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Absorption and Bioavailability of Captopril in Mice and Rats after Administration by Gavage and in the Diet

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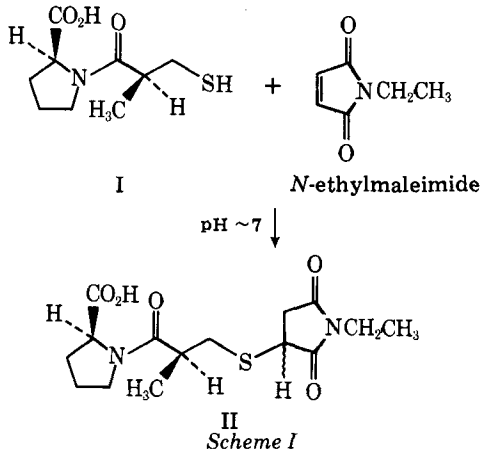
Abstract □ The absorption of captopril (I), a new antihypertensive agent, was studied in mice and rats at doses (50 and 1350 mg/kg) administered in the diet in chronic toxicological studies. ^{3}H- or ^{35}S-Labeled I was administered by gavage and in the diet to male and female animals in a two-way crossover study. Animals received daily doses of nonradiolabeled I in the diet for 25 days, except on Days 15 and 22 when radiolabeled I was administered either by gavage or in the diet. Absorption of the total radioactivity in 2-month-old mice averaged 49 and 48%, respectively, of the 50- and 1350-mg/kg doses given in the diet and 57 and 65%, respectively, of the doses given by gavage. The bioavailability of I in 2-month-old mice averaged 48 and 39% (diet) and 44 and 59% (gavage) of the 50- and 1350-mg/kg doses, respectively. In 2-month-old rats, absorption of the total radioactivity averaged 41% of the 50-mg/kg dose given in the diet. In 2- and 15-month-old rats, minimum absorption of the 1350-mg/kg dose averaged 36 and 45% (diet) and 51 and 71% (gavage), respectively; the minimum bioavailability averaged 29 and 29% (diet) and 39 and 44% (gavage), respectively. These studies demonstrate adequate absorption and bioavailability of I over a wide range of doses from the drug–diet mixtures and by young and old animals and also illustrate a useful experimental design for the estimation of relative oral absorption of a drug administered continuously in the diet over several days.

Keyphrases □ Captopril—absorption and bioavailability, gavage and dietary administration, mice and rats □ *Metabolism*—absorption and bioavailability of captopril, gavage and dietary administration, mice and rats □ Antihypertensives—absorption and bioavailability of captopril, gavage and dietary administration, mice and rats

Captopril (I), 1-[(2*S*)-3-mercapto-2-methyl-1-oxopropyl]-L-proline, is a potent and specific inhibitor of the enzyme that catalyzes the conversion of angiotensin I to angiotensin II (1) and was an orally effective antihypertensive agent in extensive clinical trials (2–4). The disposition of I in normal subjects was reported recently (5). Specific assays for determination of I as its *N*-ethylmaleimide derivative (II) also were reported (6, 7).

In oral toxicological and pathological studies over a 2-year period, I was administered daily in the diet to mice and rats at 50, 150, and 1350 mg/kg. The present study evaluated the effects of food and of repeated daily administration of 50- and 1350-mg/kg doses of I on its absorption and bioavailability in 2-month-old mice and rats and 15-month-old rats.

Investigations in animals and *in vitro* indicate that I is chemically unstable in biological fluids and undergoes rapid autoxidation to form III, the disulfide dimer of I, and other products. To prevent or minimize such processes, a procedure for immediate conversion of I to II in biological samples was established (5–7) (Scheme I). This procedure was utilized in the determination of nonlabeled I using GLC–mass spectrometry (7) and of radiolabeled I using thin-layer radiochromatography (6). Since radiolabeled I was necessary to determine absorption in the present study, thin-layer radiochromatography was used.



