Comparative Disposition Kinetics of Two Diastereomeric Pairs of Cinchona Alkaloids in the Dog

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ABSTRACT □ The comparative disposition kinetics of quinidine, quinine, cinchonine, and cinchonidine were investigated in five male, mongrel dogs after intravenous bolus injections of a 9.2-mmol/kg dose of each alkaloid base. Blood and plasma specimens were obtained at various times up to 6 h postdose and assayed for quinidine and quinine with a TLC–fluorometric procedure and for cinchonine and cinchonidine by HPLC. The plasma alkaloid concentration–time data were analyzed by weighted, nonlinear least-squares regression analysis to obtain the central compartment volume (V_c), disposition rate constants (α and β), and corresponding half-life values ($t_{1/2}$). Total body clearance (CL) and apparent volume of distribution (Vd) were estimated by nonparametric analysis. In this study, the highest plasma alkaloid concentrations were reached with quinidine and the lowest concentrations with the quinidine congener, cinchonine. The other congeneric pair, quinine and cinchonidine, exhibited plasma alkaloid concentrations that were comparable and intermediate to those of quinidine and cinchonine. With cinchonine and cinchonidine, the plasma and blood concentration–time curves were virtually superimposable. However, with quinidine and quinine, the plasma alkaloid concentrations of these diastereomers were approximately twice the corresponding blood concentrations. The total body clearance rate of quinidine was significantly slower than quinine and cinchonine clearance. No difference in clearance was observed between cinchonine and cinchonidine. The β and $t_{1/2\beta}$ for quinidine were significantly smaller and larger, respectively, than the corresponding values obtained with the other alkaloids. No significant differences in α or V_c and Vd were found between and within the two diastereomeric pairs of alkaloids. The differences in disposition kinetics observed in this study were attributable to an interaction of stereochemical and 6′-methoxy group substitution effects.

Keyphrases □ Quinine—comparative disposition kinetics in the dog, effects of stereochemistry and 6′-methoxy group substitution □ Quinidine—comparative disposition kinetics in the dog, effects of stereochemistry and 6′-methoxy group substitution □ Cinchonine—comparative disposition kinetics in the dog, effects of stereochemistry and 6′-methoxy group substitution □ Cinchonidine—comparative disposition kinetics in the dog, effects of stereochemistry and 6′-methoxy group substitution

Optical isomers as well as congeners of various drugs have been shown to exhibit different pharmacokinetic and/or pharmacological properties. Stereoselective behavior has been demonstrated for a number of drugs including disopyramide (1), fenfluramine (2), propoxyphene (3), propranolol (4, 5), quinidine/quinine (6–8), thyroxine (9), and warfarin (10). For optical isomers, the asymmetry in their molecular structure (and the resultant effects) clearly account for the differences in properties observed between isomers. Drug congeners, on the other hand, possess different properties because of the presence of different group substituents on the parent drug molecule. The various tetracycline, penicillin, and phenothiazine derivatives are well-known examples of drug congeners possessing different pharmacokinetic and pharmacological activities.

In this investigation, quinidine, quinine, cinchonine, and cinchonidine, two diastereomeric pairs of cinchona alkaloids, were used to evaluate the effects of stereochemistry and group substitution on drug disposition kinetics. Quinidine, quinine, cinchonine, and cinchonidine are the four principal naturally occurring alkaloids isolated from the bark of trees and shrubs of various species of the *Cinchona* and *Remijia* genera. Stereochemically, quinidine, quinine, cinchonine, and cinchonidine fall into two configurational and polarimetric groups. Cinchonine and its 6′-methoxy congener, quinidine, are dextrorotatory and have the same configuration at C-9. The other congeneric pair, cinchonidine and quinine, are levorotatory and have the opposite configuration at C-9 when compared with the dextrorotatory isomers.

The diastereomeric pairs, quinidine/quinine and cinchonine/cinchonidine, do not form racemates and are substances with distinct physicochemical properties (11) and therapeutic uses (7, 8). The purpose of this paper is to report our results on the disposition kinetics of quinidine, quinine, cinchonine, and cinchonidine in dogs.

