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Determination of the Bioavailability of the Quaternary Compound Trospium Chloride in Man from Urinary Excretion Data

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Dedicated to Professor Dr. Herbert Oelschläger on the occasion of his 65th birthday

Summary: *For the quaternary compound trospium chloride (Spasmex®) which is used as an anticholinergic agent a new sensitive assay method has been developed that allows the quantitative determination of the drug in human urine and plasma. Using this method it was possible to obtain pharmacokinetic data from plasma levels and urinary excretion, and to determine the bioavailability in man. Quantitative determination is performed by alkaline hydrolysis to the corresponding spiroalcohol, ion-pair extraction with dipicrylamine, subsequent derivatization with the fluorophor benoxaprofen chloride, and ion-pair chromatographic separation on a reversed-phase column with chloride as the counter-ion using a mixture of acetonitrile and water. In healthy volunteers (n = 6) the plasma concentration time curve after intravenous administration of 0.5 mg trospium chloride could be described by an open two-compartment model. The mean half-lives were 2.7 and 97 min, respectively. After oral administration of 10 mg the highest concentration found in plasma was 1.4 ng trospium chloride/ml. 55% of the given dose were excreted unchanged into urine within 48 h after i.v. administration, the corresponding value after oral administration was 1.6%. If no hydrolysis is carried out in urine samples the spiroalcohol can be detected as metabolite of trospium. Within 48 h after i.v. administration 4% and after oral administration 0.3% of the given dose are excreted into urine as spiroalcohol. From the cumulative excretion of trospium into urine within 48 h a mean bioavailability of 2.9% was calculated.*

Zusammenfassung: *Bestimmung der Bioverfügbarkeit der quartären Verbindung Trospiumchlorid aus der Urinausscheidung beim Menschen*

Für das Anticholinergikum Trospiumchlorid (Spasmex®) wurde ein empfindliches Analysenverfahren entwickelt, mit dem es möglich war, pharmakokinetische Daten aus den Konzentrations-Zeit-Kurven im Plasma und der Ausscheidung im Urin zu erhalten und daraus die Bioverfügbarkeit beim Menschen zu bestimmen. Zur quantitativen Bestimmung wird die Substanz zum entsprechenden Spiroalkohol (alkalisch) hydrolysiert, der als Ionenpaar mit Dipicrylamin extrahiert und anschließend mit dem Fluoreszenzmarker Benoxaprofenchlorid derivatisiert wird. Die chromatographische Trennung erfolgt durch Hochdruckflüssigkeitschromatographie mit einer Reversed-phase-Säule und Chlorid als Gegenion in einem Acetonitril-Wasser-Gemisch. Die Plasmakonzentrations-Zeitkurven von gesunden Probanden (n = 6) nach i.v. Gabe von 0,5 mg Trospiumchlorid ließen sich durch ein offenes zwei-Kompartiment-Modell beschreiben. Die durchschnittlichen Halbwertszeiten betragen 2,7 bzw. 97 min. Die höchste gefundene Konzentration im Plasma nach oraler Gabe von 10 mg Trospiumchlorid betrug 1,4 ng/ml. 55% der applizierten Dosis wurden nach i.v. Gabe innerhalb von 48 h unverändert im Urin ausgeschieden, der entsprechende Wert nach oraler Gabe lag bei 1,6%. Erfolgt bei der analytischen Bestimmung keine Hydrolyse, so kann der Spiroalkohol als Metabolit von Trospium im Urin nachgewiesen werden. Innerhalb von 48 h nach i.v. Gabe wurden 4% und nach oraler Gabe 0,3% der applizierten Dosis als Spiroalkohol ausgeschieden. Aus der kumulativen Trospium-Ausscheidung im Urin wurde eine durchschnittliche Bioverfügbarkeit von 2,9% errechnet.

