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CAPTOPRIL DISULFIDE CONJUGATES MAY ACT AS PRODRUGS: DISPOSITION OF THE DISULFIDE DIMER OF CAPTOPRIL IN THE RAT

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Abstract—The absorption and metabolism of the disulfide dimer conjugate of captopril has been studied in the rat following both oral and intravenous dosing and compared with that of the active monomer, captopril. Metabolism of the dimer to captopril has been shown after both oral and intravenous administration of the dimer (10 mg/kg) with peak plasma levels of captopril (154 ng/ml) occurring at 1 hr post dose. By contrast the peak plasma level of captopril after oral administration of captopril (10 mg/kg) at the same dose was much higher at 678 ng/ml and also occurred at 1 hr post dose. Plasma captopril disulfide species were much higher than the plasma levels of captopril after the administration of either dimer or captopril and tended to persist for much longer than for monomeric captopril particularly after administration of the dimer. Both the dimer and its pharmacologically active product captopril were found in relatively large amounts in lung, kidney and liver following the oral administration of the dimer.

Captopril, an orally active inhibitor of angiotensin I converting enzyme (ACE), has a relatively short elimination half life in plasma with estimates in man ranging from 1.6 hr [1] to 1.9 hr [2]. Rapid metabolism of the labile sulfhydryl group of captopril to disulfide conjugates [3] and the rapid renal excretion of this acidic drug [4] are responsible for its quick disappearance from the body.

These are undesirable pharmacokinetic properties for an antihypertensive drug. We have investigated the possibility of using a prodrug form of captopril to improve the pharmacokinetics of this drug. Metabolic biotransformations of drug analogs which liberate active drug have been shown to improve bioavailability [5] and in some cases to improve the half-life of drugs [6].

Recent reports [7–9] have shown that disulfide conjugates of captopril formed by oxidation of captopril with endogenous thiols can be reduced back to captopril by thiols such as glutathione. We have therefore investigated the pharmacokinetics of the chemically stable disulfide dimer of captopril (CPD) in rats, after both intravenous and oral administration by measuring plasma levels of captopril and captopril disulfides and have compared this to that obtained after the oral administration of captopril.

MATERIALS AND METHODS

Chemicals. Sources of chemicals were: captopril and captopril disulfide dimer, Squibb (Princeton, NJ), *N*-ethylmaleimide, Sigma (St. Louis, MO), Ethylenediamine-tetraacetic acid, di-sodium salt (EDTA), Ajax (Sydney, Australia). All other reagents were of analytical reagent grade.

Preparation of drug solutions. Captopril disulfide dimer (CPD) was dissolved in a minimum amount of 5% (w/v) sodium hydrogen carbonate and made up to a concentration of 10 mg/ml either with water for oral experiments or with 5% w/v dextrose for intravenous experiments. Solutions of CPD prepared in this manner were stable for the day of study and did not undergo spontaneous reduction back to captopril. Solutions of captopril (CP) were prepared similarly except that the addition of base was not necessary.

Stability studies. Fresh heparinized rat blood (5 ml) was incubated with captopril or captopril disulfide dimer at a concentration of 10 μg/ml at 37°, in a shaking water bath. Duplicate aliquots (200 μl) were withdrawn at various times and added to glass extraction tubes containing 1 ml potassium phosphate buffer, 100 mM, pH 7.4 and 1 mg *N*-ethylmaleimide. The contents of the tubes were then assayed by gas chromatography–mass spectrometry (GC–MS) for captopril and captopril dimer [10].

Details of animal methodology. Sprague–Dawley rats of either sex weighing 140–220 g were used for all experiments. CP and CPD were administered by gavage for oral experiments and CPD was given through the tail vein for intravenous experiments. The dose of CP and CPD for all experiments was 10 mg/kg.

