the ability of the individual drug to pass the blood-brain barrier (BBB) (Andersson, 2005; Staskin and MacDiarmid, 2006). Although most of the aforementioned antimuscarinic drugs are tertiary amines that are quite lipophilic and can easily penetrate into the brain, trospium chloride is a highly polar quaternary amine that exhibits low lipophilicity (Singh-Franco et al., 2005) (for chemical structure, see Schladitz-Keil et al., 1986). Therefore, trospium chloride can be expected to show much lower penetration through the BBB of patients than other, more lipophilic and uncharged antimuscarinic drugs (Wiedemann and Schwantes, 2007).

Apart from their physicochemical properties, brain penetration of many drugs is also affected by drug transporters expressed at the BBB. P-gp is a transmembrane protein that transports a wide range of structurally unrelated drugs, toxins, and xenobiotics, many of which are amphiphilic and/or positively charged (Marzolini et al., 2004). This observation led us to hypothesize that trospium chloride might also be a substrate of P-gp. A previous in vitro study showed that trospium chloride slightly inhibited the P-gp-mediated digoxin transport across LLC-PK1 cell monolayers that overexpressed the human P-gp (Sandage et al., 2006). Another in vitro study with P-gp-expressing Caco-2 monolayers showed that trospium chloride was transported from the basolateral to the apical direction in a verapamil-sensitive manner (Langguth et al., 1987). Both findings supported our assumption.

The present study aimed to characterize the role of P-gp in the disposition of trospium chloride by using the P-gp-deficient mdr1a,b(−/−) knockout mouse model, which, to our knowledge, has not previously been applied to any other OAB anticholinergic drug. We found that brain penetration of trospium chloride is restricted by P-gp and thus increased up to 7-fold in mdr1a,b(−/−) knockout mice. Furthermore, P-gp is significantly involved in the intestinal secretion and hepatobiliary elimination of trospium chloride in mice.

Materials and Methods

Animals. Whereas humans only have one gene encoding the drug-transporting P-gp (MDR1), in the mouse genome, two P-gp coding genes, mdr1a and mdr1b, have been identified. The tissue distribution of mouse mdr1a and mdr1b P-gps suggests that both proteins together fulfill the same function as the single MDR1 P-gp in humans (Borst and Schinkel, 1997). Therefore, we

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ABBREVIATIONS: OAB, overactive bladder; CNS, central nervous system; BBB, blood-brain barrier; P-gp, P-glycoprotein.
used mdr1a/b(−/−) double knockout mice [referred to as mdr1a,b(-/-) knockout mice] of a FVB genetic background together with wild-type FVB mice (wild-type mice) in our studies. Male wild-type mice and mdr1a,b(−/−) knockout mice were obtained from Taconic Farms (Germantown, NY). All mice were housed in isolated ventilated cages under controlled temperature with a 12-h light/dark cycle and with access to sterilized food and water ad libitum. The mice were between 12 and 19 weeks of age. All animal experiments were registered and approved by the local administration and were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication 85-23, revised in 1985).

Drug Preparation and Administration. [3H]Trospium trifluoroacetate (70 Ci/mmol) was purchased from RC TRITEC AG (Teufen, Switzerland), and unlabeled trospium chloride was kindly provided by Dr. R. Pfleger GmbH (Bamberg, Germany). For drug application, a mixture of [3H]trospium trifluoroacetate (2–2.5 μCi representing 0.06–0.07% of the total dose) and unlabeled trospium chloride was prepared in 50 μL of phosphate-buffered saline (137 mM NaCl, 2.7 mM KCl, 1.5 mM KH2PO4, and 7.3 mM Na2HPO4, pH 7.4) for intravenous administration or in 200 μL of phosphate-buffered saline (137 mM NaCl, 2.7 mM KCl, 1.5 mM KH2PO4, and 7.3 mM Na2HPO4, pH 7.4) for intravenous administration or in 200 μL of NaCl (0.9%) for p.o. application. Due to the high excess of chloride in relation to trifluoroacetate in the drug preparation, this is further referred to as [3H]trospium chloride. For oral drug administration, the animals were fasted overnight, and food was made available 3 h after drug application.

