Comparative Binding of Disopyramide Phosphate and Quinidine Sulfate to Human Plasma Proteins

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Abstract □ Disopyramide phosphate and quinidine sulfate are pharmacologically related, but chemically unrelated, antiarrhythmic agents. Two groups of independent binding sites were observed for each drug after protein binding studies in human plasma and human serum albumin solutions. The number of binding sites per protein molecule and their corresponding binding affinities were computed. The magnitude of N_1K_1, the fraction of binding, and the relative lipophilicity are quantitatively correlated. Competitive binding studies demonstrated that these two drugs do not compete for the same binding sites on protein molecules.

Keyphrases □ Disopyramide phosphate—binding to human plasma proteins compared to quinidine sulfate □ Quinidine sulfate—binding to human plasma proteins compared to disopyramide phosphate □ Binding—comparison of disopyramide phosphate and quinidine sulfate to human plasma proteins □ Protein binding—comparison of binding sites of disopyramide phosphate and quinidine sulfate, human plasma proteins

Disopyramide phosphate (I) has been reported to be two to three times as potent as quinidine sulfate in reversing cardiac arrhythmias (1). Table I lists the relative oral and intravenous mean effective dosages in canine atrial arrhythmias induced by aconitine (Scherf) or *via* crush–stimulation[1].

In a recently completed study comparing the activities of disopyramide phosphate and quinidine sulfate (II) against human ventricular and supraventricular arrhythmias, patients given 150 mg of disopyramide phosphate showed statistically equivalent therapeutic responses to patients receiving 325 mg of quinidine sulfate[1]. Both drugs were given every 6 hr, a time interval that approximates a single half-life for both drugs[1].

The interactions of drugs with plasma proteins in blood have been recognized for many years as an important parameter in drug availability, drug efficacy, and drug transport (2). It has been established that the interactions between drug and plasma proteins affect the concentration of unbound drug in the general circulation and limit the availability of drug to target tissues (3). This paper reports observations on the comparative binding of disopyramide phosphate and quinidine sulfate to human plasma proteins and discusses the potential biopharmaceutic implications of the binding profiles observed.

EXPERIMENTAL

Materials—Human plasma was collected by centrifuging[2] whole blood at 2500 rpm and 4° for 20 min. Human serum albumin[3] (fraction V, fatty acid poor) was prepared at a concentration of 7.028×10^{-4} *M* in isotonic 0.1 *M* phosphate buffer (pH 7.4). Disopyramide phosphate[4] and quinidine sulfate[5] were used as obtained.

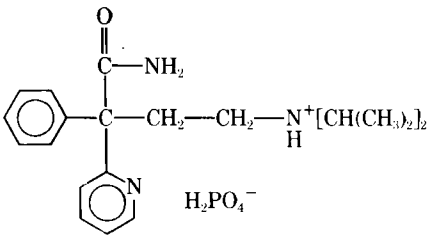
Table I—Effect of Disopyramide Phosphate and Quinidine Sulfate on Reversal of Canine Atrial Arrhythmias

Route	Drug	Mean Effective Dose, mg/kg: Aconitine	Mean Effective Dose, mg/kg: Crush–Stimulation
Intravenous	Disopyramide phosphate	2.7	5.8
Intravenous	Quinidine sulfate	8.7	11.8
Oral	Disopyramide phosphate	15	20
Oral	Quinidine sulfate	35	50

Binding Studies—Drug solutions, $1.6–8.0 \times 10^{-4}$ *M*, were prepared in isotonic 0.1 *M* phosphate buffer (pH 7.4) immediately prior to binding measurements. After mixing well, 1–5 ml of drug solution was added to 5 ml of either human plasma or human serum albumin solutions, and buffer was added to a final volume of 10 ml. The resultant mixture was equilibrated, poured into membrane ultrafilters[6], and then centrifuged[2] at 1250 rpm and 4° for 30 min.

The filtrates were assayed spectrophotometrically, and the absorbance at 260 (disopyramide phosphate) and 334 (quinidine sulfate) nm was recorded for calculating the free drug concentration. Drug solution in the absence of proteins was also investigated at the same time to correct for drug loss by membrane adsorption. The average quantities of drug adsorbed were 8 and 20% for disopyramide phosphate and quinidine sulfate, respectively.

