Pharmacokinetics of Captopril in Dogs and Monkeys

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Abstract □ [^{14}C]Captopril was given as a priming dose, followed by constant intravenous infusion for 4 or 6 hr, to three anesthetized dogs and three anesthetized monkeys. Blood, urine, and bile samples were collected during and after drug infusion. Pharmacokinetic evaluations were carried out exclusively on data obtained for unchanged captopril. The average total body clearance (Cl_T) and the renal clearance (Cl_R) of captopril, in milliliters per kilogram per hour, were 605 and 341 in the dog and 1135 and 944 in the monkey. The biliary clearance of captopril was negligible in both species. The greater difference between the Cl_R and Cl_T values in the dog compared to that in the monkey was the result of more extensive metabolism of captopril by the dog. Since almost all of the radioactive dose was recovered in urine in both species, captopril and its metabolites were almost exclusively eliminated by the kidneys. One primary reason that body and renal clearance values of captopril were much greater in the monkey than in the dog was that the net tubular secretion of captopril was about three times greater in the monkey (82%) than in the dog (28%). The volume of distribution of captopril was higher in the monkey (3.6 liters/kg) than in the dog (2.5 liters/kg); the volume of the central compartment was about the same (0.5 liter/kg) for both species. The terminal half-life value was slightly higher in the dog (2.8 hr) than in the monkey (2.2 hr).

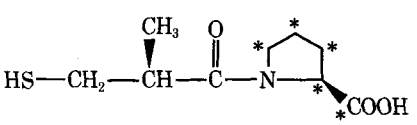
Keyphrases □ Captopril—pharmacokinetics in dogs and monkeys using radiolabeled drug, continuous intravenous infusion □ Pharmacokinetics—captopril in dogs and monkeys, continuous intravenous infusion □ Antihypertensives—pharmacokinetics of captopril in dogs and monkeys, continuous intravenous infusion

Captopril, 1-[(2*S*)-3-mercapto-2-methyl-1-oxopropyl]-L-proline, is a potent, orally active, specific inhibitor of the enzyme that catalyzes the conversion of angiotensin I to angiotensin II (1). It also was shown clinically to be an effective antihypertensive agent (2–4). This study determined the pharmacokinetics of captopril in dogs and monkeys using [^{14}C]captopril.

BACKGROUND

Captopril, a thiol compound, is chemically unstable in biological samples and undergoes rapid autoxidation to form the disulfide dimer of the parent compound and other unidentified products (5, 6). To prevent or minimize such processes, captopril was converted to its *N*-ethylmaleimide derivative in blood, urine, and bile during or immediately after sample collection (5, 6).

In previous blood level studies in animals and humans, captopril was administered orally, and difficulty was encountered in estimating its biological half-life and other pharmacokinetic parameters. This difficulty was partly because of the lack of a sensitive specific assay and partly because of an apparent nonlinearity in the terminal portions of semilogarithmic plots of blood levels *versus* time. It was anticipated that efforts to obtain pharmacokinetic parameters following a single intravenous bolus dose of captopril would have been hampered by the same problems. Consequently, to obtain better pharmacokinetic information, it was decided to study captopril pharmacokinetics in dogs and monkeys during steady-state conditions achieved by continuous intravenous infusion.



I(* = sites labeled with carbon 14)

EXPERIMENTAL

Materials—[^{14}C]Captopril was universally labeled with carbon 14 in the proline moiety. Radiochemical and chemical purities were each ~98%; ~2% of [^{14}C]captopril disulfide was present as an impurity. A nonionic surfactant[1], a tissue solubilizer[2], and *N*-ethylmaleimide[3] were used. Other chemicals were reagent grade and were obtained commercially.

Animals—Three male adult purebred beagles (9–11 kg) and three male adult rhesus monkeys (6–8 kg) were used.

Procedure—The study design was identical for dogs and monkeys. Each animal was fasted overnight, hydrated orally with water (25 ml/kg for dogs; 15 ml/kg for monkeys), anesthetized with intravenous pentobarbital sodium (30 mg/kg), intubated with an endotracheal catheter, and maintained under anesthesia by constant intravenous infusion of pentobarbital sodium (2.7 mg/kg/hr).