EXPERIMENTAL

Materials—Quinine, cinchonine, and cinchonidine bases were obtained commercially[1] and used without further purification. Chromatographic analysis of these substances revealed the presence of trace amounts (<7%) of materials that were most likely the corresponding dihydro derivatives of these alkaloids. Quinidine, free of dihydroquinidine, was prepared from quinidine sulfate USP[2] according to the method of Thron and Dirscherl (12). The separated and purified dihydroquinidine was used as the internal standard for the assay of cinchonine and cinchonidine described below.

Drug solutions suitable for parenteral administration were prepared by dissolving the alkaloid base in dilute sulfuric acid (pH 4) and aseptically filtering each solution through a 0.45-μm membrane filter[3]. All other reagents and chemicals were analytical reagent grade.

Intravenous Drug Administration and Blood Sampling—Five male mongrel dogs (15–22 kg) were used in a crossover study with a 7–10-d washout period between drug treatments. During the study, the animals were housed in separate cages with free access to water and they were fed commercial dog food[4]. Food was withheld during the actual experiments.

After placing the dog in a sling support, vein infusion sets[5] with 19-gauge hypodermic needles were inserted into a leg vein of a fore- and hindlimb to facilitate intravenous drug administration and sampling of blood for drug analysis. Patency of the blood catheter was maintained by intermittent flushing with heparinized normal saline (100 IU/mL).

Prior to drug administration, 5 mL of blood was removed as a control sample and for the determination of the hematocrit. A 9.2-mmol/kg dose of alkaloid base was given by rapid intravenous injection *via* the infusion set. Each dose was followed by 3–5 mL of normal saline to ensure complete delivery of the intended dose.

Following drug administration, 3-mL blood samples for drug analysis were obtained at 5, 10, 15, 30, 60, 90, 120, 180, 240, 300, and 360 min, using the second infusion set, and placed in 10-mL heparinized tubes[6]. Approximately 1 mL of blood was reserved for the determination of drug concentrations in whole blood. The remaining sample was centrifuged to obtain the plasma fraction. The blood and plasma samples were stored at −10°C until analyzed.

Analysis of Alkaloids in Blood and Plasma—The concentrations of quinidine and quinine in blood and plasma were determined with the TLC-fluorometric method previously described for quinidine (13).

The samples containing cinchonine and cinchonidine were extracted with benzene in the same manner as quinidine and quinine and assayed using an adaptation of the liquid chromatographic procedure for quinidine described by Drayer and associates (14). After evaporating the benzene extract to

[1] Fluka AG, Buchs SG, Switzerland.
[2] New York Quinine and Chemical Works, New York, N.Y.
[3] Millipore; Gelman Instrument Co., Ann Arbor, Mich.
[4] Wayne Pro-Mix; Applied Mills, Inc., Chicago, Ill.
[5] Travenol Laboratories, Inc., Deerfield, Ill.
[6] Becton, Dickinson and Co., Rutherford, N.J.

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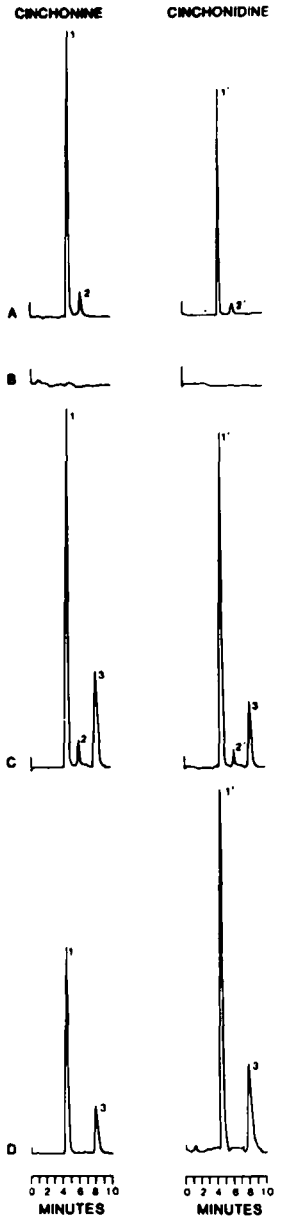


