Pharmacokinetic Drug-Drug Interactions Between Trospium Chloride and Ranitidine Substrates of Organic Cation Transporters in Healthy Human Subjects

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Abstract
Trospium chloride, a muscarinic receptor blocker, is poorly absorbed with different rates from areas in the jejunum and the cecum/ascending colon. To evaluate whether organic cation transporter (OCT) 1, OCT2 and multidrug and toxin extrusion (MATE) 1 and MATE2-K are involved in pharmacokinetics, competitions with ranitidine, a probe inhibitor of the cation transporters, were evaluated in transfected HEK293 cells. Furthermore, a drug interaction study with trospium chloride after intravenous (2 mg) and oral dosing (30 mg) plus ranitidine (300 mg) was performed in 12 healthy subjects and evaluated by noncompartmental analysis and population pharmacokinetic modeling. Ranitidine inhibited OCT1, OCT2, MATE1, and MATE2-K with half maximal inhibitory concentration values of 186 ± 25 µM, 482 ± 105 µM, 134 ± 37 µM, and 35 ± 11 µM, respectively. In contrast to our hypothesis, coadministration of ranitidine did not significantly decrease oral absorption of trospium. Instead, renal clearance was lowered by 15% (530 ± 99 vs 460 ± 120 mL/min; P < .05). It is possible that ranitidine was not available in competitive concentrations at the major colonic absorption site, as the inhibitor is absorbed in the small intestine and undergoes degradation by microbiota. The renal effects apparently result from inhibition of MATE1 and/or MATE2-K by ranitidine as predicted by in vitro to in vivo extrapolation. However, all pharmacokinetic changes were not of clinical relevance for the drug with highly variable pharmacokinetics. Intravenous trospium significantly lowered mean absorption time and relative bioavailability of ranitidine, which was most likely caused by muscarinic receptor blocking effects on intestinal motility and water turnover.

Keywords
cation transporters, drug interaction, healthy subjects, ranitidine, trospium chloride

Pharmacokinetics of drugs with high water solubility but low permeability throughout biomembranes (Biopharmaceutics Classification System, class III) are often highly variable and susceptible to influences from drug-drug and food interactions or genetic, physiological, or pathological particularities. Due to poor lipid solubility, they can pass cell membranes only by a paracellular route via water channels or as substrates of multidrug transporter proteins, and therefore often exhibit uncommon pharmacokinetic characteristics. Drugs with a low therapeutic index may consequently have efficacy and safety concerns.

One typical example is trospium chloride, a muscarinic receptor-blocking drug, which is on the market with evidence level A for the treatment of patients with a hyperactive detrusor muscle of the urinary bladder.1 The cationic drug with its quaternary ammonium structure is poorly absorbed from the gastrointestinal tract, negligibly bound to plasma proteins, and does not penetrate the brain. Trospium accumulates in the liver despite high polarity, undergoes largely full tubular secretion by the kidneys, and is substantially excreted via feces after intravenous administration.2-5 This inconsistent pharmacokinetic pattern cannot be
explained alone by the physicochemical properties of trospium. Recently, it was shown in vitro that trospium is a substrate for the organic anion transporting protein 1A2, the organic cation transporters (OCT) 1 and OCT2, and for the efflux carriers P-glycoprotein (P-gp) and the multidrug and toxin extrusion (MATE) 1 and MATE2-K, which might be involved in absorption, distribution, and renal elimination of the drug in humans. We assume that oral absorption is under the control of OCT1, which, according to our understanding, is located in the apical, brush-border membrane of the enterocytes along with P-gp. However, other research has localized OCT1 to the basolateral membrane. Trospium has a higher affinity to P-gp than to OCT1, whereby OCT1 provides higher transport capacity. Intestinal permeability of trospium can, in agreement with our hypothesis, be derived from intestinal net uptake via OCT1 minimized by apical efflux via P-gp. Moreover, we assume that this interplay is the rationale behind the known regional absorption of trospium via 2 “absorption windows,” as the protein abundance of P-gp is lower in the duodenum/jejunum and in the colon than in the ileum, whereas OCT1 is fairly uniformly abundant along the entire intestine. Experimental tools to obtain further insight into the mechanisms of intestinal drug absorption in humans are pharmacokinetic drug-drug interaction (DDI) studies with probe inhibitors. A suitable inhibitor for human intestinal OCT1 is ranitidine because the intestinal inhibitor (I2) concentration after swallowing 300 mg of the drug with 240 mL of tap water is nearly 4.0 mM, that is, >10 times that of the concentration of the substrate to which the velocity of the reaction is half maximal (Km) value of trospium (Km = 106 ± 16 μM) and the half maximal inhibitory concentration (IC50) values of ranitidine (~20-80 μM, dependent on the substrate) for human OCT1 that have been published in the literature up to now. Moreover, ranitidine is not known to modulate P-gp. It must be taken into consideration, however, that (1) trospium is also a high-affinity substrate for MATE1 and MATE2-K; (2) that basolateral OCT1 and OCT2, as well as apical MATE1 and MATE2-K, might be involved in the vectorial transport of trospium via hepatocytes and tubular cells in the kidneys; and (3) that ranitidine can also inhibit OCT2 and both MATEs.

