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Research paper

Intestinal absorption of the quaternary trospium chloride: permeability-lowering factors and bioavailabilities for oral dosage forms

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Abstract

The intestinal absorption mechanism, permeability and bioavailability of the parasympatholytic trospium chloride has been investigated in vitro and in vivo in rats, in order to gain a better mechanistic explanation for the underlying cause leading to low bioavailability of quaternary compounds following peroral dosing. Permeability determinations were done in Ussing-type chambers with rat jejunum and human Caco-2 cells. In vivo bioavailability and mass balance studies were done in rats. Absorption was studied from trospium chloride solutions in saline and from w/o microemulsions and cyclodextrin complex formulations. The absorption mechanism of trospium chloride across the intestinal epithelium is rather complex and involves *P*-glycoprotein-mediated secretion and saturable binding to intestinal mucus. Trospium permeability across the intestinal epithelium increases nonlinearly with rising drug concentrations at the apical membrane. Neither the microemulsions nor the cyclodextrin formulations increased the permeability of trospium in vitro, leading to lower or equal bioavailability of these formulations in vivo as compared with the aqueous solution control. © 1997 Elsevier Science B.V.

Keywords: Absorption; Quaternary parasympatholytics; Intestinal permeability; Bioavailability; Mucus binding; Ussing-chamber; Caco-2 cells

1. Introduction

The absolute bioavailability of perorally administered quaternary parasympatholytics is low and variable. There are numerous examples for this: trospium chloride (3α-benziloyloxynortropane-8-spiro-1′-pyrrolidiniumchloride) shows an absolute bioavailability ranging from 2.9 [1] to 11% (Krumbiegel, unpublished results); ipatropium bromide; and oxitropium bromide bioavailabilities vary from 0.9 to 6.1% (mean of 3.3%) and 0.48%, respectively [2,3], for *N*-butylscopolamin an average bioavailability of only 4.7% of the perorally administered dose has been reported [4]. There are different theories with respect to the underlying causes of this phenomenon: Firstly it is reasonable to assume that drugs from this class show only a limited intestinal membrane permeability due to their positive charge and low oil/water partition coefficient. This theory is supported by the fact that following an increase in the lipophilicity of these compounds, e.g. by ion-pair formation, the intestinal permeability may increase several-fold [5]. In addition to the low diffusivity in

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lipophilic media, there may be additional mechanisms involved. Levine [6] found that quaternary compounds may form insoluble complexes with mucopolysaccharides of intestinal mucus. There are also reports demonstrating a binding of these substances to the intestinal brush border membrane [7,8]. Other theories related to the transport mechanism of quaternary compounds involve the formation of absorbable complexes together with a phosphatic fraction of the intestinal tissue, whereas Crone and Keen [9] put forward the idea of a predominantly paracellular diffusion of quaternary parasympatolytics through aqueous filled pores. An additional mechanism was proposed by Hallen et al. [10] who claimed that these compounds may permeate the intestinal membrane by forming ion-pairs with endogenous ligands. For tetramethylammonium and choline there are reports, that carrier-mediated transport processes are operative for their intestinal membrane transfer process [11,12]. It is further reasonable to assume that the intestinal transmembrane potential may modulate the transport of cationic compounds [13,14].

The aim of the present project is to investigate the intestinal permeability and absorption mechanism of trospium which—in the form of trospium chloride—is the active ingredient of peroral formulations. Further, the absorption of trospium from newly developed galenical formulations should be elucidated in vitro and in vivo to evaluate possible absorption-enhancing properties of these formulations. The formulations include two water-in-oil microemulsions (MP94-GK004, MP94-GK005) and two cyclodextrin complex formulations (MP94-GK006, MP94-GK007).

