High-Performance Liquid Chromatographic Analysis of Captopril in Plasma

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Abstract □ A sensitive assay is described for quantitating plasma captopril levels. Captopril is an orally active inhibitor of angiotensin-converting enzyme. Blood from patients taking this drug was collected into tubes containing edetate and ascorbic acid, and the plasma was separated by centrifugation. After addition of an internal standard, the plasma was deproteinized and the supernate was adjusted to pH 6.5. *N*-(1-Pyrene)-maleimide was added to derivatize captopril and an internal standard to fluorescent adducts. These derivatives then were extracted into ethyl acetate–benzene (1:1) and separated from other derivatized thiols by high-performance liquid chromatography. The sensitivity of the assay was 150 pmoles/ml. Preliminary pharmacokinetics were obtained in patients taking captopril chronically for essential hypertension. After administration of 100 mg of captopril to patients who had fasted overnight, the plasma levels rose rapidly; peak levels were obtained at ~37 min. Thereafter, the plasma levels declined rapidly, and the terminal half-life was ~40 min. In these patients, the time course of the plasma levels did not reflect changes in blood pressure, so there appeared to be no direct relationship between plasma levels of the parent compound and blood pressure changes.

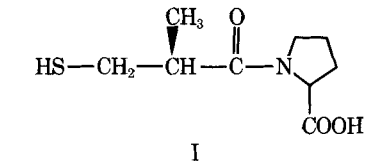
Keyphrases □ High-performance liquid chromatography—analysis of captopril in plasma □ Captopril—analysis using high-performance liquid chromatography □ Antihypertensives—high-performance liquid chromatographic method for captopril

Captopril (D-3-mercapto-2-methylpropanoyl-L-proline, I) is a potent and selective inhibitor of angiotensin-converting enzyme (kininase II) (1). Oral captopril administration lowers blood pressure in laboratory animals with experimental hypertension (2, 3) and in hypertensive humans (4, 5). Since the reductions in blood pressure apparently are related to the inhibition of angiotensin-converting enzyme (5), captopril appears to be the prototype of a new class of antihypertensive drug.

The disposition of captopril has been reported only in normotensive subjects taking an oral formulation of ^{35}S-labeled captopril (6). The present paper reports the development of a sensitive high-performance liquid chromatographic (HPLC) assay for quantitating captopril in plasma and its application to a preliminary study of captopril pharmacokinetics in patients undergoing a clinical trial for the management of essential hypertension.

EXPERIMENTAL

Instrumentation—Assays were carried out using a constant-flow, high-performance liquid chromatograph[1]. It consisted of a solvent delivery system[2], a universal injector[3], a radial compression module[4] with a reversed-phase permanently bonded octadecylsilane cartridge[5] (10 cm long × 8 mm i.d.), and a spectrophotofluorometer[6] equipped with a 20-μl flow-through quartz cell and a 150-w xenon arc lamp with excitation and emission monochromators set at 340 and 390 nm, respectively.



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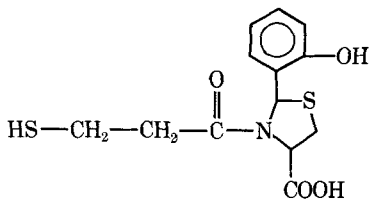

II

Reagents—Captopril[7] and an internal standard[8] [(4*R*)-2-(2-hydroxyphenyl)-3-(3-mercaptopropionyl)-4-thiazolidinecarboxylic acid, II] were used. The derivating reagent was *N*-(1-pyrene)maleimide[9]. Ethyl acetate and benzene[10] were analytical reagent grade, and methanol[1] was liquid chromatographic grade. All other reagents were the highest grade purity.

Standard Solutions—Standard solutions of captopril (1 m*M*) and II (1 m*M*) were prepared by dissolving pure drug in an aqueous solution of edetate disodium (1 m*M*) previously bubbled with nitrogen. These solutions were stored at 4° and were stable for at least 7 days. The *N*-(1-pyrene)maleimide derivatizing reagent (2 m*M*) was prepared by dissolving the powder in acetone and was stored at 4° for at least 14 days.

Patient Sampling Procedure—Four patients who had been taking captopril (100 mg, three times a day) for essential hypertension were studied. After an overnight fast, 100 mg of captopril was given; the patients then continued to fast for 4 hr. Immediately prior to dosing and at 0.25, 0.5, 0.75, 1.0, 1.5, 2, 3, and 4 hr, a 5-ml blood sample was taken from an indwelling catheter in a forearm vein and added to a heparinized tube containing 50 μl of a solution of edetate disodium (0.1 *M*) and ascorbic acid (0.1 *M*). After mixing, the samples were centrifuged immediately at 2000×*g* for 10 min to separate the plasma.

