Pharmacokinetics of the Antirheumatic Proquazone in Healthy Humans

PETER H. HINDERLING *x and ANDRÉ ROOS

Received August 5, 1980, from the *Department of Pharmacology, Biocenter, University of Basle, 4056 Basle, Switzerland.* Accepted for publication November 15, 1982. *Present address: F. Hoffmann-La Roche & Co. AG, Grenzacherstrasse 124, Basle CH-4002, Switzerland.

Abstract □ The pharmacokinetics of the antirheumatic proquazone and its conjugated and unconjugated *m*-hydroxy metabolites were investigated in five healthy male volunteers after both intravenous (75 and 122 mg) and peroral (300 and 900 mg *via* capsules) administration. For adequate intravenous dosing of the poorly water-soluble proquazone, advantage was taken of the high degree of protein binding of the drug. Proquazone was admixed with 40% sterile human albumin, and these proteinaceous drug-containing solutions were injected. The pharmacokinetics of proquazone and of the measured metabolites after intravenous administration and after the 300-mg po dose were first order, whereas deviations from linear kinetics were observed at the 900-mg dose level. The apparent half-lives of the α, β, and γ phases of proquazone in plasma were 2, 14, and 76 min, respectively, on intravenous administration. The total clearance of proquazone was 700 mL/min, which indicated a high hepatic extraction. The apparent volume of distribution at steady state was 40 L, implying extensive binding or partitioning of the lipophilic drug in the tissues. Unchanged proquazone (<0.001%), the *m*-hydroxy metabolite (<1.0%), and the conjugated *m*-hydroxy metabolite (20%) were renally excreted after intravenous administration. The extent of absorption of proquazone was ~7% and was entirely the result of a large first-pass effect. Digital computer analysis of the data after intravenous administration was performed with a linear three-compartment model. A model-independent approach was used in the analysis of the peroral data.

Keyphrases □ Proquazone—*m*-hydroxy metabolites, peroral and intravenous pharmacokinetics, humans, NONLIN, SAAM □ Pharmacokinetics—proquazone and its *m*-hydroxy metabolites, humans, oral and intravenous administration, NONLIN, SAAM □ Antirheumatics—proquazone and its *m*-hydroxy metabolites, oral and intravenous pharmacokinetics in humans, NONLIN, SAAM

Proquazone (I), a nonsteroidal anti-inflammatory drug (1, 2) which has been marketed in several countries in Europe since 1977[1], exhibits activities in animal models and humans comparable with those of classical nonsteroidal anti-inflammatory drugs (1–4). Its primary therapeutic indications are in the treatment of rheumatoid arthritis, osteoarthritis, and gouty arthritis (5, 6). Proquazone is very different chemically from other representatives of this class of drugs. It is a quinazolinone derivative and a weak base which is highly lipophilic and shows poor solubility in water (7).

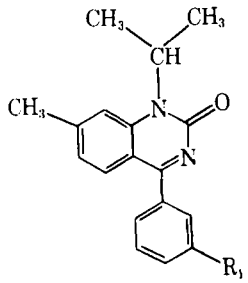
The goal of this study was to delineate the pharmacokinetics of the parent proquazone and its primary metabolites in healthy humans after intravenous and oral administration. Preliminary pharmacokinetic experiments in healthy humans had been performed only after oral administration of the drug (8, 9). These studies indicated that the drug is extensively metabolized, and seven metabolites have been identified (8). Another investigation claimed an increased bioavailability of proquazone when the drug was administered concomitantly with food (9); first-order disposition kinetics for the drug were assumed in this study. Intravenous studies have not been executed with this drug. The sensitivity of the available assay methods and the poor water solubility of the drug necessitated either administration of too small a dosage in aqueous solution or of higher dosages dissolved in excessive volumes of solubilizers[2].

The study of the plasma protein binding of proquazone showed that the drug was highly bound to albumin (98%) (7). Subsequent experiments with human albumin indicated that the apparent solubility of proquazone was significantly increased in the presence of concentrated albumin solutions (7). Preliminary pharmacokinetic experiments showed that larger intravenous dosages could be administered by means of this "biological solubilizer" (7) and that a follow-up of the plasma concentrations and urinary excretion of proquazone and metabolites over sufficient time was possible with the available fluorometric and HPLC methods[2].

EXPERIMENTAL

Materials—The following were used: proquazone[3] (I), *n*-ethylproquazone[3], and *m*-hydroxyproquazone[3] (II). Proquazone in 4-hydroxymethyl-1,3-dioxolane–5-hydroxy 1,3-dioxane (1:2)[4] (7.5% w/v) was employed in the intravenous studies, and proquazone capsules[5] (300 mg) were administered in the oral studies. The dissolution characteristics of the capsules in 0.1 M HCl using the rotating basket method were 83.1, 93.4, and 97.5% of the drug dissolved after 15, 30, and 60 min postinitiation, respectively. Human albumin[6] (20% w/v) was employed as solubilizer for the intravenous dosages of proquazone; heparin[7] was used to prevent coagulation. β-Glucuronidase–sulfatase[8] was employed for enzymic hydrolysis of conjugated *m*-hydroxyproquazone (III).

Pharmacokinetic Procedures—Five nonsmoking, healthy, male volunteers (A–E), 22–24 years old, were selected for the study. Written



	R_1
I:	H
II:	OH
III:	O_{conj}

[1] Biarison; Sandoz Ltd., Basle and Wander Ltd., Berne, Switzerland.

[2] Unpublished data.
[3] Sandoz Ltd., Basle, Switzerland.
[4] Chemie Linz, Austria.
[5] Charge number 346 L 6.
[6] Human albumin SRK, Berne, Switzerland.
[7] Liquemin,Heparin; F. Hoffmann-La Roche Ltd., Basle, Switzerland.
[8] Glusulase: β-Glucuronidase (145,474 Fishman U) and arylsulfatase: (47,830 Roy U) Endo Laboratories, Inc., Garden City, N.Y.

0022-3549/84/0300-0332$01.00/0

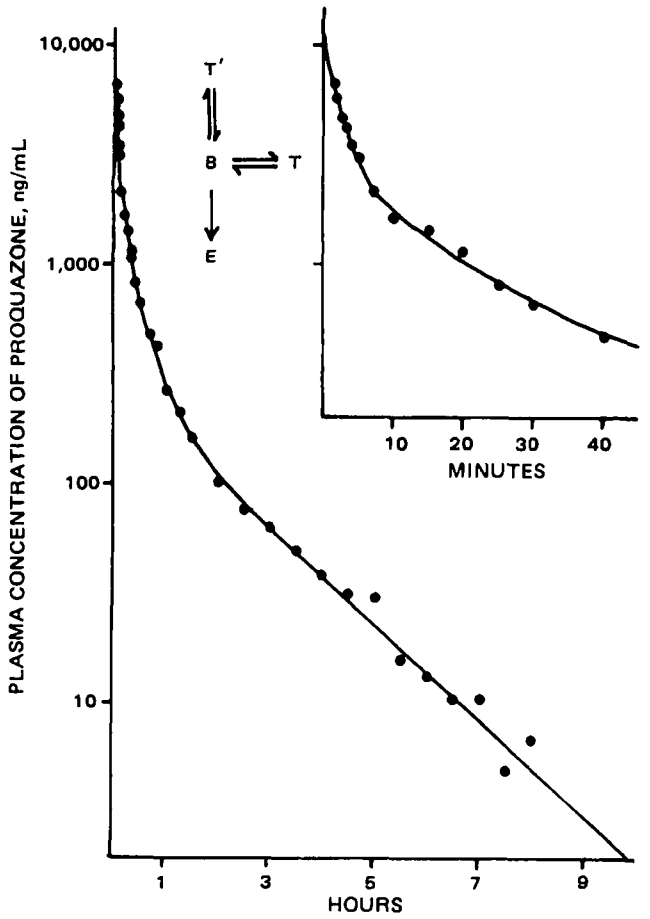


Figure 1—*Digital computer fitting (SAAM) of plasma data of I (●) in subject D after intravenous administration of 75 mg, in accordance with an intravenous infusion linear mamillary three-compartment body model. The inset is for an expanded time scale.*

