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Captopril: determination in blood and pharmacokinetics after single oral dose*

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Abstract: The study describes a specific, precise, sensitive and accurate method for determiantion of unchanged captopril, an angiotensin-converting enzyme inhibitor, in human plasma. Captopril was stabilized by forming an adduct with p-bromophenacyl bromide and this adduct was measured by high-performance liquid chromatography with UV detection. The standard curve was linear over a range of 30–800 ng ml^{-1}. The average yield of derivatization of the unchanged captopril was 73.6% and the recovery of captopril-adduct reached 93.1%. The limit of detection was 15 ng ml^{-1}, while the quantitative limit was 30 ng ml^{-1}. Inter- and intra-assay RSD was below 9%, but inter- and intra-assay accuracy was below 8%. On the basis of elaborated method, a single-dose pharmacokinetics in 12 men, in two doses (25 and 50 mg of captopril) has been investigated. The comparison of the pharmacokinetic parameters obtained from both doses of the drug have been made.

Keywords: *Captopril blood concentration; UV–HPLC detection; single dose pharmacokinetics.*

Introduction

Captopril, [1-(D-3-mercapto-2-methyl-1-oxopropyl)-L-proline) is the first orally active inhibitor of angiotensin-converting enzyme, and is used in the treatment of hypertension and congestive heart failure [1–3]. However, despite its clinical use, its pharmacological action is not yet completely understood, because it is readily converted into its disulphide dimer and forms disulphide conjugates with endogenous thiol compounds [4, 5]. Only the free captopril is pharmacologically active. Although the formation of the inactive disulphides is reversible, subsequently, they may act as a reservoir of free captopril and contribute to longer duration of action than predicted by the blood concentration of free drug [6]. Elaboration of sensitive and selective methods for monitoring the levels of captopril in body fluids to establish the relationship between the pharmacokinetics and drug responses in the body is still a current problem. Captopril has been determined in blood by several methods, including gas chromatography (GC) [7], gas chromatography–mass spectrometry (GC–MS) [8–10] and high-performance liquid chromatography (HPLC) [11–16]. The complexity of these methods, volumes of blood required or specialized equipment such as GC–MS may however limit their applicability and caused that they may not be widely accessible.

In this paper a relatively simple assay of free captopril in human plasma has been developed. Using this method, captopril kinetics on healthy volunteers after administration two doses of the drug have been studied.

Experimental

Apparatus and reagents

The HPLC system used was a Kontrol Instruments (Zurich/Switzerland) equipped with computer system for acquisition and integration of data (D 450). The apparatus were consisted of a pump (model 420), variable-wavelength UV–VIS absorbance detector (model 432), and column (Kontron Analytical S5 ODS2 250 × 4.6 mm i.d.). A mobile phase of acetonitrile–1% acetic acid (60:40, v/v), was used at a flow rate of 1.3 ml min^{-1}. The detector was set to 260 nm.

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Analysis was performed at ambient temperature.

Captopril in its free form was received from Lepharma (Landgraaf, The Netherlands). Captopril-adduct was prepared according to Klein *et al.* [11]. As a stabilizing agent, p-bromophenacyl bromide (p-BPB) (the Sigma Chemical Company, St Louis, USA) was used. Acetonitrile for HPLC was obtained from Serva (Heidelberg, Germany), Nitrazepam, used as internal standard was kindly supplied by Pharmaceutical Enterprise "Polfa-Poland". Other chemicals were of analytical reagents grade and were obtained from POCh (Gliwice, Poland). In all experiments double-distilled water have been used.

