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EFFECT OF PROBENECID ON THE DISPOSITION OF CAPTOPRIL AND CAPTOPRIL DIMER IN THE RAT

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Abstract—The urinary excretion of captopril has been studied in a bladder-cannulated rat model and compared with that obtained after co-administration with probenecid. Probenecid reduced significantly the urinary excretion of captopril from 41% to 21% of the administered dose over a 3-hr period and significantly lowered urine flow rates.

In addition, the effect of probenecid on plasma levels of captopril and total captopril (captopril plus disulfides) after oral administration of the disulfide prodrug captopril dimer (10 mg/kg) has been studied in a conscious rat preparation. Co-administration of probenecid (20 mg/kg) given either orally or intravenously increased both the plasma levels of captopril and total captopril (captopril plus captopril disulfides) over a 4-hr period. A prolonged significant inhibition of plasma ACE after co-administration of probenecid and captopril dimer suggests that probenecid may be useful to prolong the action of captopril or the prodrug captopril dimer.

Captopril, an orally active inhibitor of angiotensin I converting enzyme has a pharmacokinetic half-life in plasma of less than 2 hr in both man [1, 2] and in the rat [3]. This rapid elimination from plasma is associated with rapid urinary excretion of unchanged drug (20–30% of dose) [4, 5] and extensive metabolism to disulfides [6–8]. We have previously shown that administration of the disulfide dimer to rats gives rise to formation of captopril in plasma and tissues [3] and that disulfides of captopril have the potential to act as a prodrug form of captopril. High molecular weight disulfides of captopril such as those occurring in plasma and tissues are not excreted and are probably reduced back to captopril before elimination by the kidney.

Probenecid, a classical inhibitor of organic acid transport in the kidney [9] has been shown to reduce net tubular secretion of captopril in monkeys [5]. Since probenecid may be of use in prolonging the action of captopril or of acidic pro-drugs of captopril we have investigated the effect of co-administration of probenecid on the urinary elimination of captopril in anesthetised rats. In addition, we have studied whether probenecid co-administration with either captopril or captopril dimer increases blood levels.

MATERIALS AND METHODS

Bladder-cannulated anesthetized rat preparation. Male Wistar–Kyoto rats (weight approx. 250 g) were anesthetized with ether and a jugular vein was cannulated for administration of drugs. For maintenance anesthesia, propanidid (1% w/v in 5% dextrose w/v) was infused at a rate of 200 μl/min for the duration of the experiment. After laparotomy the bladder was opened and a polythene tubing (SP 70) with a flared end was tied into the upper portion of the bladder. Urine flow was monitored and when constant, after establishment of the cannula, a bolus injection of captopril (20 mg/kg i.v.) was given to one group of six rats and half-hourly to hourly collections of urine were taken over the next 3 hr.

In another group of four rats prepared in the same way a bolus injection of probenecid (20 mg/kg i.v.) [10] followed by a continuous infusion of probenecid (5 mg/kg per hr) were given 5 min prior to a bolus dose of captopril (20 mg/kg i.v.). Urine was collected in the same manner as for the first group. The probenecid was initially dissolved in dilute sodium hydroxide and the resulting solution adjusted back to approx. pH 9 with dilute acetic acid before administration.

Conscious rat preparation. In one group of Wistar–Kyoto rats (weight approx. 200 g) captopril dimer (10 mg/kg prepared in 5% w/v sodium bicarbonate at a concentration of 10 mg/ml) was given by gavage. In a second group of Wistar–Kyoto rats (weight approx. 200 g) probenecid (20 mg/kg) was given by gavage approximately 1 min before captopril dimer (10 mg/kg) by gavage.

In a third group of Wistar–Kyoto rats (weight approx. 200 g) probenecid (20 mg/kg) was given as a bolus intravenous injection through a tail vein previously dilated with warm water prior (1 min) to oral administration of captopril dimer (10 mg/kg).

