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Differences in the Brain Penetration of the Anticholinergic Drugs Trospium Chloride and Oxybutynin

Joachim Geyer,[1] Olga Gavrilova,[1] Ulrich Schwantes[2]

[1]Justus Liebig University, Institute of Pharmacology and Toxicology, Giessen, Germany; [2]Department of Medical Science / Clinical Research, Dr. R. Pfleger GmbH, Bamberg, Germany

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ABSTRACT

INTRODUCTION: This study was performed to compare tissue distribution and brain penetration of the anticholinergic drugs trospium chloride and oxybutynin in a mouse model. Additionally, the role of the drug efflux carrier P-glycoprotein for hepatobiliary and urinary excretion and the blood-brain barrier permeability of oxybutynin were evaluated by using knockout mice that were deficient in P-glycoprotein.

METHODS: Radio-labeled trospium chloride and oxybutynin were administered orally (1 mg/kg) to wild-type and P-glycoprotein deficient knockout mice. Tissue distribution of the drugs was analyzed after 12 hours. Additionally, oxybutynin was applied intravenously to gall bladder cannulated mice of both types. Drug excretion into bile and urine was analyzed over 2 hours by catheterization.

RESULTS: Absolute drug concentrations in the brain were almost 200-fold higher for oxybutynin (~200 ng/g) compared with trospium chloride (~1 ng/g) when applied at an equal dosage of 1 mg/kg orally, whereas concentrations in the liver were only 15-fold different (~300 ng/g for oxybutynin and ~20 ng/g for trospium chloride). P-glycoprotein deficient knockout mice after oxybutynin application showed no significant differences in brain penetration or drug excretion into bile and urine when compared with wild-type mice.

CONCLUSION: Brain penetration of oxybutynin highly exceeds that of trospium chloride at an equal dosage (1 mg/kg, given orally). In contrast to trospium chloride, brain penetration of oxybutynin is not restricted by the drug efflux carrier P-glycoprotein because oxybutynin is not a P-glycoprotein substrate *in vivo*.

KEYWORDS: Trospium chloride; Oxybutynin; P-glycoprotein; Multidrug resistance gene 1 (*mdr1*); Blood-brain barrier; Transport

CORRESPONDENCE: Prof. Dr. Joachim Geyer, Institute of Pharmacology and Toxicology, Justus Liebig University of Giessen, Frankfurter Str. 107, 35392 Giessen, Germany (Joachim.M.Geyer@vetmed.uni-giessen.de).

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INTRODUCTION

Antagonists of the acetylcholine muscarinic receptors (eg, trospium chloride, oxybutynin, tolterodine, darifenacin) are the cornerstone of pharmacotherapy for the symptoms of overactive bladder (OAB) [1]. Potential undesireable side effects involving the central nervous system (CNS) can occur. These side effects include dizziness, nervousness, sleep disorders, cognitive impairment, memory impairment, hallucination, and confusion [2,3]. The occurrence of these CNS side effects is greatly dependent on the ability of the individual drug to pass the blood-brain barrier (BBB) [4,5]. 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 permeability across biological membranes [6]. Moreover, the present authors recently showed that BBB permeability of trospium chloride

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original study

is highly restricted by the drug efflux carrier P-glycoprotein (P-gp) that is encoded by the multidrug-resistant (*mdr1*) gene [7]. The purposes of the present study were to: (1) compare tissue distribution of trospium chloride versus oxybutynin, and (2) analyze brain penetration and drug excretion of oxybutynin in P-gp deficient knockout mice.

METHODS

Animals

Male P-gp deficient *mdr1a/1b*$^{-/-}$ double knockout mice (further labeled as *mdr1*$^{-/-}$ knockout mice, n = 11) of an FVB genetic background and wild-type FVB mice (wild-type mice, n = 15) (Taconic Farms Inc., Germantown, NY, USA) were used. All mice were housed in isolated ventilated cages with a controlled temperature and a 12-hour:12-hour, light:dark cycle. Sterilized food and water were provided *ad libitum*. The mice were between 12 and 19 weeks of age. All animal experiments were registered and approved by the local administration.