Figure 1—*Chromatograms for cinchonine and cinchonidine obtained under the following test conditions: (A) parenteral drug solutions; (B) plasma blank obtained from an untreated dog; (C) plasma blank spiked with the drug and internal standard; (D) plasma sample obtained from a treated dog, with added internal standard. Peaks 1 and 1′ are cinchonine and cinchonidine, respectively; peaks 2 and 2′ are the corresponding unknown impurities found in the respective drug samples. Peak 3 is the internal standard, dihydroquinidine.*

dryness under a stream of nitrogen, the residue was reconstituted with 25 μL of mobile phase containing 40 ng of dihydroquinidine as the internal standard. An aliquot of this solution was injected into a liquid chromatograph[7] equipped with a loop injector, a C_{18} reverse-phase μ-Bondapak column[8] (3.9 mm × 30 cm; 10-μm particles), and a variable-wavelength fluorescence detector[9]. The mobile phase consisted of 15% (v/v) acetonitrile in 2.5% acetic acid, which was delivered at a rate of 2 mL/min (~2000 psi), and monitored with an excitation wavelength of 315 nm using a 418-nm cutoff filter.

Figure 1 shows the chromatographic characteristics of cinchonine and cinchonidine, as well as the internal standard, obtained under various test conditions with the assay procedure used. As shown in this figure, excellent separation and resolution of the two cinchona alkaloid peaks were achieved. Cinchonine and cinchonidine both eluted with a retention time of 4.3 min. The

[7] Model 2/2; Perkin-Elmer, Norwalk, Conn.
[8] Waters Associates, Millford, Mass.
[9] Schoeffel Model FS 970, Kratos, Inc., Westwood, N.J.

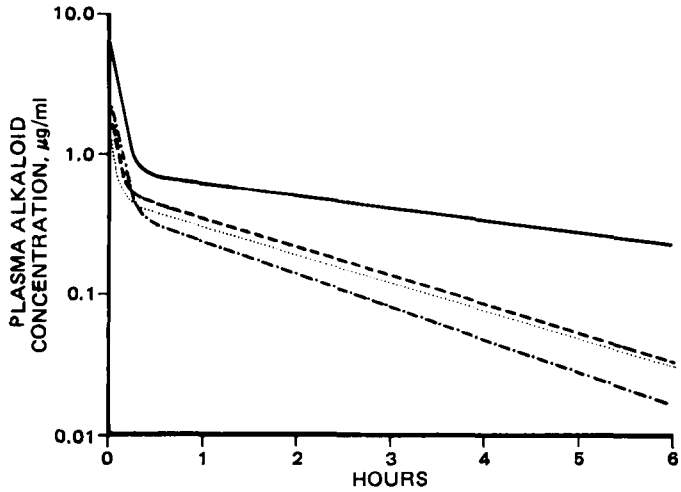


Figure 2—*Semilogarithmic graphs of the plasma alkaloid concentration–time curves for quinidine (—), quinine (· · · ·), cinchonine (- - - -), and cinchonidine (- · - · -) obtained after a 9.2 mmol/kg iv bolus dose of each alkaloid base in dog E. Each graph is the best-fit curve for each alkaloid obtained by nonlinear least-squares regression analysis of the experimental data.*

internal standard, dihydroquinidine, eluted in 8.1 min. The unidentified impurities in the drug samples eluted with an intermediate retention time of 6.0 min and, therefore, did not interfere with the assay for either drug.

Standard samples containing known concentrations of cinchonine and cinchonidine were prepared using blood obtained from untreated animals. They were processed concurrently with the plasma and blood samples to be analyzed and were used to prepare standard calibration curves using the peak height ratio of cinchonine or cinchonidine to internal standard. The unknown drug concentrations in plasma or blood were determined from the best-fit equation of the calibration curves obtained by linear regression analysis after blank correction. In all cases, the correlation coefficients were ≥0.995.