Competitive Binding Studies—The procedure for studying the competitive binding of disopyramide phosphate and quinidine sulfate to human plasma proteins was essentially the same as described earlier, except that the concentration of one drug was maintained at 8×10^{-5} *M* while the concentration of the other was



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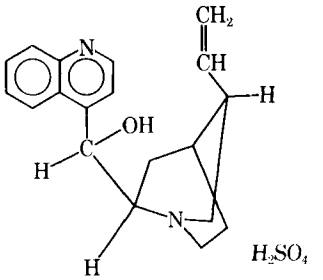

II

[1] Unpublished data.
[2] Sorvall RC-3 automatic refrigerated centrifuge.
[3] Nutritional Biochemical Corp., Cleveland, Ohio.
[4] Supplied by the Chemical Research Department of this laboratory.
[5] J. T. Baker Chemical Co., Glen Ellyn, Ill.
[6] Centriflo, model CF 50A, Amicon Corp., Lexington, Mass.

Table II—Binding of Disopyramide Phosphate and Quinidine Sulfate to Human Plasma

[Drug] $\times 10^5$ M	β Values[a], % ± SD		
	Disopyramide Phosphate	Quinidine Sulfate	Ratio[b]
1.6	30.65 ± 4.4	74.7 ± 1.4	2.44
3.2	29.8 ± 7.5	70.8 ± 3.2	2.38
4.8	26.4 ± 16.5	63.5 ± 2.0	2.41
6.4	22.3 ± 3.75	58.2 ± 3.2	2.61
			2.46 (±0.11)

[a] The fraction of drug bound, β, is defined by Eq. 1 (n = 4). [b] Ratio of the β values for quinidine sulfate over those for disopyramide phosphate at corresponding drug concentration (n = 4).

Table III—Binding of Disopyramide Phosphate and Quinidine Sulfate to Human Serum Albumin (Fraction V)

[Drug] $\times 10^5$ M	β Values[a], % ± SD		
	Disopyramide Phosphate	Quinidine Sulfate	Ratio[b]
1.6	33.01 ± 2.6	71.1 ± 5.3	2.154
3.2	30.96 ± 5.7	59.5 ± 5.5	1.922
4.8	21.1 ± 13.6	56.3 ± 1.4	2.668
6.4	22.7 ± 7.2	55.45 ± 1.33	2.443
			2.3 (±0.36)

[a] The fraction of drug bound, β, is defined by Eq. 1 (n = 5). [b] Ratio of the β values for quinidine sulfate over those for disopyramide phosphate at corresponding drug concentration (n = 4).

varied from 1.6 to 8.0 × 10^{-5} M. Since the spectrophotometric peaks for disopyramide phosphate (λ_{max} = 260 nm) and quinidine sulfate (λ_{max} = 334 nm) are well defined and clearly separated from one another, independent computation of free drug concentration of one drug in the presence of another was facilitated.

Partition Studies—A drug concentration of 8 × 10^{-5} M was freshly prepared in 1-octanol-saturated phosphate buffer (0.1 M, pH 7.4, isotonic). Ten milliliters was shaken and equilibrated with 10 ml of phosphate buffer-saturated 1-octanol until a clear phase separation was established (at least 5 hr). The drug concentrations in the buffer phase before and after equilibration were measured spectrophotometrically and utilized to estimate the magnitude of the partition coefficient.

RESULTS AND DISCUSSION

The fraction of drug bound to a given concentration of plasma proteins, β, was estimated from the difference in drug concentration recovered from the filtrate in the absence and in the presence of plasma proteins by using the following equations:

$$\beta\ (\%) = \frac{[D]_B}{[D]_T} \times 100 \qquad \text{(Eq. 1}a\text{)}$$

$$\beta\ (\%) = \frac{[D]_T - [D]_F}{[D]_T} \times 100 \qquad \text{(Eq. 1}b\text{)}$$

where $[D]_T$ and $[D]_F$ are the drug concentrations recovered in the absence and in the presence of proteins, respectively, and $[D]_B$ is the drug concentration bound to protein molecules.

As seen in Table II, both disopyramide phosphate and quinidine sulfate were bound to different degrees by human plasma proteins (final concentration of 36.3 × 10^{-5} M). When the concentration of drug was increased from 1.6 × 10^{-5} to 6.4 × 10^{-5} M, the β values for disopyramide phosphate and quinidine sulfate decreased. Table II shows that the β values for quinidine sulfate were 2.5-fold greater than those for disopyramide phosphate at all drug concentrations investigated. Since drug–protein interaction decreased the concentration of free drug and only the free drug species is membrane permeable, then the availability for permeation of quinidine sulfate at equimolar plasma concentration should be approximately 2.5-fold less than that of disopyramide phosphate.

