sampling at closer time intervals are needed to confirm these observations. The data for all patients could be described approximately by the sum of two exponentials, and the mean elimination half-life was ~40 min. The plasma concentration–time profile for one subject is shown in Fig. 2.

The pharmacokinetics of captopril in the four patients taking the drug chronically indicate that changes in plasma captopril concentrations do not reflect blood pressure changes. In all patients, captopril was undetected in their plasma 3–4 hr after dosing, yet blood pressure had not risen at this time. Blood levels of captopril metabolites, such as the disulfide, possibly may be a more useful index of the hemodynamics of this drug.

In conclusion, it was demonstrated that plasma captopril can be assayed with high sensitivity and specificity by first derivatizing this thiol compound to a fluorophore using *N*-(1-pyrene)maleimide and then separating the fluorescent adducts by HPLC. This procedure can also be used for assaying another thiol angiotensin-converting enzyme inhibitor, II, using captopril as the internal standard. This derivatization procedure should be suitable for assaying the mucolytic drug *N*-acetylcysteine after modification of the HPLC conditions to retard its elution.

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Pharmacokinetics of [^{14}C]Bretylium Tosylate in Rats

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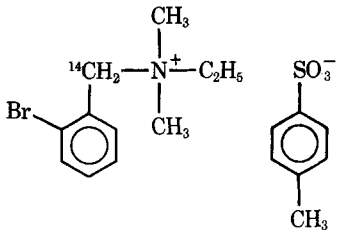
Received August 29, 1980, from the *Pharmaceutical Development Department, Research & Development, American Critical Care, McGaw Park, IL 60085.* Accepted for publication November 20, 1980.

Abstract □ The pharmacokinetics of bretylium tosylate were investigated in eight male Charles River rats. Each animal received an intravenous dose (10 mg/kg) of [^{14}C]bretylium tosylate. Serial blood samples, urine, and feces were collected for up to 72 hr. Bretylium concentrations in plasma and amounts excreted in urine and feces were determined by scintillation counting. On the average, 88 and 95% of the dose were recovered in urine and feces in 24 and 72 hr, respectively. Urinary recovery accounted for 65.6% of the dose while 29.7% was excreted in the feces. Bretylium concentrations in plasma declined triexponentially and were fitted to a three-compartment open model. Bretylium has a very high apparent volume of distribution (15 liters/kg), and its β half-life averaged 5.5 hr. Mean values of the apparent volume of the central compartment, plasma clearance, renal clearance, and excretion rate constants of bretylium in rats were 1 liter/kg, 1.93 liters/hr/kg, 1.27 liters/hr/kg, and 1.24 hr^{-1}, respectively. The results indicate that: (*a*) bretylium is strongly bound to the tissues and is eliminated by active urinary secretion and by biliary excretion in rats, and (*b*) there are strong similarities between the pharmacokinetics of bretylium in humans and rats and that this animal model might be suitable for interaction studies with other drugs.

Keyphrases □ Bretylium tosylate—pharmacokinetics, rats, compared with human studies □ Pharmacokinetics—bretylium tosylate, rats, compared with human studies □ Antiarrhythmic agents—bretylium tosylate, pharmacokinetics studied in rats, compared with human studies

Bretylium is a quaternary ammonium compound [(*o*-bromobenzyl)ethyldimethylamine] used clinically as the tosylate salt in the treatment of arrhythmias. Bretylium tosylate suppresses ventricular fibrillation in patients within minutes following intravenous administration (1). Suppression of ventricular tachycardia and other ventricular arrhythmias develops more slowly, usually 20–120 min following parenteral administration (2).



[^{14}C]bretylium tosylate

BACKGROUND

In humans, bretylium is eliminated intact mainly through the renal route (3, 4). No metabolites have been identified following administration of bretylium tosylate in humans and rats (3). The biological half-life of bretylium in normal subjects was 5.5 hr (3). Romhilt *et al.* (2) reported a half-life of 9.75 hr (range 4.2–16.9) in eight patients with cardiac disease. More recently, Adir *et al.* (4) reported a half-life of 8.1 hr for a normal subject and of 16.0 and 31.5 hr for two patients with renal impairment. In the normal subject, the renal clearance of bretylium was 1 liter/min, indicating extensive active secretion, and the urinary excretion of unchanged drug accounted for 80% of the dose (4).