The dosing of rats was staggered such that collection of blood from all dosed rats was timed to occur within 5 min of each other. Blood was collected by cardiac puncture of halothane anesthetized rats into cold heparin tubes and centrifuged immediately at 2000 *g* for 10 min in a refrigerated centrifuge (4°). This protocol was necessary to prevent loss of captopril and hence loss of ACE inhibitory activity through oxidation in blood after collection. Plasma was then immediately assayed for captopril, total captopril and angiotensin converting enzyme (ACE) activity.

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Measurement of captopril and total captopril. Captopril and total captopril (captopril present after reduction of disulfides with dithioerythritol) were measured in plasma and urine as described previously using gas chromatography–mass spectrometry [4, 6]. Blood was collected into heparinized tubes containing *N*-ethylmaleimide (1 mg per ml of blood) to prevent oxidation of captopril to disulfides. Urine was collected in plain tubes and was similarly stabilized. Urine volume was measured by weighing sample tubes before and after collection.

Measurement of plasma angiotensin 1 converting enzyme activity. An aliquot of plasma (10 μl) was assayed for angiotensin converting enzyme (ACE) activity within 10 min of collection using the fluorometric method of Friedland and Silverstein [11] with Hippuryl-histidyl-leucine as substrate. Enzyme activity was measured at 37° for 15 min, and expressed as nmole histidyl-leucine formed per min per incubation.

Materials. Sources of chemicals were: *N*-ethylmaleimide, Sigma Chemical Co. (St. Louis, MO); dithioerythritol, Calbiochem (San Diego, CA); Hippuryl-histidyl-leucine, Protein Research Foundation (Osaka, Japan). All other chemicals and reagents were analytical reagent grade. Probenecid (Gift from Merck, Sharp & Dohme), halothane (ICI, Australia) and propanidid (Bayer Pharmaceuticals, Australia) were used as described. Captopril and captopril disulfide dimer were kindly donated by the Squibb Institute (New Brunswick, NJ).

RESULTS

Anesthetized rat preparation

Rats anesthetized with an infusion of 1% propanidid in 5% glucose were found have a more constant urine flow than those anesthetized with either Inactin or Pentobarbitone.

The urine flow in rats with their bladders cannulated was found to be reasonably constant over a 3 hr period (Fig. 1), but varied from 25.7 μl/min (N = 6, S.E.M. = 5.3) in the first 30 min to 33.0 μl/min (S.E.M. = 8.8) over the third hour, with a slight but not statistically significant rise ($P > 0.05$ Student's *t*-test) occurring at 1 hr.

The urine flow of rats pretreated with an intravenous bolus injection of probenecid (20 mg/kg) followed by a maintenance infusion of 5 mg/kg per hour was significantly less ($P < 0.05$, Student's *t*-test) than control rats for the first 90 min. The urine flow over the first 30 min was 9.2 μl/min (N = 4, S.E.M. = 3.0) which was almost one-third of that obtained in the control rats given only captopril. During the third hour urine flow was 23.4 μl/min (S.E.M. = 8.3) which was not significantly different from the control group ($P > 0.05$).

Effect of probenecid on urinary excretion of captopril

Probenecid infused into anaesthetized rats with a loading dose of 20 mg/kg followed by a maintenance infusion of 5 mg/kg per hour significantly reduced the urinary excretion of captopril from 41% to 21% of the injected drug over a 3-hr period ($P < 0.05$, Student's *t*-test) (Fig. 2). The reduction in urinary excretion by probenecid was most evident in the first hour in which a reduction of 58% was observed compared to rats not receiving probenecid. The percentage of the captopril dose excreted in the first hour was 11% and 26% in the probenecid and control groups respectively (Fig. 2).

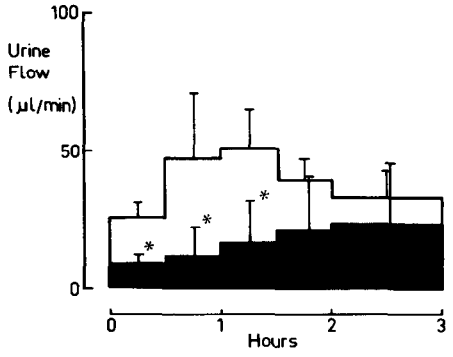

Fig. 1. Urinary flow rates in a control group treated with captopril only (20 mg/kg i.v.) or after co-administration of captopril (20 mg/kg i.v.) and probenecid (shaded portion) in bladder-cannulated rats (see Materials and Methods for dosage regimen). Means of 4–6 experiments. *$P < 0.05$ using Student's *t*-test.

