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DETERMINATION OF CAPTOPRIL IN HUMAN BLOOD BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH ELECTROCHEMICAL DETECTION

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SUMMARY

A new method for quantitation of captopril in human blood is described. Captopril was derivatized with N-(4-dimethylaminophenyl)maleimide into the electrochemically active adduct. The derivative was separated and determined by high-performance liquid chromatography with an electrochemical detector on a reversed-phase column. The proposed method was satisfactory for determination of captopril in whole blood with respect to accuracy and precision. The detection limit of captopril thereby obtained was 10 ng/ml. The blood levels of captopril in patients orally given an officinal dose were measured by the present method.

INTRODUCTION

Captopril [1-(D-3-mercapto-2-methyl-1-oxopropyl)-L-proline] (SQ-14,225) is a potent, specific and orally active inhibitor of angiotensin-converting enzyme in the renin—angiotensin system [1—4]. In recent years, the quantitation of captopril in biological fluids has been carried out by gas chromatography [5], high-performance liquid chromatography (HPLC) [6], gas chromatography—mass spectrometry (GC—MS) [7] and radiochemical methods [8, 9]. These procedures, however, are still unsatisfactory with respect to sensitivity, feasibility and reliability. An urgent need to clarify the metabolic fate of captopril given to patients prompted us to develop a method for monitoring the blood level of the unchanged drug. This paper describes a new

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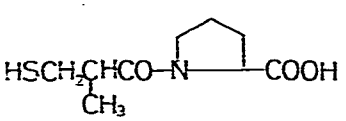
method which involves derivatization into the electrochemically active form followed by HPLC with electrochemical detection for the determination of captopril in whole blood.

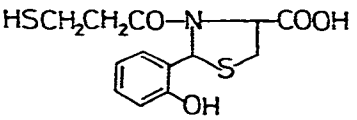
MATERIALS AND METHODS

Materials

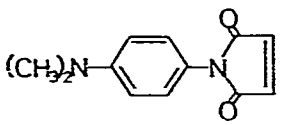
Captopril and (4*R*)-2-(2-hydroxyphenyl)-3-(3-mercaptopropionyl)-4-thiazolidinecarboxylic acid (SA 446) (Fig. 1) were kindly donated by Sankyo Co. (Tokyo, Japan) and Santen Pharmaceutical Co. (Osaka, Japan), respectively. N-(4-Dimethylaminophenyl)maleimide (DAPM) (Fig. 1) was prepared in these laboratories by the method reported by Machida et al. [10]. All other chemicals were of analytical-reagent grade. Silica gel HF_{254} (E. Merck, Darmstadt, G.F.R.) was used for thin-layer chromatography (TLC).



Captopril



SA 446



DAPM

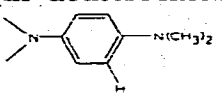
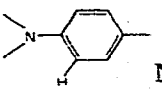
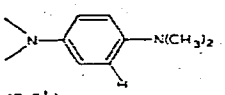
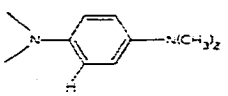
Fig. 1. Structures of captopril, SA 446 and DAPM.

Instruments

A Waters Model ALC/GPC 202 high-performance liquid chromatograph (Waters Assoc., Milford, MA, U.S.A.), equipped with a Yanagimoto Model VMD 101 electrochemical detector (Yanagimoto Co., Kyoto, Japan) was used. The applied potential of a working electrode was set at +0.9 V vs. an Ag/AgCl reference electrode. The samples were introduced by means of a Model U6K sample loop injector (Waters Assoc.) with an effective volume of 2 ml. A μBondapak C_{18} (8—10 μm) column (30 cm × 0.39 cm I.D.) (Waters Assoc.) was used under ambient conditions. Acetonitrile—0.8% $NH_4H_2PO_4$ (pH 3.0) (1 : 2) was employed as mobile phase at a flow-rate of 1 ml/min. Nuclear magnetic resonance (NMR) spectra were recorded on a JEOL Model PS-100 spectrometer at 100 MHz using tetramethylsilane as internal standard. (Abbreviations used: s = singlet and d = doublet.) Mass spectra were recorded on a Hitachi M-52 spectrometer.