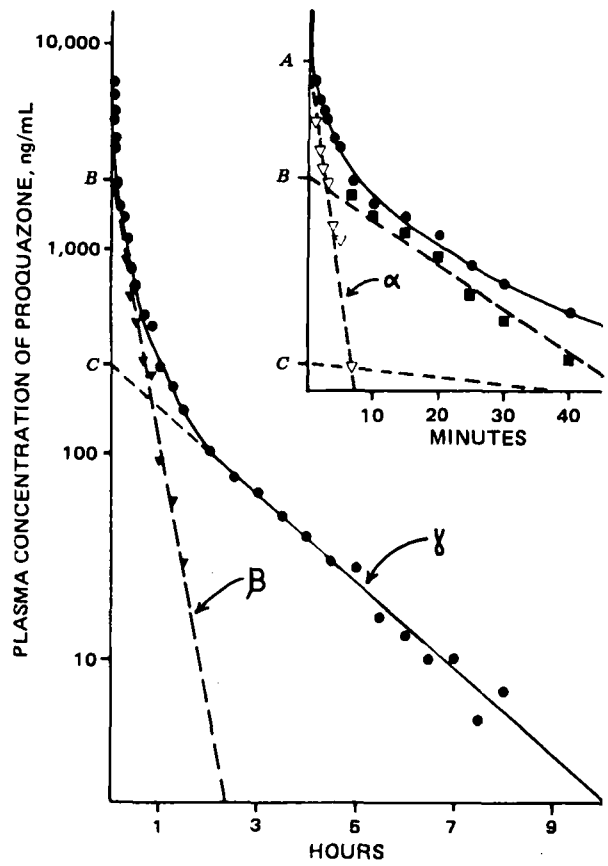


Figure 2—*Semilogarithmic plot against time of proquazone plasma concentrations following intravenous administration of 75 mg of the drug to subject D. The constants A, B, C, α, β, and γ were obtained by the method of residuals. The inset is for an expanded time scale. Key (△) original data; (▼,●,■) feathered values*

consent was obtained from all volunteers. An individual was considered healthy when he had a normal status, history, and laboratory checkup [complete blood count, hematocrit, sedimentation rate, serum electrophoresis, total proteins, creatinine clearance, urinalysis, bilirubin, serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, alkaline phosphatase, and serum electrolytes (sodium, potassium, calcium, iron, inorganic phosphate)]. These tests were repeated prior to the second, third, and fourth study on each individual to determine possible adverse drug effects or changes due to the experimental procedures. Creatinine clearance of the individuals (117 ± 24 mL/min) was within the normal range (10).

Four different studies were performed in each of the selected volunteers: intravenous administration of 75 and 122 mg in human albumin solution and oral administration of 300 and 900 mg in capsules. The 122-mg iv dose corresponded to the largest amount of the drug that could safely be administered within 45 mL of human albumin solution. The 75-mg iv dose permitted a follow-up of the plasma concentrations and urinary excretion of proquazone and its main metabolite over sufficient time. The 300- and 900-mg po dosages corresponded, respectively, to a therapeutically effective maintenance dose and a cumulative daily dose during antirheumatic treatment. A total of 20 studies were conducted, and the design of the studies was identical for each volunteer. They first received the 900- and 300-mg po dosages and then the 122- and 75-mg iv dosages in that order. Preliminary experiments with other groups of volunteers had indicated that these dosages were well tolerated[2]. Intervals of at least 2 weeks were maintained between the studies to ensure washing out of the drug and to exclude any possible enzyme induction in the subjects due to repeated administration of the drug.

The volunteers were supine during the first 24 h after administration of the drug. Following this they were ambulatory, but no exercise was allowed. The individuals were fasted 12 h before drug administration in both the intravenous and oral studies. After drug administration, the volunteers fasted 6 h. Within the first 24 h after drug administration, the food intake was restricted to fluids, *e.g.*, soups, shakes, and other beverages. The fluid intake amounted to 3000 mL/24 h for the duration of the study. No beverage or food containing caffeine or other diuretically effective substances were allowed. Three hours prior to drug administration, the volunteers received a peroral water loading (10 mL/kg). One-half hour prior to administration of the drug, a catheter[9] was placed in a cubital vein and a constant drip of 0.45% NaCl[10] (flow rate 2.0 mL/min) was started. In the intravenous studies an additional catheter[9] was inserted in a contralateral cubital vein, and a constant drip of 0.9% NaCl[10] (flow rate, 2.5 mL/min) was started. Both infusion solutions contained 1 USP U of heparin/mL to prevent coagulation. Preliminary experiments had demonstrated, that the presence of heparin did not alter the plasma protein binding of I.

The second catheter was used for administering the drug in the intravenous studies and was removed 2 h after drug administration. The first catheter was employed for blood sampling and was removed 11 h after drug administration. Later blood samples were taken by venipuncture[11]. Blood samples (10 mL) were taken through the catheter with syringes using a three-way stopcock within 10 s at 0, 1.5, 2, 2.5, 3, 5, 7, 10, 15, 20, 25, 30, 40, 50, 60, 75, and 90 min, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7.5, 8, 9, and 10 h after intravenous administration of the drug and at 0, 2.5, 5, 7.5, 10, 15, 20, 30, 45, 60, 75, and 90 min, 2, 3, 4, 5, 6, 7, 8, 10, 15, 18, 24, 36, 42, and 48 h after oral administration. A total volume of approximately 1200 mL of blood was obtained from the volunteers in the four studies within a period of three months. The initial 2 mL of blood was discarded when blood was sampled through the catheters. The blood samples were immediately transferred to heparinized centrifuge tubes and subsequently centrifuged at 300 × *g* for 10 min. Centrifugation of the samples was always accomplished within 1 h after sampling. The plasma was then immediately transferred to storage tubes and kept at −20°C until assayed. In both intravenous and peroral experiments, total volumes of urine were obtained for the following collection periods: −3–0,

[9] Viggo Venflon; Viggo AB, Helsingborg, Sweden.
[10] Kantonsspital Basel, Switzerland.
[11] Vacutainer; Becton, Dickinson and Co., Rutherford, N.J.

Table I—Pharmacokinetic Parameters of Proquazone After Intravenous Administration Derived From the Best Fit by Digital Computer Programs [a]

Parameter	Subject A		B		C		D		E		Mean ± *SD* (n = 10)	
Weight, height [b]	70, 185		83, 184		68, 177		73, 174		82, 181			
Surface area, m^2	1.92		2.06		1.84		1.87		2.02			
Dose, mg iv	74.33	121.96	74.90	122.00	74.50	122.12	74.52	121.94	74.55	121.90		
α [c], 10^2 min^{-1}	48.6	86.8	17.6	26.9	14.6	16.7	44.0	59.2	30.9	32.1	36.7	23.1
	(1.48)	(0.96)	(4.78)	(0.96)	(0.87)	(2.11)	(1.04)	(1.00)	(1.48)	(0.68)	(1.54)	(1.21)
β [c], 10^2 min^{-1}	4.90	8.20	6.88	5.09	4.18	2.78	5.10	5.50	3.74	3.32	4.97	1.63
	(1.31)	(0.97)	(0.97)	(0.94)	(1.16)	(1.45)	(1.04)	(1.03)	(1.45)	(0.97)	(1.13)	(0.20)
γ [c], 10^2 min^{-1}	1.09	1.09	1.09	0.720	1.09	0.843	0.880	0.950	0.800	0.560	0.911	0.185
	(0.97)	(0.96)	(0.87)	(0.83)	(1.27)	(1.27)	(0.96)	(0.93)	(1.15)	(0.94)	(1.02)	(0.16)
A [c], % dose/L	12.7	11.6	1.15	4.47	1.48	3.93	13.2	8.91	9.35	6.13	7.29	4.49
	(1.59)	(0.96)	(6.25)	(1.03)	(1.29)	(1.38)	(1.02)	(1.01)	(1.32)	(1.00)	(1.69)	(1.62)
B [c], % dose/L	2.16	3.43	3.51	3.45	3.24	3.22	3.10	3.68	2.40	2.32	3.05	0.543
	(1.35)	(1.01)	(1.08)	(0.97)	(0.91)	(1.16)	(0.96)	(1.01)	(1.35)	(0.99)	(1.08)	(0.158)
C [c], % dose/L	0.747	0.900	0.846	0.229	0.599	0.372	0.395	0.699	0.472	0.237	0.550	0.224
	(1.05)	(0.96)	(0.86)	(0.91)	(1.59)	(2.11)	(1.01)	(0.92)	(1.40)	(0.92)	(1.17)	(0.41)
V [d], L	6.41	6.28	18.2	12.3	18.8	13.3	5.99	7.53	8.18	11.5	10.8	4.80
V_{ps} [d], L	66.1	66.6	67.9	120	62.6	64.7	83.7	67.7	81.5	136	81.6	25.6
V_{ss} [f], L	37.6	42.7	43.2	43.0	32.4	28.4	34.5	37.2	39.1	56.4	39.5	7.69
CL [g], mL/min	745	772	727	857	702	559	730	644	641	729	711	81.6
k_{BT} [h], min^{-1}	0.235	0.435	0.014	0.081	0.020	0.053	0.194	0.284	0.136	0.149	0.160	0.132
k_{TB} [h], min^{-1}	0.125	0.285	0.152	0.148	0.116	0.094	0.130	0.228	0.099	0.116	0.146	0.063
$k_{BT'}$ [h], min^{-1}	0.056	0.102	0.027	0.019	0.011	0.006	0.041	0.043	0.028	0.020	0.035	0.028
$k_{T'B}$ [h], min^{-1}	0.019	0.023	0.021	0.009	0.015	0.010	0.012	0.016	0.012	0.008	0.015	0.005
k_{BE} [i], min^{-1}	0.112	0.116	0.041	0.070	0.036	0.041	0.123	0.085	0.080	0.066	0.077	0.032

[a] NONLIN and SAAM. [b] Weight in kg; height in cm. [c] Parameters of the sum of the tri-exponential equations to express the plasma concentration of proquazone as a function of time as obtained using the digital computer programs NONLIN and SAAM. SAAM results expressed as fractions of the estimates by NONLIN are in parentheses. [d] Calculated from Eq. 2. [e] Obtained using Eq. 6. [f] Derived from Eq. 7. [g] Computed from Eq. 5; the plasma area was obtained using Eq. 3b. [h] Rate constants of distribution calculated using the method in Ref 28. [i] Rate constant of elimination obtained using the method in Ref. 28.