Drug Preparation and Application

[^{3}H]trospium trifluoracetate (70 Ci/mmol) and [^{3}H]oxybutynin (14 Ci/mmol) were purchased from RC TRITEC AG (Teufen, Switzerland). Unlabeled trospium chloride was provided by Dr. R. Pfleger GmbH (Bamberg, Germany) and unlabeled oxybutynin was purchased from Sigma-Aldrich (Taufkirchen, Germany). Both drugs were used at a standard dosage of 1.0 mg/kg body weight in all experiments. Trospium chloride was used in a mixture of [^{3}H]trospium trifluoracetate (0.06 – 0.07 % of the total dose) and unlabeled trospium chloride, and was dissolved in 200 µl 0.9% NaCl for oral application. Due to the high excess of chloride in relation to trifluoracetate in the drug preparation, this drug is further labeled [^{3}H]trospium chloride. For oxybutynin applications, a mixture of [^{3}H]oxybutynin (0.15 – 0.5% of the total dose) and the unlabeled drug was prepared in PEG 400 and PBS (80 volume/20 volume) for intravenous (IV) administration, and in sesame oil for oral application. For oral drug administration, the animals were fasted overnight until food was made available 3 hours after drug application.

Biliary Excretion and Tissue Distribution

Wild-type mice and *mdr1*$^{-/-}$ 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, and the gallbladder was cannulated as previously described [7]. [^{3}H]oxybutynin was injected into the tail vein. Bile was collected over 120 minutes, during which time the mice were placed in a temperature-controlled hood. Additionally, [^{3}H]trospium chloride and [^{3}H] oxybutynin were administered orally at 1 mg/kg to wild-type and *mdr1*$^{-/-}$ knockout mice. At the end of the experiments, animals were euthanized by cervical dislocation. The organs were removed and homogenized in 100-6000 µl 0.05 M NaOH, depending on the tissue weight. The levels of radioactivity in the plasma and in tissue homogenates were quantified by a Wallac 1409 liquid scintillation counter.

Statistical Analysis

All data are presented as the mean and standard deviation (SD) of 3 to 4 animals. Student's two-tailed unpaired *t* test and one-way ANOVA followed by Bonferroni's *post hoc* tests were used to identify significant differences ($P < .05$) between the groups.

RESULTS

Tissue Distribution and Brain Penetration of Trospium Chloride and Oxybutynin in Mice

In order to directly compare tissue distribution and brain penetration of trospium chloride and oxybutynin, both drugs were orally applied at an equal dosage of 1 mg/kg to wild-type mice. Tissue concentrations were determined after 12 hours. Tissue concentrations of trospium chloride were in the range of 1-20 ng/g, with the lowest concentrations being in the brain and testis (1-2 ng/g) (ie, organs with tight blood-tissue barriers). Highest concentrations of trospium chloride were detected in the liver (18.5 ng/g). In contrast, tissue concentrations for oxybutynin were similar in all organs analyzed and ranged from 150-300 ng/g. Comparing both compounds, absolute brain concentrations were almost 200-fold higher for oxybutynin when compared with trospium chloride at an equal dosage (Figure 1).

