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Short communication

Identification of mebeverine acid as the main circulating metabolite of mebeverine in man

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Abstract

The intestinal spasmolytic drug mebeverine is known to undergo fast in vivo enzymatic hydrolysis into mebeverine alcohol and veratric acid. A reversed-phase HPLC method with coulometric detection was developed in order to assay the hitherto unidentified secondary metabolite mebeverine acid. After intake of a single oral dose of 405 mg mebeverine hydrochloride in four healthy human volunteers, peak plasma concentrations of mebeverine acid were found to be 1000-fold higher than those of mebeverine alcohol, i.e. ≈ 3 μg/ml versus 3 ng/ml. The appearance of mebeverine acid in plasma (median $T_{max} = 1.25$ h) as well as its disappearance (median apparent $t_{1/2} = 1.1$ h) were rapid. The urinary excretion of mebeverine acid within the first 4 h after dosing amounted to 67% of the mebeverine dose (median range: 23–107%). Mebeverine acid appears to be a valuable marker of oral exposure to mebeverine.
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1. Introduction

The spasmolytic drug mebeverine was already recognised over 30 years ago [1] in the treatment of irritable bowel syndrome (IBS) and has since been the subject of many clinical investigations [2–5]. Chromatographic methods for the determination of mebeverine, mebeverine alcohol and veratric acid have been reported [7–12]. The latter two are the direct products of enzymatic hydrolysis of mebeverine, which proceeds readily in vivo and in vitro [9]. In vitro hydrolysis in human plasma was completely inhibited by physostigmine [9], but another esterase inhibitor, pyridostigmine, was ineffective in vivo [6]. In man, no mebeverine was detected after oral administration, but appreciable concentrations of veratric acid were found [7,9]. Other animal and human data seem to support the fact that negligible concentrations of unchanged mebeverine are present in the systemic circulation after oral administration [7,9] except perhaps in the rabbit [10]. *O*-

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Demethyl-mebeverine alcohol was identified in human plasma [12] and in urine [11], together with further biotransformation products. The metabolic fate of veratric acid was studied in more detail: *O*-demethylation gave vanillic acid and isovanillic acid, which subsequently led to protocatechuic acid on *O*-demethylation [11]. The metabolic route via veratric acid could be accounted for almost quantitatively (97.6% of the dose), whereas the fate of the mebeverine alcohol moiety remained unclear (5.5% of the dose) [11]. Recently [13], a detailed mass spectrometric analysis of hepatic microsomal incubation extracts and of human urine samples allowed the identification of an additional seven biotransformation products of mebeverine alcohol resulting from *N*-deethylation, *N*-dehydroxybutylation, ring hydroxylation and conjugation. However, these metabolites were not quantified and were assumed to be part of the missing 94.5%.

In the present human study, preliminary data on the plasma pharmacokinetics and urinary excretion of mebeverine alcohol and its oxidation product mebeverine acid are described. A key element was the extraction of the ampholyte mebeverine acid at neutral pH, while mebeverine alcohol and its derivatives have to be extracted under alkaline conditions.

2. Experimental

2.1. Clinical protocol

Four healthy male subjects volunteered for the study. Their mean age was 32 years (range: 19–37), mean body weight 77 kg (range: 72–83). All gave their informed consent prior to the start of the study. The protocol and informed consent procedures were approved by the Institutional Ethical Committee prior to the start of the study. The clinical phase was conducted in compliance with the latest revision of the Declaration of Helsinki. The four volunteers received a single oral dose of 405 mg of mebeverine hydrochloride (3×135 mg Mebeverine PCH® tablets), which corresponds to the usual daily dose, with 200 ml of water and after an overnight fast. Venous blood samples were obtained prior to dosing and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12 and 24 h post-dose. Urine was collected in fractions during 24 h. Blood samples were centrifuged within 15 min and the plasma was kept frozen at −20 °C until submitted to analysis. Urine samples were also stored at −20 °C.

2.2. Measurements

Mebeverine, mebeverine alcohol and mebeverine acid were determined by HPLC, according to a modification of Hoogewijs' method [7,8]. Analytical grade mebeverine hydrochloride was obtained from Pharmachemie B.V. Mebeverine alcohol was readily prepared by hydrolysis of mebeverine in boiling aqueous alkali [7]. Mebeverine acid was synthesized using described methods [14]: ethyl-[2-(4-methoxyphenyl)-1-methyl-ethyl] amine was converted into its *N*-malonyl ethyl derivative by ethyl malonyl chloride, which was reduced by lithium aluminium hydride into ethyl-(3-hydroxypropyl)-[2-(4-methoxyphenyl)-1-methyl-ethyl]amine. The latter was reacted with thionyl chloride to yield the corresponding 3-chloropropyl derivative. Finally, Grignard reaction with carbon dioxide gave mebeverine acid, which was crystallized from boiling chloroform. Mebeverine acid had a melting point of 131 °C (dec.). Its identity was determined by NMR, IR and MS. TLC and HPLC showed no impurities.