Gallbladder Cannulation. Wild-type mice and mdr1a,b(−/−) knockout mice were anesthetized with a combination of ketamine and xylazine at a final dose of 116 mg/kg ketamine and 8 mg/kg xylazine. The anesthetic solution was injected intraperitoneally. After laparotomy and distal ligation of the common bile duct, a polyethylene catheter with an inner diameter of 0.25 mm was inserted into the incised gallbladder and was fixed to the gallbladder with an additional ligature. After intravenous injection of [3H]trospium chloride into the tail vein, bile was collected over a 120-min time period, during which the mice were placed in a temperature-controlled hood. At the end of the experiments, blood was collected by cardiac puncture, and the organs were removed and homogenized in 100 to 7000 μL of NaOH (0.05 M) (depending on the tissue weight). The levels of radioactivity in serum, bile, urine, feces, and tissue homogenates were quantified by a Wallac 1409 liquid scintillation detector (PerkinElmer Life and Analytical Sciences, Waltham, MA).

Tissue Distribution. For analysis of the tissue distribution, [3H]trospium chloride was applied i.v. and p.o. at 1 mg/kg b.wt. to wild-type and mdr1a,b(−/−) knockout mice. After 12 h, the animals were euthanized by cervical dislocation. The organs were removed and processed as described above.

Statistical Analysis. All data are presented as the mean ± S.D. of three to four animals. Student’s two-tailed unpaired t test and one-way analysis of variance followed by Bonferroni’s post hoc test were used to identify significant differences between groups.

Results

Hepatobiliary and Urinary Excretion of [3H]Trospium Chloride. Due to the significance of P-gp in determining overall body distribution and drug elimination in general, under an intravenous application regimen we analyzed the role of P-gp for the hepatobiliary elimination, urinary excretion, and intestinal secretion of trospium chloride using gallbladder-cannulated wild-type mice and mdr1a,b(−/−) knockout mice. [3H]Trospium chloride was applied intravenously at a dosage of 1 mg/kg b.wt. into the tail vein, and bile and urine samples were collected by catheterization. After 2 h, mice were euthanized and the intestinal content was recovered from the small intestine and the colon. Hepatobiliary excretion of [3H]trospium chloride was rapidly detected within a few minutes after intravenous application and tended to be higher in the wild-type mice over 50 min (Fig. 1A). The maximal drug concentrations in the bile were 12.1 ± 2.7 μg/mL and 9.5 ± 2.6 μg/mL in the wild-type mice and mdr1a,b(−/−) knockout mice, respectively. In contrast to the bile flux data, which did not reach a level of significance, the overall hepatobiliary excretion of [3H]trospium chloride during 120 min was significantly lower in the mdr1a,b(−/−) knockout mice: whereas the wild-type mice excreted 26.0 ± 0.5% of the applied dose into bile, the mdr1a,b(−/−) knockout mice only excreted 20.0 ± 1.4% of the applied dose (Fig. 1B).

Significant differences between the wild-type and the mdr1a,b(−/−) knockout mice also occurred in the liver content of [3H]trospium chloride: 120 min after drug application, only 3% of the applied dose was detected within a few minutes after intravenous application and tended to be higher in the wild-type mice and 11% of the applied dose was detected in the liver of the mdr1a,b(−/−) knockout mice, pointing to an accumulation in the liver content of [3H]trospium chloride. In contrast, 11% of the applied dose was detected in the liver of the mdr1a,b(−/−) knockout mice, pointing to an accumulation in the liver due to a reduced biliary efflux of the drug. Supporting this conclusion, the sum of drug recovered in liver and bile was nearly identical between wild-type and mdr1a,b(−/−) knockout mice, at 29 and 31%, respectively. By balancing their reduced hepatobiliary elimination, the mdr1a,b(−/−) knockout mice showed higher excretion of the drug into the urine compared with the wild-type mice, namely 39 versus 29% of the total dose within 2 h.
Intestinal Secretion of [3H]Trospium Chloride. Two hours after intravenous [3H]trospium chloride application, the intestinal content was recovered from the small intestine and colon and analyzed for intestinal drug secretion. Because we used gallbladder-cannulated and bile duct-ligated mice for this study, biliary excretion of [3H]trospium chloride could be excluded, and all gut radioactivity was derived solely from intestinal secretion. In both samples, i.e., from the small intestine and colon, significantly lower drug amounts were detected in the mdr1a,b(−/−) knockout mice compared with wild-type mice, pointing to an important role of P-gp for intestinal secretion of trospium chloride (Fig. 2A). The overall amount of trospium chloride in the intestinal content was 0.7% of the applied dose in the mdr1a,b(−/−) knockout mice and 1.7% in the wild-type mice within a 2-h investigation period. The overall amount of intestinal excretion of [3H]trospium chloride was also analyzed in mdr1a,b(−/−) knockout mice and wild-type mice without bile fistula 12 h after application of 1 mg/kg b.wt. [3H]trospium chloride (Fig. 2A). This study showed that the intestinal drug content tended to be lower in mdr1a,b(−/−) knockout mice compared with wild-type mice, but this difference did not reach the level of significance (p < 0.12).