Figure 1. Tissue Concentrations of Trospium Chloride (Green Bars) and Oxybutynin (Red Bars) in Normal Mice 12 Hours After Oral Application of an Equal Dosage of 1 mg/kg. doi: 10.3834/uij.1944-5784.2010.02.12f1

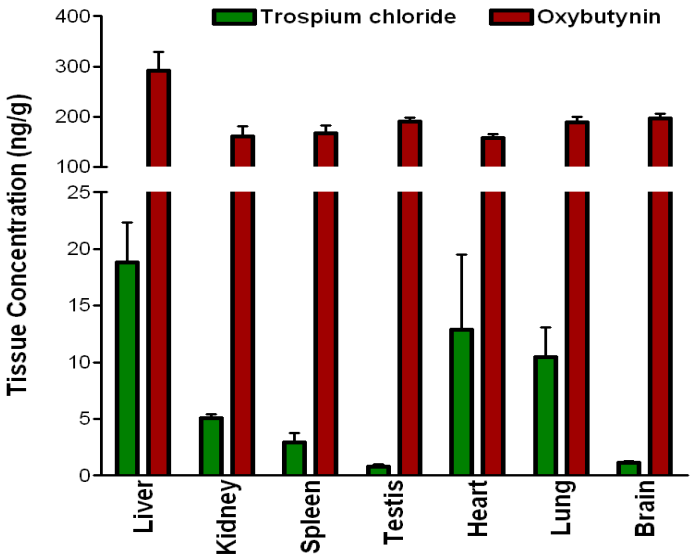

Figure 2a. Biliary Excretion of Oxybutynin (1 mg/kg, Intravenously) in Mice With Cannulated Gallbladders.
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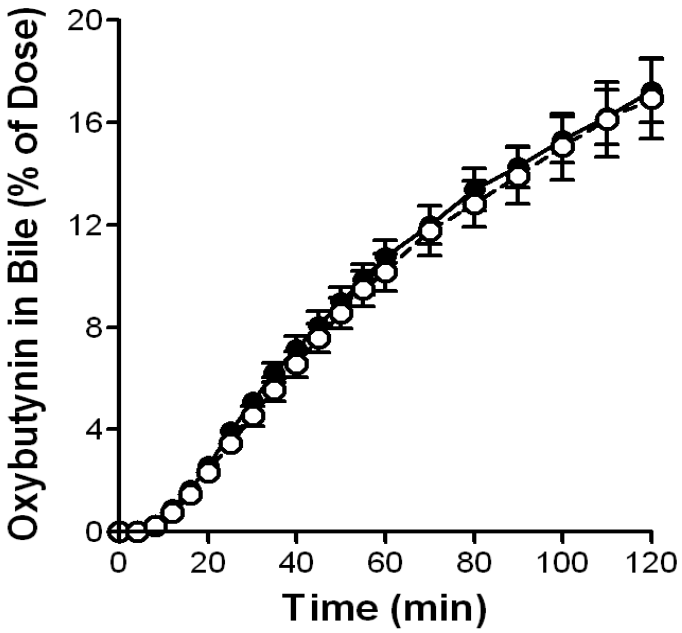

Wild-type mice, closed circles;
mdr1$^{-/-}$ knockout mice, open circles.

Brain Penetration and Elimination of Oxybutynin in mdr1$^{-/-}$ *Knockout Mice*

To analyze the role of P-gp for the hepatobiliary elimination and urinary excretion of oxybutynin, the authors used wild-type mice and *mdr1*$^{-/-}$ knockout mice with cannulated gallbladders. Oxybutynin was applied intravenously at a dosage of 1 mg/kg into the tail vein, and bile and urine samples were collected by catheterization over 2 hours. Hepatobiliary excretion of oxybutynin was rapidly detected within a few minutes following intravenous application and showed no significant differences between the wild-type mice and *mdr1*$^{-/-}$ knockout mice (Figure 2a). Over 2 hours, 17% of the applied dose was excreted into bile by both mouse strains. As was seen in bile, oxybutynin concentrations in urine showed no significant differences between the wild-type and the *mdr1*$^{-/-}$ knockout mice over 2 hours (Figure 2b). Apart from the biliary and urinary excretion, brain penetration of oxybutynin was of particular interest in this study. After intravenous and oral application of 1 mg/kg oxybutynin, absolute drug concentrations in the brain were highest 2 hour after intravenous application (~700 ng/g) and lowest 12 hours after oral application (~200 ng/g) (Figure 2c). In all application groups, brain concentrations as well as brain-to-plasma ratios of oxybutynin were not significantly different between the wild-type and the *mdr1*$^{-/-}$ knockout mice.