For the determination of mebeverine and mebeverine alcohol in plasma and urine [8], 1 ml samples were mixed with 20 μl of 1 μg/ml desipramine in water as internal standard, alkalinized with 0.1 ml of 25% ammonia and extracted by 2×8 ml of *n*-hexane, using silanized glassware. The combined extracts were reduced to dryness under vacuum, the residue was taken up in 200 μl of mobile phase and 100 μl was injected. Separation was achieved on a 25 cm × 4.6 mm, 5 μm particle size cyanopropylsilica column (Merck), using a mobile phase made of 50% acetonitrile and 50% 10 mM phosphoric acid adjusted to pH 6.0 with isobutylamine. Detection was performed by coulometry in the screen mode

(ESA Coulochem 4100 A detector: $E_g = 0.8$ V, $E_1 = 0.40$ V and $E_2 = 0.70$ V versus proprietary reference electrode). Quantification ranges were 10–100 ng/ml in plasma and in urine for mebeverine and 1–20 ng/ml in plasma and 10–500 ng/ml in urine for mebeverine alcohol.

For the determination of mebeverine acid in plasma or urine, 1 ml aliquots were mixed with ≈ 0.5 g of solid NaCl, 0.25 ml of 1 M pH 7 phosphate buffer and 10 μl of yohimbine 5 or 200 μg/ml methanolic solution (internal standard), respectively. The samples were extracted by 8 ml of chloroform, the organic phases were evaporated to dryness under vacuum and the residues were taken up in 250 μl of mobile phase and 100 μl was injected. Adequate separation was obtained on a 25 cm × 4 mm Nucleosil 120-5-C18 column (Macherey-Nagel), using a mobile phase consisting of 16% acetonitrile and 84% 50 mM phosphoric acid brought to pH 4.5 with isobutylamine. For plasma, detection was performed by coulometry in the screen mode ($E_g = 0.9$ V, $E_1 = 0.5$ V, $E_2 = 0.85$ V). For urine, UV detection was used at a wavelength of 226 nm. The quantification range was 20–1000 ng/ml in plasma and 0.5–150 μg/ml in urine. Samples above the upper limit of the quantification range were reassayed after dilution in blank matrix.

2.3. Calculations

Time to peak (T_{max}) and maximum plasma concentration (C_{max}) were obtained from the experimental data without interpolation. Areas under the plasma concentration versus time curves were computed up to the last measurable time point (AUCt) using the linear trapezoidal rule. Apparent elimination rate constants (λz) were computed as the slope of the terminal part of the log concentration versus time curves. Apparent terminal elimination half-lives ($t_{1/2}$) were then obtained as $\ln(2)/\lambda z$. Cumulated urinary excretion data were computed as the sums of the products of urinary concentration and volume in each urinary fraction. They were expressed in percent of the administered dose (Ae%), taking into account the molecular weight of each metabolite.

3. Results and discussion

The assay methods were validated in plasma but not in urine. For mebeverine and mebeverie alcohol, total accuracy and precision (4 days, five replicates per day) ranged between 106 and 99% and between 13 and 9% across the respective calibration ranges. Stability was ascertained in frozen plasma (5 months at −20°C) and in processed samples (4 days in the autosampler at room temperature).

For mebeverine acid, intra-day accuracy and precision were 97 and 17.3% at 20 ng/ml and 108 and 11.8% at 1000 ng/ml, respectively ($n = 4$). Total accuracy and precision (4 days, five replicates per day) reached 98 and 11.6% at 25 ng/ml and 101 and 9.7% at 500 ng/ml, respectively. The slope and intercept of the regression line (weighting scheme $= 1/x^2$) were 0.0034 ± 0.0003 and 0.021 ± 0.02, respectively (mean ± S.D., $n = 4$). As shown in Fig. 3, adjusting the sample pH between 5 and 7 was crucial to ensure a stable and efficient extraction yield of amphoteric mebeverine acid and internal standard yohimbine.