Preparation of captopril—DAPM adduct

To a solution of captopril (20 mg) and DAPM (25 mg) in ethanol (3 ml) was added triethylamine (3 drops), and the solution was kept at room temperature for 15 min. The resulting solution was evaporated to dryness in vacuo and the oily residue was subjected to preparative TLC using ethyl acetate—

acetic acid (20:1) as mobile phase. Elution of the gel corresponding to the spot of R_F = 0.44 with chloroform—methanol (5 : 1) gave the captopril—DAPM adduct (20 mg) as a colorless oil. NMR (in deuterochloroform) δ: 2.98 [6H, s, $-N(CH_3)_2$], 6.70 [2H, d, J = 8.6 Hz, ], 7.60 [2H, d, J = 8.6 Hz,  $N(CH_3)_2$]. MS: m/z 433 (M^+). The product was homogeneous as judged by TLC carried out in the manner described above. Subsequent treatment with diazomethane in the usual manner gave the methyl ester as a colorless oil. NMR (in deuterochloroform) δ: 1.26 (3H, d, J = 6.0 Hz, $-CHC\underline{H}_3$), 2.98 [6H, s, $-N(CH_3)_2$], 3.73 (3H, s, $-COOCH_3$), 6.75 [2H, d, J = 6.3 Hz, ], 7.11 [2H, d, J = 6.3 Hz, ]. MS: m/z 447 (M^+).

Derivatization of captopril

DAPM (20 μg) and captopril (6 μg) were dissolved in 1/30 M phosphate buffer (pH 6.85, 1 ml), and the solution was kept at 0°C in an ice-bath. The reaction was terminated by removal of excess DAPM by extraction with ether (2 × 2 ml). A 10-μl aliquot of the remaining aqueous layer was applied for HPLC.

Hydrodynamic voltammogram of captopril—DAPM adduct

The current (peak height) at each potential was divided by the current at the most positive potential to obtain the relative current ratio which was plotted against the potential.

Standard procedure for determination of blood captopril

The blood (1 ml) was drawn into Vacutainers from a suitable forearm vein just before administration of the drug and thereafter at frequent intervals during 120 min. Duplicate 0.5-ml aliquots of whole blood were immediately poured into glass tubes containing DAPM (20 μl of 1% solution in acetone) and 0.3 ml of 1/30 M phosphate buffer (pH 6.85). After addition of SA 446 (100 ng) as internal standard, the solution was vortex-mixed and allowed to stand at 0°C for 30 min. All samples were frozen in a dry-ice—acetone bath until analyzed.

Each thawed blood sample was extracted with ether (2 × 2 ml). To the aqueous layer was added glutathione (ca. 500 μg), and the solution was kept at 0°C for 20 min in an ice-bath. The resulting solution was treated with acetone (3 ml) and was then centrifuged at 1580 g for 5 min. The precipitate was washed with acetone (3 ml). The supernatant and washings were combined and concentrated under reduced pressure at room temperature to ca. 1 ml. The residue was diluted with water (6 ml) and percolated through a Sep-Pak C_{18} cartridge. After washing with water (2 ml), the desired fraction was eluted with acetonitrile (8 ml). The effluent was evaporated to dryness in vacuo below 40°C. The residue was dissolved in methanol (200 μl) and an aliquot of the solution was applied for HPLC.

Calibration curve for blood captopril

A calibration curve was constructed by assaying the human blood samples (0.5 ml each) spiked with known amounts of captopril in the manner described above. The peak height ratio of captopril to internal standard was plotted against the blood concentration of captopril.

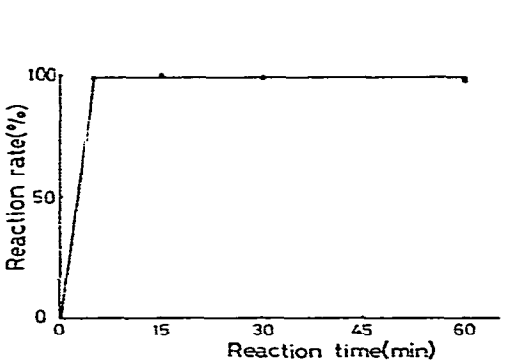
Subjects

The subjects were four male patients with essential hypertension who ranged in age from 23 to 46 years ($\bar{x}$ = 36 years). They weighed between 62.9 and 91.0 kg ($\bar{x}$ = 74.0 kg) and ranged in height from 163.5 to 173.0 cm ($\bar{x}$ = 167.9 cm). The patients received no medication for at least two weeks before the study. Each subject was given a single tablet of captopril (50 mg) with water where the dose ranged from 0.55 to 0.79 mg/kg ($\bar{x}$ = 0.69 mg/kg). The clinical study was carried out on fasted patients in the morning. The subjects were kept in the supine position during the study period.

