CHROMBIO. 2816

Note

Determination of pentopril, an angiotensin converting enzyme inhibitor, and its active metabolite in urine

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Pentopril is a new angiotensin converting enzyme (ACE) inhibitor which, like captopril and enalapril, is being developed for the treatment of hypertension. Pentopril, 1-(4-ethoxycarbonyl-2-methyl-2*R*,4*R*-pentanoyl)-2,3-dihydro-2*S*-indole-2-carboxylic acid, is a prodrug which is deesterified in vivo [1]
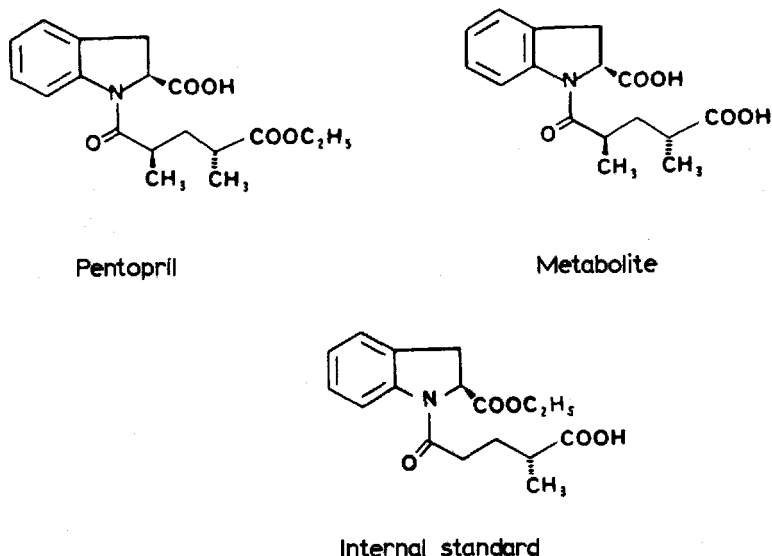

Fig. 1. Chemical structures of angiotensin converting enzyme inhibitor pentopril (CGS 13945), its active acid metabolite (CGS 13934) and the internal standard (CGS 13748).

by esterases to release its active metabolite (Fig. 1). A similar mechanism has also been reported for enalapril, another non-sulfhydryl-containing ACE inhibitor [2]. Pentopril has been found to be a very effective ACE inhibitor both in animals [3, 4] and in man [5].

To study the pharmacokinetics and disposition of this drug and its active metabolite, we needed to develop selective analytical methods for their measurements both in plasma [6] and in urine. In this report we discuss the analytical methodology developed for quantifying pentopril and its active metabolite in human urine.

EXPERIMENTAL

Reagents

Sodium bicarbonate, Baker analyzed reagent was obtained from J.T. Baker (Phillipsburg, NJ, U.S.A.), and cesium carbonate, 99.9% pure, from Aldrich (Milwaukee, WI, U.S.A.). Hydrochloric acid and methyl iodide both Baker analyzed reagents were purchased from J.T. Baker. Dichloromethane, HPLC grade, was from Fisher Scientific (Pittsburgh, PA, U.S.A.). Acetone, Photrex grade (J.T. Baker), was dried over molecular sieve Type 3A (MCB, Gibbstown, NJ, U.S.A.). Hexane, UV grade, was from Burdick and Jackson Labs. (Muskegon, MI, U.S.A.) and acetonitrile, HPLC grade, from Fisher.

Preparation of standard solutions and calibration standards

Separate standard stock solutions of drug and metabolite were prepared by dissolving pentopril (Ciba-Geigy, Summit, NJ, U.S.A.) and metabolite (Ciba-Geigy, Ardsley, NY, U.S.A.), each in acetonitrile, to a concentration of 1 mg/ml. Working standards for each of the above compounds were prepared in two different concentrations, viz., 10 and 100 μg/ml.

Internal standard stock solution was prepared by dissolving the compound (Ciba-Geigy, Ardsley, NY, U.S.A.) in acetonitrile to a concentration of 1 mg/ml. The working internal standard solution was prepared by dilution of stock solution to a concentration of 60 μg/ml. Stored at about 4°C, these solutions could be used for as long as four months.

