In Vitro to In Vivo Extrapolation of Pharmacokinetic Drug Interactions between Clarithromycin and Ranitidine with Trosipium Chloride to Evaluate Probe Drug Characteristics for P-glycoprotein and Organic Cation Transporter Functions in Human

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1. Introduction

Efficacy and safety of drugs can be significantly influenced by many physiological factors (e.g. age, gender, circadian rhythms), genetic polymorphisms, drug-drug interaction (DDI), drug-food interactions or organ diseases whereby many of the confounders influence pharmacokinetic processes. The most variable physiological mechanisms in pharmacokinetics of drugs are the drug metabolizing enzymes (e.g. cytochrome P-450 dependent monooxygenases (CYPs) and uridine diphosphate glucuronosyltransferases (UGTs)) and the multidrug transporter proteins.¹

Multidrug transporters enable the systemic uptake of drugs via the intestinal absorption barrier. They are involved into distribution of drugs toward the target sites of their desired (and undesired) actions. Transport via biological membranes is also the precondition for the metabolisms of drugs by the enterocytes of the intestine or the hepatocytes in the liver which can influence bioavailability ("first-pass effect") and systemic clearance of highly metabolized drugs. Finally, multidrug transporter function is the precondition for tubular secretion of drugs in the kidneys.²

More than 400 membrane transporters, classified into the two super-families of ATP-binding cassette (ABC) transporters and solute carriers (SLC), have been annotated in the human genome. So far, however, only ~30 of the transporters were found to be of clinical relevance.³ The ABC transporters are efflux carriers which prevent absorption and distribution, and mediate excretion of drugs out of the body. These include the P-glycoprotein (protein name: P-gp/MDR1, gene name: ABCB1), the multidrug resistance protein 2 (MRP2, ABCC2) and the breast cancer resistance protein (BCRP, ABCG2). The SLC transporters, in contrast, promote the movement of drugs into cells and thereby facilitate absorption, distribution and biotransformation of drugs. Some of the clinical relevant uptake transporters are organic cation transporter (OCT) 1 (OCT1, SLC22A1) and OCT2 (SLC22A2), the organic anion transporting polypeptide (OATP) 1B1 (SLCO1B1) and OATP1B3 (SLCO1B3), the organic anion transporter (OAT) 1 (SLC22A6), OAT2 (SLC22A7) and OAT3 (SLC22A8). The exception in the SLC transporters super-family are the multidrug and toxin extrusion transporter (MATE) 1 (MATE1, SLC47A1) and MATE2-K (SLC47A2) which mediate the excretion of drugs out of the body.⁴

It should be mentioned that various drug transporters share a broad spectrum of substrates with each other (e.g. OCTs with P-gp, OCTs with OATPs and OATPs with MRP2) and with various drug metabolizing enzymes and, therefore, can interplay during the uni-directional transfer of drugs via enterocytes, hepatocytes and renal proximal tubular cells (PTC). A typical example for the interplay of two transporters is the uni-directional transfer of the magnetic resonance imaging (MRI) contrast agent gadoxetate, which is taken up into
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hepatocytes via OATP1B1 and 1B3 and secreted into bile via MRP2. An instructive example for the interplay between transport and metabolism is ezetimibe. The intestinal uptake of ezetimibe by enterocytes is minimized by P-gp. In the enterocytes, the drug is conjugated by UGT1A1. Subsequently, the glucuronide is back-secreted into gut lumen by MRP2 to enter a long-lasting entero-intestinal and entero-hepatic circulation. In the liver, the uptake of many drugs is mediated by the sinusoidal OCT1 (e.g. morphine) or OATPs (e.g. statins, ezetimibe) and is followed by phase-I and/or phase-II metabolism in the hepatocytes. The parent drugs and/or their metabolites can be subsequently secreted by the efflux carriers P-gp, BCRP, MATE1, MRP2 or the bile-salt export pump (BSEP) into the bile.

To conclude on the pharmacokinetic role of multidrug transporters in vivo, particularly in healthy subjects and patients, the affinity of the respective drugs to candidate transporter(s) must be pre-estimated in vitro. A variety of experimental in vitro models have been suggested so far to predict the in vivo function of transporters.

Affinity of drugs to multidrug transporters is evaluated in vitro using validated and highly standardized cell lines overexpressing the respective human transporter protein. Widely accepted are the human embryonic kidney cells 293 (HEK293), Chinese hamster ovary cells (CHO), the Madin-Darby canine kidney II cells (MDCKII), porcine kidney epithelial cells (LLC-PK1) and colon cancer-2 (Caco-2) cells. In dependence on the objectives of the developmental or research program, uptake transport assay or Transwell™-Techniques can be used. The inside-out lipovesicle technique is suitable for measuring the function of efflux carriers such as P-gp or BCRP. MATEs can be studied using the ammonium prepulse technique. The inhibitory potency of a drug can be evaluated in competition assays using accepted in vitro probe substrates, e.g. digoxin for P-gp, bromosulphophthalein for OATPs or N-methylpyridinium for OCTs. The desired information are the type of inhibition (competitive, non-competitive) and the inhibition constant ($K_i$) or the half-maximal inhibitory concentration ($IC_{50}$).

The in vivo function of multidrug transporters in pharmacokinetics of drugs can be discovered in confirmative, experimental clinical studies in healthy subjects according to a DDI approach using “specific” drug-substrates, inhibitors and inducers or by using a so-called pharmacogenomics approach in subjects with loss of function or gain of function polymorphisms of the respective transporter. Suitable in vivo probe inhibitors for DDI studies are for instance ritonavir or dronedarone for P-gp, single dose rifampicin or cyclosporine for OATPs or cimetidine or ranitidine for OCTs. The substrates can be given individually as for instance digoxin in studies on P-gp, a statin in studies on OATPs or metformin in studies on OCTs. Recently, a so-called cocktail approach with several probe drugs for multiple transporters has been developed.
To predict the competition potential of drugs in man, a so called in vitro to in vivo extrapolation (IVIVE) approach can be used. IVIVE needs the competition parameters from in vitro cell model studies and the in vivo drug concentrations in the respective multidrug transporter micro-compartment (e.g. gut lumen, hepatic sinusoidal plasma, renal tubular capillaries).

Relevant genetic reduced or loss of function variants have been identified to characterize functions of OCT1, BCRP and OATP1B1 in the pharmacokinetics, efficacy and safety of many drugs (e.g. morphine, fenoterol, allopurinol). Studies in subjects with selected genetic polymorphisms provide valid evidence for the clinical role of a particular transporter. However, such studies may be challenging due to the low allele frequencies of most transporter polymorphisms. The fact that the outcome effects are largely seen in individuals who are homozygous carrier of the respective allele leads to underpowered studies. For example, the most common loss of function OCT1 alleles in Europe are OCT1*2 (Met_{420}del) and OCT1*3 (Arg_{61}Cys), but only 9 % of the population are homozygous carriers.\textsuperscript{13-15}

However, the experimental value of both clinical study approaches (DDI and pharmacogenomics) is limited by the specificity of the available in vivo probe substrates and probe inhibitors/inducers for a transporter of interest. Therefore, there is an ongoing search for more appropriate and more specific probe drugs. A new promising candidate might be the muscarinic receptor blocking agent trospium chloride (TC), as concluded from the in vitro data and the pharmacokinetics characteristics available so far.
2. Rationales and objectives

Trospium chloride is one of the most effective and safe antimuscarinic drugs for treatment of the overactive bladder syndrome (OAB). It is a quaternary ammonium compound with high and rapid water solubility (1.0 g/ml in ~11 min) and low lipid solubility at physiological pH (N-octanol/water distribution coefficient, logP = -1.2) as well as with low permeability across cell membranes (biopharmaceutical classification system (BSC) class III). At pH >4.0, trospium exhibits increased chemical degradation in the GIT.\textsuperscript{16-19}

Despite its polarity, trospium is slowly absorbed after oral administration from two ‘windows’ in the gastrointestinal tract (GIT) located at the jejunum and cecum/ascending colon (bioavailability (F) ~10 %). Trospium distributes widely (volume of distribution at steady state (V\textsubscript{SS}) ~7 l/kg) and accumulates preferentially in the liver and kidneys. But it does not cross the blood-brain barrier (BBB) and, hence, does not cause central anticholinergic side effects. Trospium undergoes only minor microsomal biotransformation and it is excreted majorly as parent compound by glomerular filtration and active tubular secretion in the kidneys (renal clearance (CL\textsubscript{R}) ~500 ml/min) and by active secretion in the intestine. Therefore, pharmacokinetic drug interaction of trospium through metabolism is unlikely.\textsuperscript{20,21}

The paradoxical pharmacokinetic characteristics of trospium could be partly explained by its affinity to membrane transporters in vitro. Trospium has high affinity to P-gp, OCT1, OCT2 and OATP1A2. It is also a high capacity substrate of MATE1 and MATE2-K. In double transfected MDCKII cells, OCT1 and OCT2 can facilitate a uni-directional, synergistic transport of trospium in interplay with MATEs.\textsuperscript{22-24} P-gp might be responsible for the poor oral absorption and limited brain distribution of trospium. The distribution of trospium into the liver, kidneys and peripheral organs could be mediated by OCT1, OCT2 and OATP1A2. The extensive active tubular secretion of trospium might be caused by OCT2, MATE1 and MATE2-K and the biliary excretion by the hepatic OCT1, MATE1 and P-gp. Therefore, trospium with its suitable features could be used as an in vivo probe drug to evaluate OCT1/P-gp controlled bioavailability, OCT1/OCT2/OATP1A2/P-gp controlled distribution volume and OCT2/MATEs controlled renal and P-gp dependent fecal clearance. The suitable features of trospium are: Firstly, it is poorly metabolized by hepatic CYP-enzymes and, hence, membrane transport is supposed to be its major disposition route. Secondly, trospium is well tolerated and a safe drug. Thirdly, reliable quantitative assays to measure trospium concentration in human blood, urine and feces are well established. Lastly, a validated three compartmental pharmacokinetic model has been developed to assess its relevant pharmacokinetic parameters with high accuracy.\textsuperscript{20,21,25}
Rationales and objectives

According to our hypothesis on trospium to be a suitable in vitro and in vivo probe drug for evaluation of the function of certain multidrug transporters, the following objectives and assignments of tasks, respectively, for the PhD research project have been defined:

Permeability, uptake and inhibition studies of trospium with/without the probe inhibitors clarithromycin and ranitidine are to be conducted in cell culture models to:

- analyze the transport-independent membrane permeability of trospium,
- re-validate whether trospium is an in vitro substrate for OCT1 and OCT2,
- assess if the transport of trospium by OCT1, OCT2, MATE1 and MATE2-K can be inhibited by clarithromycin and ranitidine,
- evaluate whether OCT1 and OCT2 genetic variants are functionally relevant to the transport of trospium,
- evaluate the additional inhibition potency of ranitidine on the function of common genetic variants of OCT1 and OCT2.

For in vivo confirmation, two independent pharmacokinetic drug interaction studies of TC with co-medication of clarithromycin as a probe inhibitor of P-gp and ranitidine as a probe inhibitor for OCT1 were conducted in healthy subjects genotyped for OCT1 in order to:

- describe the basic pharmacokinetic characteristics of trospium,
- derive IVIVE parameters to predict the interaction potential with clarithromycin and ranitidine at multidrug transporters likely involved in disposition of trospium,
- provide evidence whether clarithromycin and ranitidine influence pharmacokinetics of trospium by interactions with multidrug transporter function,
- derive evidence whether trospium might be a suitable probe drug for P-gp, OATP1A2, OCT1, OCT2, MATE1 and MATE2-K in vitro and in vivo.
3. Methods

3.1. In vitro transport studies of trospium

3.1.1. Non-ionic permeability

Non-ionic transcellular permeability of trospium was evaluated using parallel artificial membrane permeability assay (PAMPA). Chen et al. has developed a novel design of artificial membrane for improving the PAMPA model. The permeability of radiolabeled trospium ([3H]-trospium trifluoroacetate, 6.75 nM) was determined at pH 7.4 using the Corning® Gentest™ Pre-coated PAMPA plate system (Corning Life Sciences, Tewksbury, MA, USA).

3.1.2. Permeability by drug transporters

Cell Lines: the wild type and common loss of activity alleles of human OCT1, OCT2, MATE1 and MATE2-K overexpressing HEK293 cells were generated from HEK293 T-REx™ cells using the Flp-In system (Life Technologies, Darmstadt, Germany). The genetic OCT1 alleles *1A (Met408Val- wild type), *2 (Met420del), *3 (Arg61Cys), *4 (Gly401Ser), *5 (Gly465Arg/Met420del), *6 (Cys88Arg/Met420del), *7 (Ser14Phe) and OCT2 Ser270 were introduced into the transporters by targeted mutagenesis. OCT1*2 and *7 are substrate-dependent, loss of activity variants. OCT1*3 and *4 are substrate-independent, strong loss of activity proteins and OCT1*5 and *6 are substrate-independent, complete loss of activity genetic variants.

Expression of OCT1 messenger ribonucleic acid (mRNA) was 15-fold higher among all cell models transfected with the wild type and loss of function alleles compared to the clones transfected with the empty pcDNA5 vector. Uptake of the model substrate tritium-labelled 1-methyl-4-phenypyridinium ([3H]-MPP*, 10 nM) by the OCT1, OCT2, MATE1 and MATE2-K overexpressing cells were 23-, 15-, 36- and 22-fold higher than in cells transfected with the empty pcDNA5 vector. Details on generation and characterization of the transfected cells are described recently. In OCT1 and OCT2 wild type transfected cells, loss of activity was defined as an increase in activity of less than 2.5 fold of the cells transfected with empty vector, or an inability to inhibit the increased uptake by at least 50 %. In the genetic variants, loss of activity was defined as a reduction of wild type activity by more than 65 %. The extrusion transporters MATE1 and MATE2-K overexpressing HEK293 cells were transformed to uptake transporters using the prepulse technique as described elsewhere. MDCKII cells were purchased from the European collection of cell cultures (Salisbury, United Kingdom). OCT1 overexpressing MDCKII cells were generated and characterized as previously described.
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**Uptake assays:** Transporter-mediated uptake of trospium was determined in fully confluent OCT1 and OCT2 wild type and loss of activity genetic variants overexpressing HEK293 cells at 0.1, 1.0, and 290 µM. For the wild type and genetic variants without sorting defects that showed substantial trospium uptake (OCT1*1, OCT1*2 and *7, OCT2 Ala270 and Ser270), we performed more detailed, concentration-dependent uptake analyses.

**Inhibition assays:** Inhibition to the uptake of trospium (1 and 290 µM) by OCT1 *1, *2, *3, OCT2 Ala270, Ser270, MATE1 and MATE2-K overexpressing HEK293 cells was performed following incubation with ranitidine (0 - 4 mM, Sigma-Aldrich, Taufkirchen, Germany). In addition, the inhibitory effect of clarithromycin (Sigma-Aldrich, Taufkirchen, Germany) on the transport of trospium was measured in OCT1 overexpressing MDCKII cells.

**Drug analysis:** The intracellular trospium concentration was measured using the Hewlett Packard, series 1100 high performance liquid chromatography (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) with trospium-d8 chloride as internal standard (TRC Canada Inc, North York, Canada). The detailed description of the method was provided elsewhere.23

In studies involving MDCKII cells and in PAMPA, the concentration of radiolabeled trospium ([3H]-trospium) was determined by liquid scintillation beta counting (SLC) (type 1409; LKB Wallac, Turku, Finland).

**Quantitative evaluations of the in vitro studies:** The rate of uptake of transporter transfected cells were normalized to the control (pcDNA5 transfected) cells. Data was fitted to the Michaelis-Menten equation using Prism 5.01 program package (GraphPad Software, San Diego, USA) to determine affinity (Km) and maximal rate of uptake (Vmax). In the inhibition studies, the data was normalized to the uptake in the absence of inhibitor. The half-maximal inhibitory concentration (IC50) was derived by fitting the uptake data to a sigmoidal dose-response regression curve. The inhibitory constant (Ki) was calculated using nonlinear regression and visualized by the Dixon plot method.31

The effective permeability constant Pe (cm/s) of trospium in the PAMPA was calculated according to the company’s recommendation.27

Each experiment was performed in triplicate and the data points and kinetic parameters were summarized into arithmetic means and standard deviations (M ± SD).
3.2. Drug-drug interaction study in man

3.2.1. Subjects

The clinical study in 24 healthy German white subjects (14 males, 10 females, age 23 - 40 years, body mass index (BMI) 19.6 - 28.2 kg/m\(^2\)) was performed according to the international clinical harmonization (ICH) guideline for Good Clinical Practice, and to 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 (BfArM), and after registration by eudract.emea.eu.int (identifier: EudraCT 2016-002882-69) and ClinicalTrials.gov (identifier: NCT03011463). All healthy subjects were enrolled after providing written informed consent and confirmation of good health by documenting their medical history, performing a physical examination, and conducting routine clinical-chemical and hematological screenings A1, A2.\(^3\)

The subjects were admitted to the clinical study unit of the department of clinical pharmacology, center of drug absorption and transport (C\_DAT) of the University of Greifswald, Germany 12 h before, and up to 16 h after administration of the study medication. Twelve subjects (9 males, 3 females; age 25 - 34 years; BMI 19.6 - 27.7 kg/m\(^2\)) were subjected to treatment with TC and clarithromycin and another parallel group of 12 subjects (male 5, female 7, age 23 - 40 years, BMI 20.2 - 28.2 kg/m\(^2\)) to TC and ranitidine A1, A2.\(^3\)

The objective of the study was entirely descriptive. However, a sample size of N = 12 for comparisons of the area under the concentration-time curve (AUC\(_{0-\infty}\)) for trospium after oral dosing without clarithromycin or ranitidine, versus oral dosing with clarithromycin or ranitidine, was sufficient to confirm differences of ≥38 % with p <0.05 and statistical power >80 % (nQuery Advisor 7.0, Statistical Solutions, Cork, Ireland).

3.2.2. Study protocol

Pharmacokinetics of trospium, clarithromycin and ranitidine, and anticholinergic effects on salivation and accommodation of the eyes were evaluated in two parallel groups using a controlled, four-period, cross-over study design (7 days wash-out) after block-randomized administration according to ICH guidelines A1, A2.\(^3\)

The study medications in the TC-clarithromycin group were:

- 2 mg TC intravenous (TC-IV) infusion (Spasmex\textsuperscript{®} i.v. 2.0 mg Injektionslösung, Pfleger, Bamberg, Germany) with or without 500 mg clarithromycin PO (Clarithromycin-ratiopharm\textsuperscript{®} 500 mg TC Filmtabletten, ratiopharm, Ulm, Germany) and
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- 30 mg immediate release TC tablet orally (TC-PO) (Spasmex® 30 mg TC Filmtabletten, Dr. Pfleger, Bamberg, Germany) with or without 500 mg clarithromycin PO

In the TC-ranitidine group, subjects received the following treatments:

- 2 mg TC-IV infusion with or without 300 mg ranitidine PO (Ranitidin-ratiopharm® 300 mg TC Filmtabletten, ratiopharm, Ulm, Germany) and
- 30 mg TC-PO with or without 300 mg ranitidine PO

TC-IV infusion was dissolved in 20 ml saline and infused via a forearm vein within 60 minutes. TC-PO was administered with 240 ml tap water and was controlled by checking the mouths of the subjects. Intake of food and additional fluids were standardized after overnight fasting for at least 10 hours. Before and up to 36 hours after administration of drugs, venous blood was sampled in a way that ensures in-depth pharmacokinetic modeling of trospium which is devoid of bias caused by inadequate sampling time. Urine and feces were sampled for 5 days. All samples were stored at ≤ -20 °C until the quantitative analysis. Stimulated salivation was measured by controlled chewing and weighing the secretion and accommodation of the eyes using an optometer according to Schober.

3.2.3. Quantitative drug assay

All quantitative assays in plasma, urine and feces were performed under good laboratory practice (GLP) conditions. Trospium was quantified using gas chromatography-mass spectrometry with selected ion monitoring mode. The analyte was separated after liquid/liquid extraction, alkaline hydrolysis to benzilic acid and subsequent derivatization using diazomethane to generate the methyl ester. The derivative and the internal standard oxyphenonium were separated using a HP-1 reverse phase column (25 m × 0.2 mm, 0.33 µm; Agilent, Waldbronn, Germany) with helium as the carrier gas and a temperature program (90 - 280 °C in 12 min). The mass spectrometer operated in SIM modus m/z 189.1 for trospium and m/z 183.1 for oxyphenonium. The chromatograms were evaluated by means of peak-area-ratios using the internal standard method which were fitted using a regression model (1/x weighting for plasma, 1/x² for urine and feces, x = concentration). Accuracy ranged between -1.1 to 1.9 % for serum, -2.0 to 4.0 % for feces and 0.5 to 2.7 % for urine of the respective nominal values. Precision varied between 3.7 and 5.5 % (serum), 2.8 and 7.2 % (urine) and between 2.5 and 6.5 % (feces) of the respective means A1.32

Clarithromycin and ranitidine were assayed using an HPLC system coupled to a Turbo-Ion Spray mass spectrometer (LC-MS/MS) with fexofenadine as an internal standard. The samples were denaturation using acetonitrile (Carl Roth, Karlsruhe, Germany) and subjected to chromatographic separation using a filter direct-connect (PEEK) device and reverse phase
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column: Supelco Ascentis® C 18, 3 µm, 2.1 × 100 mm with isocratic elution. The electro spray ionization interface of the tandem mass spectrometer operated in the positive ion mode to monitor the following m/z transitions: clarithromycin 748.5 → 590.6 and 748.5 → 158.2; ranitidine 315.1 → 176.0 and 315.1 → 130.2; fexofenadine (internal standard) 502.2 → 466.2 and 502.2 → 484.4 A3.

The chromatograms were evaluated with the internal standard method using peak-area-ratios for calculation. Accuracy for clarithromycin ranged between 4.2 to 4.8 % of the respective nominal values for serum, between -4.4 to 2.9 % for urine and between 2.3 to 6.9 % for feces. Precision varied between 7.5 and 10.4 % for serum, between 6.8 and 13.7 % for urine, and between 5.2 and 13.7 % for feces of the respective means. Accuracy for ranitidine ranged between 0.1 to 9.5 % of the respective nominal values for plasma, between -4.9 to 0.1 % for urine and between -1.1 to 1.8 % for feces. Precision varied between 7.4 and 17.1 % for plasma, between 6.8 to 17.7 % for urine, and between 6.5 to 8.7 % for feces of the respective means A3.

3.2.4. Pharmacokinetic and statistical evaluations

Non-compartmental pharmacokinetic evaluation: The pharmacokinetics of trospium, clarithromycin and ranitidine was primarily evaluated by a non-compartmental approach. Maximum plasma concentration (C_{max}) and the time to reach C_{max} (T_{max}) were read from the plasma concentration-time curves. The area under the curve was assessed up to the last sampling time above the limit of quantification (AUC_{0-\infty}) using the trapezoidal method and extrapolated to infinity (AUC). CL_R and fecal clearance (CL_{fecal}) were derived from the cumulative amount of drug excreted into urine (A_{e_{urine}}) and feces (A_{e_{feces}}), respectively, within 5 days divided by AUC A1, A2.

Pharmacokinetic modeling: In a post-hoc analysis to the clinical study report, pharmacokinetics of trospium and ranitidine were additionally evaluated by Prof. Michael Weiss (department of clinical pharmacology, Martin Luther University of Halle, Germany) to examine the absorption and distribution profile of the drugs more in detail. The evaluation was conducted based on a three-compartmental model for disposition of trospium and ranitidine after intravenous administration A1, A2.

