Pharmacokinetics and Pharmacodynamics of Pirmenol Enantiomers in Coronary Artery Ligated Dogs

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Abstract □ The pharmacokinetics and pharmacodynamics of pirmenol enantiomers were investigated in coronary artery ligated mongrel dogs. Reduction in frequency of premature ventricular complexes (PVCs) was determined following intravenous administration of 5-mg/kg doses of racemic pirmenol ($n = 5$), (+)-pirmenol ($n = 4$), and (–)-pirmenol ($n = 4$), each given as a 5-min infusion. Electrocardiographic signals and blood samples were obtained serially over a 4-h period. Pirmenol enantiomer concentrations in plasma were determined by a stereospecific assay. Following the racemate dose, (–)-pirmenol had 47% lower clearance and 33% lower steady-state distribution volume than (+)-pirmenol. These differences could be mostly explained by stereoselective plasma protein binding, reflected in a 58% higher unbound fraction for (+)-pirmenol compared with (–)-pirmenol following racemate administration. Unbound pirmenol distribution volumes were nearly identical for both enantiomers, and unbound clearance was only 16% lower for (–)-pirmenol than (+)-pirmenol following administration of the racemate. Similar trends were observed for pirmenol enantiomers administered individually. Both pirmenol enantiomers were equally effective in arrhythmia suppression. The antiarrhythmic response of coronary artery ligated dogs to pirmenol was described by a sigmoid E_{max} model, and no statistically significant differences were observed in the pharmacodynamic parameters [i.e., EC_{50} (plasma concentration at 50% of maximum drug effect), S (constant that reflects the sigmoidal shape of the effect–concentration curve), and EC_{90} (plasma concentration at 90% of maximum drug effect)] for (+)-pirmenol, (–)-pirmenol, or pirmenol racemate.

Introduction

Stereoisomerism is an important consideration in drug development because the enantiomers of an optically active compound may differ in their interaction with other chiral molecules. Many biological molecules are chiral in nature, so the potential exists for selective interactions between these molecules and opposing enantiomers of chiral drugs. Enantiomers of a drug may show substantial differences in their pharmacological activity, and when this occurs, enantiomer differences in pharmacokinetics can become important determinants of drug response.

Pirmenol is a chiral compound, and the racemic mixture is being investigated as an antiarrhythmic agent. Pirmenol is considered as a Class I antiarrhythmic because its primary effect is blocking sodium channels in the cardiac cell membrane. Many other drugs in the same class are chiral, including disopyramide, cibenzoline, tocainide, mexiletine, flecainide, encainide, and propafenone, and are administered as racemic mixtures.

Pirmenol racemate was shown to be effective in suppression of premature ventricular contractions (PVCs) following coronary artery ligation in dogs.[1–3] However, antiarrhythmic effects may not be produced equally by both enantiomers. In dog models of ventricular arrhythmia, enantiomers of tocainide,[4] flecainide,[5] propranolol,[6] and sotalol[7] showed equivalent antiarrhythmic effects, but mexiletine demonstrated a stereoselective difference where *R*-(−)-mexiletine was more potent in prevention of ventricular tachycardia than *S*-(+)-mexiletine in dogs.[8] Furthermore, both disopyramide and propranolol showed stereoselective pharmacokinetic properties.[9–13] Clearance of *S*-(+)-disopyramide in dogs was nearly 50% higher than that of R-(−)-disopyramide, with no enantiomer difference in distribution volume.[9,14] Cook et al.[10] also observed stereoselective clearance of disopyramide in dogs, demonstrating a 40% higher renal clearance and greater first-pass metabolism for *S*-(+)-disopyramide, with *N*-dealkylation being a stereoselective metabolic pathway. In addition, several investigators have reported stereoselective hepatic metabolism of *S*-(−)-propranolol in dogs.[11–13,15]

In this study, possible differences in pharmacokinetics and pharmacodynamics of pirmenol enantiomers were investigated to provide information regarding any potential benefit in the use of either pirmenol enantiomer rather than the racemate. To determine the relative contribution of pirmenol enantiomers to the overall antiarrhythmic effect, (+)-pirmenol, (−)-pirmenol, and pirmenol racemate were each tested in the model of induced ventricular arrhythmia in dogs, which was initially used in the study of pirmenol racemate. Pharmacokinetics of pirmenol enantiomers were also studied in these animals.

Experimental Section

Study Design—Reduction in frequency of PVCs following intravenous (iv) administration of 5-mg/kg doses of pirmenol racemate ($n = 5$), (+)-pirmenol ($n = 4$), and (−)-pirmenol ($n = 4$) was determined in coronary artery ligated mongrel dogs of either gender weighing 8.1 to 13.0 kg (mean 10.4 kg). Dogs were anesthetized with pentobarbital sodium (35 mg/kg, iv), and the left anterior descending coronary artery was ligated by a two-stage procedure as described by Harris.[16] A lead II electrocardiogram (ECG) was monitored on the first day after surgery with a Gould 220 polygraph. Analysis of the ECG record was performed by the method of Moran et al.[17] in which ventricular beats of apparent sinus origin were considered normal and all other ventricular beats were classified as ectopic. Dogs were excluded from testing if >50% normal sinus beats were observed or if total ventricular rate exceeded 200 beats/min. The dogs were studied in the conscious state (1 day post-infarction) while resting comfortably suspended in slings.

