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Note

Simplified determination of captopril in plasma by high-performance liquid chromatography

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Captopril, 1-(D-3-mercapto-2-methyl-1-oxopropyl)-L-proline, is an angiotensin-converting enzyme inhibitor used in the treatment of hypertension and congestive heart failure in adults [1,2] and children [3,4]. However, the use of captopril in infants has been on an empirical basis, due to the absence of relevant kinetic data. Reasons for this absence may include the complexity of the available assay methods for captopril and/or the large volumes of blood required. Captopril has been determined by several methods, including high-performance liquid chromatography (HPLC) [5–9], gas chromatography (GC) [10], gas chromatography–mass spectrometry (GC–MS) [11,12], radioimmunoassay [13,14] and, more recently, enzyme immunoassay [15]. The complexity of these methods may limit their applicability and specialized equipment such as GC–MS may not be widely accessible. Also, the volumes of blood required may preclude the use of these methods with neonates. A simple HPLC assay has been developed and was used in a pharmacokinetic study with a 15-month-old infant with congestive heart failure.

EXPERIMENTAL

Materials

Captopril (SQ 14225) and captopril disulphide (SQ 14551) were donated by E.R. Squibb (Princeton, NJ, U.S.A.), (4*R*)-2-(2-hydroxyphenyl)-3-(3-mercap-

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topropionyl)-4-thazolidinecarboxylic acid, internal standard (I.S.) (SA 446), was donated by Santen Pharmaceutical (Osaka, Japan). N-(3-Pyrenyl)maleimide (NPM) was purchased from Fluka (Hauppauge, NY, U.S.A.) and was purified on a column (43 cm×2 cm) packed with silica gel (E. Merck, Darmstadt, F.R.G.) using chloroform as the eluent. NPM was used as a 1.5 mg/ml solution in acetonitrile. All other chemicals used were of analytical-reagent grade.

Instruments

The liquid chromatograph used was equipped with a Partisil ODS-3 C_{18} column (5 μm, 100 mm×4.6 mm I.D., Whatman, Clifton, NJ, U.S.A.). Samples were introduced by means of a Model U6K sample injector (Waters, Mississauga, Canada) and detected by an FS 970 fluorometer (Schoeffel, Oakville, Canada) with the excitation and emission wavelength set at 340 and 389 nm, respectively. The mobile phase was acetonitrile–0.1 *M* citric acid buffer at pH 3.1 (38:62) for total captopril analyses and acetonitrile–1% acetic acid (37:63) for free captopril analyses. Each was run at a flow-rate of 1.5 ml/min.

Procedures for the determination of captopril in plasma

Free captopril. Freshly drawn blood (1 ml) was mixed with 50 μl of a solution of EDTA (0.1 *M*) and ascorbic acid (0.1 *M*) [6]. The mixture was immediately centrifuged at 13 000 *g* for 2 min. A 0.5-ml aliquot of the supernate was separated, and 2 ml of 0.1 *M* phosphate buffer (pH 7), 200 ng of I.S. and 0.2 ml of a solution of the derivatizing agent NPM were added. This mixture was shaken at room temperature for 15 min and was then acidified with 0.1 ml hydrochloric acid (11 *M*) and extracted with 6 ml ethyl acetate by vortex-mixing for 20 min. After centrifuging at 2500 *g* for 5 min, the organic layer was removed and dried under nitrogen. The residue was dissolved in 50 or 200 μl of acetonitrile and aliquots of 5–15 μl were injected into the HPLC system.

Total captopril (captopril and its mixed disulphides). A 0.5-ml plasma sample was mixed with 50 μl of a solution of EDTA (0.1 *M*) and ascorbic acid (0.1 *M*), 2 ml of 0.1 *M* phosphate buffer (pH 7), 400 ng I.S. and 0.1 ml of a 2% solution of tributylphosphine (TBP) in acetonitrile. The mixture was incubated at 50 °C for 1 h. After incubation, the mixture was cooled to room temperature with water and then 0.2 ml of a solution of NPM was added. This mixture was shaken at room temperature for 15 min and then extracted and assayed as for free captopril.

Calibration curves for free and total captopril

Calibration curves for free and total captopril were constructed by spiking 0.5-ml plasma samples with known amounts of captopril and captopril disulphide, respectively, and assaying as described above. The peak-height ratio of captopril to internal standard was plotted against the concentration of captopril.