In the concentration range of 0.1–10 μg/mL for cinchonine and cinchonidine in blood and plasma, the relative standard deviations for the respective fluids were ≤7% and ≤5% for cinchonine and ≤8% and ≤5% for cinchonidine.

Data Analysis—The plasma drug concentration–time curves obtained with the bolus injections were fitted to a biexponential equation by weighted ($1/C^2$), nonlinear least-squares regression analysis using the NONLIN program (15):

$$C = Ae^{-\alpha t} + Be^{-\beta t} \qquad \text{(Eq. 1)}$$

Initial estimates of A, B, α, and β were obtained with the program CSTRIP (16); C is the plasma concentration of alkaloid at any time t, α, and β are first-order rate constants for the fast and slow disposition processes, respectively, and A and B are the corresponding ordinate intercepts for each exponential term.

Central compartment volumes (V_c) were estimated by:

$$V_c = \frac{\text{Dose}}{A + B} \qquad \text{(Eq. 2)}$$

The total body clearance rate (CL) was calculated for each alkaloid using:

$$CL = \frac{Dose}{\int_0^\infty C\,dt} \qquad \text{(Eq. 3)}$$

where $\int_0^\infty C\,dt$ is the total area under the plasma drug concentration–time curve, which was estimated by the trapezoidal rule to obtain the area up to the last measured concentration (C^*) and computing the area beyond C^* by dividing C^* by the terminal rate constant β.

The apparent volume of distribution (Vd) was determined by the relationship:

$$Vd = \frac{\text{Dose}}{\int_0^\infty C\,dt \cdot \beta} \qquad \text{(Eq. 4)}$$

Half-life values ($t_{1/2}$) were calculated as:

$$t_{1/2} = 0.693/\alpha \text{ or } \beta \qquad \text{(Eq. 5)}$$

Table I—Values for the Disposition Constants Obtained after a 9.2-mmol/kg iv Bolus Injection[a]

Parameter	Quinidine	Quinine	Cinchonine	Cinchonidine
A, mg/L	2.51 ± 0.56	2.95 ± 1.53	0.61 ± 0.20	0.66 ± 0.20
α, h^{-1}	14.2 ± 4.4	14.3 ± 7.1	14.4 ± 6.6	20.6 ± 3.4
$t_{1/2\alpha}$, h	0.09 ± 0.05	0.12 ± 0.04	0.08 ± 0.02	0.04 ± 0.01
B, mg/L	1.95 ± 0.99	1.33 ± 0.46	0.81 ± 0.21	1.05 ± 0.27
β, h^{-1}	0.179 ± 0.014	0.311 ± 0.059	0.500 ± 0.021	0.424 ± 0.024
$t_{1/2\beta}$, h	4.91 ± 0.95	2.24 ± 0.28	1.40 ± 0.06	1.86 ± 0.20
V_c, L/kg	1.10 ± 0.30	2.01 ± 0.85	2.22 ± 0.43	2.07 ± 0.51
Vd, L/kg	2.78 ± 0.69	3.93 ± 1.38	3.72 ± 0.78	3.04 ± 0.66
CL, L/h/kg	0.499 ± 0.111	1.435 ± 0.405	2.140 ± 0.436	1.333 ± 0.345

[a] Mean ± SEM; n = 5.

The two-way ANOVA test method was used to evaluate the significance of the differences observed between the four alkaloids in this study. This statistical procedure provided a means to evaluate the effects of stereochemistry, 6′-methoxy group substitution, and an interaction of both factors on drug disposition kinetics. A 0.05 significance level was used; the data are reported as the mean ±1 SE.