Statistical evaluation: Maximum likelihood expectation maximization (MLEM) population module of ADAPT 5 was used for analysis of the pharmacokinetic modeling data. Individual pharmacokinetic parameters were reported as arithmetic means ± standard deviations (SD). The Student’s t-test for paired samples was used for statistical comparison of group means with the level of statistical significance being p <0.05. Correlations between samples were evaluated using the Spearman test.
An Analysis of Variance (ANOVA) for all pharmacokinetic characteristics of the non-compartmental evaluation (except for $T_{\text{max}}$) was performed and geometric mean ratios (GMR) with 90% confidence intervals (90% CI) were assessed to conclude the clinical relevance of the results.
4. Results

4.1. *In vitro* transport of trospium

4.1.1. Non-ionic permeability of trospium

We evaluated the ability of trospium to pass cell membranes in a transporter-independent manner using PAMPA. The non-ionic permeability of [3H]-trospium was rather low (\( P_e = 1 \times 10^{-6} \text{ cm/s} \)).

4.1.2. Cellular uptake of trospium by HEK293 OCT1 and OCT2

Wild type OCT1 showed a 20-fold higher (\( p = 0.01 \)) mean transport activity of trospium uptake (\( V_{\max} = 0.8 \pm 0.1 \text{ nmol/min x mg} \)) compared to wild type OCT2 (\( V_{\max} = 0.04 \pm 0.01 \text{ nmol/min x mg} \)). But trospium had a higher affinity (\( p = 0.005 \)) to OCT2 (\( K_m = 0.5 \pm 0.1 \mu\text{M} \)) than to OCT1 (\( K_m = 17.4 \pm 2.1 \mu\text{M} \)) with intrinsic clearance (\( CL_{\text{int}} \)) 46 \pm 3.2 \text{ µl/min x mg} for OCT1 and 80 \pm 31.4 \text{ ml/min x mg} for OCT2 (\( p = 0.25 \)) (Figure 1).
4.1.3. Effect of genetic polymorphisms of OCT1 and OCT2 on trospium uptake

The genetic variants OCT1*3 and OCT1*4 caused a significant decrease in activity by 72 % and 90 %, respectively. OCT1*5 and OCT1*6 provided a complete loss of activity in the uptake of trospium at any concentration measured (Figure 2).
The variant OCT1*2 and *7, proteins without sorting defect, caused reduction of uptake activity with 43% and 42%, respectively, compared to the wild type protein.

A more detailed, concentration-dependent analysis of trospium uptake showed that OCT1*2 has a two-fold higher affinity but lower activity \((K_m = 8.7 \pm 3 \mu M, V_{max} = 0.4 \pm 0.1 \text{ nmol/min x mg})\) compared to the wild type \((K_m = 17.4 \pm 2.1 \mu M, V_{max} = 0.8 \pm 0.1 \text{ nmol/min x mg})\) with \(p < 0.000\). OCT1*7 \((K_m = 15.5 \pm 2.7 \mu M, V_{max} = 0.6 \pm 0.2 \text{ nmol/min x mg})\) has almost equivalent affinity but a lower activity than OCT1*1 \((p < 0.007)\) (Figure 3).

In addition, the effect of the common OCT2 genetic polymorphism on the uptake of trospium was analyzed in OCT2 Ala270 versus OCT2 Ser270 overexpressing HEK293 cells. The genetic variant OCT2 Ser270 provided comparable affinity \((K_m = 1.1 \pm 0.1 \mu M)\) but a 2-fold higher activity \((V_{max} = 0.08 \pm 0.01 \text{ nmol/min x mg})\) compared to the wild type OCT2 Ala270 variant \((K_m = 0.5 \pm 0.1 \mu M, V_{max} = 0.04 \pm 0.01 \text{ nmol/min x mg}), p < 0.02\) (Figure 4).
4.1.4. Inhibition of OCT1, OCT2, MATE1 and MATE2-K-mediated transport of trospium

To evaluate the inhibition effect of ranitidine in OCT1, OCT2, MATE1 and MATE2-K-mediated uptake of trospium (1.0 µM), an inhibition assay was conducted using a serial dilution of ranitidine (0 - 4,000 µM). To this end, ranitidine showed a 2.5-fold higher inhibition activity on OCT1 ($IC_{50} = 186 \pm 25$ µM) compared to OCT2 ($IC_{50} = 482 \pm 105$ µM). But
clarithromycin (0 - 1,000 µM) did not exhibit any inhibition activity on OCT1-mediated uptake of trospium. The efflux transporter MATE2-K-mediated uptake of trospium was strongly inhibited by ranitidine ($IC_{50} = 35 \pm 11$ µM) compared to the MATE1 ($IC_{50} = 134 \pm 37$ µM) (Figure 5).

**Figure 5:** Inhibitory effects of ranitidine and clarithromycin on the cellular uptake of trospium mediated by OCT1, OCT2, MATE1 and MATE2-K
4.1.5. **Effect of genetic polymorphisms on inhibition of the OCT1 and OCT2-mediated uptake of trospium**

In order to determine the effects of ranitidine on trospium uptake (1µM and 290 µM) by common loss of activity genetic variants of OCT1 and OCT2, inhibition assays were performed using serial dilutions of ranitidine (0 - 4000 µM) in OCT1*1, *2, OCT2 Ala270 and Ser270 overexpressing HEK293 cells.

The genetic variant OCT1*2 resulted in more sensitivity to inhibition by ranitidine which was confirmed with a decrease in \( IC_{50} \) by half (\( IC_{50} = 84 \pm 12 \) µM) from the wild type OCT1*1 (\( IC_{50} = 186 \pm 25 \) µM) with \( p <0.001 \). Ranitidine provided a varying inhibitory potency at different trospium concentrations. The calculated inhibitory dissociation constants (\( K_i \)) were 102 ± 15 µM for OCT1*1 and 27 ± 12 µM for OCT1*2, \( p <0.03 \).

The OCT2 Ser270 (\( IC_{50} = 515 \pm 67 \) µM) genetic variant did not cause any significant inhibition difference compared to the wild type OCT2 Ala270 (\( IC_{50} = 483 \pm 105 \)) (Figure 6).

---

**Figure 6:** Effects of ranitidine on the trospium uptake in HEK293 cells stably transfected with OCT1*1 (upper left graph), OCT1*2 (upper right graph), OCT2 Ala270 and OCT2 Ser270 (lower graph).
4.2. Pharmacokinetic interactions between trospium and clarithromycin or ranitidine

4.2.1. Influence of clarithromycin and ranitidine on pharmacokinetics of trospium

The pharmacokinetic characteristics of trospium were rather identical in values in both parallel control groups, in each case 12 subjects, of the DDI studies (Table 1 and Figure 7).

The mean plasma concentrations of trospium after intravenous infusion (2 mg) were ~20 ng/ml (range: 17 - 32 ng/ml). According to a three-compartmental model, the central compartment ($V_C$; ~0.18 l/kg, range: 0.13 - 0.26 l/kg) approximately resembled the extracellular volume of young healthy subjects. From here, trospium was distributed into intracellular water, a shallow peripheral compartment ($V_{p1}$) with a volume of ~0.5 l/kg (range: 0.4 - 0.8 l/kg), and into a deep compartment ($V_{p2}$, volume: ~4.13 l/kg, range: 2.5 - 7.4 l/kg), which corresponds to the known accumulation in parenchymatous organs (liver, kidneys). The $V_{ss}$ accounted for ~4.81 l/kg (range: 3.5 - 8 l/kg). Trospium was slowly eliminated from plasma with mean disposition residence time (MDRT) of ~6.5 h (range: 5 - 11 h) and a terminal half-life ($T_{1/2,z}$) of ~11 h (range: 6 - 16 h). The major elimination routes were glomerular filtration and substantial tubular secretion in the kidneys ($CL_{R}$; ~500 ml/min). Furthermore, there were a minor fecal secretion ($CL_{faecal}$; ~60 ml/min, range: 15 - 138 ml/min) and a substantial unspecified residual clearance of ~340 ml/min (range: 141 - 671 ml/min). About 60% of the dose was eliminated into the urine, and about 2% via feces within 5 days.

TC-PO was slowly and poorly absorbed with $T_{max}$ after ~6.5 h (range: 6 - 7 h), and bioavailability of ~10% (range: 3 - 28%). The absorption profile showed a typical biphasic pattern with the first peak concentration appeared after 2 - 3 h, and a second peak after 4 - 6 h which is in line with our hypothesis on the existence of proximal “absorption window” in the small intestine and a distal “window” in the cecum/proximal colon. The mean absorption time (MAT) was ~11 hours. About 30% of the absorbed dose was bioavailable within 2 - 4 hours, the other 70% within about 4 - 10 hours (control group in Figure 8).
Figure 7: Mean (± SD) plasma concentration-time curves of trospium after intravenous infusion (left), oral administration (30 mg TC-PO, right) alone (full circles) and with co-medications of clarithromycin (upper figures, open circles) and ranitidine (lower figures, open circles). The inserts presents plasma concentrations in log-scale. The study was performed in two parallel groups of N = 12 healthy white subjects in each group.

Figure 8: Absorption rate-time (left) and the absorbed amount-time (right) curves of trospium after oral administration of a 30 mg TC-PO alone (solid line) and after co-medication of 500 mg clarithromycin (dashed line)
As shown for the TC-clarithromycin study, bioavailability was inversely correlated to mean absorption time (MAT, rs² = 0.645, p < 0.0001), and directly correlated to the maximum absorption rate (AR_max, rs² = 0.910, p < 0.0001) independent on the co-medication (Figure 9).

![Figure 9: Correlation of mean absorption time (MAT) and maximum absorption rate (AR_max) with bioavailability (F) of trospium after oral administration (Spearman test). Full circles indicate values for the control group without clarithromycin, open circles for data after co-medication of clarithromycin.](image)

Elimination of trospium after oral administration was similar to intravenous trospium. The MDRT of trospium after oral dosing was ~4 h (range: 3 - 6 h) and the terminal half-life ~9 h (range: 6 - 14 h). Renal clearance was identical to the value after intravenous dosing (range: 383 - 707 ml/min). Approximately 6 % of the 30 mg dose was excreted via urine and ~35 % via the feces (Table 1).

**Influence of clarithromycin:** The major finding following concomitant administration of trospium with clarithromycin was elevation in the peripheral distribution volumes of trospium which led to a slight but significant increase in V_ss by ~27 %. Elimination clearances of trospium were not significantly influenced. MDRT and terminal half-life (T_½,z) significantly increased by ~25 - 30 % which was most likely the pharmacokinetic outcome of the significantly wider distribution of the drug. The maximum absorption rate (AR_max) of TC-PO was significantly lowered. Nevertheless, plasma exposure, bioavailability and amount of trospium excreted into urine were not significantly influenced by co-medication of clarithromycin. Interestingly, in nine subjects, these characteristics were lowered in the presence of clarithromycin (Figure 10). The typical biphasic time courses for absorption rate and for the cumulative amount absorbed generally laid below the respective control curves in
Results

the absence of clarithromycin (Figure 8). Due to clarithromycin, TC-PO was more poorly absorbed within the first ~3 hours as compared to the control without the co-medication: However, the statistical power of our DDI study was too low to confirm statistical significance for the whole group.

### Table 1: Pharmacokinetics of trospium after intravenous (2 mg TC) and oral administration (30 mg) before and after co-medication of clarithromycin as assessed post-hoc by population pharmacokinetic modeling. Arithmetic means with inter-subject variability and significance levels ( p <0.05, ** p <0.01) for paired Student’s t-test are given.

<table>
<thead>
<tr>
<th>Pharmacokinetic characteristics</th>
<th>Control</th>
<th>with clarithromycin</th>
<th>Control</th>
<th>with ranitidine</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intravenous infusion (2 mg/60 min)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL</td>
<td>ml/min</td>
<td>892 (14)</td>
<td>900 (4)</td>
<td>884 (18)</td>
</tr>
<tr>
<td>$V_c$</td>
<td>l</td>
<td>13.1 (19)</td>
<td>11.8 (34)</td>
<td>13.5 (19)</td>
</tr>
<tr>
<td>$V_p1$</td>
<td>l</td>
<td>35.9 (30)</td>
<td>42.9 (24)</td>
<td>42.2 (32)</td>
</tr>
<tr>
<td>$V_p2$</td>
<td>l</td>
<td>301 (27)</td>
<td>390 (23)</td>
<td>320 (27)</td>
</tr>
<tr>
<td>$V_{ss}$</td>
<td>l</td>
<td>350 (21)</td>
<td>444 (20)**</td>
<td>376 (27)</td>
</tr>
<tr>
<td>MDRT</td>
<td>h</td>
<td>6.54 (24)</td>
<td>8.23 (17)**</td>
<td>7.34 (27)</td>
</tr>
<tr>
<td>$T_{1/2,z}$</td>
<td>h</td>
<td>11.3 (10)</td>
<td>14.8 (12)**</td>
<td>15.9 (15)</td>
</tr>
<tr>
<td>CLR</td>
<td>ml/min</td>
<td>509 (13.4)</td>
<td>549 (9.7)*</td>
<td>517 (18.4)</td>
</tr>
<tr>
<td>CL (_{\text{faecal}})</td>
<td>ml/min</td>
<td>56.3 (66.2)</td>
<td>124 (210)</td>
<td>28.0 (78.1)</td>
</tr>
</tbody>
</table>

| **Oral administration (30 mg TC-PO)** | | | | |
| MAT | h | 10.8 (31) | 10.6 (26) | 9.72 (21) | 12.4 (42) |
| AR\(_{\text{max}}\) | mg/h | 4.8 (72) | 3.0 (61)* | n.d. | n.d. |
| F | % | 9.5 (48) | 7.5 (52) | 8.28 (65) | 6.75 (80) |
| CLR | ml/min | 386.1 (45) | 554.8 (55) | 476.7 (26) | 474.1 (17) |

CL, total clearance; n.d., not determined

**Influence of ranitidine:** The major finding following concomitant administration of intravenously infused trospium and orally administered ranitidine (300 mg) was the significant lowering of the renal clearance by ~15 %. All pharmacokinetic characteristics of trospium after oral administration of TC-PO were not significantly changed, including the renal clearance (Figure 1, Table 1).
4.2.2. Clinical relevance of the drug-drug interactions

In the post-hoc equivalence analysis to conclude on the clinical relevance of the trospium-clarithromycin and trospium-ranitidine interactions, the GMRs (90 % CI) for clarithromycin over trospium alone of the AUC, Cmax and CLR after oral dosing were 0.75 (0.56, 1.01), 0.64 (0.45, 0.89) and 1.00 (0.90, 1.13), respectively.

The respective GMRs (90 % CI) for ranitidine were: AUC, 0.88 (0.67, 1.15); Cmax, 0.97 (0.74, 1.26) and CLR, 1.01 0.88, 1.16) (Table 3).
Results

Table 2: Pharmacokinetic characteristics of trospium after oral administration of 30 mg TC-PO before and after co-medication of clarithromycin and ranitidine (inhibitors) as assessed by non-compartmental analysis. Arithmetic means ± SD and geometric mean ratios with 90 % confidence intervals (CI) are given.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Control</th>
<th>With inhibitor</th>
<th>GMR (90 % CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Trospium chloride-Clarithromycin</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC (ng×h/ml)</td>
<td>58.6 ± 30.2</td>
<td>44.3 ± 23.9</td>
<td>0.75 (0.56, 1.01)</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (ng/ml)</td>
<td>5.2 ± 2.9</td>
<td>3.7 ± 2.6</td>
<td>0.64 (0.45, 0.89)</td>
</tr>
<tr>
<td>T&lt;sub&gt;1/2&lt;/sub&gt; (h)</td>
<td>9.0 ± 2.2</td>
<td>9.1 ± 2.3</td>
<td>1.01 (0.85, 1.20)</td>
</tr>
<tr>
<td>CL&lt;sub&gt;R&lt;/sub&gt; (ml/min)</td>
<td>522 ± 92.6</td>
<td>521 ± 66.1</td>
<td>1.00 (0.90, 1.13)</td>
</tr>
<tr>
<td>A&lt;sub&gt;e urine&lt;/sub&gt; (mg)</td>
<td>1.8 ± 0.9</td>
<td>1.4 ± 0.7</td>
<td>0.76 (0.56, 1.02)</td>
</tr>
<tr>
<td>A&lt;sub&gt;e feces&lt;/sub&gt; (mg)</td>
<td>10.4 ± 4.4</td>
<td>10.4 ± 6.5</td>
<td>0.87 (0.55, 1.37)</td>
</tr>
<tr>
<td><strong>Trospium chloride-Ranitidine</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC (ng×h/ml)</td>
<td>60.8 ± 53.5</td>
<td>53.1 ± 39.6</td>
<td>0.88 (0.67, 1.15)</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (ng/ml)</td>
<td>5.23 ± 5.22</td>
<td>4.84 ± 3.99</td>
<td>0.97 (0.74, 1.26)</td>
</tr>
<tr>
<td>T&lt;sub&gt;1/2&lt;/sub&gt; (h)</td>
<td>9.9 ± 2.2</td>
<td>9.7 ± 2.5</td>
<td>0.97 (0.87, 1.08)</td>
</tr>
<tr>
<td>CL&lt;sub&gt;R&lt;/sub&gt; (ml/min)</td>
<td>501 ± 141</td>
<td>494 ± 97.8</td>
<td>1.01 0.88, 1.16)</td>
</tr>
<tr>
<td>A&lt;sub&gt;e urine&lt;/sub&gt; (mg)</td>
<td>1.69 ± 1.50</td>
<td>1.55 ± 1.24</td>
<td>0.88 (0.69, 1.14)</td>
</tr>
<tr>
<td>A&lt;sub&gt;e feces&lt;/sub&gt; (mg)</td>
<td>7.51 ± 3.82</td>
<td>7.97 ± 5.73</td>
<td>0.82 (0.45, 1.51)</td>
</tr>
</tbody>
</table>

In case of the trospium-clarithromycin interaction, the 90 % CI of the GMR for the elimination characteristics T<sub>1/2</sub> and CL<sub>R</sub> were within the span of equivalence for a drug with high variable pharmacokinetics (0.75 - 1.33). The spans for the exposure characteristics AUC and C<sub>max</sub> were only slightly exceeded. In case of the trospium-ranitidine interaction, all 90 % CI of the respective GMRs were even within the narrow span of 0.80 - 1.25.

4.2.3. Influence of trospium on pharmacokinetics of clarithromycin and ranitidine

Unbiased data on the influence of trospium on pharmacokinetics of clarithromycin and ranitidine cannot be presented here because placebo control groups without inhibitors were not planned for the secondary intention of the DDI study.

Clarithromycin was markedly slowly absorbed when combined with intravenous TC relative to TC-PO co-medication as indicated by significantly longer T<sub>max</sub> and mean body residence time (MBRT) as well as tendency for lower C<sub>max</sub>. Consequently, the GMR (90 % CI) (oral over intravenous trospium) for AUC [1.10 (0.98, 1.23)] and CL<sub>R</sub> [1.01 (0.89, 1.14)] were within the standard equivalence range of 0.80 - 1.25 contrarily to C<sub>max</sub> [1.17 (0.86, 1.61)] (Figure 5).

Ranitidine was significantly less absorbed after intravenous relative to oral co-medication of TC whereas all elimination characteristics (T<sub>1/2</sub>, CL<sub>R</sub>) remained unchanged. The mean absorption time (MAT) decreased by 1.4 h and the amount of ranitidine excreted via the urine by ~25 %. The typical erratic absorption profile of ranitidine was not changed by either co-
medication (Figure 11 and Table 4). Consequently, the 90% CI of the GMR for AUC and $C_{\text{max}}$ were outside but for $CL_R$ within the stipulated standard bioequivalence range (0.80 - 1.25).

**Figure 11:** Mean ($\pm$ SD) plasma concentration-time curves of clarithromycin (left) and ranitidine (right) after intravenous co-infusion (full circles) and oral co-medication (open circles) of trospium chloride

**Table 3:** Pharmacokinetic characteristics of ranitidine after intravenous infusion of 2 mg and oral co-medication of 30 mg trospium chloride (TC) as assessed by post-hoc population pharmacokinetic modeling (arithmetic means with inter-subject variability) and non-compartmental evaluation (arithmetic means $\pm$ SD). Geometric mean ratios (GMR) with 90% confidence intervals (CI) are given for the characteristics of the non-compartmental analysis.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>TC infusion</th>
<th>TC per os</th>
<th>GMR (90% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>population pharmacokinetic modeling</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC $\mu$g$\times$h/ml</td>
<td>4.7 (27)</td>
<td>5.8 (38) *</td>
<td>-</td>
</tr>
<tr>
<td>MBRT h</td>
<td>2.6 (29)</td>
<td>4.1 (21) *</td>
<td>-</td>
</tr>
<tr>
<td>$\Delta$MAT$^a$ h</td>
<td>-</td>
<td>-1.4 (76)</td>
<td>-</td>
</tr>
<tr>
<td><strong>non-compartmental analysis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC $\mu$g$\times$h/ml</td>
<td>4.5 ± 1.4</td>
<td>6.3 ± 2.8*</td>
<td>1.34 (1.16, 1.54)</td>
</tr>
<tr>
<td>$C_{\text{max}}$ $\mu$g/ml</td>
<td>0.7 ± 0.4</td>
<td>1.0 ± 0.6</td>
<td>1.31 (1.11, 1.70)</td>
</tr>
<tr>
<td>$T_{\text{max}}$ h</td>
<td>4.1 ± 1.7</td>
<td>4.3 ± 1.9</td>
<td>-</td>
</tr>
<tr>
<td>$T_{1/2}$ h</td>
<td>4.0 ± 2.0</td>
<td>3.7 ± 0.6</td>
<td>0.98 (0.84, 1.14)</td>
</tr>
<tr>
<td>$CL_R$ ml/min</td>
<td>436 ± 144</td>
<td>409 ± 82.5</td>
<td>0.95 (0.81, 1.11)</td>
</tr>
<tr>
<td>$A_e$ urine mg</td>
<td>111 ± 25.7</td>
<td>146 ± 40.5*</td>
<td>1.08 (0.96, 1.22)</td>
</tr>
<tr>
<td>$A_e$ feces mg</td>
<td>6.1 ± 13.8</td>
<td>3.3 ± 6.5</td>
<td>0.74 (0.47, 1.16)</td>
</tr>
</tbody>
</table>

$MBRT = MDRT + MAT$; $^a\Delta$MAT = $MBRT_{TC-IV} - MBRT_{TC-PO}$, $s_0, s_1$

*p <0.05 compared to “with TC-PO”, paired Student’s t-test
4.2.4. Safety and pharmacodynamic effects

Single dose intravenous and oral administrations of the TC test doses were safe and well tolerated by our healthy subjects. The most frequent adverse events expected were dry mouth, tachycardia and urinary retention. There were no evident differences in tolerability between mono-use or during concomitant administration with clarithromycin or ranitidine.

Treatment with TC caused the expected pharmacodynamical changes in salivation after intravenous and oral administration. Accommodation of the eyes was not markedly influenced. Co-medication of clarithromycin tended to minimize the changes in salivation (3 h after i.v. administration, p = 0.067). Conclusive results on pharmacodynamics interactions cannot be drawn because a placebo control was not planned in the DDI study.
5. Discussion

In our study on the potential functional meaning of multidrug transporters in the pharmacokinetics of trospium, data from experimental *in vitro* studies using transfected HEK293 cells were provided to predict the outcome of a hypothesis-driven DDI study with suitable probe inhibitors (competition approach) in healthy subjects. The DDI study was initiated to confirm the potential clinical meaning of the respective transporter for efficacy and safety of trospium chloride in treatment of patients with overactive bladder syndrome.

