predicted for an oral dose, assuming linear kinetics, would be 10–20% (20). First-pass metabolism would also account for the higher mean oral clearance observed for both solution and tablet relative to the mean plasma clearance after intravenous and intramuscular administrations. Since ~70% of a parenteral dose and 60% of an oral dose are excreted as intact drug in the 0–24-h urine samples, the 10% difference as a function of the oral route of administration is consistent with the predicted limited extent of first-pass metabolism of the oral dose. These findings are consistent with the results of previous studies (10, 12), which have reported urinary recoveries for intact bumetanide of 60 ± 3% (10) and 66 ± 3% (12) from the intravenous dose, but only 49 ± 5% (10) and 59 ± 3% (12) from the oral dose.

The mean urinary excretion of intact bumetanide and the corresponding mean urinary volume following the four treatments are shown in Fig. 3. The diuretic effect of bumetanide following 1-mg iv, im, oral solution, and oral tablet doses was clearly evident in the first 2-h period, with mean urine volumes ranging from 1270 mL (tablet) to 1600 mL (oral solution). The diuretic activity lasted for ~4–8 h for all treatments, and the mean cumulative diuresis after 24 h was the same for the four treatments. The diuresis profile of bumetanide parallels the urinary excretion profile of intact bumetanide in that the major portion of intact bumetanide was excreted during the first 2 h following drug administration (Fig. 3). This confirms previous observations (9, 21) that the diuretic activity of bumetanide is closely associated with the concentration of intact drug in the kidney. The fact that the oral formulations are as effective diuretics as the parenteral preparations presumably reflects the maintenance of a minimum effective plasma or renal tubule concentration of bumetanide (22).

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Determination of Total Captopril in Human Plasma by Gas Chromatography–Mass Spectrometry with Selected-Ion Monitoring After Reduction of Disulfides

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Abstract □ Captopril is liberated from covalently protein-bound disulfides and other disulfide metabolites in human plasma by reduction with tri-*n*-butyl-phosphine. The captopril is then treated with *N*-ethylmaleimide, purified on XAD-2 resin, eluted with ethyl acetate, and methylated prior to its determination by gas chromatography–mass spectrometry with selected-ion monitoring. The limit of detection is 20 ng/mL of plasma.

Keyphrases □ Captopril—reduction of disulfides, GC–MS, selected-ion monitoring □ GC MS—selected-ion monitoring, total captopril in human plasma, reduction of disulfides

Captopril (I), an orally active angiotensin I-converting enzyme inhibitor (1, 2), is currently marketed for the treatment of hypertension (3, 4). Because of the extreme reactivity of thiols in biological systems, captopril is quantitatively converted to a derivative, such as the *N*-ethylsuccimide derivative (II), prior to analysis (5). The reaction product was determined by a gas chromatographic–mass spectrometric (GC–MS) method (6, 7) with selected-ion monitoring for captopril in whole blood and by a radiometric-thin-layer chromatographic (RTLC) procedure for establishing the biological disposition of captopril (8, 9). The latter method has been used in various metabolism and pharmacokinetic studies (10–12). Other reported methods for the determination of captopril employ GC (13), HPLC (14–17), and most recently GC–MS with selected-ion monitoring (18).

Metabolites of captopril identified so far include captopril disulfide (III), the mixed cysteine and glutathione disulfides of I (IV and V, respectively), and the *S*-methyl (VI) and *S*-methyl sulfoxide (VII) metabolites (9, 10, 12, 19). A substantial proportion of the captopril radioactivity in human blood after oral administration of radioactively labeled I was associated with the plasma fraction (8), which was covalently bound as plasma protein disulfides. It is believed that the plasma proteins and mixed disulfides with endogenous thiol-

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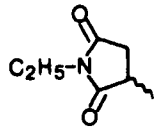
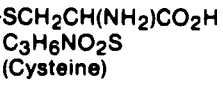
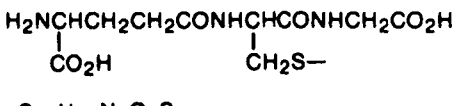
containing compounds (*e.g.*, glutathione) may act as a reservoir from which captopril is liberated over time to exert pharmacological effects (12, 20). It would not be practical to measure each metabolite separately, nor would it be possible to adequately measure the protein-bound levels by a nonradiometric method. Consequently, a method for measuring total captopril in plasma has been developed by treating the plasma fraction with the disulfide-reducing agent tri-*n*-butylphosphine (21), followed by reaction with *N*-ethylmaleimide to form II, which is then methylated and measured by GC–MS with selected-ion monitoring (7).