2. Materials and methods

2.1. Materials

Trospium chloride (batch # 44404), water in oil microemulsions MP94-GK004 (batch # 940424/GK) and MP94-GK005 (batch # 940604/GK) cyclodextrin complexes MP94-GK006 (batch # 940416/GK) and MP94-GK007 (batch # 940208/GK) were supplied by Madaus AG. The composition of MP94-GK004 was trospium chloride, demineralized water, PEG-8-caprylic/capricglyceride, polyglyceryl-6-dioleat and glyceroltricaprilocaprat. MP94-GK005 consisted of trospium chloride, demineralized water, PEG-8-caprylic/capricglyceride, polyglyceryl-6-dioleat and polyglyceryl-oleinglyceride. MP94-GK006 was a complex of trospium chloride and β-cyclodextrin and MP94-GK007 of trospium chloride and hydroxypropyl-β-cyclodextrin. Complexation was assumed to occur as a result of the disappearance of the trospium signal in the complex as determined by thermal analysis. The trospium content (w/w) in the formulations was 1.92% in MP94-GK004, 1.99% in MP94-GK005, 14.04% in MP94-GK006 and 12.45% in MP94-GK007. For the mass balance studies in rats, trospium chloride was specifically labelled by reduction of dehydrotrospium chloride (Amersham Buchler, Braunschweig, FRG). The specific activity was 2.35 GB/mg, the radiochemical purity 98–99%. For dilution non radioactive trospium chloride (Lot 7049, Madaus AG) was used. Radioactivity was measured using Unisolve™ 100 (Zinsser, Frankfurt, FRG) as cocktail in a liquid scintillation counter (Tri-Carb 3380, Packard, Frankfurt, FRG). Urine and bile were directly added to the cocktail. faeces and the intestinal tract were extracted with methanol. Dulbecco's modified Eagle's medium (DMEM), MEM non essential amino acids (NEAA) solution, 0.05% trypsin/0.025% ethylenediamine tetra-acetic acid (EDTA) solution, Hank's balanced salt solution (HBSS), Dulbecco's modified phosphate-buffered saline with and without Ca^{2+} and Mg^{2+} (PBS) were obtained from Gibco BRL-Life Technologies (Paisley, UK). Fetal calf serum (FCS) was purchased from PAA Labor und Forschung GmbH (Linz, A). 4′,6-diamidino-2-phenylindole-dihydro-chloride (DAPI), 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT), fluorescamine and sodium dodecyl sulfate (SDS) were obtained from Sigma (St. Louis, US). 2-[*N*-morpholino]ethane-sulfonic acid (MES) was from Fluka Chemie AG (Buchs, CH). For HPLC, solvents of HPLC quality were used. All other chemicals were of analytical grade from Fluka (Buchs, CH) and Merck (Zurich, CH).

2.2. Methods

2.2.1. In vitro studies

The intestinal permeability of trospium was investigated by measuring its transport across rat jejunum and also across Caco-2 cell monolayers, a human intestinal cell line. Rat jejunum was obtained immediately following sacrification of Wistar rats which were fasted overnight but had free access to water and freed of serosal tissue. During the preparation, the tissue was kept in an ice-cold Krebs-Ringer bicarbonate buffer (composition see below). The isolated jejunum was freed of serosal tissue. During that preparation the tissue was maintained in an ice-cold Krebs-Ringer bicarbonate buffer (composition see below). The Caco-2 cells used in this study were from passages 69 to 72. They were grown in 80 cm^2 flasks, purchased from Nunc (Roskilde, DK), at 37°C in a 5% CO_2 atmosphere using DMEM containing 20% FCS, 1% L-glutamine, 1% NEAA, 4.5 g/l glucose and 0.11 g/l sodium pyruvate. The medium was changed every other day. After having reached confluence, cells were washed with PBS and removed from the flasks by incubating the monolayers with 0.25% trypsin in EDTA (1 mmol/l) solution