As stated previously, disopyramide phosphate and quinidine sulfate are pharmacologically related, but chemically unrelated, antiarrhythmic agents. A series of binding studies with several concentrations of one drug in the presence of a fixed concentration (8 × 10^{-5} M) of the other was performed to evaluate the potential for competitive binding interactions (4). Plots of the reciprocal of the binding ratio *versus* the reciprocal of the free drug concentration (5) were constructed to determine if disopyramide phosphate and quinidine sulfate were competing for the same binding site (Figs. 1 and 2). The same magnitude of slope and the different extrapolated $1/\gamma$ intercepts in the absence and the presence of disopyramide phosphate (vice versa) clearly demonstrated that these two drugs do not compete for the same binding site (6). Therefore, the probability for displacement of one drug by the other upon simultaneous administration is markedly diminished.

The correlation between the binding data in Table III (human serum albumin) and those in Table II (human plasma) indicates that albumin was the primary molecule responsible for the observed binding of both disopyramide phosphate and quinidine sulfate.

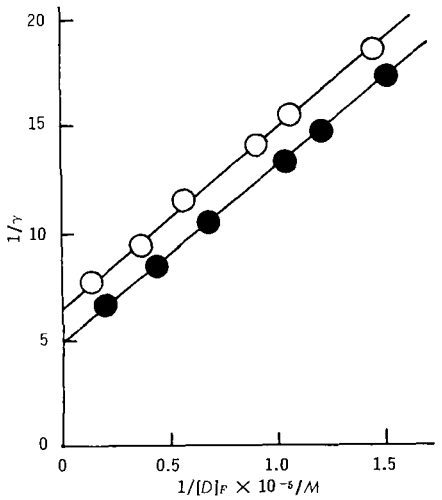

Figure 1—*Relationship between the reciprocal of the binding ratio ($1/\gamma$) and the reciprocal of the free quinidine sulfate concentration ($1/[D]_F$) at low γ values ($\gamma \leq 0.15$) in the absence (○) and in the presence (●) of disopyramide phosphate (8 × 10^{-5} M). Equivalent slopes were obtained.*

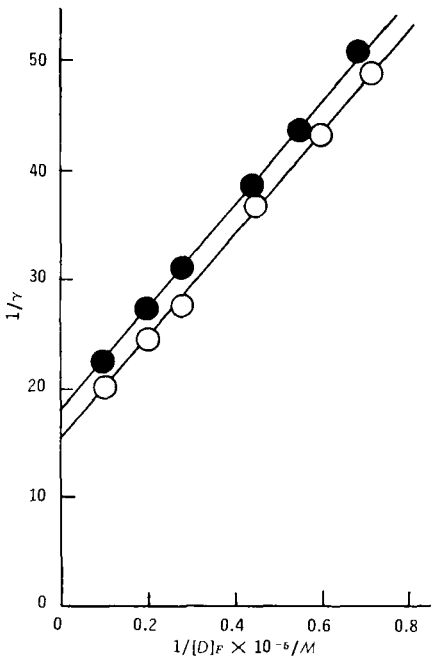

Figure 2—*Relationship between the reciprocal of the binding ratio ($1/\gamma$) and the reciprocal of the free disopyramide phosphate concentration ($1/[D]_F$) at low γ values ($\gamma \leq 0.05$) in the absence (○) and in the presence (●) of quinidine sulfate (8 × 10^{-5} M). Equivalent slopes were obtained.*

Table IV—Human Serum Albumin Binding Parameters for Disopyramide Phosphate and Quinidine Sulfate

[Drug] × 10^5 M[a]	Disopyramide Phosphate		Quinidine Sulfate	
	γ[b]	$\gamma/[D]_F$[c] ($\times 10^{-2}/M$)	γ[b]	$\gamma/[D]_F$[c] ($\times 10^{-2}/M$)
40	0.120	3.354	0.336	11.94
24	0.0945	4.568	0.247	16.11
16	—	—	0.199	22.06
8	0.0581	9.746	0.119	31.15
6.4	0.0497	10.684	0.100	34.4
4.8	0.0468	14.817	0.078	37.9
3.2	0.0330	16.164	—	—
2.4	—	—	0.041	43.4
1.6	—	—	0.031	60.8

[a] The drug concentration added initially. [b] The binding ratio, γ, is given by $[D]_B/[HSA]$, where $[D]_B$ and [HSA] are the concentrations of drug bound and of human serum albumin added, respectively. [c] $[D]_F$ is defined as the concentration of free drug in the drug–protein mixture.

fate. The data also support the use of aqueous solutions of human serum albumin (HSA) for the mechanistic analysis of drug–protein interaction between disopyramide phosphate and quinidine sulfate.