For determination of creatinine clearance, a priming dose of 60 mg of creatinine/kg was injected intravenously followed by 18 mg/kg/hr in the infusate. The infusate also contained phosphate-buffered 5% mannitol (pH 7.4) to maintain adequate urine flow. After the start of the infusion, the animal was surgically prepared for the collection of bile and urine. The cystic duct was ligated, and the common bile duct was cannulated near its junction with the duodenum; then both ureters were cannulated for urine collection. Urine and bile were collected for consecutive 30-min periods, and a blood sample was withdrawn at the midpoint of each collection period. After two consecutive control periods, [^{14}C]captopril priming and constant infusion doses were started simultaneously.

For the first dog and monkey, the priming and infusion doses (Tables I and II) were selected using data obtained from preliminary studies; the infusions were continued for 4 hr. Subsequently, the doses were determined based on results from the first animal of each species such that the steady-state concentrations of unchanged captopril in the blood of the next two animals of each species were approximately the same as the first (0.2 μg/ml). The doses for the last two dogs and monkeys were as follows: a priming dose of 0.4 mg/kg (dog) and 1.0 mg/kg (monkey) over a 1-min period and constant infusion of 0.1 mg/kg/hr (dog) and 0.2 mg/kg/hr (monkey) for 6 hr. [^{14}C]Captopril was infused using a constant infusion pump[4]. After the end of the infusion of [^{14}C]captopril, the 5% mannitol infusion was continued, and the animal was kept under anesthesia for an additional 8 hr. The 5% mannitol infusate contained creatinine and pentobarbital sodium and was infused throughout the study using another constant infusion pump[4].

Urine and bile were collected every 30 min during [^{14}C]captopril infusion, for 3 hr after the end of infusion, and then every 1 hr for the next 5 hr. A blood sample was withdrawn at the midpoint of each collection. All samples were treated with *N*-ethylmaleimide and analyzed for total radioactivity and unchanged captopril.

Analytical Methods—A thin-layer radiochromatographic assay for captopril in blood and urine was described previously (5, 6). Bile and urine samples were analyzed in an identical manner. This method is quite specific for unchanged captopril and has a limit of detection of ~10 ng/ml. The scintillation cocktail of Anderson and McClure (7) was used to count blood and bile samples. The tissue solubilizer[2] was added to solubilize whole blood and bile samples. Urine samples were counted directly in Bray's liquid scintillation counting solution[5].

All samples were counted in liquid scintillation spectrometers[6]. These counters were regularly normalized for valid utilization of automatic external standardization ratios for determination of disintergrations per minute. Known standards were counted to verify the accuracy of the instruments. Counting efficiency was determined using automatic ex-

[1] Triton X-114, Ruger Chemical Co., Irvington, N.J.
[2] Soluene-350, Packard Instrument Co., Downers Grove, Ill.
[3] Eastman Kodak, Rochester, N.Y.
[4] Sigmamotor, Middleport, N.Y.
[5] National Diagnostics, Parsippany, N.J.
[6] Packard Tri-Carb liquid scintillation spectrometers, model 2425 or 3380, Packard Instrument Co.

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Table I—Pharmacokinetic Parameters Obtained in Anesthetized Dogs during and after Infusion of [^{14}C]Captopril

Pharmacokinetic Parameter	Infusion Data[a] Animal H8-113	H6-117	H8-23	Average	Postinfusion Data Animal H8-113	H6-117	H8-23	Average	Overall Average, ±SD
Steady-state blood level, μg/ml	0.20	0.17	0.21	0.19	—	—	—	—	0.19 ± 0.02
Priming dose, mg/kg	1.37	0.42	0.44	—	—	—	—	—	—
Infusion dose, mg/kg/hr	0.10	0.12	0.12	—	—	—	—	—	—
Body clearance, ml/kg/hr	516	718	545	593	560	754	540	618	605 ± 103
Renal clearance, ml/kg/hr	364	444	233	347	343[b]	428[b]	235[b]	335	341 ± 91
Biliary clearance, ml/kg/hr	0.40	0.41	0.55	0.45	—	—	—	—	0.45 ± 0.08
Creatinine clearance, ml/kg/hr	282	328	148	253	—	—	—	—	253 ± 94
Renal clearance/body clearance	0.71	0.62	0.43	0.59	0.61	0.57	0.44	0.54	0.56 ± 0.11
Renal clearance/creatinine clearance	1.29	1.35	1.57	1.40	—	—	—	—	1.40 ± 0.15
Net tubular secretion, %[c]	22	26	36	28	—	—	—	—	28 ± 7
Apparent volume of distribution, liters/kg	4.06[d]	2.41	2.86	2.63	2.18	2.59	2.22	2.33	2.45 ± 0.28
Volume of central compartment, liters/kg	—	—	—	—	0.63	0.43	0.40	0.49	0.49 ± 0.13
Half-life ($t_{1/2}\beta$), hr	5.44[d]	2.33	3.61	2.97	2.71	2.38	2.84	2.64	2.77 ± 0.51
α, hr^{-1}	—	—	—	—	1.52	2.60	2.62	2.25	2.25 ± 0.63
β, hr^{-1}	—	—	—	—	0.26	0.29	0.24	0.26	0.26 ± 0.03
k_{12}, hr^{-1}	—	—	—	—	0.45	0.71	1.03	0.73	0.73 ± 0.29
k_{21}, hr^{-1}	—	—	—	—	0.44	0.43	0.47	0.45	0.45 ± 0.02
k_{el}, hr^{-1}	—	—	—	—	0.89	1.75	1.37	1.34	1.34 ± 0.43
k_e, hr^{-1}	—	—	—	—	0.54	1.00	0.59	0.71	0.71 ± 0.25