Patient study

CAP (1 mg/kg total body weight) was administered as an oral solution to a 15-month-old infant during cardiac catheterization, as part of treatment for congestive heart failure. Blood samples (2 ml) were drawn at 0, 0.25, 0.5, 1.0, 1.5, 2, 4,

6 and 8 h after the initial dose. Free and total captopril levels were measured. Informed parental consent was obtained before the study.

RESULTS AND DISCUSSION

A simple HPLC assay for captopril and its mixed disulphides in plasma has been developed. This method makes it possible to obtain pharmacokinetic data for paediatric patients, since only 1 ml of blood or 0.5 ml of plasma is required to determine free or total captopril. Other methods [5,6,8,9,11–13] require 2–15 ml of blood and this precludes their use with infants due to the small volumes of blood available. Sample preparation is considerably faster and easier than with previous methods [5–7, 9–15]. Sample treatment in our method involves only one extraction and no other clean-up steps, as compared with two to eight extractions required by other methods [5,7,9,10,12]. Nevertheless, typical chromatograms (Figs. 1 and 2) show captopril and the I.S. to be well separated from peaks due to endogenous substances. Plasma volumes ranging from 0.1 to 1.0 ml can be analyzed without modification to the method. Concomitantly administered drugs including chlorpromazine, promethazine, chloral hydrate (pre-catheterization sedative mixture), furosemide and digoxin did not interfere with this assay.

Calibration curves (Fig. 3) were linear in the range 10–400 ng/ml for free and 50–750 ng/ml for total captopril. The coefficients of variation ($n=4$ for all points) were 6.6 and 2.8% with 13.6 and 408 ng/ml captopril, respectively, for free captopril and 6.7 and 2% with 55.5 and 740 ng/ml captopril, respectively, for total

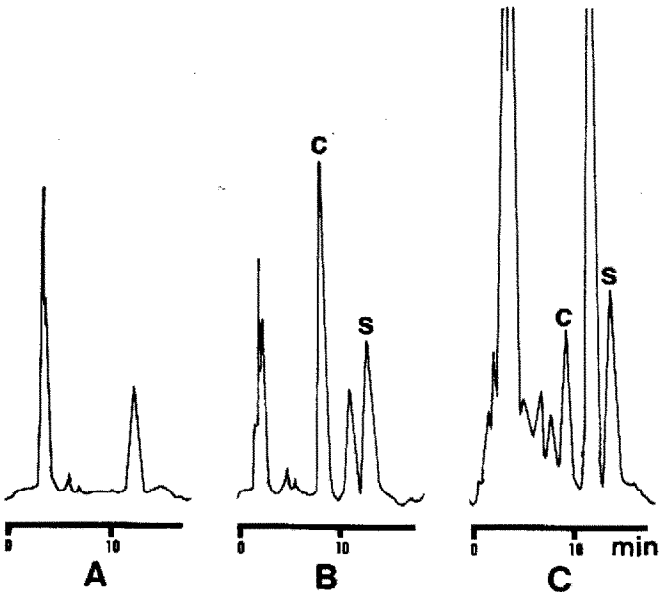

Fig. 1. Typical chromatograms for free captopril in plasma samples. (A) Blank; (B) spiked with 476 ng/ml captopril; (C) patient. Peaks: c = captopril, s = internal standard.

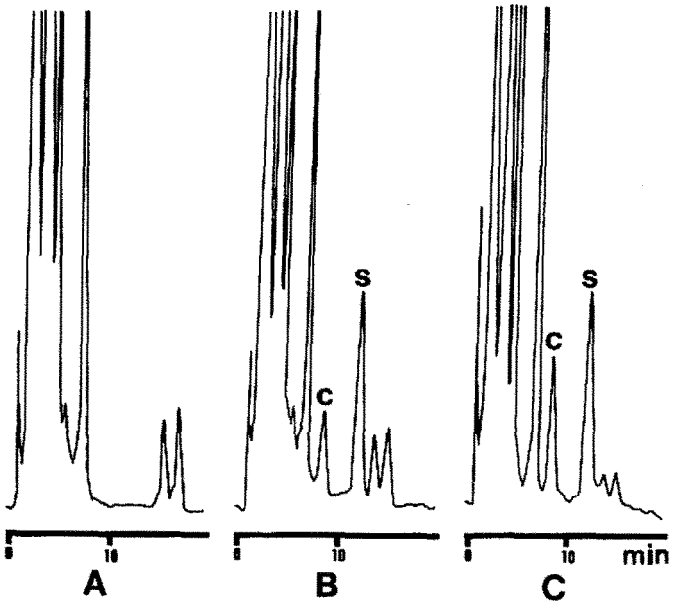

Fig. 2. Typical chromatograms for total captopril in plasma samples. (A) Blank; (B) spiked with 162 ng/ml free captopril equivalent; (C) patient. Peaks: c = captopril, s = internal standard.

captopril. The limit of determination was 10 ng/ml for free captopril and 50 ng/ml for total captopril using 0.5 ml plasma.

