In Vivo Evaluation of the Michaelis–Menten Constant For a Medium Extraction Ratio Drug: Application to Cinromide in the Rhesus Monkey

ELIZABETH A. LANE * **and RENÉ H. LEVY** ˣ

Received August 27, 1982, from the *University of Washington, Department of Pharmaceutics, Seattle, WA 98195.* Accepted for publication October 14, 1982. *Present address: Laboratory of Chemical Pharmacology, National Heart, Lung and Blood Institute, Bethesda, MD 20205.

Abstract □ The dose-dependent nonlinearity of the clearance of cinromide, a medium extraction ratio drug, has been established in two monkeys. Special problems encountered in evaluation of nonlinearity of such drugs were resolved by the experimental design: cinromide was infused to steady state *via* the portal vein. A linearized form of the Michaelis–Menten equation was used to determine v_{max} and K_m. In addition, cinromide was administered to one of the monkeys *via* a femoral vein to verify the overestimation of K_m by administration at a peripheral venous site.

Keyphrases □ Cinromide—*in vivo* evaluation of Michaelis–Menten constant, medium extraction ratio drug, application to the rhesus monkey □ Michaelis–Menten constant—*in vivo* evaluation, medium extraction ratio drug, application to cinromide in the rhesus monkey □ Medium extraction ratio drug—*in vivo* evaluation of Michaelis-Menten constant, application to cinromide in the rhesus monkey

The accurate determination of *in vivo* Michaelis–Menten parameters is dependent on a number of considerations. For any drug it is a function of the compartmental model to which the data are fitted (1). For drugs with medium or high extraction ratios, the relationship between site of administration and site of elimination is important (2). For example, it has been pointed out that an overestimation of the Michaelis–Menten constant (K_m) is theoretically expected for drugs with high hepatic extraction ratios administered by a peripheral venous site (3). Oral administration presents the advantage of an estimation of intrinsic clearance, but it allows no control over the rate of absorption. Furthermore, it requires an independent determination of fraction absorbed. A review of the literature indicates that the problems associated with the *in vivo* determination of Michaelis–Menten parameters for a medium or high extraction ratio drug have not yet been resolved.

An investigation of the pharmacokinetic characteristics of cinromide in the rhesus monkey established that this drug has a medium extraction ratio (4). Administration of this drug by intravenous (femoral vein) infusion at three zero-order rates for 5 hr suggested the presence of nonlinearity. At the highest infusion rate (~100 mg/hr) steady state was achieved within 5 hr (half-life = 0.92 ± 0.23 hr) in only two of the five monkeys, and the postinfusion half-life of cinromide was significantly longer after the highest infusion rate (4). The object of the present study was to establish the nonlinearity of cinromide and to attempt an *in vivo* determination of Michaelis–Menten parameters. The experimental design was based on the following considerations: (*a*) the problems of extraction ratio, site of administration, and fraction absorbed were resolved by placement of a catheter in the hepatoportal vein; (*b*) the bias of compartmental models was avoided, and the rate of presentation of the drug to the liver was controlled by selection of a steady-state approach.