[a] Mean of the last three (Dog H8-113) or eight (Dogs H6-117 and H8-23) consecutive 30-min infusion periods, considered to be at steady-state. [b] Calculated using all data obtained during and after the infusion of [^{14}C]captopril (see Fig. 2). [c] Represents minimum net tubular secretion since captopril protein binding was assumed to be zero (see text for details). [d] These values were considered to be unreliable because true steady-state was not achieved in this dog; not included in the average.

Table II—Pharmacokinetic Parameters Obtained in Anesthetized Monkeys during and after Infusion of [^{14}C]Captopril

Pharmacokinetic Parameter	Infusion Data[a] Animal 5-31	8-7	8-1	Average	Postinfusion Data Animal 5-31	8-7	8-1	Average	Overall Average ±SD
Steady-state blood level, μg/ml	0.22	0.16	0.27	0.22	—	—	—	—	0.22 ± 0.06
Priming dose, mg/kg	2.52	0.93	0.97	—	—	—	—	—	—
Infusion dose, mg/kg/hr	0.20	0.20	0.22	—	—	—	—	—	—
Body clearance, ml/kg/hr	915	1316	823	1018	1262	1551	941	1251	1135 ± 285
Renal clearance, ml/kg/hr	997	1174	672	948	1017[b]	1150[b]	653[b]	940	944 ± 229
Biliary clearance, ml/kg/hr	0.20	0.16	0.18	0.18	—	—	—	—	0.18 ± 0.02
Creatinine clearance, ml/kg/hr	200	197	114	170	—	—	—	—	170 ± 49
Renal clearance/body clearance	1.09	0.89	0.82	0.93	0.81	0.74	0.69	0.75	0.83 ± 0.14
Renal clearance/creatinine clearance	4.98	5.84	6.04	5.56	—	—	—	—	5.56 ± 0.56
Net tubular secretion, %[c]	80	83	83	82	—	—	—	—	82 ± 1.7
Apparent volume of distribution, liters/kg	3.92	2.55	2.52	3.00	4.67	5.19	2.91	4.26	3.63 ± 1.14
Volume of central compartment, liters/kg	—	—	—	—	0.70	0.50	0.34	0.51	0.51 ± 0.18
Half-life ($t_{1/2}\beta$), hr	2.97	1.33	2.12	2.14	2.57	2.32	2.15	2.35	2.24. ± 0.55
α, hr^{-1}	—	—	—	—	2.66	3.64	3.91	3.40	3.40 ± 0.66
β, hr^{-1}	—	—	—	—	0.27	0.30	0.32	0.30	0.30 ± 0.03
k_{12}, hr^{-1}	—	—	—	—	0.73	0.49	1.03	0.75	0.75 ± 0.27
k_{21}, hr^{-1}	—	—	—	—	0.40	0.35	0.46	0.40	0.40 ± 0.06
k_{el}, hr^{-1}	—	—	—	—	1.80	3.10	2.74	2.55	2.55 ± 0.67
k_e, hr^{-1}	—	—	—	—	1.45	2.30	1.92	1.89	1.89 ± 0.21

[a] Mean of the last four (Monkey 5-31) or six (Monkeys 8-7 and 8-1) consecutive 30-min infusion periods, considered to be at steady-state. [b] Calculated using all data obtained during and after the infusion of [^{14}C]Captopril (see Fig. 2). [c] Represents minimum net tubular secretion since captopril protein binding was assumed to be zero (see text for details).

ternal standardization. The color and chemical quenching of the samples were corrected by using appropriately prepared quench curves. All samples were prepared and analyzed in duplicate and were counted for a minimum of 3000 counts, which resulted in a counting error of <1.8%.