Subjects and procedure

The study was carried out on two groups of 12 healthy volunteers, aged 20–24 years, who were in good health and taking no concurrent medications or alcohol. All gave prior informed written consent. Because coadministration of captopril with food decreased the bioavailability of the drug [18], the investigations have been performed on fasting volunteers. After fasting overnight, each volunteer was administered orally 25 or 50 mg of captopril. The meals they had 3 h after drug administration. Venous blood samples (2 ml) were withdrawn to the tubes containing 0.05 ml of EDTA (0.1 M) and 0.05 ml of ascorbic acid (0.1 M), prior to the dose and again at 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0 and 8.0 h after drug ingestion. The blood samples were centrifuged and 0.5 ml of plasma was added immediately (time <15 min) to the screw-cap glass tube containing 0.03 ml of p-BPB (1 mg ml^{-1}) in acetonitrile and 0.05 ml of sodium hydroxide (0.1 M). The sample was shaken for 15 min to essentially complete the process of derivatization. Resulting captopril-adduct was stabilized by adding 0.075 ml of HCl (1 M). The obtained mixture was stored at -15°C and has been stable for about 3 months.

Assay procedure

Prior to the extraction of captopril-adduct, to acidified sample was added internal standard (500 ng ml^{-1}), and 0.15 ml of acetic buffer, pH 4.0 (0.2 M). Extraction was made by 4.0 ml of benzene. After centrifugation, the organic phase was evaporated to dryness under the stream of nitrogen and reconstituted in 0.2 ml of mobile phase. A 20-μl aliquot was injected into the column. Typical chromatograms of blank plasma (A) and plasma spiked with 400 ng ml^{-1} of captopril and 500 ng ml^{-1} of internal standard are shown in Fig. 1.

Results

Validation of analytical method

Linearity of the standard curves with accuracy below 10% was found in the range from 30 to 800 ng ml^{-1}. Relative standard deviations (RSDs) for seven independent calibration curves slopes, made between-days was 10.2%, mean correlation coefficient of those curves was 0.9924 ± 5.0%, the mean Y intercept is related to 10.8 ng ml^{-1}. The limit of detection of captopril, which gave a peak three times that the baseline noise, was 15 ng ml^{-1}, whereas quantitative limit was 30 ng ml^{-1}. Precision of the assay, calculated as a coefficient of variation for intra assay variability ranged from 4.9% for 600 ng ml^{-1} to 6.0% for 150 ng ml^{-1}, and for inter-assay validation from 6.5% for 150 ng ml^{-1} to 12.3% for 50 ng ml^{-1}.

Mean recoveries of extraction for three different concentrations of captopril in human plasma (150, 300 and 600 ng ml^{-1}) were 96.8, 92.2 and 90.4%, respectively, whereas mean recovery for internal standard at concentration 500 ng ml^{-1} was 88.5% ($n = 6$). The results of method validation are summarized in Table 1.

Extraction recovery of captopril-adduct in three different concentrations (150, 300, 600 ng ml^{-1}) was calculated by dividing the peak height after extraction by the peak height of captopril-adduct in the same concentrations injected directly into the column. The mean value is equal to 93.1%. The mean value of the conversion of free captopril to captopril-adduct was 73.6% (Table 1).

Pharmacokinetic studies

Kripalani *et al.* suggested the possibility of captopril complex nonlinear pharmacokinetics [17]. Other authors did not confirm this phenomenon [14, 18, 19]. We have examined the single-dose pharmacokinetics of captopril given in two doses (25 and 50 mg) on a 12 volunteers group.

After oral administration, captopril has been shown to be rapidly absorbed, reaching peak concentration (C_{max}) after 0.5–1.0 h (Table 2). Double increase of the dose from 25 to 50 mg