5.1. Affinity of trospium to multidrug transporters and pharmacokinetic conception

Using OCT1 and OCT2 overexpressing HEK293 cells, we found that OCT1 is a high capacity transporter of trospium as compared to OCT2. The OCT1 genetic alleles *3 and *4 resulted in significant loss of activity and the sorting defect alleles *5 and *6 caused complete loss of uptake of trospium compared to the wild type OCT1*1. The loss of function alleles OCT1*2 and OCT1*7 provided reduced but still considerable activity. OCT1*2 ($K_m = 8.7 \pm 3 \mu M$) reveals a higher affinity compared to OCT1*7 ($K_m = 15.5 \pm 2.7 \mu M$). The common OCT2 genetic allele Ser270 caused slight but significant increase in activity of OCT2 with $V_{max}$ value of $0.08 \pm 0.01$ nmol/min × mg.

Recent studies by other authors have shown that the efflux carrier P-gp (inside-out lipovesicles of MDCKII cells) and the uptake transporter OATP1A2 (MDCKII cells) were shown to be of high affinity but low capacity transporters for trospium compared to OCT1. The efflux transporters MATE1 and MATE2-K, which were overexpressed in HEK293 cells using the Flp-In system, possess comparable affinity and activity in trospium uptake compared to OCT1 (Table 4).

<table>
<thead>
<tr>
<th>Transporter</th>
<th>Experimental systems</th>
<th>$K_m$ (µM)</th>
<th>$V_{max}$ (nmol/min × mg)</th>
<th>$CL_{int}$ (µl/min × mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-gp</td>
<td>A&lt;sup&gt;22&lt;/sup&gt;</td>
<td>34.9 ± 7.5</td>
<td>0.1 ± 0.01</td>
<td>8.9 ± 7.8</td>
</tr>
<tr>
<td>OATP1A2</td>
<td>B&lt;sup&gt;22&lt;/sup&gt;</td>
<td>6.9 ± 1.3</td>
<td>0.04 ± 0.002</td>
<td>4.2 ± 1.1</td>
</tr>
<tr>
<td>OCT1</td>
<td>B&lt;sup&gt;22&lt;/sup&gt;</td>
<td>106 ± 16</td>
<td>0.3 ± 0.02</td>
<td>2.9 ± 0.7</td>
</tr>
<tr>
<td>OCT2</td>
<td>C</td>
<td>17.4 ± 2.1</td>
<td>0.8 ± 0.1</td>
<td>46 ± 3.2</td>
</tr>
<tr>
<td>MATE1</td>
<td>C&lt;sup&gt;23&lt;/sup&gt;</td>
<td>5.0 ± 0.1</td>
<td>0.04 ± 0.01</td>
<td>80 ± 31.4</td>
</tr>
<tr>
<td>MATE2-K</td>
<td>C&lt;sup&gt;23&lt;/sup&gt;</td>
<td>15.4 ± 2.4</td>
<td>1.1 ± 0.1</td>
<td>71.6 ± 7.9</td>
</tr>
</tbody>
</table>

A: Inside-out lipovesicles; B: identical MDCKII cells; C: identical HEK293 cells
Discussion

In double-transfected MDCKII cells, OCT1 and OCT2 were shown to function in interplay with MATE1 and MATE2-K in the transcellular transport of trospium.\cite{24} Therefore, a functional interplay can be assumed between OCT1 and MATEs in hepatocytes and OCT2 with the MATEs in renal PTC (synergistic uni-directional processes). Theoretically, trospium may be orally absorbed via enterocytes by the interplay of OCT1-mediated uptake minimized by the P-gp-mediated back-secretion. Such antidromic, bidirectional processes caused by OATP1A2 (uptake) and P-gp (efflux) in BCEC might also be the rationale behind the observation that trospium does not pass the BBB and does not exert CNS effects.\cite{3,19,21}

### 5.2. Transporter derived hypothesis for the pharmacokinetics of trospium

The efflux transporter P-gp is highly expressed in the apical membrane of enterocytes, canalicular membrane of hepatocytes, basolateral membrane of the capillary endothelial cells in organ barriers (e.g. BBB, testes, and ovary) and apical membrane of PTC. The activity of P-gp therefore minimize oral absorption (e.g. digoxin)\cite{4} and the transfer via organ barriers (e.g. loperamide in the BBB)\cite{34} and is involved in renal and hepatic elimination of drugs (e.g. talinolol).\cite{35} OCT1 is the major uptake carrier for organic cations (e.g. metformin) from the sinusoidal blood into hepatocytes.\cite{36,37} The canalicular MATE1 is involved in biliary secretion of drugs and metabolites (e.g. cimetidine).\cite{4} In the kidneys, the basolateral OCT2 and the apical MATE1 and MATE2-K mediate the uni-directional excretion of cationic compounds via the PTC (e.g. metformin).\cite{38} OATP1A2 is the major uptake carrier at the basolateral membrane of BCEC and is associated with the presence/absence of central nervous effects of drugs (e.g. deltorphin II).\cite{39} It is also expressed in the kidneys (apical membrane of distal nephrons), liver (apical membrane of cholangiocytes), lung, testes, and placenta.\cite{40}

The chimeric pharmacokinetic characteristics of the highly polar quaternary ammonium compound trospium can be related to the physiological function of the multidrug transporters to which trospium has high affinity as described above. The interplay between the uptake carrier OCT1 and the efflux carrier P-gp at the apical membrane of enterocytes along the small and large intestine may explain the slow and poor absorption of trospium after oral administration. Uptake by OCT1 and/or OCT2 can be the rationale for accumulation of trospium in the liver and in kidneys and, in turn, the high distribution volume of the drug. The interplay between OCT1 with P-gp and MATE1 in the liver is in line with enterohepatic circulation of trospium, between OATP1A2 and P-gp with the poor distribution into the brain and between OCT2 and the MATEs with the substantial tubular secretion in the kidneys.

To our hypothesis, P-gp, OATP1A2, OCT1, OCT2, MATE1 and MATE2-K might be involved in the absorption and disposition of trospium as shown in Figure 12.
According to our transporter-driven hypothesis on the pharmacokinetics of trospium, any confounder with influence on the function of the involved uptake and efflux carriers might be relevant for absorption, distribution and elimination of the poorly metabolized drug, in turn, may influence efficacy and safety such as pharmacogenetic polymorphisms, interaction with inducers/inhibitors or physiological/pathophysiological confounders (e.g. circadian rhythms, organ diseases, dietary ingredients, xenobiotic compounds).

Some of the confounders can also be intentionally utilized to gain deeper insight into the hypothesized role of the transporters in overall disposition of trospium by hypothesis-driven, experimental clinical studies. The widely accepted approaches include pharmacogenomics studies in selected healthy subjects with loss of function polymorphisms or gain of function polymorphisms and DDI studies with suitable, selective probe inhibitors of the respective transporter.

Based on the available information from the literature at the beginning of our research project on trospium chloride, clarithromycin and ranitidine were selected to be suitable as probe drugs according to their specificity to inhibit intestinal P-gp and OCT1, respectively, and according to their in vitro competition parameters from transporter cell model studies in line.

Figure 12: Conception on the role of multidrug transporters in pharmacokinetics of trospium after oral and intravenous administrations in man.
with the known \textit{in vitro/in vivo} characteristics for prediction of the DDI studies as recommended in the current U.S. food and drug administration (FDA) and European medicines agency (EMA) guidelines.\textsuperscript{41,42}

5.3. Translation of \textit{in vitro} data into understanding the outcome of clinical relevant drug-drug interactions with probe inhibitors

For prediction of the outcome of the influence of inhibitors of drug transporters on the pharmacokinetics of trospium according to the statistical recommendations of regulatory authorities (FDA and EMA),\textsuperscript{9,11} parameters on the pharmacokinetics of the drug in healthy subjects ([I]$_1$, [I]$_2$, [I]$_3$) and inhibition constants (IC$_{50}$, Ki) are required. [I]$_1$, [I]$_2$ and [I]$_3$ are defined to be the unbound maximum concentrations of the drugs (substrate, inhibitor) in venous blood, in the small intestine and in the sinusoidal blood of the liver, respectively. [I]$_1$ is taken from the plasma concentration-time curve, [I]$_2$ is derived from the given therapeutic oral dose dissolved in 240 ml water for administration and [I]$_3$ was assessed in our study for trospium using the pharmacokinetic data and for clarithromycin and ranitidine from the data after oral administration and individual literature data after intravenous administration of ranitidine\textsuperscript{43-45} and clarithromycin\textsuperscript{46,47} using the equation suggested by Ito et al (Table 5).\textsuperscript{48}

In light of the $K_m$ values of trospium for P-gp and OCT1 and the [I]$_2$ values for trospium after oral administration of 30 mg TC-PO, both transporters along the small intestine should be highly saturated. Only ~10% of the administered dose is absorbed from gut. However, there is analytical evidence for degradation of trospium by the alkaline pH of intestinal fluid (pH >4.0) because only 10 mg of the ~27 mg remaining, non-absorbed dose fraction could be balanced in the feces. Therefore, the transporters might be susceptible for major inhibition at least in the “wider absorption window” for trospium in cecum/ascending colon under the precondition that the probe inhibitors (clarithromycin and ranitidine) are still available in the lumen of the distal absorption region.\textsuperscript{16}

The probe inhibitors clarithromycin and ranitidine showed quite different \textit{in vitro} inhibition profiles on trospium transporters. Clarithromycin inhibits the P-gp efflux (IC$_{50}$ = 9.0 ± 0.5 µM) and the OATP1A2-mediated uptake of trospium (IC$_{50}$ = 93 ± 1.5 µM) but not OCTs and MATEs. Ranitidine, on the other hand, inhibits OCT1 ($K_i$ = 102 ± 15), MATE1 (IC$_{50}$ = 134 ± 37 µM) and MATE2-K (IC$_{50}$ = 35 ± 11 µM). It is also a weak inhibitor of OCT2 (IC$_{50}$ = 482 ± 105 µM). But it does not completely inhibit OCT1-mediated uptake of trospium (~65% inhibited) at its [I]$_2$ concentration and is not known to inhibit P-gp and OATP1A2.\textsuperscript{41,42,49}
Table 5: Unbound maximal concentrations of trospium, clarithromycin and ranitidine in venous blood ([I]₁), intestinal fluid ([I]₂) and hepatic sinusoidal blood ([I]₃).

<table>
<thead>
<tr>
<th>Pharmacokinetic characteristics</th>
<th>Trospium</th>
<th>Clarithromycin</th>
<th>Ranitidine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Route of administration</td>
<td>IV (2 mg)</td>
<td>PO (30 mg)</td>
<td>TC-IV</td>
</tr>
<tr>
<td>PPB</td>
<td>50 %²⁰</td>
<td>50 %²⁰</td>
<td>65 %⁵⁰</td>
</tr>
<tr>
<td>F</td>
<td>-</td>
<td>0.1</td>
<td>0.55⁵⁰</td>
</tr>
<tr>
<td>[I]₁ (µM)</td>
<td>0.03 ± 0.008</td>
<td>0.007 ± 0.005*</td>
<td>0.8 ± 0.5</td>
</tr>
<tr>
<td>[I]₂ (µM)</td>
<td>-</td>
<td>290</td>
<td>2,790</td>
</tr>
<tr>
<td>[I]₃ (µM)</td>
<td>0.03 ± 0.008</td>
<td>0.009 ± 0.005*</td>
<td>7.6 ± 0.5</td>
</tr>
</tbody>
</table>

*paired Student’s t-test p < 0.000; $paired Student’s t-test p < 0.055

Unbound hepatic sinusoidal blood concentrations ([I]₃) were calculated from the maximum portal venous blood concentrations (Cₘₐₓ,portal) and the peripheral venous blood concentrations (Cₘₐₓ,plasma) of the drugs assuming that 60% of the sinusoidal blood supply is contributed by the portal vein and 40% by the hepatic artery, and was normalized to plasma protein binding (PPB).

Cₘₐₓ,portal was estimated by the formula Cₘₐₓ,portal = Cₘₐₓ,plasma + \( \frac{K_a \times D \times F_a \times Q_h}{Q_h} \) \( K_a \) is the absorption rate constant, D the oral dose, \( F_a \) the oral bioavailable and \( Q_h \) the hepatic blood flow of 1,500 ml/min in fasting subjects.³⁵

\( K_a \) values were estimated by the formula \( K_a = \frac{1}{MAT} \). MAT for ranitidine and clarithromycin was calculated by the formula MAT = MBRT - MDRT. The mean ± SD of MDRT values for clarithromycin (4.0 h) and ranitidine (2.51 ± 0.52 h) were derived from \( V_{ss}/CL \) using individual data from historical intravenous studies with clarithromycin⁴⁶,⁴⁷ and ranitidine.⁴³ - ⁴⁵

According to the statistical prediction model of the authorities for IVIVE of DDIs, \([I]_1/IC_{50} \geq 0.1\) for basolateral uptake and efflux transporters or \([I]_2/IC_{50} \geq 10\) for apically expressed intestinal transporters (FDA), and \([I]_1/IC_{50}(K_i) > 0.02\) for hepatic efflux transporters or \([I]_3/IC_{50}(K_i) \geq 0.04\) for hepatic uptake transporters (EMA) is considered to be of clinical importance. For renal efflux transporters (e.g. MATE1, MATE2-K), a threshold of \([I]_1/IC_{50} \geq 0.02\) is recommended.⁹,¹¹

The respective ratios for ranitidine and clarithromycin with additional consideration of influence of trospium after oral and intravenous administration on the pharmacokinetics of clarithromycin and ranitidine are given in Table 6.
## Discussion

### Table 6: Prediction of transporter-mediated DDI of trospium with ranitidine and clarithromycin based on *in vitro* IC$_{50}$/K for basolateral uptake and efflux transporters

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Kinetic parameters</th>
<th>OCT1</th>
<th>OCT2</th>
<th>MATE1</th>
<th>MATE2-K</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trospium <em>in vitro</em></td>
<td>IC$_{50}$/K, ranitidine (µM)</td>
<td>186 ± 25  (102 ± 15)</td>
<td>482 ± 105</td>
<td>134 ± 37</td>
<td>35 ± 11</td>
</tr>
<tr>
<td>Ranitidine with TC-IV <em>in vivo</em></td>
<td>IC$_{50}$/K</td>
<td>0.01 ± 0.01 (0.02 ± 0.01)</td>
<td>0.004 ± 0.002</td>
<td>0.014 ± 0.009</td>
<td>0.05 ± 0.03</td>
</tr>
<tr>
<td>Ranitidine with TC-PO <em>in vivo</em></td>
<td>IC$_{50}$/K</td>
<td>21 (39)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>IC$_{50}$/K</td>
<td>0.18 ± 0.01 (0.3 ± 0.01)</td>
<td>NA</td>
<td>0.24 ± 0.009</td>
<td>NA</td>
</tr>
<tr>
<td>Ranitidine with TC-PO <em>in vivo</em></td>
<td>IC$_{50}$/K</td>
<td>0.03 ± 0.02</td>
<td>0.005 ± 0.004</td>
<td>0.02 ± 0.01</td>
<td>0.07 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>IC$_{50}$/K</td>
<td>21 (39)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Ranitidine with TC-PO <em>in vivo</em></td>
<td>IC$_{50}$/K</td>
<td>0.023 ± 0.01 (0.04 ± 0.02)*</td>
<td>NA</td>
<td>0.03 ± 0.01*</td>
<td>NA</td>
</tr>
</tbody>
</table>

### P-gp OATP1A2

<table>
<thead>
<tr>
<th>Treatment</th>
<th>IC$_{50}$/clarithromycin (µM)</th>
<th>OCT1</th>
<th>OCT2</th>
<th>MATE1</th>
<th>MATE2-K</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trospium <em>in vitro</em></td>
<td>8.9 ± 0.5</td>
<td>92.6 ± 1.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clarithromycin with TC-IV <em>in vivo</em></td>
<td>IC$_{50}$/</td>
<td>0.1 ± 0.06</td>
<td>0.001 ± 0.0006</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clarithromycin with TC-PO <em>in vivo</em></td>
<td>IC$_{50}$/</td>
<td>1 ± 0.06</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clarithromycin with TC-PO <em>in vivo</em></td>
<td>IC$_{50}$/</td>
<td>1.4 ± 0.08*</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* paired Student’s t-test p <0.000; § paired Student’s t-test p <0.055

The *in vitro* interactions predicted to cause clinical DDI according to FDA: [I]$_i$/IC$_{50}$ ≥0.1 for basolateral uptake and efflux transporters, [I]$_i$/IC$_{50}$ ≥0.02 for renal efflux transporters or [I]$_i$/IC$_{50}$ ≥10 for apically expressed intestinal transporters5, and EMA: [I]$_i$/IC$_{50}$/K >0.02 for hepatic efflux transporters or [I]$_i$/IC$_{50}$/K ≥0.04 for hepatic uptake transporters5 are shown in bold faces. NA, not applicable

Accordingly, the IVIVE of trospium uptake inhibition has shown that clarithromycin could cause DDI by inhibiting P-gp-mediated efflux of trospium at the intestine with [I]$_i$/IC$_{50}$ of ~310, and hepatocytes with [I]$_i$/IC$_{50}$ = 1.4 ± 0.08.9,11,42 Therefore, co-medication of oral clarithromycin was expected to result in an increase in oral absorption and hepatic clearance of trospium but not changes in distribution.

Ranitidine was predicted to have a potential *in vivo* inhibition effect on intestinal OCT1 ([I]$_i$/K ~40), renal MATE1 ([I]$_i$/IC$_{50}$ ~0.02) and MATE2-K ([I]$_i$/IC$_{50}$ ~0.1). The uptake of trospium by the basolateral OCT1 into hepatocytes and OCT2 into renal PTC was not expected to be inhibited by ranitidine. Therefore, it was predicted that oral co-medication of ranitidine with 30 mg TC could result in a decrease of oral bioavailability and renal clearance of trospium. Change in distribution volume was not expected.
5.4. Proof of concept by the results of the clinical studies and clinical relevance

Trospium provides ample of pharmacokinetic characteristics by which the function of drug transporters can be well quantified.

The drug is poorly (F ~10 %) and slowly absorbed (T_{max} ~6.5 h) in the intestine by two distinguishable uptake processes with different rates. We have shown by pharmacokinetic modelling that 30 % of the bioavailable dose are absorbed within 2 to 4 h after administration and further 70 % in the next 4 to 10 hours. Additionally, the maximum absorption rate (AR_{max}) and the mean absorption time (MAT) are good measures for quantification of the uptake processes and prediction of trospium bioavailability. The AR_{max} of trospium occurs in the second absorption phase. From the known gastrointestinal transit times of immediate-release drugs after oral administration,^{53} it can be concluded that the proximal “absorption window” must be located in the small intestine and the distal window in the cecum/ascending colon.

Tadken et al. have already hypothesized in a previous study that interplay of OCT1 and P-gp along the gut could be responsible for the biphasic oral absorption kinetics of trospium.^{21}

According to that hypothetical model, inhibitors of P-gp and OCT1 such as clarithromycin and ranitidine should have maximum effects on rate and extent of absorption in the cecum/ascending colon, provided that they reach this region still in parent form and adequate concentration.

The P-gp inhibitor clarithromycin carries a high potential for in vivo interaction with the oral absorption of trospium in the case of a concomitant use, i.e., to increase its bioavailability as predicted by IVIVE. Unexpectedly, we measured no significant or clinical relevant effects of clarithromycin on plasma exposure, maximum plasma concentration or bioavailability of trospium. In our paper in Appendix A1^{32} we discussed the following reasons for absence of the expected DDI result:

1. The intestinal P-gp obviously is highly saturated by oral administration of 30 mg trospium chloride swallowed with 240 ml water. Rapid water absorption may additionally increase the concentration at the P-gp receptor site.^{54,55} Therefore, competition with 500 mg clarithromycin might not have influenced P-gp function in relevant manner.
2. Clarithromycin exerts a strong prokinetic effect which may lead to faster gastric emptying of the dosage form and bypassing of the “narrow absorption window” in the small intestine. Up to 30 % of the absorptive capacity for trospium seemed to be more or less bypassed in all subjects after clarithromycin co-medication.
3. The inhibitor clarithromycin does not reach the distal “wider absorption window” with adequate concentrations for inhibition of the colonic P-pg because the parent
compound is chemically degraded in the neutral or slightly alkaline medium or by the microbiota.

It was also predicted by our IVIVE that oral absorption of trospium in healthy subjects can be lowered by interaction with ranitidine, an inhibitor of OCT1 in vitro. The following major deliberations are useful in explaining the unexpected rejection of our working hypothesis as discussed in detail in the appended paper A2:

1. After oral administration of 30 mg trospium chloride, intestinal OCT1 is highly saturated as already discussed above for P-gp.
2. OCT1 is not the right uptake transporter for trospium in the intestine because our study hypothesis was derived from evidence by Han et al. 2013 who localized OCT1 to the apical membrane of enterocytes which was in contrast to the previously accepted basolateral localization.
3. There is many doubt that ranitidine reaches the distal absorption window for trospium in sufficient concentration as ~50 % of the dose is absorbed along the small intestine, and an additional fraction is degraded by the colonic microbiota. The distal “wider absorption window” in the cecum/ascending colon is the place for maximal absorption rate for trospium.
4. OCT1 might not be the major uptake carrier for trospium in the human intestine. Alternative candidates are the plasma membrane monoamine transporter (PMAT), the serotonin reuptake transporter (SERT), or any others which share similar features with the substrates and inhibitors of OCTs.

On the contrary to our IVIVE prediction, we found in our DDI with clarithromycin a non-expected but significant expansion of the shallow and deep distribution spaces for trospium by ~27 %. Nonetheless of the IVIVE prediction, clarithromycin seems to inhibit P-gp in organs with a tight endothelium (brain, testes, ovary), as well as in organs with fenestrated epithelium (heart, skeletal and smooth muscles). Distribution into the brain seemed not be influenced in clinically relevant manner because clarithromycin is also an inhibitor of OATP1A2, the major uptake carrier for trospium in the BBB.

The major elimination route of trospium is renal excretion by glomerular filtration and substantial tubular secretion. Its renal clearance in both parallel groups of healthy subjects accounted for ~ 500 ml/min, i.e., about 4-times of the glomerular filtration rate and nearly 80 % of the total renal blood flow. To our knowledge, clarithromycin and ranitidine do not influence the glomerular filtration rate in the kidneys (e.g. by effects on blood perfusion). Therefore, any change in the renal clearance of trospium by our probe inhibitors must have been caused by their influence on the active tubular transport processes as resulting from the interplay of OCT2 with MATE1, MATE2-K and P-gp, or with other transporters with so far
unknown affinity to trospium. Renal elimination processes can be best quantified after intravenous administration of the TC (lowest variability).

5.5. Suitability of trospium chloride to be a probe drug of P-gp, OATP1A2, OCTs and MATEs

From the in vitro data as published in the literature and from the results of the experimental studies with HEK293, it can be concluded that trospium is a suitable probe drug for the in vitro evaluation of the transport function of P-gp, OATP1A2, OCT1 and OCT2, MATE1 and MATE2-K. The drug substrate has high affinity to the transporter proteins and can easily be quantified using an LC-MS/MS assay with adequate sensitivity and quality in respective experimental media. The drug can also successfully be used for evaluation of the interplay between OCT1 and 2 with MATE1 in double transfected cell models. Whether it is also suitable for evaluation of uni-directional transport processes in cells double-transfected with OCT2/MATE2-K and OATP1A2 or OCTs/P-gp must be studied.