0–1, 1–2, 2–4, 4–6, 6–8, 8–10, 10–12, 12–18, 18–24, 24–36, 36–48, 48–60, 60–72, and 72–96 h after drug administration. The urinary pH was monitored, and aliquots of 20–30 mL were transferred to storage containers and kept at −20°C until assayed. Heart rate and blood pressure were monitored at hourly intervals in all the studies up to 10 h after administration.

Preliminary experiments investigated the possible binding of I to syringes, stopcocks, and urine containers. No significant drug binding was found for the drug with these materials.

Drug Administration—*Intravenous*—Freshly prepared sterile solutions of 1.6 and 1.0 mL of I in 4-hydroxymethyl-1,3-dioxolane-5-hydroxy 1,3-dioxane (1:2) (7.5% w/v) in 2-mL syringes were slowly added to 45 mL of human albumin solution kept in 50-mL syringes[12] using a three-way stopcock[13]. The solutions had been brought to a temperature of 37°C previously. After mixing the combined solutions thoroughly, 2-ml aliquots were taken for the determination of the dosages administered. The drug-containing albumin solutions were injected at a constant rate over a period of 60 ± 5 s through one of the catheters. The catheter was immediately flushed with 10 mL of blood which had been taken previously from the volunteer and had been heparinized subsequently. This time was considered as time zero.

Peroral—The capsules (1 or 3) were swallowed together with 100 mL of water. The time of swallowing was considered as time zero.

Analytical Procedures—Proquazone in plasma and urine was determined by a fluorometric method[2]. The main metabolite (II) and its conjugated product (III) were measured in urine by an HPLC assay[2].

The fluorometric assay involved extraction under alkaline conditions into n-heptane and back-extraction under acidic conditions into an aqueous phase. Fluorescence was determined at an emission wavelength of 510 nm (excitation at 326 nm). Unknown concentrations of I were determined by means of standard curves derived from samples spiked with known amounts of the drug. The individual time zero samples obtained from each volunteer served as blanks. The fluorometric assay was specific for I, and its lower level of sensitivity was at 10 ng/mL in plasma and urine. The mean percentage recovery (±*SD*) of the assay determined from samples which were spiked with known amounts of I was 108 ± 8% (n = 25) and 104 ± 5% (n = 7), respectively, for concentrations between 50 and 100 ng/mL in plasma and 95 ± 7% (n = 11) at a concentration of 50 ng/mL in urine. The mean percent precision (10^2 *SD*/Mean) of the fluorometric assay estimated from measurements of samples spiked with known amounts of I was 3% (n = 6) for the concentration range 10–1000 ng/mL in plasma and 9% (n = 6) for the concentration range 10–10,000 ng/mL in urine.

The HPLC method developed for the measurement of II in urine included an extraction into carbon tetrachloride–chloroform (1:1) prior to injection into the HPLC and measurement of the UV absorbance at 237 nm. HPLC conditions were as follows. A stainless steel column (25 × 0.4-cm i.d.)[14] prepacked with silica gel[15] was used for separation. It was protected by a stainless steel precolumn (4.0 × 0.4-cm i.d.) which was dry-packed with porous beads of silica gel[16]. A ternary mixture of isooctane–ether–ethoxyethanol (650:350:50, v/v/v) was employed as the mobile phase at flow rates of 1.6–2.0 mL/min. n-Ethylproquazone was used as internal standard. The respective retention times for II and n-ethylproquazone were 6.6 and 7.8 min.

For the determination of total [conjugated + unconjugated (II + III)] metabolites, the urine samples were exposed to enzymic hydrolysis at 37°C for 2 h at pH 5.35. The enzymic digests were then extracted into benzene–isopropyl alcohol (5:1) and subsequently injected into the HPLC. The amounts of III were obtained from the difference between the individually determined total metabolites and the amount of II. The lower level of sensitivity of the HPLC method in urine was 20 ng/mL. The mean percent recovery (±*SD*) for II plus III with this method was 98 ± 10 (n = 22) for the concentration range 0.2–1.0 μg/mL. The respective mean percent precisions of the method for II plus III and II obtained from samples spiked with known amounts of the metabolites were similar and were 7 and 11% (n = 6) for the above concentration ranges.

Estimates for the relative precision of the fluorometric and HPLC methods were also calculated from the replicate determinations of the samples obtained in the pharmacokinetic studies. In plasma the relative precision of the fluorometric method was constant over a large concentration range (8000–50 ng/mL) and was high (2%). In the lower concentration range (50–5 ng/mL), the relative precision of the method was clearly smaller (35%). In urine the variability of the fluorometric method for I in the concentration range of 50–5 ng/mL was large, and the relative precision was 60%. Similarly obtained estimates for the relative precision of II and III measurements by the HPLC method yielded average values of 7% (4.0–0.01 μg/mL) and 6% (250–0.01 μg/mL), respectively. All measurements in plasma and urine by fluorometry or HPLC were performed in duplicate. The means of these determinations were used for graphical and digital computer analyses.

Pharmacokinetic Data Analysis—*Proquazone*—The data analyses

[12] Disposable Syringes; ASIK I/S Hospital Supplies, Denmark.
[13] Pharmaseal Laboratories, Glendale, Calif.
[14] Hibar; Merck Ltd., Darmstadt, West Germany.
[15] Lickrosorb; Merck Ltd., Darmstadt, West Germany.
[16] Perisorb; Merck Ltd., Darmstadt, West Germany.

Table II—Urine Data of Proquazone and Its *m*-Hydroxy Metabolites

Subject	Dose, mg	$10^4\ U_\infty/D$[a]	$10^2\ U_\infty^{II}/D$[b]	$10^2\ U_\infty^{III}/D$[c]	$10^2\ U_\infty^{(II+III)}/D$[d]	$10^2\ U_\infty^{II}/U_\infty^{III}$[e]
A	75 iv				14.53	
	122 iv	0.616	0.201	14.36	14.56	1.40
	300 po		0.078	15.62	15.69	0.496
	900 po		0.068	10.35	10.42	0.657
B	75 iv				23.32	
	122 iv	1.749	0.176	18.75	18.93	0.936
	300 po		0.163	23.29	23.45	0.700
	900 po		0.040	11.05	11.11	0.391
C	75 iv				24.00	
	122 iv	0.346	0.363	27.85	28.21	1.30
	300 po		0.371	28.03	28.40	1.32
	900 po		0.116	19.35	19.46	0.598
D	75 iv				19.19	
	122 iv	0.733	0.358	18.78	19.14	1.91
	300 po		0.208	17.10	17.32	1.21
	900 po		0.150	10.68	10.84	1.40
E	75 iv				21.16	
	122 iv	0.600	0.149	18.28	18.43	0.815
	300 po		0.043	15.85	16.28	0.274
	900 po		0.049	13.55	13.61	0.359
Mean ± *SD* (*n* = 5)	75 iv				20.44 ± 3.80	
	122 iv	0.781 ± 0.492	0.250 ± 0.010	19.60 ± 4.97	19.85 ± 5.03	1.27 ± 0.430
	300 po		0.173 ± 0.129	19.97 ± 5.47	20.23 ± 5.50	0.800 ± 0.450
	900 po		0.085 ± 0.046	13.00 ± 3.77	13.08 ± 3.78	0.680 ± 0.420
Paired *t* test	300 po/900 po		$p < 0.14$	$p < 0.02$	$p < 0.02$	
	122 iv/300 po					$p < 0.05$
	122 iv/900 po					$p < 0.005$

[a] Fraction of dose renally excreted as unchanged proquazone at infinite time, obtained using Eq. 9. [b] Fraction of dose renally excreted as *m*-hydroxyproquazone at infinite time, obtained using Eq. 17. [c] Fraction of dose renally excreted as conjugated *m*-hydroxyproquazone at infinite time, obtained using Eq. 17. [d] Fraction of dose renally excreted at infinite time as the sum of unconjugated and conjugated *m*-hydroxyproquazone. [e] Ratio of the renally excreted amounts at infinite time of unconjugated to conjugated *m*-hydroxyproquazone.

