Quinidine and Dihydroquinidine Interactions in Human Plasma

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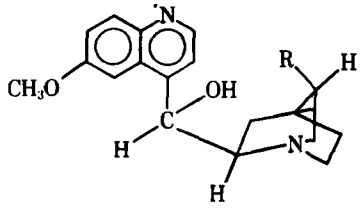
Abstract □ The protein-binding characteristics of dihydroquinidine, a known impurity in drug grade quinidine, in human plasma and the effects of dihydroquinidine on quinidine interactions with these plasma constituents were studied by equilibrium dialysis. In the plasma concentration range of 1.75–23.0 mg/liter, dihydroquinidine binding was similar to the binding observed with quinidine. The data suggested the presence of a single class of binding sites for both compounds in the plasma drug concentration range and samples studied. The mean values for the association constant, K, and the total concentration of binding sites, nP_t, for dihydroquinidine were $4.75 \pm 0.67 \times 10^4\ M^{-1}$ and $5.78 \pm 0.17 \times 10^{-5}\ M$, respectively. The corresponding values for quinidine were $4.78 \pm 1.00 \times 10^4\ M^{-1}$ and $5.65 \pm 0.48 \times 10^{-5}\ M$. In the presence of 5 and 10% (of total alkaloid content) dihydroquinidine, the plasma concentration of unbound quinidine did not change significantly. At a 20% level of dihydroquinidine, however, an increase in unbound quinidine was observed ($p < 0.05$). The elevations in free quinidine concentrations were directly related to the level of dihydroquinidine present. The results of this study indicate that the interactions between dihydroquinidine and quinidine for binding sites on human plasma proteins are competitive.

Keyphrases □ Quinidine—interactions with plasma constituents, effects of dihydroquinidine on binding □ Dihydroquinidine—protein binding, effects on quinidine interactions with plasma constituents □ Interactions—quinidine and dihydroquinidine in human plasma □ Protein binding—quinidine and dihydroquinidine, interactions in plasma

It is well known that by effectively retarding drug movement out of the vascular system, interactions of a drug with plasma proteins can greatly affect the drug's disposition behavior and pharmacological activity (1–3). Quinidine (I), a widely used cardiac antiarrhythmic agent, interacts with various protein components of human plasma. In its therapeutic plasma concentration range of 2–5 μg/ml (4), approximately 80% of the drug is reportedly bound to plasma proteins (5–7). Many studies demonstrated that quinidine interacts with serum albumin (5, 8–11). Nilsen and Jacobsen (12) showed that the drug binds to low and high density plasma lipoproteins. In addition, Skuterud *et al.* (7) suggested the possible interaction of quinidine with α_1- and α_2-serum globulins. Quinidine binding to γ-globulin is reportedly negligible (5).

Although data are available on quinidine binding to plasma proteins, the binding properties of dihydroquinidine (II, a common impurity in drug grade quinidine) to these plasma constituents as well as the effects of dihydroquinidine on quinidine binding in the vascular compartment are not known. The USP (13) presently allows dihydroquinidine levels of up to 20% of the total alkaloidal content in quinidine preparations. In addition, the disposition kinetics of dihydroquinidine and quinidine were similar after intravenous administration of quinidine gluconate containing relatively low levels of the impurity (14).



I: R = CH_2=CH
II: R = CH_3—CH_2

These observations suggest that substantial quantities of the impurity can be present in the blood after quinidine administration. More important, the structural similarities between the two compounds suggest that dihydroquinidine can potentially affect the disposition and activity of quinidine through alterations in drug binding to plasma proteins.

This paper discusses the protein-binding characteristics of dihydroquinidine in human plasma and the effects of this impurity on quinidine interactions with these plasma constituents.

EXPERIMENTAL

Materials—Quinidine base, free of dihydroquinidine, was prepared from quinidine sulfate USP[1] according to the method of Thron and Dirscherl (15).

The dihydroquinidine base, which was separated from the quinidine base, was used after catalytic reduction of the trace levels of quinidine to the dihydro derivative. The hydrogenation method described by Heidelberger and Jacobs (16) was used for reduction. A pure sample of dihydroquinidine was obtained after separation on a silica gel[2] chromatographic column and recrystallization with hot methanol and ether (1:1). This material was identical in its melting point, specific rotation in chloroform, NMR, IR, and fluorescence spectra, and chromatographic behavior in various thin-layer systems to those of a pure dihydroquinidine reference sample[3].

Aqueous solutions of quinidine and dihydroquinidine were prepared with sulfuric acid to facilitate drug solution.

Fresh plasma[4] was obtained from 12 healthy and drug-free adult volunteers after separation of the red blood cell fraction.