Tissue preparation and assay details. Animals were anaesthetized with halothane and aortic blood collected in EDTA-coated tubes. Various tissues including kidneys, lung and liver were also excised at various times after a single dose of drug. Blood was immediately centrifuged at 2000 g_{max} for 10 min to separate the plasma. Tissues were immediately cooled in ice, weighed, and as soon as practicable (always less than 30 min after dissection) homogenized in 5 vol. by weight of tissue with ice-cold 10 mM potassium phosphate buffer, pH 7.4, con-

taining 1 mM EDTA. An aliquot of plasma or tissue homogenate were assayed as soon as possible for captopril (400 μl), for total captopril (200 μl) (captopril and disulfide conjugates) by GC–MS as previously described [10]. Protein was measured by the method of Lowry *et al.* [11].

Measurement of angiotensin 1 converting enzyme activity. Angiotensin converting enzyme (ACE) activity was measured in plasma samples (collected in heparin tubes and stored on ice) using the fluorimetric method of Friedland and Silverstein [12]. Plasma samples were always assayed within 20 min of collection to avoid loss of inhibition [13].

Calculation of area-under-curve. The area under the plasma concentration–time curve (AUC) was calculated by the trapezoidal method on a benchtop calculator. AUC estimates extrapolated to infinity were calculated from the formula AUC $(t\text{–}\infty) = C_t/B_t$ where C_t is the concentration at time t and B_t is the exponent describing the decay in the concentration–time curve at time t. An estimate of this parameter was obtained using an exponential curve-fitting routine on the last 3 data points. Although estimation of B_t is inaccurate due to the limited number of points and the assumption of linear kinetics it does provide an estimate of the AUC for comparative purposes beyond 6 hr and its likely contribution to the overall AUC.

RESULTS

Stability of captopril and captopril dimer in rat blood

Captopril disappeared rapidly from rat blood when incubated at 37° with a half-life of disappearance of approximately 30 min. At 2 hr only 1.4% captopril was left. In contrast, the disulfide dimer of captopril was relatively stable in rat blood showing only a small (5%) loss over 2 hr. Only trace amounts of captopril were present in plasma after blood had been incubated with dimer for 2 hr.

Plasma levels in rats after administration of the dimer

Plasma levels of captopril were readily detected at 15 min (first time point studied) following oral administration of captopril dimer and reached a peak level of 157 ng/ml at 60 min post dose (Fig. 1). Plasma levels of total captopril disulfide species were much higher at all the time points studied and showing a peak level at 2 hr (Fig. 1). The amount of the captopril dimer present in the total captopril levels after oral administration of the dimer to rats was not detectable using a GC–MS procedure suggesting that most or all of the disulfide forms of captopril were mixed disulfide conjugates.

Area-under-curve estimates (AUC) for both free and total forms of captopril after oral administration of the dimer showed that free captopril represented only 28.2% of total circulating captopril species up to 6 hr post dose (Table 1).

Intravenous administration of the dimer also resulted in formation of monomeric captopril. Captopril levels appeared rapidly peaking at 15 min post dose (0.619 μg/ml) and rapidly declined thereafter (Fig. 2).

Plasma levels of total captopril were many times higher than for captopril (Fig. 2). AUC calculations

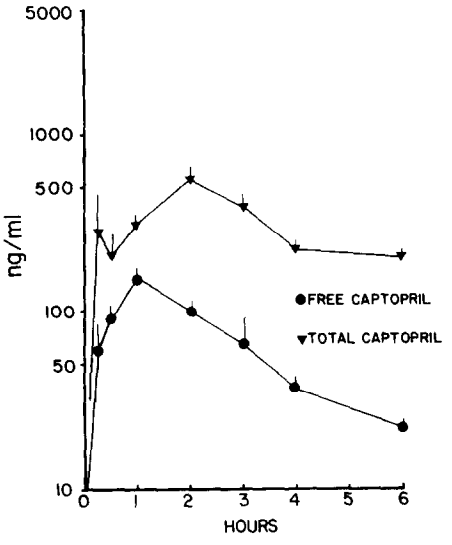

Fig. 1. Plasma levels of captopril and total captopril in rats following 10 mg/kg p.o. captopril disulfide dimer. Mean of 4–6 experiments, ± S.E.M.

show that 10.4% of all captopril species over 6 hr was represented by monomeric captopril (Table 1).