Extraction Procedure—The internal standard (10 nmoles) was added to 1.0 ml of plasma in a polypropylene tube (9.5 × 2 cm), and then 200 μl of perchloric acid (2 *M*) was added. After centrifugation at 2000×*g* for 10 min, 0.5 ml of the supernate was removed and its pH was adjusted to 6.5 with tribasic potassium phosphate (1 *M*). The derivatizing reagent was added (50 nmoles), and the solution was left at room temperature for 15 min. The solution then was extracted with 3 ml of ethyl acetate–benzene (1:1) by vortex mixing for 30 sec. Following centrifugation to separate the phases, the upper layer was transferred to a clean glass-stoppered conical tube and evaporated to dryness under nitrogen. The residue was dissolved in 100 μl of methanol and was either subjected immediately to liquid chromatography or stored overnight at 4°.

Chromatography—Ten-microliter injection volumes were used. The mobile phase was methanol–potassium phosphate buffer (5 m*M*, pH 6.5) (52:48), and the flow rate was maintained at 2 ml/min at a back pressure of ~1000 psi.

Quantitation—Since captopril is known to oxidize spontaneously into disulfides, ascorbic acid and edetate were added to the blood immediately upon sampling. Preliminary experiments demonstrated that this procedure prevented any loss of captopril when it was added to control blood and analyzed as already described.

[1] Waters Associates, Chippendale, Australia, 2008.
[2] Model 6000A, Waters Associates.
[3] Model U6K, Waters Associates.
[4] Model RCM-100, Waters Associates.
[5] PAK A cartridge, Waters Associates.
[6] Farrand Optical Co., New York, N.Y.
[7] E. R. Squibb & Sons Pty. Ltd., Melbourne, Australia.
[8] Santen Pharmaceuticals, Osaka, Japan.
[9] Polysciences.
[10] Merck, Darmstadt, West Germany.

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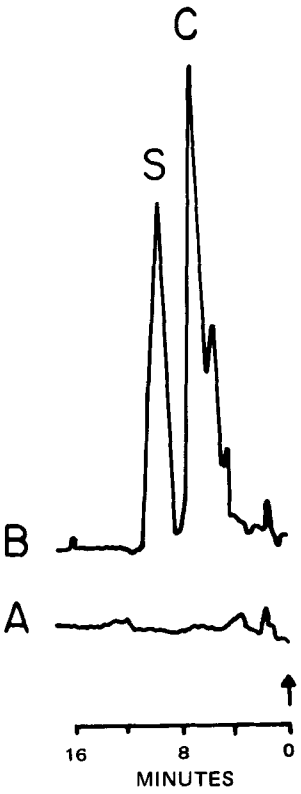

Figure 1—*Elution of fluorescent material from HPLC column after derivatization and extraction of control plasma (A) and plasma from a patient who had taken 100 mg of captopril 1 hr previously (B). Key: C, captopril (I); and S, internal standard (II).*

A calibration curve was constructed by adding varying amounts of captopril and a constant amount of internal standard to 1 ml of control plasma and analyzing the samples as already described. To assess linearity, the peak height ratio of captopril to the internal standard was plotted against the added captopril.

Recoveries of sulfhydryl compounds were calculated by first adding the same amount of each compound to plasma and then deproteinizating, derivatizing, extracting, evaporating, and reconstituting in methanol. The methanol extract then was injected, and the peak height was calculated. The same amount of sulfhydryl compound in phosphate buffer was derivatized and directly injected, and the peak height was measured. The percent recovery was calculated by dividing the peak height after solvent extraction by the peak height without solvent extraction and multiplying by 100.

Pharmacokinetic Analysis—Plasma captopril level–time data were fitted to a polyexponential equation using the nonlinear least-squares regression analysis computer program AUTOAN 2 (7). The estimated parameters were obtained assuming first-order absorption and elimination.

RESULTS AND DISCUSSION

Captopril disposition in humans (6) and laboratory animals (8) has only been studied using a single dose of radiolabeled captopril. Thus, a sensitive and specific assay is needed for measuring blood levels of captopril in patients so that its pharmacokinetics can be defined for developing an optimal dosage regimen.

Since captopril has no prominent absorption properties in the UV or visible spectrum, methods were examined for derivatizing captopril through its thiol group to yield a suitable fluorophore. A suitable reagent

Table I—Summary of Captopril Pharmacokinetics in Patients [a]

Patient	T_{max}, hr	C_{max}, nmoles/ml	AUC, nmoles hr/ml	$T_{1/2}$, hr
1	0.75	18.5	18.66	0.92
2	0.67	20.8	20.12	0.52
3	0.58	18.3	17.34	0.70
4	0.50	9.9	10.99	0.52
Mean	0.63	16.9	16.77	0.66
SE	0.05	2.4	2.01	0.09

[a] T_{max} = time when maximum plasma concentration, C_{max}, is reached. AUC = area under curve, and $T_{1/2}$ = elimination half-life.