In this paper, we provide experimental in vitro data on the inhibition of OCT1, OCT2, MATE1, and MATE2-K by ranitidine in stably transfected HEK293 cells (targeted chromosomal integrations) for which the Michaelis-Menten kinetic parameters for the transport of trospium had been provided previously. Determination of the inhibition potency of ranitidine in vitro is required because inhibition of OCT1 is known to be highly substrate dependent, and data on inhibition of the trospium uptake by ranitidine were lacking so far. Additionally, we provide the pharmacokinetic characteristics for intravenous and oral trospium chloride without and after oral comedication of ranitidine in healthy subjects. These were evaluated in a descriptive investigator-initiated DDI study in healthy subjects and described by in vitro to in vivo extrapolation (IVIVE) characteristics for the prediction of the pharmacokinetic outcome.

Methods

In Vitro Inhibition Assays

HEK293 cells overexpressing OCT1, OCT2, MATE1, or MATE2-K were generated by targeted chromosomal integration using the Flp-In system (Life Technologies, Darmstadt, Germany) as described elsewhere. The competition experiments were performed as described previously. Briefly, 6 × 10⁵ cells were plated per single well on a 12-well plate and cultured for 2 days. The transport experiments were performed at 37°C in Hank’s Balanced Salt Solution buffer (Life Technologies, Darmstadt, Germany) supplemented with 10 mM HEPES (pH 7.4) using 1.0 or 290 μM trospium as the substrate and increasing concentrations of ranitidine as the inhibitor (0-4 mM; Sigma-Aldrich, Taukirchen, Germany). When effects on MATE1 and MATE2-K uptake were measured, the direction of transport was changed by the ammonium prepulse technique as described elsewhere. The cells were lysed with 80% acetonitrile containing 5 ng/mL of trospium-d8 chloride (TRC Canada Inc, North York, Canada) as the internal standard, and the intracellular concentrations of trospium were determined by liquid chromatography–tandem mass spectrometry using the Hewlett Packard series 1100 HPLC system (Agilent Technologies, Waldbronn, Germany), and a Perkin Elmer Series 200 autosampler (Perkin Elmer, Darmstadt, Germany) coupled to the API 4000 QTRAP Turbo-Ion Spray mass spectrometer (AB Sciex, Darmstadt, Germany) as already described. The data were normalized to the uptake of trospium in the absence of ranitidine. The IC50 was derived by fitting the uptake data to a sigmoidal dose-response regression curve (Prism, version 5.01, GraphPad Software, San Diego, California). The inhibitory constant (Ki) was calculated using the Dixon plot.

Pharmacokinetic Drug Interaction Study in Healthy Subjects

The pharmacokinetic DDI study in 24 healthy subjects was performed according to the International Council for Harmonisation guideline for Good Clinical Practice and the regulations of the German Medicines Act after being approved by the Independent Ethics Committee of the University of Greifswald and by the German...
Federal Department of Drugs and Medicinal Products, and after registration by eudract.emea.eu.int (identifier: EudractCT 2016-002882-69) and ClinicalTrials.gov (identifier: NCT030114463). The study was designed for 2 parallel subgroups, each with 12 healthy subjects, who were used to study the interactions of trospium chloride with clarithromycin\(^{26}\) and with ranitidine, the results of which are reported here.

**Subjects.** The DDI of trospium chloride with ranitidine was performed in 12 healthy German white subjects (5 men, 7 women; aged 23-41 years; body mass index, 20.2-28.2 kg/m\(^2\)) who were enrolled after providing written informed consent, and after confirmation of good health by documenting their medical history, following a physical examination, and routine clinical-chemical and hematologic screenings. All subjects had negative results at the time of screening for drugs, human immunodeficiency virus, hepatitis B virus, and hepatitis C virus. All subjects were nonsmokers or smokers of <10 cigarettes/day, and 7 subjects occasionally consumed alcohol. None was on a special diet (eg, vegetarian). The subjects did not take any medication. The 7 female subjects used safe, nonhormonal methods for birth control. None had a positive pregnancy test result at any screening time. Intake of grapefruit-containing food or beverages and poppy seed–containing products was not allowed from 14 days before and during the study. Alcohol consumption was forbidden during the study. The subjects were hospitalized 12 hours before and up to 16 hours after administration of the study medications.