Data Analysis—Pharmacokinetic parameters for captopril were calculated from data obtained during the infusion and postinfusion phases of the study, using relationships given in the *Appendix*.

RESULTS

In all animals, blood pressure, heart rate, and respiration rate were monitored; no major changes were observed in any of these parameters during the infusion of [^{14}C]captopril. The creatinine clearance, a measure of the glomerular filtration rate, also was found to be reasonably constant during the infusion period.

Pharmacokinetic evaluations were carried out exclusively on data obtained for unchanged captopril in blood, urine, and bile during the steady-state period and the postinfusion phase. A typical semilogarithmic plot of concentrations of unchanged captopril in blood *versus* time is shown in Fig. 1. In all animals, the blood levels followed a similar pattern as a function of time; the levels were initially high due to the priming dose, steady-state levels were reached toward the end of the infusion period, and the blood levels declined biexponentially during the postinfusion period. This biexponential decline of blood captopril concentrations indicates that the drug exhibits characteristics of a two-compartment open model, with elimination occurring from the central compartment.

The priming and infusion doses of [^{14}C]captopril given to the first dog (H8-113) and the first monkey (5-31) were selected using limited data available from previous blood level studies, which were not designed to estimate pharmacokinetic parameters. Because of the relatively short duration of infusion and deficiencies in the priming and infusion doses initially selected, true steady-state conditions were either just approached or attained for only a limited time in the first dog and first monkey. Based on the information derived from these two animals, the priming and infusion doses of [^{14}C]captopril were modified to attain rapid steady-state conditions in an additional two dogs (H6-117 and H8-23) and two monkeys (8-7 and 8-1).

For the latter animals, the infusion period for [^{14}C]captopril was extended from the 4 hr used in the earlier study to 6 hr to maintain steady-state conditions for a longer period. As a result, good steady-state conditions were attained for the last two dogs and two monkeys. Most results for these animals were comparable to those obtained for the first animal of each species. Therefore, with few exceptions, the results of the various pharmacokinetic parameters were averaged for all three animals

Table III—Excretion of Total Radioactivity and Unchanged Captopril in the Urine and Bile of Anesthetized Dogs and Monkeys following Infusion of [^{14}C]Captopril

Excretion	Component	Percent of Dose Excreted[a]							
		Dog				Monkey			
		H8-113	H6-117	H8-23	Average ± *SD*	5-31	8-7	8-1	Average ± *SD*
Urine	Total radioactivity	92.8	95.2	83.8	90.6 ± 6.0	91.7	99.9	90.6	94.1 ± 5.1
	Captopril	58.1	61.3	39.4	52.9 ± 11.8	80.6	86.7	75.8	81.0 ± 5.5
Bile	Total radioactivity	0.78	2.65	3.45	2.29 ± 1.37	0.31	0.49	0.47	0.42 ± 0.10
	Captopril	0.04	0.05	0.09	0.06 ± 0.03	0.01	0.02	0.02	0.017 ± 0.006
Total (urine and bile)	Total radioactivity	93.6	97.9	87.3	92.9 ± 5.3	92.0	100.4	91.1	94.5 ± 5.1
	Captopril	58.1	61.4	39.5	53.0 ± 11.8	80.6	86.7	75.8	81.0 ± 5.5

[a] Cumulative excretion for entire duration of the study (4- or 6-hr infusion period plus 8-hr postinfusion).

(Tables I and II). Since pharmacokinetic parameters calculated from both steady-state and postinfusion data were comparable for each animal, overall averages of all the parameters also were calculated.