Key words: *Anticholinergics · Spasmex® · Spiroalcohol chloride, bioavailability, clinical studies · Trospium chloride, bioavailability, clinical studies*

1. Introduction

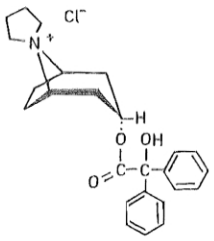
The quaternary compound trospium chloride (Spasmex®[2]; see formula diagram) is used as an anticholinergic agent. It has atropine-like effects, effects in the ganglion and a direct effect in the muscle-fibre, the latter being as strong as that of papaverine. The spasmolytic activity is predominant. Other anticholinergic effects are of little or no importance (central effects) [1].

The drug was postulated to be mainly excreted as the corresponding "spiroalcohol" (see formula diagram) [2]. As the compound and its metabolite could not be determined in plasma and urine until recently, the bioavailability was estimated from pharmacodynamic properties. Albrecht et al. [3] found an oral bioavailability of 5–10% using the so-called pupillometry.

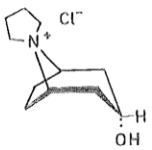
It is also known from other quaternary compounds that they are only poorly absorbed from the human gastrointestinal

[1] Part of the Ph.D. thesis of G. Schladitz-Keil.

[2] Manufacturer: Dr. R. Pfleger Chemische Fabrik GmbH, Bamberg (Federal Republic of Germany).



trospium chloride



spiroalcohol (chloride salt)

tract. The oral bioavailability of ipratropium bromide is for example 12.9% [4]. The intestinal absorption of ^{3}H-N-butyl scopolamine bromide in man appears to amount to a maximum of 10%, the urinary excretion within three days was found to be 1.7 ± 0.3% of the given dose [5].

As the doses of trospium chloride are low, plasma and urine concentrations were expected to be low, too. Therefore, a new sensitive procedure for the quantitative determination of trospium and its metabolite in urine (and plasma) was developed by our group [6] containing hydrolysis, ion-pair extraction, and fluorescent derivatisation with benoxaprofen chloride.

Using this method it was possible to determine the concentrations of trospium and the corresponding spiroalcohol in human urine after intravenous and oral administration and thus to determine the bioavailability of this quaternary compound from urinary excretion data. Plasma levels of trospium in man after i.v. and oral administration were also investigated.

2. Experimental

2.1. Chemicals

The reference compounds trospium chloride and spiroalcohol chloride were supplied by Dr. R. Pfleger Chemische Fabrik GmbH. Benoxaprofen was made available by Eli Lilly (Bad Homburg, FR Germany). Benoxaprofen chloride was synthesized according to [7].

Solvents (analytical grade or LiChrosolv®) and reagents were obtained from E. Merck (Darmstadt, FR Germany). Dipicrylamine contains 50% of water. Ingotest® water (Boehringer Ingelheim KG, Ingelheim/Rhein, FR Germany) was used for HPLC.

2.2. Instruments

A chromatograph LC 601 with a fluorescence detector 605–10 S and a recorder 56 (all three instruments from Perkin Elmer, Überlingen, FR Germany) were used for HPLC.

The analytical columns (12.5 cm × 4.6 mm ID) were purchased from Dr. Herbert Knauer (Bad Homburg, FR Germany) and filled with LiChrosorb RP-8 material, particle size 5 µm (for urine samples), and Nucleosil C-8 material, particle size 5 µm (for plasma samples).