Concerning the suitability of trospium as an in vivo probe drug for any of the transporters mentioned above, final conclusions cannot be derived on the basis of the IVIVE data and the results of the pharmacokinetic DDI studies with clarithromycin and ranitidine alone.

Trospium might probably be a probe drug for intestinal P-gp and OCT1 for certain, very specific study objectives even though interactions of clarithromycin and ranitidine with its oral absorption could not be confirmed by the DDI studies. The reasons for the negative results (e.g. saturation of P-gp and OCT1, localization of OCT1 in the apical membrane, prokinetic effect of clarithromycin and availability of clarithromycin and ranitidine in the colon) were already discussed above (chapter 5.4). To our opinion, trospium can only be selected for DDI studies with new chemical entities (NCE) with predicted inhibitory potential on OCT1 and P-gp and which are available after oral absorption along the small intestine and in the cecum/ascending colon. A second kind of application might be pharmacogenomics studies in subjects with functionally relevant polymorphisms of P-gp and OCT1 or in patients with suspected transport failure due to intestinal diseases. In all studies with trospium chloride as a probe drug, the investigational oral dose should be <30 mg to avoid substantial saturation of the transporters. An appropriate dose might be 3 mg ([I]₀~30 µM) in immediate release dosage form which provide unsaturated and first-order uptake kinetics for competitive drug interaction with P-gp (Kᵣ = 35 ± 7.5 µM) and OCT1 (Kᵣ = 17.4 ± 2.1 µM). With that oral microdose, pharmacodynamic (muscarinic receptor blocking) effects of trospium on the pharmacokinetics of NCEs are most likely not expected.

Intravenously administered trospium (2 mg TC) might be a suitable probe drug to evaluate the effects of a NCE with P-gp inhibiting potential on distribution of a drug. To our knowledge, trospium is the first drug candidate at all for which increase in distribution volume following
oral co-administration of a P-gp inhibitor (clarithromycin) could be confirmed in a controlled, randomized clinical study in healthy subjects. This result, however, was not predicted by our IVIVE analysis. On the contrary to P-gp, the IVIVE predicted lowering in the trospium distribution volume by the OCT1 inhibitor (uptake into the liver) and the OCT2 inhibitor (uptake into the kidneys) ranitidine was, due to several reasons, not a surrogate for the function of OCTs in organ distribution of intravenously administered trospium.

The function of the efflux transporters MATE1 and MATE2-K in the PTC of the kidneys can be well assessed with the probe drug trospium by measuring its renal clearance which is significantly lowered after ranitidine co-medication. This has been predicted by our IVIVE data.

In future experimental in vitro and in vivo studies, special emphasis should also be spent to the apical re-uptake transporters in the PTC which might be inhibited by clarithromycin as a hypothesized reason for the increased renal clearance of intravenous trospium after oral co-medication of 500 mg clarithromycin. This very spectacular result should be preferentially evaluated to discover the rationale behind the incidental phenomenon. Probably, a new renal target involved in renal tubular drug elimination can be identified. Please remember that clarithromycin inhibited in our DDI study the uptake of trospium in the small intestine in all subjects by a so far unknown transport mechanism. Probably, the same transporter protein is concerned.

Generally, further pharmacokinetic evaluation is required before final conclusion on the suitability of trospium as a probe drug for the function of cation transporters in man can be drawn.

5.6. Limitations

In this study, prediction of in vivo DDI was conducted using the EMA and FDA statistical models based on the work of Fahmi OA et al. The prediction criteria have been employed by the regulatory authorities in order to recruit NME for clinical trials and identify an interaction potential during the early phase of drug development. But this mechanistic model, unlike PBPK model, uses one maximum inhibitor concentration and does not consider temporal change in concentration. Moreover, it is a highly sensitive (~80 %) but moderately specific (~60 %) statistical test that could hamper accuracy of prediction (positive prediction value ~65 %). 68,69

With competitive inhibition, unlike $K_i$, $IC_{50}$ value is dependent on the substrate concentration and method used (e.g. transporter expression) and hence varies from laboratory to laboratory. With the exception of OCT1, we used $IC_{50}$ values of transporters from different laboratories for the interpretation and extrapolation of in vitro to in vivo inhibition of trospium uptake by clarithromycin and ranitidine. 70,71
Unlike pharmacogenomics, use of drug interaction as a tool to study function of transporters is constrained by the absence of selective inhibitors. We do not know yet the effects of clarithromycin and ranitidine in other organic cation transporters (e.g. PMAT, SERT) which might involve in trospium uptake in the intestine, kidney and other organs.\textsuperscript{8,72} 

In the evaluation and discussion of our \textit{in vitro-in vivo} results, it must be considered that TC exerts gastrointestinal prokinetic effects which are relevant for the pharmacokinetics of the probe inhibitors clarithromycin and ranitidine because they may significantly change the substrate concentrations of trospium in the transporter binding compartments (e.g. gut lumen, peripheral blood, sinusoidal blood in the liver) as provided in Tables 5 and 6.
6. Conclusion

Trospium is a drug with poor non-ionic permeability via biological lipid membrane layers.

The cationic compound has high affinity to the human multidrug transporters P-glycoprotein, OATP1A2, OCT1, OCT2, MATE1 and MATE2-K. The in vitro transport by P-glycoprotein and OATP1A2 can be inhibited with clarithromycin and the transport by the OCTs and MATEs with ranitidine.

Co-medication of clarithromycin and ranitidine, respectively, in single therapeutic doses does not significantly influence the oral absorption of trospium. Instead, clarithromycin leads to significant increase of its distribution volume and ranitidine to significant lowering of its renal clearance.

In vitro to in vivo extrapolation (IVIVE) using the study data is recommended by authorities to predict the inhibition potential of a drug in human subjects. However, only the in vivo effects of ranitidine on renal clearance of trospium can be predicted by in vitro competition assays with MATE1 and MATE2-K. The effects of clarithromycin on distribution of trospium were not predictable by IVIVE using experimental data with P-gp.

The overall influence of clarithromycin and ranitidine on exposure characteristics of trospium is not of clinical relevance when considering the highly variable pharmacokinetics of the drug.

For pharmacokinetic studies in human subjects, trospium might be used as a probe drug to evaluate P-glycoprotein-dependent distribution changes and influences on tubular secretion by confounders of MATE1 and MATE2-K. Trospium after oral micro-dosing might likely be suitable for evaluation of drug absorption via P-glycoprotein and OCT1. Generally, additional clinical research is needed before final conclusion on the suitability of trospium to be a probe drug for transporter studies in vivo can be drawn.
7. Summary

Oral administration of drugs is the most common, convenient, safest and economical route of drug administration. There is lack of established tools to study the function of transporters in the intestinal absorption of drugs. Because of its favorable physico-chemical, pharmacokinetic and pharmacodynamic characteristics, trospium could be potentially used as a probe substrate to study the function of drug transporters. Therefore, this study was conducted to examine the suitability of trospium chloride as a probe drug to study the function of multidrug transporters in the human body. To this end, two randomized, controlled, four-period, cross-over pharmacokinetic drug interaction studies of oral and intravenous trospium with co-medication of oral clarithromycin or ranitidine were performed in 24 healthy subjects to mechanistically characterize the role of P-gp, OATP1A2, OCT1, OCT2, MATE1 and MATE2-K in the absorption and disposition of trospium. The contribution of the drug transporters in the absorption and disposition of trospium were examined in isolated systems using in vitro uptake and inhibition assays in transporter transfected human cell lines.

OCT1 ($V_{\text{max}} = 0.8 \pm 0.1 \text{ nmol/min} \times \text{mg}$) is a high capacity transporter of trospium compared to OCT2 ($V_{\text{max}} = 0.04 \pm 0.01 \text{ nmol/min} \times \text{mg}$). But the OCT2 ($K_m = 0.5 \pm 0.1 \text{ µM}$) transporter demonstrated a high affinity in the transport of trospium compared to OCT1 ($K_m = 17.4 \pm 2.1 \text{ µM}$). OCT1 genetic alleles *2, *3, *4 and *7 resulted in significant loss of activity and the alleles *5 and *6 caused complete loss of uptake of trospium. The common OCT2 genetic allele Ser270 caused slight but significant increase in activity of OCT2.

Ranitidine inhibits OCT1 ($IC_{50} = 186 \pm 25 \text{ µM}$), MATE1 ($IC_{50} = 134 \pm 37 \text{ µM}$) and MATE2-K ($IC_{50} = 35 \pm 11 \text{ µM}$)-mediated uptake of trospium in vitro. But it is a weak inhibitor of OCT2 transporter ($IC_{50} = 482 \pm 105 \text{ µM}$). Using FDA and EMA in vitro to in vivo extrapolation models, ranitidine was predicted to have a potential inhibition effect on intestinal OCT1 ($[I]_2/IC_{50} \sim 40$), renal MATE1 ($[I]_1/IC_{50} \sim 0.02$) and MATE2-K ($[I]_3/IC_{50} \sim 0.1$) transporters in vivo. Clarithromycin was predicted to cause DDI by inhibiting P-gp-mediated efflux of trospium at the intestine ($[I]_2/IC_{50} \sim 310$) and hepatocytes ($[I]_3/IC_{50} \sim 1$). Therefore, co-medication of oral clarithromycin was expected to result in an increase in oral absorption and hepatic clearance of trospium but not changes in distribution volume.

In healthy subjects, oral trospium is slowly (MAT $\sim 10$ h) and poorly (F $\sim 10\%$) absorbed from the jejunum and cecum/ascending colon, widely distributed into the body ($V_{ss} = 5 - 6 \text{ l/kg}$) and slowly eliminated ($t_{1/2} = 9 - 10$ h) majorly via renal glomerular filtration and tubular secretion (CL$_R \sim 500$ ml/min). After co-medication of clarithromycin (inhibitor of P-gp), on the contrary to our IVIVE prediction, we found a non-expected but significant expansion of the shallow and deep distribution spaces for trospium by $\sim 27\%$. A single dose administration of
Summary

trospium with co-medication of ranitidine (inhibitor of OCT1) resulted in no effect on the intestinal absorption of trospium. But the renal clearance of trospium decreased slightly (15\%) but significantly.

Intravenously administered trospium (2 mg TC) might be a suitable probe drug to evaluate the effects of a P-gp inhibitor on distribution of a drug. Oral trospium chloride can be selected for DDI studies with new chemical entities (NCE) with predicted inhibitory potential on OCT1 and P-gp and which are available after oral absorption along the small intestine and in the cecum/ascending colon. Another kind of application of trospium chloride might be pharmacogenomics studies in subjects with functionally relevant polymorphisms of P-gp and OCT1 or in patients with suspected transport failure due to intestinal diseases. The function of the efflux transporters MATE1 and MATE2-K in the PTC of the kidneys can be well assessed with the probe drug trospium by measuring its renal clearance.
8. Reference


### 9. Abbreviation

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABC</td>
<td>ATP-binding cassette</td>
</tr>
<tr>
<td>Ae feces</td>
<td>Amount excreted into feces</td>
</tr>
<tr>
<td>A&lt;sub&gt;e&lt;/sub&gt; feces</td>
<td>Amount excreted into feces</td>
</tr>
<tr>
<td>A&lt;sub&gt;e&lt;/sub&gt; urine</td>
<td>Amount excreted into urine</td>
</tr>
<tr>
<td>AR&lt;sub&gt;max&lt;/sub&gt;</td>
<td>Maximum absorption rate</td>
</tr>
<tr>
<td>AUC</td>
<td>Area under the concentration-time curve</td>
</tr>
<tr>
<td>BBB</td>
<td>Blood-brain barrier</td>
</tr>
<tr>
<td>BCEC</td>
<td>Brain capillary endothelial cells</td>
</tr>
<tr>
<td>BCRP</td>
<td>Breast cancer resistant protein</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>Caco-2</td>
<td>Colon cancer-2 cells</td>
</tr>
<tr>
<td>CL&lt;sub&gt;fecal&lt;/sub&gt;</td>
<td>Fecal clearance</td>
</tr>
<tr>
<td>CL&lt;sub&gt;fecal&lt;/sub&gt;</td>
<td>Fecal clearance</td>
</tr>
<tr>
<td>CL&lt;sub&gt;int&lt;/sub&gt;</td>
<td>Intrinsic clearance</td>
</tr>
<tr>
<td>CL&lt;sub&gt;R&lt;/sub&gt;</td>
<td>Renal clearance</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt;</td>
<td>Maximum plasma concentration</td>
</tr>
<tr>
<td>DDI</td>
<td>Drug-drug interaction</td>
</tr>
<tr>
<td>F</td>
<td>Bioavailability</td>
</tr>
<tr>
<td>FDA</td>
<td>U.S. Food and drug administration</td>
</tr>
<tr>
<td>FEX</td>
<td>Fexofenadine</td>
</tr>
<tr>
<td>GIT</td>
<td>Gastrointestinal tract</td>
</tr>
<tr>
<td>GMR</td>
<td>Geometric mean ratio</td>
</tr>
<tr>
<td>HEK293</td>
<td>Human embryonic kidney cells293</td>
</tr>
<tr>
<td>IC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>Half-maximal inhibitory concentration</td>
</tr>
<tr>
<td>[I&lt;sub&gt;p&lt;/sub&gt;]&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Unbound plasma drug concentration</td>
</tr>
<tr>
<td>[I&lt;sub&gt;p&lt;/sub&gt;]&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Intestinal drug concentration</td>
</tr>
<tr>
<td>[I&lt;sub&gt;p&lt;/sub&gt;]&lt;sub&gt;3&lt;/sub&gt;</td>
<td>Unbound liver sinusoidal drug concentration</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>ICH</td>
<td>International council for harmonization</td>
</tr>
<tr>
<td>IVIVE</td>
<td>in vitro to in vivo extrapolation</td>
</tr>
<tr>
<td>$K_i$</td>
<td>Inhibitory constant</td>
</tr>
<tr>
<td>$K_m$</td>
<td>Michaelis constant</td>
</tr>
<tr>
<td>LC-MS/MS</td>
<td>Liquid chromatography tandem mass spectroscopy</td>
</tr>
<tr>
<td>MAT</td>
<td>Mean absorption time</td>
</tr>
<tr>
<td>MATE1</td>
<td>multidrug and toxic compound extrusion 1</td>
</tr>
<tr>
<td>MATE2-K</td>
<td>multidrug and toxic compound extrusion 2-K</td>
</tr>
<tr>
<td>MBRT</td>
<td>Mean body residence time</td>
</tr>
<tr>
<td>MDCKII</td>
<td>Madin-Darby canine kidney II cells</td>
</tr>
<tr>
<td>MDRT</td>
<td>Mean disposition residence time</td>
</tr>
<tr>
<td>MRP2</td>
<td>Multidrug-resistance protein 2</td>
</tr>
<tr>
<td>NCE</td>
<td>New chemical entity</td>
</tr>
<tr>
<td>OATP1A2</td>
<td>Organic anion transporting polypeptide 1A2</td>
</tr>
<tr>
<td>OATP1B1</td>
<td>Organic anion transporting polypeptide 1B1</td>
</tr>
<tr>
<td>OCT1</td>
<td>Organic cation transporter 1</td>
</tr>
<tr>
<td>OCT2</td>
<td>Organic cation transporter 2</td>
</tr>
<tr>
<td>PAMPA</td>
<td>Parallel artificial membrane permeability assay</td>
</tr>
<tr>
<td>$P_e$</td>
<td>Effective permeability</td>
</tr>
<tr>
<td>P-gp</td>
<td>Permeability-glycoprotein (P-glycoprotein)</td>
</tr>
<tr>
<td>PMAT</td>
<td>Plasma membrane monoamine transporter</td>
</tr>
<tr>
<td>PPB</td>
<td>Plasma protein binding</td>
</tr>
<tr>
<td>PTC</td>
<td>Proximal tubular cells</td>
</tr>
<tr>
<td>SERT</td>
<td>Serotonin reuptake transporters</td>
</tr>
<tr>
<td>SLC</td>
<td>Solute Carrier</td>
</tr>
<tr>
<td>$T_{\frac{1}{2},z}$</td>
<td>Terminal half-life</td>
</tr>
<tr>
<td>TC</td>
<td>Trospium chloride</td>
</tr>
<tr>
<td>TC-IV</td>
<td>Intravenous trospium chloride</td>
</tr>
<tr>
<td>TC-PO</td>
<td>Oral trospium chloride tablet</td>
</tr>
<tr>
<td>$T_{\text{max}}$</td>
<td>Time to reach maximum plasma concentration</td>
</tr>
<tr>
<td>$V_c$</td>
<td>Volume of the central compartment</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td>$V_{\text{max}}$</td>
<td>Maximum rate of uptake</td>
</tr>
<tr>
<td>$V_{p1}$</td>
<td>Volume of the shallow peripheral compartment</td>
</tr>
<tr>
<td>$V_{p2}$</td>
<td>Volume of the deep peripheral compartment</td>
</tr>
<tr>
<td>$V_{SS}$</td>
<td>Volume of distribution at equilibrium</td>
</tr>
</tbody>
</table>
10. Publications


My own contribution:

Collaborated on planning and conduction of the clinical study titled:

“Pharmacokinetic interaction between trospium chloride after intravenous (2 mg) and oral administration (30 mg) with ranitidine (300 mg p.o.) as an inhibitor of OCT1 and with clarithromycin (500 mg p.o.) as an inhibitor of P-glycoprotein in 32 healthy subjects genotyped for OCT1”

- Materials and methods
  - Creation of the study documents
  - Statistical planning of the study subjects
  - Organizing the conduction of the clinical study
  - Performed pharmacodynamic measurements under physician guidance
  - Sampling (urine and stool) and preparation of biomaterial and bio-banking (plasma, urine and stool)
  - Quantification of clarithromycin according to GLP criteria

- Results
  - Performed pharmacokinetic and biometric evaluation of the study data

- Preparation of the study reports
- Conducted the *in vitro* study
- wrote manuscript

Michael Weiss

- Performed pharmacokinetic modeling and wrote manuscript
Christiane Modess

- Designed and performed the clinical study.

Tarek Roustom

- Performed and analyzed the study

Danilo Wegner

- Designed the study and analyzed data.

Ulrich Schwantes

- Designed the study, contributed analytical tools and wrote manuscript.

Hans-Ulrich Schulz

- Contributed analytical tools.

Claudia Neumeister

- Performed the study.

Werner Siegmund

- Designed, supervised and performed the study, analyzed data and wrote manuscript.

_______________________  _______________________
Prof. Dr. Werner Siegmund  Bayew Tsega Abebe
Effects of the P-Glycoprotein Inhibitor Clarithromycin on the Pharmacokinetics of Intravenous and Oral Tropium Chloride: A 4-Way Crossover Drug-Drug Interaction Study in Healthy Subjects

Bayew Tsega Abebe, MSc¹, Michael Weiss, PhD², Christiane Modes, MD¹, Tarek Roustom, MD¹, Tobias Tadken, MD¹, Daniol Wegner¹, Ulrich Schwantes, PhD³, Claudia Neumeister, PhD³, Hans-Ulrich Schulz, PhD¹, Eberhard Scheuch, PhD¹, and Werner Siegmund, MD¹

Abstract

The quaternary ammonium compound tropium chloride is poorly absorbed from 2 "absorption windows" in the jejunum and cecum/ascending colon, respectively. To confirm whether intestinal P-glycoprotein (P-gp) is involved, a 4-period, crossover drug interaction study with tropium chlorides after intravenous (2 mg) and oral administration (39 mg) without and after coadministration of clarithromycin (500 mg), an inhibitor for P-gp, was initiated in 11 healthy subjects. Pharmacokinetics of tropium was evaluated using gas chromatography-mass spectrometry, noncompartmental evaluation, and pharmacokinetic modeling. Tropium chloride was poorly absorbed after oral administration. Subcutaneous bioavailability was 11% (P<0.01). About 30% of the bioavailable dose fraction was absorbed from the "narrow window." Coadministration with clarithromycin increased steady-state distribution volumes by ~27% (P<0.01). Bioavailability was not increased as hypothesized. The geometric mean ratio (90% confidence interval) at area under the plasma concentration-time curve, maximum concentration, and renal clearance accounted for 0.75 (0.56-1.01), 0.84 (0.45-0.89), and 1.00 (0.90-1.13), respectively. The amount of tropium absorbed from the "narrow window" was reduced in all subjects but from the "wider window" in only 9 of them. Bioavailability was strongly predicted by the maximum absorption rate of tropium in the "cisal window" (r² = 0.913, P < .0001). In conclusion, the P-gp inhibitor clarithromycin significantly increases distribution volumes but not oral absorption of tropium. The amount absorbed from the "narrow window" was lowered in all subjects. However, the extent of all influences seems not to be of clinical relevance.

Keywords

clarithromycin, drug interaction, P-glycoprotein, pharmacokinetics, tropium chloride

Tropium chloride is an efficient and safe muscarinic receptor-blocking agent for the treatment of patients with overactive bladder syndrome with evidence level A as confirmed by controlled, randomized clinical trials. The drug is superior to other approved drugs for overactive bladder syndrome because it does not penetrate the blood-brain barrier, and therefore the much-feared cognitive impairment, in the mostly elderly patients who are potentially at risk of dementia, is absent. However, tropium chloride also seems to be a good probe drug for clinical pharmacologists to evaluate the function of multidrug transport proteins in humans by using mechanistic clinical-pharmacokinetic studies. From a pharmacokinetic perspective, tropium chloride is a drug with somewhat chimeric pharmacokinetic properties. In line with the quaternary chemical structure of the ammonium compound, which has high water solubility and hence minor nonionic permeability via physiologic lipid membranes (Biopharmaceutics Classification System class III), tropium is poorly absorbed from the gastrointestinal tract (F ~8%-10%), negligibly bound to plasma proteins, and practically nonmetabolized. Despite its polarity, tropium accumulates in parenchymatous organs with the exception

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of the brain (steady-state distribution volume $V_{ss}$, ~7 L/kg), undergoes glomerular filtration and largely full tubular secretion by the kidneys (renal clearance $CL_R$, ~540 mL/min), and is substantially excreted via feces ($C_{FD}$, ~100 mL/min). There is convincing experimental evidence from in vitro studies using transfected human embryonal kidney 293 (HEK) cell models that trospium is a substrate for the organic cation transporters 1 (OCT1) and 2, which are highly abundant in the liver and kidneys, respectively, organs into which trospium preferentially penetrates. Trospium also has affinity to P-glycoprotein (P-gp), an efflux carrier in the apical membrane of capillaries that protects organs that have a tight endothelial barrier, such as the brain. Therefore, trospium clearly does not pass the blood-brain barrier despite its affinity to the organic anion-transporting polypeptide 1A2 (OATP1A2), a unique uptake carrier in the brain.

In the human gut, P-gp serves as an efflux carrier that can exert a barrier function and thereby prevent the unrestricted absorption of drugs. This might be a major reason for the poor bioavailability of the drug. The intestinal uptake mechanism for trospium is still unknown, as OATP1A2 is not expressed in enterocytes, and localization and function of OCT1 as a candidate for the intestinal uptake of trospium is still a subject of controversial discussion. However, we assume in accordance with Han et al., that intestinal OCT1 is localized in the apical layer of the intestinal epithelium and serves as an uptake transporter for the cationic drug. Trospium has higher affinity to P-gp than to OCT1, whereby OCT1 provides higher transport capacity. The intestinal permeability, although poor, appears to be the net amount from uptake by OCT1, minus that caused by efflux via P-gp. As the protein abundance of OCT1 is fairly uniform along the intestinal tract, whereas P-gp is less abundant in the duodenum/upper jejunum and in the colon compared to the ileum, these appear to be sites with differing permeability. In line with this observation, we identified a "narrow absorption window" during the small intestinal transit, and a "wider window" in the ileum/proximal ascending colon following administration of immediate release (IR) trospium chloride in a mechanistic pharmacokinetic study in healthy subjects.