Scheme I

EXPERIMENTAL

Materials—^{3}H-Labeled I (uniformly labeled in the proline moiety) and ^{35}S-labeled I had radiochemical and chemical purities of at least 95%, with a maximum of 5% of III present as an impurity. No other impurities were detected. To administer sufficient radioactivity for good quantitation, each dose contained a minimum of 5 μCi (range of 5–40 μCi). Since the doses ranged from 50 to 1350 mg/kg and since there was an approximate 10-fold difference between the average weights of mice and rats, the specific activities of radiolabeled I also varied greatly, from 0.06 to 1.0 μCi/mg for ^{3}H-labeled I and from 2.7 to 8.9 μCi/mg for ^{35}S-labeled I.

Animals—Male and female CD-1 outbred albino mice and CD outbred albino rats[1] were used. All mice were ~2 months old at the start of the study and weighed ~35 (males) and 27 (females) g. Two-month-old rats weighed ~260 (males) and 170 (females) g, and 15-month-old rats weighed 550 (males) and 330 (females) g. All animals had access to food and water *ad libitum* and were housed individually in metabolic cages throughout the study.

Experimental Design—For each dose level and age group, 16 animals (eight males and eight females) initially were given 14 consecutive daily doses of either 50 or 1350 mg of I/kg (nonradioactive) admixed with the diet. On Day 15, half of the animals (four males and four females) received radiolabeled drug (tritium or sulfur 35) in the diet; the other half received radiolabeled drug in aqueous solution by gavage. Administration of the nonradioactive drug–diet preparation was resumed on Day 16. On Day 22 (crossover), the animals that had received the radioactive drug in the diet on Day 15 were given the radioactive drug in aqueous solution by gavage; those that had received the drug in aqueous solution by gavage on Day 15 were given the radioactive drug in the diet. Administration of the nonradioactive drug–diet preparation was resumed for 3 days. The animals were given a drug-free diet on the days they received the radioactive drug by gavage.

Urine and feces were collected every 24 hr for 4 days after each radioactive dose. To minimize I oxidation, urine samples for the first 2 days after drug administration were collected in vessels containing 2 ml of a 2.5% aqueous solution of *N*-ethylmaleimide (5).

In an early study using 2-month-old rats, single 50-mg/kg doses of radiolabeled I were administered in the diet to six (three males and three females) animals and by intravenous route to four (two males and two females) animals. Thus, in that study, repeated administration of nonradiolabeled drug was not employed, and the animals did not receive radiolabeled I by gavage. Additionally, in separate studies in 2-month-old mice, single 50-mg/kg doses of radiolabeled I were given intravenously to eight (four males and four females) animals, and single 650-mg/kg doses of radiolabeled I were given intravenously to four males. In these studies, urine and feces also were collected for 4 days.

In experiments where 16 animals were used initially, a few animals that received the drug by gavage died of trauma due to misdosing during the first or the second phase of the study. Data were obtained for less than 16 animals.

Preparation, Administration, and Assay of Dose—*Nonradioactive I*—The drug–diet preparation was prepared fresh each week. Compound I was mixed with an appropriate amount of ground diet[2] in a mixer[3] to give a drug concentration that provided an estimated daily dose of 50 or 1350 mg/kg. Homogeneity and stability of the drug–diet mixture prepared by this procedure were satisfactory. The animals were offered this drug–diet preparation each day, except when receiving radioactive drug. Food consumption and body weights were determined daily.

Radioactive I—Animals were weighed just prior to dosing, and doses were administered based on those weights. For each animal, an accurately weighed amount of radiolabeled I, corresponding to 50 or 1350 mg of drug/kg, initially was combined with an appropriate amount of feed. The feed and drug were mixed thoroughly on glassine paper with a spatula. To facilitate essentially complete consumption of the drug–diet preparation, the total weight of the feed mixed with the radiolabeled drug was ~0.5 g less than the average daily consumption of feed during the 3 days prior to dosing with radiolabeled drug.

