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Plasma concentrations and excretion of zipeprol in man under acidic urine conditions

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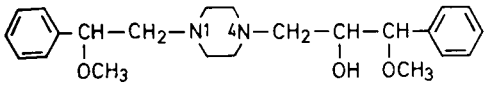
Plasma concentrations of zipeprol, and the urinary excretion of it and two of its basic metabolites, compound II and compound III were examined after oral administration of zipeprol hydrochloride to man. The drug was rapidly and extensively metabolized, and the amount of unchanged drug excreted in the acidic urine varied from 1–5% of the dose; the two basic metabolites accounted for 19–38% of the dose.

Zipeprol (Respilene) (I) is a new, non-opiate, antitussive agent. In animals, it did not cause any respiratory or blood pressure depression (Cosnier, Hache & others, 1976). It also has slight antihistaminic and bronchospasmolytic properties (Rispat, Burgi & others, 1976).

The metabolism of the drug has been reported (Beckett & Achari, 1977), but the present study investigates the quantitative aspect of its excretion and that of two of its *N*-dealkylated products, compounds II and III, in man.



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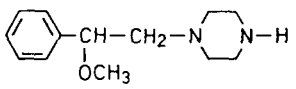

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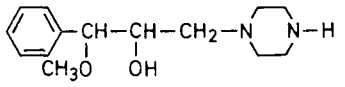

III

MATERIALS AND METHODS

Gas liquid chromatography (g.l.c.). A Perkin-Elmer Model F.11 gas chromatograph equipped with dual flame ionization detector was used. Columns 1 m, 3 mm i.d. glass tubing. Air and hydrogen pressures were 175 and 140 kNm^{-2}, respectively. Column A: 3% XE60 on 80–100 mesh Chromosorb G (HP); column temperature, 220°; injection port temperature, 300°; nitrogen, 110 kNm^{-2} (1·25 $cm^3\ s^{-1}$). The column was silanized *in situ* with HMDS. Column B: 0·2% Carbowax 20M on 60–80 mesh DMCS treated glass beads; column and injection port temperatures, 135° and 175°, respectively, for compound II and 175° and 200°, respectively for compound III; nitrogen, 70 kNm^{-2} (0·95 $cm^3\ s^{-1}$).

Volunteer study. Three healthy male volunteers were used. They were not allowed to take alcoholic beverages or any other drug and refrained from smoking during the trial. The urine was maintained acidic (pH 4·8 ± 0·2) by the administration of enteric coated ammonium chloride tablets (Lilly) (see Beckett, 1966; Beckett & Brookes, 1967; Beckett, Salmon & Mitchard, 1969; Testa & Beckett, 1974). Zipeprol hydrochloride (175 mg) was administered orally as an aqueous solution (70–80 ml). A repeat trial was performed on subject 3 with a 200 mg dose. Urine samples were taken half hourly for 2 h, 1 hourly for 6 h, then 2 hourly for 6 h, and then at convenient intervals until the completion of the 24 h trial. The exact time of voiding and the volume and pH of the urine was noted.

Blood samples were taken at the following intervals: 0·25, 0·75, 1·25, 1·75, 2·5, 3·5, 4·5, 5·5, 6·5 and 7·5 h, after the administration of the drug; each sampling time was the mean of the urine collection times before and after the time of blood collection. Plasma was separated by centrifugation and the cells were washed with isotonic sodium chloride solution. 'Blank' blood and urine samples were taken before the administration of drug and all samples were stored at 4° until analysis.

Analysis of zipeprol in urine. 5·0 ml of urine was transferred into a glass centrifuge tube, 0·5 ml of sodium hydroxide (20%) and 7 ml of freshly distilled ether was added. The tube was shaken gently (5 min), centrifuged (2 min) to clarify the layers and the ether layer was withdrawn into an evaporating tube containing 1·0 ml of internal standard (0·2 μmol^{-1} of antazoline in n-pentane). The extraction was repeated with further 2 × 7 ml of ether and the

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extracts were combined. The ethereal extract was concentrated in a water bath (45°) and with nitrogen to a small volume (20 μl). An aliquot (3 μl) was examined on g.l.c. column A. The peak height ratio of zipeprol to antazoline was calculated and the amount of zipeprol was determined with reference to the previously constructed calibration curve. A 'blank' extraction was carried out in a similar manner.

Analysis of zipeprol in plasma. 1·0 ml of plasma was diluted with 3 ml of water, 0·5 ml of sodium hydroxide (20%) and 2 g of sodium chloride (to obtain a clear ethereal extract) was added. The mixture was extracted as described above. In this case, however, 0·5 ml (0·1 μmol ml^{-1} of antazoline in n-pentane) internal standard was used and the ethereal extract was concentrated to about 10 μl.