A previous study in the rat (3) demonstrated that 63% of the intramuscular dose (5 mg/kg) was excreted unchanged in the urine and that 31% was excreted in the feces. Most radioactivity was excreted within 24 hr. An average of 16% of the intravenous dose (2.5 mg/kg) in four rats was excreted in the bile within 5 hr.

This investigation studied the pharmacokinetics of distribution and elimination of bretylium in the rat following an intranveous dose of ra-

Table I—Percent of Dose of Radioactivity Recovered in Urine and Feces following Intravenous Administration of [^{14}C]-Bretylium Tosylate (10 mg/kg) in Eight Rats

Sample	Dose Recovered, % Mean	CV
Urine		
0–24 hr	62.8	7.90
0–72 hr	65.6	6.43
Feces		
0–24 hr	25.7	9.73
0–48 hr	29.7	9.70
Total	95.3	5.49

diolabeled bretylium and examined the suitability of the rat model for further *in vivo* investigation of the drug.

EXPERIMENTAL

Bretylium tosylate (labeled at benzyl carbon with carbon 14, specific activity 27.3 μCi/mg) was obtained commercially[1]. The radioactive drug was diluted with the cold drug[2] to a final specific activity of 3.242 μCi/mg.

Eight male Charles River CD[3] rats, 390–460 g, were kept in individual metabolism cages during the study. One day before the drug administration, a cannula was inserted surgically into the jugular vein of each rat under light ether anesthesia to allow intravenous dosing and systemic blood sampling. The preparation of the cannulas and the surgical procedure were described previously (5). At time zero, a 10-mg/kg dose of [^{14}C]bretylium tosylate in solution was given intravenously through the jugular vein cannula after an overnight fast. The cannula was flushed with 0.5 ml of heparinized saline.

Food was withheld for 3 hr after drug administration, but water was freely available at all times. Serial blood samples (0.4 ml) were drawn at 0 (just before drug administration), 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, and 10 hr following dosing and were placed in heparinized microcentrifuge tubes; the plasma was separated immediately. The blood volume withdrawn from the rat was replaced with an equal volume of heparinized saline. Urine and feces were collected for 72 and 48 hr, respectively.

The total radioactivity in plasma was determined by counting an aliquot of the plasma or urine in a liquid scintillation counter[4]. The total radioactivity in the feces was measured after homogenization and combustion of an aliquot of the homogenate by a sample oxidizer[5].

Plasma concentration *versus* time data of each rat were fitted to a three-compartment open model (6), with elimination from the central compartment, using the NLIN procedure available in SAS[6] package (7). Marquardt's iterative method was used with a convergence criterion of 10^{-4}. Each observation was weighted by the reciprocal of its square to estimate the β-phase parameters, B and β, which were used to estimate P, π, A, and α without weighing. Derived constants and parameters then were calculated from the estimated parameters using standard equations (6).

RESULTS AND DISCUSSION

Table I summarizes the recovery of bretylium measured as radioactivity in urine and feces following intravenous administration of [^{14}C]-bretylium tosylate to eight rats. On the average, >95% of the dose was recovered in urine and feces in 72 hr. Urinary recovery was 65.6% of the dose, and the recovery in the feces was 29.7%. These values are in good agreement with the previously reported data for rats (3). An average of 88% of the dose was excreted within the first 24 hr. In humans, however, the urinary excretion of unchanged drug in 3 days accounted for 80–90% of the dose (3).

The method used in this study to fit the plasma concentration data to the exponential curve represented by the three-compartment open model was modified slightly. Because of wide and prolonged distribution of the drug, the β phase (4 hr after drug administration) consisted of only three or four data points. It was found that simultaneous determination of all six least-squares parameters may lead to a bias in the characterization

[1] New England Nuclear, Boston, Mass.
[2] American Critical Care, McGaw Park, Ill.
[3] Charles River Breeding Laboratories, Wilmington, Mass.
[4] Model 2425, Packard Instrument Co., Downers Grove, Ill.
[5] Model B306, Packard Instrument Co., Downers Grove, Ill.
[6] Statistical Analysis System, SAS Institute, Raleigh, N.C.