RESULTS AND DISCUSSION

A comparison of the plasma concentrations of quinidine, quinine, cinchonine, and cinchonidine obtained after intravenous administration of equimolar bolus doses is shown in Fig. 2 for a representative dog. In all dogs, the plasma concentration–time curves for each alkaloid declined biexponentially according to Eq. 1. A rapid distribution phase followed by a slower, terminal disposition phase was observed with all four alkaloids following the bolus injections. It was also noted in all animals studied that the highest plasma concentrations of alkaloid observed were achieved with quinidine. On the other hand, of the four alkaloids studied, the lowest plasma concentrations were always seen with the quinidine congener, cinchonine. The two remaining congeneric pair of alkaloids, quinine and cinchonidine, exhibited plasma concentrations that were comparable and intermediate between those of quinidine and cinchonine.

The rapid and brief distribution phase seen in this study in dogs with the four alkaloids ($t_{1/2\alpha} \simeq 5$ min) has also been observed for quinidine in rabbits (17, 18) and monkeys (19). Moreover, a similar distribution characteristic has been found with quinidine in humans (20–22).

Analysis of the plasma concentration-time data for each drug indicated that the relative differences in plasma alkaloid concentrations seen in this study were attributable to differences in total body clearance rates (CL) for the four alkaloids (Table I). Total body quinidine clearance (0.499 ± 0.111 L/h/kg) was significantly slower than the observed clearance rates of quinine and cinchonine of 1.435 ± 0.405 and 2.140 ± 0.436 L/h/kg, respectively. Interestingly, while there was a difference in CL between quinidine and quinine, it was observed that the cinchonine/cinchonidine diastereomers had similar clearance rates.

The terminal first-order elimination rate constant obtained for quinidine of 0.179 ± 0.014 h^{-1} was significantly smaller than the observed values for the remaining alkaloids ($p < 0.01$), which included the l-isomer of the diastereomeric pair, quinine (Table I). This finding indicated that the fractional removal rate of quinidine was the slowest of the four alkaloids studied. Further analysis of the results revealed that, while there was no difference in β between the cinchonine and cinchonidine diastereomers (0.500 ± 0.021 and 0.424 ± 0.024 h^{-1}), the terminal rate constants for this diastereomeric pair were significantly greater than the rate constant of quinine (0.311 ± 0.059 h^{-1}).

With the corresponding elimination half-life determinations (Table I), the cinchonine/cinchonidine pair were eliminated with similar $t_{1/2\beta}$ values of 1.40 ± 0.06 and 1.86 ± 0.20 h, respectively. On the other hand, the elimination half-lives of the quinidine/quinine diastereomers of 4.91 ± 0.95 and 2.24 ± 0.28 h, respectively, were significantly different ($p < 0.01$).

The results of the volume measurements are also summarized in Table I. In this study, no significant differences were observed between or within the two diastereomeric pairs of alkaloids in their central compartment volumes or overall apparent volumes of distribution.

In a recent investigation, Clohisy and Gibson (6) also observed that the total body clearance rate of quinidine was slower than the clearance rate of quinine in dogs. They found that the clearance of quinidine of 0.215 L/h/kg was about 44 or 56% slower than the quinine clearance rate of 0.487 L/h/kg. Although these clearance rates are less than the respective values observed in the present study, the results of the two studies are in agreement in that we observed a clearance rate of quinidine that was 63% slower than the corresponding rate of quinine clearance.

