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Lack of bioavailability of mebeverine even after pretreatment with pyridostigmine

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Abstract *Objective*: After the oral administration of mebeverine to animal or human, measurable concentrations of the drug have never been found in the plasma. The ex vivo hydrolysis of mebeverine can be blocked by esterase inhibitors. In the present study, human volunteers were pretreated with pyridostigmine to attempt to improve the bioavailability of the parent drug.
Methods: Following a single-blind, random design, 12 normal human volunteers received orally either placebo or 60 mg pyridostigmine, followed 2 h later by 405 mg mebeverine. Blood samples were drawn intermittently for 4 h and were spiked immediately with neostigmine in order to block ex vivo hydrolysis.
Results: Even after pretreatment with pyridostigmine, the plasma samples failed to reveal detectable concentrations of mebeverine. Pyridostigmine pretreatment mediated a significantly higher peak concentration of veratric acid, the acid moiety resulting from hydrolysis of mebeverine.
Conclusion: As mebeverine seemingly undergoes complete presystemic hydrolysis, it seems unlikely that the effects of the drug could be mediated centrally. Furthermore, as it is unlikely that sufficient mebeverine traverses the intestine to exert a local effect on the colon (i.e., the time-course of veratric acid plasma levels does not support such a conclusion), the therapeutic effect of the drug, if any, has to be ascribed to an active metabolite. However, the hydrolysis products of mebeverine are not known to be pharmacologically active.

Key words Bioavailability, Mebeverine, Pyridostigmine, Veratric acid

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Introduction

Mebeverine is an antispasmodic agent that has been used for 3 decades in the treatment of irritable bowel syndrome (IBS). There are no published data on its pharmacokinetics in humans, and it is possible that mebeverine is not bioavailable due to rapid and extensive presystemic hydrolysis in the gut and/or the liver [3]. When administered i.v., mebeverine decreases heart rate by 5–33% of the resting rate [1], but this has never been described for oral administration. Furthermore, the hydrolysis products of mebeverine are not known to be pharmacologically active [3]. The esterase inhibitor physostigmine sulfate effectively blocks ex vivo hydrolysis of mebeverine to veratric acid in fresh human plasma [3].

The aim of the present study was to ascertain whether pretreatment with pyridostigmine, another esterase inhibitor, would prevent the biotransformation of mebeverine, thereby making the parent drug bioavailable.

Materials, methods and subjects

Twelve healthy ambulatory volunteers, six male and six female, were the subjects. None had cardiac, hepatic, endocrine, or renal disease. The protocol was approved by the ethics committee of the University of Pretoria and the volunteers gave their informed consent.

A single-blind, randomized, two-way crossover study was followed. After an overnight fast, volunteers received either placebo or 60 mg pyridostigmine (Mestinon, Roche), followed 2 h later by 405 mg mebeverine (Colofac, Solvay). A baseline blood sample was obtained immediately before and at 10, 20, 30, 60, 120 and 240 min after oral administration of mebeverine. Immediately after sampling, 100 µl of a 500-$\mu g \cdot ml^{-1}$ solution of neostigmine (Prostigmin, Roche) was added to the 7-ml blood samples to block ex vivo hydrolysis of mebeverine by plasma esterases. A neostigmine concentration as low as 1$\mu g\ ml^{-1}$ inhibited the in vitro hydrolysis of mebeverine by more than 90%.

Sample preparation and assay method

Blood was collected into non-heparinized tubes and centrifuged for 15 min at 2000 *g*. Plasma was decanted and then precipitated in an

equal volume of acetonitrile. The precipitate was removed first by centrifuging at 1000 *g* and then in Pettendorff tubes at 10 000 *g* prior to injecting the sample directly onto the HPLC column system.

HPLC solvents were Chromasolv HPLC grade from Riedl de Haën. Heptafluorobutyric acid (HFBA) and veratric acid were from Sigma. The liquid chromatograph was a Hewlett Packard system consisting of a 1050 pump system and manual injector with a loop of 100 µl, a 1050 multiple-wavelength detector, a 1046A programmable fluorescence detector and a HPLC2D Chem-Station data analysis system. The column used was a Nova Pak C18, 3.9 × 150 mm (Waters, Mass., USA) fitted with a Spherisorb S10 ODS2 precolumn (Phase Separations, Conn., USA). An isocratic ion-pair method, with 50 $mmol \cdot l^{-1}$ hepafluorobutyric acid in 50% acetonitrile in water, as mobile phase, was used at ambient temperature. Mebeverine and veratric acid were detected by fluorescence (exitation at 246 nm and emission at 317 nm wavelength). The mobile phase was ultrasonicated for 15 min and degassed with helium during use. The minimal detectable concentration of spiked mebeverine in plasma using this technique was 0.2 $ng \cdot ml^{-1}$. The coefficient of variation for mebeverine was 11.5% among different days.