Predose baseline arrhythmia was determined as the average number of PVCs/min during the final 20 min before dosing. The ECG readings (20 s each) were obtained every minute during the infusion and for the first 5 min after the infusion, every 5 min for the first hour post-infusion, and every 15 min from 1 to 4 h following the end of the infusion. Additional ECG readings were obtained 1 min before and 1 min after each blood sampling time, and the three ECG values (PVCs/min) were averaged. Pirmenol doses were dissolved in 10 mL of 0.9% saline and administered as a 5-min infusion via the femoral artery. Blood samples (1.5 mL) were obtained before dosing (blank), at the end of the 5-min infusion, and at 5, 10, 20, 30, 45, 60, 90, 120,

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180, and 240 min post-infusion. Plasma was separated and assayed the same day or stored frozen at −20 °C until analysis.

Quantitation of Pirmenol Enantiomers—Plasma pirmenol enantiomer concentrations were determined by a stereospecific liquid chromatographic assay,[18] except that a 0.25-mL sample (diluted with 0.25 mL of blank dog plasma) was used. Briefly, racemic pirmenol and internal standard [(+)-propranolol] were isolated from dog plasma by a three-step extraction procedure using toluene, 0.1 N HCl, and hexane, respectively. A cellulose-bonded chiral analytical column (Chiralcel OJ) was used, with a mobile phase consisting of hexane: isopropanol:diethylamine (98.9:1.0:0.1) and with detection by ultraviolet (UV) absorbance at 262 nm. Linear calibration curves were obtained in the concentration range of 0.0200 to 5.00 μg/mL for each enantiomer. The precision of the method was ≤7.1% for both enantiomers, and bias was ±2.2% for (+)-pirmenol and ±1.5% for (−)-pirmenol. Neither enantiomer was observed in chromatograms following administration of the opposite enantiomer to coronary artery ligated dogs. Therefore, pirmenol enantiomer conversion did not occur in vivo nor did it occur during sample analysis.

Unbound concentrations were determined in each study sample by equilibrium dialysis. Plasma containing pirmenol (0.75 mL) was dialyzed against an equal volume of isotonic pH 7.4 phosphate buffer in a 37 °C shaking water bath for 4 h (equilibrium was reached, as indicated by preliminary experiments). Plasma and buffer samples were assayed for pirmenol enantiomer concentrations by a liquid chromatographic method.[18]

Pharmacokinetic Data Analysis—Postinfusion plasma pirmenol concentration (C_p)−time (t) data were fitted to the following polyexponential equation:

$$C_p = \sum_{i=1}^{n} Y_i e^{-\lambda_i t} \tag{1}$$

in which parameter estimates (Y_i, λ_i) were obtained by nonlinear least-squares regression (RSTRIP, Version 4, MicroMath Scientific Software, Salt Lake City, UT). The number of exponents used in the equation and weighting factor (1, 1/concentration, or 1/concentration^2) were selected based on coefficient of determination (r^2), standard deviation of the parameter estimates, and visual inspection of the residuals. Coefficients were corrected for infusion time (T) with the following equation:

$$C_i = \frac{Y_i \lambda_i T}{e^{\lambda_i T} - 1} \tag{2}$$

Pharmacokinetic parameters were then calculated with standard equations.[19,20] The pharmacokinetics of unbound pirmenol were determined by the same methods.

Linear or nonlinear binding was assessed from a plot of Cb'' as a function of Cf. For linear binding, fraction unbound in plasma (f_u) was calculated with eq 3[21]:

$$f_u = \frac{Cf}{Cf + Cb''} \tag{3}$$

in which Cf is the unbound concentration of pirmenol in buffer after dialysis, and Cb'' is the volume-corrected bound concentration of pirmenol in plasma after dialysis.

For nonlinear binding, Cb'' versus Cf data were best fit to a single Langmuir equation:

$$Cb'' = \frac{P(1) \cdot Cf}{P(2) + Cf} \tag{4}$$

where $P(1)$ and $P(2)$ are capacity and dissociation constants, respectively. Nonlinear regression was performed with the program MINSQ II (Version 1.02, MicroMath Scientific Software, Salt Lake City, UT), with a weighting factor of unity. Goodness of fit criteria included coefficient of determination (r^2), standard deviation of parameter estimates, and visual inspection of residuals. Since total concentration (Cp) is the sum of bound (Cb) and unbound (Cf) concentrations in plasma, total concentration can be expressed as follows:

$$Cp = \frac{P(1) \cdot Cf}{P(2) + Cf} + Cf \tag{5}$$

Equation 5 can be rearranged to a quadratic equation (eq 6) that was solved for unbound concentration (Cf) using the total concentration in the original plasma sample (Cp) and estimates of $P(1)$ and $P(2)$:

$$Cf^2 + (P(1) + P(2) - Cp) \cdot Cf - Cp \cdot P(2) = 0 \tag{6}$$

The time-averaged fraction unbound in plasma (f_u) was then estimated as follows:

$$f_u = \frac{AUC(0\text{-}\infty)_{unbound}}{AUC(0\text{-}\infty)_{total}} \tag{7}$$