A possible explanation for these discrepancies could be the different dosages

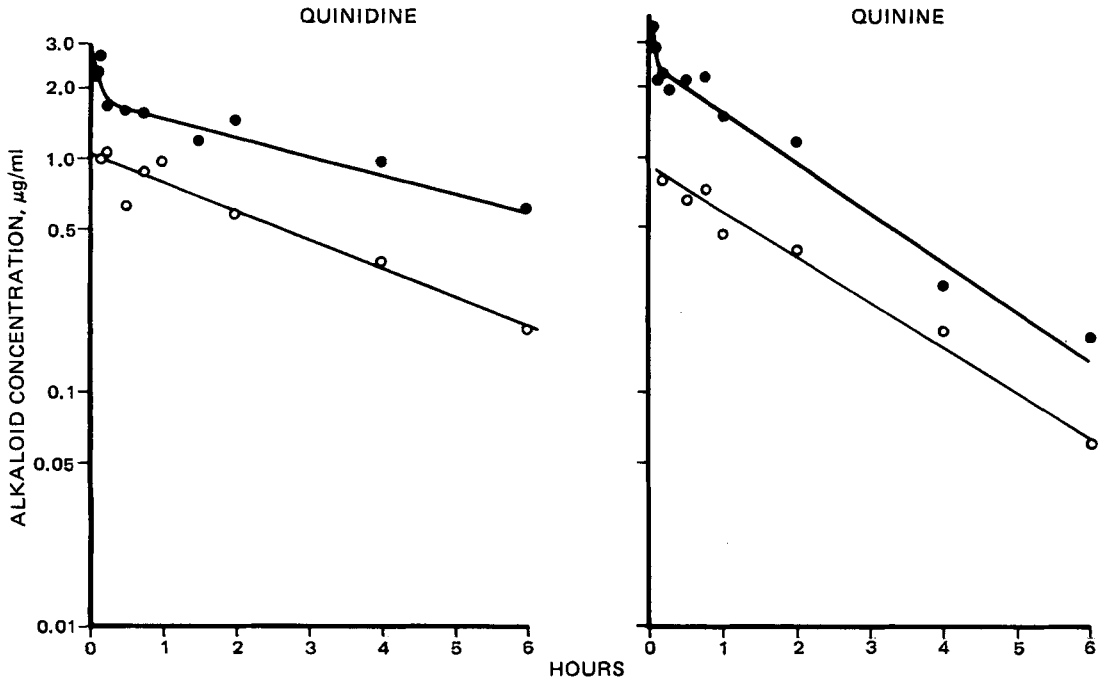


Figure 3—*Semilogarithmic graphs comparing the blood and plasma drug concentration–time curves of quinidine and quinine obtained after a 9.2 mmol/kg iv bolus dose of alkaloid base to dog C. The solid lines are the best-fit curves.*

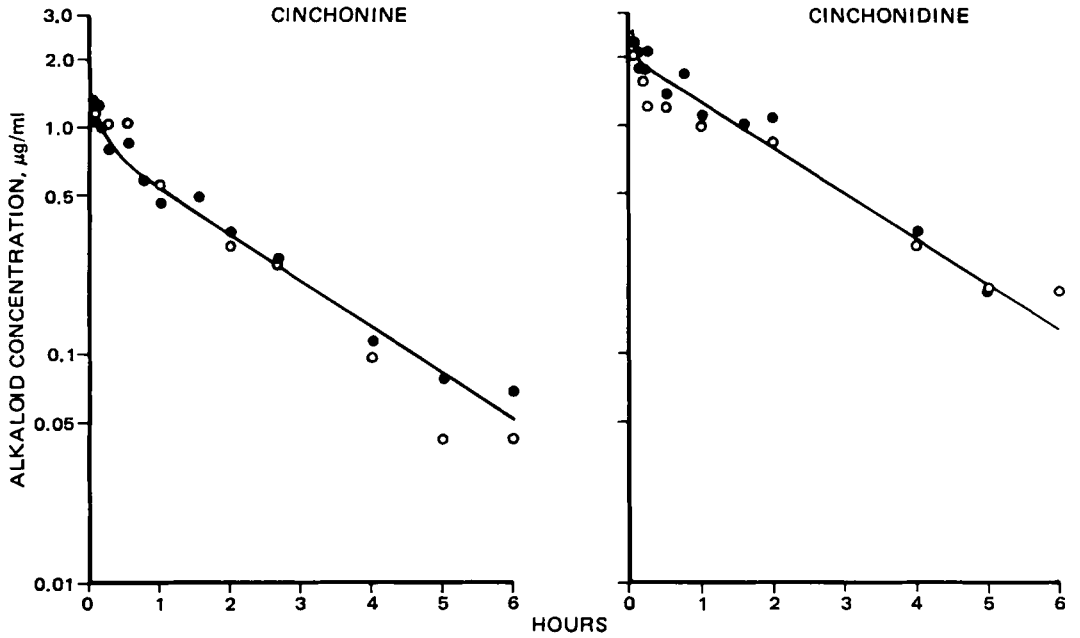
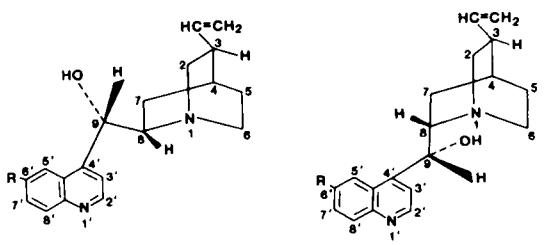