Average clearance values (milliliters per kilogram per hour) obtained in the dog were 605 (body clearance, Cl_T), 341 (renal clearance, Cl_R), and 0.45 (biliary clearance, Cl_B) (Table I). Average creatinine clearance (Cl_{Cr}) was 253 ml/kg/hr. A comparison of the Cl_R and Cl_{Cr} values indicated an average minimum net tubular secretion of captopril of 28% in the dog. Net tubular secretion values were calculated by assuming no reversible (physicochemical) binding of captopril to plasma proteins and, therefore, represent minimum estimates. Since *N*-ethylmaleimide used for stabilizing captopril hemolyzes blood, plasma could not be obtained for determination of protein binding. However, *in vitro* studies done in this laboratory showed that, while captopril forms chemically reversible covalent bonds with plasma proteins, its physicochemical (noncovalent) binding to plasma proteins is negligible (8). The average volume of distribution (V_d) in the dog was 2.5 liters/kg, while the volume of the central compartment (V_1) averaged ~0.5 liter/kg. The average terminal half-life ($t_{1/2}\beta$) in blood in the dog was ~2.8 hr (Table I).

In the monkey, the average clearance values, in milliliters per kilogram per hour were 1135 (Cl_T), 944 (Cl_R), 0.2 (Cl_B), and 170 (Cl_{Cr}) (Table II). With the assumption of negligible physicochemical binding of captopril to plasma proteins, a comparison of Cl_R and Cl_{Cr} indicated an average minimum net tubular secretion of 82%. The average V_d and V_1 values were 3.6 and 0.5 liters/kg, respectively. The average $t_{1/2}\beta$ value in the monkey was 2.2 hr (Table II).

Renal clearance (Cl_R) values for all animals also were calculated by using all data obtained and plotting the urinary excretion rate of unchanged captopril *versus* the blood concentration of captopril at the midpoint of urinary collection periods. A typical plot is shown in Fig. 2. These data provided excellent linearity, as indicated by correlation coefficient (r^2) values of >0.97 for all animals. The slopes of these lines represented the renal clearance values, and the intercept values were close to the expected value of zero. The values obtained for Cl_R by this method were similar to those obtained using only the steady-state data (Tables I and II).

The excretion data (Table III) showed that averages of 91% (dog) and 94% (monkey) of the administered dose were recovered in urine, while biliary excretion accounted for only 2.3% (dog) and 0.4% (monkey) of the administered dose.

DISCUSSION

The disposition of captopril in humans after oral administration of [^{14}C]captopril was reported recently (5); at the time the present study in animals was conducted, captopril pharmacokinetics in humans following intravenous administration of the drug had not been determined. Based on the disposition data obtained in humans after oral administration of [^{14}C]captopril, there was a lack of correlation between the pharmacokinetics and pharmacodynamics of the drug (5). Although unchanged captopril was not detected in the blood after 6 hr, other studies in hypertensive patients had demonstrated good control of blood pressure with a three-times-daily dosing regimen (5). Thus, this study in dogs and monkeys was initiated to gain a better understanding of captopril pharmacokinetics and to provide information for properly designing pharmacokinetic studies in humans.

Captopril appears to exhibit characteristics of a two-compartment open model in both the dog and monkey. In both species, the values for biliary clearance were negligible. Although the hepatic clearance of captopril could not be directly calculated, negligible biliary clearance of captopril indicated that the difference between the total body clearance and renal clearance of captopril in both species was primarily due to the metabolic

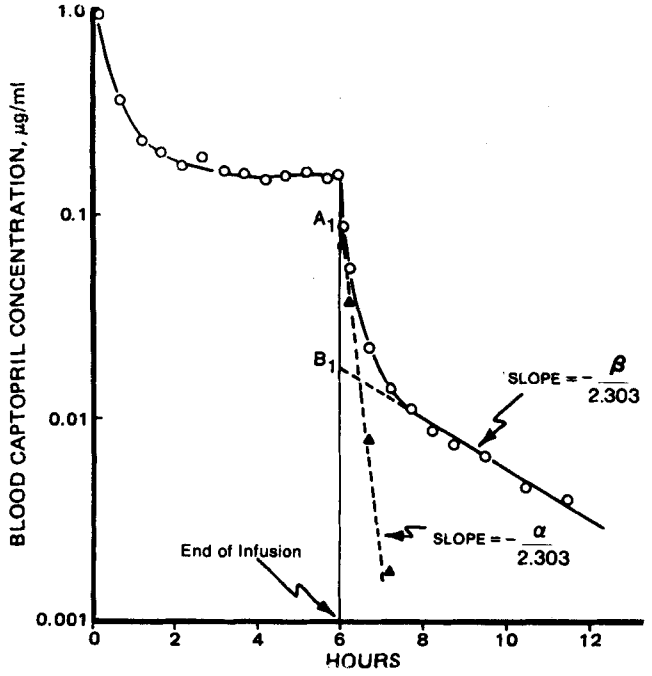

Figure 1—*Concentration of unchanged captopril in blood during and after infusion of [^{14}C]captopril into an anesthetized monkey (8-7).*