Methods—The binding of quinidine and dihydroquinidine to plasma proteins was studied by equilibrium dialysis. The apparatus[5] used consisted of 20 polytef cells, each of which was divided into two 1-ml compartments (half-cells) by cellulose tubing[6]. Plasma, 1 ml, was dialyzed against 1 ml of pH 7.4 Krebs–Ringer bicarbonate buffer at 37 ± 0.5° for 3.5 hr. Preliminary investigations indicated that the distribution equilibrium was reached in 3 hr and that the binding of quinidine or dihydroquinidine to the dialysis membrane and/or cell surfaces was less than 7%.

After time for equilibration, the solutions in the buffer and plasma half-cells were removed and assayed for their drug content. The unbound drug fraction was determined by dividing the concentration of drug in the buffer half-cell, which contained unbound drug only, by the drug concentration in the corresponding plasma half-cell.

[1] New York Quinine and Chemical Works, New York, N.Y.
[2] EM Reagents, Brinkmann Instruments, Westbury, N.Y.
[3] Supplied by Fluka AG, Buch SC, Switzerland. This material can be purchased commercially from this company.
[4] Blood Bank, University Hospital, Omaha, Neb.
[5] Dianorm equilibrium dialyzing system, Innovati-Medizin AG, CH-Esslingen, Switzerland.
[6] Spectrapor 1 membrane tubing, Spectrum Medical Instruments, Los Angeles, Calif.

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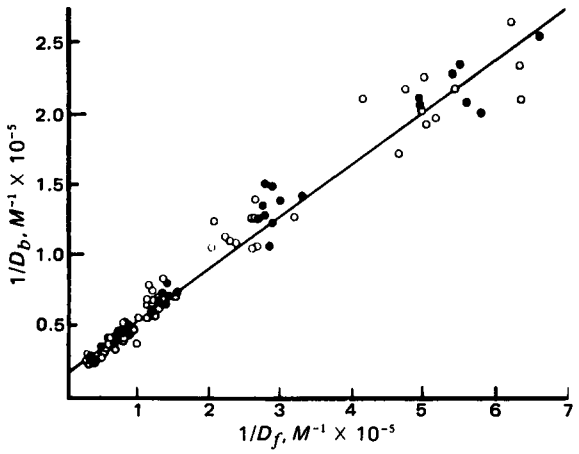

Figure 1—*Relationship between the reciprocal of the bound drug concentration, $1/D_b$, and the reciprocal of the free drug concentration, $1/D_f$, of dihydroquinidine (●) and quinidine (○). Slope and intercept values were 0.36 ± 0.04 and 1.73 ± 0.17 × 10^4 M^{-1} for dihydroquinidine and 0.37 ± 0.05 and 1.77 ± 0.29 × 10^4 M^{-1} for quinidine, respectively.*

Dihydroquinidine binding to plasma proteins was studied with predialysis plasma drug concentrations in the range of 9.2 × 10^{-3}–9.2 × 10^{-2} mM. These concentrations correspond to 3–30 mg/liter of plasma. Double reciprocal plots (17) in the form of Eq. 1 were used to evaluate the binding data:

$$\frac{1}{D_b} = \frac{1}{nKP_t}\frac{1}{D_f} + \frac{1}{nP_t} \qquad \text{(Eq. 1)}$$

where D_b and D_f are the molar concentrations of bound and unbound drug, respectively; n is the number of binding sites on each protein molecule; K is the association constant for the drug–protein complex; and P_t is the total protein concentration.

Equation 1, which assumes the existence of a single class of binding sites, shows that a linear graph is obtained when $1/D_b$ is plotted as a function of $1/D_f$. Therefore, the binding parameters (K and nP_t) can be obtained from the slope and $1/D_b$ axis intercept values of the line. In this way, no direct knowledge of the total protein concentration is required for the analysis of the binding data; the product nP_t reflects the total concentration of binding sites in the plasma sample.

Two types of binding experiments were performed to investigate the competitive influences of dihydroquinidine on the interactions of quinidine with plasma proteins. In the first series, the effects of 0, 5, 10, and 20% (of the total alkaloidal content) dihydroquinidine on quinidine binding were examined. The data were evaluated by assessing the unbound quinidine fraction as a function of the total plasma drug concentration in the 1.2–23.7-mg/liter range.

To evaluate the potential for competitive binding interactions, constant dihydroquinidine concentrations of 2.25 × 10^{-6}, 4.5 × 10^{-6}, and 6.0 × 10^{-6} M were used in the second series with predialysis plasma quinidine concentrations varying between 9.2 × 10^{-3} and 9.2 × 10^{-2} mM. The results were analyzed as previously reported (11, 18, 19).

Dihydroquinidine and quinidine (in the absence of the impurity) in buffer and plasma were assayed by the double-extraction method of Cramer and Isaksson (20). In the presence of the impurity, quinidine concentrations were determined with the previously reported TLC–fluorometric procedure (21).

The data are reported as the mean ± SD. Statistical evaluations were performed using the Student t test.