Figure 4—*Semilogarithmic graphs comparing the blood and plasma drug concentration-time curves of cinchonine and cinchonidine obtained after a 9.2 mmol/kg iv bolus dose of alkaloid base to dog C. The solid lines are the best-fit plasma curves.*

that were used in the two studies. In 1948, the findings of Taggart *et al.* (23) suggested that the disposition kinetics of all of the four alkaloids examined in this study were dose dependent. For quinidine, quinine, cinchonine, and cinchonidine, they reported that there was a disproportionate relationship between dose and mean plasma drug concentration attained. More recently, Bolme and Otto (24) also suggested that the pharmacokinetics of quinidine in humans is dose dependent. Thus, dose-dependent pharmacokinetics for quinidine and quinine in the dog is a distinct possibility. In this investigation, quinidine and quinine doses of 3 mg of alkaloid base/kg of body weight were given. Clohisy and Gibson (6) administered doses of the two alkaloids of 6.5 mg/kg. In support of the present findings, after the administration of a quinidine dose of 2.5 mg/kg, the estimated rate of quinidine clearance observed in dogs by Jellett *et al.* (25) was 0.435 L/h/kg (compared with 0.499 ± 0.111 L/h/kg in this study).

The observed difference in clearance between quinidine and quinine suggests that the disposition characteristics of these two diastereomers are stereoselective, as recently reported by Clohisy and Gibson (6). With the similarities in the apparent distribution volumes of these two alkaloids that were observed, these findings suggest that the stereoselective disposition behavior of quinidine and quinine is most likely associated with mechanisms for the metabolism and/or excretion of these drugs rather than a distribution process. In support of this conclusion, it was demonstrated in this study that the other diastereomers, cinchonine and cinchonidine, exhibited similar total body clearance rates. The two diastereomeric pair of alkaloids differ only in the 6′-position of the quinoline ring system, quinidine and quinine having a methoxy group in this position. Drayer *et al.* (26) have shown that a minor metabolite of quinidine in humans is the *o*-demethylated derivative of the drug. Therefore, the present data suggest that the 6′-position might be a site on the drug molecule that is involved in the stereoselective clearance of the quinidine/quinine diastereomers.



	R	
CINCHONINE	-H	CINCHONIDINE
QUINIDINE	$-OCH_3$	QUININE

With the observation that quinidine, quinine, cinchonine, and cinchonidine exhibited similar volumes of distribution, the differences in terminal rate constants and corresponding elimination half-lives found between quinidine and quinine were expressions of the differences in total body clearance rates that these diastereomers possess. A similar observation has been seen recently with the optical isomers of disopyramide (1) as well as by Clohisy and Gibson (6) in their study with quinidine and quinine.

In this study, there was no apparent effect of stereochemistry or the presence of the methoxy group in the 6′-position of the quinoline nucleus on the two measured volumes (V_c and Vd). *A priori*, this finding was quite unexpected since the 6′-methoxy group would have been expected to increase the volume of distribution by imparting greater lipid solubility to the parent drug molecule[10]. It is possible that the distribution volumes of the four alkaloids are similar because the stereochemical difference and 6′-methoxy substitution do not materially affect the manner and extent to which these alkaloids interact with tissue proteins.