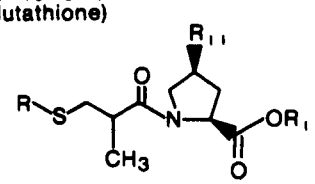
$C_6H_8NO_2$



$-SCH_2CH(NH_2)CO_2H$
$C_3H_6NO_2S$
(Cysteine)



$C_{10}H_{16}N_3O_6S$
(glutathione)



I:R = $R_1 = R_{11} = H$
II:R = $C_6H_8NO_2$ $R_1 = R_{11} = H$
III:R = $C_9H_{14}NO_3S$ $R_1 = R_{11} = H$
IV:R = $C_3H_6NO_2S$ $R_1 = R_{11} = H$
V:R = $C_{10}H_{16}N_3O_6S$ $R_1 = R_{11} = H$
VI:R = CH_3 $R_1 = R_{11} = H$
VII:R = CH_3(*S*-oxide) $R_1 = R_{11} = H$
VIII:R = H $R_1 = H\ R_{11} = F$
IX:R = $C_6H_8NO_2$ $R_1 = H\ R_{11} = F$
X:R = $C_6H_8NO_2$ $R_1 = CH_3\ R_{11} = H$
XI:R = $C_6H_8NO_2$ $R_1 = CH_3\ R_{11} = F$

EXPERIMENTAL SECTION

Apparatus—A mass spectrometer[1] with a data acquisition system, gas chromatograph, and an interface was used as reported by Cohen *et al.* (7). A centrifuge[2] with stainless steel adapters, drug-screen columns[3], a water bath[4] set at 50°C, two sample concentrators[5], dry-block heating baths[6,7], a column mounting plate[8], an aspirator trough[8], 1-mL reaction vessels[9], vial seals[10], 100-μL micropipets[11], and 1-, 3-, 5-, and 10-mL dispensers[12] were used. Samples were agitated on a heavy-duty shaker[13]. Screw-capped 150-mm culture tubes with polytef-faced liners, 20-mL scintillation vials, 21-gauge syringe needles, a vial crimper, a desiccator, 15-cm disposable Pasteur pipets, a vortex mixer, 13 × 100-mm disposable glass tubes, and 1- and 5-mL disposable serological pipets were obtained commercially[13].

Reagents—Acetone, ethyl acetate, hydrochloric acid, phosphoric acid, *N*-ethylmaleimide, dimethylformamide, Tris, urea, dibasic sodium phosphate, sodium chloride, EDTA (disodium salt), neutral alumina Brockman Activity I (80–200 mesh), and sodium bicarbonate were reagent grade. An *N*-ethylmaleimide solution was prepared daily by dissolving 0.5 g of reagent in 1.5 mL of dimethylformamide. A 20% v/v solution of tri-*n*-butylphosphine was prepared with redistilled reagent[14] and acetone. A buffer was prepared by dissolving 12.1 g of Tris in 980 mL of distilled water, and the pH was adjusted to 8.2 with phosphoric acid. To 26 g of urea, was added 65 mL of the pH 8.2 buffer. This solution was prepared each day as required. A pH 7.0 buffer was prepared by dissolving 40 g of dibasic sodium phosphate heptahydrate in 1960 mL of distilled water, adjusting the pH with phosphoric acid. A diluted column activator solution[3] was prepared by diluting 35–500 mL with distilled water. The solution was refrigerated and used for not more than 1 week. The plasma used for experiments was prepared from commercially obtained blood[15]. Methanolic hydrochloride was prepared as described previously (6, 7). Captopril (I), captopril disulfide (III), and the fluoro analogue internal reference (VIII) were pharmaceutical-grade materials[16].