RESULTS AND DISCUSSION

It is well known that in biological fluids captopril undergoes oxidation resulting in the formation of the disulfide [11]. Although captopril itself is electrochemically active [12], the sulfhydryl group must be protected prior to analysis. The use of a derivatization reagent having both functions reactive toward the sulfhydryl group and responsive for electrochemical detection seemed to be promising. From this point of view, DAPM, readily obtainable by the known method [10], was chosen as a suitable derivatization reagent for this purpose.

When treated with DAPM in phosphate buffer, captopril underwent easily the Michael addition yielding the adduct. The structural assignment of the adduct and its methyl ester was unequivocally established on the basis of their mass and NMR spectral data. First, the effect of reaction time on the formation of the adduct was investigated. As illustrated in Fig. 2, derivatization of captopril was completed in 5 min at 0°C. This derivatization procedure appeared to be suitable for determination of captopril. The DAPM



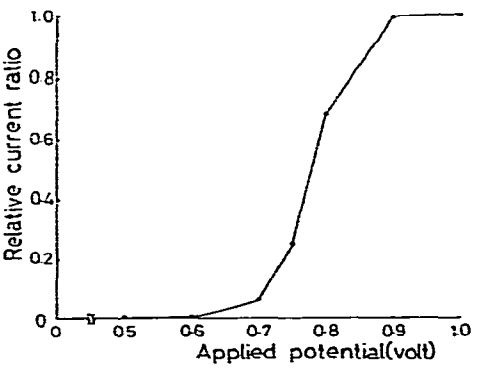


Fig. 2. Time course for derivatization of captopril with DAPM.

Fig. 3. Hydrodynamic voltammogram of the captopril—DAPM adduct.

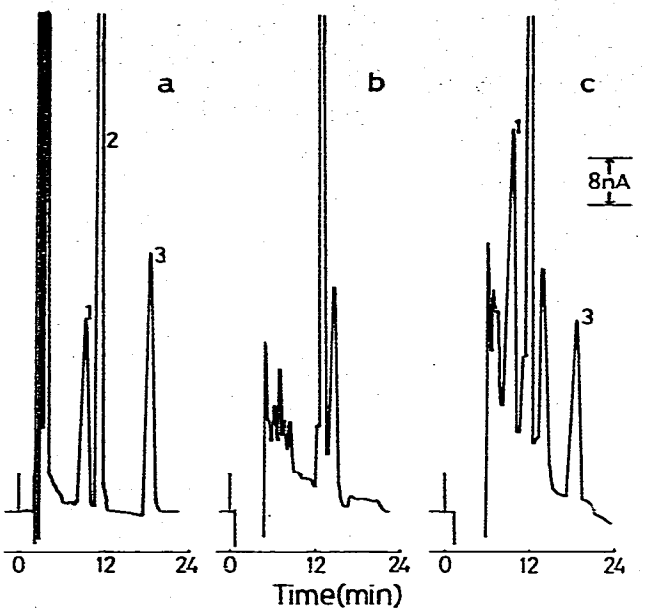


Fig. 4. High-performance liquid chromatograms obtained with (a) standard samples (each 10 ng), (b) blank blood sample, (c) blood sample of a patient orally given 50 mg of captopril. Peaks: 1 = captopril—DAPM adduct; 2 = DAPM, 3 = SA 446—DAPM adduct. Interference from endogenous polar substances due to saturation of the output of the detector was overcome by removing the connector for ca. 5 min after injection.

adduct was similarly formed from SA 446 used as internal standard. Among several columns tested, μBondapak C_{18} was most favorable for efficient separation. A hydrodynamic voltammogram of the captopril—DAPM adduct was obtained in the usual manner (Fig. 3). The detector gave a linear response up to +0.9 V vs. the Ag/AgCl electrode and then showed a plateau. The applied potential was therefore set at +0.9 V against the reference electrode. A typical chromatogram of the adducts formed from captopril and internal standard is illustrated in Fig. 4a. The detection limit (signal-to-noise ratio = 2 at 4 nA full scale) was estimated to be 200 pg for captopril.