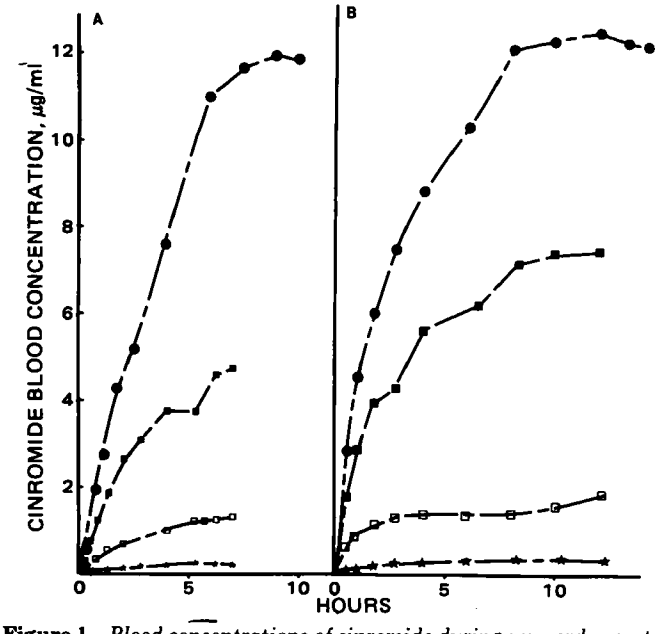

Figure 1—*Blood concentrations of cinromide during zero-order portal vein infusions. Key: (A) Monkey 306 dosed at (★) 11.5, (□) 34.0, (■) 70.0, and (●) 93.3 mg/hr; (B) monkey 307 dosed at (★) 12.3, (□) 30.6, (■) 69.9, and (●) 85.6 mg/hr.*

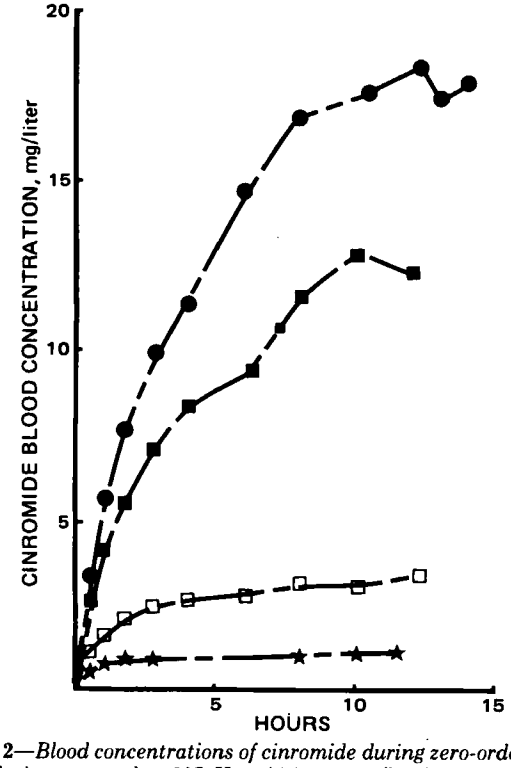

Figure 2—*Blood concentrations of cinromide during zero-order femoral vein infusions to monkey 307. Key: (★) 15.2 mg/hr; (□) 32.8 mg/hr; (■) 76.6 mg/hr; (●) 90.2 mg/hr.*

0022-3549/83/1100-1352$01.00/0

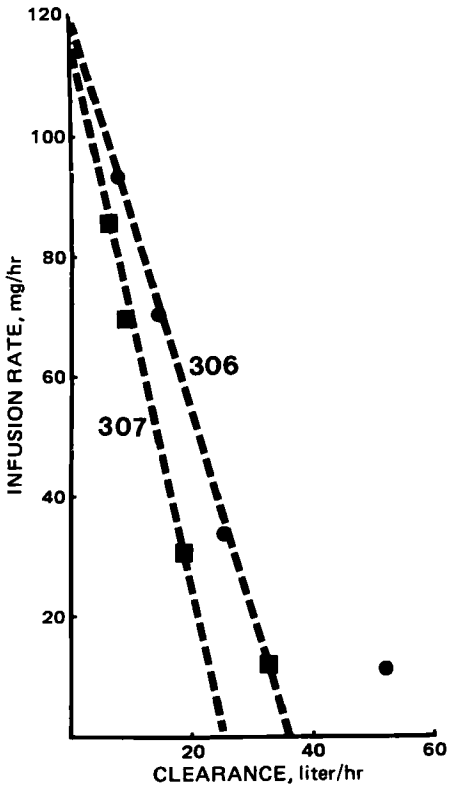

Figure 3—*Graphic evaluation of Michaelis–Menten constants for cinromide administered* via the *portal vein.*