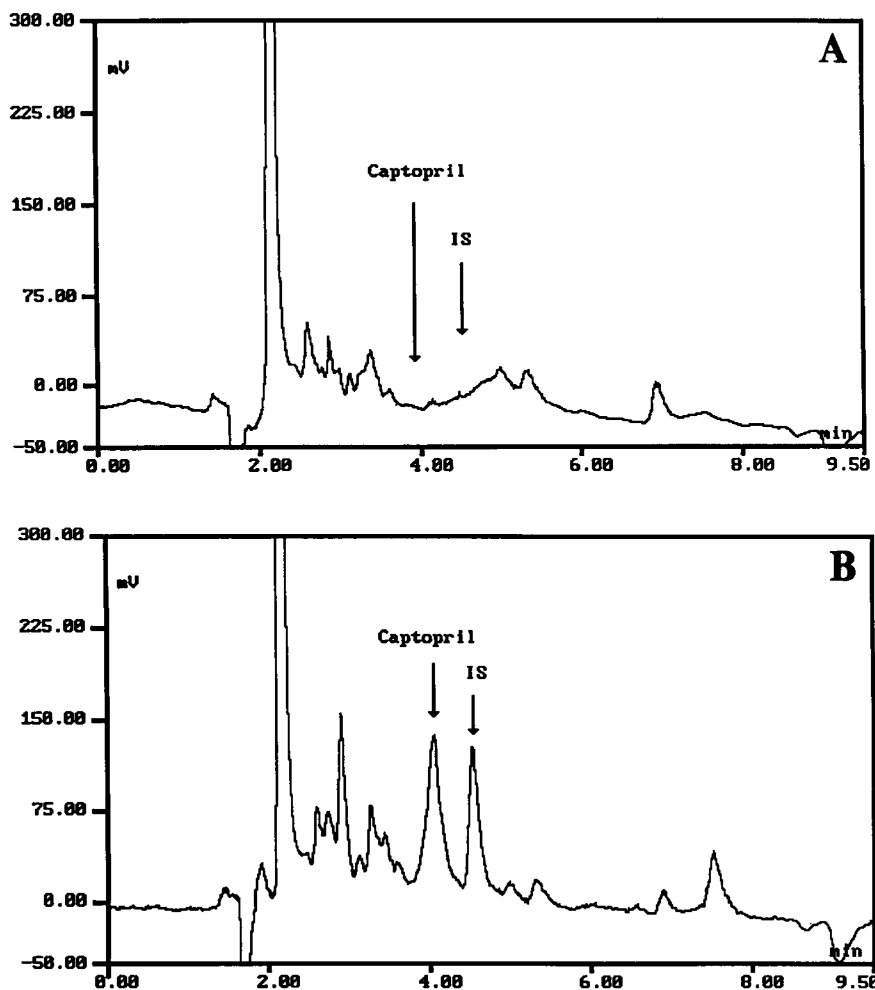

Figure 1
Typical chromatograms of blank plasma (A) and plasma spiked with captopril (300 ng ml^{-1}) and internal standard (500 ng ml^{-1}) (B).

Table 1
Validation of analytical method

	Concentration of captopril (ng ml^{-1})			
Parameter	50	150	300	600
Intra-assay variability				
n	—L	6	6	6
Mean	—	152.7	308.4	598.5
SD	—	8.6	15.0	29.3
RSD%	—	6.0	4.9	4.9
Acc%	—	4.9	6.0	5.6
Inter-assay validation				
n	6	11	12	11
Mean	55.0	147.6	302.3	572.3
SD	6.8	9.5	25.6	40.0
RSD%	12.3	6.5	8.5	7.0
Acc%	11.7	4.3	6.8	7.1
Mean recovery of extraction captopril-adduct (ER) and mean conversion yield of captopril to captopril-adduct (CY) (*n* – 6)				
ER%		96.8 ± 7.8	92.2 ± 7.1	90.4 ± 6.7
CY%		72.5 ± 8.2	74.4 ± 7.2	73.8 ± 8.6

