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Note

Determination of two metoprolol metabolites in human urine by high-performance liquid chromatography

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Pharmacokinetic studies in man have demonstrated that the β-blocker metoprolol is eliminated from the body mainly in the form of metabolites; only a minor fraction of the given dose is recovered as unchanged drug in urine [1]. Four urinary metabolites were identified (compounds I—IV in Fig. 1) and quantified after administration of a single dose of ^{3}H-labelled metoprolol [2]. Two of them, α-hydroxymetoprolol (I) and O-desmethylmetoprolol (II) possess β_1-receptor blocking activity in the cat [2] and dog [3] but are five to ten times less potent than the parent drug. Metabolite I, whose plasma concentrations are about 50% of those of metoprolol in healthy subjects [4, 5], accounts for 10% of the dose in urine. Metabolite II is rapidly oxidized to an inactive amino acid (III) and recovered in only minor amounts in human body fluids [2, 4, 5]. The other two metabolites, III and IV, have no pharmacological activity; they account, respectively, for about 65% and 10% of an oral dose [2].

Several methods have been described for the assay of the active metabolite (I) in plasma or urine [6—10].

Metabolite III is highly water-soluble so that extraction from biological fluids cannot be achieved. The only method available involves three evaporation steps and two derivatization stages before gas chromatography [5]. Its limit of quantitation is 0.5 μg/ml of urine.

We describe a simpler high-performance liquid chromatographic (HPLC) method for the major metabolite, III, involving direct injection of diluted urine. It allows the assay also of α-hydroxymetoprolol which is the major biotransformation product in plasma. The applicability of the method is demonstrated.

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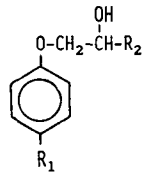

COMPOUND	R_1	R_2	RENAL ELIMINATION (% OF DOSE) (2)
METOPROLOL	$CH_2-CH_2-O-CH_3$	$CH_2-NH-CH(CH_3)_2$	⩽ 5
METABOLITE I α-HYDROXY-METOPROLOL (H 119/66)	$CH(OH)-CH_2-O-CH_3$	$CH_2-NH-CH(CH_3)_2$	10 (ACTIVE)
METABOLITE II O-DESMETHYL-METOPROLOL (H 105/22)	CH_2-CH_2-OH	$CH_2-NH-CH(CH_3)_2$	< 0.4 (ACTIVE)
METABOLITE III (H 117/04)	CH_2-COOH	$CH_2-NH-CH(CH_3)_2$	60 - 65 (INACTIVE)
METABOLITE IV (H 104/83)	$CH_2-CH_2-O-CH_3$	COOH	10 - 15 (INACTIVE)

Fig. 1. Chemical structure of metoprolol and of its known metabolites.

EXPERIMENTAL

Chemicals and reagents

Metoprolol tartrate was supplied by Ciba-Geigy (Basle, Switzerland). Metabolites I—IV were supplied by Hässle (Mölndal, Sweden). All solvents and reagents were of analytical grade. Solution A for the mobile phase was made of 2.9 mmol (238 mg) of anhydrous sodium acetate and 40 mmol (2.3 ml) of acetic acid in 1 l of water (pH 3.5 buffer).

Reference solutions

These were prepared in water from the hydrochloride of III and the *p*-hydroxybenzoic acid salt of I.

Sample preparation

A 0.5-ml volume of urine is diluted to 20 ml with distilled water. A 1-ml aliquot of the diluted urine and 100 μl of water or of aqueous reference solutions are mixed in a glass tube; 60 μl are injected onto the column.

Chromatography

The chromatography was performed on a Hewlett-Packard instrument, Model 1081 B, equipped with a variable-wavelength detector (Schoeffel SF 770) set at 222 nm.

The column was a stainless-steel tube (25 cm × 4.7 mm I.D.) filled with LiChrosorb RP-8, 5 μm (E. Merck, Darmstadt, F.R.G.). The slurry, made of 3.6 g of LiChrosorb (preliminarily dried at 110°C for 2 h) dispersed in a mixture of 10.8 ml of *n*-heptane and 10.8 ml of isopropyl alcohol, was forced into the column with *n*-heptane. The column was rinsed with 50 ml of ethanol before use. The degassed mobile phase acetonitrile—solution A (70:30, v/v) was used at a flow-rate of 1 ml/min. The mobile phase and the column were at room temperature.

The retention times were about 10 and 13 min for metabolites III and I, respectively. The top pressure was about 90 bars.

Calibration curves

Calibration samples were prepared by adding 100 μl of reference solutions containing both metabolites I and III as described in the sample preparation procedure. The added amounts corresponded to concentrations ranging from 16.5 μmol/l (5 μg/ml) to 329.5 μmol/l (100 μg/ml) for metabolite III and from 11.9 μmol/l (5 μg/ml) to 237.5 μmol/l (100 μg/ml) for metabolite I.

The calibration curves were obtained by plotting the peak height of each metabolite versus the concentrations. Their equations were calculated by the least-squares method using linear regression. For routine analysis, a calibration curve is established every day from five to seven calibration samples, each sample being injected once.

RESULTS AND DISCUSSION

Urine interferences

Metabolites I and III are well separated from the urine components. Urine from several volunteers was tested. A typical chromatogram of blank urine and spiked urine is shown in Fig. 2.