The bioavailability was assessed by dividing the AUC for captopril obtained from oral experiments by the AUC obtained from the intravenous study. This showed that the bioavailability of the dimer was approximately 4.8% when all captopril species were taken into account, and 57.0% when only free captopril was considered after p.o. and i.v. administration.

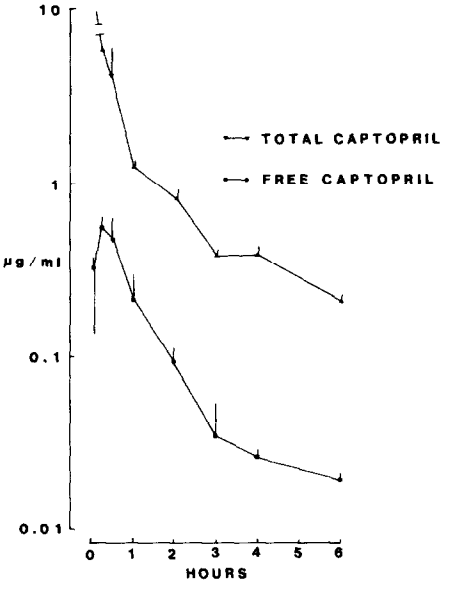

Fig. 2. Plasma levels of captopril and total captopril in rats following 10 mg/kg i.v. captopril disulfide dimer. Mean of 4 experiments, ± S.E.M.

Table 1. Pharmacokinetic parameters for captopril and total captopril species following oral (gavage) and intravenous administration of captopril dimer (10 mg/kg) to conscious rats

Parameter	Oral		Intravenous	
	Captopril	Total captopril	Captropril	Total captropril
C_{max} (μg/ml)	0.157 ± 0.030*	0.546 ± 0.117	0.619 ± 0.025	—
T_{max} (min)	60	120	15	—
AUC (μg/ml per min)	23.9	84.8	44.8	428
AUC (μg/ml per min) (0–∞ hr)	26.6	184.8	46.6	552
Bioavailability	4.8†	33‡	—	—

Total captopril refers to the total levels of captopril and captopril disulfide species measured by the reduction assay.

* Mean ± S.E.M. of 4–6 experiments.

† Bioavailability $= \frac{AUC_{po}\ (Cap)}{AUC_{iv}\ (Total\ Cap)}$.

‡ Bioavailability $= \frac{AUC_{po}\ (Cap)}{AUC_{iv}\ (Cap)}$.

Plasma levels after administration of monomeric captopril

The plasma levels of captopril and total captopril species in the conscious rat after administration of monomeric captopril were higher than what was observed after administration of the dimer (Fig. 3). The mean plasma level of captopril was 678 ng/ml at 1 hr post dose. This was over fourfold higher than the peak captopril levels observed after the dimer. Total plasma captopril levels were again much higher than captropril with a peak level of 3.10 μg/ml also occurring at 1 hr (Fig. 3).

AUC calculations showed that 19.7% of the total captopril species over 6 hr was captopril and 18.2% when the AUCs were extrapolated to infinity (Table 2).