for 10 min at 37°C. The cells were resuspended in medium and seeded onto polycarbonate filters, having a diameter of 1.13 cm^2 and a mean pore diameter of 0.4 μm (Snapwell cell culture chamber inserts, Costar, Cambridge, US) at a density of 100 000 cells/cm^2 for 15–18 days. Permeability measurements were performed in Ussing-type chambers, each consisting of two acrylic half-cells [15]. Following the mount of the tissue to one half-cell, the matching other half was joined to seal the diffusion apparatus and the chambers were immediately placed in an aluminum block heater. Trospium chloride was dissolved in concentrations ranging from 0.5 to 45 mmol/l in modified Krebs-Ringer bicarbonate buffer solution and was added either to the mucosal (donor solution) or the serosal side of the membrane (receiver solution). The buffer solution contained (mmol/l) sodium chloride (125), potassium chloride (5.0), calcium chloride (1.4), magnesium chloride (1.0), monobasic sodium phosphate (1.2), sodium bicarbonate (10.0), D-glucose (5.0), glutamine (5.0). The osmolarity of the solutions was adjusted to 290 ± 10 mosm with D-mannitol, the pH was adjusted to 6.5. The other half-cell of the diffusion apparatus (receiver solution) was filled with plain buffer solution without trospium. The volumes in each half-cell were 2.5 ml, the permeation area was 0.29 cm^2 for the experiments with rat jejunum and 1.13 cm^2 in the experiments with the cell cultures. The volume in each half cell was circulated by gas lift (oxycarbon, 95% O_2, 5% CO_2). As a leakage marker lucifer-yellow was used in the donor solutions at a concentration of 1 mmol/l. Samples (100–350 μl) were taken at appropriate time points with replacement of the sampled volume. Lucifer yellow was determined quantitatively in the acceptor solution by fluorimetry. The concentration of trospium chloride in donor and acceptor solutions was determined by RP-HPLC on a LiChrospher RP C-18 column (4 mm × 30 cm). The mobile phase consisted of 10 mM aqueous sodium heptanesulfonate solution and acetonitrile (40:60; v/v) to which 1.5% (v/v) ortho-phosphoric acid (85%) had been added. The flow rate was 1.0 ml/min. Detection was by measuring the UV absorbance at 210 nm. Routinely the viability of the jejunal preparation was evaluated by measuring concentrations of the leakage marker, electrophysiological parameters and D-glucose concentrations in both compartments at the end of the experimental period. A significant increase in the serosal glucose concentration was taken as one viability indicator. In addition, on-line electrophysiological recordings of tissue potential difference, short-circuit current and tissue resistance confirmed the viability and integrity of the preparation over the investigation period (120 min).

Mucus binding studies were performed at room temperature by equilibrium dialysis and by ultrafiltration. Equilibrium dialysis measurements were done in Dianorm® teflon diffusion cells (Diachema, Munich, Germany). Each half-cell had a volume of 5 ml. A cellulose membrane (Diachema) with a molecular weight cutoff of 10 kD—which allowed the diffusion of small molecules but not of intestinal mucus—separated the two compartments. One half of the diffusion cell was filled with partially purified and lyophilized mucus from porcine intestine (Mucin Typ III, Sigma), dissolved at 2% concentrations (w/v) in 0.1 M hydrochloric acid or in phosphate buffer pH 7.5 (2%, w/v), trospium chloride (0.7×10^{-6}–2.4×10^{-5} mol/l) and in some experiments with hexamethoniumbromide (1.5 mmol/l) as a cationic displacer molecule. The other half cell was filled with buffer only. The volume shift (Donnan-effect) during the dialysis period was quantified by determining the density of the mucus solution before and after the dialysis period (Paar DMA 46 Densitometer, Graz, Austria). The volume shift at 1, 2 and 3% mucus concentrations was less than 1% and was neglected in the calculations. The system was also evaluated for potential binding of trospium to its components, and for an optimum equilibration time. There was no measurable binding of trospium to the dialysis membrane and to the diffusion cell components. The dialysis experiments were run over a 2 h period after it had been shown that within that time frame a concentration equilibrium of trospium in both chambers was reached. The concentration of trospium in both cells was determined by RP-HPLC.

The ultrafiltration was performed in Microsep® microconcentrators (Skan AG, Basel) with a sample volume of 3 ml, a filtration area of 0.46 cm^2 and a molecular cutoff of 3 kD. Centrifugation was done for 4 h at 5000 rpm.