**Study Protocol.** Pharmacokinetics of trospium chloride and ranitidine, and the anticholinergic effects of trospium on salivation and accommodation of the eyes, were evaluated in a controlled, 4-period, crossover study (7-day washout) after block-randomized administration of the following study medications in fasting subjects: (1) intravenous infusion of 2.0-mg trospium chloride (Spasmex IV 2.0 mg Injektionslösung, Pfleger, Bamberg, Germany) in 20 mL of saline within 60 minutes and consumption of 240 mL of tap water; (2) oral administration of a 30-mg immediate release tablet of trospium chloride (Spasmex 30-mg coated tablets, Pfleger, Bamberg, Germany) with 240 mL of tap water; (3) intravenous infusion of 2.0-mg trospium chloride in 20 mL of saline within 60 minutes together with 300 mg ranitidine (Ranitidin-ratiopharm, ratiopharm, Ulm, Germany) swallowed with 240 mL of tap water; (4) 30 mg of immediate release trospium chloride together with 300 mg of ranitidine swallowed with 240 mL of tap water. Further details on administration, fasting periods, standard diet, and time points and intervals, respectively, of sampling and storage of plasma, urine, and feces are described in a recent publication.\(^{26}\)

Stimulated salivation was measured by chewing a 5 × 5-cm piece of PARAFILM “M” (American Can Company, Luton, Bedfordshire, England) and accommodation using an optometer according to Schober as described previously.\(^{5}\)

**Quantitative Drug Assays**

All quantitative assays for the study were performed under the conditions of Good Laboratory Practice. Trospium was quantified in plasma, urine, and feces using gas chromatography–mass spectrometry with selected ion monitoring mode (Agilent, Waldbronn, Germany) as described in previous papers.\(^{5,26}\) Accuracy ranged from 98.9% to 101.9% of the respective nominal values for plasma, 98.0% to 104.0% for feces, and 99.5% to 102.7% for urine. Precision varied between 3.7% and 5.5% (plasma), 2.8% and 7.2% (urine), and 2.5% and 6.5% (feces) of the respective means.

Ranitidine was assayed in plasma, urine, and feces using liquid chromatography–tandem mass spectrometry with positive mass transition mode (AB Sciex API 2000 with turbo-ion spray, AB Sciex, Darmstadt, Germany) with fexofenadine (Sigma-Aldrich, Munich, Germany) as an internal standard. The samples were centrifuged after denaturation of proteins using acetoni-trile (Carl Roth, Karlsruhe, Germany), and 10 µl of the supernatant were injected into the chromatographic system (column: Supelco Ascentis C 18, 3 µm, 2.1 × 100 mm, Sigma-Aldrich, Munich, Germany). The chromatograms were evaluated online with the internal standard method using peak-area ratios for calculation (Analyst 1.4.2 software, AB Sciex, Darmstadt, Germany). The calibration functions were constructed with a linear regression model weighted by 1/x (x = concentration) for plasma between 0.005 and 2.0 µg/mL, and for urine and feces between 0.005 and 10.0 µg/mL. Accuracy ranged from 99.9% to 109.5% of the respective nominal values for plasma, from 94.1% to 100.1% for urine, and from 98.9% to 101.8% for feces. Precision of the respective means varied from 7.4% to 17.1% for plasma, 6.8% to 17.7% for urine, and 6.5% to 8.7% for feces.

**Pharmacokinetic Evaluation**

The pharmacokinetics of trospium and ranitidine were primarily evaluated by a noncompartmental approach: Maximum plasma concentration (C\(_{\text{max}}\)) and the time to C\(_{\text{max}}\) (t\(_{\text{max}}\)) were read from the plasma concentration–time curves. The area under the curve (AUC) was assessed up to the last sampling time above the limit of quantification (AUC\(_{\text{0-t}}\)) using the trapezoidal method and extrapolated to infinity. For IVIVE, the unbound peripheral venous plasma concentrations (I\(_{1}\)), ...
the maximum drug concentration in the small intestine (dose/240 mL, I$_2$), and the unbound hepatic sinusoidal plasma concentrations (I$_1$) were derived from the data of the study. Unbound plasma concentrations were calculated considering a plasma protein binding for trospium of 50% and for ranitidine of 15%.\textsuperscript{2,27} Unbound hepatic sinusoidal plasma concentrations (I$_1$) were calculated from the maximum portal venous plasma concentrations ($C_{max, portal}$) and the respective I$_1$ values of the drugs assuming that 60% of the sinusoidal blood supply comes from the portal vein and 40% from the hepatic artery. $C_{max, portal}$ was estimated by $C_{max, portal} = C_{max, plasma} + \frac{K_a \times D \times F_o}{Q_h}$ with $K_a$ as the absorption rate constant, $D$ the oral dose, $F_o$ the oral bioavailable, and $Q_h$ the hepatic blood flow of 1500 mL/min in fasting healthy subjects.\textsuperscript{28} $K_a$ values were estimated by $K_a = \frac{1}{MAT}$ with $MAT$ being the mean absorption time. MAT was derived by $MAT = MBRT - MDRT$ (MBRT, mean body residence time after oral administration; MDRT, mean drug disposition residence time after intravenous dosing). The MDRT values of trospium were taken from the data of the study. MDRT for ranitidine (2.51 ± 0.52 hours) was assessed by volume of distribution at steady state ($V_{ss}$)/clearance (CL) using individual data (N = 25) from historical studies with intravenous ranitidine in healthy subjects.\textsuperscript{29,30} Bioavailability for ranitidine (Fa = 50%) was also taken from the literature.\textsuperscript{29,30}

In a post hoc analysis to the clinical study report, the plasma concentrations–time curves of trospium were additionally subjected to a population pharmacokinetic modeling to obtain a more in-depth insight into the oral absorption and distribution processes as described previously. Based on a 3-compartment model for trospium chloride disposition after intravenous administration, the parameters CL, distribution clearances (CL$_{d1}$, CL$_{d2}$) and the volumes of the central ($V_c$) and the peripheral compartments ($V_{p1}$, V$_{p2}$) were measured. The steady-state distribution volume is given as $V_{ss} = V_c + V_{p1} + V_{p2}$ and the MDRT is calculated as $MDRT = V_{ss}/CL$ as described above.