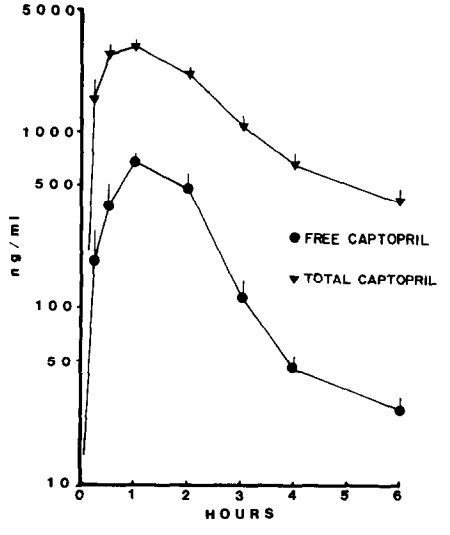

Fig. 3. Plasma level of captopril and total captopril in rats following 10 mg/kg p.o. captopril. Mean of 4 experiments, ± S.E.M.

Plasma half-life calculations

The plasma half-life of captopril and total captopril are difficult to obtain even under conditions which give more data points [14], however a comparison of the half-lives of the dimer and captopril can be obtained by estimating the half-life by fitting the last 3 points to an exponential curve-fitting routine. The half-life for captopril (98 min) and total captopril (341 min) after administration of the dimer were longer than the half-lives for captopril (60 min) and total captopril (101 min) after administration of captopril. This analysis assumes linear kinetics which is unlikely since the disposition of captopril is probably best fitted by a complex bi-phasic relationship [14].

Tissue levels of captopril and disulfide conjugates

The tissue levels of captopril and captopril conjugates were measured in kidney, liver and lung following administration of both captopril and captopril dimer (Fig. 4). These tissues have previously been shown to contain the highest concentration of captopril after oral captopril [3].

The highest concentration of captopril was present in kidney both as monomeric and conjugated captopril. Liver contained intermediate amounts of cap-

Table 2. Pharmacokinetic parameters for captopril and total captopril species following oral (gavage) administration of captopril (10 mg/kg) to conscious rats

Parameter	Captopril	Total captopril
C_{max} (μg/ml)	0.678 ± 0.466	3.10 ± 1.33
T_{max} (min)	60	60
AUC (μg/ml per min) 0–6 hr	82.7	419.9
AUC (μg/ml per min) 0–∞ hr	85.3	467.6

Results are means of 4 experiments (±S.E.M.).

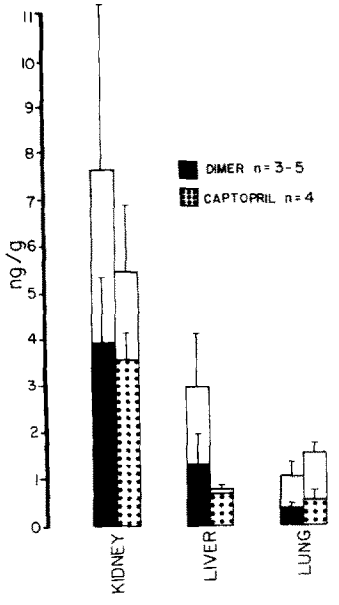

Fig. 4. Tissue levels of captopril (hatched bars) and total captopril disulfide conjugates (unhatched bars) in rats following 10 mg/kg p.o. of either captopril or captopril disulfide dimer. Each point is the mean of 4 experiments ± S.E.M.

topril species whilst lung displayed the lowest level of captopril species. The amount of captopril disulfides in lung and kidney were similar to that obtained after administration of captopril; however, liver contained a relatively large amount of captopril disulfides after administration of the dimer which were not present after oral administration of captopril.

Plasma angiotensin I converting enzyme activity

Angiotensin I converting enzyme activity in plasma was inhibited by the oral administration of both captopril and the captropril dimer ($P < 0.05$, Student's *t*-test). Peak inhibition for both compounds occurred at 60 min and was still significantly different from control activity at 2 hr ($P < 0.05$, Student's *t*-test). Activity had returned to control levels at 4 hr post dose (Fig. 5). The inhibition observed was greater with captopril than for the dimer ($P < 0.05$) which was consistent with the higher plasma levels of captopril observed after oral administration of captopril.