The next effort was focussed on establishing a clean-up procedure for captopril in biological fluids. A whole-blood sample was immediately poured into a glass tube containing DAPM in phosphate buffer. The excess DAPM was eliminated by extraction with ether, and also by treatment with glutathione because the glutathione—DAPM adduct was eluted at the solvent front on the chromatogram. The deproteinized sample was applied to a Sep-Pak C_{18} cartridge, and the captopril and internal standard fraction was eluted with acetonitrile. No interfering peaks were recognized on the chromatogram obtained with the blood specimen (Fig. 4b).

A known amount of captopril was added to the blood, and the overall recovery was estimated. Captopril spiked at five levels was recovered at the rate of more than 70% (Table I). In addition, the mean value of recovery for 100 ng of SA 446 was 71.8 ± 2.4%. When the peak height ratio of captopril to internal standard was plotted against the concentration of captopril spiked to the human blood, a linear correlation was observed between these two in

TABLE I

RECOVERY OF CAPTOPRIL ADDED TO HUMAN BLOOD

Captopril (ng/ml)		Recovery (%) ± S.D.*
Added	Found	
100	70.5	70.5 ± 3.4
200	142	70.9 ± 2.3
400	282	70.6 ± 3.2
800	561	70.1 ± 2.9
1600	1120	70.2 ± 2.9

*$n = 6$.

TABLE II

ACCURACY AND PRECISION OF THE PROPOSED METHOD FOR DETERMINATION OF CAPTOPRIL IN HUMAN BLOOD

Captopril (ng/ml)		C.V. (%)
Added	Found ± S.D.*	
100	102 ± 3.3	3.2
400	395 ± 11.2	2.8

*$n = 6$.

the range of 60—1600 ng. It is evident from the data in Table II that the proposed method was also satisfactory in both accuracy and precision. In addition, the present method provided quantitative results for blood levels of captopril of not less than 10 ng/ml. The detection limit obtained by this procedure was comparable to that obtained by GC—MS [7] or the radiochemical method [8].

The present method was then applied to the determination of captopril

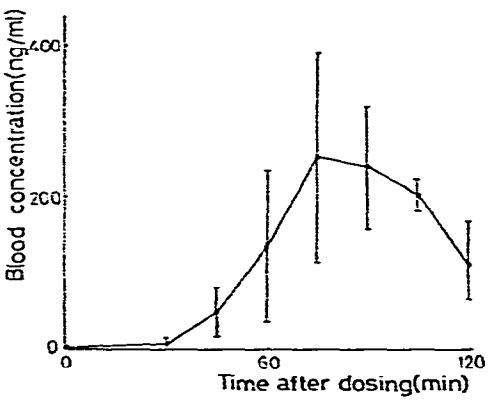


Fig. 5. Change in blood level of captopril in human blood. Each point and bar indicate the respective mean and standard deviation obtained for four patients with essential hypertension. Each subject was orally given 50 mg of captopril in a single dose.

in blood samples taken from four patients who had received orally 50 mg of captopril. A typical chromatogram of the unchanged drug together with internal standard is illustrated in Fig. 4c. A change in the blood level of captopril during 120 min after administration is shown in Fig. 5. The blood captopril reached its maximum at 75 min at a level of 297 ± 133 ng/ml. The result is in accord with that previously obtained by the radiochemical method [9]. The proposed method for determination of captopril in human blood is satisfactory with respect to both sensitivity and reliability. It should be noted that derivatization of captopril into the DAPM adduct is useful for preventing oxidative degradation and providing high sensitivity for electrochemical detection.

The application of a newly established method to the determination in biological fluids of other hypotensive drugs having a sulfhydryl group is at present being conducted in these laboratories, and the details will be reported in the near future.

ACKNOWLEDGEMENTS

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