In order to fit the data after oral administration of trospium, a sum of 2 inverse Gaussian density functions was used as absorption time distribution, that is, the normalized time course of absorption rate into the systemic circulation.\textsuperscript{32,33}

\[ f_{2IG}(t) = \frac{p f_1(t) + (1-p) f_2(t)}{\sqrt{2\pi RD^2t^3}} \exp\left[\frac{(t-MT)^2}{2RD^2MTt^3}\right], \quad 0 < p < 1 \] (1)

where

\[ f_i(t) = \frac{MT_i}{\sqrt{2\pi RD^2t^3}} \exp\left[\frac{(t-MT_i)^2}{2RD^2MT_i t^3}\right], \quad 0 < i < 1 \] (2)

The absorption rate (input rate into the central compartment) is then given by

\[ AR(t) = DF f_{2IG}(t) \] (3)

$F_o$ denotes the bioavailability of the orally administered dose D. The mean absorption time then becomes

\[ MAT = pMT_1 + (1-p)MT_2 \] (4)

After fitting the intravenous data, the individual estimates of disposition parameters CL, CL$_{d1}$, CL$_{d2}$, V$_c$, V$_{p1}$, and V$_{p2}$ were held fixed in fitting the oral data.

For pharmacokinetic modeling of oral ranitidine pharmacokinetics, data after intravenous administration were unfortunately not available as a reference treatment. Therefore, the plasma concentration–time curves were directly fitted by a 3 inverse Gaussian density function model:

\[ C(t) = AUC \sum_{i=1}^{3} p_i f_i(t), \quad 0 < p_i < 1 \] (5)

where the functions $f_i(t)$ are given by Equation (2), $p_i$ is a mixing parameter, and $AUC$ denotes the area under the curve.\textsuperscript{32} The objective was to estimate the model parameters by fitting Equation (5) to the oral data to calculate AUC and the mean body residence time (MBRT after oral administration),

\[ MBRT = \sum_{i=1}^{n} p_i MT_i \] (6)

Note that $MBRT = MAT + MDRT$.

Renal clearance (CL$_{R}$) and fecal clearance (CL$_{f}$) were derived from the cumulative amount (A$_c$) excreted into urine and feces, respectively, within 5 days, then divided by AUC.

Maximum likelihood expectation maximization population module of ADAPT 5 was used for data analysis.\textsuperscript{34} The maximum likelihood expectation maximization program provides estimates of the population mean and intersubject variability, as well as of the individual subject parameters (conditional means). We assumed log-normally distributed model parameters and that the measurement error has a standard deviation, which is a linear function of the measured quantity:

\[ VAR = [\sigma_0 + \sigma t C (t)]^2 \] (7)

All population pharmacokinetic models fitted the concentration-time data very well as demonstrated by the goodness of fit and standardized residual plots, as well as by examples of individual fits (see Figure S1).
Table 1. Pharmacokinetic Characteristics of Trospium Chloride After Intravenous and Oral Administrations Before and After Comedication

| Pharmacokinetic Characteristics | Control | Comedication with Ranitidine |
|---------------------------------|---------|-----------------------------|
| **Intravenous infusion (2 mg/60 min)** |         |                             |
| CL (mL/min)                     | 880 (18) | 830 (19)                    |
| **Vp1 (L)**                     | 14 (19)  | 12 (19)                     |
| **Vp2 (L)**                     | 42 (32)  | 40 (37)                     |
| **Vss (L)**                     | 320 (27) | 320 (36)                    |
| **CL1 (mL/min)**                | 380 (27) | 370 (34)                    |
| **CL2 (mL/min)**                | 440 (29) | 410 (40)                    |
| **CL3 (mL/min)**                | 470 (21) | 430 (25)                    |
| **MDRT (h)**                    | 7.3 (27) | 8.0 (39)                    |
| **T1/2 (h)**                    | 16 (15)  | 16 (11)                     |
| **CLR (mL/min)**                | 520 (18.4) | 440 (26.0) |
| **CL fecal (mL/min)**           | 28 (7.1)  | 31 (96.1)                   |
| **S0**                          | 0.02     | 0.07                        |
| **S1**                          | 0.14     | 0.11                        |
| **Oral administration (30 mg immediate release tablets)** |         |                             |
| MAT (h)                         | 9.7 (21) | 12.0 (42)                   |
| F (%)                           | 83.3 (65) | 6.8 (80)                    |
| **S0**                          | 0.02     | 0.07                        |
| **S1**                          | 0.14     | 0.11                        |

**Statistical Evaluation**

The results shown in Table 1 were obtained by population pharmacokinetic analysis as described above. All other parameters were reported as arithmetic means ± standard deviations. Student’s t-test for paired samples was used for statistical comparison of group means. The sample size of N = 12 was sufficient to confirm AUC differences of trospium of ≥38% with P < .05 and statistical power >80% (nQuery Advisor 7.0, Statistical Solutions, Cork, Ireland). In a post hoc pharmacokinetic analysis, an analysis of variance for all pharmacokinetic characteristics of the noncompartmental evaluation (except for tmax) was performed, and geometric mean ratios (GMRs) with 90% confidence intervals (CIs) were assessed. All noncompartmental pharmacokinetic and statistical evaluations were carried out using the SAS statistical package (SAS 9.4 TS Level 1M3, SAS Institute Inc., Cary, North Carolina).