The drug–diet preparation was transferred to individual feeding cups and offered to the animal. The material adhering to the glassine paper and spatula and that remaining in the feeding cup after 24 hr were transferred with methanol to a volumetric flask and assayed in duplicate for total radioactivity. The amount of radiolabeled drug consumed with the feed by each animal was determined by subtracting the total amount of radioactivity found in the combined washings from the total radioactivity added initially to the feed. For dosing by gavage, a solution was prepared fresh by dissolving radiolabeled I in distilled water.

Liquid Scintillation Counting—The scintillation cocktail of Anderson and McClure (8) was used to count all samples, except feces, in studies using [^{3}H]captopril. This cocktail contained 0.2 g of 1,4-bis[2-(5-phenyloxazolyl)]benzene, 3 g of 2,5-diphenyloxazole, and 250 ml of a nonionic surfactant[4], which were brought to volume (1 liter) with xylene. A saturated solution of sodium pyruvate in methanol, acetic acid, and methanol in a ratio of 4:3:1 (by volume) was used to neutralize the contents of the vials before counting. Individual samples were prepared as will be described.

Urine (0.1 ml) was mixed with 0.5 ml of a tissue solubilizer[5], 15 ml of scintillation cocktail, and 0.1 ml of the neutralizing solution. In studies using [^{35}S]captopril, fecal samples were shaken with ~2–3 volumes of water for 16 hr. A portion of the suspension (0.2 g) was digested by shaking for 16 hr with 1.0 ml of the solubilizer. The digested sample was bleached with 1.0 ml of 20% benzoyl peroxide and mixed with 0.1 ml of the neutralizing solution and 15 ml of the scintillation cocktail.

Silica gel scrapings from TLC plates were mixed with 1.0 ml of water and counted in 15 ml of the scintillation cocktail.

Fecal homogenates of animals dosed with [^{3}H]captopril were combusted in a sample oxidizer[6]. The resulting tritiated water was trapped in 20 ml of scintillation cocktail[7].

All samples were counted in liquid scintillation spectrometers[8]. Counting efficiencies were determined with automatic external standardization and the use of previously prepared quench curves. To correct for the decay of sulfur 35 radioactivity (half-life of 87 days), suitably diluted aliquots of the dosing solutions were counted with each set of biological samples.

Thin-Layer Radiochromatography—A thin-layer radiochromatographic assay of I was described previously (5, 6). In the present study, aliquots (20–100 μl) of urine were chromatographed on 0.25-mm silica gel GF plates[9] in chloroform–ethyl acetate–acetic acid (4:5:3). Compounds I, II, and III were used as reference standards and were visualized by exposure to iodine vapor; their R_f values were 0.55, 0.42, and 0.34, respectively. Each TLC plate was divided into three zones based on the location of the reference standards. The zone in the 0.4–0.6 R_f range, which included I and II, was used to determine unchanged I in the sample.

Data Analysis—Estimates of absolute absorption of total radioactivity and absolute bioavailability of I were obtained using urinary excretion data; the percentages of the dose excreted in urine as total radioactivity and as unchanged I after oral administration of I were divided by similar data obtained following administration of a single comparable intravenous dose of radiolabeled I.

Statistical analyses were performed using the Student *t* test and analysis of variance. Where necessary, the overall means were adjusted for the unequal number of animals in the different groups.

RESULTS

For rats and mice at 50- and 1350-mg/kg doses in the diet and by gavage, there were no statistically significant differences in amounts of radioactivity recovered in urine and feces between the sexes or between the two phases of the crossover. Therefore, the data for all animals (males and females) and for both phases of the crossover were combined for each mode of administration, and the overall mean excretion values were determined for mice (Table I) and rats (Table II). For both modes of administration and in both species, most excretion in urine and feces occurred during the first 48 hr after dosing. Between 48 and 96 hr, <3% of the administered radioactivity was excreted in the urine and feces of mice and <8% was excreted in the urine and feces of rats.