To perform a Scatchard analysis of the drug–protein interaction, several studies were carried out for both drugs over a wide concentration range (more than 20-fold) with a physiological concentration (3.514×10^{-4} M) of human serum albumin. A representative set of data is illustrated in Table IV. As expected, with a fixed number of albumin binding sites, when the concentration of drug was decreased, the magnitude of the binding ratio, $\gamma = ([D]_B/[HSA])$, decreased and the value of $\gamma/[D]_F$ increased. A computer program (6) based on Eq. 2 (7) was applied to calculate the number of binding sites in (N_1 and N_2) binding groups and their corresponding binding affinities (K_1 and K_2):

$$\gamma = \frac{N_1K_1[D]_F}{1 + K_1[D]_F} + \frac{N_2K_2[D]_F}{1 + K_2[D]_F} \qquad \text{(Eq. 2)}$$

The results are shown in Table V. It is apparent that disopyramide phosphate was bound mainly to the binding sites in the N_1 binding group while quinidine sulfate showed predominant binding to the N_2 group. Furthermore, quinidine sulfate had much higher binding affinities (K_1 = 12,844.5 and K_2 = 3422.5) than disopyramide phosphate (K_1 = 4618.1 and K_2 = 457.2). These observations are in agreement with the observation reported earlier (Tables II and III) that quinidine sulfate was bound to protein molecules 2.4-fold more than disopyramide phosphate.

In the dosage range (3–10 mg/kg) used for antiarrhythmic therapy, the resultant blood levels (10^{-5}–10^{-6} M) of disopyramide phosphate and quinidine sulfate[1] will interact primarily with the N_1 binding group. The results in Table V indicate that the affinity between the binding sites in the N_1 group and quinidine sulfate is 2.78-fold stronger than disopyramide phosphate (from 12,844.5 to 4618.1), even though serum albumins have approximately the same number of binding sites in the N_1 group available to both disopyramide phosphate (3.51) and quinidine sulfate (3.42). Therefore, the magnitude of the N_1K_1 value for quinidine sulfate (43,928.2) is about 2.7-fold greater than that for disopyramide phosphate (16,209.5). The 2.46-fold higher β value for quinidine sulfate than for disopyramide phosphate (Tables II and III) is a quantitative reflection of the 2.7-fold difference in the magnitude of the N_1K_1 values.

Table V—Comparison on Binding Characteristics to Human Serum Albumin between Disopyramide Phosphate and Quinidine Sulfate

Binding Parameters	Disopyramide Phosphate	Quinidine Sulfate
Groups of binding sites		
N_1	3.51	3.42
N_2	0.81	9.88
Binding affinities		
K_1	4618.1	12,844.5
K_2	457.2	3,422.5
N_1K_1[a]	16,209.5	43,928.2

[a] Ratio of N_1K_1 value for quinidine sulfate over that for disopyramide phosphate was 2.7.

Table VI—Physicochemical Parameters for Disopyramide Phosphate and Quinidine Sulfate

Drug	Partition Coefficient[a]	Lipophilicity[b]
Disopyramide phosphate	0.66 (±0.03)	−0.181
Quinidine sulfate	129.9 (±4.5)	+2.114

[a] Measured in 1-octanol–phosphate buffer (pH 7.4) (n = 3). [b] Lipophilicity = log (partition coefficient).

The results of partitioning studies in the system, 1-octanol–phosphate buffer at pH 7.4 (Table VI), demonstrate that quinidine sulfate had a much higher lipophilicity (2.114) than disopyramide phosphate (−0.181). The relative lipophilicity (8), Hansch parameter (π), was estimated as follows:

$$\pi = \log (\text{p.c.})_X - \log (\text{p.c.})_H = 2.114 - (-0.181) = 2.295 \qquad \text{(Eq. 3)}$$

The 2.3–2.5-fold higher drug–protein interaction observed for quinidine sulfate when compared to disopyramide phosphate (Tables II and III) may possibly be correlated with the higher π value for the former (9).

The ratio of percent drug retained by the membrane ultrafilters (quinidine sulfate–disopyramide phosphate) was also 2.5 (20%:8%). Further work with a series of disopyramide derivatives is underway to evaluate the correlation between hydrophobic, nonspecific protein binding and adsorption to polymer membranes.

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