**Results**

**Inhibitory Potency of Ranitidine In Vitro**

When using 1.0-µM trospium as a substrate, ranitidine was able to inhibit OCT1-mediated trospium uptake with IC50 of 186 ± 25 µM (Figure 1). No significant inhibition was observed with 1.0-µM ranitidine, a concentration that is close to the observed I2 concentration of ranitidine, but a complete inhibition was observed with 4.0-mM ranitidine, a concentration that corresponds to the estimated I2 concentration for ranitidine. However, at 290-µM substrate concentration of trospium, a concentration corresponding to the I2 concentration, only 64% inhibition was achieved with 4.0-mM ranitidine as the inhibitor. From the competition assays, a Ki = 102 ± 15 µM was derived.

Regarding potential effects of ranitidine in the kidney, the most potent inhibition was observed for MATE2-K followed by MATE1 and OCT2 (IC50: 35 ± 11 µM, 134 ± 37 µM, and 482 ± 105 µM, respectively).

**Effects of Ranitidine on the Pharmacokinetics of Trospium**

The plasma concentration–time curves of intravenous trospium could be best fitted to a 3-compartment open model (Figure 2). The drug was widely distributed (Vss: ~4.81 L/kg) with a central compartment of ~0.18 L/kg, a shallow peripheral compartment (Vp1) of ~0.5 L/kg) and a deep compartment (Vp2) of ~4.13 L/kg. Trospium disposition was characterized by a mean residence time of ~7.3 hours and a terminal half-life of ~16 hours. The major elimination routes were renal (CLR; ~500 mL/min) and fecal excretion (CL fecal; ~30 mL/min). About 65% of the dose was eliminated into the urine, and about 3% via feces within 5 days (Tables 1 and 2).

After oral administration, the individual plasma concentration–time curves of trospium were highly variable (Figure S1). Trospium was slowly and poorly absorbed (tmax; ~6.7 hours; MAT, ~9.7 hours; F, ~8.3%). The absorption rate-time curve showed the typical biphasic pattern with a maximum after 2 to 3 hours, and a second peak after 4 to 6 hours. About 25% of the absorbed dose was bioavailable within 2 to 4 hours after oral dosing (Figure 3). The drug was slowly eliminated, with a terminal half-life of ~10 hours and renal clearance of ~500 mL/min. About 6% of the oral dose was eliminated via urine and 25% via feces (Tables 1 and 2).

The unbound trospium concentrations in the peripheral venous plasma (S1) and the hepatic sinusoidal plasma (S2) after intravenous infusion and oral dosing were 0.03 ± 0.008 µM vs 0.007 ± 0.005 µM (P < .001) and 0.03 ± 0.005 µM vs 0.009 ± 0.005 µM (P < .001), respectively.

Oral comedication of ranitidine significantly decreased the renal clearance of trospium by ~15%. After oral comedication of 300 mg of ranitidine, all pharmacokinetic characteristics of trospium were not significantly changed. The 90% CI of the GMR for the
Figure 1. In vitro analyses of the inhibition of trospium uptake by ranitidine. HEK293 cells overexpressing OCT1, OCT2, MATE1, or MATE2-K were incubated with 1 µM (filled circles) or 290 µM (open squares) trospium in the presence of increasing concentrations of ranitidine. The uptake is represented as percentage of transporter-mediated ranitidine uptake without inhibition. Shown are means ± SD of at least 3 experiments. MATE, multidrug and toxin extrusion; OCT, organic cation transporter; SD, standard deviation.

elimination characteristic half-life was within the span of equivalence. The ranges for the exposure characteristics AUC and C\text{max} were only slightly exceeded (Table 2).

Effects of Intravenous and Oral Trospium on the Pharmacokinetics of Ranitidine
With regard to the influence of trospium on the pharmacokinetics of ranitidine, we cannot provide unbiased pharmacokinetic characteristics, as a placebo control group without trospium comedication was not involved in the study. However, the plasma concentration–time curves of ranitidine after oral comedication of 30 mg of trospium chloride were relatively similar to profiles described in the literature. The orally administered drug was slowly and erratically absorbed. The plasma concentration–time curves of all subjects showed a shoulder or a typical double-peak phenomenon during absorption (Figure 4). Maximum concentrations were observed on average after ~4.0 hours. However, the mean plasma concentration–time profile of ranitidine (shown in the insert of Figure 4) and the mean t\text{max} value might be misleading, as they do not illustrate the large intersubject variability during absorption of orally administered ranitidine. In some subjects, there were additional absorption peaks up to 6 to 8 hours after dosing. Ranitidine was eliminated with a terminal half-life of ~4.0 hours and renal clearance of ~440 mL/min indicating glomerular filtration and tubular secretion as the excretion mechanisms. Approximately 55% of the parent drug was eliminated into the urine, but only <2% via feces (Table 3).