Absorption and Bioavailability in Mice—For the 50-mg/kg oral dose, mean recoveries of radioactivity in urine were 37.2% (diet) and 43.8% (gavage) of the dose (Table I). Following a single intravenous dose (50 mg/kg), excretion of radioactivity in the 0–96-hr urine was 76.3% of the dose. Thus, the absolute absorption values for the oral radioactive doses

[1] Charles River Breeding Laboratories, Wilmington, Mass.
[2] Purina Laboratory Chow, Ralston-Purina Co., St. Louis, Mo.
[3] Hobart Manufacturing Co., Troy, Ohio.

[4] Triton X-114, Ruger Chemical Co., Irvington, N.J.
[5] Soluene-350, Packard Instrument Co., Downers Grove, Ill.
[6] Packard model 306 automatic Tri-Carb oxidizer, Packard Instrument Co., Downers Grove, Ill.
[7] Monophase-40, Packard Instrument Co., Downers Grove, Ill.
[8] Packard Tri-Carb model 2425 or 3380, Packard Instrument Co., Downers Grove, Ill.
[9] Analtech Inc., Newark, Del.

Table I—Absorption and Excretion of Total Radioactivity after Administration of Radiolabeled Captopril to Mice

Dose, mg/kg	Number of Mice	Route/Mode of Administration	Percent of Dose Excreted in 0–96 hr[a]			Absorption of Radioactivity, %
			Urine	Feces	Total	
50	8 (4 M, 4 F)	Intravenous	76.3 ± 2.29	13.8 ± 1.97	90.1 ± 1.56	—
	12 (7 M, 5 F)	*per os* (in diet)	37.2 ± 5.16	48.0 ± 1.46	85.2 ± 5.71	48.8 ± 6.92
	12 (7 M, 5 F)	*per os* (by gavage)	43.8 ± 3.68	47.3 ± 3.30	91.1 ± 1.43	57.4 ± 5.12
1350	4 M	Intravenous (650 mg/kg)	89.8 ± 1.19	8.84 ± 0.72	98.7 ± 0.95	—
	16 (8 M, 8 F)	*per os* (in diet)	42.8 ± 1.18	47.6 ± 1.61	90.4 ± 1.13	47.6 ± 1.46[b]
	16 (8 M, 8 F)	*per os* (by gavage)	58.7 ± 1.43	34.8 ± 2.22	93.5 ± 1.22	65.4 ± 1.81

[a] All values are the mean ± *SEM*. [b] Significantly less than the mean for the gavage dose ($p < 0.05$).

Table II—Excretion of Total Radioactivity after Administration of Radiolabeled Captopril to Rats

Age, months	Dose, mg/kg	Number of Rats	Route/Mode of Administration	Percent of Dose Excreted in 0–96 hr[a]		
				Urine[b]	Feces	Total
2	50	4 (2 M, 2 F)	Intravenous	93.0 ± 1.34	9.0 ± 1.15	102.0 ± 0.23
		6 (3 M, 3 F)	*per os* (in diet)	37.7 ± 1.30	58.9 ± 2.55	96.5 ± 1.93
	1350	16 (8 M, 8 F)	*per os* (in diet)	35.5[c] ± 1.19	61.5 ± 2.68	97.0 ± 1.96
		13 (7 M, 6 F)	*per os* (by gavage)	51.2 ± 1.80	41.4 ± 1.45	92.6 ± 1.02
15	1350	13 (7 M, 6 F)	*per os* (in diet)	45.1[c] ± 1.66	50.8 ± 2.71	95.9 ± 2.64
		12 (6 M, 6 F)	*per os* (by gavage)	71.2 ± 2.98	24.5 ± 3.21	95.7 ± 2.23

[a] All values are the mean ± *SEM*. [b] These values represent minimum absorption of the orally administered dose. [c] Significantly less than the mean for the gavage dose ($p < 0.05$).

were 48.8% (diet) and 57.4% (gavage). These values were not statistically significantly different ($p > 0.05$). Unchanged I excreted in the 0–24-hr urine accounted for 19.4% (diet) and 18.0% (gavage) (Table III). After intravenous administration of a comparable dose, unchanged I in the urine accounted for 40.5% of the dose. Therefore, the absolute bioavailability values for I after oral administration of 50 mg/kg were 47.9% (diet) and 44.4% (gavage). These values were not significantly different ($p > 0.05$).