on I after intravenous administration used graphical and digital computer methods. Initial (0–2.5 min) and terminal (10 h→∞) concentrations were not available. However determinations of clearance and volume of distribution parameters are based on plasma concentrations from time zero to infinity. Hence, plasma concentrations in the initial and terminal intervals were generated by fitting the experimentally determined plasma concentrations of I (C_p) after intravenous administration to polyexponential functions. The fittings were performed with the individual data using the nonlinear least-squares estimation programs SAAM (11) and NONLIN (12). Best results were obtained with both SAAM and NONLIN when the experimental data were fitted to the class (13) of three-compartment intravenous infusion models (without any assumptions regarding topology of the compartments) in accordance with:

$$C_p = A'e^{-\alpha t'} + B'e^{-\beta t'} + Ce^{-\gamma t'} \qquad \text{(Eq. 1)}$$

where A', B', and C' are the coefficients and α, β, and γ are exponents of the three-exponential equation and t' is the postinfusion time. Different weighting procedures were evaluated in preliminary fitting attempts with both programs. Weighting according to $w = 1/K \cdot C_p$, where K corresponds to a constant, and $w = 1/C_p$ was most adequate for the definitive fitting of the data by SAAM and NONLIN, respectively. The assumption of an intravenous infusion model was justified since the large value of the rate constant of the early disposition phase ($t_{1/2,\alpha}$ = 2 min) necessitated consideration of the 1-min infusion time. Fitting the data to a three-exponential equation, but not to a two-exponential equation, yielded random scatter of the data points about the regression line in all 10 data sets (by NONLIN and SAAM); a typical example (SAAM) is given in Fig. 1. Fitting the data to a four-exponential equation did not produce a significant reduction of the weighted sum of squared deviations (14), although four phases were separable by graphical analysis in some plots.

In fitting the data to the class of three-compartment models, no assumptions were made regarding the topology of the compartments. However, when clearance and steady-state volume of distribution values were derived from the coefficients and exponents of the fits, it was presumed that elimination of the drug occurred exclusively from the measured site, *i.e.*, plasma compartment (15). Only a few of the class of three-compartment models fulfill this requirement. Among those, a mamillary three-compartment model was arbitrarily chosen, and its volume and rate constant parameters calculated from the coefficients and exponents obtained in the fits. Mamillary models, which presume elimination to occur exclusively from the measured site, *i.e.*, the central compartment (15), represent the most often used model type in pharmacokinetic data analysis. The study of a particular type of model permits one to follow the time course of the drug in the tissue compartments and to evaluate depth and capacity of the different body compartments.

The peroral data were analyzed in a different way; they were not fitted to polyexponential equations. Only the terminal logarithmic linear phase plasma concentrations were fitted to obtain plasma concentrations over the entire time course. The plasma concentrations of I after peroral administration were much smaller and showed more scatter than the intravenous data. In addition, a possible deviation from first-order kinetics of I at the 900-mg dose level was implied by the metabolite data and could not be rejected.

m-Hydroxy Metabolites—The urinary data on metabolites II and III after both modes of administration were subjected to a model independent analysis.

Pharmacokinetic Calculations—*Proquazone*—The apparent volume of distribution of the central compartment, V, was estimated by:

$$V = D/C_0 = D/(A + B + C) \qquad \text{(Eq. 2)}$$

where D corresponds to the dose, C_0 is the drug concentration at time zero (intravenous bolus model), and A, B, and C represent the coefficients of the corresponding model equation (16). The total area under the plasma concentration time curve, AUC, was obtained from:

$$\text{AUC} = A/\alpha + B/\beta + C/\gamma \qquad \text{(Eq. 3a)}$$

$$\text{AUC} = \text{AUC}(t_0,t_1) + \text{AUC}(t_1,t_2) + \text{AUC}(t_2,t_\infty) \qquad \text{(Eq. 3b)}$$

where $\text{AUC}(t_0,t_1)$ and $\text{AUC}(t_1,t_2)$ correspond, respectively, to the partial area during the time of infusion and to the partial area during the time interval after the infusion was stopped to the last measured sample, and $\text{AUC}(t_2,t_\infty)$ is the partial area from the time of the last measured sample (10 h) to infinity on the presumption of terminal logarithmic linear decay. $\text{AUC}(t_0,t_1)$ and $\text{AUC}(t_1,t_2)$ were obtained by numerical integration (linear trapezoidal rule) after the plasma concentration at the end of infusion was estimated from Eq. 1. $\text{AUC}(t_2,t_\infty)$ was determined by algebraic integration in accordance with:

$$\text{AUC}(t_2,t_\infty) = C_{10\text{ h}}/\gamma \qquad \text{(Eq. 4)}$$

where $C_{10\text{ h}}$ corresponds to the concentration of the last measured sample. The ratio of the $\text{AUC}(t_0,t_1)$ to AUC was, on average, 0.04; the ratio of $\text{AUC}(t_2,t_\infty)$ to AUC was negligibly small (<0.004). The total clearance of drug, CL, was estimated according to:

$$CL = D/\text{AUC} \qquad \text{(Eq. 5)}$$

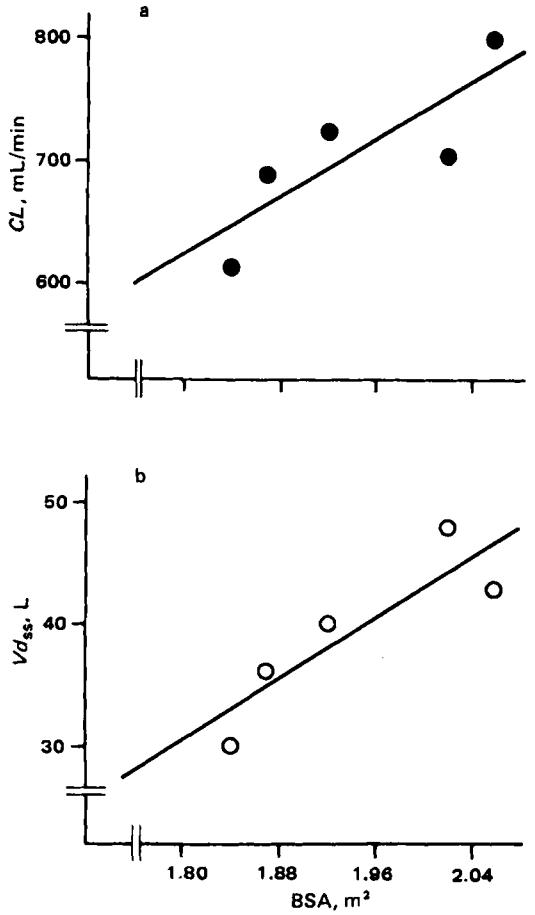


Figure 3—*Linear plots of the clearance (a) and steady-state volume of distribution (b) against body surface area in volunteers A–E, using the mean values obtained in the intravenous experiments for each individual. There are apparent linear relationships between* CL *and BSA [y = 435 (±440) + 588 (±226)·x, r = 0.832, p < 0.05] and* V_{ss} *and BSA [y = 83.6 (±38.5) + 63.4 (±19.8)·x, r = 0.880, p < 0.05].*

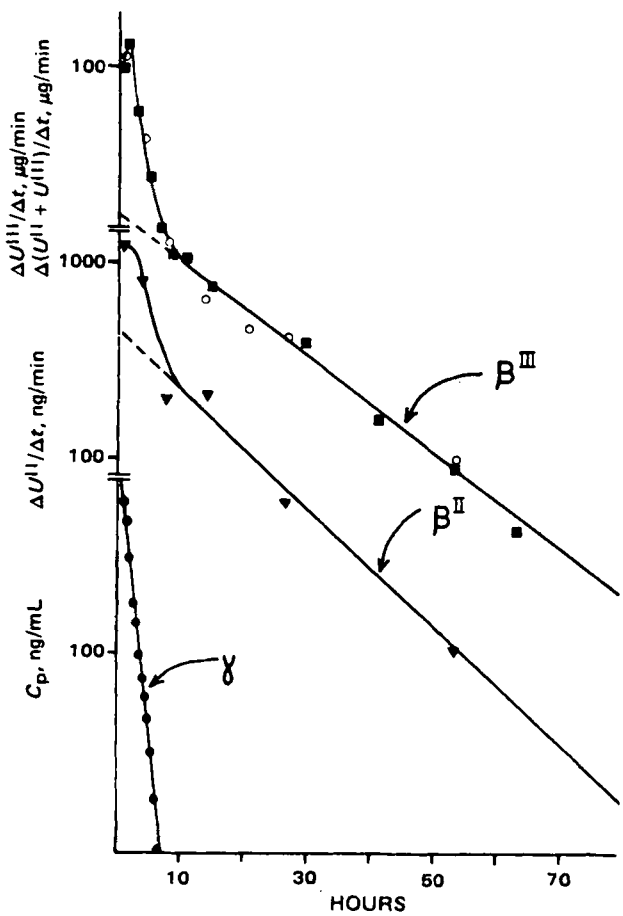


Figure 4—*Semilogarithmic plots against time after intravenous administration of the rates of urinary excretion per minute (*dU/dt*) for II (▼), III (○), and II plus III (■). A semilogarithmic plot of plasma concentration against time of I (●) is included for comparison. The metabolites II and III have similar triphasic profiles with peak rates at 1 h after administration and apparent terminal logarithmic linear phases with slopes of* $-\beta^{II,III}$. *Data were obtained in subject C after an intravenous dose of 122 mg.*