Pharmacodynamic Data Analysis—The pharmacologic effect of pirmenol was measured as the percent reduction in PVCs/min from the pre-study baseline value. At each blood sampling time, PVCs were averaged for ECG readings obtained 1 min before, during, and 1 min after the sampling time. Effect measurements obtained postinfusion without simultaneous blood sample collection were paired with plasma drug concentrations that were calculated with parameter estimates obtained from pharmacokinetic data analysis. Effect−concentration profiles were fitted to a sigmoid E_{max} equation, as shown in eq 8:

$$E = \frac{E_{max} \cdot C^S}{EC_{50}{}^S + C^S} \tag{8}$$

where E is the percent reduction in PVCs from baseline; E_{max} is the maximum drug effect; C is the plasma pirmenol concentration (μg/mL); EC_{50} is the plasma concentration at 50% of E_{max} (μg/mL); and S is a constant that reflects the sigmoid shape of the effect−concentration curve. The E_{max} value was held constant at 100% because complete abolition of PVCs was evident with pirmenol. The EC_{50} and S values were estimated by nonlinear least squares regression with the program MINSQ II and a weighting factor of unity. Because effect is defined as percent reduction in PVCs, the maximum possible effect that can be achieved is 100%. The effective therapeutic concentration was determined as the plasma drug concentration at 90% PVC reduction (EC_{90}, μg/mL). The EC_{90} values were calculated with the parameter estimates and a rearrangement of eq 8, such that $EC_{90} = EC_{50}/(0.111)^{1/S}$.

Statistical Analysis—Enantiomer differences in mean pharmacokinetic parameters were evaluated by a paired t test following administration of pirmenol racemate and an unpaired t test following administration of the individual enantiomers. Mean pharmacokinetic parameters following the racemate dose were compared with parameters obtained following the enantiomer dose by unpaired t tests. Mean pharmacodynamic parameters were compared by analysis of variance. A p value of ≤0.05 was considered significant in all statistical tests.

Results and Discussion

Pharmacokinetics of Pirmenol Enantiomers—Pirmenol plasma concentration−time data were fitted to eq 1 with either 1, 2, or 3 exponents. In each case, a weighting factor of 1/concentration^2 was used. Coefficients of determination ranged from 0.968 to 0.998 for those analyses using total pirmenol, and from 0.893 to 0.999 for those analyses using unbound pirmenol. Pharmacokinetic parameters for total and unbound pirmenol enantiomers following the racemic dose and following individual enantiomer administration are given in Table 1. Plasma total (−)-pirmenol concentrations exceeded total (+)-pirmenol concentrations following administration of the racemate as well as following separate administration of each enantiomer at equal doses (Figure 1). The AUC(0-∞) values were approximately twofold greater for total (−)-pirmenol than for total (+)-pirmenol following pirmenol racemate and this difference was statistically signifi-

Table 1—Mean (±SD) Pharmacokinetic Parameters of Pirmenol Enantiomers in Coronary Artery Ligated Dogs Following a Single Intravenous Dose of 5 mg/kg Given as Pirmenol Racemate ($n = 5$), (+)-Pirmenol ($n = 4$), or (–)-Pirmenol ($n = 4$)[a]

	Racemic Dose				Enantiomeric Dose			
Parameter[b]	(+)-Pirmenol	(–)-Pirmenol	Significance	*p* Value	(+)-Pirmenol	(–)-Pirmenol	Significance	*p* Value
				Total Pirmenol				
$t_{1/2}$	1.93 (0.62)	2.46 (1.13)	NS	0.094	2.30 (1.66)	2.40 (0.86)	NS	0.916
AUC(0–∞)	1.77 (0.28)	3.44 (0.82)	S, (–) > (+)	0.0025	3.31 (0.97)	4.76 (1.05)	NS	0.088
MRT	2.46 (0.52)	3.20 (1.08)	NS	0.062	2.74 (1.76)	3.11 (0.89)	NS	0.723
CL	24.0 (4.0)	12.7 (3.3)	S, (+) > (–)	0.0001	26.9 (7.9)	18.3 (5.0)	NS	0.118
V_1	1.95 (0.62)	1.36 (0.43)	S, (+) > (–)	0.0036	2.47 (0.62)	2.24 (0.30)	NS	0.528
V_{ss}	3.49 (0.62)	2.35 (0.68)	S, (+) > (–)	0.0010	4.40 (2.79)	3.28 (0.57)	NS	0.460
V_{area}	3.92 (1.10)	2.60 (1.04)	S, (+) > (–)	0.0006	5.42 (3.89)	3.64 (0.87)	NS	0.406
				Unbound Pirmenol				
$t_{1/2}$	1.33 (0.37)	1.57 (0.50)	S, (–) > (+)	0.020	1.52 (0.48)	2.00 (0.70)	NS	0.305
AUC(0–∞)	0.601 (0.078)	0.727 (0.139)	S, (–) > (+)	0.028	1.03 (0.19)	1.34 (0.36)	NS	0.166
MRT	1.85 (0.49)	2.21 (0.68)	S, (–) > (+)	0.017	1.93 (0.47)	2.71 (0.93)	NS	0.185
CL	70.3 (9.8)	59.4 (13.7)	S, (+) > (–)	0.021	83.2 (13.8)	65.4 (17.3)	NS	0.159
V_1	4.84 (0.72)	5.09 (0.95)	NS	0.364	6.87 (1.69)	7.66 (1.38)	NS	0.495
V_{ss}	7.64 (1.74)	7.57 (1.75)	NS	0.864	9.73 (3.33)	10.0 (1.0)	NS	0.902
V_{area}	7.97 (2.02)	7.78 (1.87)	NS	0.649	11.2 (4.8)	10.5 (0.9)	NS	0.797
f_u	0.343 (0.040)	0.217 (0.051)	S, (+) > (–)	0.0001	0.319 (0.055)	0.297 (0.108)	NS	0.725