Based on relative urinary excretion (as a percentage of the radioactive dose) after oral (1350 mg/kg) and intravenous (650 mg/kg) administration, absorption of the oral radioactive 1350-mg/kg dose was 47.6% (diet) and 65.4% (gavage) (Table I)[10]. Thus, absorption of the 1350-mg/kg dose given in the diet was ~73% of that given by gavage. Based on the excretion of unchanged I in urine (0–48 hr) after oral and intravenous administration, the absolute bioavailability of I from the 1350-mg/kg oral dose was 39.2% (diet) and 58.6% (gavage) (Table III). The differences in the absorption and bioavailability values for the two modes of administration were statistically significant ($p < 0.05$).

Absorption and Bioavailability in Rats—The absolute absorption of the oral radioactive dose (50 mg/kg) in 2-month-old rats was 40.5% based on recoveries of radioactivity in 0–96-hr urine of 37.7% (diet) and 93.0% (intravenous) of the administered dose (Table II). At the time this study was done, an assay for I was not available and bioavailability estimates could not be obtained.

Excretion in the 0–96-hr urine (representing minimum absorption) after administration of 1350 mg/kg in 2-month-old rats accounted for 35.5% (diet) and 51.2% (gavage) of the dose (Table II). Unchanged I excreted in the 0–48-hr urine (representing minimum bioavailability) accounted for 28.9% (diet) and 39.4% (gavage) of the dose (Table IV).

In 15-month-old rats (1350-mg/kg dose), excretion in the 0–96-hr urine (representing minimum absorption) accounted for 45.1% (diet) and 71.2% (gavage) of the dose (Table II). Unchanged I excreted in the 0–48-hr urine accounted for 29.2% (diet) and 44.0% (gavage) of the dose (Table IV). The differences in minimum absorption values for the two modes of administration were statistically significant in both age groups ($p < 0.05$). The differences in minimum bioavailability values for the two modes of administration were statistically significant for the 2-month-old rats ($p < 0.05$) but not for the 15-month-old rats ($p > 0.05$).

Since urinary excretion of total radioactivity and unchanged I after parenteral administration of 1350 mg of the drug/kg was not determined in rats, the values reported for absorption and bioavailability of I represent minimum estimates.

DISCUSSION

During chronic oral toxicological studies, test substances are administered to rodents either by gavage or by incorporation in the diet. Boyd (9) reported that administration of the entire daily dose to animals by gavage reveals the maximum toxic potential of chronic human dosing better than administration of a compound in the diet. However, in a more recent pharmacokinetic study in rats, continuous dietary administration of an orally absorbed cephalosporin, cefatrizine, produced higher peak plasma concentrations, longer exposure, and greater drug bioavailability than administration of the same daily dose given once a day orally by gavage (10). Furthermore, in many cases, the convenience of administering a drug in the diet and the elimination of trauma and dangers of pulmonary complications caused by intubation make dietary drug administration the preferred mode during long-term toxicological studies. Moreover, for a drug that is given more than once a day for therapeutic efficacy, dietary administration in toxicological studies mimics more closely the therapeutic dosage regimen and, thus, has a better chance of showing potential accumulation than single daily doses administered by gavage. On the other hand, administration by gavage provides maximum control over quantitation and the dosing time and might be more suitable for drugs with long half-lives and whose absorption is severely impaired by the presence of food.

The present studies demonstrate that the absorption and bioavailability of I in mice and rats were satisfactory at doses of 50 and 1350 mg/kg given in the diet during repeated daily administration. There were no apparent differences in the absorption and bioavailability of I between males and females of either species. The data suggest that the absorption

[10] The comparison between 1350-mg/kg po and 650-mg/kg iv doses was considered to be valid since (in separate studies) absorption of I in the dose range of 50–650 mg/kg was found to be dose independent. The maximum nontoxic intravenous dose in mice was 650 mg/kg.