Accuracy, precision, reproducibility, limit of quantitation

Metabolites I and III can be measured with good precision and accuracy at concentrations down to 5 μg/ml (11.9 μmol/l for I and 16.5 μmol/l for III) (Table I).

Selectivity

The parent drug metoprolol and the other known metabolites (Fig. 1) did not interfere in the assay of metabolites I and III.

The acidic metabolite (IV) was eluted in the solvent front. The relative retention times were 1, 1.19, 1.77 for III, I and metoprolol, respectively. Two peaks were observed for metabolite II (relative retention times 1 and 1.19). As this metabolite, which is an intermediate in the formation of metabolite III, accounts for less than 0.4% of the dose excreted in urine [4], it will not interfere significantly in the assay of I and III.

Provided the concentrations of metoprolol are higher than 14.6 μmol/l (5 μg/ml), the unchanged drug can be simultaneously assayed with the two metabolites, with comparable precision and accuracy (Table I).

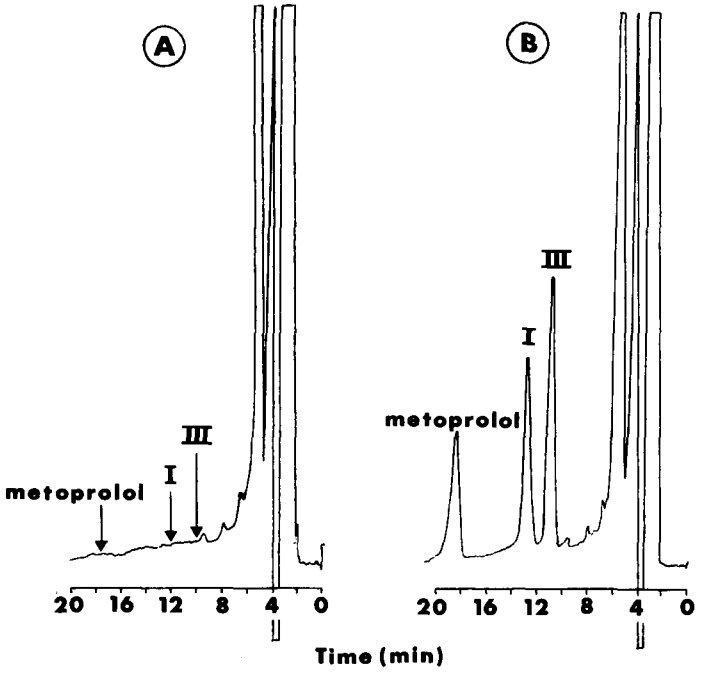

Fig. 2. Chromatograms corresponding to 1 ml of diluted blank urine (A) and to 1 ml of diluted urine spiked with 5 nmol of metabolite III, 3.6 nmol of metabolite I and 4.4 nmol of metoprolol (B).

TABLE I

ACCURACY, PRECISION, REPRODUCIBILITY AND QUANTITATION (SPIKED SAMPLES)

	Metabolite III					α-Hydroxymetoprolol (metabolite I)			Metoprolol		
Amount added (μmol/l)*	16.5	32.9	65.9	131.8	197.7	11.9	47.5	118.8	14.6	29.2	87.7
Amount found (μmol/l)*	17.5	33.3	65.5	134	198.3	12.1	46.3	116.5	12.7	28.9	86.2
Number of replicates	6	18	18	18	18	6	6	8	6	6	6
Coefficient of variation (%)	4.5	5.2	3.4	3.4	2.7	7.0	1.5	1.8	6.8	4.9	1.5
Mean recovery (%)	106	101	100	102	100	102	97	98	88	99	99

*The data are expressed in μmol of free base per l.

Application

The urinary excretion data given in Fig. 3 indicate that the present method is suitable for the assay of these metabolites after administration of metoprolol. The results given were in agreement with those already reported [2, 5]. The limit of quantitation for α-hydroxymetoprolol is higher than that obtained by Lennard and Silas [10] by HPLC with fluorimetric detection (200 ng/ml). However, it appeared sufficient for pharmacokinetic studies with metoprolol [11, 12].

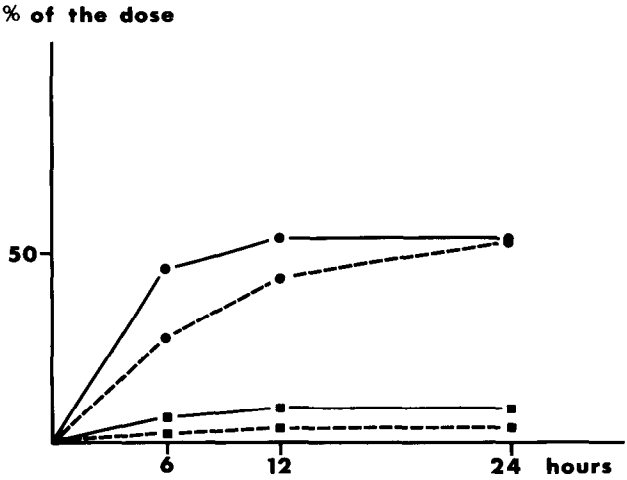

Fig. 3. Urinary elimination (24-h) of metoprolol metabolites I (■) and III (●) after administration of a single oral dose of 100 mg of metoprolol tartrate (292 μmol of metoprolol base). (——), Subject 1; (- - -), subject 2.

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