[a] Body weight = 10.0 ± 1.2 kg for racemate dose group (mean ± SD); 9.7 ± 1.5 kg for (+)-pirmenol dose group (mean ± SD); and 11.6 ± 1.1 kg for (–)-pirmenol dose group (mean ± SD). [b] Parameter definitions: $t_{1/2}$ = terminal-phase half-life (h); AUC(0-∞) = area under the plasma concentration–time curve extrapolated to infinity (μg·h/mL); MRT = mean residence time (h); CL = plasma clearance (mL/min/kg); V_1 = volume of the central compartment (L/kg); V_{ss} = volume of distribution steady-state (L/kg); V_{area} = volume relating plasma drug concentration to amount in the body during the log-linear terminal phase (L/kg); f_u = fraction unbound in plasma.

cant (S). The difference between AUC(0-∞) values following individual enantiomer administration was not statistically significant (NS) even though total (−)-pirmenol AUC(0-∞) was 44% greater than for total (+)-pirmenol. Clearance of total drug was 47% lower (S) for (−)-pirmenol than for (+)-pirmenol following the racemate dose and 32% lower (NS) for (−)-pirmenol following individual enantiomer doses. Similar to the significantly higher plasma clearance for (+)-pirmenol than for (−)-pirmenol, stereoselective clearance was also observed for disopyramide and propranolol in dogs, where clearance of *S*-(+)-disopyramide and *S*-(−)-propranolol exceeded their *R*-enantiomers by 40 to 50% and by two- to threefold, respectively.[9−12,14] Volume of distribution parameters (V_1, V_{ss}, V_{area}) were ~50% larger for total (+)-pirmenol than for total (−)-pirmenol (S) following the racemate, and 10−49% larger for (+)-pirmenol than for (−)-pirmenol (NS) following individual enantiomer administration. There were no significant differences between enantiomers in elimination half-life and mean residence time whether pirmenol was given as the racemate or the individual enantiomers.

Although the trends in pharmacokinetic differences were similar following both individual pirmenol enantiomer and racemate administration, none of these enantiomer differences were statistically significant following individual enantiomer doses. This result was not unexpected because enantiomers could be compared by a paired t test following racemate administration and an unpaired t test was necessary to compare the two separate groups of dogs receiving individual enantiomers. The power of a paired t test for enantiomer comparisons following pirmenol racemate made the detection of statistically significant differences more likely relative to enantiomer comparisons following individual enantiomer doses.

Plasma protein binding was more extensive for (−)-pirmenol than for (+)-pirmenol. Mean fraction unbound was 58% higher (S) for (+)-pirmenol than for (−)-pirmenol following the racemate dose, and 7% higher (NS) for (+)-pirmenol following individual enantiomer doses. Plasma protein binding was linear for most dogs, with only three of the 13 dogs tested showing nonlinear protein binding. Of these three dogs, one received pirmenol racemate and the other two received (+)-pirmenol alone.

Higher (−)-pirmenol concentrations observed for total pirmenol can, at least in part, be explained by differences in extent of plasma protein binding. Differences in total pirmenol enantiomer concentrations were minimized when unbound concentrations were compared. Clearance of unbound (−)-pirmenol was only 16% lower (S) following administration of the racemate and 21% lower (NS) following administration of the individual enantiomers, relative to unbound (+)-pirmenol. Distribution volume terms for unbound drug were similar for both (+)- and (−)-pirmenol, whether administered as pirmenol racemate or as the individual enantiomers. As a result of lower clearance for unbound (−)-pirmenol with no enantiomer difference in unbound pirmenol distribution volume, half-life was longer for unbound (−)-pirmenol than for unbound (+)-pirmenol.

Possible interaction between enantiomers was assessed by comparing pharmacokinetic parameters for each enantiomer given as the racemate or as the individual enantiomer by an unpaired t test. Several distribution volume terms (i.e., unbound (+)-pirmenol V_1, total (−)-pirmenol V_1, and unbound (−)-pirmenol V_1, V_{ss}, and V_{area}) were 30−60% greater (S) following individual enantiomer administration compared with racemate. Lower distribution volume following the racemate compared with the individual enantiomers may suggest the possibility of decreased tissue binding when both enantiomers are present compared with when each enantiomer is administered alone. In contrast, there were no significant differences between enantiomer alone and enantiomer in the presence of its optical antipode with respect to clearance, half-life, and plasma protein binding.

Pharmacodynamics of Pirmenol Racemate and Enantiomers—Baseline arrhythmia ranged from 138 to 172 PVCs/min, 118 to 199 PVCs/min, and 149 to 207 PVCs/min in dogs receiving (+)-pirmenol, (−)-pirmenol, and pirmenol racemate, respectively. Expressed as percent of total beats, baseline values ranged from 89−97% PVCs, 92−100% PVCs, and 80−100% PVCs in dogs receiving (+)-pirmenol, (−)-pirmenol, and pirmenol racemate, respectively. Therefore, similar degrees of arrhythmia were used for comparison