Comedication of trospium by intravenous infusion resulted in a significantly lower exposure (AUC) relative to the data after oral dosing. The mean absorption time decreased by 1.4 hours and the amount of ranitidine excreted via the urine by ~25%. The typical erratic absorption profile of ranitidine was not changed by the intravenous comedication of trospium (Figure 4). The 90%CI of the GMR for AUC and C\text{max} were outside of the stipulated standard span for bioequivalence (0.80-1.25).

Parameters for IVIVE
From the pharmacokinetic data, it can be derived that trospium reaches substrate concentration in the gut lumen after oral administration (S\text{2} = 290 µM), which saturates OCT1 in vitro. On the contrary, the unbound plasma concentrations after oral (S\text{1} = 0.007 ± 0.005 µM) and intravenous administration (S\text{1} = 0.03 ± 0.008 µM) are notably below the respective K\text{m} values for OCT1, OCT2, MATE1,
and MATE2-K in vitro (Table 4). From the IVIVE parameters for potential inhibition of OCT2, MATE1, and MATE2-K in the kidneys, as recommended by the recent guidance of the US Food and Drug Administration, it can be concluded that ranitidine has the potential to inhibit renal secretion of trospium by MATE1 and MATE2-K in vivo.36 Ranitidine also has the potential to inhibit the hepatic OCT1 according to the I_{50}/K_{r} ≥ 0.04 suggested by the European Medicine Agency Guideline on the investigation of drug interactions.37 From the EMA guideline, it can also be derived that ranitidine has the potential to inhibit intestinal OCT1 in vivo because of the I_{50}/K_{r} ratio of 39.0. Interestingly, the route of trospium administration significantly influenced the IVIVE parameters for ranitidine. For MATE1, there was a relevant interaction potential only after oral comedication of trospium chloride. The potential for interaction with MATE2-K in vivo was statistically higher after oral dosing of trospium. In the case of OCT1, there was a substantial interaction potential only after intravenous comedication compared to the borderline potential following oral comedication (P < .001).

Safety and Pharmacodynamics Effects
Both intravenous and oral trospium chloride were safe and well tolerated by our healthy subjects. The typical expected adverse drug reactions such as dry mouth, tachycardia, and urinary retention occurred in a minority of subjects. Trospium chloride also caused the expected changes in salivation after intravenous and oral administration. Accommodation of the eyes was not markedly influenced. There was no evident difference in tolerability between mono use or during concomitant administration with ranitidine. Comedication of ranitidine did not modify the changes in salivation by trospium. However, conclusive results cannot be drawn because a placebo control was not involved in the study.

Discussion
Changes in Pharmacokinetics of Trospium
Contrary to the primary hypothesis of our descriptive pharmacokinetic DDI study in healthy human subjects, a clinically relevant influence of ranitidine, an inhibitor of OCT1, on the oral absorption of trospium could not be confirmed. The 90% CIs of the GMRs for AUC
and $C_{\text{max}}$ (trospium with ranitidine over control) were within a range of 0.75 to 1.33. This range is exceeded by several confounders of the pharmacokinetics of trospium, which are well tolerated in clinical practice (eg, circadian time rhythm, food effects).\(^2\)

Before analysis of the results of this study, we expected that comedication of ranitidine would decrease oral bioavailability of trospium chloride. This assumption was derived from the in vitro evidence available at the time of study initiation and from our experimentally derived IVIVE parameters (Table 4). The following major deliberations are useful in explaining the unexpected rejection of our working hypothesis:

First, our hypothesis was derived from evidence by Han et al.,\(^10\), who localized OCT1 to the apical membrane of Caco-2 cell monolayers and mouse and human enterocytes. This was in contrast to the previously accepted basolateral localization in enterocytes.\(^{11-14}\)

However, evidence that OCT1 is an apical uptake carrier for trospium also came from the results of a DDI study with the OCT1 substrate metformin. Oral comedication of metformin lowered the bioavailability of trospium by $\sim 30\%$.\(^38\) Further evidence for apical localization of OCT1 in the human intestine also came from the data of a pharmacogenomics association study in patients with type 2 diabetes with gastrointestinal intolerance to metformin (GoDARTS substudy).\(^39\) It was hypothesized that metformin intolerance is related to high metformin exposure in the gut lumen as caused, for example, by lowered intestinal uptake.\(^{40,41}\) Therefore, it is plausible that reduced-function gene polymorphisms, and comedication of strong inhibitors of OCT1 (eg, verapamil, proton pump
inhibitors, codeine, citalopram), are high-risk factors for the metformin intolerance, provided that OCT1 acts as an apical uptake transporter in the intestine.