Analysis of (metabolites) compounds II and III in urine (separate portions of urine were extracted for each compound). To 5·0 ml of urine in a glass centrifuge tube was added 0·5 ml of sodium hydroxide (20%) and 1·0 ml of internal standard (0·1 μmol ml^{-1} of benzylamphetamine HCl for compound II and 0·1 μmol^{-1} of mepivacaine HCl for compound III). The aqueous phase was saturated with sodium chloride and extracted with 4 × 7 ml of freshly distilled ether as described under the analysis of zipeprol in urine. An aliquot (3 μl) of the concentrated ethereal extract was examined on g.l.c. column B at the appropriate instrument conditions.

RESULTS AND DISCUSSION

When zipeprol was extracted with ether from water, urine and plasma at a basic pH (12–13), 3 extractions were sufficient for its complete extraction. For compounds II and III however, complete extraction was also achieved at pH 12–13 when the aqueous phase was saturated with sodium chloride and extracted 4 times with ether.

Linear and reproducible calibration curves were obtained in the range 0·0001–0·004 μmol for the drug in plasma, and in the range 0·02–0·6 μmol for the drug and compounds II and III in urine. Regression analysis of the data gave correlation coefficients of not less than 0·997 for the plasma curves and not less than 0·999 for the urine curves. Intercepts for all the calibration curves were very small and the curves almost passed through the origin.

The absolute recovery of the drug was ascertained by comparing its calibration curve from water with that from the alcoholic solution of the base; these were identical.

The urinary excretion rates (Figs 1 and 2) of zipeprol and its metabolites, compounds II and III were influenced by changes in urine flow rates and in urine pH despite the effort to keep a controlled pH below 5·0. The influence of urine pH on the excretion rates of zipeprol and compounds II and III can be

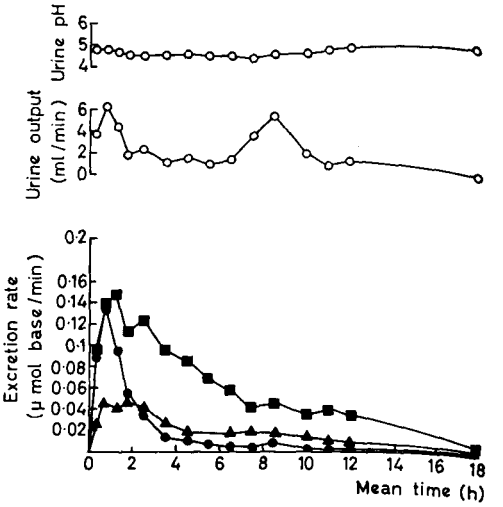

FIG. 1. Urinary excretion of zipeprol (●) and its metabolites, compound II (▲) and compound III (■) after oral administration of 175 mg of zipeprol hydrochloride as an aqueous solution to subject 1 under acidic urine conditions.

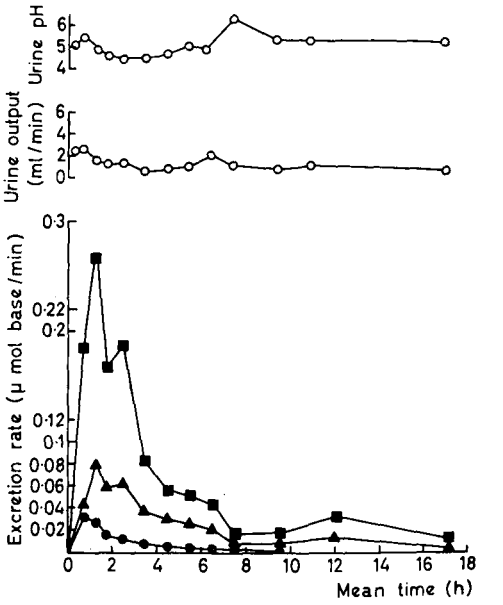

FIG. 2. Urinary excretion of zipeprol (●) and its metabolites, compound II (▲) and compound III (■) after oral administration of 175 mg of zipeprol hydrochloride as an aqueous solution to subject 3 under acidic urine conditions.

seen clearly from Fig. 2. After 7·5 h the urine pH increased to 6·2 and the excretion rates fell sharply. This would mean reabsorption of the compounds back into the circulation, and further metabolism. It also indicates that under uncontrolled urine pH, unchanged drug would not probably be detected in the urine. Hence, if the excretion data were to be used for the evaluation of the rates of metabolism and of the recovery of the unchanged drug, the urine pH must be closely controlled.