Standard Solutions—Captopril disulfide (III) solutions were prepared at concentrations of 10 and 40 μg/mL in methanol and stored in a refrigerator. A solution of II was prepared by treating 50 mg of I with 250 mg of *N*-ethylmaleimide in 10 mL of pH 7.0 buffer for 15 min, diluting to 100 mL with acetone, and filtering through a fine-porosity sintered glass filter. An unextracted control solution was prepared from II and IX, with respective final concentrations equivalent to 40 μg of I and VIII per mL of solution. The solution was stored in a refrigerator. For calibration, appropriate solution concentrations of III in methanol were prepared.

Preparation of Standard Curves—To each of six 20-mL scintillation vials, was added 1 mL of plasma. With 100-μL syringes, varying amounts (between 0 and 1 μg) of III and 1 μg of internal reference standard VIII were added. To another set of six 20-mL scintillation vials containing 1 mL of plasma, between 0 and 8 μg of III and 4 μg of VIII were added. These standards were immediately reduced or stored in a freezer with the samples until required.

Reduction—Aliquots (1 mL) of a thawed, well-mixed plasma sample were transferred to separate 20-mL scintillation vials and 5 mL of urea pH 8.2 buffer solution was added to each vial. To vial 12, 1 mL of blank plasma and the appropriate amount of control solution containing III and VIII were added. To plasma samples collected from 0.25 to 6 h after oral administration of a 100-mg tablet of captopril, was added 4 μg of VIII; 1 μg of VIII was added to all other plasma samples. The respective additions to controls were 4 μg of III and VIII and 1 μg of III and VIII. To each vial (located under a hood), 0.1 mL of 20% v/v tri-*n*-butylphosphine solution was added. The vials, covered with tin foil-lined caps, were mixed on a vortex mixer. These samples and standards were allowed to stand in the dark at 50°C for 1 h.

Reaction of Reduced Captopril with *N*-Ethylmaleimide—The aforementioned vials were cooled in the hood for 15 min and uncapped; to each vial, was added 25 mg of *N*-ethylmaleimide in 0.1 ml of dimethylformamide, and the vials were recapped. The samples were vortexed for 10 s, the vials were set aside for 5 min, and then the samples were treated with 5 mL of 0.67 M H_3PO_4.

Isolation and Purification of Samples—Prior to use, the required number of XAD-2 columns were activated with 5 mL of diluted activator solution, treated with 5 mL 0.1 M HCl, and 21-gauge syringe needles were attached. Each sample solution was decanted into an individual column, and the solution was allowed to percolate through the column; 5 ml of 0.1 M HCl was added to a scintillation vial, mixed, and decanted to the column after the original solution had eluted. The syringe needle was removed, and the column was washed first with a 10-mL aliquot and then with a 20-mL aliquot of 0.1 M HCl[17]. The columns were placed on a column mounting plate and dried under reduced pressure for 1 h; this was followed by eluting II and the fluoro analogue (IX) first with 10 mL and then with 15 mL of purified ethyl acetate. To each test tube containing the ethyl acetate eluant was added 3 mL of 5% sodium bicarbonate solution; this was shaken for 5 min to effect the transfer of II and IX into the aqueous layer, followed by centrifugation at 2000 rpm for 5 min. The upper ethyl acetate layer was discarded and 1 mL of 2 M phosphoric acid and 3 g of sodium chloride were added and the sample was mixed for 5 min. The sample was reextracted into ethyl acetate by adding 5 mL of the purified solvent, shaking, and centrifuging for the required times as indicated above. The entire contents of each test tube were decanted to new 13- × 100-mm test tubes, and the upper ethyl acetate layer (~4.8 mL) was transferred to a 20-mL scintillation vial with a 5-mL serological pipet. The solvent was then evaporated with a sample concentrator set at 65°C. The sample was transferred quantitatively, first with 0.5 mL and then with 0.2 mL of purified ethyl acetate, to a 1-mL reaction vial with a Pasteur pipet. An unextracted control solution containing 4 μg each of II and IX was added to a clean reaction vial and evaporated to dryness with a concentrator, along with extracted samples and the control. The vials were then dried under reduced pressure in a desiccator for 15 min. Samples were redissolved and methylated to X and XI as previously described (6, 7).