To confirm the functional role of intestinal P-gp in oral absorption of trospium chloride, we designed a drug-drug interaction (DDI) study with clarithromycin, a probe inhibitor for P-gp. We selected this macrolide antibiotic because it is ethically justified to be used as an experimental tool in healthy subjects and due to its high affinity to P-gp (half maximal inhibitory concentration $IC_{50} = 8.9 \pm 0.5 \mu M$) relative to the concentrations of clarithromycin in gut lumen, which are to be expected after taking the standard dose of 500 mg with 240 mL of tap water ($I_{2} = 2.79 \mathrm{mM}$; $I_{2}/C_{50} > 10$) without influencing intestinal OCT1. We hypothesized that concomitant use with clarithromycin decreases fecal clearance of trospium following intravenous administration, and its bioavailability after oral dosing. Systemic effects, such as an increase in distribution volume or a lowering of the renal clearance, were not expected, due to the fact that trospium is taken up in large organs by uptake transporters for which clarithromycin is not an inhibitor, for example, OATP1A2 in the brain or OCT1 in the liver and kidneys, and because of the expected low plasma concentrations of clarithromycin (maximum concentration $C_{max}$ during chronic treatment, ~4–8 nM) relative to its inhibitory potency P-gp ($IC_{50} = 8.9 \pm 0.5 \mu M$).

**Methods**

The clinical study in healthy subjects was performed according to the International Council for Harmonisation guidelines for Good Clinical Practice and the regulations of the German Medicines Act after being approved by the Independent Ethics Committee of the University Medicine of Greifswald and by the German Federal Department of Drugs and Medicinal Products, and after registration by eudraCT (identifier: EudraCT 2016-002882-69) and ClinicalTrials.gov (identifier: NCT03011463). All healthy subjects were enrolled after providing written informed consent. The DDI study was performed in the clinical study unit of the Department of Clinical Pharmacology, Center of Drug Absorption and Transport (CDAT) of the University of Greifswald, Greifswald, Germany.

**Subjects**

The study was performed in 12 healthy German white subjects (9 men, 3 women, aged 25 to 44 years; body mass index, 19.6–27.7 kg/m$^2$). The subjects were enrolled after confirmation of good health by documenting their medical history, performing a physical examination, and conducting routine clinical-chemical and hematologic screenings. All subjects had negative results at the time of screening for drugs, HIV, hepatitis B virus, and hepatitis C virus. Three subjects were smokers (<=10 cigarettes/day) and 9 subjects occasionally consumed alcohol. None was on a special diet (e.g., vegetarian). The subjects did not take any medication. The 3 female subjects used safe, nonhormonal methods for birth control. None had a positive pregnancy test 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 medication.

Study Protocol
Pharmacokinetics of tropium chloride and clarithromycin, and anticholinergic effects 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 (IV) infusion of 2.0 mg tropium chloride (Spasmex iv 2.0 mg, Injection solution, Pfeifer, Barnberg, Germany) in 20 mL of saline within 60 minutes and consumption of 240 mL of tap water; (2) oral administration of a 30-mg IR tropium chloride tablet (Spasmex 30 mg tropium chloride; Filmtabletten, Pfeifer, Barnberg, Germany) with 240 mL of tap water; (3) IV infusion of 2.0 mg of tropium chloride in 20 mL of saline within 60 minutes together with 500 mg of clarithromycin (Clarithromycin-ratiopharm 500 mg clarithromycin; Filmtabletten, ratiopharm, Ulm, Germany) swallowed with 240 mL of tap water; (4) 30 mg of IR tropium chloride together with 500 mg clarithromycin swallowed with 240 mL tap water.

Correctness of oral administration was controlled by checking the mouths of the subjects. The clinical investigators ensured that the subjects rested thereafter in their beds with the upper part of the body raised about 30 degrees for 6 hours until time for the standard lunch.

Intake of food and additional fluids was standardized during the in-house stay. The overnight fasting period before medications lasted 10 hours. Lunch, afternoon snack, and dinner were eaten 6, 9, and 12 hours after administration of the respective study medication. The subjects consumed the same individual amount of food in all study periods. Venous blood was collected via an indwelling forearm cannula before and 0.33, 0.66, 1.33, 1.66, 2.3, 4, 5, 6, 8, 10, 12, 16, 24, 30, and 36 hours after IV infusion, and before and 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 11, 12, 16, 24, 30, and 36 hours after oral administration of tropium chloride. Plasma was stored for quantitative analysis at ≤−20°C within 2 hours after sampling. Urine and feces were sampled at daily intervals for 5 days, and respective aliquots were stored at ≤−20°C until the quantitative analysis.

Stimulated salivation was measured by chewing a 5 × 5 cm piece of PARAFILM "M" (American Can Company, Bedfordshire, UK) for 5 minutes and weighing the secretion before and 3, 5, and 11 hours after administration. Accommodation of the eyes was measured before and 3, 5, and 11 hours after administration using an optometer according to Schober.

Quantitative Drug Assays
All quantitative assays were performed under the conditions of Good Laboratory Practice. Tropium was quantified in plasma, urine, and feces using gas chromatography–mass spectrometry (5890 Series II Plus with mass selective detector HP 5971; Hewlett Packard, Palo Alto, California) with selected ion monitoring mode (Agilent, Waldbronn, Germany). The analyte was separated after ion-pair extraction with perchlorate, reextraction with tetraethyl ammonium as a counter ion, alkaline hydrolysis to benzaldehyde, and subsequent derivatization using diazomethane to generate the methyl ester. Tropium and the internal standard oxyphephonium were chromatographically separated using an HP-1 column (25 m × 0.2 mm, 0.33 μm; Agilent, Waldbronn, Germany) with helium as the carrier gas and a temperature program (90–280°C in 12 minutes). The mass spectrometer operated in SIM mode m/z 189.1 for tropium and m/z 183.1 for oxyphephonium. The chromatograms were evaluated by means of peak-area ratios using the internal standard method (Hewlett Packard GC-MS Station G1791 BA; Agilent, Waldbronn, Germany). The calibration curves were fitted using a regression model (1/x weighting for plasma, 1/x² for urine and feces, x = concentration) for plasma between 0.03 and 5.0 ng/mL, and for urine and feces between 10.0 and 1000 ng/mL. The quantitative assays were performed under the conditions of Good Laboratory Practice. Accuracy ranged from −1.1 to 1.9% for plasma, −2.0 to 4.0% for feces, and 0.5 to 2.7% for urine of the respective nominal values. Precision varied between 3.7 and 5.5% (plasma), 2.8 and 7.2% (urine), and between 2.5 and 6.5% (feces) of the respective means.

Clarithromycin was assayed in plasma, urine, and feces using the Agilent 1100 series HPLC system (Agilent Technologies, Waldbronn, Germany) and a Perkin Elmer Series 200 Autosampler (Perkin Elmer, Darmstadt, Germany) coupled to the API2000 Turboln Spray mass spectrometer (AB Sciex, Darmstadt, Germany) with fexofenadine (Sigma-Aldrich, Munich, Germany) as an internal standard. The samples were centrifuged after desaturation of proteins using acetonitrile/water (50:50, v/v) (Carl Roth, Karlsruhe, Germany) and 10 μL of the supernatant were injected into the chromatographic system (0.5 μm Supelco pre-column filter direct-connect device and Supelco Ascentis C18 column, 3 μm, 2.1 × 100 mm, Sigma-Aldrich, Munich, Germany). The chromatography was performed with isocratic elution using ammonium acetate buffer (5 mM; pH 3.0)/acetonitrile (40:60, v/v) as the mobile phase and 200 μL/min flow rate. The electro spray ionization Turboln interface of the mass spectrometer operated in the positive ion mode to monitor the following m/z transitions: clarithromycin
748.5 → 590.6 and 748.5 → 158.2; fexofenadine
502.2 → 466.2 and 502.2 → 484.4. The following gas
parameters were used: 400ºC; gas 1, 60 psi; gas 2, 60 psi; voltage, 3000 V; collision-activated dissociation
40 psi (all nitrogen). The chromatograms were
evaluated online with the internal standard method
using peak-area ratios for calculation (software Analyst
1.4.2, AB Sciex, Dassault, 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
4.2% to 4.8% of the respective nominal values for
plasma, from -4.4% to 2.9% for urine and between 2.3%
to 6.9% for feces. Precision varied between 7.5% and
10.4% for plasma, between 6.8% and 13.7% for urine,
and between 5.2% and 13.7% for feces of the respective
means.

Pharmacokinetic Evaluation

Noncompartmental Evaluation. The pharmacokinetics
of t9spongium chloride and clarithromycin was primarily
evaluated by a noncompartmental approach. Cmax and
the time of Cmax (tmax) were read from the plasma
calculation-time curves (AUC). The AUC was
assessed up to the last sampling time above the limit of
corticosterone (AUC0-tmax) using the trapezoidal
method and extrapolated to infinity (AUC). CLR and
CLRinc were derived from the cumulative amount excreted
into urine and feces, respectively, within 5 days
divided by AUC.

Pharmacokinetic Modeling. In a post hoc analysis
to the clinical study report, pharmacokinetics of
t9spongium chloride was additionally evaluated by
population pharmacokinetic modeling to obtain a
more in-depth insight into the oral absorption and
distribution processes. Based on a 3-compartment
model for t9spongium disposition after intravenous
administration, the drug amounts in the central (xC)
and peripheral compartments (xp1 and xp2) could be
described using the following differential equations:

$$\frac{dx}{dt} = I(t) - (CL + CL_{d1} + CL_{d2})x_c/V_c$$

$$+ CL_{d1}xp1/V_{p1} + CL_{d2}xp2/V_{p2}$$

$$dx_{p1}/dt = CL_{d1}xp1/V_{p1} - CL_{d1}xp1/V_{p1}$$

$$dx_{p2}/dt = CL_{d2}xp2/V_{p2} - CL_{d2}xp2/V_{p2}$$

(1)

(2)

(3)

with the parameters clearance (CL), distribution
clearances (CLd1, CLd2) and volumes of the central (Vc) and
peripheral compartments (Vp1, Vp2). It should be noted
that I(t) represents the infusion rate for data following
intravenous administration, and the absorption rate
AR (t) for data after oral dosing (see below). The
steady-state distribution volume is given by Vss = Ve +
Vp1 + Vp2 and the mean disposition residence time
(MDRT) is calculated as

$$MT = \sqrt{\frac{MT}{2\pi RD^2}} \exp \left(\frac{(t - MT)^2}{2RD^2MT}\right)$$

(4)

MT and RD2 denote the mean and the relative
dispersion (normalized variance) of absorption time,
respectively. Since the single IG did not fit the data
sufficiently well, a sum of 2 IGs was finally used:

$$f(t) = \int p f_1(t) + (1 - p) f_2(t), \quad 0 < p < 1$$

(5)

The functions f0(t) are given in Equation (4), and p is
a mixing parameter. The absorption rate is then given by

$$AR(t) = DF \int f(t)$$

(6)

F denotes the bioavailability of the orally adminis-
tered dose D. The mean absorption time then becomes

$$MAT = pMT_1 + (1 - p)MT_2$$

(7)

Maximum likelihood expectation maximization
population module of ADAPT 5 was used for data
analysis.11 The maximum likelihood expectation maxi-
mization program provides estimates of the population
mean and intersubject variability as well as of the
individual subject parameters (conditional means).
Random effects associated with pharmacokinetic
c parameters were assumed to have a log-normal
distribution around a population mean parameter value.
A normally distributed residual error model was adopted
whereby measurement error has a standard deviation
that is a linear function of the measured quantity:

$$VAR_i = [\sigma_0 + \sigma_1 t_i]^2$$

(8)

After fitting the IV data, the individual estimates of
disposition parameters CL, CLd1, CLd2, Vc, Vp1, and
Vp2 were held fixed in fitting the oral data. The model
fits to the data were assessed by the goodness-of-fit plot,
Table 1. Pharmacokinetic Characteristics of Trospium Chloride After Intravenous and Oral Administration Before and After Concomitant Use of Clarithromycin by Population Pharmacokinetic Modelling

<table>
<thead>
<tr>
<th>Pharmacokinetic Characteristics</th>
<th>Control</th>
<th>Clarithromycin Concomitant Use</th>
<th>Clarithromycin Concomitant Use and Dose Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV Infusion (2 mg/50 min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL (mL/min)</td>
<td>892</td>
<td>680</td>
<td></td>
</tr>
<tr>
<td>V (L)</td>
<td>13.1</td>
<td>11.5</td>
<td></td>
</tr>
<tr>
<td>V (L)</td>
<td>35.9</td>
<td>47.5</td>
<td></td>
</tr>
<tr>
<td>V (L)</td>
<td>20.1</td>
<td>29.0</td>
<td></td>
</tr>
<tr>
<td>V (L)</td>
<td>350.0</td>
<td>444.0</td>
<td></td>
</tr>
<tr>
<td>CL (mL/min)</td>
<td>443.7</td>
<td>533</td>
<td></td>
</tr>
<tr>
<td>CL (mL/min)</td>
<td>420.3</td>
<td>579</td>
<td></td>
</tr>
<tr>
<td>MDRT (h)</td>
<td>6.34</td>
<td>8.12</td>
<td></td>
</tr>
<tr>
<td>T1/2 (h)</td>
<td>11.3</td>
<td>14.8</td>
<td></td>
</tr>
<tr>
<td>s</td>
<td>0.12</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td>Oral Administration (30 mg IR tablet)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PMT (h)</td>
<td>10.0</td>
<td>10.6</td>
<td></td>
</tr>
<tr>
<td>ARmax (mg/h)</td>
<td>4.8</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>F (%)</td>
<td>53.4</td>
<td>74.5</td>
<td></td>
</tr>
<tr>
<td>s</td>
<td>0.11</td>
<td>0.22</td>
<td></td>
</tr>
<tr>
<td>s</td>
<td>0.37</td>
<td>0.13</td>
<td></td>
</tr>
</tbody>
</table>

ARmax, maximum absorption rate; CLint, intercompartmental clearance; IM, bioavailability; IM, immediate release; PMT, mean absorption time; MDRT, mean residence time; s, residual variability (variance of the measurement error); IM = (1 + s) × CLint/MDRT; T1/2 terminal half-life; V, volume of central compartment; V, volume of shallow and deep compartments; V, distribution volume at steady state.

Arithmetic means with intersubject variability and significance levels (P < .05, *P < .01) for Student’s paired t-test are given.

standardized residuals vs predicted concentration, and individual fits. Model discrimination was carried out using Akaike’s information criterion.

The predictive properties of the model were evaluated by performing visual predictive check after 1000 Monte Carlo simulations using ADAPT 5. Median and 95% confidence intervals for simulated data were plotted. The model fitted the concentration-time data reasonably well as demonstrated by the goodness-of-fit, examples of individual fits, and visual predictive check (see Figures S1-S8).

Statistical Evaluation
The results shown in Table 1 were obtained by population pharmacokinetic analysis, with the exception of maximum absorption rate (ARmax), which was taken from the simulated absorption rate curves using individual parameters (conditional means). Individual pharmacokinetic parameters were reported as arithmetic means ± standard deviations. The Student’s t-test for paired samples was used for statistical comparison of group means, with the level of statistical significance being at least P < .05. Correlations between samples were evaluated using the Spearman test.

The objective of the study was entirely descriptive. However, a sample size of N = 12 for comparisons of the AUC0-∞ for trospium after oral dosing with or without clarithromycin, vs. oral dosing with clarithromycin, was sufficient to confirm differences of ≥38% with P < .05 and statistical power >80% (nQuery Advisor 7.0, Statistical Solutions, Cork, Ireland). In a post hoc statistical analysis to the study protocol, an analysis of variance for all pharmacokinetic characteristics of the noncompartmental evaluation (except for t1/2) was performed and geometric mean ratios (GMRs) with 90% confidence intervals (90% CIs) were assessed. All noncompartmental pharmacokinetic and statistical evaluations were performed using the SAS statistical package (SAS 9.4 TS Level 1M3, SAS Institute Inc., Cary, North Carolina).

Results
Pharmacokinetics
After IV infusion of 2 mg of trospium chloride, mean plasma concentrations of ~20 ng/mL were reached, which was about 4 times the concentration after administration of a 30-mg IR tablet. The volume of the central compartment (Vc; ~0.18 L/kg) approximately resembled the volume of the extracellular space of our subjects. From here, trospium was widely distributed (Vd = 48.1 L/kg) into a shallow peripheral compartment (Vp1; ~0.5 L/kg), which corresponds to the intracellular water space, and into a deeper compartment (Vp2; ~4.13 L/kg), which corresponds to the known accumulation in parenchymatous organs (liver, kidneys). The distribution clearance into both compartments was high and practically identical in value. Trospium disposition was characterized by a mean disposition residence time of ~6.5 hours and a terminal half-life (T1/2) of ~11 hours. The major elimination routes were glomerular filtration and substantial tubular secretion in the kidneys (ClR; ~500 mL/min), and additionally a minor fecal secretion (CLisal; ~60 mL/min). There was substantial unspecified residual clearance for trospium because the total body clearance accounted for ~900 mL/min. About 60% of the dose was eliminated into the urine and about 2% via feces within 36 hours (Tables 1 and 2, Figure 1).

Trospium chloride was slowly and poorly absorbed along the intestine with t1/2 after ~6.5 hours, and bioavailability of ~10%, after oral administration of a 30-mg IR tablet. 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, which is in line with increasing absorption along the small intestine, and with maximum absorption in the ceacum/proximal colon. About 30% of the absorbed dose was bioavailable within 2 to 4 hours following
administration (Figure 2). The MDRT of trospium after oral dosing was ～6.5 hours and the T_{1/2} ～9 hours (Table 1).

The major finding following concomitant administration of intravenously infused trospium and orally administered clarithromycin was an elevation in the peripheral distribution volumes V_{Pl} and V_{D2} of trospium, which led to a slight but significant increase in V_{AUC} of ～27%. All clearances of trospium were not significantly influenced. MDRT and T_{1/2} significantly increased after concomitant administration of clarithromycin by ～25% to 30%, which was clearly a result of the increase of V_{AUC}. After oral concomitant administration of clarithromycin in our group of 12 healthy subjects, A_{max} of IR trospium chloride were significantly lowered. Plasma exposure, bioavailability, and amount of trospium excreted into urine were not significantly influenced by comodulation with clarithromycin. In 9 subjects, however, AUC, bioavailability, and cumulative urinary excretion were lowered in the presence of clarithromycin (Figure 3). The typical biphasic time courses for absorption rate and for the cumulative amount absorbed generally lay below the respective control curves in the absence of clarithromycin. Due to clarithromycin, IR trospium chloride was more poorly absorbed within the first ～3 hours as compared to the control without the comodulation (Figure 2). Bioavailability was inversely correlated to mean absorption time (r^2 = 0.645, P < .0001), and directly correlated to the A_{max} (r^2 = 0.910, P < .0001, Figure 4). In the post hoc equivalence analysis was found that the GMRs (90% CI) for clarithromycin over trospium alone of the AUC, C_{max}, and CIR after oral dosing were 0.75 (0.56–1.01), 0.64 (0.45–0.89) and 1.00 (0.90–1.13), respectively.

In our DDII study with trospium chloride, we cannot provide unbiased pharmacokinetic characteristics for clarithromycin because a placebo control group with clarithromycin alone was not planned. However, clarithromycin after intravenous comodulation with 2 mg of trospium chloride was absorbed with a markedly lower rate than following oral comodulation of the 30-mg IR dosage form. This was indicated by significantly longer t_{1/2} values and a tendency for lower C_{max} concentrations. The median residence time was significantly prolonged. The GMR (90% CI) (trospium per os over infusion) for AUC, C_{max}, and CIR were 1.10 (0.98–1.23), 1.17 (0.86–1.61), and 1.01 (0.89–1.14), respectively (Table 3, Figure 5).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Control</th>
<th>Clarithromycin</th>
<th>GMR (90% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC mg/L</td>
<td>36.6 ± 5.0</td>
<td>35.7 ± 2.4</td>
<td>0.96 (0.90–1.04)</td>
</tr>
<tr>
<td>C_{max} mg/L</td>
<td>21.8 ± 3.2</td>
<td>20.6 ± 2.4</td>
<td>0.95 (0.89–1.02)</td>
</tr>
<tr>
<td>t_{max} h</td>
<td>10.9 ± 2.2</td>
<td>12.6 ± 2.4</td>
<td>1.12 (0.99–1.28)</td>
</tr>
<tr>
<td>CIR mL/min</td>
<td>325 ± 73.6</td>
<td>371 ± 63.4</td>
<td>1.10 (1.02–1.18)</td>
</tr>
<tr>
<td>C_{max} mL/min</td>
<td>579 ± 98.5</td>
<td>521 ± 66.1</td>
<td>0.90 (0.76–1.06)</td>
</tr>
<tr>
<td>A_{max} ng</td>
<td>4.3 ± 0.2</td>
<td>3.9 ± 0.1</td>
<td>1.07 (1.01–1.14)</td>
</tr>
<tr>
<td>A_{max} ng</td>
<td>0.1 ± 0.1</td>
<td>0.3 ± 0.6</td>
<td>1.26 (0.65–2.40)</td>
</tr>
</tbody>
</table>

A_{max} cumulative amount excreted into urine, A_{max} cumulative amount excreted into urine, AUC area under the plasma concentration-time curve, Q confidence interval, CIR clearance, C_{max} maximum plasma concentration, IR immediate release, n.d. not determined, t_{1/2} terminal half-life, t_{max} time to maximum plasma concentration. Arithmetic means ± SD and geometric mean ratios with 90% confidence intervals are given.
inhibitor of P-gp—would lead to a higher bioavailability of troposum. Systemic effects due to P-gp inhibition, such as increase of distribution volumes or lowering of the CLint, were not expected.

Surprisingly, we observed no significant effects of clarithromycin on plasma exposure (AUC), Cmax, or bioavailability of troposum. The 90%CI of the GMRs for AUC (0.56–1.01) and Cmax (0.45–0.89) even exceeded the lower limit of the equivalence range that is accepted by regulatory bodies (US Food and Drug Administration, European Medicines Agency) for drugs with highly variable pharmacokinetics (0.75–1.33). Because the GMR span for the CLint (0.90–1.13), the major route of troposum elimination, did not exceed the standard range of 0.80 to 1.25, the failure of the equivalence limits for AUC and Cmax is most likely to be attributed to lower absorption rates of the drug in the small intestinal (narrow absorption window) as observed in all subjects. However, the influence on absorption does not appear to be practically relevant for the clinical use of troposum chloride, as the changes were smaller than the known circadian time differences and the influence of eating a fat-rich breakfast.14

To understand the outcome of our DDI study, the influence of clarithromycin on the transport processes for troposum in the small and large intestine and the known potential motilin receptor-blocking, prokinetic effects of the macrolide antibiotic must be considered as major confounders.15-22

First, the prokinetic effect of clarithromycin obviously leads to faster gastric emptying of the possibly not fully disintegrated and dissolved dosage form (mean dissolution time in vitro, 10.7 minutes; own data), and a faster passage along the small intestine, hence, the narrow absorption window for troposum. Up to 30% of the absorptive capacity for troposum in the small intestine seemed to be more or less bypassed in all subjects after clarithromycin comedication. The wider absorption window is not influenced, as 14-membered macrolides are unable to enhance colonic motility.18 Interestingly, the absorption after comedication of clarithromycin was quite similar to the absorption of Eudragit enteric coated troposum chloride, which does not disintegrate upon reaching the distal jejunum ileum.23,24 The enteric coated tablets also generated maximum plasma concentrations after being absorbed from the wider window following a lag time of ~2 to 3 hours. Bioavailability was ~20% lower.25 Second, the major prokinetic effect of clarithromycin is most likely not or only slightly overshadowed by inhibition of intestinal P-gp. We have recently shown that troposum is a likewise high-affinity substrate for P-gp (ABCB1 HEK-293 liposomes, \( \text{I}_{50} = 34.9 \pm 7.5 \, \mu \text{M} \)). Taking a therapeutic IR troposum chloride dose (30 mg) with 240 mL water yields a so called \( f_2 \)-concentration of ~0.3 mM.26

![Graph 1](image1.png)

**Figure 2.** Absorption rate time (above) and the absorbed amount (below) of troposum after oral administration of a 30-mg IR tablet alone (solid line) and after comedication of 500 mg of clarithromycin (dashed line).