Second, we have previously shown that the bioavailability of trospium is largely correlated to the maximum absorption rate in the "wider absorption window," which has been located in the cecum/ascending colon; that is, the major site of trospium absorption is in the colon. However, it is unknown whether the parent ranitidine reaches the cecum/ascending colon along the small intestine, and an additional fraction is degraded by the colonic microbiota. We recovered only 1% to 2% in the feces (Table 3). It must also be considered that OCT1 is likely saturated by trospium along both "absorption windows," as only 10% of the administered dose is absorbed, and that OCT1 is inhibited by ranitidine in vitro with the saturating I2 concentrations of ~4.0 mM by 35% only (Figure 1).

Therefore, it is possible that we have overseen an involvement of OCT1 in trospium due to the inhibition of OCT1 by trospium chloride because of the much lower intrasubject variability of the AUC values and, in turn, the greater statistical power of the evaluation compared to oral dosing.

However, we did not find any pharmacokinetic evidence for this (eg, increase of bioavailability, lower distribution volume). It seems that ranitidine reaches maximum plasma levels in the hepatic sinusoidal blood after completion of the initial fast distribution phase of trospium following intravenous administration, and distinctly earlier than the sinusoidal maximum of trospium after oral administration. This might be the reason why the predicted interaction was not observed in vivo.

Interestingly, in our study, the major pharmacokinetic change after ranitidine coadministration was the decrease of the renal clearance of trospium. Trospium is eliminated in the kidneys by glomerular filtration and substantial tubular secretion. The unidirectional transfer from medullary blood via proximal tubular epithelial cells into urine apparently results from the interplay of the basolateral OCT2 with the apical/luminal MATE1 and MATE2-K. The in vivo potential of ranitidine for the inhibition of renal MATE2-K, and most likely for the renal MATE1, could clearly be predicted by the IVIVE indices that were derived from our study data. The small decrease in the renal clearance could only be confirmed for intravenous infusion of trospium chloride because of the much lower intrasubject variability of the AUC values and, in turn, the greater statistical power of the evaluation compared to oral dosing.

### Table 3. Pharmacokinetic Characteristics of Ranitidine After Intravenous Infusion of 2 mg (TC-IV) and Oral Comedication of 30 mg of Trospium Chloride (TC-PO) as Assessed by Post Hoc Population Pharmacokinetic Modeling (Arithmetic Means With Intersubject Variability) and Noncompartmental Evaluation (Arithmetic Means ± SD)

| Characteristics | TC-IV | TC-PO | GMR (90%CI) |
|-----------------|-------|-------|-------------|
| **Population pharmacokinetic modeling** |       |       |             |
| AUC μg × h/mL  | 4.7 (27) | 5.8 (38) |             |
| MBRT h         | 2.6 (29) | 4.1 (21) |             |
| ΔMAT h         | 1.1 | 3.1 |             |
| S0             | 1.1 | 3.1 |             |
| S1             | 0.1 | 0.2 |             |
| **Noncompartmental analysis** |       |       |             |
| AUC μg × h/mL  | 4.5 ± 1.4 | 6.3 ± 2.8 | 1.34 (1.16-1.54) |
| Cmax μg/mL     | 0.7 ± 0.4 | 1.0 ± 0.6 | 1.31 (1.11-1.70) |
| tmax h         | 4.1 ± 1.7 | 4.3 ± 1.9 | ND           |
| t1/2 h         | 4.0 ± 2.0 | 3.7 ± 0.6 | 0.98 (0.84-1.14) |
| CLa mL/min     | 440 ± 140 | 410 ± 83 | 0.95 (0.81-1.11) |
| Aextrospium mg | 110 ± 26 | 150 ± 41 | 1.08 (0.96-1.22) |
| Ae,feces mg    | 6.1 ± 14 | 3.3 ± 6.5 | 0.74 (0.47-1.16) |
| **For in vitro/in vivo prediction** | | | |
| I1 μM          | 1.9 ± 1.1 | 2.6 ± 1.7** |             |
| I2 μM          | 3.980 | 3.980 |             |
| I3 μM          | 32.7 ± 1.1 | 43.1 ± 1.7 |             |

Ae,feces, cumulative amount excreted into feces; Aextrospium, cumulative amount excreted into urine; AUC, area under the plasma concentration–time curve; CI, confidence interval; CLa, renal clearance; tmax, maximum plasma concentration; t1/2, unbound plasma concentration; t2, drug concentration in the small intestine (dose/240 mL); I1, unbound hepatic sinusoidal plasma concentration; MBRT, MDRT + MAT, mean body residence time (MRTtr); ΔMAT = MBRT TC-IV – MBRT TC-PO; So, Si, residual variability (variance of the measurement error): VAR = [S0 + S1 (C(t))²]; t1/2, half-life; tmax, time to maximum concentration.

Geometric mean ratios (GMR) with 90% confidence intervals are given for the characteristics of the noncompartmental analysis. Additionally, means ± standard deviations for parameters of in vitro/in vivo prediction are given.