3. Analytical methods

3.1. Urine

3.1.1. Determination of total compounds (trospium and spiroalcohol)

5 ml of urine were mixed with 1 ml of 1 mol/l sodium hydroxide solution and kept at 140–145 °C for 90 min. After cooling 0.2 ml of 25% HCl and 0.6 ml of dipicrylamine solution (32.9 mg dipicrylamine + 10 ml 0.1 mol/l NaOH) were added. After centrifugation (10 min, 5000 rpm) 5 ml of this mixture were extracted into 4.5 ml of chloroform and centrifuged (20 min, 6000 rpm). 4 ml of the organic phase were re-extracted into 2.3 ml of 0.1 mol/l HCl. After centrifugation 2 ml of the aqueous phase were mixed with 2 ml of methanol and evaporated to dryness in a vacuum centrifuge. The residue was washed several times with methanol (6–7 ml in total) by evaporating the methanol to dryness under a nitrogen stream. Then 0.2 ml of benoxaprofen chloride solution (10 mg benoxaprofen chloride + 1 ml of dried acetonitrile) was added and heated in a glycerin bath (140–145 °C) for 30 min. The solvent was evaporated in vacuum and the residue was dissolved in 1 ml of water and 1.2 ml of ethyl acetate. After shaking and centrifugation the organic phase was discarded. Again the aqueous phase was extracted with 1 ml of ethyl acetate. 1 ml of the aqueous phase was evaporated to dryness.

The residue was dissolved in 100 µl of water-acetonitrile (60/40, v/v). Injection volume: 20 µl; column temperature: 50 °C; flow rate: 2 ml/min (105 bar = 10.4 MPa); mobile phase: mixture of water-acetonitrile (60/40, v/v) containing 0.08 mol/l sodium chloride and 0.031 mol/l choline chloride, to 1 l of this mixture 10 ml of 1 mol/l HCl were added; detection: excitation wavelength 313 nm, emission wavelength 370 nm; retention time of the derivative: 6 min.

3.1.2. Determination of spiroalcohol

5 ml of urine were mixed with 0.6 ml of dipicrylamine solution (32.9 mg dipicrylamine + 10 ml 0.1 mol/l NaOH + 200 mg dried Na_2CO_3) and treated in the same way as described for total compounds.

3.2. Plasma

5 ml of plasma were hydrolyzed for 105 min as described for urine. After cooling to about 40 °C 0.2 ml of 25% HCl were added and centrifuged for 10 min (5000 rpm). Again the mixture was heated up for 10 min (120 °C) and centrifuged after cooling. 5 ml of the hydrolyzed plasma were mixed with 0.5 ml of dipicrylamine solution (98.7 mg dipicrylamine + 10 ml 0.1 mol/l NaOH + 600 mg dried Na_2CO_3). 5 ml of this mixture were extracted into 4.8 ml of chloroform and centrifuged (30 min, 6000 rpm). The further extraction and derivatisation procedure was the same as described for urine samples except one step: the amount of methanol to wash the residue after re-extraction was 10–11 ml in total. After derivatisation the residue was dissolved in 100 µl of water-acetonitrile (69/31, v/v). This mixture was also used for the mobile phase containing the same amounts of salts and HCl as described for urine samples. Injection volume, flow rate (91 bar = 9.0 MPa), and detection were the same as for urine samples; column temperature: 55 °C; retention time of the derivative: 7.5 min.

3.3. Subjects and procedure

The study was carried out with a group of six healthy male volunteers. The average age of the group was 24.7 years (22–27 years). The average weight was 73.3 kg (65–80 kg).

After collection of blank urine and blank plasma samples 10 mg trospium chloride (2 tablets à 5 mg) were administered orally with water. 1 h after administration a light breakfast was taken.

Urine was collected during 48 h at the following intervals: 0–2, 2–4, 4–6, 6–8, 8–10, 10–12, 12–24, 24–36, and 36–48 h. The urine fractions were tested for pH-value and total volume. 50 ml portions were stored at –20 °C until analysis.

12 ml of blood were taken at the following times: 20, 40, 60, 120, 180, 240, 360, and 480 min after administration. The actual times of sampling were noted on the record sheets. The blood samples were collected in heparin treated tubes and the plasma was separated by centrifugation. The samples were stored at –20 °C until analysis.

After a wash-out period of at least three days, 0.5 mg trospium chloride (Spasmex Injektionslösung, Dr. R. Pfleger Chemische Fabrik GmbH) were administered intravenously.