Effect of probenecid on plasma levels of captopril following administration of the dimer

Plasma levels of captopril in the rat following oral administration of the dimer (10 mg/kg) peaked at 1 hr post dose (C_p = 109 ng/ml, S.E.M. = 18, N = 8) and showed a gradual decline thereafter with a plasma level of 42 ng/ml (S.E.M. = 6) at 4 hr (Fig. 3). Pre-treatment with oral probenecid (20 mg/kg) increased plasma levels of captopril at all time points beyond 30 min ($P < 0.05$) with the peak level also occurring at 1 hr post dose (C_p = 155 ng/ml, S.E.M. = 36, N = 4). Intravenous administration of the same dose of probenecid (20 mg/kg), however, had a greater effect on plasma levels, particularly at 30 min and 1 hr (N = 4). The peak level of captopril

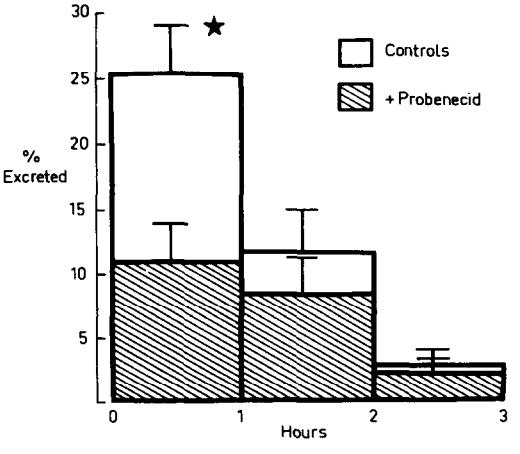

Fig. 2. Urinary excretion of captopril in a control group and after co-administration of probenecid and captopril (see methods for dosage regimen) in bladder-cannulated rats. Error bars are S.E.M. *$P < 0.05$ using Student's *t*-test.

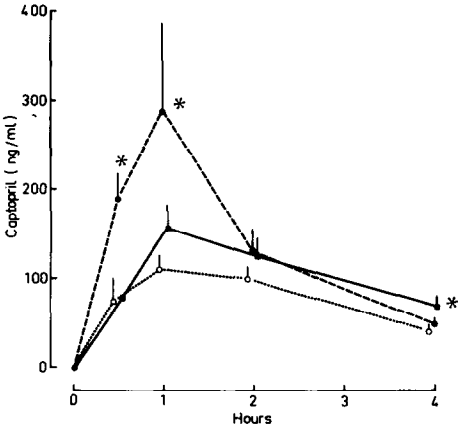

Fig. 3. Plasma levels of captopril after oral administration of captopril dimer (○. . .○), co-administration of oral captopril dimer and intravenous probenecid (●. . .●) or co-administration of oral captopril dimer and oral probenecid (■——■). See methods for details of doses used. Means S.E.M. of 4 experiments for the probenecid treated groups and mean of 8 experiments for the control group. *P < 0.05 using Student's *t*-test.