The apparent pseudo-steady-state volume of distribution, V_{ps}, was obtained from:

$$V_{ps} = CL/\gamma \qquad \text{(Eq. 6)}$$

The apparent steady-state volume of distribution was estimated according to:

$$V_{ss} = D\left(\frac{A}{\alpha^2} + \frac{B}{\beta^2} + \frac{C}{\gamma^2}\right) \Big/ \left(\frac{A}{\alpha} + \frac{B}{\beta} + \frac{C}{\gamma}\right)^2 \qquad \text{(Eq. 7)}$$

Amounts of unchanged drug excreted in urine at infinite time, U, were obtained from:

$$U = U_{6\text{ h}} + (dU/dt)_{6\text{ h}}/\gamma \qquad \text{(Eq. 8)}$$

where $U_{6\text{ h}}$ and $(dU/dt)_{6\text{ h}}$ correspond, respectively, to the amounts excreted up to 6 h and to the urinary excretion rate 6 h after drug administration; γ was estimated from semilogarithmic plots of the excretion rate against time. Renal clearance, CL_{ren}, was calculated according to:

$$CL_{ren} = U/\text{AUC} \qquad \text{(Eq. 9)}$$

Glomerular clearance, CL_{glom}, was predicted from:

$$CL_{glom} = CL_{cr} \cdot f_u \qquad \text{(Eq. 10)}$$

where CL_{cr} corresponds to the individually measured creatinine clearance, and f_u is the fraction of drug unbound in the plasma of the individual (7).

On the assumption that all nonrenal elimination occurred in the liver, the hepatic extraction efficiency (hepatic first-pass effect) was computed from:

$$\epsilon^{I,1} = \frac{CL_{hep}}{\hat{V}_{hep}} = \frac{CL - CL_{ren}}{\hat{V}_{hep}} \qquad \text{(Eq. 11)}$$

where CL_{hep} and $\hat{V}_{hep}$ correspond to the liver clearance and liver plasma flow [825 mL/min (17)], respectively.

The percentage of an oral dose of I that reached the systemic circulation unchanged [extent of absorption (18)], E, was estimated according to:

$$E = 10^2 \frac{(AUC/D)_{po}}{(AUC/D)_{iv}} \qquad \text{(Eq. 12)}$$

The fraction of the dose eliminated by first pass on oral administration, ϵ, was estimated from the urinary data of II and III using:

$$\epsilon^{II} = R^{II}\{(U^{II}/D)_{po} - [(U^{II}/D)_{iv} \cdot E/10^2]\} \qquad \text{(Eq. 13a)}$$

$$\epsilon^{III} = R^{III}\{(U^{III}/D)_{po} - [(U^{III}/D)_{iv} \cdot E/10^2]\} \qquad \text{(Eq. 13b)}$$

where $R^{II} = [(D - U^{I})/U^{II}]_{iv}$ and $R^{III} = [(D - U^{I})/U^{II}]_{iv}$ on the assumption that the ratio of drug metabolized to other metabolite(s) or eliminated by routes other than renal to the amount of II and III formed is invariant with route of administration. Estimates for ϵ were also obtained from proquazone data in accordance with:

$$\epsilon^{I,2} = 1 - (E/10^2) \qquad \text{(Eq. 14)}$$

The total extent of absorption of unchanged plus changed drug at the 300-mg dose level, E^{tot}, was estimated from:

$$E^{tot} = 10^2\epsilon^{I,1} + E \qquad \text{(Eq. 15)}$$

m-*Hydroxy Metabolites*—The respective amounts of II or III excreted in urine at infinite time, $U^{II,III}$, were obtained from:

$$U^{II,III} = [U_{72\text{ h}} + (dU/dt)_{72\text{ h}}/\beta]^{II,III} \qquad \text{(Eq. 16)}$$

where $U^{II,III}_{72\text{ h}}$ corresponds to the excreted amounts of II or III at 72 h and

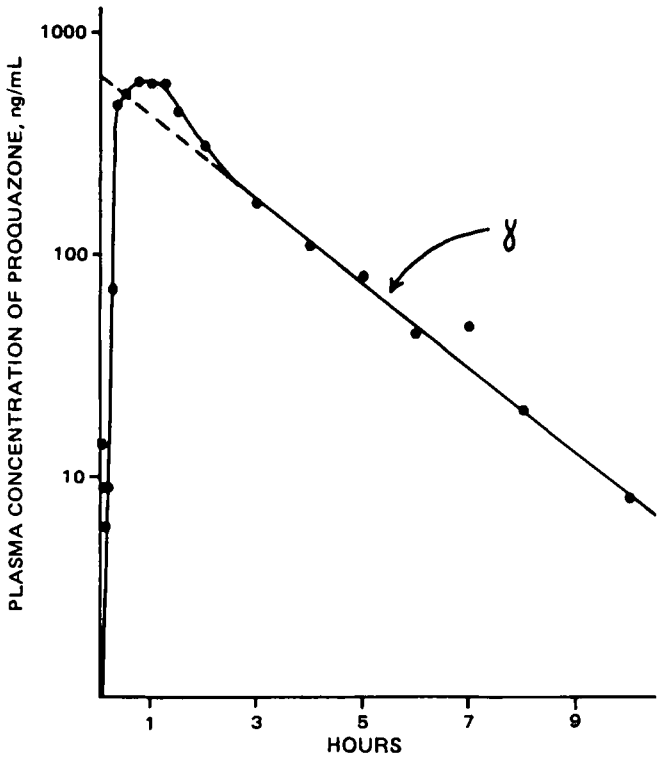


Figure 5—*Semilogarithmic plot against time of proquazone plasma concentration following administration of 900 mg po to subject A showing a triphasic profile. The apparent terminal logarithmic phase is linear with a slope of* $-\gamma$.

$(dU/dt)^{II,III}_{72\ h}$ to the excretion rates of II and III 72 h after drug administration; $\beta^{II,III}$ was estimated from semilogarithmic plots of the respective urinary excretion rates against time. The ratios of $(U_{72\ h-\infty}/U)^{II}$ and $(U_{72\ h-\infty}/U)^{III}$ were insignificant (<0.01).

Statistics—Paired *t* test statistics were performed with the proquazone and metabolite data after peroral administration of the drug.

RESULTS

Procedural and Adverse Effects—The values of the clinical laboratory data that were obtained prior to each of the four administrations of proquazone were always within the normal range for males of this age group (19). Proquazone at the four dosage levels was well tolerated, and no adverse drug effects were observed. Blood pressure and heart rate did not show significant changes in the interval up to 10 h after administration of the drug.

Pharmacokinetics of Proquazone and Its *m*-Hydroxy Metabolites—Mean values for the parameters as estimated by NONLIN are given hereafter in the text; the plus and minus values for these means refer to the standard deviation. The number of values considered was 10 unless otherwise specified. The volume and clearance parameters of I are referenced to total (bound plus unbound) drug concentration in plasma. It was assumed that the concentration ratio of total to unbound drug in plasma was maintained during the passage of I through the capillaries of the liver. Negligible concentrations of I were found in erythrocytes in whole blood (7).

Intravenous Administration—*Proquazone*—The NONLIN and SAAM estimates for the coefficients and exponents of the fitted three-exponential equation were equivalent (Table I). The values respectively obtained for A and α varied the most. There was good agreement between the estimated total clearance (NONLIN, 708 mL/min; SAAM, 689 mL/min) and steady-state volume of distribution (NONLIN, 39.5 L; SAAM, 39.7 L) obtained by the two programs.