*P < .001; **P < .055 compared to "TC-IV." Student’s paired t-test.
Ratios in bold letters can predict a clinically relevant drug-drug interaction, that is, $I_1/IC_{50}$
distribution. The velocity of the reaction is half maximal; MATE, multidrug and toxin extrusion; NA, not
applicable; OCT, organic cation transporter; Vmax, maximum volume of distribution.

Changes in Pharmacokinetics of Ranitidine

The hydrophilic cationic ranitidine is, similar to trospium, widely distributed and eliminated by tubular
secretion in the kidneys. Ranitidine is slowly but very erratically absorbed with a typical “double peak” or
“multi peak” phenomena in the majority of subjects, and undergoes a significant “first-pass” metabolism.
The intestinal uptake seems to be rather complex and includes paracellular transport mechanisms, the uptake transporter OCT1, and the efflux carrier P-gp. Ambitious research in healthy subjects carried out in 1994 showed that the ranitidine absorption rates are highest in the proximal regions of the small intestine. The protein abundance of cytochrome P450 3A4, the major enzyme for ranitidine demethylation, is higher in the proximal small intestine than in the more distal areas. Therefore, ranitidine probably undergoes more intensive intestinal “first-pass” metabolism after intravenous comedication of trospium, which leads to lower bioavailability. This hypothesis, however, is not in agreement with the findings that ranitidine is not affected by food. A second explanation could be that the intravenous trospium has substantially changed intestinal water uptake rates, which are associated with the paracellular transport of ranitidine. However, we cannot make any final conclusions because urinary and fecal excretion of the metabolites was not quantified.

Conclusions

Comedication of ranitidine leads to significantly lower renal clearance but not to changes in oral absorption and distribution of trospium in healthy subjects. The in vivo effects of ranitidine on renal clearance of trospium can be predicted by in vitro competition assays with MATE1 and MATE2-K overexpressing cell models. However, the overall influence of ranitidine on exposure characteristics were not of clinical relevance when considering the highly variable pharmacokinetics of the drug.

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### Table 4. Parameters for In Vitro/In Vivo Prediction of the Pharmacokinetic Outcome of the Drug-Drug Interaction Study Between Trospium Chloride After Intravenous (TC-IV) and Oral Administration (TC-PO) and Ranitidine in Healthy Subjects as Recommended by the Recent FDA Guideline

| Treatment                                                                 | OCT1     | OCT2     | MATE1    | MATE2-K  |
|---------------------------------------------------------------------------|----------|----------|----------|----------|
| Tropium in vitro                                                          | IC50 (µM) | 15.1 ± 3.1 | 0.6 ± 0.1 | 15.4 ± 2.4 | 8.2 ± 1.8 |
|                                                                           | Vmax (µmol/min × mg) | 1.14 ± 0.16 | 0.10 ± 0.02 | 1.08 ± 0.14 | 0.30 ± 0.01 |
|                                                                           | I2/IC50  | 186 ± 25 (102 ± 15) | 482 ± 105 | 134 ± 37 | 35 ± 11 |
| Ranitidine with TC-IV in-vivo                                             | I1/IC50  | 0.01 ± 0.01 (0.02 ± 0.01) | 0.004 ± 0.002 | 0.014 ± 0.009 | 0.05 ± 0.03 |
|                                                                           | I1/C50(Ki) | 21 (39) | NA | NA | NA |
|                                                                           | I2/C50(Ki) | 0.18 ± 0.01 (0.3 ± 0.01) | NA | 0.24 ± 0.009 | NA |
| Ranitidine with TC-PO in vivo                                             | I1/IC50  | 0.03 ± 0.02 | 0.005 ± 0.004 | 0.02 ± 0.01 | 0.07 ± 0.05 |
|                                                                           | I1/C50(Ki) | 21 (39) | NA | NA | NA |
|                                                                           | I1/IC10  | 0.023 ± 0.01 (0.04 ± 0.02) | NA | 0.03 ± 0.01 | NA |

FDA, US Food and Drug Administration; IC50, half maximal inhibitory concentration; Ki, inhibitory constant; Km, concentration of the substrate to which the velocity of the reaction is half maximal; MATE, multidrug and toxin extrusion; NA, not applicable; OCT, organic cation transporter; Vmax, maximum volume of distribution.

Ratios in bold letters can predict a clinically relevant drug-drug interaction, that is, $I_1/IC_{50} ≥ 0.02$ for transporters in the apical membrane of the proximal tubule cell, $I_2/IC_{50} ≥ 10$ for enterocytes and $I_3/Ki ≥ 0.04$.

*Paired Student’s t-test, $P < .001$; **$P < .055$ compared to RAN with TC-IV.

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Conflicts of Interest
C.N. and U.S. are employees of Dr. Pfleger Arzneimittel GmbH, Bamberg. H.-U.S. is the managing director of the Laboratory for Contract Research in Clinical Pharmacology and Biopharmaceutical Analytics, Bad Schwartau, Germany. All other authors have no conflict of interest.

Data Sharing
All data that were evaluated in our paper are filed by Dr. Pfleger Arzneimittel GmbH, Bamberg, Germany, and can be obtained by contacting Dr. Ulrich Schwantes (ulrich.schwantes@dr-pfleger.de).

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