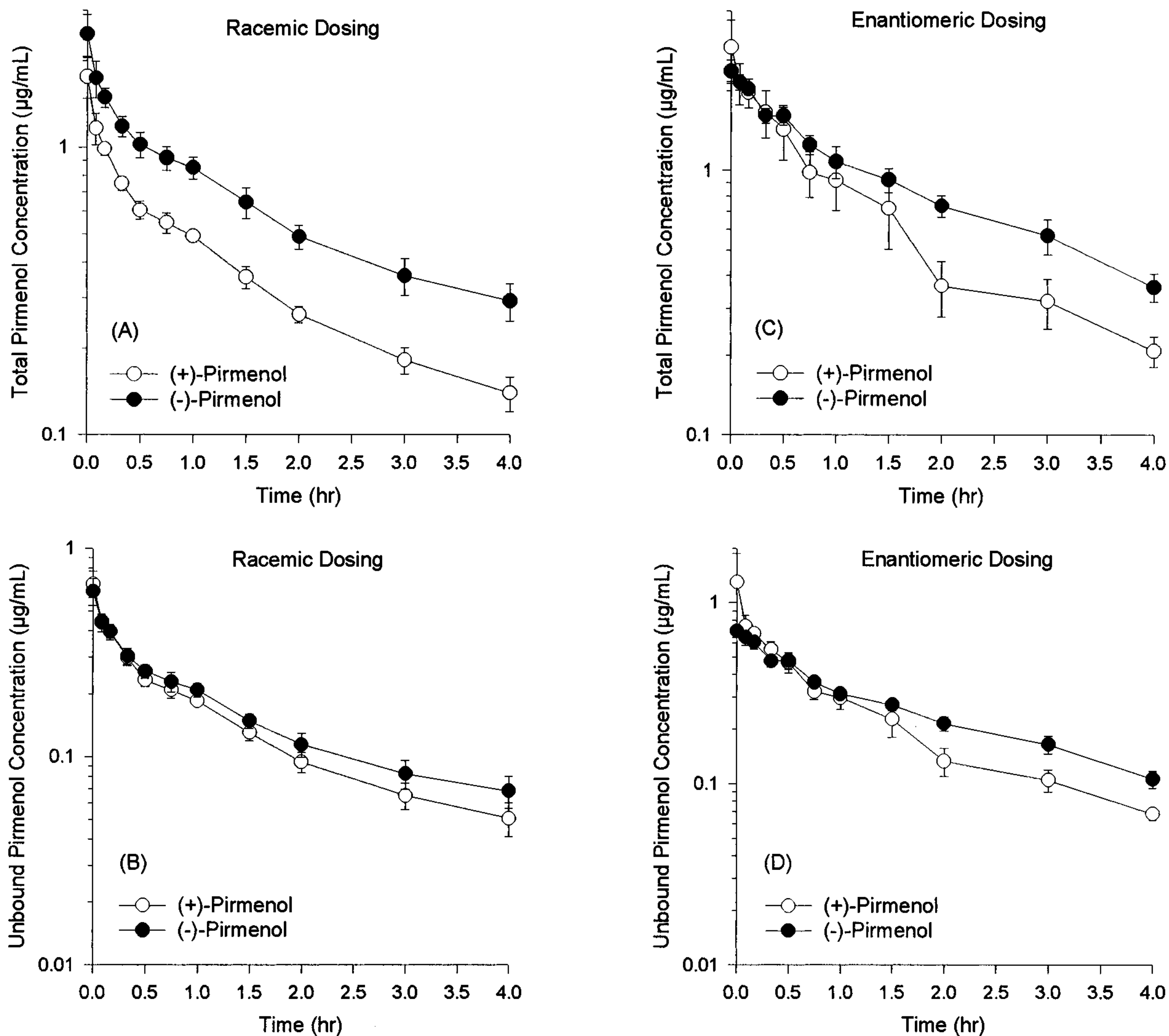


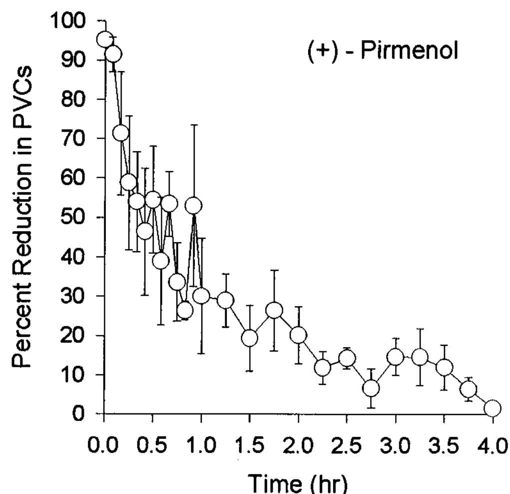
Figure 1—Mean (±SE) plasma concentrations of total (A) and unbound (B) pirmenol enantiomers following a single iv 5-mg/kg racemic pirmenol dose to coronary artery ligated dogs ($n = 5$); mean (±SE) plasma concentrations of total (C) and unbound (D) pirmenol enantiomers following a single iv 5-mg/kg (+)-pirmenol dose ($n = 4$) and (–)-pirmenol dose ($n = 4$) to coronary artery ligated dogs.