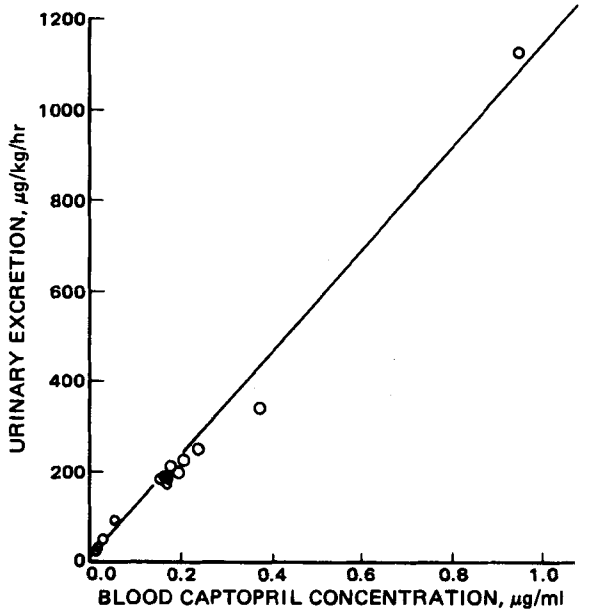

Figure 2—*Renal clearance of captopril in an anesthetized monkey (8-7) during and after infusion of [^{14}C]captopril. Slope (Cl_R) = 1150 ml/kg/hr, y-intercept = 1.8 μg/kg/hr, and correlation coefficient (r^2) = 0.990.*

clearance of captopril. Since almost all of the radioactive dose was recovered in urine in both species, captopril and its metabolites were almost exclusively eliminated by the kidneys.

A comparison of the disposition data in humans (5) and in dogs and monkeys (this study) indicated that the majority of the radioactivity excreted in urine in these species was unchanged captopril. In addition, the major elimination route of captopril in humans was *via* the kidneys. Based on a comparison of fecal excretion of radioactivity in normal subjects (5) and in patients with impaired renal function (9) after oral administration of [^{14}C]captopril, nonrenal elimination of captopril and its metabolites did not increase under conditions of reduced kidney function. Thus, to avoid any toxic side effects from accumulation of captopril or its metabolites, smaller doses or longer intervals between doses of captopril may be necessary in patients with renal impairment.

The volume of distribution of captopril was higher in the monkey (3.6 liters/kg) than in the dog (2.5 liters/kg); the volume of the central compartment (V_1) was about the same (0.5 liter/kg) for both species. The values for distribution rate constants (k_{12} and k_{21}) were comparable for both species. However, the overall elimination rate constant (k_{el}) in the monkey (2.6 hr^{-1}) was about twice that found in the dog (1.3 hr^{-1}) due to the higher renal clearance in the monkey. The renal excretion rate constant (k_e) in the monkey (1.9 hr^{-1}) was also more than twice that found in the dog (0.7 hr^{-1}).

The body and renal clearance values of captopril in the monkey were much greater than the corresponding values for the dog, primarily because the net tubular secretion of captopril was three times higher in the monkey than in the dog. The volumes of distribution of captopril in the dog (2.5 liters/kg) and monkey (3.6 liters/kg) indicated extensive distribution of captopril throughout the body.

A comparison of the urinary excretion data between the dog and monkey (Table III) indicated that, while the average amount of total radioactivity excreted in urine during the entire experiment was approximately the same for both animals, the average amount of unchanged captopril excreted in urine was much higher in the monkey. In fact, the average ratios of Cl_R and Cl_T in the dog (0.56, Table I) and monkey (0.83, Table II) were in excellent agreement with the fractions of the administered dose excreted in urine as unchanged drug in these animals (0.53 and 0.81, respectively) (Table III). Thus, the greater difference between Cl_R and Cl_T values in the dog compared to that in the monkey can be explained by the fact that a relatively larger fraction of a given dose of captopril was eliminated by a nonrenal mechanism (metabolism) in the dog than in the monkey. Biliary excretion accounted for only 2.3% (dog) and 0.4% (monkey) of the dose (Table III). Average fecal excretion of radioactivity in unanesthetized dogs and monkeys after single intravenous doses of [^{14}C]captopril (2.5 mg/kg) was also <2%[7]. These data indicate that the low biliary excretion observed in anesthetized animals was not due to the administration of a general anesthetic. Thus, in both species, the major route for elimination from the body was *via* the kidneys.