Brain Penetration and Organ Distribution of [3H]Trospium Chloride. In both studies involving intravenous [3H]trospium chloride application to gallbladder-cannulated mice (2 h) and mice without bile fistula (12 h), we also analyzed drug penetration into the brain. In addition, mdr1a,b(−/−) knockout mice and wild-type mice received 1 mg/kg b.wt. [3H]trospium chloride orally because this is the approved route of application for this drug in humans. The overall drug concentrations in the brain were highest 2 h after intravenous application and extremely low 12 h after p.o. application. In the mdr1a,b(−/−) knockout mice, [3H]trospium chloride brain concentrations were 2 times higher (36.5 ± 7.3 versus 20.6 ± 3.0 ng/g) in the 2 h/i.v. group, 7 times higher (26.2 ± 3.3 versus 3.8 ± 2.1 ng/g) in the 12 h/i.v. group, and 4 times higher (2.3 ± 1.6 versus 0.6 ± 0.6 ng/g) in the 12 h/p.o. application group compared with wild-type mice (Fig. 2B). In addition, the brain-to-plasma ratio 2 h after application was higher for the mdr1a,b(−/−) knockout mice (0.27 ± 0.03) compared with the wild-type mice (0.17 ± 0.05) (Table 1). Unfortunately, [3H]trospium chloride concentrations in the plasma were below the detection level 12 h after intravenous and p.o. applications. Therefore, brain-to-plasma ratios could not be calculated from these application groups.

Brain Penetration of Trospium Chloride. Trospium chloride, a quaternary ammonium compound, which is hydrophilic and highly polar, is considered to have low ability to diffuse passively across the BBB (Scheife and Takeda, 2005; Wiedemann and Schwantes, 2007). These physicochemical properties may therefore limit the extent of CNS side effects of trospium chloride, which are expected to occur as a result of the nonselective interaction of anticholinergics with CNS muscarinic receptors (Kay et al., 2005). In the present study, we confirmed the very low ability of trospium chloride to penetrate the BBB and demonstrate that in mice, as an additional and biological protective barrier, the P-gp-mediated drug efflux at the BBB highly limits brain penetration of this drug. Because the transport data for many drugs closely correlate between the human and mouse P-gp (Feng et al., 2008), the same protective effect will probably occur also at the human BBB. This result is clearly consistent with data from clinical studies in man that did not find any undesirable CNS side effects under OAB treatment with trospium chloride (Singh-Franco et al., 2005).

The Role of P-gp for Overall Trospium Chloride Pharmacokinetics. Apart from the prominent role of P-gp in limiting brain penetration of trospium chloride, we found significant effects of the P-gp efflux pump on the intestinal secretion and hepatobiliary elimination and hence on the overall pharmacokinetics of this drug. A comparable effect of P-gp has also been previously shown for other P-gp transported drugs such as cyclosporine A, digoxin, paclitaxel, and loperamide (Schinkel et al., 1996; Sparreboom et al., 1997). Regarding trospium chloride clearance from the brain and liver, clear differences were found: 2 h after application, [3H]trospium chloride accumulated by a greater extent in the liver (ratio 3.2) than in the brain of the mdr1a,b(−/−) knockout mice (ratio 1.8). This result is likely caused by the impaired hepatobiliary elimination route in the absence of P-gp. This order was reversed 12 h after application, where 6.9-fold higher drug concentrations were detected in the brain of the mdr1a,b(−/−) knockout mice compared with the wild-type mice. Liver concentrations were no longer different between the mouse strains at this later time point. This effect can be explained by a slow rate of elimination of trospium chloride from the brain in the absence of P-gp, which caused a decline from 37 to 26 ng/g from 2 to 12 h, respectively. Such reduced clearance from the brain in the absence of
P-gp has been described before for other P-gp drugs such as vinblastine and digoxin (Schinkel et al., 1994; Mayer et al., 1996). In contrast, [3H]troposium chloride was quite rapidly cleared from the brain of the wild-type mice (21 to 4 ng/g from 2 to 12 h, respectively) (Table 1).