[1] Model HP 5985B; Hewlett Packard, Palo Alto, Calif.
[2] Model IEP 2741; International, Scientific Products Co.
[3] XAD-2 with Column Activator A; Brinkmann Instruments.
[4] Organomation Associates, Inc.
[5] Model SC 248; Brinkmann Instruments.
[6] Techne, SC-3.
[7] Techne, DB-3.
[8] Biochemical Diagnostics.
[9] Supelco Inc., Bellefonte, Pa.
[10] Hewlett-Packard Instruments, Palo Alto, Calif.
[11] Scientific Manufacturing Industries.
[12] Lab Industries.
[13] Fisher Scientific Co.
[14] Strem Chemicals, Inc.
[15] New Jersey Blood Services.
[16] E. R. Squibb & Sons, Princeton, N.J.
[17] Sometimes it is necessary to initiate flow with the palm of the hand or a rubber bulb.

Table I—Covalent Binding of Captopril and Its Metabolites in Plasma as a Function of Storage Temperature and Time

Drug	Sample	Storage Temp., °C	Extractable Drug with Methanol, ng/vial[a]		
			Initial	1 Week	4 Weeks
[^{14}C]I	1	5	3240	2230 (68)	1250 (38)
	2	5	3390	2250	1290
	1	−20	—	940 (28)	430 (13)
	2	−20	—	920	420
[^{14}C]III	1	5	3920	3960 (101)	2250 (58)
	2	5	3940	2710 (69)	2280
	1	−20	—	3650 (93)	2140 (55)
	2	−20	—	3820 (100)	2180
[^{14}C]IV	1	5	3420	1980 (59)	nil
	2	5	3450	2040	nil
	1	−20	—	2030 (57)	870 (23)
	2	−20	—	1910	720
[^{14}C]VI	1	5	1630	1580 (97)	1590 (99)
	2	5	1600	1560	1600
	1	−20	—	1560 (98)	1590 (98)
	2	−20	—	1610	1590

[a] Values in parentheses are the percentages of the initial values.

Table II—Reduction of Captopril Disulfide As Function of pH

Solution	pH	Reduction, %
0.1 M HCl	1.1	47.5
~0.67 M H_3PO_4	1.6	67.8
0.1 M phosphate Buffer	7.0	79.5
	8.0	81.9
0.1 M Tris buffer	8.2	90.5
8 M Urea in 0.1 M Tris buffer	8.2	97.0
Tris buffer–propanol, 1:1	8.2	98.0
Tris buffer–propanol, 3:1	8.2	97.8
(~0.1 M NaOH)	12.5	64.0

Selected-Ion Monitoring—Approximately 1–2 μL of solution added to the tip of a GC solids injector[18] was allowed to evaporate. The appropriate ion profiles (m/z 230.1, m/z 248.1) were obtained by selected-ion monitoring. The maximum peak heights were measured by using a program written especially for the batch processing of the data (7). The program selects the baseline-corrected maximum peak heights of the requested ions. The mean ratio (R_n^*), and standard deviation for three to five injections of the control sample bracketing the samples were determined for subsequent use in correcting the data for differences in response from the calibration slope (k_n). The adjustment factor (A) is related to the calibration slope and the measured ratio of the control (R_n^*), by:

$$A = \frac{R_n^*}{I_n + k_n}$$

The data were processed as previously described (7).

RESULTS AND DISCUSSION

Captopril undergoes extensive biotransformation to form covalently protein-bound disulfides (21) in addition to the disulfides III, IV, and V (9, 10, 12, 21). When captopril is added to plasma and stored at either 5°C or −20°C, 62 and 87% of I is bound to protein (Table I). The greater binding at the lower temperature may result from an increase in protein thiols from freezing and thawing of the plasma. Approximately 43% of the disulfide III was bound to the protein, presumably due to interchange and disulfide formation with protein. The only captopril-related product not bound was *S*-methyl captopril (VI), a potential metabolite. The use of *N*-ethylmaleimide as a thiol alkylating agent in whole blood, plasma, and urine is the most effective method of preventing the formation of protein-bound captopril (5–7). It is not feasible to measure unbound captopril, because a significant amount of captopril is converted to protein-bound compound during the production of serum or plasma from whole blood and during storage prior to analysis.