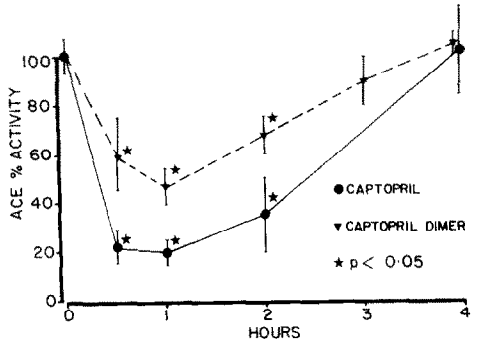

Fig. 5. Inhibition of plasma ACE in rats following 10 mg/kg p.o. of either captopril or captopril disulfide dimer. Each point is the mean of 4–6 experiments ± S.E.M. Asterisks indicate statistical significance from control activity ($P < 0.05$).

DISCUSSION

The metabolism of the antihypertensive drug captopril is rather unique compared to other drugs. Oxidation of the free sulfhydryl group is rapid resulting in a number of products particularly disulfides with cysteine, glutathione [14, 15] and high M.W. thiols which are most likely proteins [7].

Although oxidation of captopril occurs rapidly, particularly in plasma [3, 8] there is also a rapid renal excretion of captopril [4]. Typically about 50% of the available captopril is excreted unchanged, mainly in the first few hours after oral dosing [14]. As a consequence plasma levels of captopril decline rapidly and are generally low or undetectable 6 hr after a single dose [14, 16].

The metabolism of captopril is further complicated by recent reports which show that the formation of disulfide conjugates are reversible. Reduction of disulfide conjugates *in vitro* have been shown to occur in a number of tissue homogenates including liver, kidney, lung and intestine as well as RBC hemolysates [9]. Indirect evidence now suggests that disulfides of captopril may play a role in the maintenance of the hypotensive action of captopril probably by acting as a pool of drug which is capable of reduction back to captopril [18, 19].

We have shown in this study that oral and intravenous administration of CPD to conscious rats results in the rapid appearance of captopril in the systemic circulation and in tissues such as lung, kidney and liver. The disposition of CPD, expressed as free and total disulfide forms of captopril, is in this respect similar to that obtained following the administration of the same dose of captopril although the plasma levels after the dimer were lower than after captopril. However, plasma levels of total captopril declined at a slower rate when the dimer was given orally which may have advantages in reducing rather large variations in plasma levels of captopril after administration of captopril. The reduced clearance of the dimer may be a result of a reduced renal excretion as a result of a greater proportion of captopril present as disulfides which are not subject to renal excretion or due to a slower gastro-intestinal absorption of the dimer compared to captopril.

The overall bioavailability of the dimer expressed as free or total captopril was surprisingly good (43%) when the AUCs were extrapolated to infinity. This is similar to that obtained for captopril in the rat [17].

However, since estimates of the elimination half-life of captopril and dimer are subject to a large error due to the limited number of data points and the complex disposition of captopril the calculation of the bioavailability derived from extrapolated AUC can only be regarded as estimates. It was of interest that the inhibition of plasma ACE after the dimer was greater than expected relative to captopril since the plasma levels of captopril after the dimer were

much lower. Since the dimer does not inhibit ACE *in vitro* and requires prior reduction to captopril for activity it is likely that an interpretation of this result is the non-linear inhibition of plasma ACE when operating at the top end of the dose–response curve [20].

This study therefore raises the possibility of using a disulfide conjugate of captopril as a means of delivering captopril as a prodrug. The use of such a prodrug of captopril may increase the pharmacokinetic half-life of captopril in man and increase the duration of action of this rapidly metabolised drug. Use of such a compound may also reduce the rather large variations in plasma levels of captopril that are observed over an 8 hr period in man and as a consequence lessen the risk of developing potentially serious side effects. We are currently investigating the reduction of other disulfides of captopril *in vivo* to ascertain which conjugate possesses the most suitable characteristics for a prodrug.

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