Calibration standards having concentrations of 1, 2.5, 5 and 10 μg/ml were prepared from the 10 μg/ml working standards, while concentrations of 25 and 100 μg/ml were prepared from the 100 μg/ml working standards. The concentration of internal standard in each tube was 15 μg/ml.

Appropriate volumes of each working standard solution of pentopril, metabolite and internal standard were mixed in a test tube, acetonitrile was evaporated under nitrogen and the residue was reconstituted in drug-free human urine to give the final concentrations of 1, 2.5, 5, 10, 25 and 100 μg/ml each for pentopril and metabolite.

Sample preparation and recovery

Place 1 ml of a urine sample in a 150 × 16 mm screw-capped centrifuge tube containing 15 μg of internal standard (solvent removed under nitrogen). Add 1 ml of 10% sodium bicarbonate; vortex thoroughly. The resultant pH should be alkaline and about 9—10. Add 7 ml of dichloromethane, mix on rotary

mixer at medium speed for 20 min, then centrifuge for 10 min at 500 g.

The aqueous layer is transferred to a clean tube, 1 ml of 20% hydrochloric acid is added slowly. Resultant pH should be acidic and between 2 and 3. Add 7 ml of dichloromethane, mix on rotary mixer at medium speed for 20 min, then centrifuge for 10 min at 500 g. The aqueous layer is completely aspirated and the organic solvent is evaporated under nitrogen at about 45°C.

Derivatization

The dry residue is dissolved in 1 ml of dry acetone. Anhydrous cesium carbonate (150—200 mg) is added followed by 1 ml of methyl iodide. The tightly capped tube is heated at 75°C for 2 h, thoroughly vortexing intermittently.

The tube is brought to room temperature and organic solvents are evaporated under a stream of nitrogen. The dried residue is dissolved in 3 ml of distilled water and extracted twice with 8 ml of 2% acetone in hexane. The organic layer is decanted after freezing the lower aqueous layer in dry ice—acetone mixture. The combined organic solvent is evaporated to dryness under nitrogen at about 45°C. The dry residue is dissolved in 50 μl ethyl acetate of which 3 μl are injected on-column.

Chromatographic conditions and instrumentation

The chromatographic system consists of a Varian Model 3700 gas chromatograph with a flame-ionization detector and a Spectra Physics Model SP4100 integrator.

The column used is a 1.83 m $\times$ 2 mm I.D. glass column packed with 3% OV-101 on Chromosorb W HP 80—100 mesh (Supelco, Bellefonte, PA, U.S.A.). The following temperatures are used: column 190°C, injector and detector 250°C. Nitrogen is used as carrier gas at a flow-rate of 25 ml/min. The sensitivity of the electrometer is set at 10^{-10} a.u.f.s. and attenuation 1.

RESULTS

Fig. 1 shows the structures of drug, metabolite and internal standard. Chromatograms of urine from a male volunteer before and after (12—24 h aliquot) oral administration of 250 mg of pentopril are shown in Fig. 2. The elution sequence (retention times between parentheses) for derivatives was: metabolite (5 min), pentopril (6 min) and internal standard (7.5 min). Since no peaks were observed after the internal standard peak, total chromatography time was 9 min.