Again, urine was collected. Collection periods were identical with those described for oral administration. Plasma was collected at the following times after administration: 5, 10, 20, 30, 60, 120, 180, and 240 min.

3.4. Data analysis

From the amounts of trospium, spiroalcohol, and total compounds excreted into urine during the different collection periods, the cumulative urinary excretions could be calculated. The cumulative amount of trospium excreted after 48 h ($Ae_{48\ h}$) was used to determine the absolute bioavailability of trospium. The values are expressed as arithmetical mean ($\bar{x}$) and standard deviation.

Maximal plasma concentrations (C_{max}) observed after oral administration were determined.

If possible terminal half-lives were estimated by linear regression analysis including the values of the terminal log-linear phase; from the slope of this phase (λ_z) $t_{1/2}$ was calculated. $T_{1/2\lambda l}$ was determined using the residual method. The constants C_l, λ_l, and C_z, λ_z were also estimated using the BMDP (Software Development, Middlebury, USA) program (two-compartment open model) on a DEC 10 (Digital Equipment Corp., Maynard, MA, USA).

Other pharmacokinetic parameters (area under the curve: AUC, total clearance: CL, and renal clearance: CL_R) were calculated for total compounds according to the following equations [8]:

$$t_{½\lambda l} = \frac{0.693}{\lambda_l};$$

$$t_{½} = \frac{0.693}{\lambda_z};$$

$$AUC\ (0-\infty) = \frac{C_1}{\lambda_1} + \frac{C_2}{\lambda_2}$$

$$CL = \frac{dose}{AUC\ (0-\infty)}\ ;$$

$$CL_R = \frac{Ae\ (48\ h)}{AUC\ (0-48)}\ .$$

4. Results

Cumulative urinary excretion data within 48 h for each volunteer and administration are given in Table 1. Fig. 1 illustrates the mean cumulative urinary excretion curves of trospium, spiroalcohol, and total compounds after i.v. and oral administration.

Table 1: Cumulative urinary excretion of trospium, spiroalcohol, and total compounds within 48 h after administration of 0.5 mg trospium chloride i. v. and 10 mg trospium chloride orally (values calculated as trospium chloride and spiroalcohol chloride).

Volunteer	Trospium		Spiroalcohol		Total
	μg	nmol	μg	nmol	nmol
I. v.					
1	257.44	601.5	15.20	69.8	671.3
2	227.51	531.6	14.18	65.1	596.7
3	263.03	614.6	8.82	40.5	655.1
4	393.05	918.4	6.63	30.4	948.8
5	274.53	641.4	7.58	34.8	676.2
6	251.87	588.5	8.74	40.2	628.7
Oral					
1	136.23	318.3	30.25	138.9	457.2
2	141.10	329.7	29.22	134.2	463.9
3	171.85	401.5	3.08	14.2	415.7
4	181.16	423.2	7.77	35.7	458.9
5	96.69	225.9	5.73	26.3	252.2
6	219.47	512.8	17.92	82.3	595.1

There is no significant difference between the excretion of trospium and total compounds after administration of 0.5 mg trospium chloride i.v. and after 10 mg orally, whereas the differences in the excretion of spiroalcohol are statistically significant (non-parametric Wilcoxon matched-pairs signed rank test) [9]. The average amount of metabolite found after oral administration is higher than after i.v. administration and amounts 15.80% of total compounds excreted into urine. The range is 3.4 to 30.4%. The corresponding value for i.v. administration is 7.12% (range 3.2–10.9%).

Bioavailability (F) was determined from the trospium excretion into urine within 48 h. From the data that are given in Table 2, a mean bioavailability value of 2.91% was calculated. If F is determined from the total amount of compounds excreted into urine within 48 h a mean bioavailability value of 3.25% results.