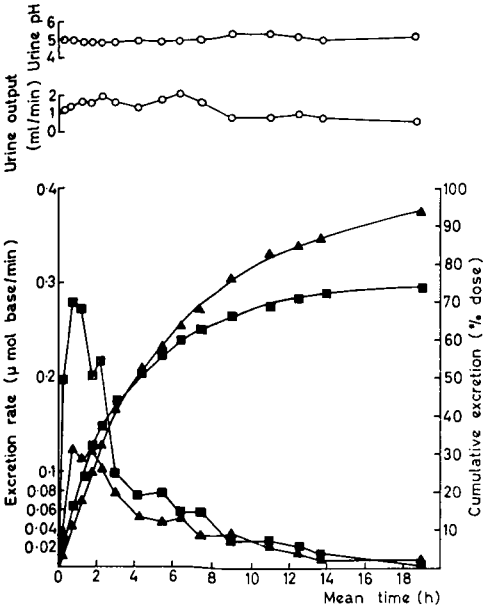

FIG. 3. Urinary excretion of compound II (▲) and compound III (■) after oral administration of an aqueous solution of a mixed dose of 12 mg of compound II and 27 mg of compound III (both as hydrochloride) to subject 2 under acidic urine conditions.

The excretion rate of zipeprol was maximum about 1 h after its administration; it then diminished exponentially. The peak excretion rates for both compound II and compound III occurred about 1–1·5 h after the administration of drug. Zipeprol

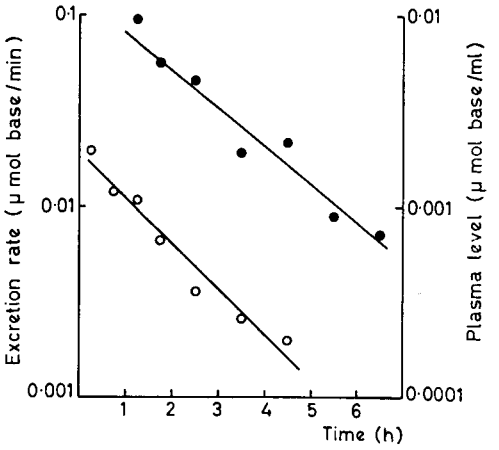

FIG. 4. Plasma concentration (○) and urinary excretion (●) of zipeprol after oral administration of 175 mg of the hydrochloride as an aqueous solution to subject 2 under acidic urine conditions.

was extensively metabolized and was rapidly eliminated from the body. The 24 h urinary recovery based on the unchanged drug and compounds II and III varied from 21–43% (Table I); 1–5% of the dose was recovered unchanged, 4–13% as compound II and 14–25% as compound III.

After an oral dose of zipeprol, the urinary output of compound III was always greater than that of compound II (Table 1). This could result either from the faster metabolism of compound II, or from the preferential oxidation of the exocyclic carbon atom α to the nitrogen 1 giving more of compound III. The α-carbon oxidation leads to unstable carbinolamines which are converted to the amine and aldehyde. However, when subject 2 was given orally a mixed dose of compounds II and III under acidic urine control, very little metabolism of these compounds were observed and about 95% of compound II and 74% of compound III was recovered unchanged in the urine (Fig. 3). These results indicate that possibly the exocyclic carbon atom α to the

Table 1. *Plasma concentration of zipeprol, and the urinary excretion of drug and two of its metabolites, compounds II and III after an oral dose of zipeprol hydrochloride as aqueous solution under acidic urine conditions.*

Subject	Sex	Age	Weight (kg)	Dose (mg)	Max. plasma concn of drug (μmol ml^{-1})	% excreted in urine over 24 h period			% dose recovered
						Drug	Compound II	Compound III	
1	M	25	66	175	0·0005 (0·19 μg ml^{-1})	4·2	4·5	14·4	23·1
2	M	30	67	175	0·002 (0·76 μg ml^{-1})	5·2	12·8	25·0	43·0
3	M	26	77	175	not detected	1·0	5·7	15·0	21·7
				200		3·0	6·7	16·3	26·0

nitrogen 1 was oxidized preferentially, rather than that α to the nitrogen 4.

The plasma concentration of zipeprol was low in subjects 1 and 2, and it was not detected in the plasma of subject 3. Low plasma concentration suggests that the drug was extensively metabolized in the liver or gut wall before entering the general circulation. However, the urinary excretion rate of the drug was proportional to its plasma concentration as shown by the almost parallel relation (Fig. 4) between the plasma concentration of drug and its urinary excretion rate.

Zipeprol was not detected in the red cells (analysed in the same way as the plasma).

Since the amount of unchanged drug was small, and compounds II and III were produced directly and rapidly, the bioavailability of zipeprol can be followed by measuring the urinary excretion rates of either compound II or compound III (Beckett & Hossie, 1969).

Acknowledgements

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