Table 2
Mean pharmacokinetic parameters and statistical analysis

Parameter	D = 25 mg	D = 50 mg	*t*-test
AUC_{ot}	363.8 ± 92.3	536.7 ± 125.0	—
$AUC_{ot}/1\ mg$	14.55 ± 3.69	10.74 ± 2.50	$P < 0.05$
C_{max}	162.9 ± 72.1	290.1 ± 143.0	—
$C_{max}/1$ mg	6.52 ± 2.88	5.80 ± 2.86	n.s.
tm_{ax}	0.63 ± 0.23	0.79 ± 0.32	n.s.
$t_{0.5}$	1.11 ± 0.34	1.03 ± 0.19	n.s.
K_{el}	0.672 ± 0.176	0.688 ± 0.112	n.s.
Cl/F	72.92 ± 18.53	97.77 .005 22.34	—

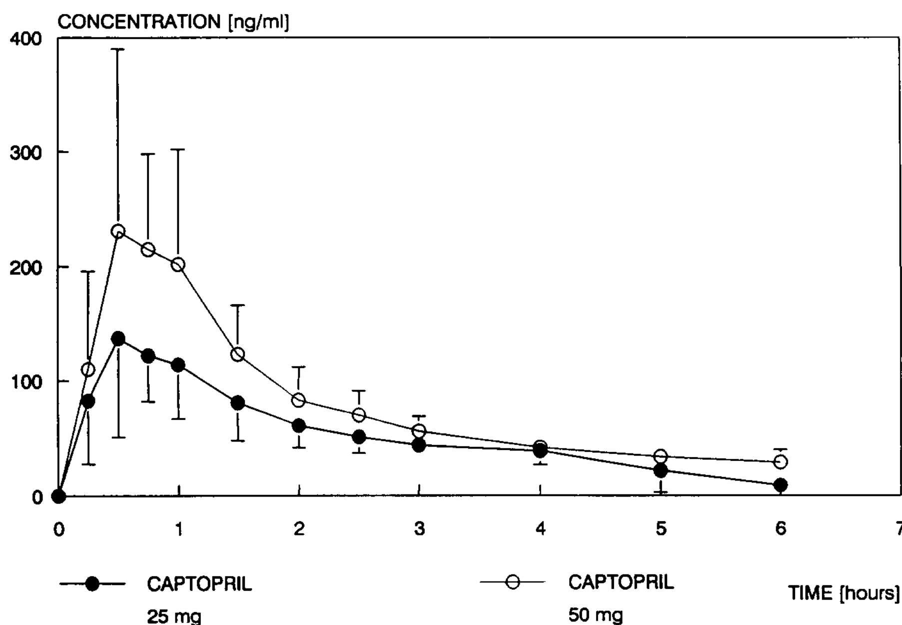

Figure 2
Concentration–time curves after single oral dose of 25 and 50 mg of captopril.

made about double increase C_{max} ratio (C_{max50}/C_{max25} = 1.914). After reaching peak concentration, blood levels of captopril declined rapidly and was generally undetectable at 6–8 h post-dose. Mean plasma captopril levels after administration 25 and 50 mg doses of captopril is presented in Fig. 2.

The increase of the captopril dose from 25 to 50 mg gave lower than two ratio of *AUC* (AUC_{50}/AUC_{25} = 1.639). For calculation of elimination parameters we assumed a single exponential elimination phase. Elimination rate constant after 25 mg dose is 0.651 ± 0.171 h^{-1} and after 50 mg, 0.699 ± 0.151 h^{-1}. For calculation of the total captopril clearance after oral administration, we assumed 60%, as the fraction reaching the systemic circulation (F = 0.6) according to [19]. The values of pharmacokinetic parameters of captopril given in two doses are presented in Table 2.

Discussion

Analytical method

The determination of captopril in blood is hampered by two analytical problems: lack of chromophores in the molecule to allow direct UV–HPLC detection and a limited stability of captopril in biological fluids. To develop suitable assay procedure, the sulphhydryl group had to be stabilized by using a variety of antioxidants, chelating agents, or derivatizing agents [11–16].