The maximum sensitivity of reported methods [5–15] ranges from 0.5 to 1000

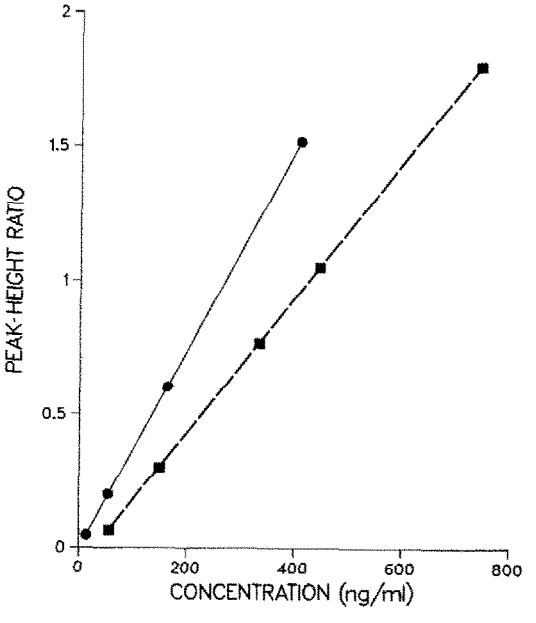

Fig. 3. Calibration curves for free (●) and total (■) captopril.

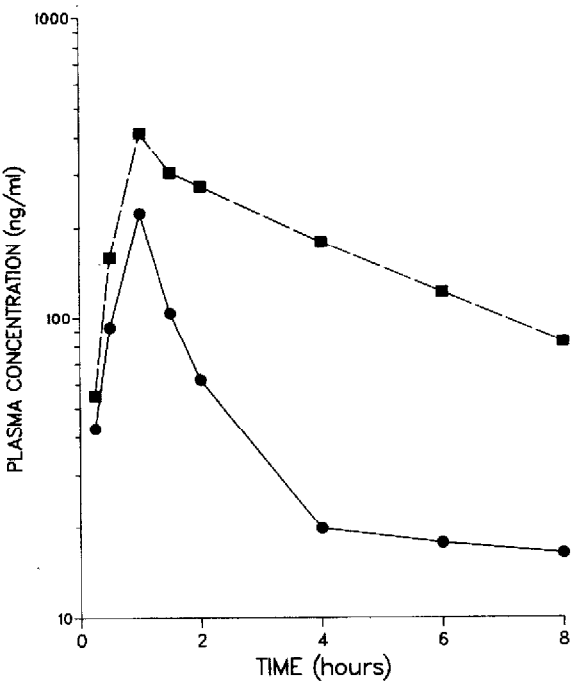

Fig. 4. Plasma concentration versus time curves for free (●) and total (■) captopril after a 1 mg/kg oral dose to an infant.

ng/ml. The coefficients of variation of these assays ranged from 0.6 to 13%. Hence the sensitivity and reproducibility of our method are comparable with available methods. Note that, although other assays have similar quantitation limits, the use of a smaller sample volume provides a definite advantage. Some methods [5,12,13,15] have detection limits (0.5–5 ng/ml) lower than ours. However, these methods are more cumbersome and time-consuming. The methods of Kawahara et al. [5] and Drummer et al. [12] also require larger volumes of blood (2–3 ml). The method of Perret and Drury [8] is simpler than ours and has similar detection limits, but it requires 1 ml of plasma and total captopril is not measured.

Derivatization with N-pyrenyl maleimide of thiol compounds in general and of captopril in particular has been described previously [6,16]. The derivatives of captopril and I.S. were found to be stable for at least one month when stored at −20°C. The reduction of the captopril dimer and mixed disulphides with TBP has been shown to be effective [5,9].

The present assay was used in a study with a 15-month-old infant with congestive heart failure. The plasma concentration versus time curves for free and total captopril are shown in Fig. 4. After a 1 mg/kg oral dose, the time to reach maximum plasma concentration for both free and total captopril was 1 h. The maximum plasma concentration was 223 ng/ml for free captopril and 412 ng/ml for total captopril. More than three times the adult dose (0.3 mg/kg) was required to achieve comparable plasma captopril levels and significant haemodynamic ef-

fects in this infant. Further investigation is needed in order to confirm this observation.

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