After oral administration the highest concentration (C_{max}) found in plasma was 1.4 ng trospium (calculated as chloride) per ml plasma (volunteer 6). Most of the maximum concentrations were already in the range of the detection limit (0.5–1 ng trospium chloride/ml plasma). Therefore it was not possible to quantify trospium at all sampling times. Thus the plasma concentration time curves are incomplete.

5 min after i.v. administration a mean plasma concentration of 15.7 ng trospium chloride/ml plasma was found (range: 7.8–20.1 ng/ml).

Some pharmacokinetic parameters (C_1, λ_1, C_2, λ_2, $t_{1/2\lambda 1}$, $t_{1/2}$, AUC $(0-\infty)$, CL, and CL_R) estimated for the plasma concentration time curves after i.v. administration are summarized in Table 3. If half-lives were determined using the residual method the following values result:

E.g.: volunteer 4: $t_{1/2\lambda 1} = 5.5$ min; $t_{1/2} = 222$ min
volunteer 6: $t_{1/2\lambda 1} = 5.0$ min; $t_{1/2} = 108$ min

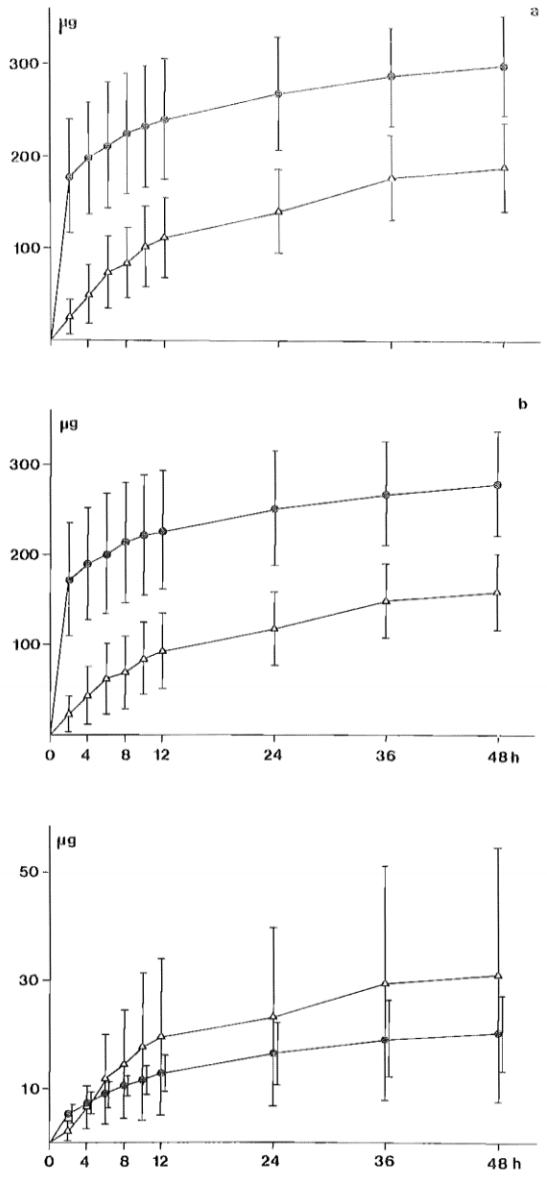


Fig. 1: Mean cumulative urinary excretion curves of total compounds (a), trospium (b), and spiroalcohol (c) after administration of 0.5 mg trospium chloride i.v. (●————●) and 10 mg orally (△————△); all compounds calculated as trospium chloride equivalents.

Table 2: Bioavailability values (F) for trospium chloride (TrCl) and total compounds (t.c.) for oral administration of 10 mg trospium chloride from cumulative urinary excretion within 48 h.