DISCUSSION

Trospium chloride, a quaternary ammonium compound, is highly hydrophilic (logP = -1.22) [6] and shows very low permeability across biological membranes [5]. Additionally, the authors recently demonstrated drug efflux by P-gp at the BBB for this drug [7]. Both of these factors (ie, the physicochemical properties and drug efflux by P-gp) highly restrict trospium chloride entry into the brain. Therefore, the CNS side effects that are expected to occur as a result of nonselective interaction with CNS muscarinic receptors are limited for this drug [8]. In the present study, the authors directly compared tissue distribution and brain penetration of trospium chloride with another anticholinergic drug (oxybutynin) in young mice. In contrast to trospium chloride, oxybutynin is highly lipophilic with a logP of 4.3 (www.drugbank.ca). As a consequence, total brain concentrations of trospium chloride were almost 200-fold lower than brain concentrations of the more lipophilic oxybutynin when orally applied at an identical dosage (1 mg/kg). Even after intravenous application of 1 mg/kg and a given identical bioavailability, absolute brain concentrations of oxybutynin (679 ± 162 ng/g) (Figure 2c) were more than 30-fold higher than that of trospium chloride (21 ± 3 ng/g) at comparable plasma concentrations (261 ± 99 ng/mL for oxybutynin and 127 ± 47 ng/mL for trospium chloride) [7].

In contrast to trospium chloride, brain penetration and drug excretion of oxybutynin were not affected by P-gp deficiency in the *mdr1*$^{-/-}$ knockout mice. This is clearly consistent with data from clinical studies in humans, where oxybutynin showed central side effects such as influencing rapid-eye movement (REM) sleep [9,10] and CNS electrical activity [11,12], whereas trospium chloride did not impair such CNS functions.

It is reasonable to expect that in elderly patients, who represent the majority of patients with OAB who are treated with antimuscarinic drugs, drug brain penetration might be increased due to histological and functional changes at the BBB. Indeed, several age-related changes in the structure of the cerebral microvasculature have been reported including changes in the cross-sectional area of the capillary wall, gliofibrillar proliferation, increased basement membrane thickness, and reduced number of endothelial cells [13]. However, whether these changes actually affect penetration of antimuscarinic drugs across the BBB is unclear and needs additional investigation. Furthermore, one has to consider that in a number of disease conditions, including acute hypertension [14], cerebral ischemia [15], type II diabetes [16], and Alzheimer's disease [17], the permeability barrier function of the cerebral vasculature is altered. Therefore, in patients with multiple comorbidities, brain penetration of peripherally

Figure 2b. Urinary Excretion of Oxybutynin (1 mg/kg, Intravenously) in Mice With Cannulated Gallbladders.
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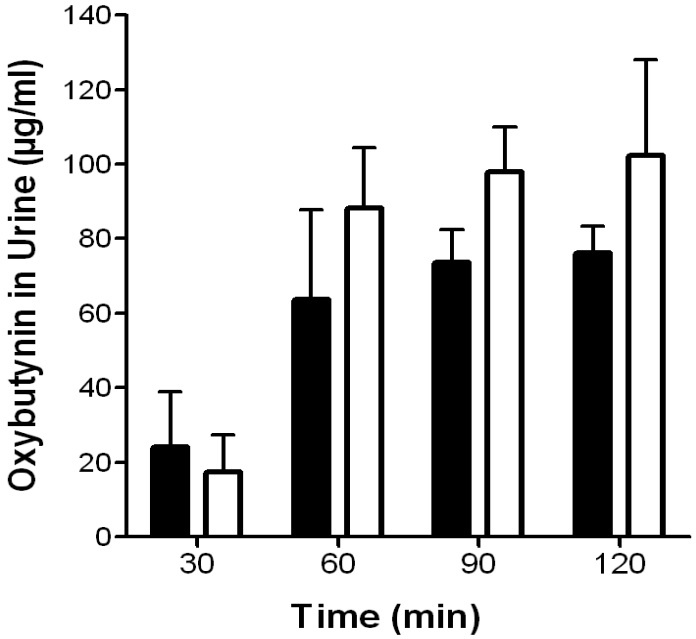