The standard curves showed good linearity ($r^2 = 0.999$) over the concentration range of 1—100 μg/ml employing 1 ml of sample volume. The day-to-day coefficients of variation (C.V.) for slopes of the standard curves (range 1—100 μg/ml, $n = 10$) during five weeks was 5.9% for pentopril and 8.0% for metabolite (Table I). Based on the analysis of replicate drug-supplemented samples, the within-day C.V. of the assay was between 1.8 and 10.1% for both compounds (Table II). Results of blind analysis of supplemented urine samples, containing 2.5—90 μg/ml, gave mean values that range from 90 to 100% of those expected for pentopril and 88 to 100% for metabolite (Table III). Fig. 3
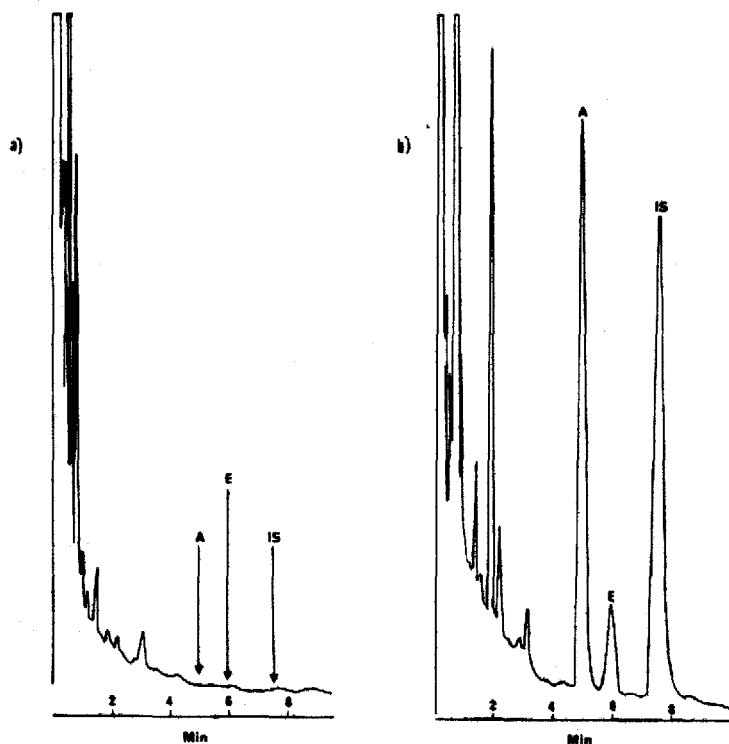
Fig. 2. Chromatograms of urine from a male volunteer (a) at 0 h and (b) after (12—24 h aliquot) oral administration of 250 mg of pentopril. Peaks: A = acid metabolite; E = ester (pentopril); IS = internal standard.

TABLE I

BETWEEN-DAY VARIABILITY OF THE SLOPES AND COEFFICIENTS OF DETERMINATION OF STANDARD CURVES FOR PENTOPRIL AND ITS METABOLITE IN HUMAN URINE

	Mean*	C.V. (%)
Pentopril		
Slope	0.0815	5.9
y-Intercept	0.0335	—
Coefficient of determination (r^2)	0.9998	0.02
Metabolite		
Slope	0.0823	8.0
y-Intercept	0.0519	—
Coefficient of determination (r^2)	0.9997	0.02

*Determined on ten different days during five weeks.

illustrates the application of this analytical method. The rates of urinary excretion for pentopril and the active metabolite are shown against time at midpoint of urinary collection interval in a normal subject after a single 125-mg oral dose of pentopril.

TABLE II

WITHIN-DAY REPRODUCIBILITY OF MEASUREMENTS OF PENTOPRIL AND ITS METABOLITE IN DRUG-SUPPLEMENTED HUMAN URINE

Compound	Concentration added (μg/ml)	Mean concentration found ($n = 4$) (μg/ml)	Coefficient of variation (%)
Pentopril	2	1.85	10.1
	10	8.81	1.8
	100	97.7	5.0
Metabolite	2	1.79	7.0
	10	8.82	2.0
	100	98.9	4.4

TABLE III

ACCURACY OF BLINDED ASSAY OF PENTOPRIL AND ITS METABOLITE ADDED TO HUMAN URINE

Compound	Concentration added (μg/ml)	Mean concentration found ($n = 9$) (μg/ml)	Accuracy (%)	Coefficient of variation (%)
Pentopril	2.5	2.23	90	9.4
	45	41.1	91	6.7
	90	90.3	100	5.8
Metabolite	2.5	2.32	93	8.8
	45	39.8	88	8.1
	90	89.6	100	8.4

Stability of urine samples

Both drug- and metabolite-supplemented urine samples containing 2.5, 45 or 90 μg/ml of each compound were frozen and stored at about $-5°$C for 60 days. Recovery of drug at the end of 60 days for concentrations of 2.5, 45 and 90 μg/ml was 84, 87 and 94%, respectively, while recovery of the metabolite for the same concentrations was 84, 80 and 71%, respectively.