Volunteer	$F_{(TrCl)}$ (%)	$F_{(t.c.)}$ (%)
1	2.65	3.41
2	3.10	3.89
3	3.27	3.17
4	2.30	2.42
5	1.76	1.87
6	4.36	4.73
$\bar{x}$	2.91	3.25
SD	28.19	31.46

The arithmetical means of the half-lives found are 2.7 and 97 min, the mean total clearance amounts to 1405.3 ml/min and is significantly higher than the mean renal clearance which is 806.6 ml/min.

Plasma concentration time curves obtained from volunteers after i.v. and oral administration are shown in Fig. 2. As the curves are incomplete, especially after oral administration, the absolute bioavailability could only be determined from urine and not from plasma data.

Table 3: Pharmacokinetic parameters from the plasma concentration-time curves after i.v. administration of 0.5 mg trospium chloride.

Volunteer	C_1 (ng/ml)	λ_1 (h^{-1})	C_2 (ng/ml)	λ_2 (h^{-1})	$t_{1/2\lambda 1}$ (min)	$t_{1/2}$ (min)	AUC (0–∞) (ng ml^{-1} h)	CL (ng/ml)	CL_R (ng/ml)
1	46.0	16.4316	2.2	0.4264	2.5	96	8.0	1041.7	598.6
2	28.5	19.0118	1.7	1.4128	2.2	30	2.7	3086.4	1576.5
3	43.4	12.1587	2.1	0.4162	3.4	102	8.6	969.0	543.4
4	92.0	20.0706	1.6	0.4299	2.1	96	8.3	1004.0	815.4
5	49.9	19.5115	1.5	0.3749	2.1	108	6.6	1262.6	730.9
6	39.9	11.1073	1.2	0.2888	3.7	144	7.8	1068.4	574.9

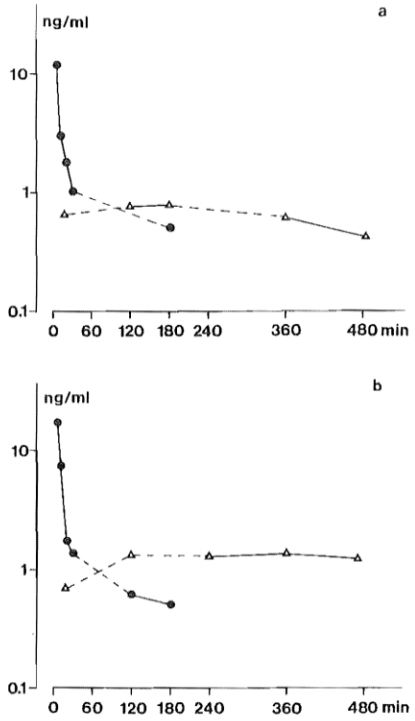


Fig. 2: Plasma concentration time curves after i.v. (●——●) administration of 0.5 mg and oral (△——△) administration of 10 mg trospium chloride. **a)** Subject 5; **b)** subject 6.

5. Discussion

The investigations clearly show that trospium is mostly excreted unchanged into urine. The concentrations found in plasma are "total concentrations", as the components can only be quantified if the plasma is hydrolyzed; i.e., the portion of spiroalcohol in plasma remains unknown. However, it is supposed to be rather low, because of the low urinary excretion rate of this metabolite, especially after i.v. administration. The concentration time curve of total compounds in plasma after i.v. administration can be described by an open two-compartment model. The oral bioavailability of about 3% for trospium chloride is in the same range as the one described for other quaternary compounds (e.g. scopolamine N-butyl bromide).

In a recent investigation an oral dose of 5 mg trospium chloride thrice daily was shown to be effective in patients [10]. These results agree with those obtained from the pharmacokinetic investigations. An oral bioavailability of 2.9% means that 0.29 mg reach the systemic circulation, if 10 mg are administered, and 0.145 mg, if 5 mg are administered. The spasmolytic activity of trospium chloride was found to be four times higher than that of atropine sulfate [1]. 0.145 mg trospium chloride would therefore be equivalent to 0.58 mg of atropine sulfate, which is an effective dose.

6. Literature

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