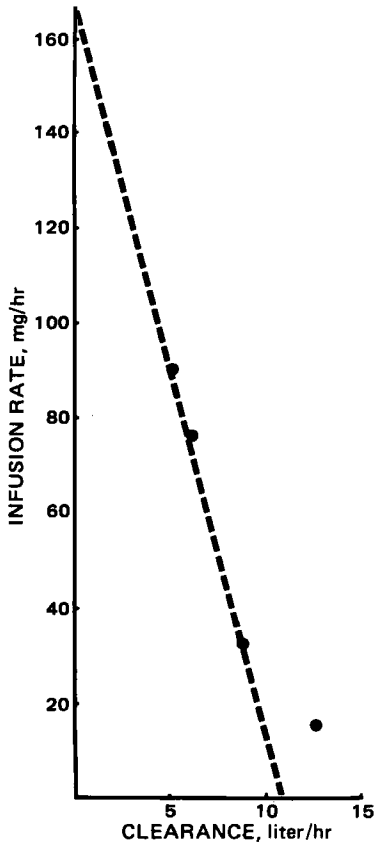

Figure 4—*Graphic evaluation of Michaelis–Menten constants for cinromide administered* via *a femoral vein (nonportal route) to monkey 307.*

EXPERIMENTAL

Two male rhesus monkeys [306 (5.7 kg) and 307 (7.9 kg)] were conditioned in primate chairs prior to the beginning of this study. Two catheters were surgically implanted in each monkey; one in the portal vein and one in the jugular vein. Patency of catheters was assured by infusion of saline (1 ml/hr). The monkeys were maintained on fresh fruit and monkey food.

Cinromide[1] was administered in 60% polyethylene glycol 400 solution by constant-rate infusion at 4 ml/hr *via* the portal vein. Four different concentrations of cinromide were infused to give dosage rates of ~10-, 30-, 70-, and 90-mg/hr. Infusions were aimed at achieving steady state and were maintained for at least 7 hr for the 10-, 30-, and 70-mg/hr doses, and at least 10 hr for the 90-mg/hr dose rate. Eleven blood samples were collected *via* the jugular vein catheter during each infusion. Blood samples were analyzed by high-performance liquid chromatography (HPLC) as previously described (4). To verify the effect of administration route on determination of the Michaelis–Menten constant, cinromide was administered to one monkey (307) *via* a femoral vein at the same four infusion rates.

RESULTS AND DISCUSSION

The concentrations achieved by four portal vein infusions to two monkeys are shown in Fig. 1. In every case, steady state appears to have been achieved. In both monkeys, the increase in steady-state concentration was more than proportional to the increase in infusion rate, illustrating the decrease in intrinsic clearance with increase in dose rate. A sevenfold increase in dosing rate corresponded to a 30-fold increase in steady-state concentration. The concentrations achieved by infusion of cinromide at the same rates *via* a femoral vein into monkey 307 are shown in Fig. 2. A sixfold increase in dosing rate corresponded to a 15-fold increase in steady-state concentration. Thus, the nonlinearity of cinromide was less pronounced when administered *via* the femoral vein than when administered *via* the portal vein. These results demonstrate the buffering effect of blood flow on systemic clearance.

Determination of parameters from the data of Figs. 1 and 2 was based on the following theoretical considerations. For a low extraction ratio drug, the dose-dependent clearance (CL) may be expressed as:

$$CL = \frac{R_0}{C^*} = \sum_{i=1}^{n} \frac{v_{\max_i}}{K_{m_i} + C^*} \qquad \text{(Eq. 1)}$$

where C^* is the steady-state total drug concentration, R_0 is the drug infusion rate, $v_{\max_i}$ is the maximum velocity of the reaction for the ith enzyme, and K_{m_i} is the Michaelis constant for the ith enzyme, uncorrected for blood protein binding. It is also assumed that the free fraction in blood is constant.