Table II—Pharmacokinetic Parameters of Bretylium in Rats: Plasma Concentration *versus* Time following Intravenous Administration Fitted to Three-Compartment Open Model

Parameter	Mean	SD	CV
Model Parameters and Rate Constants[a]			
P, μg/ml	4.85	0.925	19.1
π, hr^{-1}	6.77	2.48	36.6
A, μg/ml	0.742	0.645	86.9
α, hr^{-1}	1.28	0.603	47.3
B, μg/ml	0.231	0.033	14.2
β, hr^{-1}	0.131	0.024	18.2
Derived Parameters and Constants[b]			
$t_{1/2}$, hr	5.45	1.01	18.6
AUC, (μg hr)/ml	3.07	0.264	8.59
V_c, liters/kg	1.05	0.242	23.1
$V_{d,\beta}$, liters/kg	15.1	2.71	17.9
Cl_p, liters/hr/kg	1.93	0.166	8.62
Cl_r, liters/hr/kg	1.27	0.170	13.4
k_{12}, hr^{-1}	1.84	0.949	51.6
k_{21}, hr^{-1}	2.18	1.54	70.6
k_{13}, hr^{-1}	1.97	0.484	24.5
k_{31}, hr^{-1}	0.288	0.038	16.1
k_{10}, hr^{-1}	1.89	0.305	23.6
k_{ex}, hr^{-1}	1.24	0.210	16.9

[a] n = 8. [b] See text for definitions.

of the pharmacokinetic parameters of bretylium. This problem could be overcome by first fitting only the points in the β phase to the monoexponential equation and by weighing each observation by the reciprocal of its square. The influence of the α phase thus was minimized, and the best possible estimates of the β-phase parameters, B and β, were obtained. By using these two predetermined parameters, all data points, including those in the β phase, were simultaneously fitted again to the triexponential equation. This time no weighting factor was used. Through this procedure, the nonlinear least-squares estimates of P, π, A, and α were found, describing the distribution phase.

The mean values of the pharmacokinetic parameters estimated by this procedure, as well as the derived parameters calculated from the estimated parameters, are presented in Table II. The standard deviation and coefficient of variation of each parameter also are listed. A representative semilogarithmic plot of the plasma concentration *versus* time data from an individual rat is shown in Fig. 1.

In rats, bretylium is distributed rapidly in the body into two peripheral

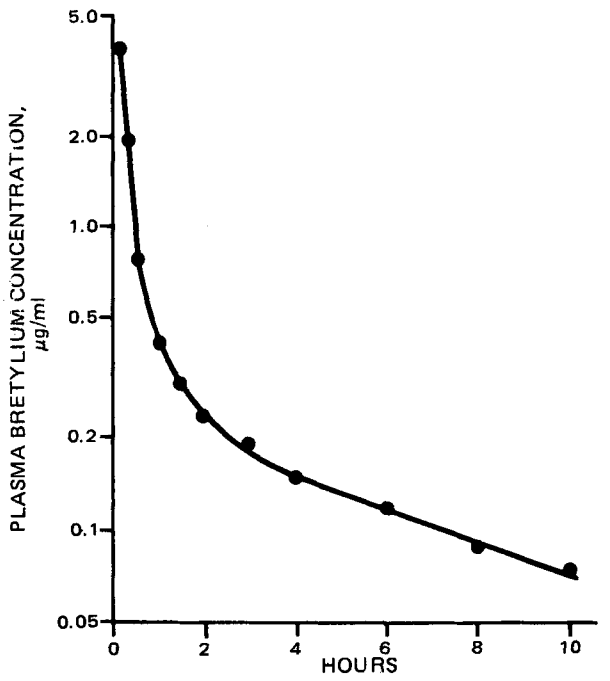


Figure 1—*Representative semilogarithmic plot of concentration of bretylium in plasma* versus *time following intravenous administration of bretylium tosylate (10 mg/kg) to a rat. The curve represents the data fitted to the three-compartment open model.*

compartments. The apparent equilibrium between the compartments was achieved within 3 hr. The apparent volume of the central compartment (V_c) was 1 liter/kg. The distribution half-life of the shallow and deep compartments (Compartments 2 and 3) were 6.14 and 32.5 min, respectively. In the postdistribution phase, the plasma levels declined with a terminal half-life of 5.45 hr (range 4.0–7.4). The plasma clearance (Cl_p) and the apparent volume of distribution ($V_{d,\beta}$) averaged 1.93 liters/hr/kg and 15 liters/kg, respectively. This unusually high apparent volume of distribution and the significant drop in plasma levels from 3–4 μg/ml at 6 min after injection to <200 ng/ml within 3 hr indicated extensive tissue uptake of bretylium in rats. Similar binding characteristics of bretylium were demonstrated in humans (8).