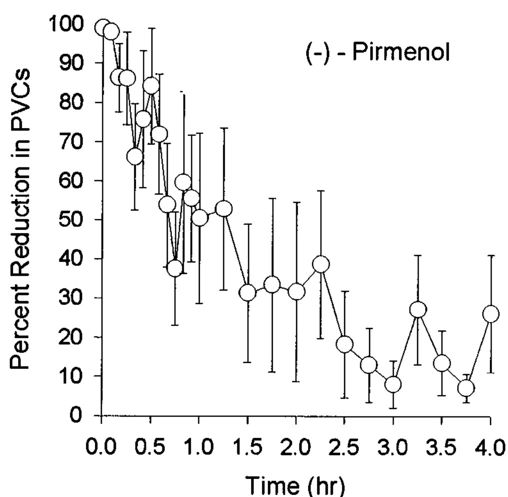
Table 2–Statistical Comparison of Mean (±SD) Pharmacodynamic Parameters of Pirmenol Enantiomers and Pirmenol Racemate in Coronary Artery Ligated Dogs Following a Single Intravenous Dose of 5 mg/kg Given As Pirmenol Racemate ($n = 5$), (+)-Pirmenol ($n = 4$), or (–)-Pirmenol ($n = 4$)

Parameter[a]	Dose			Significance	*p* Value
	Racemate	(+)-Pirmenol	(–)-Pirmenol		
		Baseline Arrhythmia			
% of Total Beats	94 (8)	94 (3)	96 (4)	NS	0.804
PVCs/min	172 (24)	149 (15)	158 (45)	NS	0.550
		Total Pirmenol			
EC_{50}	1.43 (0.65)	1.20 (0.28)	1.08 (0.33)	NS	0.532
S	2.77 (2.75)	2.27 (0.74)	4.76 (2.82)	NS	0.325
EC_{90}	5.72 (3.75)	3.51 (0.82)	1.96 (0.77)	NS	0.116
r^2	0.566 (0.148)	0.745 (0.108)	0.769 (0.181)		
		Unbound Pirmenol			
EC_{50}	0.41 (0.16)	0.43 (0.10)	0.35 (0.18)	NS	0.743
S	2.17 (1.78)	2.18 (0.67)	4.70 (2.59)	NS	0.124
EC_{90}	1.82 (1.20)	1.33 (0.47)	0.62 (0.34)	NS	0.145
r^2	0.550 (0.152)	0.748 (0.107)	0.767 (0.180)		

[a] EC_{50} = plasma drug concentration at 50% of E_{max} (µg/mL); EC_{90} = plasma drug concentration at 90% of E_{max} (µg/mL).

between treatment groups (Table 2). In previous studies of pirmenol racemate, Mertz and co-workers[1,2] showed that the arrhythmia produced with the Harris dog model was stable for a treatment period of at least 6 h on the day after surgery. Arrhythmia was monitored for 4 h post-dose in this study, so the observed reduction in PVCs can be attributed to a drug





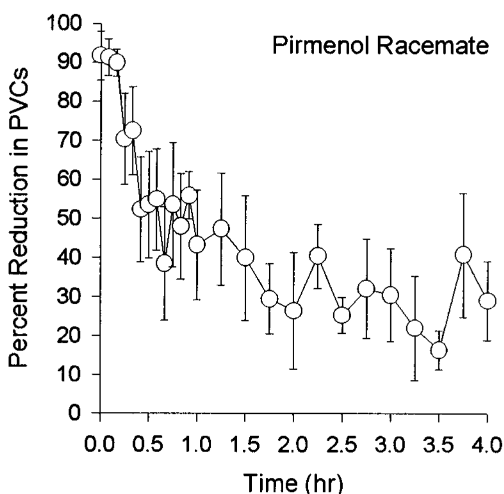
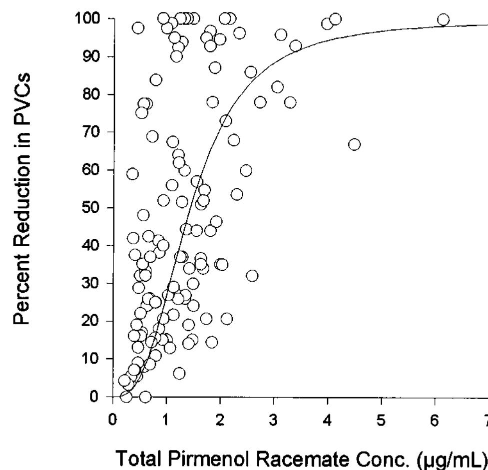


Figure 2—Mean (±SE) effect versus time profiles in coronary artery ligated dogs following a single iv dose of 5 mg/kg of (+)-pirmenol, (−)-pirmenol, or pirmenol racemate.

effect without significant contribution from a natural return of sinus rhythm with time following surgery.

Both pirmenol enantiomers were equally effective in arrhythmia suppression. Pirmenol racemate also showed PVC reduction similar to that of the individual enantiomers at equivalent doses. For all three treatments, suppression of the arrhythmia was complete or nearly complete by the end of the 5-min infusion. Following the infusion, PVCs returned



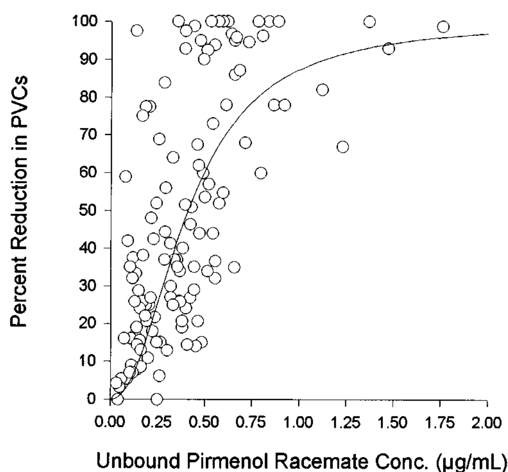


Figure 3—Effect–concentration profiles of total and unbound pirmenol racemate in coronary artery ligated dogs following a single iv dose of 5 mg/kg of pirmenol racemate. The solid line represents the hypothetical effect–concentration curve based on mean EC_{50} and S values as displayed in Table 2. Individual data are superimposed on this curve.

with time as drug was being cleared from the systemic circulation (Figure 2).