The enzymatic hydrolysis pathway of the ester linkage of mebeverine was already known [7,8] and it was suspected that mebeverine alcohol generated in vivo by the action of esterases could be further oxidised by the mixed oxidase system yielding mebeverine acid, which could explain the very low plasma concentrations of the alcohol. Synthesis of mebeverine acid made analysis of this compound in plasma and urine possible. Plasma and urine levels of mebeverine, mebeverine alcohol and mebeverine acid were determined in four subjects after a single oral dose of 405 mg of mebeverine hydrochloride. Pharmacokinetic data are summarised in Table 1. Unchanged mebeverine was undetected in plasma as well as in urine. Mebeverine alcohol was detected in the plasma of three subjects and reached a median C_{max} of 3 ng/ml. Its cumulated urinary excretion over 24 h represented 0.02–0.3% of the dose. These findings were consistent with previous reports [9–13]. On the other hand, mebeverine acid plasma levels were three orders of magnitude higher: a median C_{max} of 2.88 μg/ml (range: 0.78–5) was rapidly obtained 1–1.5 h after administration. The area

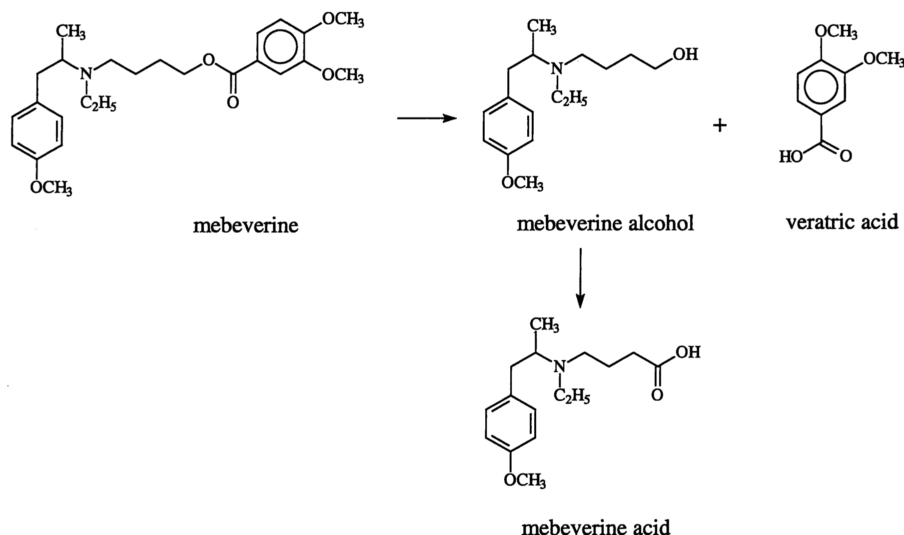

Fig. 1. In vivo hydrolysis of mebeverine into mebeverine alcohol and veratric acid and the subsequent oxidation of mebeverine alcohol to mebeverine acid.

under the plasma curve exhibited an important inter-subject variability (median: 8.22 and range: 2.24–11.4 μg·h/ml). The apparent plasma elimination half-life was short and identical in all four subjects within a narrow range (0.96–1.3 h). The cumulated urinary excretion of mebeverine acid was rapid and extensive, but variable among subjects: It already amounted to 43% of the dose after 2 h, 67% after 4 h and reached 80% after 24 h. The in vivo biotransformation scheme of mebeverine is summarized in Fig. 1. Globally, these results confirm that mebeverine does not enter the systemic circulation at detectable levels after a single dose. Mebeverine undergoes fast hydrolysis to mebeverine alcohol and veratric acid. The metabolic fate of veratric acid has been described previously and its excretion has been shown to account for nearly 100% of the administered dose [11]. The results presented here document the fast conversion of mebeverine alcohol into mebeverine acid by oxidation probably mediated by the hepatic mixed oxidase system, the acid being subject to rapid systemic clearance and amounting also for most of the dose recovered in the urine. The renal clearance of mebeverine acid (Cl_R = Ae/AUC) was of the order of 390 ml/min, indicating active tubular secretion. It is also noteworthy that subject two exhibited the lowest plasma levels of mebeverine alcohol and the highest levels of mebeverine acid, while in subject three, the highest concentrations of mebeverine alcohol were correlated with the lowest levels of mebeverine acid.