The intercompartmental distribution rate constants (k_{12} and k_{21}) between the central compartment and Compartment 2 in the same rat were close to each other and ranged from 0.8 to 5.1 hr^{-1} between rats. However, the transfer rate constant (k_{31}) from the third compartment to the central compartment was considerably smaller (mean 0.288 hr^{-1}) than either k_{13} (the intercompartmental rate constant in the opposite direction, 1.97 hr^{-1}) or the elimination rate constant (k_{10}, 1.89 hr^{-1}). This finding suggests that tissue binding in Compartment 3 (the deep compartment) is rate limiting in the elimination of bretylium from the body.

The excretion rate constant (k_{ex}) and the renal clearance (Cl_r) of bretylium in rats were 1.24 hr^{-1} and 1.27 liters/hr/kg, respectively. This renal clearance corresponds to 21 ml/min/kg and represents 66% of the total body clearance; it is about three times greater than the glomerular filtration rate of 6.64–8 ml/min/kg reported for normal rats (9, 10). It also indicates that active secretion is the major mechanism of urinary excretion of bretylium in rats. A similar mechanism of active secretion of bretylium in urine also was demonstrated for humans (4).

The major portion of the nonrenal excretion of bretylium probably occurs through the bile, as indicated by the recovery of ~30% of the dose in feces in 48 hr. If excretion in the bile is rapid immediately after dosing, there is a possibility of enterohepatic cycling. The extent of biliary excretion of bretylium has not been investigated in humans. However, on the basis of urinary excretion data, a much smaller quantity is expected to be eliminated *via* the biliary route.

In summary, these results indicate that bretylium is extensively distributed in rats, bound to the tissues, and excreted mainly unchanged in the urine, mostly by active secretion, similar to the findings in humans. Biliary excretion is the second major route of elimination of bretylium in rats. The data also show striking similarity in the pharmacokinetics of distribution and elimination of bretylium in humans and rats. This similarity might allow the use of the animal model for further *in vivo* investigations, including efficacy and drug–drug interaction studies in the rat.

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High-Performance Liquid Chromatographic Measurement of Cloprednol in Human Plasma

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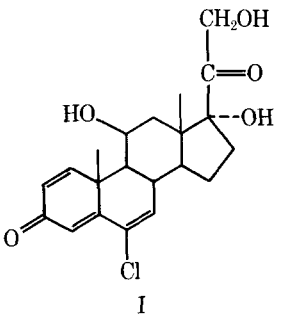
Received October 6, 1980, from the *Department of Analytical and Metabolic Chemistry, Syntex Research, Palo Alto, CA 94304.* Accepted for publication December 2, 1980.

Abstract □ A rapid and specific high-performance liquid chromatographic method for the quantitative determination of cloprednol in human plasma is described. Samples were extracted using methylene chloride–ether (40:60) and then purified further by solvent and pH partitioning techniques. Cloprednol was analyzed using normal-phase chromatography and UV detection at 254 nm. The final recovery after losses during the cleanup procedure for cloprednol from human plasma was 80.8%. The lowest concentration that could be measured with confidence was 8 ng/ml.

Keyphrases □ Cloprednol—high-performance liquid chromatographic analysis, human plasma □ High-performance liquid chromatography—analysis, cloprednol in human plasma □ Anti-inflammatory agents—cloprednol, high-performance liquid chromatographic analysis, human plasma

Cloprednol (6-chloro-11β,17α,21-trihydroxypregna-1,4,6-triene-3,20-dione, I) is a fast-acting corticoid used in the treatment of collagen and allergic diseases (1).

Recently published analytical techniques for the quantitative determination of 17-hydroxycorticoids in



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plasma include radioimmunoassays (2–5), competitive protein binding (6–8), GLC–mass spectrometry (9, 10), and high-performance liquid chromatography (HPLC) (11–15). The specificity in radioimmunoassays and competitive protein binding methods remains questionable due to the cross-reactivity by structurally similar compounds (16, 17). The GLC–mass spectrometric technique is specific but

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