Statistical analysis

The veratric acid concentrations in serum were plotted against time and t_{max} and C_{max} were estimated by visual methods. The Wilcoxon matched-pairs test was used to compare groups with one another. Changes within each individual and not between them were compared. As this is a test for non-parametric data, the means and standard deviations were used for graphic display only and were not used for statistical analysis.

Results

Analysis of the plasma samples from the 12 fasted volunteers failed to reveal detectable concentrations of mebeverine, even after pretreatment with pyridostigmine. However, veratric acid, the acid moiety resulting from hydrolysis of mebeverine, achieved considerable concentrations (Fig. 1). As ex vivo hydrolysis in the blood and plasma samples was prevented by the addition of the esterase inhibitor neostigmine, these results suggest that the ester functional group of mebeverine was rapidly and extensively hydrolyzed in vivo.

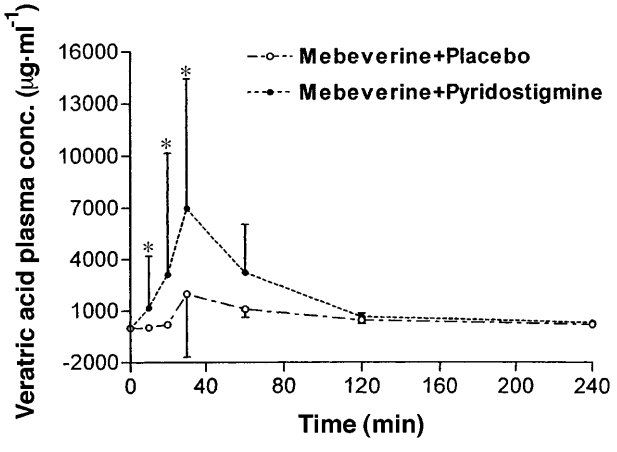

Fig. 1 Mean with SD plasma concentrations of veratric acid over 240 min. *Significantly different from mebeverine plus placebo pretreatment ($P \leq 0.03$)

Table 1 Mean (SD) maximum concentration C_{max} and time to reach C_{max} (t_{max}) of veratric acid

Treatment	Mebeverine + placebo	Mebeverine + pyridostigmine
C_{max} ($ng \cdot l^{-1}$)	2.5 (3.5)	9.5* (8.5)
t_{max} (min)	51.8 (14.0)	50.9 (28.1)

*Significantly different from mebeverine plus placebo pretreatment ($P = 0.036$)

Mebeverine with pyridostigmine pretreatment mediated a significantly higher peak concentration of veratric acid than mebeverine without pretreatment (Table 1).

Discussion

The results of the present study show that mebeverine is completely metabolized following oral administration, as no unaltered mebeverine is found in the plasma. This is in agreement with the results reported by Dickinson et al. [3] and Kristinsson et al. [5]. The gut and/or liver have been suggested to be the most probable sites of hydrolysis [5]. The pyridostigmine-induced faster rise in veratric acid plasma levels, probably caused by an increase in gastric and intestinal motility, does not help to pinpoint the specific site of the rapid presystemic hydrolysis of mebeverine.

It has been suggested that the effects of mebeverine might be explained by the local action of some unabsorbed drug on the bowel [3]. This is supported by the finding that infusion of mebeverine into the sigmoid colon of healthy volunteers stopped the postprandial increase in the contractions recorded with multiple manometric sensors situated in the rectosigmoid region [2]. The time-course of veratric acid plasma levels after oral intake of mebeverine makes it unlikely that sufficient drug traverses the intestine to exert a luminal effect in the colon; i.e the T_{max} of veratric acid is approximately 50 min, while the mean ORO caecal transit time is about 6 h.

It has been reported that over one third of patients with IBS benefit from placebos [8]. There are few controlled studies on mebeverine and these were conducted with relatively small numbers. Two of the studies indicating that the therapeutic effect of mebeverine is superior to placebo [1, 7] have been criticized because of the statistical methods used [4]. In a double-blind trial with 80 patients and a treatment period of 16 weeks, there was no significant difference between the symptomatic effect of mebeverine and placebo [6].

To conclude, no measurable plasma concentrations of mebeverine were found after oral administration to human volunteers, even after pretreatment with pyridostigmine. As it is unlikely that sufficient mebeverine traverses the intestine to exert a local effect on

the colon, the therapeutic effect of the drug, if any, has to be ascribed to an active metabolite. However, the hydrolysis products of mebeverine have never been known to be pharmacologically active.

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