Most commonly a single $v_{\max}$ and K_m are evaluated as if a single enzyme is responsible for the elimination of the drug. If the drug is eliminated by more than one enzyme having similar values of K_m, they cannot be distinguished by this approach (5). A useful linear transformation of this relationship (Eq. 1) has been applied to phenytoin (6) and other drugs:

$$R_0 = v_{\max_1} - K_{m_1} \frac{R_0}{C^*} \qquad \text{(Eq. 2)}$$

For a medium to high extraction ratio drug eliminated only in the liver, it is appropriate to apply the above equations when drug is administered by a portal route. When drug is administered *via* a peripheral vein and the well-stirred model of the liver is assumed, the clearance is related to the Michaelis–Menten parameters by:

$$CL = \frac{R_0}{C^*} = \frac{Q_H \sum_{i=1}^{n} \frac{v_{\max_i}}{K_{m_i} + C^*}}{Q_H + \sum_{i=1}^{n} \frac{v_{\max_i}}{K_{m_i} + C^*}} \qquad \text{(Eq. 3)}$$

where Q_H is the liver blood flow. If drug is eliminated by a single enzyme, Eq. 3 may be rearranged to give:

$$R_0 = v_{\max_1} - \left(K_{m_1} + \frac{v_{\max_1}}{Q_H}\right) \frac{R_0}{C^*} \qquad \text{(Eq. 4)}$$

Comparison of Eqs. 2 and 4 demonstrates that the evaluation of K_m is dependent on administration route, while the determination of $v_{\max}$ is independent. When drug is administered by a peripheral venous site, K_m is overestimated by the value of $v_{\max}/Q_H$, as suggested by other authors (3).

The data from the portal vein infusions were plotted according to the

[1] Supplied by Burroughs Wellcome Co., Research Triangle Park, N.C.

linearized form of the Michaelis–Menten equation (Eq. 2). These data appeared to be nonlinear for both monkeys (Fig. 3). This nonlinearity is of the type observed in cases involving two saturable pathways with different values of K_m (5). Nonlinear least-squares fitting of data to acquire the best estimates of parameters requires at least one more datum point than the number of parameters to be estimated. In this case, there are only four data points to estimate four parameters; therefore, no estimates of the parameters for the pathway having the lower K_m were attempted. The v_{max} and K_m for the higher capacity pathway could be approximated by fitting the data from the three higher infusion rates to a straight line. The values of v_{max} and K_m were 120 mg/hr and 3.35 mg/liter for monkey 306 and 114 mg/hr and 4.43 mg/liter for monkey 307. A plot of data from the femoral vein infusions according to the linearized form of the Michaelis–Menten equation shows nonlinearity similar to that of the portal vein data (Fig. 4). The estimated values of v_{max} and K_m are 170 mg/hr and 15.6 mg/liter, respectively.

These values of v_{max} and K_m for femoral vein administration may be compared with those obtained for the same monkey dosed *via* the portal vein. The observed difference in v_{max} was not expected. It may be a result of the metabolism of cinromide by more than one enzyme, as indicated by the nonlinearity of the data from all four infusions. Also, the clearance values obtained from the three higher infusion rates into the femoral vein span a narrow range (5.1 to 9.3 liter/hr), which can result in uncertainty in the intercept. According to theory (Eqs. 2 and 4) the value of K_m obtained by femoral vein administration (15.6 mg/liter) should exceed that obtained by portal vein administration (4.4 mg/liter) by the ratio v_{max}/Q_H. This ratio can be roughly estimated if the hepatic blood flow in monkey 307 is calculated using the portal and femoral infusion data and the equation of Wilkinson and Shand (7). Although this equation was developed for a dose-independent intrinsic clearance, it can be applied in the dose-dependent case, provided that the steady-state concentration of drug at the enzyme is equal for the two routes of administration. This is accomplished by the administration of drug by the femoral and portal routes at equal rates. The average value obtained from the four pairs of infusions was 21.0 ± 2.8 liter/hr. Division of the two values of v_{max} from monkey 307 by this value of blood flow yields 5.4 and 8.4 mg/liter, within 50–70% of the difference between the two values of K_m (11.2 mg/liter). This calculation is compatible with the theoretical prediction. Equality between these two estimates of the difference in K_m arising from route of administration is not expected, since the determination of K_m, v_{max}, and Q_H involve error. In fact, the degree of compatibility suggests that the well-stirred model of the liver can be used to explain the effects of administration route on the disposition of cinromide.