2.2.2. In vivo studies in rats

Absorption studies were performed in male rats, strain SIVZ (Institut für Zuchthygiene, Veterinärmedizinische Fakultät der Universität Zürich), weighing 300–350 g. The animal experiments were approved by the 'Veterinäramt des Kantons Zürich'. The animals were fasted 15–20 h prior to the experiment. Anaesthesia was induced by an i.m. injection of 1.5 g/kg body weight of urethane. The rats were put on a heating pad to maintain body temperature. The peritoneum was opened by a midline incision. Segments of the proximal jejunum starting approximately 15 cm caudal of the ligament of Treitz, were isolated. The trospium preparation was dissolved in approximately 0.5 ml of 0.9% saline solution (in the case of trospium chloride and the cyclodextrin complexes) or undiluted (in the case of the water in oil microemulsions) and directly injected into the jejunum (10 mg/kg). For the i.v. study, the trospium chloride dose (1 mg/kg) was dissolved in 0.9% saline solution and injected as a bolus dose into one femoral vein of the rat. Following drug dosing, approximately

200–400 μl of blood was withdrawn at different time points from a permanent jugular vein catheter and introduced into a heparinized tube to prevent coagulation. The plasma was separated by centrifugation at 2000 rpm. The red blood cells were diluted with an equal volume of physiological saline solution and injected back into the animal. At the end of the experimental period, the animals were killed by an intracardial injection of urethane. Trospium and its metabolite azoniaspironortropanol (trospium alcohol) was analyzed from plasma by HPLC [16]. For the mass balance studies, rats were anaesthetized with ether, lapratomized and a polyethylene catheter was inserted into the common bile duct. Injection of trospium was performed into the penis vein with doses of 5 or 50 μg/kg corresponding to 1.85 MBq for both doses. After suturing, the rats were placed in modified Bolman cages allowing the separate collection of urine, bile and faeces for 24 h.

2.3. Data analysis

The permeability coefficient (P_{eff}) was calculated according to the following equation:

$$P_{eff} = \frac{V\mathrm{d}C/\mathrm{d}t}{AC_0}$$

where $V\mathrm{d}C/\mathrm{d}t$ is the change in mass of drug transported per unit time in the receiver chamber, A is the surface area of the intestinal tissue or the monolayers, respectively, and C_0 is the starting concentration of the permeating species in the donor chamber.

The pharmacokinetic parameters were calculated following non-compartmental analysis. Areas under the blood concentration-time curves ($AUC_{0\rightarrow t}$) were calculated according to the linear trapezoidal rule. Extrapolated areas ($AUC_{0\rightarrow\infty}$) were calculated for the i.v. administration by dividing the calculated C_{last} by the terminal elimination rate constant (λ_z) obtained from log-linear regression analysis of the terminal three–four concentration time-points. The total plasma clearance (Cl_{tot}) was calculated by dividing the dose by the $AUC_{0\rightarrow\infty}$. Terminal half-lives were determined from log-linear regression of the three–four terminal concentration-time points. The absolute bioavailability, F, of trospium after intestinal application was calculated according to

$$F = \frac{AUC_{test}}{AUC_{iv}} \frac{D_{iv}}{D_{test}}$$

Average bioavailabilities are expressed as geometric means of ln-transformed data. Confidence intervals, 90%, were calculated for each formulation with respect to the i.v. administration following ln-transformation of the area under the curves.

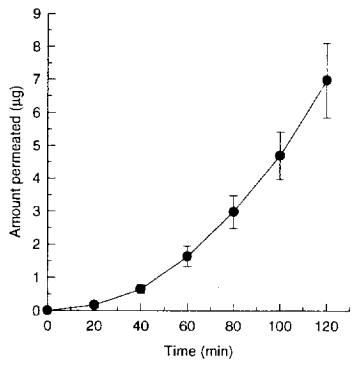

Fig. 1. Accumulation of trospium on the basolateral side in a modified Ussing chamber apparatus. The permeation across rat jejunum was in the apical to basolateral direction. The initial concentration of trospium chloride on the apical side was 7.5 mmoles/l; means ± S.E.M., $n = 12$.