The terminal half-life value was slightly higher in the dog than in the monkey. Problems in estimating the captopril half-life were encountered in previous studies in which apparent curvilinearity of semilogarithmic plots of blood concentrations *versus* time had been observed after oral administration of captopril in humans and animals (5, 10). This curvilinearity was tentatively attributed to formation of reversible disulfide bonds of captopril with proteins. By using a steady-state design for the present study, meaningful estimates of the half-life and other pharmacokinetic parameters were obtained, and the problems of curvilinearity in the semilogarithmic plots of blood concentrations *versus* time encountered after administration of single captopril doses were avoided.

APPENDIX

Total body clearance (Cl_T) is the volume of blood cleared of drug per unit time, which is a measure of the rate at which infused compound is cleared or removed from the blood by all mechanisms:

$$Cl_T = \frac{\text{infusion rate}}{\text{blood concentration}} \qquad \text{(Eq. A1)}$$

Renal clearance (Cl_R) is the volume of blood cleared of drug by the kidney and excreted in the urine per unit time:

$$Cl_R = \frac{\text{renal excretion rate of compound}}{\text{blood concentration}} \qquad \text{(Eq. A2)}$$

Equation A2 is also used to calculate the creatinine clearance (a measure of glomerular filtration rate) when creatinine is infused along with the drug.

Biliary clearance (Cl_B) is the volume of blood cleared of drug by the bile per unit time:

$$Cl_B = \frac{\text{biliary excretion rate}}{\text{blood concentration}} \qquad \text{(Eq. A3)}$$

Renal clearance/body clearance reflects the contribution of renal excretion to the body clearance of drug. The deviation from 1.0 reflects the contribution of nonrenal mechanisms to the body clearance of the compound.

Renal clearance (Cl_R)/creatinine clearance (Cl_{Cr}) reflects the mode of drug excretion by the kidney. A ratio of <1.0 suggests glomerular filtration and net tubular reabsorption. A ratio of >1.0 suggests glomerular filtration and net tubular secretion.

Apparent volume of distribution (V_d) is the theoretical volume of water occupied by drug in the body, assuming uniform distribution and a concentration equal to that found in the blood:

$$V_d = \frac{\text{total compound in body}}{\text{blood concentration}} \qquad \text{(Eq. A4)}$$

Terminal half-life ($t_{1/2}\beta$) is an estimate of the time required by all mechanisms to clear half of the drug from the body:

$$t_{1/2}\beta = \frac{\text{volume of distribution}}{\text{body clearance}} \times 0.693 \qquad \text{(Eq. A5)}$$

Postinfusion blood concentration data can also be used to calculate some parameters calculated by using steady-state data as well as some parameters that cannot be calculated by using data obtained during the infusion period. The following relationships were used to calculate various pharmacokinetic parameters (with the assumption that the compound shows characteristics of a two-compartment open model):

$$C = A \exp(-\alpha t') + B \exp(-\beta t') \qquad \text{(Eq. A6)}$$

$$A = \frac{A_1 \alpha T}{1 - \exp(-\alpha T)} \qquad \text{(Eq. A7)}$$

$$B = \frac{B_1 \beta T}{1 - \exp(-\beta T)} \qquad \text{(Eq. A8)}$$

$$k_{21} = \frac{A\beta + B\alpha}{A + B} \qquad \text{(Eq. A9)}$$

$$k_{el} = \frac{\alpha\beta}{k_{21}} \qquad \text{(Eq. A10)}$$

$$k_{12} = \alpha + \beta - k_{21} - k_{el} \qquad \text{(Eq. A11)}$$

$$V_1 = \frac{\text{total dose infused}}{A + B} \qquad \text{(Eq. A12)}$$

$$V_d = \frac{\text{total dose infused}}{AUC \times \beta} \qquad \text{(Eq. A13)}$$

$$Cl_T = V_1 \times k_{el} = V_d\beta \qquad \text{(Eq. A14)}$$

$$k_e = \frac{Cl_R}{V_1} \qquad \text{(Eq. A15)}$$

$$Cl_T = Cl_R + Cl_H + Cl_0 \qquad \text{(Eq. A16)}$$

$$Cl_H = Cl_B + Cl_M \qquad \text{(Eq. A17)}$$

$$t' = t - T \qquad \text{(Eq. A18)}$$

where A_1 and B_1 are intercepts on the concentration axis at the point when the infusion is stopped (Fig. 1) and:

C = concentration of drug at any time during the postinfusion period
T = time of infusion
t = time since the start of the infusion
V_1 = volume of the central compartment
V_d = apparent volume of distribution
Cl_H = hepatic clearance
Cl_M = metabolic clearance
Cl_0 = clearance by other unknown routes
AUC = area under the blood concentration *versus* time curve
k_{12} and k_{21} = distribution rate constants
k_{el} = overall elimination rate constant
k_e = renal excretion rate constant

[7] S. M. Singhvi and K. J. Kripalani, unpublished data.

Renal or biliary clearance can be calculated by plotting the rate of urinary excretion or biliary excretion against the blood concentration at the midpoint of each urinary or biliary collection, respectively. The resulting slopes represent each corresponding clearance value (Fig. 2 illustrates the calculation of renal clearance). However, Cl_H, Cl_M, and Cl_0 cannot be calculated by conventional methods.

Percent net tubular secretion can be calculated using the following relationship:

$$\text{percent net tubular secretion} = \frac{Cl_R - Cl_{cr} \times \text{fraction of unbound compound}}{Cl_R} \times 100 \quad \text{(Eq. A19)}$$

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Quantitation of Daunorubicin, Doxorubicin, and Their Aglycones by Ion-Pair Reversed-Phase Chromatography

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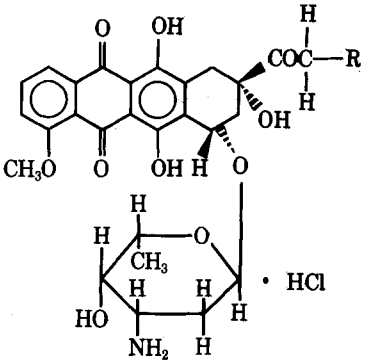
Abstract □ A fast and sensitive high-pressure liquid chromatographic method was developed for the quantitation of doxorubicin, daunorubicin, and their aglycones in pharmaceutical preparations. Because its higher pH extends column life while permitting determination of impurities, this system represents an improvement over previously published methods. It utilizes a C_{18} bonded silica gel column and a solvent system consisting of methanol–0.01 *M* monobasic ammonium phosphate aqueous solution (65:35) at pH 4.0 and 1600 psi of pressure. The accuracy of the doxorubicin and daunorubicin determinations, expressed as the coefficient of variation, is 1.65 and 1.27%, respectively. The aglycones can be determined with a precision of <1.3%.

Keyphrases □ Doxorubicin—high-pressure liquid chromatographic assay, quantitation of impurities □ Daunorubicin—high-pressure liquid chromatographic assay, quantitation of impurities □ High-pressure liquid chromatography—quantitation of doxorubicin, daunorubicin, and their aglycones □ Antibiotics—quantitation of doxorubicin, daunorubicin, and their aglycones by high-pressure liquid chromatography

Doxorubicin (adriamycin) is an anthracycline antibiotic (I) consisting of the tetracyclic quinoid aglycone doxorubicinone (adriamycinone) in a glycosidic linkage to the amino sugar daunosamine (1, 2). Doxorubicin has been shown to be effective against various human neoplasms (3, 4). Pharmaceutical preparations of doxorubicin contain several impurities such as daunorubicin, daunorubicinone, and doxorubicinone, as well as other minor contaminants. Some of these impurities, particularly daunorubicin and daunorubicinone, are present because doxorubicin is prepared from daunorubicin by chemical synthesis (5), and the parent compound and its impurities are carried through to the product during the manufacturing process.

Daunorubicin (II) is a recently approved drug used in the treatment of acute leukemia and certain solid tumors in humans (6, 7). Daunorubicin preparations contain impurities such as daunorubicinone, which may be carried through the isolation process of the drug from the fermentation broth (8). It is important that the potency of both doxorubicin and daunorubicin be determined because clinical treatment relies on an accurate dosage schedule (9). It is equally important to determine the impurities in these pharmaceutical preparations, particularly the aglycone content, because these impurities are mutagenic and have no antitumor activity (10).



I: R = OH
II: R = H

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