**Safety and Pharmacodynamics Effects**

Single-dose IV and oral administration of the troposum chloride test dose was safe and well tolerated by our healthy subjects. The most frequent adverse events were expected, such as dry mouth, tachycardia, and urinary retention. There were no evident differences in tolerability between mono-use or during concomitant administration with clarithromycin. Treatment with troposum chloride caused the expected pharmacodynamic changes in salivation after IV and oral administration. Accommodation of the eyes was not markedly influenced. Comedication of clarithromycin tended to minimize the changes in salivation (3 hours after IV administration; \( P = 0.067 \)). Conclusive results on pharmacodynamic interactions cannot be drawn because a placebo control was not planned in the DDI study.

**Discussion**

**Changes in Pharmacokinetics of Troposum**

According to the working hypothesis of the DDI study, we expected that comedication of troposum chloride with clarithromycin—that is, a substrate as well as an
inhibitor of P-gp—would lead to a higher bioavailability of trospium. Systemic effects due to P-gp inhibition, such as increase of distribution volumes or lowering of the CLR, were not expected.

Surprisingly, we observed no significant effects of clarithromycin on plasma exposure (AUC), Cmax, or bioavailability of trospium. The 90% CIs of the GMRs for AUC (0.56–1.01) and Cmax (0.45–0.89) even exceeded the lower limit of the equivalence range that is accepted by regulatory bodies (US Food and Drug Administration, European Medicines Agency) for drugs with highly variable pharmacokinetics (0.75–1.33). Because the GMR span for the CLR (0.90–1.13), the major route of trospium elimination, did not exceed the standard range of 0.80 to 1.25, the finding of the equivalence limits for AUC and Cmax is most likely to be attributed to lower absorption rates of the drug in the small intestinal (narrow absorption window) as observed in all subjects. However, the influence on absorption does not appear to be practically relevant for the clinical use of trospium chloride, as the changes were smaller than both the known circadian time differences and the influence of eating a fat-rich breakfast.14

To understand the outcome of our DDI study, the influence of clarithromycin on the transport processes for trospium in the small and large intestine and the known powerful motilin receptor-blocking, prokinetic effects of the macrolide antibiotic must be considered as major confounders.19–22

First, the prokinetic effect of clarithromycin obviously leads to faster gastric emptying of the possibly not fully disintegrated and dissolved dosage form (mean dissolution time in vitro, 10.7 minutes; own data), and a faster passage along the small intestine, hence, the narrow absorption window for trospium. Up to 30% of the absorptive capacity for trospium in the small intestine seemed to be more or less bypassed in all subjects after clarithromycin comedication. The wider absorption window is not influenced, as 14-membered macrolides are unable to enhance colonic motility.19 Interestingly, the absorption after comedication of clarithromycin was quite similar to the absorption of Eudragit enteric coated trospium chloride, which does not disintegrate upon reaching the distal jejunum/ileum.23,24 The enteric coated tablets also generated maximum plasma concentrations after being absorbed from the wider window following a lag time of ~2 to 3 hours. Bioavailability was ~20% lower.25 Second, the major prokinetic effect of clarithromycin is most likely not or only slightly overshadowed by inhibition of intestinal P-gp. We have recently shown that trospium is a likewise high-affinity substrate for P-gp (ABCA1 HEP-293 liposomes, Km = 34.9 ± 7.5 μM). Taking a therapeutic IR trospium chloride dose (30 mg) with 240 mL water yields a so called \([L_2]\)-concentration of ~0.3 mM, 26

![Figure 2. Absorption rate time (above) and the absorbed amount (below) of trospium after oral administration of a 30-mg IR tablet alone (solid line) and after comedication of 500 mg of clarithromycin (dashed line).](image-url)
by which P-gp in the small and large intestine must be highly saturated ($f_{u2}/f_{u3} > 8$), as only a small fraction is absorbed ($<10\%$). Rapid water absorption additionally leads to enrichment in the gut lumen.\(^{21,27}\) Clarithromycin has a similar affinity to P-gp (\textit{ABCBI} 
HEK-293 liposomes, IC\textsubscript{50} = 8.9 ± 0.5 \mu M)\(^{13}\), and the $f_{u2}$ concentration accounts for ~2.78 mM ($f_{u2}/f_{u3}$, ~8%). However, this cannot have greatly influenced the small intestinal net absorption of tropism due to the bypassing, prokinetic influence of clarithromycin in the small intestine as discussed above.

Third, the influence of clarithromycin on the absorption of tropism in the cecum/ascending colon (wider absorption window) is most important for the outcome of the study because bioavailability was greatly correlated to the AR\textsubscript{max} ($r^2 = 0.910$, $P < .0001$) that occurred in this region. In our opinion, the competition of the drugs with the complex transport processes in the distal window is poorly predictable for the following reasons: The maximum stool concentrations of tropism ranged between 34.2 and 21.6 \mu M (oral dose, 30 mg) and of clarithromycin between 25.3 \mu M and 692 \mu M (oral dose, 500 mg; fecal recovery of the radiolabeled compound, ~60%),\(^{28}\) as only ~2 mg of the 500-mg clarithromycin dose were recovered in an unchanged form in the feces (0.4%; Table 3). However, we know from several clinical studies in healthy human subjects that the time of defecation, stool volume, and fecal drug concentrations in stool samples are entirely arbitrarily distributed and not predictable by any method. In the study, we also found no plausible correlations between the patterns of drug concentrations in the fecal samples or AR\textsubscript{max} and bioavailability, respectively.

Fourth, lower bioavailability of tropism in the presence of clarithromycin could also be explained by inhibition of an apical uptake carrier in enterocytes, such as OCT\textsubscript{1}, or of a basolateral efflux pump, such as MRP\textsubscript{3}.\(^{5,12}\) MRP\textsubscript{3} inhibition, for example, in the liver, would also be in line with an increase in the distribution.
volume at steady state (see below). However, there is no evidence that clarithromycin is an inhibitor of OCT1 and MRPI.\(^3,11\) Furthermore, the apical localization of OCT1 in enterocytes is still a subject of an ongoing controversial discussion.\(^3,11\)

A second major effect of clarithromycin in our study was the significant expansion of the shallow and deep distribution space for trosipom by \(\sim 27\%\), which was unexpected due to the low plasma concentrations of clarithromycin (\(C_{\text{min}}\), \(0.32 - 7.49\) \(\mu\text{g}/\text{mL}\)) compared to the in vitro \(IC_{50}\) for P-gp (\(C_{\text{min}}/IC_{50}\), 0.03-0.84). Nonetheless, clarithromycin seems to inhibit P-gp in organs with a tight endothelium (brain, testes, ovarium), as well as in organs with fenestrated epithelium (heart, skeletal, and smooth muscles).\(^25,30\) Distribution into the liver and kidney, which are the major deep compartments for trosipom, cannot rationally be affected by clarithromycin, as P-gp is an apical efflux carrier in tubular cells and hepatocytes, respectively. Therefore, neither renal nor intestinal clearance was influenced by clarithromycin.

It is not known whether an increase in the distribution volume for trosipom is of any clinical relevance, for example, for the penetration into the bladder smooth muscle cells and to subsequent availability to muscarinic receptors (M\(_2\), M\(_3\)).\(^1,3\) The \(C_{\text{max}}\) of trosipom was around 8.65 \(\text{nmol/L}\) (range, 3.04-20.8 \(\text{nmol/L}\)); that is, there is no large excess above the concentrations necessary to antagonize M\(_2\) and M\(_3\) receptors (\(C_{\text{K}}\), \(\sim 0.5-0.7\) \(\text{nmol/L}\)), also when taking into consideration that the plasma-protein binding for trosipom is 40\% to 60\%.\(^1,3\) Therefore, the decrease of plasma levels after clarithromycin co-medication might be compensated by facilitated penetration.

Because of P-gp, it is assumed that trosipom does not penetrate into the brain and therefore does not exert clinically relevant anticholinergic (adverse) drug reactions, not even at high doses in elderly patients.\(^2,3,3-36\) However, there are numerous doubts as to whether inhibition of P-gp by clarithromycin is of clinical relevance because trosipom has only low affinity to the uptake transporter OATP1A2 (\(K_{\text{m}}\), 6.9 ± 1.3 \(\mu\text{M}\)) in the blood-brain barrier.

### Changes in Pharmacokinetics of Clarithromycin

The trosipom chloride-clarithromycin DDS study was not designed with the primary intention of investigating the changes in pharmacokinetics of clarithromycin. Because of the nonexistence of an unbiased control for clarithromycin, final conclusions on potential interactions with trosipom chloride cannot be made. However, the pharmacokinetic characteristics after co-medication of a single therapeutic IR trosipom chloride tablet.
Figure 5. Mean (± SD) plasma concentration-time curves of clarithromycin (500 mg per ao) after oral administration of 30 mg of trosopium chloride (open circles) and after intravenous infusion of 2 mg of trosopium chloride (full circles).

are rather similar to data that can be found in the literature. Therefore, orally given trosopium chloride seems not to be clinically relevant for treatment with clarithromycin. On the contrary, an intravenous infusion of 2 mg of trosopium chloride, which results in a similar plasma exposure (AUC) to that of a 30-mg IR tablet, significantly delayed oral clarithromycin absorption by ~4 hours before reaching Cmax. The Cmax and bioavailability, however, were not significantly influenced. An infusion of trosopium chloride leads to the central compartment, which includes extracellular spaces, to active concentrations in excess of those needed to antagonize muscarinic receptors in the gastrointestinal tract. Therefore, clarithromycin reaches the absorption sites in the small intestine later due to a significant delay in gastric emptying. In our opinion, the outcome of the DDI seems not likely to be of any clinical relevance.

Limitations of the Discussion
In our discussion, we reviewed data that were derived from in vitro studies using transporter transfected (HEK) cell models, although we were aware that there are great interlaboratory differences regarding the enzyme-kinetic characteristics (e.g., km, kcat, IC50). Furthermore, we included the complex individual physiological situation in healthy subjects (e.g., gastric emptying, intestinal transit times, luminal water volumes, gastro-ileo-cecal reflux, regional transporter abundance[31,37,40,41]) which is difficult to predict, as our clinical study was only partially designed to measure such physiologic parameters in addition to the major outcome characteristics (e.g., determination of renal and fecal clearance, pharmacokinetic modeling). Therefore, although parts of the discussion remain rather speculative, its content might encourage future mechanistic clinical studies in humans to gain more in-depth insight into this rather complex issue.

Conclusions
Concomitant treatment with single therapeutic doses of clarithromycin and trosopium chloride leads to a significantly wider distribution of trosopium but not to the hypothesized increase in bioavailability. However, evidence was also seen for lower absorption of trosopium in the small intestine. The rationale behind these findings seems to be related to the prokinetic characteristics of clarithromycin and to the inhibition of P-gp in blood-organ barriers. However, the extent of the influence seems not to be of clinical relevance.

IV infusion but not oral administration of trosopium chloride in approximately identical bioavailable doses leads to a significant delay in clarithromycin absorption.

Acknowledgments
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Bad Schwartau, Germany, and Dr. Anna Derr for proofreading the manuscript (Department of Medical Science/Clinical Research, Dr. Pfleger Arzneimittel GmbH, Bamberg, Germany).

Data-Sharing Statement
All data 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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Declaration of Conflicting Interests
Claudia Neunmeister and Ulrich Schwantes are employees of the Dr. Pfleger Arzneimittel GmbH, Bamberg. Hans-Ulrich Schulz is the managing director of the Laboratory for Contract Research in Clinical Pharmacology and Biopharmaceutical Analytics, Bad Schwartau, Germany. All other authors declare no conflict of interest.

References

Supporting Information
Additional supporting information may be found online in the Supporting Information section at the end of the article.
A2 Pharmacokinetic drug-drug interactions between trospium chloride and ranitidine substrates of organic cation transporters in healthy human subjects


My own contribution:

1. Collaborate on planning and conduction of the clinical study:
   - Materials and methods
     - Creation of the study documents
     - Statistical planning of the study subjects
     - Organizing the clinical study
     - Performed pharmacodynamic measurements under physician guidance
     - Sampling (urine and stool) and preparation of biomaterial and bio-banking (plasma, urine and stool)
     - LC-MS/MS measurement of ranitidine according to GLP criteria.
   - Results
     - Performed pharmacokinetic and biometric evaluation of the study data
   - Preparation of the study reports and wrote manuscript

2. Performed the in vitro study in human cell lines
   - Materials and method
     - Planning and design of the study
     - Preparation of transporter wild type and genetic variant transfected cells in cell culture media
     - Preparation of probe substrate and inhibitor drugs
     - Conducted uptake and inhibition assays
     - Protein measurement and normalization
     - Collaborated on the analytical measurement of the study drugs
   - Results
- Evaluation of the study data
  - Wrote manuscript

Michael Weiss
- Performed pharmacokinetic modeling and wrote manuscript

Christiane Modess
- Designed and performed the clinical study.

Tarek Roustom
- Performed the study and analyzed data.

Tobias Tadken
- Performed the study and analyzed data.

Danilo Wegner
- Designed the study and analyzed data.

Ulrich Schwantes
- Designed the study, contributed analytical tools and wrote manuscript.

Claudia Neumeister
- Performed the study and contributed quality assurance.

Mladen Tvettekow
- Designed, supervised and performed the in vitro study, analyzed data and wrote manuscript

Werner Siegmund
• Designed, supervised and performed the clinical study, analyzed data and wrote manuscript.

Prof. Dr. Werner Siegmund

Bayew Tsega Abebe
Pharmacokinetic Drug-Drug Interactions Between Tropium Chloride and Ranitidine Substrates of Organic Cation Transporters in Healthy Human Subjects

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Abstract
Tropium 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 excision (MATE) 1 and MATE2-K are involved in pharmacokinetics, comparisons with ranitidine, a probe inhibitor of the cation transporters, were evaluated in transfected HEK293 cells. Furthermore, a drug interaction study with tropium 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 tropium. 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 tropium 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, tropium 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 tropium 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. Tropium accumulates in the liver despite high polarity, undergoes largely full tubular secretion by the kidneys, and is substantially excreted via feces after intravenous administration.2,3 This inconsistent pharmacokinetic pattern cannot be

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explained alone by the physicochemical properties of trosupin. Recently, it was shown in vitro that trosupin 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,\(^{6,9}\) 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\(^{10}\) along with P-gp. However, other research has localized OCT1 to the basolateral membrane.\(^{1-14}\)

Trosupin has a higher affinity to P-gp than to OCT1, whereby OCT1 provides higher transport capacity. Intestinal permeability of trosupin 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 trosupin 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.\(^{6,15}\) 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 (I) 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 (K\(_{\text{m}}\)) value of trosupin (K\(_{\text{m}} = 106 \pm 16 \mu M\)) and the half maximal inhibitory concentration (IC\(_{50}\)) value of ranitidine (\(\sim 20-80 \mu M\), dependent on the substrate) for human OCT1 that have been published in the literature up to now.\(^{6,13,16,17}\) Moreover, ranitidine is not known to modulate P-gp.\(^{18}\) It must be taken into consideration, however, that (1) trosupin is also a high-affinity substrate for MATE1 and MATE2-K,\(^{19}\) (2) that basolateral OCT1 and OCT2, as well as apical MATE1 and MATE2-K, might be involved in the vectorial transport of trosupin via hepatocytes and tubular cells in the kidney,\(^{9,15}\); and (3) that ranitidine can also inhibit OCT2 and both MATEs.\(^{16,20-22}\)

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 trosupin had been provided previously.\(^{19,23}\) Determination of the inhibition potency of ranitidine in vitro is required because inhibition of OCT1 is known to be highly substrate dependent,\(^{24}\) and data on inhibition of the trosupin uptake by ranitidine were lacking so far. Additionally, we provide the pharmacokinetic characteristics for intravenous and oral trosupin 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 Flip-In system (Life Technologies, Darmstadt, Germany) as described elsewhere.\(^{19,23}\) The competition experiments were performed as described previously.\(^{15}\) Briefly, 6 x 10\(^5\) 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 trosupin as the substrate and increasing concentrations of ranitidine as the inhibitor (0-4 mM; Sigma-Aldrich, Taufkirchen, 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.\(^{15}\) The cells were lysed with 80% acetonitrile containing 5 mg/mL of trosupin-d8 chloride (TRC Canada Inc, North York, Canada) as the internal standard, and the intracellular concentrations of trosupin 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.\(^{19}\) The data were normalized to the uptake of trosupin in the absence of ranitidine. The IC\(_{50}\) 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 (K\(_{i}\)) was calculated using the Dixon plot.\(^{25}\)

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: EudraCT 2016-002882-69) and ClinicalTrials.gov (identifier: NCT03011463). 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 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/m²) 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 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 medication.

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.

Stimulated salivation was measured by chewing a 5 x 5-cm piece of PARAFILM "M" (American Can Company, Luton, Bedfordshire, England) and accommodation using an optometer according to Schober as described previously.

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. 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 acetonitrile (Carl Roth, Karlsruhe, Germany), and 10 µL of the supernatant were injected into the chromatographic system (column: Supelco Ascentis C18, 3 µm, 2.1 x 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 (Cmax) and the time to Cmax (tmax) 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 (AUClast) using the trapezoidal method and extrapolated to infinity. For IVIVE, the unbound peripheral venous plasma concentrations (I1),
the maximum drug concentration in the small intestine (dose/240 mL, $I_2$), and the unbound hepatic sinusoidal plasma concentrations ($I_3$) were derived from the data of the study. Unbound plasma concentrations were calculated considering a plasma protein binding for trosiptin of 50% and for ranitidine of 15%.\textsuperscript{3,27} Unbound hepatic sinusoidal plasma concentrations ($I_1$) were calculated from the maximum portal venous plasma concentrations ($C_{\text{max, portal}}$) 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. $C_{\text{max, portal}}$ was estimated by $C_{\text{max, portal}} = C_{\text{max, plasma}} + \frac{K_a \times D}{Q_h} \times 0.6$, with $K_a$ as the absorption rate constant, $D$ the oral dose, $F_a$ the oral bioavailability, and $Q_h$ the hepatic blood flow of 1500 mL/min in fasting healthy subjects.\textsuperscript{28} $K_a$ values were estimated by $K_a = \frac{M}{MAT}$ 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 trosiptin 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{29-31} Bioavailability for ranitidine ($F_a = 50\%$) 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 trosiptin 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 trosiptin chloride disposition after intravenous administration, the parameters $CL$, $CL_{d1}$, 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 by $V_{ss} = V_c + V_{p1} + V_{p2}$ and the MDRT is calculated as $MDRT = V_{ss}/CL$ as described above.

In order to fit the data after oral administration of trosiptin, 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{22,33}

\begin{equation}
 f(t) = pf_1(t) + (1-p)f_2(t), \quad 0 < p < 1 
\end{equation}

where

\begin{equation}
 f_i(t) = \frac{MT_i}{\sqrt{2\pi R D_i^2 t}} \exp \left[ -\frac{(t - MT_i)^2}{2 R D_i^2 MT_i} \right] 
\end{equation}

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

\begin{equation}
 AR(t) = DF f_2(t) f(t) 
\end{equation}

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

\begin{equation}
 MAT = p MT_1 + (1 - p) MT_2 
\end{equation}

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:

\begin{equation}
 C(t) = AUC \sum_{i=1}^{3} p_i f_i(t) \quad 0 < p_i < 1 
\end{equation}

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),

\begin{equation}
 MBRT = \sum_{i=1}^{n} p_i MT_i 
\end{equation}

Note that $MBRT = MAT + MDRT$.

Renal clearance ($CL_r$) and fecal clearance ($CL_{fa}$) were derived from the cumulative amount ($A_0$) 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:

\begin{equation}
 VAR = [\sigma_0 + \sigma_1 C(t_i)]^2 
\end{equation}

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 Tropism Chloride After Intravenous and Oral Administrations Before and After Concomitant With 300 mg of Ranitidine Evaluated Post Hoc Using Population Pharmacokinetic Modeling

<table>
<thead>
<tr>
<th>Pharmacokinetic Characteristics</th>
<th>Control</th>
<th>Concomitant of Ranitidine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intravenous infusion (0.2 mg/60 min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL (mL/min)</td>
<td>880 (18)</td>
<td>830 (19)</td>
</tr>
<tr>
<td>V (L)</td>
<td>14 (19)</td>
<td>12 (19)</td>
</tr>
<tr>
<td>Vd (L)</td>
<td>27 (27)</td>
<td>40 (37)</td>
</tr>
<tr>
<td>Vd (L)</td>
<td>330 (27)</td>
<td>330 (34)</td>
</tr>
<tr>
<td>Vd (L)</td>
<td>330 (27)</td>
<td>370 (34)</td>
</tr>
<tr>
<td>CL (mL/min)</td>
<td>440 (29)</td>
<td>410 (43)</td>
</tr>
<tr>
<td>CL (mL/min)</td>
<td>470 (11)</td>
<td>430 (25)</td>
</tr>
<tr>
<td>MDRT (h)</td>
<td>7.3 (27)</td>
<td>8.6 (33)</td>
</tr>
<tr>
<td>T1/2 (h)</td>
<td>16 (15)</td>
<td>16 (11)</td>
</tr>
<tr>
<td>CL (mL/min)</td>
<td>520 (18)</td>
<td>440 (20)</td>
</tr>
<tr>
<td>CL (mL/min)</td>
<td>520 (18)</td>
<td>540 (20)</td>
</tr>
<tr>
<td>Vd (L)</td>
<td>28 (7.1)</td>
<td>31 (7.8)</td>
</tr>
<tr>
<td>S1</td>
<td>0.12</td>
<td>0.13</td>
</tr>
<tr>
<td>S2</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Oral administration (30 mg immediate release tablets): MA; a 9.7 (11) 12 (42)
% f 83 (65) 86 (60)
S1 0.02 0.07
S2 0.14 0.11

CLClmax, intercompartmental clearances; CL/Clmax, renal clearance.
E; bioavailability; MAT, mean absorption time; MDRT, mean disposition residence time; S; S1, residual variability (variance of the measurement error; VAR = [S1 1 S2 (a)2]; T1/2, terminal half-life; Vd, volume of central compartment; Vd120, volumes of shallow and deep compartments; Vd, distribution volume at steady state.
Model parameters and intersubject variability (%) are shown. Sample differences were tested using Student's paired t-test.
P < .05

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 tropism of ≥38% with P < 0.05 and statistical power >80% (nQuery Advisor 7.6, Statistical Solutions, Cork, Ireland). In a post hoc statistical analysis to the study protocol, an analysis of variance for all pharmacokinetic characteristics of the noncompartmental evaluation (except for Imax) 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 tropism as a substrate, ranitidine was able to inhibit OCT1-mediated tropism uptake with I50 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 μM ranitidine, a concentration that corresponds to the estimated I2 concentration for ranitidine. However, at 290 μM substrate concentration of tropism, a concentration corresponding to the I1 concentration, only 64% inhibition was achieved with 4.0 μM ranitidine as the inhibitor. From the competition assay, 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 Tropism
The plasma concentration-time curves of intravenous tropism could be best fitted to a 3-compartment open model (Figure 2). The drug was widely distributed (Vd1/2; ~481 L/kg) with a central compartment of ~0.18 L/kg, a shallow peripheral compartment (Vp) of ~0.5 L/kg) and a deep compartment (Vd2) of ~4.13 L/kg. Tropism 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 (CLREN; ~500 mL/min) and fecal excretion (CLFEC; ~30 mL/min). About 55% of the dose was eliminated into the urine, and about 40% via feces within 5 days (Tables 1 and 2).