3. Results

Trospium can permeate the intestinal epithelium in intact form. The analysis of the chromatographic data did not indicate biotransformation of the compound prior to or during absorption. A typical permeation-time profile is shown in Fig. 1. The concentration dependence of trospium permeability was investigated at donor concentrations ranging from 0.5–45 mmol/l. There was a clear dependence of trospium effective permeability (P_{eff}) on its donor concentration (Fig. 2). At a donor concentration of 0.5 mmol/l the permeability was 8.2×10^{-7} cm/s, whereas it increased to 2.2×10^{-6} cm/s at 45 mmol/l. The increase in permeability with concentration showed a tendency to follow a saturation profile, since permeabilities were not significantly different at donor concentrations ≥ 3 mmol/l but significantly higher than permeabilities at less than 3 mmol/l donor concentrations. In Caco-2 cells, the apical to basolateral permeability of trospium

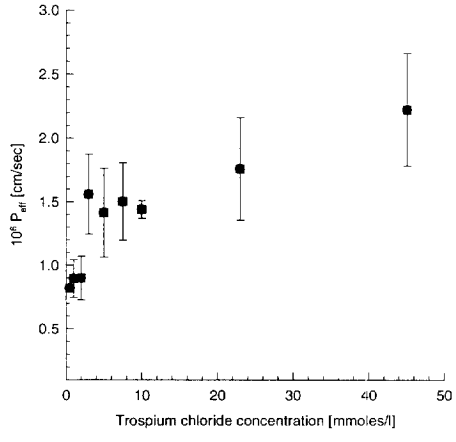

Fig. 2. The dependence of trospium permeability from the apical to the basolateral side on its apical concentration; means ± S.E.M., $n = 4–14$.

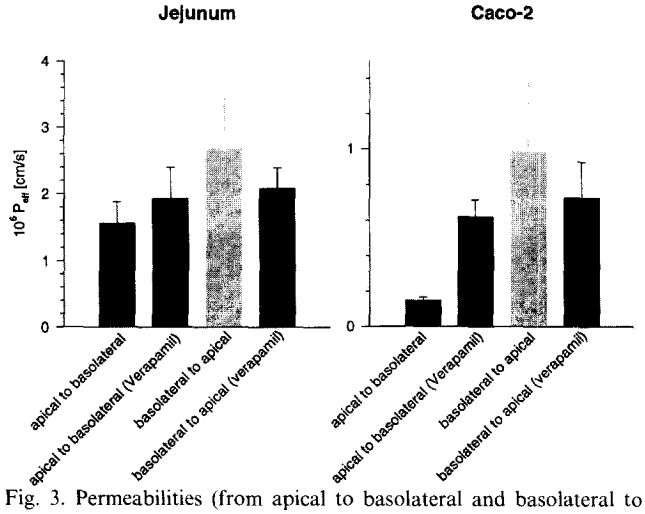

Fig. 3. Permeabilities (from apical to basolateral and basolateral to apical) of trospium in the presence and absence of verapamil in rat jejunum and Caco-2 cell monolayers. The initial apical concentration of trospium was 3 mmoles/l for rat jejunum and 5 mmoles/l in the Caco-2 cell model; means ± S.E.M., $n = 4–10$.

amounted to 1.48×10^{-7} cm/s which was roughly 10 fold lower than its permeability in rat jejunum. In both models, rat jejunum and Caco-2 cells, the transport of trospium was direction specific. Permeabilities were always and significantly higher from basolateral to apical as compared with the reverse direction (Fig. 3). This effect was very pronounced in the Caco-2 cell model, where the apical to basolateral permeability increased from 1.5×10^{-7} to 9.9×10^{-7} cm/s in the reverse direction at donor concentrations of 5 mmol/l. In the presence of verapamil, an inhibitor of the multidrug-resistance gene product *P*-glycoprotein, the permeabilites in both directions tended to equalize (Fig. 3). Additional evidence for a net intestinal secretion of trospium is given by the in vivo mass-balance studies following i.v. drug administration in bile-cannulated rats. At a dose of 5 μg/kg, 82.6% of the dose could be recovered of which 11.1% was found in the faeces and the intestinal tract and therefore directly secreted from the blood via the intestinal wall into the intestinal lumen and 25.4% in the bile (Table 1. The other main elimination pathway is via the kidney (≈ 50% of the dose). At the