Plasma or serum was subsequently processed to liberate I bound to proteins and mixed disulfides by reduction with tri-*n*-butylphosphine[19]. This reagent was previously used to reduce captopril disulfides in urine (14, 21). The great depot of protein disulfides required a tri-*n*-butylphosphine concentration of 20 mg/mL of plasma to achieve 98% reduction. Only 85 and 50% reduction could be obtained at tri-*n*-butylphosphine concentrations of 10 and 2 mg/mL, respectively. Nearly complete reduction is obtained in solutions more basic than pH 8 (Table II). The rate of reduction and the yield are also influenced by temperature. At 50°C, it takes ~30 min to reduce III with 20 mg/mL of tri-*n*-butylphosphine and 45 min to reduce protein-bound I. At temperatures greater than 60°C, III is reduced in <15 min, but some sample decomposition occurs. Up to 10 mL of urea buffer, used as a plasma-solubilizing agent, did not effect the reduction efficiency when 1 mL of plasma was used. When >1 mL of plasma was used, the yield decreased. Only 90% disulfide reduction could be achieved with whole blood samples, but decreased whole blood volumes yielded satisfactory reduction efficiency. Although excellent reductions have been obtained from 50% propanol solutions (22), the solvent causes interferences in the isolation procedure. When added to plasma, I, III, IV, and V are essentially quantitatively converted and subsequently measured as the methyl ester of II by the GC–MS procedure with selected-ion monitoring (Table III). Plasma samples of I, III, and IV stored for 4 weeks either at 5°C or −20°C yield substantially all of the available reducible compound (Table IV).

The precision of measurement was improved by adding 1000 ng of internal reference to the samples with lower concentrations (within a range of 0–1000 ng/mL); 4000 ng was added to those samples expected to have >1000 ng of reducible captopril. Samples collected from human subjects at 0.25 to 6 h after administration of a single 100-mg tablet of captopril were treated as high-concentration samples, whereas the 0-h samples and those collected at ≥8 h were treated as low-concentration samples. The methylated sample extracts were measured by essentially the same methods as used for free I (6, 7). Calibration curves of standard disulfide III, extracted from plasma, demonstrated a linear response–concentration relationship for reduction and extraction. The slope and intercept, 2.084 and 0.0667, respectively, are identical to the values reported for free I in whole blood (6). The extracted control was used as a single-point calibration (6, 7). The mean of three to five values was

Table III—Recovery of Captopril and Disulfides from Plasma [a]

	Plasma, μg/mL[b]		
	Added	Found	Reduction, %
Captopril Disulfide (III)	5	4.82	96.4
	2.5	2.41	96.4
	1.0	0.98	98.0
	0.5	0.48	97.0
	0.25	0.23	92.0
Captopril (I)	5.12	4.89	95.5
	2.56	2.48	96.7
	1.02	0.9	96.0
	0.51	0.48	93.0
	0.25	0.26	102.
Captopril–cysteine (IV)	8	7.79	97.4
	4	3.87	96.8
	2	1.93	96.0
Captopril–glutathione (V)	12	11.7	97.7
	6	5.75	95.8
	3	2.96	98.5
Plasma Blank	0	0	—

[a] Captopril was used as standard. [b] As percentage of captopril equivalents.

[18] SI-IRDS; Scientific Glass Equipment Co., Penzias Associates, Roslyn Heights, N.Y.

[19] Tri-*n*-butylphosphine is a toxic substance, and all procedures should be carried out in a hood. As it is readily oxidized by air, it should be refrigerated under nitrogen, with acetone solutions prepared daily.

Table IV—Recovery of Captopril from Plasma Stored at 5°C and −20°C for 4 Weeks

Drug	Sample	Storage Temp., °C	Recovery, ng/vial		
			Initial	Final	Percent of Initial
[^{14}C]I	1	5	3240	3020	91
	2	5	3390	3270	99
	1	−20	—	2950	90
	2	−20	—	2920	88
[^{14}C]III	1	5	3920	3730	95
	2	5	3940	3830	97
	1	−20	—	3900	99
	2	−20	—	3890	99
[^{14}C]IV	1	5	3420	3170	92
	2	5	3450	3090	90
	1	−20	—	3260	95
	2	−20	—	3160	92

Table V—Bioavailability Data for Captopril[a]