In the present investigation, corresponding blood samples were collected and analyzed for their alkaloid content to evaluate the relative differences in drug concentrations between plasma and whole blood. It was reasoned that this information might give some insight into how the four drugs might differ in disposition in the body. With the quinidine/quinine diastereomers, after pseudodistribution equilibrium conditions were achieved, the blood concentrations of both diastereomers were demonstrably lower than the corresponding alkaloid concentrations in the plasma (Fig. 3). Nevertheless, the terminal disposition rate constants assessed with the blood and plasma data were similar for the respective isomers and, therefore, the blood and plasma elimination curves were parallel. For cinchonine and cinchonidine, the blood and plasma concentration-time curves were virtually superimposable after distribution equilibrium conditions were reached (Fig. 4).

In the plasma alkaloid concentration range of ~0–5 μg/mL, the overall plasma-to-blood concentration ratios observed for quinidine, quinine, cinchonine, and cinchonidine were 2.15 ± 0.26, 2.11 ± 0.28, 0.92 ± 0.10, and 1.26 ± 0.13, respectively. From these results and Fig. 4 it can be seen that the cinchonine/cinchonidine diastereomers are essentially distributed and cleared to the same extent whether assessed by measuring blood or plasma concentrations of these two alkaloids in the dog. On the other hand, the plasma-to-blood concentration ratios for quinidine and quinine suggest that these diastereomers are cleared twice as fast from the blood as from the plasma. In addition, based on blood quinidine and quinine concentration determinations, the apparent volumes of distribution for both of the diastereomers are twice as large as the corresponding plasma-estimated values.

Two possible explanations would account for the differences in plasma and blood concentrations observed for the two pairs of structurally related diastereomers in this investigation. First, quinidine and quinine may have had a higher affinity and/or capacity for binding to plasma proteins. In the vas-

[10] In three different solvent systems, 1-octanol, chloroform, and benzene–0.1 M phosphate buffer (pH 7.4), the partition coefficients of the methoxy diastereomers were significantly greater than the corresponding cinchonine/cinchonidine diastereomers in each of these systems (27).

cular system, a drug that is highly bound to plasma proteins will tend to remain in the plasma fraction. In separate *in vitro* experiments, the binding of quinidine, quinine, cinchonine and cinchonidine to bovine serum albumin was studied by equilibrium dialysis (28). In these studies, the bound fraction was highest for quinidine (followed by cinchonine > quinine > cinchonidine) when the plasma alkaloid concentrations were in the range of $0\text{-}25 \times 10^{-6}$ M. Moreover, the affinity constant (K_A) of quinidine for bovine serum albumin was three times greater than the K_A of quinine, and the affinity constants for the methoxy-substituted diastereomers were found to be 4–8 times larger than the corresponding unsubstituted diastereomers. It is possible that qualitatively similar conditions exist for the interactions of the four alkaloids to plasma proteins in the dog.

Alternatively, the differences in the relationship between blood and plasma alkaloid concentrations for the two diastereomeric pairs may have been due to differences in the binding to the red blood cell membrane and/or relative affinity for components within the erythrocytes (*e.g.*, hemoglobin). A lower affinity for erythrocytes could also result in a lower overall concentration of an alkaloid in the blood. The red blood cell/plasma ratios (K_r) were estimated for each alkaloid with the following expression:

$$K_r = \frac{K_b - (1 - H)}{H} \qquad \text{(Eq. 6)}$$

where K_b is the ratio of the concentration of drug in the blood to the corresponding plasma drug concentration and H is the hematocrit. According to Eq. 6, it was observed that the ratios of the cinchonine/cinchonidine diastereomers were significantly larger than the ratios obtained with the quinidine/quinine diastereomers.

Stereoselective drug disposition kinetics have been seen with a number of different optical isomers including disopyramide (1), propranolol (4,5), and warfarin (9). These differences in disposition kinetics undoubtedly affect the quantitative differences in pharmacological activities observed between isomers. In this study with the quinidine/quinine and cinchonine/cinchonidine diastereomers, the two-way ANOVA test method indicated that the disposition characteristics of these alkaloids were due to an interaction of stereochemical and 6′-methoxy group substitution effects and could not be attributed to a single effect. However, the stereochemical influences on the disposition kinetics of these four alkaloids appeared to be weaker than the effects contributed by the 6′-methoxy substitution.

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