**Drug-Drug Interactions at the Level of P-gp Transport.** Because the prevalence of OAB increases markedly with age (Stewart et al., 2003), patients treated with antimuscarinic drugs are likely to take several concomitant medications, and thus it is important to consider the potential for drug-drug interactions in this situation. Most of the available antimuscarinic drugs such as oxybutynin, tolterodine, and darifenacin are extensively metabolized by the cytochrome P450 enzymes CYP3A4 and/or CYP2D6 (Michel and Hegde, 2006). In contrast, troposium chloride is not a substrate of the cytochrome P450 isoenzymes and is almost entirely excreted into the urine as the active drug (Schladitz-Keil et al., 1986; Singh-Franco et al., 2005). Apart from troposium chloride, many other drugs often used in elderly patients are substrates or inhibitors of P-gp. Therefore, drug-drug interactions with troposium chloride could occur at the level of transport (Pal and Mitra, 2006). However, in recent clinical studies, it has been shown that troposium chloride comedication did not significantly alter the pharmacokinetics of the P-gp substrate digoxin, and interactions with other drugs have also not been identified in vivo (Singh-Franco et al., 2005; Sandage et al., 2006). Therefore, from our study of the available literature, we postulate that drug-drug interactions with troposium chloride via P-gp transport are probably of minor clinical importance, if at all.

In conclusion, this study adds a new drug to the list of P-gp substrates investigated in the mdr1a,b(−/−) knockout mouse model. Our results have shown that the drug efflux transporter P-gp at the BBB highly restricts the entry of troposium chloride into the brain. This mechanism is likely to be at least part of the reason for the reduced CNS side effect problems for troposium chloride during OAB treatment in humans.

### References

-mannose 6-phosphate receptor
-lation in humans.

### Table 1

<table>
<thead>
<tr>
<th>Tissue</th>
<th>2 h, i.v., with Bile Fistula</th>
<th>12 h, i.v., without Bile Fistula</th>
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<tr>
<td>Wild-Type</td>
<td>Wild-Type</td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>21 ± 3</td>
<td>4 ± 2</td>
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<tr>
<td>Liver</td>
<td>942 ± 458</td>
<td>422 ± 276</td>
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<tr>
<td>Kidney</td>
<td>3086 ± 1001</td>
<td>104 ± 33</td>
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<td>Stomach</td>
<td>267 ± 53</td>
<td>106 ± 39</td>
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<tr>
<td>Lung</td>
<td>99 ± 21</td>
<td>12 ± 9</td>
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<tr>
<td>Spleen</td>
<td>95 ± 5</td>
<td>46 ± 21</td>
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<tr>
<td>Testis</td>
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<td>12 ± 4</td>
</tr>
<tr>
<td>Plasma</td>
<td>127 ± 47</td>
<td>135 ± 35</td>
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<tr>
<td>Brain/Plasma</td>
<td>0.17 ± 0.05</td>
<td>0.27 ± 0.03</td>
</tr>
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</table>

Data are presented as means ± S.D. of n = 3 (2 h) or n = 4 (12 h) animals per group. Ratios are obtained by dividing the drug concentration in mdr1a,b(−/−) knockout mice by the concentration in wild-type mice.


**Schinkel AH, Smit JJ, van Tellingen O, Beijnen JH, Wagenaar E, van Deemter L, Mol CA, van der Valk MA, Robanus-Maandag EC, and te Riele HP (1994) Disruption of the mouse mdr1a and mdr1b glycoprotein gene leads to a deficiency in the blood-brain barrier and to increased sensitivity to drugs. *Cell* 77:491–502.**


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