The CL estimates by NONLIN and SAAM were close to those obtained by application of the trapezoidal rule (711 mL/min), suggesting that the polyexponential fittings by both programs were adequate. The dose-normalized plasma concentration–time data at the lower and higher dose levels were superimposable and indicated first-order kinetics of I. The dose percentages of unchanged I eliminated by the renal route were extremely small (<0.001%) and could be measured reliably only after the higher intravenous dose (Table II). Three phases with mean apparent half-lives of 2 (±1) min ($t_{1/2,\alpha}$), 14 (±5) min ($t_{1/2,\beta}$), and 76 (±15) min ($t_{1/2,\gamma}$) were separable in the curves in plasma (Fig. 2, Table I). The latter value was confirmed by $t_{1/2,\gamma}$ = 98 (±30) min (n = 5) estimated from semilogarithmic plots of the urinary excretion rates against time. In all the experiments, the plasma samples taken at 1.5 min after the end of the infusion yielded maximum concentrations and indicated fast initial mixing in plasma. The apparent volume of distribution of the central compartment (V) was, on average, 11 (±4.8) L (Table I) and exceeded the plasma volume [3 L (20)] significantly. This suggested fast extravascular distribution of the highly plasma protein-bound drug (7). Total clearance of I, CL, averaged 707 (±83.7) mL/min. The extraction efficiency of the liver for I (ϵ) was 0.86 (±0.101) and suggested that I can be classified as a highly extracted compound (21, 22). There was an apparent linear correlation between CL and body surface (BSA), but not between CL and weight, for the individuals (Fig. 3a). The renal clearance, CL_{ren}, averaged 0.06 (±0.05) mL/min and was smaller than the estimated glomerular clearance, CL_{glom} = 2.5 (±0.50) mL/min, indicating tubular reabsorption in excess of possible tubular secretion for the highly lipophilic drug.

Table III—Pharmacokinetic Parameters of Proquazone Derived from Plasma Data After Peroral Administration

Subject	Dose, mg	γ^a, 10^2 min^{-1}	$t_{max}{}^b$, min	$C_{max}{}^c$, % dose/L	$AUC_{0\text{-}10\ h}/D^d$, 10^2 min/L
A	300	0.75	45	0.087	10.45
	900	0.72	45	0.068	10.07
B	300	0.84	180	0.030	5.45
	900	0.81	240	0.016	4.14
C	300	0.65	90	0.027	8.44
	900	0.44	90	0.036	7.88
D	300	0.77	60	0.020	3.60
	900	1.24	30	0.056	3.94
E	300	0.87	60	0.088	13.77
	900	0.83	30	0.241	32.50
Mean ± *SD* (n = 5)	300	0.78 ± 0.08	87 ± 55	0.051 ± 0.034	8.43 + 4.02
	900	0.81 ± 0.29	87 ± 89	0.083 ± 0.090	11.71 ± 11.91

[a] Slopes of the apparent terminal logarithmic linear γ phase. [b] Times of the maximum plasma concentration of proquazone. [c] Maximum plasma concentration of proquazone at time t_{max} after peroral administration. [d] Dose corrected quasi total areas under the plasma concentration time curve from time zero to 10 h after administration, obtained using Eq. 3.

There was no indication for urine flow or urine pH dependency of the renal elimination of I. The volumes of distribution at pseudo-steady-state (V_{ps}) and at steady state (V_{ss}) were 82 (±26) and 39 (±7.7) L, respectively, (Table I). The large difference between these values indicated that the microscopic rate constants of distribution into the tissue compartment T′ ($k_{BT'}$ and $k_{T'B}$) were small relative to the rate constant of elimination (k_{BE}) (Table I). The slower equilibrating tissue compartment T′ (Fig. 1) must be regarded as a deep compartment. Its capacity for the drug is much larger than that of the shallow compartment T (Table I). The volume of distribution at steady state, V_{ss}, depended linearly on the BSA of the individuals (Fig. 3b). In contrast, no such correlation existed between V_{ss} and weight of the individuals, indicating that the widely used principle of weight normalization of kinetic parameters may be questionable.

m-Hydroxy Metabolites—Semilogarithmic plots against time of the urinary excretion rates of II and III showed triphasic profiles with terminal half-lives $t_{1/2\beta}$ of 6.7 (±3.1) h (n = 5) for II and 10.6 (±3.0) h (n = 5) for III (Fig. 4). This implied that the *m*-hydroxy metabolites have a considerably larger time of residence in the body than the parent drug. Metabolite II is reportedly pharmacologically active[2]. The percentages of the administered dose renally excreted as *m*-hydroxy metabolites (II plus III) were 20.4 ± 3.80% and 19.9 ± 5.03% (n = 5) (Table II) after the lower and higher intravenous dosages, respectively. This implied first-order kinetics of the metabolites. The combined urinary recoveries of I, II, and III amounted to 20%, indicating that 80% of the dose was eliminated by alternative routes. The renally recovered dose percentages of III [19.6 ± 4.97% (n = 5)] clearly exceeded those of II [0.25 ± 0.01% (n = 5)] (Table II).

A model comprising all the experimental findings after intravenous administration is given in Scheme I. As mentioned previously, a model-dependent data analysis was performed with the parent drug data,

Table IV—Extent of Absorption and First-Pass Effect of Proquazone

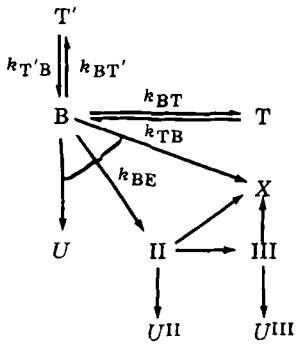
Subject	Dose mg	Extent of Absorption: Unchanged Drug E^a	Extent of Absorption: Unchanged + Changed Drug $E^{tot\ b}$	First-Pass Effect: $\epsilon^{I,1c}$	First-Pass Effect: $\epsilon^{III d}$	First-Pass Effect: $\epsilon^{II e}$	First-Pass Effect: $\epsilon^{I,2f}$
A	300	8.2	109	0.92	1.01	0.38	0.78
	900	7.7					
B	300	4.5	124	0.96	1.20	0.88	0.95
	900	3.3					
C	300	5.4	101	0.76	0.95	0.97	0.94
	900	5.1					
D	300	2.7	91	0.83	0.89	0.56	0.95
	900	2.7					
E	300	9.7	87	0.83	0.78	0.19	0.66
	900	22.4					
Mean ± *SD*	300	6.1 ± 2.8	103 ± 15	0.86 ± 0.13	0.96 ± 0.16	0.60 ± 0.33	0.86 ± 0.13
	900	8.2 ± 8.2					

[a] *Calculated from Eq. 12.* [b] *Computed according to Eq. 15.* [c] *Obtained using Eq. 11.* [d] *Estimated from Eq. 13a using III data.* [e] *Calculated from Eq. 13b using II data.* [f] Obtained in accordance with Eq. 14 after rearrangement.

whereas a model-independent approach was effected with the metabolite data. Scheme I reflects this approach. The model depicted is the most simple that can account for all the kinetic events observable with the type of data analysis chosen. The parent drug is represented by a three-compartment body model with extrarenal [formation of II, formation of unidentified metabolite(s), and biliary excretion (X)] and renal (U) elimination. The identified metabolites II and III are represented each by a body compartment (II, III) with renal (U_{II}, U_{III}) and extrarenal [formation of III, formation of unidentified metabolite(s), and biliary excretion (X)] elimination. As can be seen, the amounts of I, II, and III passing through all routes of elimination, which were not monitored directly, are lumped together (X).

Peroral Administration—*Proquazone*—Maximally, three (Fig. 5) or two phases were separable in the plasma concentration–time profiles of I after administration of the two peroral dosages. The triphasic profiles peaked earlier than the biphasic profiles. These findings indicated that absorption was faster than early disposition (β) in the former, but slower than early disposition in the latter experiments. The times of peak plasma concentration (t_{max}), the dose-normalized peak plasma concentrations ($10^2C_{max}/D$), and the dose-normalized areas under the plasma concentration time curves ($10^2AUC_{0-10\ h}/D$) were not significantly different for the two dose levels (Table III). The slopes of the terminal logarithmic linear phase (γ) after oral dosing agreed with those previously found after intravenous dosing. These results suggest that first-order kinetics were operative for I after oral dosing at both dosage levels. In four of the five individuals tested (A–D), the dose-normalized AUC values were remarkably similar at both dosage levels, whereas in one individual (E) this parameter was clearly larger after 900 mg than after 300 mg (Table III). The extent of absorption (18) averaged 6.1 ± 2.8% (n = 5) and did not differ significantly from E = 8.2 ± 8.2% (n = 5) after the 900-mg dose (Table IV). The small dose fractions of drug reaching the systemic circulation unchanged implied a poor absorption or a large first-pass effect for I.

m-*Hydroxy Metabolites*—Semilogarithmic plots of the urinary excretion rates against time after oral dosing showed profiles similar to those observed after intravenous administration except that they peaked later (Figs. 4 and 6). The percentages of the administered dose renally excreted as II and III after the 900-mg dose tended to be smaller than after the 300-mg dose, representing respectively 65 and 50% of the recoveries found at the lower dose level (Table II). The ratios of the urinary recoveries of II to III were equivalent after administration of the two peroral dosages (Table II), suggesting that the observed dose-dependent, nonlinear kinetics were associated with the formation of II. This was in contrast to the previously observed linear kinetics of disposition of I after peroral dosing, which implied linear kinetics of formation for II. When the dose fractions of I eliminated during first pass at the 300-mg dose level were estimated from II and III data assuming linear kinetics, average values of ϵ^{II} = 0.60 and ϵ^{III} = 0.96 were obtained (Table IV). Only the latter value was in accordance with ϵ estimates by proquazone data [$\epsilon^{I,1}$ = 0.86, $\epsilon^{I,2}$



Scheme I—Model for intravenous administration of proquazone.