In our experiments, concentrations of captopril in blood plasma were determined by a specific HPLC assay with UV detection which is the modification of Klein *et al.*, method [11]. The modifications comprised different internal standard, alkalization just before derivatization of free captopril which rises its yield, finding out the suitable pH

conditions for plasma storage and additional buffering of the sample before the extraction. Also the chromatographic conditions, i.e. column and mobile phase, were modified.

In assay procedure described by Klein *et al.* [11] no information is given about pH conditions in which captopril is transformed to captopril-adduct. On the other hand, Kawahara *et al.* [15] have found that the addition of a base to the reaction mixture apparently increases the yield of derivatization. In general, our observations confirm these results. The total yield of derivatization of free captopril to captopril adduct for three different concentrations, i.e. 150, 300 and 600 mg ml^{-1} in six independent replications increased from 58.8%, without alkalization, to 73.6%, after alkalization (Table 1).

Additionally, since in Klein method, assay procedure have been done immediately after blood taken from the patient, they have not established the storage conditions. Owing to the great number of plasma samples in pharmacokinetic studies, in our case, suitable storage conditions had to be elaborated. In our experiment, acidification of captopril-adduct with 1 N HCl and storage in −20°C preserved stability during 6 weeks. We also have found that buffering in pH 4.0 improved the extraction yield more than 90%, see Table 1, while without buffering the results of extraction were lower and not stable.

Similar to Klein *et al.* [11] we chose p-BPB as derivatizing agent, to form captopril-adduct. The product, relatively simple to obtain, is stable within time and, simultaneously, exhibit UV-absorbing properties, useful in HPLC detection. Although, the sensitivity is not so high as in Klein method [11], in our conditions much lore lower endogenous interferences and better accuracy in low concentrations have been observed. The average Y intercept of all the calibration curves are related to 10.8 ng ml^{-1}, whereas in Klein method Y intercept value is related to about 100 ng ml^{-1}. Since plasma concentrations of captopril in terminal part are frequently less than 100 ng ml^{-1}, low accuracy can limit usefulness of the method in clinical practice or pharmacokinetic investigations. The sensitivity of the elaborated method proved to be high enough to measure drug concentrations in time within 0.25 to 6–8 h in all volunteers, after administration of a single dose of 25 and 50 mg of captopril. There were some problems with evaluation and validation of the results from the last part of terminal profiles, where sometimes even the raising of drug concentration can be observed. This phenomenon, which complicates also pharmacokinetics of captopril, can be caused by conversion of captopril in blood into dimmer, as well as re-conversion to free captopril over the time. So, the observed concentrations can represent endogenous balance between free and disulphides form of captopril in blood.

Pharmacokinetic parameters

In order to compare the pharmacokinetics of captopril, given in two doses, the calculations of C_{max} and AUC were standardized in relation to 1 mg of the dose (Table 2). As it can be seen, only normalized AUC value is statistically higher for 25 mg dose. Also C_{max} is slightly higher for the lower dose, but the difference is not statistically significant.

The AUC values can be affected by the differences on absorption level, i.e. dose, preparation and so on, or differences on elimination level which are related to inter-subject variability. As it can be seen in Table 2 the elimination parameters (K_{el}, $t_{0.5}$) do not differ statistically significant, what proved, that the differences in AUC were not caused by endogenic subject reasons or inter-subject differences.

Only clearance after oral administration (Cl/F), given by equation 1

$$Cl/F = D/AUC_{tot} \quad (1)$$

where D — dose, Cl — absolute clearance after intravenous administration and F — fraction or absorption, differs significantly. These differences however, are determined by differences in AUC_{tot} and, therefore, were not caused by endogenic subject reasons or inter-subject variabilities.

Conclusions

Satisfactory qualitative separation and quantitative determination of captopril in plasma within its therapeutic range has been described. The method applied in human investigation suggests the possibility of non-linear pharmacokinetics of captopril, which would be in compliance with the observations of Kripalani *et al.* [17].

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