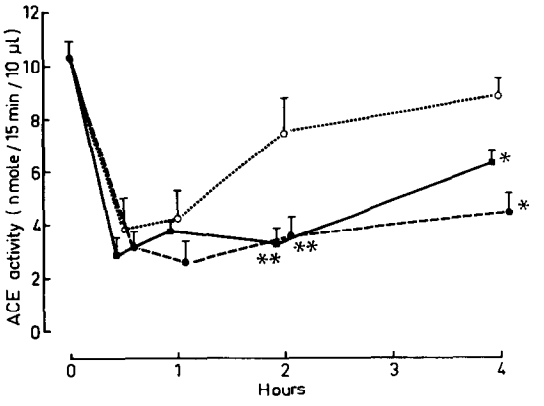

Fig. 5. Inhibition of plasma angiotensin converting enzyme after oral administration of captopril dimer (○——○), co-administration of oral captopril dimer and intravenous probenecid (●- - -●), and co-administration of oral captopril dimer and oral probenecid (■——■) in conscious rats. See methods for details of doses used. Means of 4–5 experiments ± S.E.M. Activity of ACE in the absence of drugs was 10.2 ± 0.6 nmole/15 min per 10 µl plasma (N = 18). *P < 0.05, **P < 0.01 using Student's *t*-test.

at 1 hr was almost 3-fold higher than in the control group; however, plasma captopril declined rapidly after i.v. administration of probenecid and at 4 hr was not different (Student's *t*-test, P > 0.05) to either the control group or the oral probenecid treated group (Fig. 3).

Plasma levels of total reducible captopril species which include those bound to proteins showed a similar profile to captopril, although peak levels occurred at 2 hr post dose in the control group (C_p = 546 ng/ml, S.E.M. = 117). These were significantly (P < 0.05) elevated by prior administration of oral probenecid (20 mg/kg) (Fig. 4); the peak plasma level of total captopril in the probenecid treated group was 747 ng/ml (S.E.M. = 119) and also occurred at 2 hr post dose. In contrast, intravenous administration of the same dose of probenecid (20 mg/kg) just prior to oral dimer (10 mg/kg) gave peak plasma levels of total captopril at 1 hr post dose of 871 ng/ml (S.E.M. = 208). By 4 hr plasma levels of total captopril following either oral or intravenous probenecid were not statistically higher than the control group (P > 0.05, Student's *t*-test).

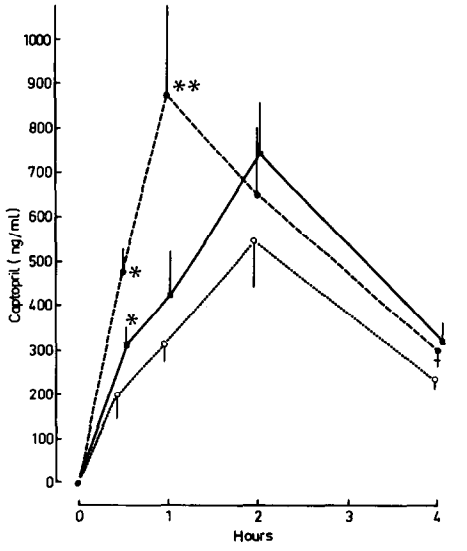

Fig. 4. Plasma levels of total captopril (total amount of captopril formed after reduction of disulfides) after oral administration of captopril dimer (○——○), co-administration of oral captopril dimer and intravenous probenecid (● ●), or co-administration of oral captopril dimer and oral probenecid (■——■) in conscious rats. See Methods for details of doses used. Means of 4 experiments ± S.E.M. for the probenecid treated group and 5–7 experiments for the control group. *P < 0.05, **P < 0.01 using Student's *t*-test.

Inhibition of angiotensin converting enzyme

Inhibition of plasma ACE which is a measure of the pharmacological activity of captopril showed a significant inhibition after a single oral dose of dimer. Peak inhibition occurred at 30 min (24% of control activity) and ACE activity remained depressed for at least 4 hr (Fig. 5). Oral and intravenous administration of probenecid significantly prolonged the duration of inhibition of plasma ACE (Student's *t*-test, P < 0.05). At 4 hr the inhibition of plasma ACE after probenecid was significantly less than the activity seen in the control group at 4 hr (Student's *t*-test, P < 0.05).

DISCUSSION

Probenecid [*p*-(dipropylsulfamoyl)benzoic acid] has been used to enhance plasma levels of penicillin antibiotics [12] and has been found to interact with a number of acidic drugs such as indomethacin [13], Naproxen [14], methotrexate [15] and salicylic acid [16] as well as uric acid [17]. Studies in rats have shown that probenecid reduces the renal clearance of frusemide [10] probably by inhibition of renal tubular secretion.