After oral administration, the individual plasma concentration-time curves of tropism were highly variable (Figure S1). Tropism was slowly and poorly absorbed (tmax ~6.7 hours; MAT, ~9.7 hours; t1/2; ~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 50% of the oral dose was eliminated via urine and 25% via feces (Tables 1 and 2).

The unbound tropism concentrations in the peripheral venous plasma (Sv) and the hepatic sinusoidal plasma (Sh) 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 tropism by ~15%. After oral comedication of 300 mg of ranitidine, all pharmacokinetic characteristics of tropism were not significantly changed. The 90% CI of the GMR for the
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 concomidation was not involved in the study. However, the plasma concentration–time curves of ranitidine after oral concomitance of 30 mg of trospium chloride were relatively similar to profiles described in the literature. 29-31 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).

Concomidation 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 concomitance 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 ($S_g = 290 \mu M$), which saturates OCT1 in vitro. On the contrary, the unbound plasma concentrations after oral ($S_t = 0.90 \pm 0.05 \mu M$) and intravenous administration ($S_t = 0.05 \pm 0.008 \mu M$) are notably below the respective $K_m$ 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 tropism by MATE1 and MATE2-K in vivo.\(^{39}\) Ranitidine also has the potential to inhibit the hepatic OCT1 according to the \(I_2K_1\) ratio of 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 \(I_2K_1\) ratio of 39.0. Interestingly, the route of tropism administration significantly influenced the IVIVE parameters for ranitidine. For MATE1, there was a relevant interaction potential only after oral comedication of tropism chloride. The potential for interaction with MATE2-K in vivo was statistically higher after oral dosing of tropism. 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 tropism 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. Tropism 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 tropism. However, conclusive results cannot be drawn because a placebo control was not involved in the study.

### Discussion

#### Changes in Pharmacokinetics of Tropism

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 tropism could not be confirmed. The 90% CIs of the GMRs for AUC

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Control</th>
<th>With ranitidine</th>
<th>GMR (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intravenous infusion (2 mg/60 min)</td>
<td>AUC</td>
<td>37.2 ± 8.8</td>
<td>39.5 ± 8.7</td>
</tr>
<tr>
<td></td>
<td>Cmax</td>
<td>21.5 ± 2.9</td>
<td>21.7 ± 6.2</td>
</tr>
<tr>
<td></td>
<td>t1/2</td>
<td>12 ± 5.0</td>
<td>12 ± 3.3</td>
</tr>
<tr>
<td></td>
<td>Cmax</td>
<td>530 ± 19</td>
<td>460 ± 127</td>
</tr>
<tr>
<td> </td>
<td>AUC</td>
<td>1.2 ± 0.3</td>
<td>1.1 ± 0.3</td>
</tr>
<tr>
<td> </td>
<td>AUC</td>
<td>0.06 ± 0.04</td>
<td>0.08 ± 0.08</td>
</tr>
<tr>
<td>Oral administration (30 mg immediate release tablets)</td>
<td>AUC</td>
<td>608 ± 33.3</td>
<td>511 ± 39%</td>
</tr>
<tr>
<td></td>
<td>Cmax</td>
<td>51 ± 12</td>
<td>48 ± 4.0</td>
</tr>
<tr>
<td></td>
<td>t1/2</td>
<td>67 ± 12</td>
<td>64 ± 1.19</td>
</tr>
<tr>
<td></td>
<td>Cmax</td>
<td>99 ± 12</td>
<td>7.7 ± 2.5</td>
</tr>
<tr>
<td> </td>
<td>AUC</td>
<td>501 ± 44</td>
<td>494 ± 97</td>
</tr>
<tr>
<td> </td>
<td>AUC</td>
<td>1.7 ± 0.5</td>
<td>1.6 ± 1.2</td>
</tr>
<tr>
<td> </td>
<td>AUC</td>
<td>75 ± 16</td>
<td>8.0 ± 5.7</td>
</tr>
</tbody>
</table>

\(A_{\text{max}}\) = cumulative amount excreted into feces; \(A_{\text{auc,act}}\) = cumulative amount excreted into urine; AUC, area under the plasma concentration-time curve; CI, confidence interval; Cmax, peak concentration; Cmin, minimal plasma concentration; GMR, geometric mean ratio; ND, not determined; t1/2, half-life; \(V_{\text{ss}}\), time to maximum concentration. Standard errors and geometric means with 90% confidence intervals are given.

\(P < .05\) compared to control: Student’s paired t-test.
Figure 3. Absorption rate-time (top) and the amount absorbed (bottom) of tropium after oral administration of 30 mg of tropium chloride alone (solid line) and after comedication of ranitidine (stained line). The curves were simulated using the mean parameter estimates of the double inverse Gaussian function.

Figure 4. Individual plasma concentration-time curves of oral ranitidine (360 mg) after oral comedication of 30 mg of tropium chloride (top), and after intravenous comedication of 2 mg of tropium chloride (bottom). The inset shows mean (±SD) plasma concentration-time curves of ranitidine with oral (open circles) and intravenous comedication (full circles) of tropium chloride. SD, standard deviation.

and $C_{\text{max}}$ (tropium 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 tropium, which are well tolerated in clinical practice (eg, circadian time rhythm, food effects).

Before analysis of the results of this study, we expected that comedication of ranitidine would decrease oral bioavailability of tropium 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.\textsuperscript{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.\textsuperscript{11-16}

However, evidence that OCT1 is an apical uptake carrier for tropium also came from the results of a DDI study with the OCT1 substrate metformin. Oral comedication of metformin lowered the bioavailability of tropium by $\sim$50%.\textsuperscript{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).\textsuperscript{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.\textsuperscript{40,41} Therefore, it is plausible that reduced-function gene polymorphisms, and comedication of strong inhibitors of OCT1 (eg, verapamil, proton pump
Table 3. Pharmacokinetic Characteristics of Ramitidine After Intravenous Infusion of 2 mg (TC-IV) and Oral Co-medication of 36 mg of Tropium Chloride (TC-PO) as Assessed by Post Hoc Population Pharmacokinetic Modelling (Arithmetic Means With Inter-subject Variability) and Noncompartmental Evaluation (Arithmetic Means ± SD)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>TC-IV</th>
<th>TC-PO</th>
<th>GMx (10%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population pharmacokinetic modelling</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC µg × h/mL</td>
<td>4.5 ± 1.4</td>
<td>6.3 ± 2.8</td>
<td>1.34 (1.06-1.65)</td>
</tr>
<tr>
<td>Cmax mg/L</td>
<td>3.7 ± 3.2</td>
<td>1.0 ± 0.6</td>
<td>1.31 (1.11-1.70)</td>
</tr>
<tr>
<td>tmax h</td>
<td>4.1 ± 1.7</td>
<td>4.3 ± 1.9</td>
<td>ND</td>
</tr>
<tr>
<td>b0 h</td>
<td>4.0 ± 2.0</td>
<td>3.7 ± 0.6</td>
<td>0.98 (0.64-1.54)</td>
</tr>
<tr>
<td>CLH mL/min</td>
<td>440 ± 140</td>
<td>410 ± 83</td>
<td>0.95 (0.81-1.11)</td>
</tr>
<tr>
<td>AUC0 last mg</td>
<td>110 ± 26</td>
<td>150 ± 41</td>
<td>1.28 (0.96-1.22)</td>
</tr>
<tr>
<td>AUC0-∞ mg</td>
<td>5.1 ± 14</td>
<td>12.3 ± 6.5</td>
<td>0.74 (0.47-1.16)</td>
</tr>
<tr>
<td>For in vitro vivo prediction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b1 µM</td>
<td>1.9 ± 1.1</td>
<td>2.6 ± 1.7</td>
<td>0.77</td>
</tr>
<tr>
<td>b2 µM</td>
<td>3.980</td>
<td>3.980</td>
<td>0.77</td>
</tr>
<tr>
<td>b3 µM</td>
<td>27.2 ± 7.1</td>
<td>4.3 ± 1.7</td>
<td>0.77</td>
</tr>
</tbody>
</table>

AUC0-∞, cumulative amount excrusted into feces; AUC0-∞, cumulative amount excrusted into urine; AUC, area under the plasma concentration-time curve; CI, confidence interval; CLH, renal clearance; Cmax, maximum plasma concentration; t1/2, half-life of plasma concentration; CLH, hepatic plasma concentration; MRT, mean residence time; MRT, mean residence time; Cmax, maximum concentration; Cmax, half-life of Cmax, time to maximum concentration.

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

*p < .05; **p < .05 compared to TC-IV. Student’s pairs t-test.

Table 3 shows that there is a significant difference in the pharmacokinetic characteristics between TC-IV and TC-PO. The AUC of TC-IV is higher than that of TC-PO, indicating that TC-IV may have a greater bioavailability. The tmax of TC-IV is also shorter than that of TC-PO, suggesting a faster absorption rate. However, the CLH of TC-IV is lower than that of TC-PO, indicating a slower drug elimination rate.

Therefore, it is possible that we have overestimated the involvement of OCT1 in tropism uptake because the inhibitor ranitidine did not reach active concentrations at the major absorption sites in the colon.

Third, OCT1 might not be the right uptake carrier for tropism in the human intestine. Alternative candidates are the plasma membrane monoamine transporter (PMAT), OCT3, the serotonin reuptake transporter (SERT), or any others which share similar features with the substrates and inhibitors of OCRT. The results of the metformin-tropism DDI study mentioned above can also be discussed in the light of PMAT and SERT, for which metformin is a high-affinity substrate. Metformin possibly impairs oral absorption of tropism by competition with PMAT, whereas the OCT1 inhibitor ranitidine lacks clinically relevant effects in the complex in vivo situation. A suitable approach to distinguish between OCT1 and PMAT in the oral absorption of tropism and/or metformin would be DDI studies with lopiniavir, a strongly selective inhibitor of PMAT that has no influence on OCT1 to OCT3. It should be mentioned that PMAT is not expressed in the kidneys.

Potential interaction of ranitidine was also predicted for the OCT1-mediated uptake of tropism in the liver by the IVIVE characteristics (Table 4). However, we did not find any pharmacokinetic evidence for this (e.g., increase in 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 tropism following intravenous administration, and distinctly earlier than the sinusoidal maximum of tropism 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 tropism. Tropism 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 tropism 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 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.26

<table>
<thead>
<tr>
<th>Treatment</th>
<th>OCT1</th>
<th>OCT2</th>
<th>MATE1</th>
<th>MATE2-K</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trospium in vivo</td>
<td>15.5 ± 3.1</td>
<td>3.4 ± 3.1</td>
<td>15.4 ± 3.4</td>
<td>8.2 ± 1.8</td>
</tr>
<tr>
<td>( \text{IC}_{50} ) (\mu M)</td>
<td>1.1 ± 0.16</td>
<td>0.10 ± 0.03</td>
<td>1.03 ± 0.14</td>
<td>0.30 ± 0.01</td>
</tr>
<tr>
<td>Ranitidine with TC-IV in vivo</td>
<td>1.12 ± 0.25 (102 ± 15)</td>
<td>40 ± 105</td>
<td>114 ± 37</td>
<td>35 ± 11</td>
</tr>
<tr>
<td>I/IC50</td>
<td>0.01 ± 0.01 (0.02 ± 0.01)</td>
<td>0.001 ± 0.002</td>
<td>0.014 ± 0.001</td>
<td>0.05 ± 0.03</td>
</tr>
<tr>
<td>I/IC50 (%K)</td>
<td>21 (9)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>IC50/IC90</td>
<td>3.18 ± 0.01 (0.2 ± 0.01)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Ranitidine with TC-PO in vivo</td>
<td>2.02 ± 0.02</td>
<td>0.005 ± 0.004</td>
<td>0.02 ± 0.01</td>
<td>0.37 ± 0.05</td>
</tr>
<tr>
<td>I/IC50</td>
<td>21 (9)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>IC50/IC90</td>
<td>0.022 ± 0.01 (0.01 ± 0.02)</td>
<td>NA</td>
<td>0.03 ± 0.01</td>
<td>NA</td>
</tr>
</tbody>
</table>

FDA, US Food and Drug Administration; IC90, half maximal inhibitory concentration; K, inhibitory constant; K0, concentration of the substrate to which the velocity of the reaction is half maximal; MATE, multidrug and toxic compound efflux transporters; NA, not applicable; OCT, organic cation transporter; IC50, 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 erantically absorbed with a typical “double peak” or “multi peak” phenomena in the majority of subjects, and undergoes a significant “first-pass” metabolism.27,29,31 The intestinal uptake seems to be rather complex and includes paracellular transport mechanisms, the uptake transporter OCT1, and the efflux carrier P-gp.15,17,35,45-51 Ambitious research in healthy subjects carried out in 1994 showed that the ranitidine absorption rates are highest in the duodenum/proximal jejunum and the most distal jejunal ileum, and are significantly correlated to water uptake, whereas a marked secretion occurs in the mid-jejunum independent of water fluxes.52

After oral administration of 30 mg of trospium chloride, competition with oral absorption of ranitidine was not expected because the \( K_0 \) concentration of trospium (~0.3 mM) is much lower than the \( K_0 \) level after 300 mg of ranitidine (~4.0 mM), and when considering the similar \( K_0 \) values for OCT1 (trospium, 156 ± 16 μM; ranitidine, ~60-70 μM).53,16,17 The pharmacokinetic parameters of ranitidine were well comparable to literature data. Following intravenous coadministration of trospium, however, mean absorption time, plasma exposure, and amount of ranitidine excreted into urine were significantly lowered.

In agreement with our hypothesis, gastric emptying is delayed and intestinal motility is lowered only after single-dose, intravenous infusion, but not after oral administration of the mucosacaric receptor-blocking trospium chloride.53-55 Consequently, gastric emptying of ranitidine is delayed, and smaller dose fractions of ranitidine are absorbed predominantly in the more 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 coadministration of trospium, which leads to lower bioavailability.15,36,27 This hypothesis, however, is not in agreement with the findings that ranitidine is not affected by food.58 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

Coadministration 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.

Acknowledgments

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in Clinical Pharmacology and Biopharmaceutical Analytics, Bad Soden, Germany), and Dr. Anita Derr for proofreading the manuscript (Department of Medical Sciences/Clinical Research, Dr. Pfleger Arzneimittel GmbH, Bamberg, Germany).

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 Sowden, 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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References
28. Ito K, Iwanabu T, Kameyama S, Ueda K, Suzuki H, Sugiura Y. Prediction of pharmacokinetic alterations caused by...


**Supplemental Information**

Additional supplemental information can be found by clicking the Supplements link in the PDF toolbar or the Supplemental Information section at the end of web-based version of this article.

My own contribution:

- Materials and methods
  - Sample preparation (plasma, urine and stool) for analytical measurement
  - Collaboratively developed and validated an LC-MS/MS method to quantify clarithromycin and ranitidine according to GLP criteria
  - Collaborated on LC-MS/MS measurement of ranitidine and clarithromycin

- Results
  - Performed pharmacokinetic and biometric evaluation of the study data

- Preparation of the study reports and wrote manuscript

Eberhard Scheuch

- Design and performed the study, analyzed the study data and wrote manuscript

Werner Siegmund

- Designed, supervised and performed the study, analyzed data and wrote manuscript.

Prof. Dr. Werner Siegmund

Bayew Tsega Abebe
Simultaneous Quantitative Analysis of Clarithromycin and Ranitidine, Probe Inhibitors of P-Glycoprotein and OCT1, to Evaluate Potential Pharmacokinetic Influence of Potential Transporter Substrates

Eberhard Scheuch1, Bayew Tsega Abebe1, Werner Siegmund1

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Abstract
Clarithromycin and ranitidine are accepted probe inhibitors in drug-drug interaction studies (DDI) with substrates of the multidrug transporters P-glycoprotein and OCT1. We assayed both drugs in human plasma, urine, and feces using LC-MS/MS with positive mass transition mode (AB Sciex API 2000 with turbo-ion spray) with fexofenadine as an internal standard. After protein denaturation with acetonitrile/water (30:70, v/v), the samples were centrifuged and the supernatants (10 μL) were injected into the chromatographic system (column: Sapeleo Ascentis® C18, 3 μm, 2.1×100 mm). The chromatography was performed with isocratic elution plied using ammonium formate buffer ([5 mM; pH 3.0])/acetonitrile, 40:60, v/v) as mobile phase at a flow rate of 200 μL min⁻¹. The chromatograms were evaluated online with the internal standard method using peak-area-ratios for linear regression analysis weighted by 1/x (x = concentration) for the validation ranges in plasma between 0.005 and 2.0 μg mL⁻¹, and for urine and feces between 0.005 and 10.0 μg mL⁻¹. The method was shown to possess sufficient specificity, accuracy, precision and stability without matrix effects, thereby fulfilling current bioanalytical guidelines. The assay was suitable to simultaneous quantitative analysis of clarithromycin and ranitidine in plasma, urine, and feces of a DDI with tropsium chloride to exclude major influence of tropsium on the pharmacokinetics of the probe inhibitors. The assay is superior to other methods as it enables for the first time, quantification of the drugs in feces.

Keywords: LC-MS/MS - Clarithromycin - Ranitidine - Feces - P-Glycoprotein - OCT1

Introduction
Translation of knowledge from basic research into clinical practice requires often a toolbox with non-invasive methods which can be applied in healthy subjects and patients without ethical reservations. One example is evaluation of meaningful drug-metabolizing enzymes or multidrug transporters for pharmacokinetics in man using a pharmacogenomics or a drug-drug interaction (DDI) approach with probe drug substances, probe inhibitors and/or inducers for the respective variable. Often, a so-called cocktail approach for several metabolizing enzymes and transporters is chosen [1–4].

Many analytical methods are described for simultaneous measurement of the respective probe-drug substances in human plasma and mostly also in urine [5–7]. Less emphasis is given to the quantitative analytics of the respective probe inhibitors and probe inducers (modulators) which are selected to evaluate the clinical role of drug-metabolizing enzymes and transporters in mechanism-driven DDIs although it is known, that major experimental bias can be caused by the influences of the probe-drug substances on pharmacokinetics of the respective probe modulators.

Therefore, we formerly already published a validated LC-MS/MS assay for simultaneous quantification of clarithromycin (CLA) and rifampicin, a probe inhibitor for cytochrome P450 (CYP) 3A4 and a pregnane-X-receptor-type probe inducer of CYPs, respectively, to evaluate mutual DDIs in foals [8].

In a recent project with the muscarinic receptor-blocking tropsium chloride (TC), we needed quantitative analytics in human matrices for clarithromycin (CLA) and ranitidine (RAN), selective probe inhibitors for the efflux carrier P-glycoprotein (P-gp) [9] and organic cation transporters...
(OCTs), respectively [http://transportal.compbio.ucsf.edu/compounds/rani-tidine; access date 12.06.2019]. The highly water-soluble TC is a substrate for human P-gp and OCT1 in vitro [10]. The efflux carrier P-gp was located in enterocytes of the intestinal tract to the apical membrane next to the uptake transporter OCT1 [11, 12]. Therefore, we hypothesized that the cellular interplay of the transporters along the small and large intestine might be the rationale for poor and slow absorption of trospium via a “narrow absorption windows” in the small intestine and a “wider window” in the colon/ascending colon (bioavailability − 19%) [13]. To prove this hypothesis, we initiated a DDI study in healthy subjects to measure the effects the probe inhibitors CLA and RAN on pharmacokinetics of TC. However, TC exhibits strong anti-muscarinic effects and can influence itself the absorption of the probe inhibitors, e.g., by delay of gastric emptying and intestinal transit time or intestinal water turnover. Therefore, the effects of the investigational drug TC on the pharmacokinetics and, in turn, on the inhibitory potency of the probe inhibitors have to be evaluated. As TC is mainly absorbed via the “absorption window” in the colon, data on the availability of the probe inhibitors in the proximal colon are required to conclude on the mechanism of a potential DDI.

In the paper, we describe a quantitative assay for simultaneous measurement of CLA and RAN after oral administration of single therapeutic doses in human plasma, urine, and feces and provide for the first time, dependent quantitative data on intestinal elimination of the probe inhibitors in healthy subjects.

Materials and Methods

Chemicals

Methanol and acetonitrile were obtained in ultra LC-MS quality (>99.98%) from Carl Roth (Karlsruhe, Germany). Deionized water (conductance: ≤0.05 μS cm⁻¹, pH 5.0–6.0) was generated with the Astacus® system (membrapure, Hennigsdorf, Germany). The analytes CLA, RAN and the internal standard fexofenadine were purchased from Sigma-Aldrich (Steinheim, Germany). The stock solutions were prepared using a mixture of acetonitrile/water (50:50 v/v) and stored at −22 ± 4 °C maximally for 8 weeks. The working solutions were weekly freshly prepared with the same mixture and stored at 2–8 °C. All other chemicals were of analytical grade.

Sample Preparation

All plasma, urine, and feces samples from the clinical study had been kept at least at −20 °C until the beginning of the analysis. Then, they were slowly thawed at room temperature. To 0.2 mL of the samples of all matrices, 0.02 mL of the internal standard solution containing fexofenadine (0.1 mg mL⁻¹ for plasma, 0.5 mg mL⁻¹ for urine and feces), 0.02 mL citric acid (1.0%) and 0.5 mL acetonitrile/water (50:50 v/v) were added. After thorough mixing, the samples were centrifuged at 3200×g (4 °C) for 10 min (Heraeus Fresco 21, Thermo Fischer Scientific, Langenselbold, Germany) and manually decanted. The obtained supernatant was concentrated down to 0.3 mL by vacuum centrifugation (3400×g; 25 °C; 25 min) using the vacuum concentrator ScanVac (LaboGene, Allerød, Denmark). 0.2 mL of the concentrate was subjected to LC–MS/MS analysis 10 μL of which was injected into the chromatographic device.

LC-MS/MS Analysis

The LC–MS/MS analysis was done with the Agilent 1100 series HPLC system (Agilent Technologies, Waldbronn, Germany) and a Perkin Elmer Series 200 Autosampler (Perkin Elmer, Darmstadt, Germany) coupled to the API2000 TurbIOn Spray mass spectrometer equipped with the Analyst 1.4.2 software (AB Sciex, Darmstadt, Germany). We selected fexofenadine as an internal standard because of practical reasons. In our clinical department, the drug is used as a suitable probe drug in vitro experiments and in clinical studies in animals and man for evaluation of multidrug transporter functions together with rifampicin, clarithromycin, ranitidine and other drugs. Depending on the research application of the respective analyses, the compounds can be used both as analytes and as an internal standard without repetition of the complete validation procedures.