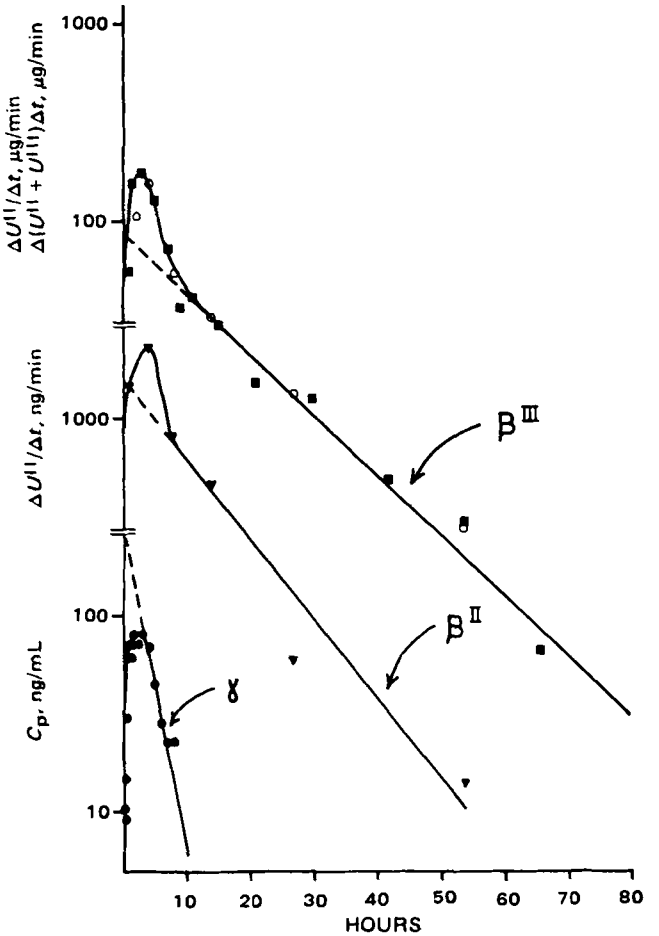


Figure 6—*Semilogarithmic plots against time after oral administration of the rates of urinary excretion per minute, (dU/dt) for II (▼), III (○), and II plus III (■). A semilogarithmic plot of plasma concentration against time of I (●) is included for comparison. The metabolites II and III have similar triphasic profiles with peak rates at 3 h after administration and apparent logarithmic linear phases with slopes of $-\beta^{II,III}$. Data were obtained in subject C after an oral dose of 300 mg.*

= 0.92, (Table IV)]. The underestimated values for ϵ obtained from II data (Table IV) implied that a significant percentage of I (~40%) was metabolized not only to II but subsequently to III during the first pass. The total extent of absorption (unchanged plus changed drug) of I was complete [E^{tot} = 103 ± 15% (n = 5), Table IV]. These results suggested that (*a*) the total first-pass effect should be assessed from III data, not from II data; (*b*) the low extent of absorption found for I was due to a large first-pass effect, and not due to poor absorption in accordance with the lipophilic nature of the compound; and (*c*) the assumption of first-order kinetics at the 300-mg dose level was reasonable.

DISCUSSION

The plasma and urinary data of proquazone and its *m*-hydroxy metabolites after administration of 75 and 122 mg iv and 300 mg po were indicative of first-order kinetics. Nonlinear, dose-dependent kinetics of formation for II were evident from the metabolite data at the 900-mg po dose level, whereas the parent drug data implied first-order kinetics of disposition for I (and hence first-order kinetics of generation for II) at this dose level. These findings were irreconcilable only if presystemic or systemic metabolism of I to II were the sole or major route of elimination for the parent drug, as equivalent 35% deviations from linear kinetics should then be found with both parent drug and metabolite data. However, if the percentage of I metabolized to II were smaller (≤40%), the parent drug data would deviate from linear kinetics by ≤14% (0.40·35%), whereas a unvariable 35% (1.0·35%) deviation from linear kinetics would be found with the metabolite data. Two other studies with administrations of 100 and 400 mg po of drug to 15 healthy volunteers determined quantitatively not only II and III, but all the metabolites which could be derivatives of II and III[2]. These studies used urinary data and permitted a quantitative assessment of the *m*-hydroxy pathway. The results showed that the percentage of I metabolized to II was ≤35%. Hence, the findings obtained at the 900-mg po dose level in the present study can be rationalized, and that the nonlinearity of the kinetics of I were recognized only with the metabolite data is reasonable. Considering the variability of the peroral plasma data of I, deviations on the order of ≤14% were too discrete to become apparent.

A major fraction of II (and III) after peroral administration of I was generated during first pass of drug through the liver; only a minor fraction of II (and III) was formed systemically from I (Table IV). The observed dose-dependent kinetics of formation of II (and III) at the 900-mg dose level was largely the result of a saturation of this first-pass effect. Whether or not the kinetics of systemically formed II (and III) were dose dependent at the higher peroral dose level could not be decided based on the available experimental evidence. A delineation as to the type of the nonlinear kinetics observed, *i.e.*, Michaelis–Menten kinetics (23), product inhibition kinetics (24), or blood flow-dependent kinetics (21, 22) (pharmacologically induced temporal decrease of the liver blood flow), was not possible with the available experimental data. However it is interesting to note that primarily hydroxy metabolites cause "product inhibition" (25).

The statistical design of the present study was not balanced. However, it is improbable that the nonlinearity of the kinetics observed at the 900-mg po dose level was a period- (procedure-) rather than a treatment- (dose-) related effect. First, the laboratory data gave no evidence for the existence of procedural effects. Second, the 900-mg dose was the first treatment given to the volunteers and procedural effects are more likely to become significant in the later experiments (300 mg po and 122 and 75 mg iv) as a result of a cumulative (carry over) effect. However, the kinetics of the parent drug and metabolites were first order in these experiments. Third, the intervals of at least 2 weeks which were maintained between the studies should have minimized possible carry-over effects.

Reports on data-fitting obtained by different computer programs can rarely be found in the literature (26, 27). The results of the present study indicated that there was reasonable agreement for the parameter estimates obtained by NONLIN and SAAM. There is a need for more of these comparative fitting efforts, so that the reliability and interchangeability of pharmacokinetic data obtained with different computer programs can be assessed definitively.

APPENDIX: GLOSSARY

D = Dose of proquazone (mg)
C_p = Concentration of proquazone in plasma (ng/mL)
C_0 = Concentration of proquazone in plasma at time zero after intravenous injection (ng/mL)
C_{max} = Maximum plasma concentration of proquazone at time t_{max} after peroral administration (ng/mL)
B = Amount of proquazone in the central compartment
T = Amount of proquazone in the shallow peripheral compartment
T′ = Amount of proquazone in the deep peripheral compartment
II = Amount of *m*-hydroxyproquazone in the body compartment
III = Amount of the conjugated *m*-hydroxyproquazone in the body compartment
U_t, U = Respective amounts of proquazone renally excreted unchanged at time t and at infinite time after administration
$U_t^{II,III}$, $U^{II,III}$ = Respective amounts of *m*-hydroxyproquazone and conjugated *m*-hydroxyproquazone renally excreted at time t and at infinite time
X = Amounts of proquazone, *m*-hydroxyproquazone, and conjugated *m*-hydroxyproquazone eliminated by routes that were not monitored directly: amounts of I, II, III excreted in bile and/or metabolized to unidentified metabolites
dU/dt = Amount of drug or metabolite excreted in urine, dU, (μg or ng) during a time interval, dt (min)
A', B', C', α, β, γ = Parameters of the three-exponential fit of the plasma concentrations of proquazone according to an intravenous infusion three-compartment model, where A', B', and C' are in ng/mL and α, β, and γ are in min
A, B, C = Parameters of the corresponding intravenous injection three-compartment model for proquazone, where A, B, and C are in % of dose/L
k_{ij} = Generalized form for the microscopic first-order rate constant from the ith to the jth compartment for proquazone (min^{-1})
$\beta^{II,III}$ = Slope of the apparent terminal logarithmic linear phase of *m*-hydroxyproquazone and conjugated *m*-hydroxyproquazone
V = Volume of distribution of proquazone in the central compartment (L)
V_{ps} = Apparent pseudo-steady-state volume of distribution of proquazone (L)
V_{ss} = Apparent steady-state volume of distribution of proquazone (L)
$AUC_{0-t'}$ AUC = Respective areas under the plasma concentration–time curve from time zero to time t and from time zero to infinity of proquazone
CL = Total clearance of proquazone (mL/min)
$\dot{V}_{hep}$ = Hepatic plasma flow (mL/min)
CL_{ren} = Renal clearance of proquazone (mL/min)
CL_{glom} = Glomerular clearance of proquazone (mL/min)
f_u = Fraction of proquazone in plasma unbound
E = Extent of absorption of unchanged proquazone
E^{tot} = Total extent of absorption of unchanged plus changed proquazone
ϵ^I, ϵ^{II}, ϵ^{III} = Respective estimates of first-pass effect (hepatic extraction efficiency) for proquazone from proquazone, *m*-hydroxy metabolite, and conjugated *m*-hydroxy metabolite data
t' = Postinfusion time (min)
BSA = Body surface area (m^2)

REFERENCES

(1) R. V. Coombs, R. P. Dauna, M. Denzer, G. E. Hardtmann, B. Huegi, G. Koletar, H. Ott, E. Junkiewicz, J. W. Perrine, and E. I. Takesue, *J. Med. Chem.*, **16**, 1237 (1973).