Table III—Bioavailability of Captopril after Administration of Radiolabeled Captopril to Mice

Dose, mg/kg	Number of Mice	Route/Mode of Administration	Percent of Dose Excreted in Urine: Total Radioactivity	Percent of Dose Excreted in Urine: Unchanged Captopril	Bioavailability, %
50[a]	8 (4 M, 4 F)	Intravenous	74.0 ± 2.75	40.5 ± 4.26	—
	12 (7 M, 5 F)	*per os* (in diet)	33.0 ± 5.10	19.4 ± 3.11	47.9 ± 9.18
	12 (7 M, 5 F)	*per os* (by gavage)	41.0 ± 3.13	18.0 ± 1.98	44.4 ± 6.76
1350[b]	4 M	Intravenous (650 mg/kg)	89.4 ± 1.28	73.4	—
	16 (8 M, 8 F)	*per os* (in diet)	41.6 ± 1.15	28.8 ± 1.77	39.2 ± 2.43[c]
	16 (8 M, 8 F)	*per os* (by gavage)	57.6 ± 1.35	43.1 ± 0.52	58.6 ± 0.73

[a] All values are the mean ± *SEM* for individual animals and represent the 0–24-hr urine. [b] The 0–48-hr urine was pooled for males and females separately. [c] Significantly less than the mean for the gavage dose ($p < 0.05$).

Table IV—Urinary Excretion of Unchanged Captopril after Oral Administration of Radiolabeled Captopril (1350 mg/kg) to Rats

Age, months	Number of Rats	Route/Mode of Administration	Percent of Dose Excreted in 0–48-hr Urine[a]: Total Radioactivity	Percent of Dose Excreted in 0–48-hr Urine[a]: Unchanged Captopril[b]
2	16 (8 M, 8 F)	*per os* (in diet)	34.3 ± 0.98	28.9 ± 2.08[c]
	13 (7 M, 6 F)	*per os* (by gavage)	50.2 ± 1.78	39.4 ± 1.79
15	13 (7 M, 6 F)	*per os* (in diet)	44.4 ± 1.62	29.2 ± 4.04
	12 (6 M, 6 F)	*per os* (by gavage)	67.5 ± 2.50	44.0 ± 3.75

[a] All values are the mean ± *SEM;* the 0–48-hr urine was pooled for males and females separately in each group for thin-layer radiochromatography. [b] These values represent minimum bioavailability of the administered dose. [c] Significantly less than the mean for the gavage dose ($p < 0.05$).

and bioavailability of I were actually higher in 15-month-old rats than in 2-month-old rats. This observation indicates that animals were exposed to substantial amounts of I throughout the 2-year toxicological and pathological studies. In general, the absorption and bioavailability of I were greater after gavage doses than after the same doses given in the diet.

Although <19% (mice) and <39% (rats) of the administered dose were excreted as unchanged I in urine after oral administration, there was no evidence for any first-pass biotransformation or biliary excretion (and recycling) of I. In fact, the data in Table III indicate that the ratios of unchanged I to total radioactivity excreted in urine were comparable after oral and intravenous administration. In addition, fecal excretion after intravenous administration of I in rats (~9% of the dose) and in mice (~14% of the dose), in conjunction with biliary excretion of radioactivity determined in bile-cannulated rats (~10% of the dose) after oral administration of radiolabeled I^{11}, indicated little biliary excretion of I and its metabolites. Therefore, the lesser amount of unchanged I excreted in urine after oral administration as compared to intravenous administration appeared to be due to incomplete I absorption rather than first-pass biotransformation.

Repeated I administration did not significantly affect the extent of absorption or bioavailability of I in mice, based on a comparison of results obtained in the present studies with results obtained in previous studies in which single oral doses of I were given to fasted mice. The two-way crossover studies also illustrate a useful experimental design for the estimation of relative absorption of a drug administered continuously in the diet over several days. The effect of repeated administration on the absorption rate of I was not determined. However, all absorption and excretion measurements were carried out in animals that had received the drug for a minimum of 2 weeks.

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