The dose-dependent nonlinearity of cinromide, a medium extraction ratio drug, has been demonstrated. Catheterization of the portal vein for chronic drug administration provided a means of evaluating the intrinsic clearance of cinromide independently of hepatic blood flow and, thereby, a means of examining the dose dependence of cinromide. Comparison of values obtained for the whole body Michaelis–Menten constant for the peripheral and portal routes of administration confirmed the theoretically predicted effects of flow limitation on clearance.

REFERENCES

(1) A. J. Sedman and J. G. Wagner, *J. Pharmacokinet. Biopharm.*, **2**, 161 (1974).

2 J. L. Rheingold, R. E. Lindstrom, and P. K. Wilkinson, *J. Pharmacokinet. Biopharm.*, **9**, 261 (1981).

(3) K. S. Pang, M. Rowland, and T. N. Tozer, *Drug. Metab. Dispos.*, **6**, 197 (1978).

(4) E. A. Lane and R. H. Levy, *J. Pharm. Sci.*, **72**, 493 (1983).

(5) A. J. Sedman and J. G. Wagner, *J. Pharmacokinet. Biopharm.*, **2**, 149 (1974).

(6) T. M. Ludden, D. W. Hawkins, J. P. Allen, and F. Hoffman, *Lancet*, **i**, 307 (1976).

(7) G. R. Wilkinson and D. G. Shand, *Clin. Pharmacol. Ther.*, **18**, 377 (1975).

ACKNOWLEDGMENTS

This study was supported in part by NINCDS Research Contract N01-NS-1-2282. E. A. Lane was supported by National Institutes of Health National Research Service Awards GM-07348 and GM-07750 from the National Institute of General Medical Sciences.

The authors acknowledge the work of Ms. Sally Nickelson in animal surgery and maintenance.

Tick Repellents II: *N*-Substituted Azacyclopentanones and Azacyclopentenones

W. A. SKINNER ˣ, U. ROSENTRETER*, and T. ELWARD

Received May 10, 1982, from *Life Sciences Division, SRI International, Menlo Park, CA 94025.* Accepted for publication September 3, 1982. *Present address: Waldstrasse 11, 5600 Wuppertal 1, West Germany.

Abstract □ Several *N*-substituted azacyclopentanones and azacyclopentenones were synthesized and evaluated as repellents for the brown dog tick *Rhipicephalus sanguineus*. Several of these compounds were more effective in our test system than were the standard repellents, *N,N*-diethyl-*m*-toluamide and butopyranoxyl.

Keyphrases □ *N*-Substituted azacyclopentanones—synthesis, structure–activity relationships, evaluation as tick repellents □ *N*-Substituted azacyclopentenones—synthesis, structure–activity relationships, evaluation as tick repellents □ Tick repellents—potential, *N*-substituted azacyclopentanones and azacyclopentenones, synthesis

Tick-borne diseases still represent a problem and the need for a safe means of controlling ticks exists. Compounds useful for repelling mosquitoes are not necessarily those that are the most effective for ticks. Screening of repellents for ticks required the development of a rapid, simple assay system, which has been completed in our laboratories. This method, which was described previously (1), involved the use of a plastic vial containing the ticks with a filter paper cap impregnated with the test substance. The common behavior of ticks to travel upward is used to compare the control behavior with that in a vial treated at the top with repellent.

Most repellents reported for ticks have been amides or esters (2). We decided to explore azacyclopentanones and azacyclopentenones as a group of cyclic amides for their ability to repel the brown dog tick, *Rhipicephalus sanguineus*.

Compounds reported in Tables I–III were prepared by the methods described in *Experimental* for selected compounds.

EXPERIMENTAL[1]

Preparation of 1-Decyl-azacyclopentane-2-one (Ib)—A mixture of 22.1 g of 1-bromodecane (0.1 mole), 8.5 g (0.1 mole) of 2-pyrrolidone,

[1] Elemental analyses were performed by the Microanalytical Laboratory, Department of Chemistry, Stanford University, Stanford, Calif.

0022-3549/83/1100-1354$01.00/0