Table 1
Urinary, biliary and faecal excretion and recovery rates in male rats within 24 hours after i.v. injection of 5 and 50 μg/kg trospium chloride as percentage of the given dose

	Dose 5 μg/kg	Dose 50 μg/kg
Urine	45.01 ± 15.15	47.44 ± 9.40
Faeces	2.02 ± 1.98	0.63 ± 0.51
Intestinal tract	9.06 ± 2.08	5.61 ± 0.97
Bile	25.41 ± 3.88	25.55 ± 3.16
Animal without intestinal tract	3.75 ± 1.72	3.35 ± 1.20
Sum	85.25 ± 13.80	82.58 ± 7.81

Means ± S.E.M., $n = 6$.

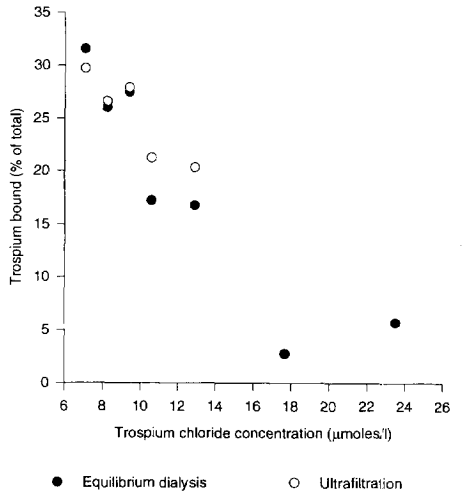

Fig. 4. Binding of trospium to gastrointestinal mucus at pH 7.5 at increasing trospium chloride concentrations. The mucus concentration was 2% (w/v). Equilibrium dialysis method and ultrafiltration gave similar results; mean data are given, CV less than 5%.

higher dose of 50 μg/kg 6.2% of the dose was found in the intestine and the faeces and 25.6% in the bile (Table 1). Another factor contributing to the nonlinear intestinal permeability of trospium is the binding of the molecule to intestinal mucus at neutral pH values, as shown in Fig. 4. The binding is saturable, since at higher trospium concentrations the percentage of mucus-bound trospium decreases. Both methods, the equilibrium dialysis and the ultrafiltration method gave similar results. The mucus binding of trospium could be partially inhibited by the addition of excess cationic hexamethonium bromide to the incubation solution. At 1.17×10^{-5} and 0.953×10^{-5} molar concentrations, 61.3 and 40.4%, respectively of the bound trospium were released at a hexamethonium bromide concentration of 1.5×10^{-2} mmol/l. There was no measurable mucus binding of trospium at pH 1.

Permeability studies of trospium from aqueous solutions and various formulations across rat jejunum showed that none of the formulations improved the permeability of trospium as compared with an aqueous solution of trospium chloride (Fig. 5). For the two microemulsions MP94-GK004 and MP94-GK005, average permeabilities of 1.5×10^{-7} and 2.1×10^{-7} cm/s were measured, as compared with a permeability of 2.2×10^{-6} cm/s for the standard aqueous solution of trospium chloride at the same concentration (45.1 mmol/l). For the two cyclodextrin complexes MP94-GK006 and MP94-GK007, average permeabilities amounted to 7.6×10^{-7} and 6.1×10^{-7} cm/s, respectively, with comparative permeabilities of 7.4×10^{-7} for the aqueous solution at the same concentration (2 mmol/l).

In the in vivo studies in rats similar although not quantitatively identical results were obtained. Follow-

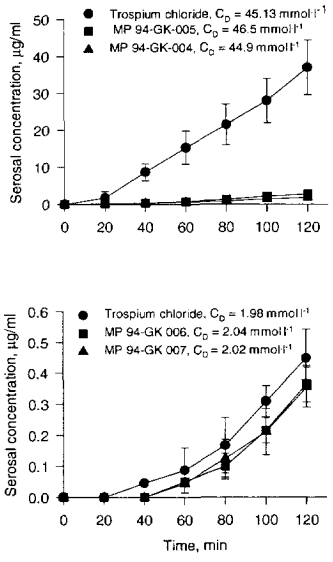

Fig. 5. Accumulation of trospium on the basolateral side in a modified Ussing chamber apparatus as a function of time. Tospium chloride solutions and trospium formulations were applied to the apical side and samples were taken from the basolateral side; data represent means ± S.E.M., $n = 4–8$; error bars are partly within symbols.