The chromatography was performed with isotropic elution using ammonium formate buffer (5 mM; pH 3.0)/acetonitrile, 40(60), v/v as mobile phase at a flow rate of 200 μL min⁻¹ and the reverse phase column Supelco® Ascentis (C18, 3 μm, 2.1×100 mm, Sigma-Aldrich, Darmstadt, Germany) both tempered at 40 °C. The chromatographic flow was directed to a 0.5 μm PEEK, Supelco® precolumn filter direct-connect (PEEK) device (Sigma-Aldrich, Darmstadt, Germany) to avoid contamination with solid particles. The HPLC was coupled to the mass spectrometer equipped with the electro spray ionization (ESI) TurboIon® interface and operated in the positive ion mode to monitor the following mix transitions: CLA 748.5 → 590.6 and 748.5 → 158.2; RAN 315.1 → 175.0 and 315.1 → 130.2; FEX (internal standard) 502.2 → 466.2 and 502.2 → 484.8. The following gas parameters have been used: temperature, 400 °C; gas 1, 60 ps; gas 2, 60 ps; voltage, 5000 V; collision-activated dissociation (CAD) gas, 10 ps (all nitrogen). The detailed mass spectrometry parameters are given in Table 1. The validated Analyst 1.4.2 software (AB Sciex, Darmstadt, Germany) was applied to evaluate the
Table 1: Mass spectrometry parameters for detection of clarithromycin, ranitidine, and the internal standard fenofibrate in the positive multiple reaction monitoring mode.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Q1 mass</th>
<th>Q3 mass</th>
<th>DP</th>
<th>EP</th>
<th>FP</th>
<th>CE</th>
<th>CXP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clarithromycin</td>
<td>748.5</td>
<td>590.6</td>
<td>56</td>
<td>10.5</td>
<td>250</td>
<td>29</td>
<td>26</td>
</tr>
<tr>
<td>Ranitidine</td>
<td>315.1</td>
<td>176.0</td>
<td>31</td>
<td>6.5</td>
<td>330</td>
<td>21</td>
<td>26</td>
</tr>
<tr>
<td>Fenofibrate</td>
<td>592.2</td>
<td>486.6</td>
<td>61</td>
<td>9.5</td>
<td>90</td>
<td>41</td>
<td>20</td>
</tr>
</tbody>
</table>

Dwell time was in each case 150 ms. Declustering potential (DP), entrance potential (EP), collision energy (CE), focusing potential (FP) and collision cell exit potential (CXP) are given in V (V).

Validation

The newly developed LC–MS/MS assay was validated in accordance with the EMA guideline of for bioanalytical method validation (https://www.cema.europa.eu/documents/scientific-guideline/guideline-bioanalytical-method-validation_en.pdf). The method was validated for selectivity in serum, urine, and feces by comparing six different batches of the respective blank matrix samples containing CLA, RAN, and FEX. The calibration curves consisted of a matrix sample without analytes and internal standard (double blank), a matrix sample with spiked with the internal standard (blank) and in each case seven matrix samples for calibration spiked with 0.005, 0.02, 0.1, 0.3, 0.8, 1.5, 2.0 µg mL⁻¹ CLA and RAN in plasma (validation range 0.005–2.0 µg mL⁻¹) and 0.005, 0.02, 0.1, 0.5, 2.0, 6.0, 10.0 µg mL⁻¹ urine and feces (validation range 0.005–10.0 µg mL⁻¹). Accuracy and precision were assessed using quality control matrix samples (QC) spiked with 0.01, 1.0, and 1.8 µg mL⁻¹ CLA and RAN in plasma and 0.01, 5.0, and 8.0 µg mL⁻¹ in urine and feces. The respective concentrations covered the low, mean, and high analytical range. Within-day accuracy and precision were assessed by comparison of the measured concentrations with the nominal concentrations in the QC samples sets which were measured on different days and expressed as relative error of the nominal concentrations (accuracy) and the respective coefficients of variation of the mean values (precision). Inter-day accuracy and precision were determined by repeated measurements of the respective calibration and QC samples on different days. Intra-day accuracy and precision were determined by measurements of six QC sample sets on 1 day.

To assess recovery from biological samples, the MS-signal (peak area) obtained with samples which were extracted as mentioned above were compared with the signals obtained with the diluted stock solution without extraction (100% value). QC sample concentrations were used for evaluation of recovery. Matrix effects were evaluated by comparison of the peak-area ratios obtained from extracted blank samples which were post-hoc spiked with the analytes (QC concentrations) with the peak-area ratios from samples obtained with the stock solutions after respective dilution (100% values).

Stability and freeze–thaw stability were determined by measurement of six QC sample sets before and after storage at 10°C for 8 h (stability at) and after two freeze–thaw cycles (−20°C) prior to extraction. Long-term stability has been evaluated for 4 weeks only, because all assays were performed within this span after sampling. Generally, adequate stability was assumed if the analyte concentrations under the defined storage conditions did not deviate more than 15% from the initial values.

Measurement of Biological Samples

On each analytical day, freshly prepared sample sets for calibration and QC were prepared using identical biological blank matrices for both issues as described above. At least 10% of all samples measured during an analytical run (calibration, QC, study samples) were samples used for quality control. To accept the validity of an analytical run, at least two of the three QC samples had to be within an accuracy range of ±15% of the respective nominal values as suggested by the international recommendations.

Clinical Study

The validated method for quantitative determination of CLA and RAN was applied for the quantitative evaluation of the drugs in human plasma, urine, and feces as samples in a hypothesis-driven DDIs study with TC. The effects TC after intravenous (2 mg) and oral (30 mg) administration on the pharmacokinetics of CLA and RAN, respective, were measured post hoc using the newly developed LC–MS/MS assay. The subjects were subjected either to single oral dose administration of 500 mg CLA (Claritromycin-ratiopharm® 500 mg TC Filmtabletten, ratiopharm, Ulm, Germany) or to 300 mg RAN (Ranitidin-ratiopharm® 300 mg Filmtabletten,
Simultaneous Quantitative Analysis of Clarithromycin and Ranitidine, Probe Inhibitors of...

<table>
<thead>
<tr>
<th>Drug</th>
<th>Q1 mass</th>
<th>Q3 mass</th>
<th>DP</th>
<th>EP</th>
<th>FP</th>
<th>CE</th>
<th>CXP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clarithromycin</td>
<td>748.5</td>
<td>590.6</td>
<td>56</td>
<td>10.5</td>
<td>250</td>
<td>29</td>
<td>25</td>
</tr>
<tr>
<td>Ranitidine</td>
<td>315.1</td>
<td>176.0</td>
<td>31</td>
<td>6.5</td>
<td>330</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>502.2</td>
<td>486.2</td>
<td>61</td>
<td>9.5</td>
<td>90</td>
<td>41</td>
<td>20</td>
</tr>
</tbody>
</table>

Dwell time was in each case 150 ms. Declustering potential (DP), entrance potential (EP), collision energy (CE), focusing potential (FP) and collision cell exit potential (CXP) are given in volt (V).

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Rack-stability and freeze-thaw stability were determined by measurement of six QC sample sets before and after storage at 10 °C for 8 h (rack stability) and after two freeze-thaw cycles (−20 °C) prior to extraction. Long-time stability has been evaluated for 4 weeks only, because all assays were performed within this span after sampling. Generally, adequate stability was assumed if the analytic concentrations under the defined storage conditions did not deviate more than 15% from the initial values.

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Clinical Study

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Fig. 1 MS/MS fragmentation spectra of a clarithromycin, b ranitidine and c the internal standard fexofenadine showing the parent and product ions.

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<table>
<thead>
<tr>
<th></th>
<th>Serum</th>
<th>Urine</th>
<th>Feces</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
</tr>
<tr>
<td>b</td>
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<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
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<tr>
<td>c</td>
<td><img src="image7.png" alt="Image" /></td>
<td><img src="image8.png" alt="Image" /></td>
<td><img src="image9.png" alt="Image" /></td>
</tr>
</tbody>
</table>

Fig. 2: Total ion chromatograms of a double blank (a), blank spiked with internal standard, b) and a calibrator sample spiked with clarithromycin (pink), ranitidine (green) and the internal standard loxofenadine (blue) in plasma, urine and feces.

ratiopharm, Ulm, Germany) and to concomitant co-medication of a 30 mg TC film tablet p.o. and, after a washout of at least 7 days, to intravenous co-infusion of 2 mg TC in 20 mL saline for 60 min (Spasmex®, Pfleger, Bamberg, Germany). The oral administrations were performed with 240 mL tap water after at least 10 h of overnight fasting. A standard dinner was provided 5 h after the oral administrations. Venous blood was collected via an indwelling forearm cannula before and 0.33, 0.66, 1, 1.33, 1.66, 2, 3, 4, 5, 6, 8, 10, 12, 16, 24, 30, 36 h after intravenous infusion, and before and 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 11, 12, 16, 24, 30, 36 h after oral administrations of TC. Plasma was stored for quantitative analysis at ≤ −20 °C within 2 h after sampling. Urine and feces were sampled at daily intervals for 5 days and respective aliquots were also stored at ≤ −20 °C until the quantitative analyses. Feces were completely defecated in plastic containers which fit into the sink of a standard water closet and which could be odorlessly closed (Vacunnex, Tutow, Germany). Each portion was diluted with adequate amounts of distilled water via a narrow, closely foliate opening and thoroughly homogenized using a hand blender. Aliquots of the freshly prepared mixture were stored as described.

Pharmacokinetic evaluation: Maximum plasma concentration (Cmax) and the time of Cmax (Tmax) were read from the plasma concentration-time curves. The area under the curve was assessed up to the last sampling time above the limit of quantification (AUC0–∞) using the trapezoidal method and extrapolated to infinity (AUC). Renal clearance (CLr) was derived from the cumulative amount (A) excreted into urine divided by AUC.

Results and Discussion

The newly developed LC-MS/MS method enabled for the first time simultaneous quantification of CLA and RAN which are suitable probe inhibitors in clinical DDI studies with potential substrates (e.g., TC) of the membrane transporters P-glycoprotein and OCT1 [9]. Beside the primary objectives of a DDI study, a potential bias coming from the known pharmacokinetic and/or pharmacodynamics effects of an investigational product (e.g., the muscarinic receptor blocking of TC) on the known pharmacokinetics of the selected competitors (e.g., the probe inhibitors CLA and RAN) must be evaluated with a secondary intention. The secondary objectives must be obtained using the same quality criteria as defined for evaluation of the primary objectives, e.g., the guidance on bioanalytical method validation of the EMA (https://www.ema.europa.eu/documents/scientific-guideline/guideline-bioanalytical-method-validation_en.pdf). To our knowledge, an analytical method for the
Table 2. Quality characteristics, matrix effects and recovery (\%) for clarithromycin (CLA) and roxithromycin (RAN) in human plasma, urine, and feces

<table>
<thead>
<tr>
<th>Drug</th>
<th>Accuracy Calibration</th>
<th>QC</th>
<th>Precision Calibration</th>
<th>QC</th>
<th>Matrix effects</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLA (plasma)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inter-day</td>
<td>-6.3 to 3.5</td>
<td>-8.6 to 8.4</td>
<td>4.5 to 10.9</td>
<td>2.7 to 14.1</td>
<td>105 to 112</td>
<td>14.7 to 18.7</td>
</tr>
<tr>
<td>Intra-day</td>
<td>0.9 to 12.5</td>
<td>9.9 to 12.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CLA (urine)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inter-day</td>
<td>-4.2 to 6.2</td>
<td>-4.4 to 6.5</td>
<td>1.9 to 8.2</td>
<td>3.4 to 9.2</td>
<td>97.8 to 114</td>
<td>60.7 to 63.8</td>
</tr>
<tr>
<td>Intra-day</td>
<td>-13.4 to 6.0</td>
<td>2.7 to 14.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CLA (feces)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inter-day</td>
<td>-7.1 to 14.5</td>
<td>-2.8 to 0.3</td>
<td>2.5 to 6.1</td>
<td>4.8 to 8.5</td>
<td>95.7 to 104</td>
<td>n.d.</td>
</tr>
<tr>
<td>Intra-day</td>
<td>-4.5 to 0.0</td>
<td>5.1 to 15.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAN (plasma)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inter-day</td>
<td>-6.4 to 12.4</td>
<td>-10.1 to 1.2</td>
<td>5.4 to 13.7</td>
<td>3.7 to 9.5</td>
<td>99.3 to 114</td>
<td>12.1 to 18.9</td>
</tr>
<tr>
<td>Intra-day</td>
<td>-5.6 to 8.7</td>
<td>5.1 to 5.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>RAN (urine)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inter-day</td>
<td>-4.2 to 5.1</td>
<td>-3.6 to 0.7</td>
<td>1.5 to 7.1</td>
<td>3.6 to 9.4</td>
<td>99.2 to 114</td>
<td>6.5 to 12.7</td>
</tr>
<tr>
<td>Intra-day</td>
<td>-14.3 to 8.6</td>
<td>1.7 to 7.5</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>RAN (feces)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inter-day</td>
<td>-4.1 to 4.9</td>
<td>-3.9 to 9.3</td>
<td>2.3 to 8.0</td>
<td>5.5 to 13.6</td>
<td>97.5 to 199</td>
<td>n.d.</td>
</tr>
<tr>
<td>Intra-day</td>
<td>0.7 to 12.3</td>
<td>3.8 to 10.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Within-day and between-day accuracy are given as relative error of the mean concentrations and precision as coefficients of variation of the nominal concentrations.

n.d. not determined

Simultaneous quantification of the probe inhibitors CLA and RAN was not available so far. Sensitive assays for quantification of both drugs in combination with other analytes were already described in the literature [3, 14, 15]. For the first time, however, our assay enabled quantitative analysis of CLA and RAN in all biological matrices (plasma, urine, and feces) needed to obtain complete pharmacokinetic data sets, including parameters to characterize fecal excretion.

LC–MS/MS Analysis

All analytes generated in the positive ionization mode high analytical signals for the protonated molecule ([M + H]+) (Fig. 1) using the Electro-Spray Turbulon® interface (ESI), which generated markedly higher signals than the Heated Nebulizer® source (APCI). The respective mass-to-charge transitions were manually generated and optimized to obtain maximum mass peak intensities (Table 1). Isocratic elution with the reversed-phase column resulted in retention times of 1.9 min, 1.4 min, and 1.7 min for CLA, RAN, and FEX, respectively (Fig. 2). Thus, one analytical run needs approximately 6 min for accurate separation of CLA, RAN, and the internal standard which is comparable to previously published LC–MS/MS assays for CLA and RAN [8, 14, 15].

Validation

The newly developed LC–MS/MS for simultaneous quantification in human plasma, urine, and feces was selective for CLA and RAN as confirmed by absence of the expected analytical signals in both blank and double blank samples of the respective matrix (Fig. 2). The peak-area ratios of the analytical signals were highly correlated to the concentration of the drugs. The coefficients of linear correlation (r) for the calibration curves of CLA were 0.9971–0.9998 for plasma, 0.9994–0.9999 for urine, and 0.9976–0.9999 for feces. The respective values for RAN were: plasma 0.9861–0.9994, urine 0.9994–0.9999 and feces 0.9988–0.9998. Therefore, the validation ranges of the assay for CLA and RAN in plasma (0.005–2.0 µg mL⁻¹) and urine and feces (0.005–10.0 µg mL⁻¹) are confirmed by the data of the linear regression analysis. The lower limit of detection (LLOD) for CLA and RAN in all matrices was 2.0 µg mL⁻¹. The newly developed assay for simultaneous quantification can be considered to be at least comparably with the sensitivity and analytical range of previous analytical methods for CLA and RAN [8, 14, 15]. The quality of the analytical assay fulfilled the international requirements. For quantitative measurements of CLA and RAN in plasma, urine and feces,
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![Graph showing concentration-time curves of clarithromycin and ranitidine](image)

**Fig. 3** Plasma concentration-time curves of clarithromycin (500 mg po, above) and ranitidine (300 mg po, below) after intravenous co-infusion (60 min) of 2 mg troleandom chloride (open circles, TC-IV) and oral medication of 30 mg troleandom chloride (closed circles, TC-PO) in one subject

intra-day and inter-day accuracy and intra-day and inter-day precision were confirmed to be within the accepted range of ±15% of the nominal concentrations and <15% for the respective coefficients of variation of the mean values. Considerable matrix effects could not be observed as concluded from the absence of significant ion suppression or enhancement, i.e., signal intensity remained nearly unchanged compared to matrix-free samples. The extraction rates of both CLA and RAN as performed using acetone/ether/water were rather identical along the entire validation range (Table 2).

The known stability of CLA and RAN during the time spans required for the analyses of all samples of a clinical study had been confirmed, i.e., rack-time stability plasma at 10 °C over a period of 8 h (in percentage of the concentrations in the initial QC samples) was between 103 and 104% for CLA and 98.9 and 106% for RAN, in urine between 101 and 105% for CLA and 91.7 and 107% for RAN, and in feces between 92.5 and 105% for CLA and 93.8 and 103% for RAN. Freeze-thaw stability in plasma ranged between 102 and 111% for CLA and 101 and 122% for RAN and in urine between 91.9 and 111% for CLA and 90.5 and 117% for RAN. Long-term stability (4 weeks) ranged between 89 and 113% for CLA and 83.4 and 119% for RAN, in urine between 88.5 and 101% for CLA and 90.5 and 118% for RAN and in feces between 92.8 and 104% for CLA and 88.5 and 110% for RAN.

**Application of the Method**

The newly developed method was used in a DDI study with TC and CLA/RAN in healthy subjects which was designed with primary intention to the influence of CLA and RAN on pharmacokinetics of TC in healthy human subjects. With a secondary intention, the effect of TC on the concentrations time profiles and basic pharmacokinetic parameters for the probe inhibitors CLA and RAN in two female healthy subjects (No. 029: 30 years, body mass index 19.6 kg m⁻², No. 012: 25 years, body mass index 25.2 kg m⁻²) were presented here to confirm the applicability of the LC-MS/MS method in ICH-GCP-conform clinical studies (Fig. 3). For the first time, our assay enabled quantification of CLA and RAN in feces. Therefore, we provide all individual fecal concentration-time profiles of CLA and RAN from the DDI study (Fig. 4). The full results of the DDI are published elsewhere.

All data of the clinical study are on file (Pfleger, Bamberg, Germany).

The pharmacokinetic characteristics of CLA and RAN after co-medication of 30 mg TC in the two selected subjects presented in Fig. 3 were rather similar to overall data in the literature without co-medication of TC [16-21]. It must be considered that intravenous infusion of 2 mg TC leads within 60 min to many times higher maximum plasma levels than the same bioavailable portion of a 30 mg immediate-release TC tablet after ~5-6 h [13]. Therefore, orally given TC seems not to be of major relevance for competition with the absorption of the probe inhibitors. On the contrary, an intravenous infusion of 2 mg TC, which results in a similar plasma exposure (AUC) to that of a 30 mg TC-tablet, marked delayed oral absorption of CLA and RAN. Bioavailability, maximum concentrations and elimination of CLA and RAN, however, were not markedly changed. Therefore, we believe that the rationale behind these findings are delay of gastric emptying and lower intestinal motility as caused by the strong muscarinic receptor blocking effects of TC [22].

Because the place of the maximum TC absorption is the distal “absorption window” in the cecum/ascending colon, the luminal concentrations of the inhibitors CLA and RAN should be of major importance for the outcome of the DDI between TC and CLA/RAN. In Fig. 4, it is shown that the maximum concentrations of both CLA and RAN in the defecated feces occur within 48 h after administration in all almost all subjects which have fasted at least 10 h before up.
to 5 h after oral administration of the drugs. The cumulative fecal excretion of RAN (300 mg, po) was 6.1 ± 13.8 mg after TC co-infusion and 3.3 ± 6.9 mg after oral TC co-medication. The respective data for CLA (500 mg po) were 1.7 ± 1.0 mg and 2.1 ± 1.3 mg (all differences were not significant). More than 90% of the fecal amount was excreted within 48 h after administration.

The whole gut transit time in healthy subjects under our study conditions accounts for −40 h (recto-cecal transit time; −4 h, colon transit time; −36 h) as measured in a former study using a radiopaque marker technique [23]. Because the colonic water content in fasting healthy subjects is less than 100 mL and does not significantly change after eating a high caloric meal [34], the fecal drug concentrations might be a surrogate for the local concentrations of the inhibitors in the “distal absorption window” for TC. The local concentrations might even be higher because CLA and RAN undergo chemical degradation in the neutral or slightly alkaline medium or by the colonic microbiota [25]. It will be highly interesting to evaluate whether both drugs can still influence under these conditions the absorption of TC in the cecum/ascending colon and whether the outcome can be predicted by determination of the inhibitor concentrations in the feces. Thus, the maximum fecal concentration of CLA after swallowing of 500 mg tablet was, in all of our subjects below the IC50 for inhibition of P-gp (8.9 ± 0.5 µM) [9]. In case of ranitidine, the maximum fecal concentrations of most subjects were below the IC50-value for inhibition of human OCT1 (~20–80 µM, dependent on the substrate) which have been published in the literature up till now [10, 26–28].
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Conclusions

The developed LC-MS/MS assay for simultaneous quantification of CLA and RAN in human matrices is in sensitivity and quality comparable with the known assays for measurement of the probe drugs simultaneously with other drugs. The assay is superior to other methods as it enables for the first time quantification of CLA and RAN in feces.

Acknowledgements

The authors thank Sabine Bade, Gitta Schamacher and Daniä Wegner for excellent technical assistance.

Compliance with Ethical Standards

Conflict of Interest

The authors declare that they have no conflict of interest.

Ethical Standards

The clinical study in healthy subjects was performed according to the ICH-guideline for Good Clinical Practice (ICH-GCP), and to the regulations of the German Medicines Act after being approved by the Independent Ethics Committee of the University of Greifswald, the German Federal Department of Drugs and Medicinal Products (BfArM), and after registration by eudraCT.eu.int (identification: EudraCT 2016-002828-69) and ClinicalTrials.gov (identifier: NCT03011463). All healthy subjects were included into the study after giving written informed consent. This work was supported by an institutional grant for the Department of Clinical Pharmacology, University Medicine Greifswald from the Dr. Pflieger GmbH, Bamberg.

References


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Eigenständigkeitserklärung

Hiermit erkläre ich, dass diese Arbeit bisher von mir weder an der Mathematisch-
Naturwissenschaftlichen Fakultät der Universität Greifswald noch einer anderen
wissenschaftlichen Einrichtung zum Zwecke der Promotion eingereicht wurde.

Ferner erkläre ich, dass ich diese Arbeit selbstständig verfasst und keine anderen als die
darin angegebenen Hilfsmittel und Hilfen benutzt und keine Textabschnitte eines Dritten
ohne Kennzeichnung übernommen habe.

11. Declaration of Independence

I hereby declare that this work has not yet been submitted by me to the Faculty of
Mathematics and Natural Sciences at the University of Greifswald nor to any other scientific
institution for the purpose of the doctorate.

Furthermore, I declare that I have written this work independently and have not used any
other means or aids other than the ones specified therein and have not taken any text
sections of a third person without identification.

______________________________________________
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12. Curriculum Vitae

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12/2003 – 07/2007 B.Sc. study (Pharmacy), School of Pharmacy, University of Gondar, Ethiopia

08/2007 – 02/2009 Research Assistant, University of Gondar, Ethiopia

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    Title: “Assessment of drug use in public and private health facilities in Southwest Ethiopia.”

06/2011 – 05/2014 Lecturer of clinical pharmacy, University of Gondar, Ethiopia

06/2014 – 04/2016 Assistant professor & Head, the School of Pharmacy, University of Gondar, Ethiopia

    Qualified as expert pharmacist (clinical pharmacy)

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__________________________________________

Bayew Tsega Abebe
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