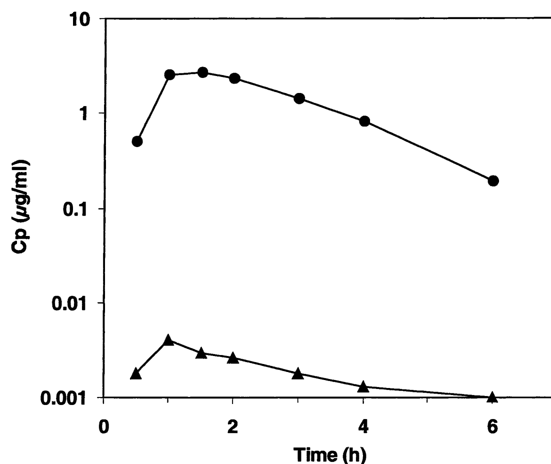

Fig. 2. Median plasma concentration versus time profile of mebeverine acid (circles) and mebeverine alcohol (triangles) observed in four healthy human volunteers after a single intake of an oral dose of 405 mg of meverine hydrochloride.

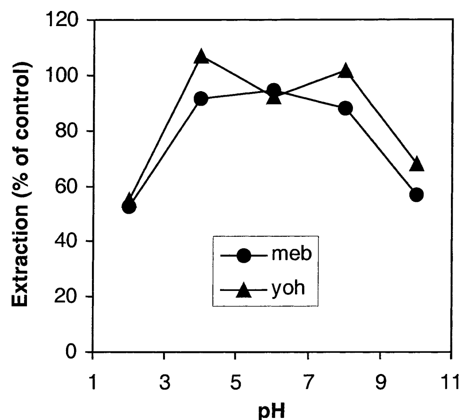

Fig. 3. Effect of sample pH on extraction yield of mebeverine acid and yohimbine (internal standard).

Mebeverine acid was successfully detected under the present experimental conditions due to neutral pH extraction conditions, while mebeverine and mebeverine alcohol derivatives require alkaline pH, as used in previous investigations [7–13]. Further, in the mass spectrometric study [13] of mebeverine biotransformation products, positive ionization conditions were used, while negative ionization would probably be more suitable for mebeverine acid.

Mebeverine itself shows clear antispasmodic activity, but its metabolites mebeverine alcohol and veratric acid do not seem to possess any action on smooth muscles and non-myelinated nerve fibres [15–17]. Mebeverine administered i.m. exhibits a stronger effect on colon motility in man than when given via the oral route [1]. The possibility that mebeverine is absorbed in patients after repeated dosing cannot be excluded. The absorbed amount of unchanged mebeverine is, however, expected to be low. In addition, the possibility that other metabolic products display some antispasmodic activity cannot be ruled out. It has been proposed that mebeverine exerts its effect locally in the colon [18].

4. Conclusion

The extraction at neutral pH and the chromatographic conditions described in this report permit-

Table 1
Pharmacokinetic parameters of mebeverine, mebeverine alcohol and mebeverine acid, after oral administration of 405 mg of mebeverine hydrochloride to four healthy human volunteers

	Subject No.				Median	Range
	1	2	3	4		
*Mebeverine**						
Plasma (ng/ml)	<10	<10	<10	<10		
Urine (ng/ml)	<10	<10	<10	<10		
Mebeverine alcohol						
C_{max} (ng/ml)	4.9	<1	11.2	1.1	3.0	<1–11.2
T_{max} (h)	2	–	1	1	1	1–2
AUC (0-t) (ng·h/ml)	20.4	<1	24.4	0.57	10.5	<1–24.4
Ae (0–24 h) (% dose)	0.11	0.03	0.32	0.02	0.07	0.02–0.3
Mebeverine acid						
C_{max} (μg/ml)	4.02	5.00	0.78	1.73	2.88	0.78–5.0
T_{max} (h)	1	1	1.5	1.5	1.25	1–1.5
AUC (0-t) (μg·h/ml)	11.4	11.2	2.24	5.22	8.22	2.24–11.4
$T_{1/2}$ (h)	1.3	1.1	0.96	0.99	1.1	0.96–1.3
Ae (0–2 h) (% dose)	61	74	15	26	43	15–74
Ae (0–4 h)	83	107	23	52	67	23–107
Ae (0–24 h)	102	132	26	58	80	26–132

* Non detectable.

ted the isolation and quantification of a hitherto unknown major metabolite of mebeverine resulting from hydrolysis into mebeverine alcohol followed by oxidation to the corresponding carboxylic acid. This metabolite appears rapidly in plasma in high concentrations and is rapidly cleared in the urine. The proposed mebeverine marker should be applicable to human pharmacokinetic studies on mebeverine, as shown in Table 1 and in Fig. 2.

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