(2) G. E. Hardtmann, B. Huegi, G. Koletar, S. Kroin, J. W. Perrine, and E. I. Takesue, *J. Med. Chem.*, **17**, 636 (1974).

(3) E. I. Takesue, J. W. Perrine, and J. H. Trappold, *Arch. Int. Pharmacodyn.*, **221**, 122 (1976).

(4) U. Gubler and M. Baggiolini, *Scand. J. Rheum., Suppl.*, **21**, 8 (1978).

(5) R. Allan and M. Bleicher, *J. Int. Med. Res.*, **5**, 253 (1977).

(6) M. Nissilä and A. Kajander, *Scand. J. Rheum., Suppl.*, **21**, 36 (1978).

(7) A. Roos and P. H. Hinderling, *J. Pharm. Sci.*, **70**, 252 (1981).
(8) H. Ott and J. Meier, *Scand. J. Rheum., Suppl.*, **21**, 12 (1978).
(9) E. E. Ohnhaus, *Int. J. Clin. Pharmacol.*, **18**, 136 (1980).
(10) K. Siersbäck-Nielsen, J. Mølholm Hansen, J. Kampmann, and M. Kristensen, *Lancet*, **i**, 1133 (1971).
(11) M. Berman and M. F. Weiss, "User's Manual for SAAM," Public Service, Publication No. 1703, National Institutes for Health, Bethesda, Md. (1967).
(12) C. M. Metzler, "A User's Manual for NONLIN," The Upjohn Co., Technical Report 7292/69/7292/005, Kalamazoo, Mich. (1969).
(13) J. G. Wagner, *J. Pharmacokinet., Biopharm.*, **3**, 457 (1975).
(14) H. G. Boxenbaum, S. Riegelman, and R. M. Elashoff, *J. Pharmacokinet. Biopharm.*, **2**, 123 (1974).
(15) L. Z. Benet and R. L. Galeazzi, *J. Pharm. Sci.*, **68**, 1071 (1979).
(16) M. Gibaldi and D. Perrier, "Pharmacokinetics," Dekker, New York, N.Y., 1975, pp. 72–73.
(17) A. C. Guyton, "Textbook of Medical Physiology," 3rd ed., Saunders, Philadelphia, Pa., 1968, p. 325.
(18) M. Gibaldi and D. Perrier, "Pharmacokinetics," Dekkler, New York, N.Y., 1975, p. 145.
(19) K. Diem and C. Lentner (Eds), "Documenta Geigy, Scientific Tables," 7th ed. Geigy Pharmaceuticals, Division of Ciba-Geigy Corp., Ardsley, N.Y., 1975, pp. 617, 579.
(20) A. C. Guyton,"Textbook of Medical Physiology," 3rd ed., Saunders, Philadelphia, Pa., 1968, p. 419.
(21) A. S. Nies, D. G. Shand, and G. R. Wilkinson, *Clin. Pharmacokinet.*, **1**, 135 (1976).
(22) G. R. Wilkinson and D. G. Shand, *Clin. Pharmacol. Ther.*, **18**, 377 (1977).
(23) E. R. Garrett, J. Brés, K. Schnelle, and L. L. Rolf, *J. Pharmacokinet. Biopharm.*, **2**, 43 (1974).
(24) J. J. Ashley and G. Levy, *J. Pharmacokinet. Biopharm.*, **1**, 99 (1973).
(25) D. Perrier, J. J. Ashley, and G. Levy, *J. Pharmacokinet. Biopharm.*, **1**, 231 (1973).
(26) P. Veng Pedersen, *J. Pharmacokinet. Biopharm.*, **5**, 513 (1977).
(27) H. Wijnand and C. J. Timmer, *J. Pharmacokinet. Biopharm.*, **7**, 681 (1979).
(28) M. Gibaldi and D. Perrier, "Pharmacokinetics," Dekker, New York, N.Y. 1975, pp. 93–94.

ACKNOWLEDGMENTS

Supported in part by a grant provided by Sandoz Ltd., Basle, for which the authors are grateful.

We thank Ms. R. Leder and the nursing staff of the Merian Iselin Hospital in Basle for their expert technical assistance in performing the pharmacokinetic studies and Dr. W. Pacha, Dr. H. P. Keller, and the Sandoz Laboratories in Basle for performing the assays in plasma and urine.

Quantitative Determination of Captopril in Blood and Captopril and Its Disulfide Metabolites in Plasma by Gas Chromatography

M. S. BATHALA ˣ, S. H. WEINSTEIN, F. S. MEEKER, JR., S. M. SINGHVI, and B. H. MIGDALOF

Received December 8, 1982, from the *Department of Drug Metabolism, The Squibb Institute for Medical Research, Princeton, NJ 08540.* Accepted for publication February 11, 1983.

Abstract □ A sensitive, quantitative gas chromatographic–electron capture (GC–EC) method for the determination of captopril in blood and captopril and its disulfide metabolites (collectively) in plasma was developed. After addition of an internal standard and *N*-ethylmaleimide to the biological samples, excess *N*-ethylmaleimide and naturally occurring interfering substances were removed by extraction with benzene followed by acidification and extraction with hexane. The *N*-ethylmaleimide adducts of captopril and of the internal standard were then extracted with benzene and converted to their hexafluoroisopropyl esters. For the assay of captopril and its disulfide metabolites, tributylphosphine was used to reduce the disulfide metabolites to captopril prior to derivatization. The hexafluoroisopropyl esters of the *N*-ethylmaleimide adducts of captopril and of the internal standard, the 4-ethoxyproline analogue of captopril, were separated by GC on a column packed with 3% OV-101 on Chromosorb W-HP. The lower limits of sensitivity were 20 ng/mL for captopril in blood and 50 ng/mL for captopril and its disulfide metabolites in plasma. Linearity, precision, and accuracy were excellent. The method was validated by comparison of results obtained for total captopril in dog plasma by the GC–EC assay with results obtained by a published GC–MS method. The assay was applied to dog and human samples to explore its general utility.

Keyphrases □ Captopril—quantitative determination in blood and plasma, disulfide metabolites, gas chromatography □ Metabolites, disulfide—captopril, plasma, quantitative determination by gas chromatography □ Gas chromatography—captopril, quantitative determination in blood and plasma, disulfide metabolites

Captopril, 1-[(2*S*)-3-mercapto-2-methylpropionyl]-L-proline (I), the first orally active inhibitor of angiotensin-converting enzyme (peptidyl dipeptide hydrolase, kininase II, E.C. 3.4.15.1) to be used clinically, is a potent antihypertensive agent used to treat both renovascular and essential hypertension (1, 2). It is of particular value in treating hypertension resistant to conventional drugs (3), and shows great promise for use in congestive heart failure (2, 4).

The predominant route of excretion of captopril is *via* the kidneys (5–7). Since kidney function is often impaired in patients with hypertension, a sensitive assay for the drug in blood or plasma using readily accessible analytical equipment would be of value in optimizing dosage. Assays for both unchanged captopril and its disulfide metabolites would be useful, since interconversions occur among these compounds in rats, dogs, and humans (8–11). Metabolites of captopril include the symmetrical disulfide (IIA), captopril–L-cysteine mixed disulfide, captopril–glutathione mixed disulfide, and mixed disulfides of captopril with plasma proteins (IIB). Current methods for measurement of the drug in biological fluids are either not sensitive enough for monitoring therapeutic levels (12–14) or involve analytical equipment not available to the average clinical laboratory (15–17). Therefore, a sensitive method has been developed for quantifying captopril in blood and captopril

0022-3549/84/0300-0340$01.00/0