ing intravenous bolus dosing, trospium disposition showed a biphasic decline of plasma concentrations versus time. The total clearance amounted to 34.9 ± 10.8 ml/min per kg with a terminal half-life of 4.4 ± 2.2 h and a steady-state volume of distribution, V_{ss}, of 9.2 ± 4.1 l/kg (Table 2). Following intrajejunal administration, the bioavailabilities were quite variable as can be seen from the wide confidence intervals in Table 3. This phenomenon, however, is quite commonly seen with low-permeable compounds. The highest bioavailability based on a comparison of the $AUC_{0\rightarrow240}$ values was obtained with the trospium chloride reference solution (15.4%) and the β-cyclodextrin complex formulation MP94-GK006 (11.7%). The bioavailability of the other formulations were 8.5% for the glyceroltricaprilocaprat-containing microemulsion MP94-GK004, 5.6% for the hydroxypropyl-β-cyclodextrin complex MP94-GK007 and 3.6% for the β-cyclodextrin complex

Table 2
Summary of pharmacokinetic parameters of trospium in rats following i.v. bolus administration of 1 mg/kg of trospium chloride as an aqueous solution

$AUC_{0\rightarrow240}$ (ng min/ml)	17 327 ± 5006
$AUC_{0\rightarrow\infty}$ (ng min/ml)	28 608 ± 7243
λ_z regr./min	0.00253 ± 0.001095
$t_{1/2}$ regr. (min)	274.4 ± 131.4
CL_{tot} (ml/min per kg)	34.9 ± 10.8
V_{ss} (l/kg)	9.2 ± 4.1

Means ± S.E.M., $n = 3–4$.

Table 3
Absolute bioavailability of trospium in rats following intrajejunal administration of a trospium chloride aqueous solution (saline), a trospium-β-cyclodextrin complex (MP94-GK006), a trospium-hydroxypropyl-β-cyclodextrin complex (MP94-GK007) and two microemulsion formulations MP94-GK004 and MP94-GK005

Formulation	AUC (ng/ml per min)	F^a (%)	CI[b] (%)
Saline	26731 ± 11469	15.4	4.8–49.9
MP94-GK004	14729 ± 1880	8.5	4.4–16.2
MP94-GK005	6152 ± 1047	3.6*	1.8–7.0
MP94-GK006	20264 ± 22940	11.7	2.4–57.4
MP94-GK007	9873 ± 2979	5.6	2.4–13.8

Geometric means ± S.E.M., $n = 3–4$. The trospium dose was 10 mg/kg for all formulations.
[a] Geometric mean bioavailability based on $AUC_{0\rightarrow240}$ data.
[b] 90% confidence interval for $\frac{AUC_{test}}{AUC_{iv}}\frac{D_{iv}}{D_{test}}$ following logarithmic data transformation.
* Significantly different from the saline reference formulation ($P < 0.05$).

MP94-GK005. The confidence intervals of these absolute bioavailabilities are given in Table 3. The absorption rates of trospium from the formulations were slow and thus correspond well to the observed low intestinal permeability of trospium.

4. Discussion

In this study the intestinal absorption of trospium was characterized in vitro and its absolute bioavailability from an aqueous solution, which amounts up to 11% in humans, was compared with two w/o microemulsions and two cyclodextrin complex formulations in rats. The in vitro permeability of trospium across rat jejunum was in the range of 10^{-6} cm/s which supports the interpretation that the low trospium bioavailability in vivo is mainly due to its limited permeability across the intestinal epithelium. Atenolol, a biotransformation-stable β-receptor antagonist for example, shows five-fold higher permeabilities in the in vitro model and a bioavailability of 50 in humans. Some of the transport barriers responsible for the low intestinal permeability of trospium have been elucidated in this study. Trospium may be bound to gastrointestinal mucus which reduces the effective concentration gradient of unbound drug across the intestinal membrane. The nature of this interaction is presumably of electrostatic origin and the cation trospium may interact with the negatively charged carboxylic groups of the mucus-composing sialic acids and can also be competitively displaced by excess concentrations of other cations. This may also serve to explain the observation that at low pH the fraction of trospium bound to mucus is negligible.