We have shown here using an *in vivo* rat model in

which the bladder is cannulated to allow continuous sampling of urine that probenecid at doses needed to inhibit tubular secretion [10] reduces the renal excretion of captopril. The reduction in renal excretion was most apparent in the first hour post dose in which a reduction of almost 60% was apparent. Over a 3 hr period the amount of captopril excreted as a percentage of dose was reduced from 41% to 21% by probenecid. The reduced excretion of captopril by probenecid was also accompanied by a significant reduction in urine flow rate. This was most pronounced in the first 2 hr post dose and was almost back to control flow rates in the third hour.

These observations are consistent with an earlier report in man which has shown that a single dose of probenecid reduces renal excretion of captopril over 24 hr from 45.8% to 28.9% [18] and is associated with an estimated reduction of net tubular secretion of captopril from 78% to 57%. Since captopril is excreted to a large extent by a tubular secretion process this effect of probenecid on reducing the urinary excretion of captopril does not seem to be related to any alteration in urinary pH by probenecid since tubular secretion of weak acids is largely pH independent.

We have extended our studies with probenecid to investigate its effect on the plasma levels of captopril and captopril disulfides after administration of the disulfide dimer of captopril. The dimer is being investigated as a potential pro-drug of captopril in which metabolic biotransformation to captopril is required for biological activity [3]. Probenecid significantly increased plasma levels of both captopril and total captopril (disulfides) after a single dose of dimer with the maximum increase occurring at 1 hr post dose. The increase was most apparent with disulfides of captopril and this is probably related to the fact that a captopril disulfide is given instead of captopril and probably also by an increased metabolism of captopril to disulfides caused by an increased residence time of captopril in plasma and tissues. Captopril has previously been shown to be extensively metabolized in plasma and tissues mostly to high molecular weight disulfides [8, 19] which require conversion back to captopril or lower molecular weight forms before renal excretion [20–22]. Oral administration of captopril dimer results in rapid conversion to captopril and mixed disulfides during its 'first-pass' [3]. Hence the profile of captopril and captopril disulfides in plasma is similar after either administration of captopril or captopril dimer. This is reflected by a similar profile of metabolites in the urine of rats treated either with captopril or captopril disulfide dimer (Drummer, unpublished observations). Therefore any possible selective effect of probenecid on blocking the urinary excretion of low molecular weight captopril disulfides appears unlikely.

Administration of probenecid intravenously had a much more marked effect on plasma levels of both captopril and total captopril (disulfides) than oral administration of the same dose. Although comparison with oral and intravenous probenecid has not been critically reported in the literature our data would suggest a low bioavailability of probenecid in the rat (Drummer, unpublished observations) probably caused by extensive first-pass metabolism rather than poor absorption [23].

The increase in plasma levels of captopril and particularly captopril disulfides by probenecid in this study is consistent with a study in man which has shown a reduction in tubular secretion of captopril and a concomitant increase in plasma levels of captopril species during continuous intravenous administration of [^{14}C]-captopril [18].

Measurement of inhibition of plasma ACE as an index of the biological action of captopril has shown that probenecid significantly increases the duration of inhibition rather than increasing the peak inhibition. This is similar to the effect seen with inhibition of pressor responses to Angiotensin I where increasing the dose of captopril increased the duration of action without a further reduction in pressor response to angiotensin I [24]. This may be related to a saturation of the ACE inhibition (top end of the dose–response curve) rather than a true effect on peak responses.

Co-administration with probenecid, therefore, decreases urinary excretion of captopril and increases plasma levels of both captopril and total disulfides after oral dosing with captopril dimer in the rat as well as increasing the duration of action of the dimer as assessed by inhibition of plasma ACE. This is consistent with an inhibition of renal tubular secretion of captopril by probenecid. However, it has yet to be established whether probenecid co-administration has any real advantages clinically in the use of either captopril or the disulfide pro-drug when either of these drugs are given chronically.

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