Time, h	Bioavailability (F) in Blood, ng/mL[b]	$^1/_2T$, ng/mL[c]	Disulfides ($^1/_2T - F$), ng/mL[d]	Bioavailability in Plasma, ng/mL[b]
0.5	350 ± 100	390	40	780 ± 230
1.0	900 ± 140	1300	400	2590 ± 420
1.5	720 ± 110	1480	760	2960 ± 390
2.0	360 ± 50	1240	880	2480 ± 320
2.5	230 ± 40	980	750	1960 ± 220
3.0	170 ± 40	800	630	1600 ± 120
4.0	100 ± 20	570	470	1130 ± 80
5.0	80 ± 50	490	410	980 ± 220
6.0	30 ± 10	370	340	740 ± 110
8.0	10 ± 3	250	240	490 ± 50
12.0	4 ± 1	130	130	260 ± 20
16.0	1 ± 0.3	80	80	160 ± 10
24		40	40	70 ± 10

[a] Single oral administration of 100 mg of captopril; nine healthy male subjects were treated. [b] ± *SEM* for nine healthy male subjects. [c] One-half total volume for plasma (blood equivalent). [d] Difference is equivalent to disulfides in blood.

used to adjust for the small variation in the response (6, 7). The data for total captopril are of the same quality as those for free captopril in whole blood (7). For total captopril values, the estimated precision from 0 to 100 ng/mL of plasma is 20 ng/mL; between 100 and 1000 ng/mL the precision is ~50 ng/mL; and at >1000 ng/mL the precision is 3%. The distribution of the measured total plasma levels of 27 plasma samples taken at 0, 12, 16, and 24 h is a demonstration of the sensitivity of the method (Fig. 1). Recorded to the nearest decade, 90% of the 0-h samples were determined to have 0 ng/mL, whereas the range of the 24-, 16-, and 12-h samples fell into groups of 40–100, 110–210, and 180–370 ng/mL, respectively. The mean values were also nearly equally spaced, with respective values of 70 (σ_n = 20), 130 (σ_n = 25), and 240 (σ_n = 50) ng/mL. A limit of detection of 20 ng/mL more than adequately reflects these data, and a sensitivity of 30 ng/mL appears to be readily achievable.

The mean bioavailability of I in whole blood and the total reducible captopril plasma levels of nine healthy subjects given a single oral 100-mg dose of captopril is shown as a typical example (Table V). The maximum concentration occurs at 1 h for free captopril, which is 900 (±140) ng/mL of whole blood, and at 1.5 h for total captopril in plasma, which is nearly 3000 ng/mL. To relate the total captopril levels in plasma to the whole blood equivalent, the plasma values were divided by 2 to adjust for the volume difference between the whole blood and plasma fractions. The difference between one-half the total captopril value and the free captopril value is the reducible captopril in whole blood. This parameter peaks at 2 h after drug administration. In a recent kinetic study of captopril in humans (20), it was suggested that captopril bound to plasma proteins and mixed disulfides with endogenous thiol-containing compounds may act as a reservoir from which captopril is slowly released. This possibility has also been cited in a captopril binding study (12). Future studies in which the total captopril method combined with the method for free captopril (7) are used should help in the refinement of the pharmacokinetics of captopril.

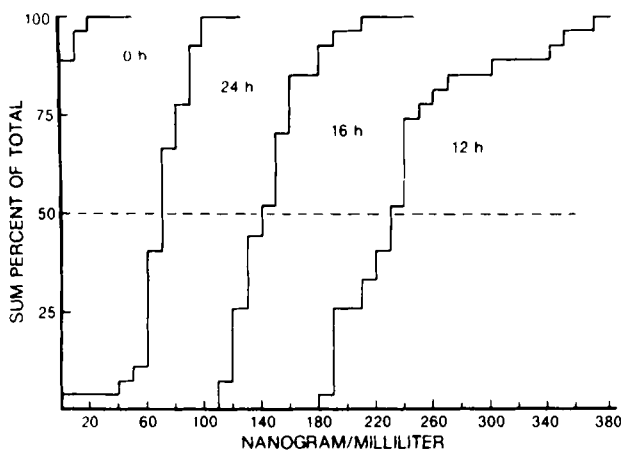


Figure 1—*Distribution and concentration range of total captopril in 0-, 24-, 16-, and 12-h plasma samples.*

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Pharmacokinetics of Pentobarbital, Quinidine, Lidocaine, and Theophylline in the Thermally Injured Rat