Wild-type mice, closed bars; *mdr1*$^{-/-}$ knockout mice, open bars.

Figure 2c. Brain Tissue Concentrations of Oxybutynin (1 mg/kg) After Intravenous and Oral Applications.
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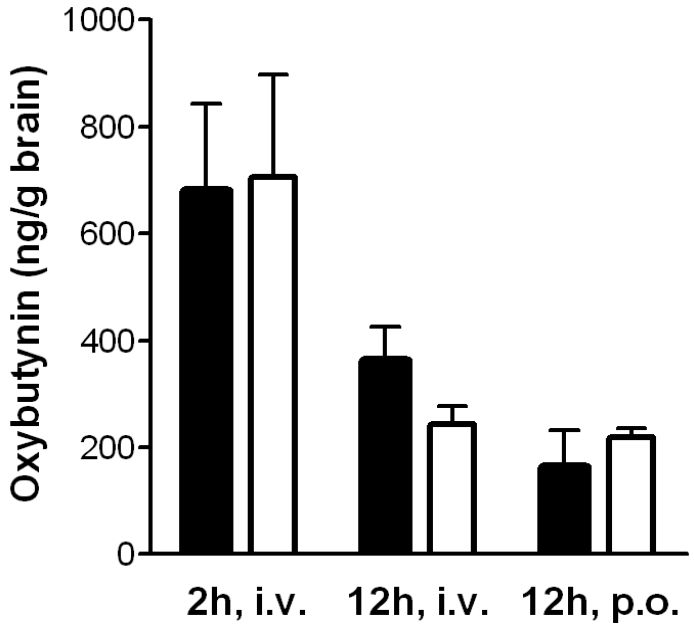

Wild-type mice, closed bars; *mdr1*$^{-/-}$ knockout mice, open bars.
Abbreviations: h, hours; i.v., intravenous; p.o., orally

acting antimuscarinic drugs might be increased. This potential difference in drug effects in the presence of comorbidities should be considered during OAB treatment.

Besides trospium chloride, interactions with P-gp have previously been shown for some other antimuscarinic drugs in different *in vitro* assays. Darifenacin stimulated the ATPase activity in membrane preparations of Sf9 cells over-expressing the human P-gp with an apparent K_M of 54 µM, indicating that darifenacin could be another transported substrate of P-gp [18]. Furthermore, fesoterodine professed to be a substrate of P-gp with K_M of 56 µM [19], and solifenacin was a weak inhibitor of P-gp on cultured LLC-PK_1 cells with IC_{50} of 5.1 µM, but it was not directly transported via the human P-gp [20]. However, these *in vitro* data cannot be transferred directly to the *in vivo* situation. The data need to be elucidated in further studies to determine whether P-gp restricts the penetration across the BBB for one of these compounds, as is the case for trospium chloride.

CONCLUSION

The drug efflux transporter P-gp at the BBB highly restricts the entry of trospium chloride into the brain, but similar results are not found for oxybutynin. Moreover, because oxybutynin is much more lipophilic than trospium chloride, absolute brain concentrations of oxybutynin highly exceed those of trospium chloride at an equal oral dosage. These significant differences in BBB permeability help to explain the reduced CNS-side effects experienced by humans with OAB who are treated with trospium chloride.

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Conflict of Interest: Ulrich Schwantes is employee of Dr. R. Pfleger GmbH.

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