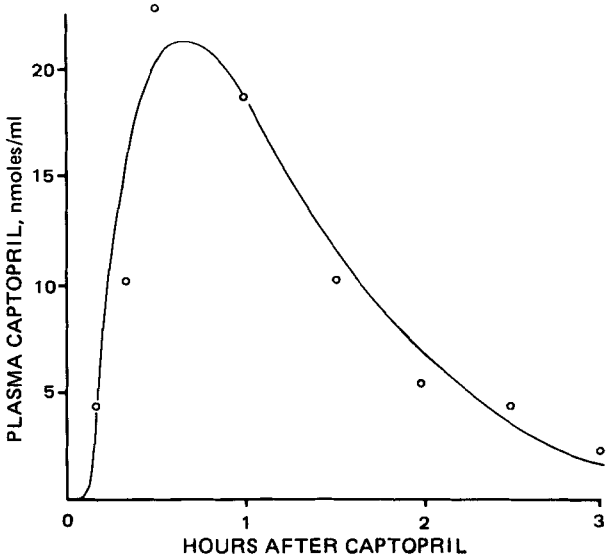

Figure 2—*Time–plasma concentration curve for captopril after oral administration of 100 mg to a hypertensive patient.*

was *N*-(1-pyrene)maleimide since this reagent did not fluoresce in aqueous solution but formed fluorescent adducts, under mild conditions, with thiol compounds or proteins containing such groups (9, 10). While this reagent has been used for fluorescence polarization studies of high molecular weight proteins, in the present study it formed a fluorescent adduct with captopril. However, other low molecular weight thiols such as cysteine, *N*-acetylcysteine, and glutathione also formed fluorescent derivatives that were indistinguishable from each other by their excitation and emission spectra.

Since glutathione and cysteine are present in the blood, it was apparent that an HPLC technique would be necessary to separate these derivatized thiols. Since such an assay could be improved by the incorporation of an internal standard, another thiol angiotensin-converting enzyme inhibitor (II) was included in initial studies to define the HPLC conditions for separation of the fluorescent adducts of glutathione, cysteine, captopril, and II. The conditions given under *Experimental* achieved satisfactory separations with retention times of 2.4, 3.2, 5.6, and 7.6 min, respectively. Further specificity was obtained by examining various solvents to extract the fluorescent adducts differentially after derivatization. The most suitable solvent was an equal mixture of ethyl acetate and benzene (3 ml), and the percent recovery of the succinimide derivatives at pH 6.5 was as follows: cysteine, 3%; glutathione, <1%; captopril, 68%; and II, 91%.

Figure 1 illustrates a typical chromatogram obtained after extraction of drug-free plasma (curve A) and of plasma from a patient who had taken captopril 1 hr earlier (curve B). The peak at 5.6 min represents captopril, and the peak at 7.6 min was the internal standard (II). The minor peak on the shoulder of the captopril peak was always seen when captopril was derivatized and chromatographed on the radial compression column. However, it was not seen in earlier studies when a C_{18} reversed-phase column was used, presumably due to the lower efficiency of this column. Since the former column has a higher number of plates (~5000) than the latter column (~2000 plates) and, therefore, achieves a higher resolution, this minor component could be an isomer of captopril.

The threshold of captopril detection in plasma corresponding to a peak height approximately three times that of baseline noise was 150 pmoles/ml. A calibration curve for the peak height ratio of captopril to the internal standard was linear over the concentration range of 150–10,000 pmoles/ml. The within-run precision of the assay (coefficient of variation) at a concentration of 1.5 nmoles/ml was 8.1% (n = 4), and the between-run precision was 9.0% (n = 6).

Because of recent reports of toxicity, including neutropenia (11) and proteinuria (12), permission has not been given for single-dose pharmacokinetic studies in normotensive subjects. A limited study of captopril kinetics in patients taking this drug chronically for essential hypertension was made. Patients fasted overnight, and a predose blood sample was taken; no captopril could be detected in any predose blood sample. After the administration of a standard dose of 100 mg of captopril, absorption was very rapid in all patients; the average time for achieving peak plasma concentrations of captopril was 37 min (Table I). In two patients, the appearance rate of captopril in the blood was so rapid that it did not appear to be following a first-order process. Further experiments with

sampling at closer time intervals are needed to confirm these observations. The data for all patients could be described approximately by the sum of two exponentials, and the mean elimination half-life was ~40 min. The plasma concentration–time profile for one subject is shown in Fig. 2.