Pharmacodynamic parameters are listed in Table 2 for total and unbound pirmenol enantiomers and racemate. Percent reduction in PVCs was correlated with drug concentration with a sigmoid E_{max} model (eq 8). Coefficient of determination (r^2) values ranged from 0.405 to 0.901, and were similar for all drug species. Low r^2 values were probably a result of spontaneous variability in the effect parameter (PVCs/min), which has been described by Harris.[16] Mean EC_{50} values were similar for (+)-pirmenol, (−)-pirmenol, and pirmenol racemate, ranging from 1.08 to 1.43 μg/mL for total drug (NS) and from 0.35 to 0.43 μg/mL for unbound drug (NS). Values for the slope factor were approximately twofold greater (NS) for (−)-pirmenol than for (+)-pirmenol or pirmenol racemate. Similarly, therapeutic plasma concentrations reflected by the EC_{90} values were nearly two- to threefold lower (NS) for (−)-pirmenol as compared with (+)-pirmenol and pirmenol racemate; however, these differences were not statistically significant (Table 2). Effect−concentration curves simulated with mean EC_{50} and S values reflect the totality of individual data reasonably well for both total and unbound pirmenol racemate (Figure 3). Similar effect−concentration curves were also observed following (+)-pirmenol and (−)-pirmenol. To better highlight these treatment comparisons, the general profiles of effect−concentration curves based on mean param-

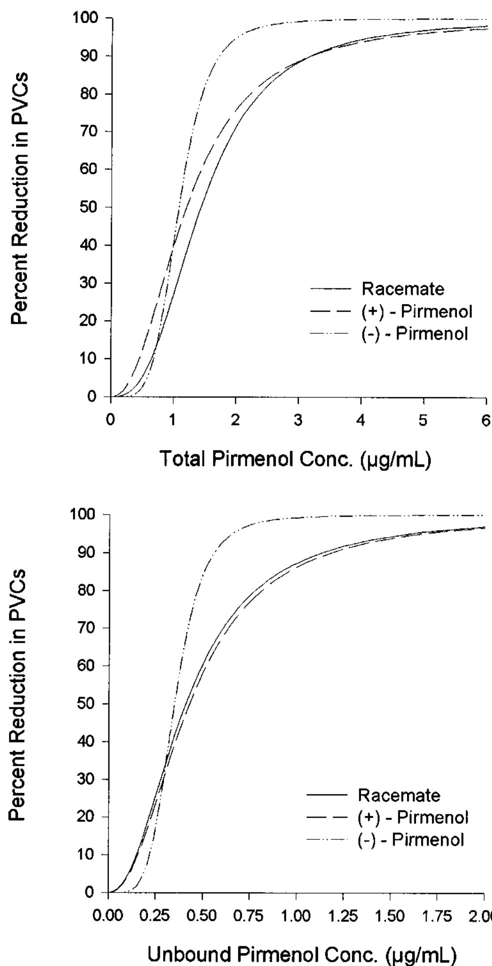


Figure 4—Comparison of fitted effect–concentration curves using mean EC_{50} and *S* estimates from Table 2 for total and unbound drug following a single iv dose of 5 mg/kg of (+)-pirmenol, (−)-pirmenol, or pirmenol racemate to coronary artery ligated dogs.

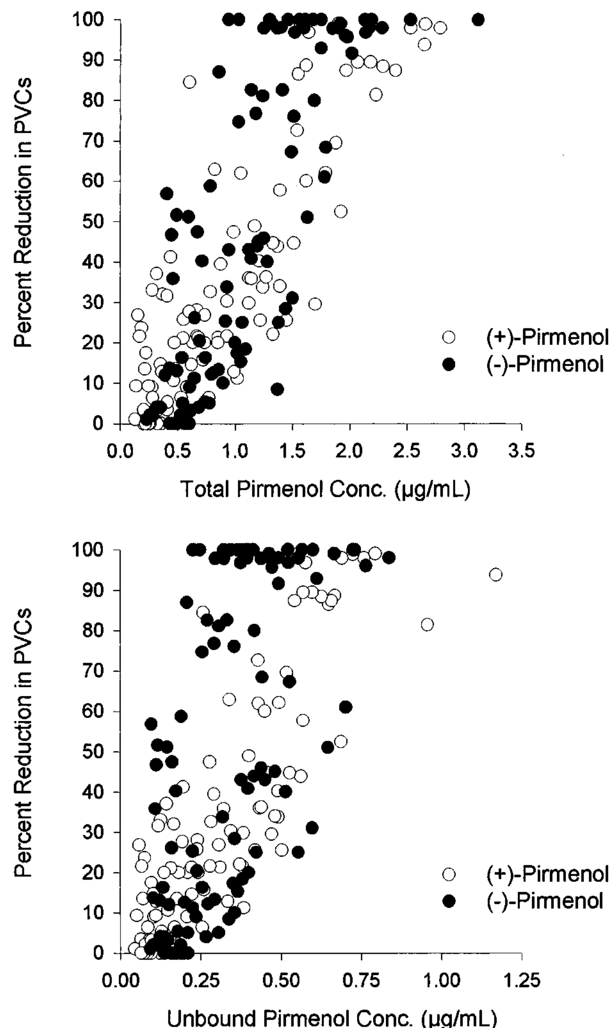


Figure 5—Comparison of pooled effect–concentration data for total and unbound drug following a single iv dose of 5 mg/kg of (+)-pirmenol or (−)-pirmenol to coronary artery ligated dogs.

eter estimates for (+)-pirmenol, (−)-pirmenol, and pirmenol racemate are displayed in Figure 4.