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Abstract □ Previous studies have shown that rats with 15% third-degree burns show a severe depression in various *in vitro* hepatic drug-metabolizing enzymes. This effect was assessed *in vivo* by measuring the disposition of four liver-metabolized drugs in 16% third-degree burned rats at 7 d postburn. Compared with pair-fed control rats, pentobarbital demonstrated a significantly prolonged clearance and elimination half-life without a change in volume of distribution. Quinidine demonstrated a significantly increased volume of distribution and a significantly decreased clearance without a change in elimination half-life. Lidocaine showed a significantly increased volume of distribution. Theophylline, which is only 50% metabolized in the rat, showed no changes in any pharmacokinetic parameters. The free drug fractions of quinidine and lidocaine were found to be significantly decreased at 1 d postburn and normal at 7 d postburn. These results warrant pharmacokinetic studies in human burn patients.

Keyphrases □ Pentobarbital—pharmacokinetics in thermally injured rats □ Quinidine—pharmacokinetics in thermally injured rats □ Lidocaine—pharmacokinetics in thermally injured rats □ Theophylline—pharmacokinetics in thermally injured rats □ Thermal injury—phramacokinetics of pentobarbital, quindine, lidocaine, and theophylline, rats

Thermal injury can produce numerous pathophysiological alterations in an organism which may modify the absorption, distribution, metabolism, and renal excretion of therapeutic agents (1). Such changes would be directly reflected in the derived pharmacokinetic parameters for a given drug (2). Recently, our laboratory has presented evidence of a generalized depression in various *in vitro* liver drug-metabolizing enzymes in rats subjected to 15–16% third-degree burns (3–5). Also, this phenomenon may occur in human thermal injury, as demonstrated by significantly decreased levels of D-glucaric acid in human burn victims (6). However, such data on drug metabolism in which *in vitro* liver homogenates and D-glucaric acid are used may not reflect the pharmacokinetic characteristics of a drug in an intact organism. Besides the activity of hepatic microsomal drug-metabolizing enzymes, other factors such as alterations in hepatic blood flow and changes in drug binding by plasma proteins may significantly contribute to the overall process of hepatic drug clearance (7). Thus, the present study was undertaken in a rodent model with a 16% third-degree burn to assess the effects of thermal injury on the *in vivo* disposition of four liver-metabolized drugs commonly used for burn patients.

EXPERIMENTAL SECTION

Animals—Adult male Sprague-Dawley rats[1] (weight, 220–250 g) received 16% full-thickness burns as described below. They were housed in plastic cages in groups of one or two over corncob bedding in a room maintained at 24 ± 0.5°C with a constant 12-h light and dark cycle. Pharmacokinetic experiments were done 7 d after the burning. Control rats housed alone were carefully pair-fed[2] daily with the burned rats to adjust for the slight anorexic effect of the burn wound.

Burn Procedure—A full-thickness burn was produced on the rats by the method of Arturson (8). This type of burn completely destroys all dermal appendages and epithelial elements, including sensory nerve endings, resulting in a virtually pain-free preparation (9). The animals were anesthetized with sodium pentobarbital (50 mg/kg) and shaved with electric clippers. Each rat was placed in a plastic mold with a hole cut out that corresponded to ~16% of the total body surface area. This value was arrived at by skinning burned rats and measuring the area of the burn and the total body area by tracing these areas onto graph paper and counting the squares. It was found that this particular mold consistently delivered an approximate 16% burn for animals in the weight range of 220–250 g. The burns were inflicted by placing the mold (containing the bare rat skin protruding through the hole) in 90 ± 1°C water for 20 s. This procedure produced a burn with sharp edges that did not impair the mobility of the animals. The animals awoke from the procedure in no apparent distress and did not require analgesics. At no time during the study did any burned animal exhibit gross signs of infection. Control animals were shaved and anesthetized but not immersed in water.

Biochemical Parameters—Estimations of the serum levels of proteins, enzymes, and other constituents were done on six 16% burned and six pair-fed control rats by sending 5 mL of serum from the pooled blood of two rats to a commercial laboratory[3]. The serum was analyzed with a standard serum multiple analyzer, with normal rat serum used as standards. Serum was ob-

[1] Charles River Breeding Laboratories, Wilmington, Mass.
[2] Rodent Chow; Ralston Purina Inc., St. Louis, Mo.
[3] Vet Path Veterinary Laboratories, Teterboro, N.J.

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