DISCUSSION

The relatively simple plasma method [6] using high-performance liquid chromatography (HPLC) could not be successfully utilized for determination of pentopril and its metabolite in urine due to interfering endogenous substances. An alkali wash prior to extraction under acidic condition helped in removing some of the interference, but could not remove the majority of the polar interfering peaks close to the diacid metabolite. Two steps were therefore taken to develop the method in urine. First, it seemed necessary to esterify the carboxyl functionality to increase the retention of the acid metabolite. Second, comparison of HPLC with UV detection and gas chromatography with flame ionization detection at this step showed a cleaner chromatogram from the gas chromatographic method [6].

Selective esterification was achieved under mild conditions by reacting com-
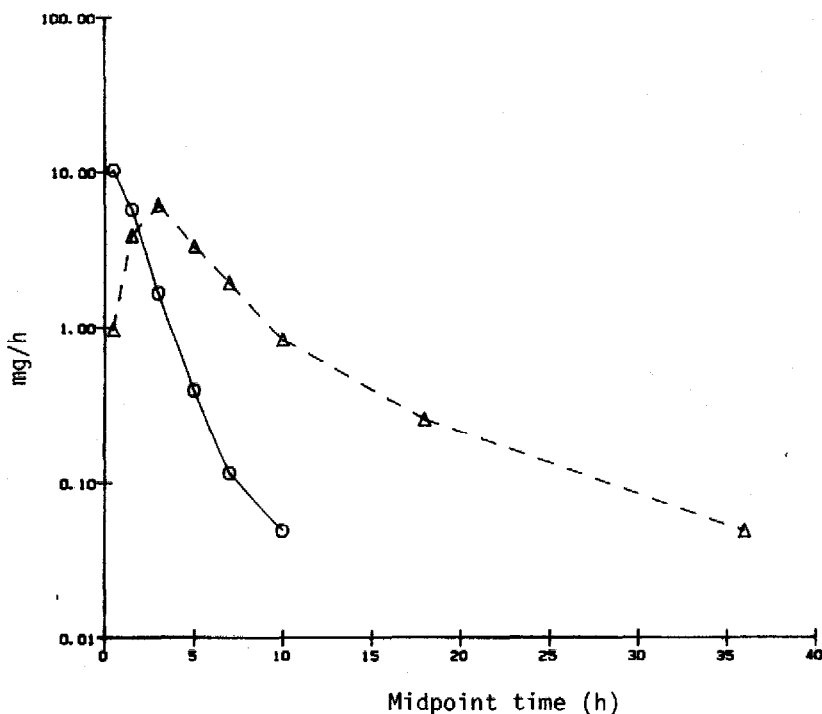
Fig. 3. Changes in urinary excretion rate with midpoint time of urinary collection interval for pentopril (○) and its active metabolite (△) in one male volunteer after a single oral 125-mg dose of pentopril.

pounds with cesium carbonate and alkyl halides [7]. Reaction is simple with no observable racemization. A similar reaction using potassium carbonate in place of cesium carbonate failed to yield ester peaks on gas chromatography. All three esters are easily separated on 3% OV-101 in about 7.5 min, while on 3% OV-17 a delayed separation of internal standard and ester peaks is observed. Low oven temperature considerably increased column life.

The chromatography was tested for selectivity against other commonly prescribed drugs such as indomethacin, cimetidine, digoxin, aspirin, probenecid, phenobarbital and furosemide. These drugs do not interfere with the chromatogram of pentopril, metabolite or the internal standard.

This reported assay is accurate, reproducible and selective for pentopril and its major active metabolite, and allows quantification of these compounds in urine for clinical and pharmacokinetic studies.

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