An additional explanation for the low trospium permeability across the intestinal epithelium may be derived from the unfavourable physicochemical properties of the molecule—log partition coefficient between *n*-octanol and buffer at pH 7.4: −1.22; solubility in water: > 50 mg/ml at RT; solubility in light mineral oil: 9.2×10^{-3} mg/ml [5]—which might prevent its efficient uptake into the lipophilic membranes of the enterocytes and thus reduces the effective permeation area for trospium in the intestine to the area of the paracellular space.

An additional mechanism, which has recently been discovered to be limiting the intestinal permeability of a variety of drug molecules also seems to interfere with an efficient intestinal absorption of trospium [17–19]. The fact that trospium apical to basolateral permeability was increasing to a saturation level and that its transport from the basolateral to the apical side was significantly higher than in the reverse direction in Caco-2 cells and in rat jejunum and equalized in the presence of verapamil points to the involvement of an intestinal verapamil-sensitive secretory process mediated by *P*-glycoprotein (*P*-gp). *P*-gp is a 170 kD plasma membrane protein found in apical membranes of enterocytes and in Caco-2 cells [20,21] as well as in bile-cannalicular membranes and in brain endothelial cells and tumor cells showing the multidrug-resistance phenomenon. The characteristic feature of this ATP-dependent system is that it secretes drugs back into the intestinal lumen as soon as they have entered the enterocyte. As this intestinal secretion system begins to saturate at higher drug concentration, the overall permeability of the secreted drug thus, increases with possible increases in bioavailability [18]. Once secretion is totally saturated, passive diffusion dominates and a new equilibrium is reached with pseudo-linear permeability now evident. Another indicator for the *P*-gp-mediated secretion of trospium observed in vitro is its secretion into the bile and into the intestine in vivo. It is well known today that the *P*-gp-secretory protein is expressed at the cannalicular membrane of the hepatocytes as well as in the brush-border membrane of enterocytes, thus facilitating hepato-biliary as well as intestinal secretory transport of compounds with affinity towards *P*-gp. This may be taken as a mechanistic interpretation for the disposition of trospium. In addition to the fact that trospium permeability differed between the rat jejunum and the Caco-2 monolayer, the ratio of the apical to basolateral and basolateral to apical permeability was significantly greater in the Caco-2 system, which may be due to the overexpression of *P*-gp in the cell cultures as compared with the jejunum. The low permeability of trospium across the intestinal epithelium was also reflected in the in vivo studies in rats where generally slow input rates of trospium were found following its jejunal administration.

Neither the microemulsions nor the cyclodextrin complex formulations showed an improved permeability or bioavailability of trospium in vitro and in vivo. In vitro, the permeability of trospium from the two microemulsion formulations decreased from 2.2×10^{-6} to 1.5×10^{-7} and 2.1×10^{-7} cm/s for MP94-GK004 and MP94-GK005, respectively. In vivo, the bioavailability of trospium from these formulations was 8.5 and 3.6% and reached only 55 and 23% of the bioavailability of the control solution. The difference observed in both model systems may be attributed to the more complicated situation in vivo, where the presence of surface active bile salts and enzymes may additionally modify the behaviour of the formulation in the intestine. For the two cyclodextrin formulations the permeability of trospium changed from 7.4×10^{-7} (saline solution) to 7.6×10^{-7} and 6.1×10^{-7} cm/s for MP94-GK006 and MP94-GK007, respectively. In vivo, the bioavailability of trospium from these formulations was 11.7 and 5.6% and reached 75.8 and 36.9% of the bioavailability of the control solution.

5. Conclusions

Trospium chloride undergoes a complex absorption mechanism in the intestine involving intestinal secretion and reversible mucus binding. Its overall permeability across the intestinal epithelium is low and could not be significantly improved by microemulsion and cyclodextrin complex formulations, thus providing a mechanistic explanation for its low bioavailability in man.

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