Although the mean EC_{90} appeared to be lower for (−)-pirmenol (NS), greater sensitivity of sodium channels to (−)-pirmenol is not likely because Nakaya et al.[22] showed no stereoselective interaction of pirmenol enantiomers with the sodium channel in guinea pig papillary muscle. Similarity of the phenyl and pyridine rings at the chiral center of the chemical structure of pirmenol was suggested by Nakaya et al.[22] to be responsible for the lack of stereoselectivity in sodium channel blocking activity. Parameter differences between pirmenol enantiomers may have resulted from different dogs being used in each treatment group and the relatively small number of animals studied. A larger sample size may be necessary to determine if either pirmenol enantiomer was actually more potent. However, it is unlikely that the data for (+)-pirmenol and (−)-pirmenol reflect an actual difference in EC_{90} between enantiomers. If this were true, then one would predict the EC_{90} of racemate to be intermediate between the (+)-pirmenol and (−)-pirmenol values. In fact, EC_{90} values for racemate were apparently larger (NS) than for individual enantiomers, which probably reflects the different animals studied. In addition, individual effect–concentration data for the (+)-pirmenol and (−)-pirmenol doses show a large degree of overlap, with each enantiomer dose extending over a wide range of effect and concentration values (Figure 5).

The severity of arrhythmia may also be a factor in determining the therapeutic concentration required for PVC suppression because Steffe et al.[2] showed that higher doses were required for treatment of more severe arrhythmia occurring on the first day as compared with the second day after coronary artery ligation. In another study, minimum effective therapeutic concentration for 100% PVC suppression by total pirmenol racemate was estimated as 1.0 μg/mL for low-rate arrhythmias (average baseline, 53% PVCs) and 2.3 μg/mL for high-rate arrhythmias (average baseline, 96% PVCs) during continuous iv infusion in coronary artery ligated dogs.[1] In this context, high EC_{90} values were generally associated with the most severe arrhythmias, and the highest mean baseline PVC rate (PVCs/min) was in the group receiving pirmenol racemate (NS). Although not statistically different, mean EC_{90} values for the racemate were higher than for the (+)-pirmenol and (−)-pirmenol groups with lower mean baseline PVC rates (Table 2).

Mean (±SD) minimum effective plasma concentration, defined as the concentration at the last minute of statistically significant decrease in %PVCs from the predose value, was reported as 1.1 ± 0.3 μg/mL (corresponding to 65% reduction in PVCs) in coronary artery ligated dogs; complete arrhythmia suppression was observed at concentrations ranging from 0.7 to 4 μg/mL.[3] These literature values, as well as those reported by Mertz and Steffe,[1] correspond favorably with the mean effective therapeutic concentration (EC_{90}) of 5.72 μg/mL (range

1.19 to 8.83 μg/mL) for total racemic pirmenol obtained from fitting effect−concentration data to a sigmoid E_{max} model in this study.

Conclusions—Plasma total (−)-pirmenol concentrations exceeded those of (+)-pirmenol, with statistically different values for AUC(0-∞) [94% higher for (−)-pirmenol], *CL* [47% lower for (−)-pirmenol], and V_{ss} [33% lower for (−)-pirmenol] following the pirmenol racemate dose. Similar trends were observed for pirmenol enantiomers administered individually; however, the differences did not achieve statistical significance. (+)-Pirmenol showed a 58% higher unbound fraction in plasma than (−)-pirmenol following racemate administration, so differences between pirmenol enantiomer pharmacokinetic parameters based on unbound plasma drug concentrations were dramatically reduced. Unbound pirmenol distribution volumes were nearly identical for both enantiomers, and small statistical differences were observed for unbound AUC(0-∞) [17% higher for (−)-pirmenol] and unbound clearance [16% lower for (−)-pirmenol] following the racemate. Therefore, differences between total pirmenol enantiomer pharmacokinetics can be mostly explained by stereoselective plasma protein binding.

The antiarrhythmic response of coronary artery ligated dogs to pirmenol was similar for both enantiomers and for racemate. Reduction in PVCs as a function of plasma pirmenol concentration was adequately described by a sigmoid E_{max} model. The EC_{50} values were similar for (+)-pirmenol, (−)-pirmenol, and pirmenol racemate, but minimum effective plasma concentration (90% PVC suppression) was nearly two- to threefold lower for (−)-pirmenol than for (+)-pirmenol or pirmenol racemate. The EC_{90} differences may be a consequence of different animals being used in each treatment group and the limited number of animals studied. Overall, no statistically significant differences were observed in EC_{50}, *S*, and EC_{90} between dogs treated with (+)-pirmenol, (−)-pirmenol, or pirmenol racemate.

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