The pharmacokinetics of captopril in the four patients taking the drug chronically indicate that changes in plasma captopril concentrations do not reflect blood pressure changes. In all patients, captopril was undetected in their plasma 3–4 hr after dosing, yet blood pressure had not risen at this time. Blood levels of captopril metabolites, such as the disulfide, possibly may be a more useful index of the hemodynamics of this drug.

In conclusion, it was demonstrated that plasma captopril can be assayed with high sensitivity and specificity by first derivatizing this thiol compound to a fluorophore using *N*-(1-pyrene)maleimide and then separating the fluorescent adducts by HPLC. This procedure can also be used for assaying another thiol angiotensin-converting enzyme inhibitor, II, using captopril as the internal standard. This derivatization procedure should be suitable for assaying the mucolytic drug *N*-acetylcysteine after modification of the HPLC conditions to retard its elution.

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Pharmacokinetics of [^{14}C]Bretylium Tosylate in Rats

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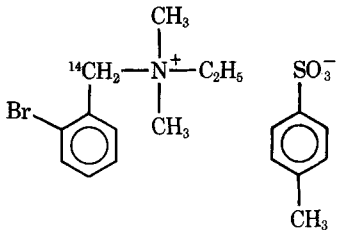
Received August 29, 1980, from the *Pharmaceutical Development Department, Research & Development, American Critical Care, McGaw Park, IL 60085.* Accepted for publication November 20, 1980.

Abstract □ The pharmacokinetics of bretylium tosylate were investigated in eight male Charles River rats. Each animal received an intravenous dose (10 mg/kg) of [^{14}C]bretylium tosylate. Serial blood samples, urine, and feces were collected for up to 72 hr. Bretylium concentrations in plasma and amounts excreted in urine and feces were determined by scintillation counting. On the average, 88 and 95% of the dose were recovered in urine and feces in 24 and 72 hr, respectively. Urinary recovery accounted for 65.6% of the dose while 29.7% was excreted in the feces. Bretylium concentrations in plasma declined triexponentially and were fitted to a three-compartment open model. Bretylium has a very high apparent volume of distribution (15 liters/kg), and its β half-life averaged 5.5 hr. Mean values of the apparent volume of the central compartment, plasma clearance, renal clearance, and excretion rate constants of bretylium in rats were 1 liter/kg, 1.93 liters/hr/kg, 1.27 liters/hr/kg, and 1.24 hr^{-1}, respectively. The results indicate that: (*a*) bretylium is strongly bound to the tissues and is eliminated by active urinary secretion and by biliary excretion in rats, and (*b*) there are strong similarities between the pharmacokinetics of bretylium in humans and rats and that this animal model might be suitable for interaction studies with other drugs.

Keyphrases □ Bretylium tosylate—pharmacokinetics, rats, compared with human studies □ Pharmacokinetics—bretylium tosylate, rats, compared with human studies □ Antiarrhythmic agents—bretylium tosylate, pharmacokinetics studied in rats, compared with human studies

Bretylium is a quaternary ammonium compound [(*o*-bromobenzyl)ethyldimethylamine] used clinically as the tosylate salt in the treatment of arrhythmias. Bretylium tosylate suppresses ventricular fibrillation in patients within minutes following intravenous administration (1). Suppression of ventricular tachycardia and other ventricular arrhythmias develops more slowly, usually 20–120 min following parenteral administration (2).



[^{14}C]bretylium tosylate

BACKGROUND

In humans, bretylium is eliminated intact mainly through the renal route (3, 4). No metabolites have been identified following administration of bretylium tosylate in humans and rats (3). The biological half-life of bretylium in normal subjects was 5.5 hr (3). Romhilt *et al.* (2) reported a half-life of 9.75 hr (range 4.2–16.9) in eight patients with cardiac disease. More recently, Adir *et al.* (4) reported a half-life of 8.1 hr for a normal subject and of 16.0 and 31.5 hr for two patients with renal impairment. In the normal subject, the renal clearance of bretylium was 1 liter/min, indicating extensive active secretion, and the urinary excretion of unchanged drug accounted for 80% of the dose (4).

A previous study in the rat (3) demonstrated that 63% of the intramuscular dose (5 mg/kg) was excreted unchanged in the urine and that 31% was excreted in the feces. Most radioactivity was excreted within 24 hr. An average of 16% of the intravenous dose (2.5 mg/kg) in four rats was excreted in the bile within 5 hr.

This investigation studied the pharmacokinetics of distribution and elimination of bretylium in the rat following an intranveous dose of ra-