RESULTS AND DISCUSSION

Dihydroquinidine Binding Studies—Figure 1 summarizes the binding of dihydroquinidine in human plasma when the observed drug concentration ranged from 1.75 to 23.0 mg/liter and the bound drug fraction varied between 0.75 and 0.45. The linearity of this double reciprocal plot supported the assumption of a single class of binding sites for dihydroquinidine in the plasma drug concentration range and samples studied and, hence, the appropriateness of Eq. 1 to describe the binding data. After linear regression analysis of the data obtained for each individual plasma sample, the best values of K and nP_t were 4.75 ± 0.67 × 10^4 M^{-1} and 5.78 ± 0.17 × 10^{-5} M, respectively. The corresponding values for quinidine were 4.78 ± 1.00 × 10^4 M^{-1} and 5.65 ± 0.48 × 10^{-5} M.

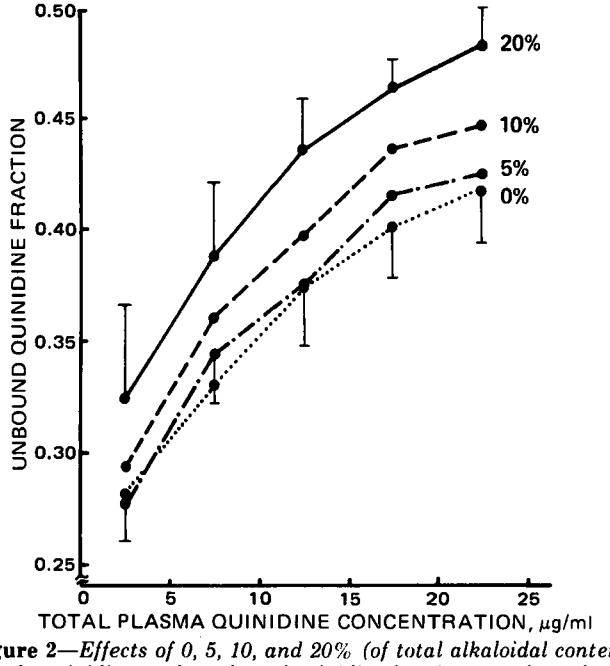

Figure 2—*Effects of 0, 5, 10, and 20% (of total alkaloidal content) dihydroquinidine on the unbound quinidine fraction at various plasma quinidine concentrations.*

The binding characteristics of dihydroquinidine and quinidine in human plasma were virtually identical (Fig. 1). Furthermore, no differences in the binding parameters were observed between the two drugs. The data suggest that both compounds interact with the same sites and protein fractions in plasma. Moreover, these observations suggest that the free plasma quinidine concentration could be elevated in the presence of dihydroquinidine and that the increases would be directly related to the levels of impurity in the initial quinidine sample (*e.g.*, tablet or capsule).

Competitive Binding Studies—The effects of 0, 5, 10, and 20% (of total alkaloid content) dihydroquinidine on the interactions of quinidine with plasma proteins are presented in Fig. 2. Plasma quinidine concentrations in the ranges of 0–4.99, 5–9.99, 10–14.99, 15–19.99, and 20–24.99 mg/liter were graphed as 2.5, 7.5, 12.5, 17.5, and 22.5 mg/liter, respectively.

In the drug concentration range of 2–23 mg/liter and in the absence of impurity, the free, unbound quinidine fraction varied between 0.28 ± 0.02 and 0.42 ± 0.02. In the presence of 5 and 10% dihydroquinidine, although the unbound quinidine fraction was generally slightly greater,

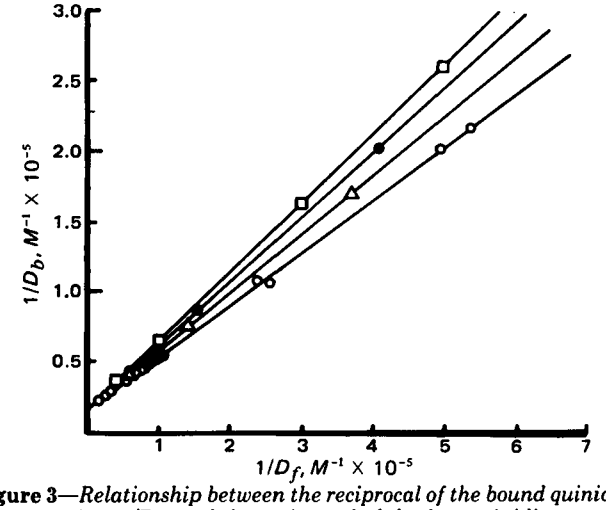

Figure 3—*Relationship between the reciprocal of the bound quinidine concentration, $1/D_b$, and the reciprocal of the free quinidine concentration, $1/D_f$, in the absence (○) and presence of 2.25 × 10^{-6} (△), 4.5 × 10^{-6} (●), and 6.0 × 10^{-6} (□) M of dihydroquinidine.*

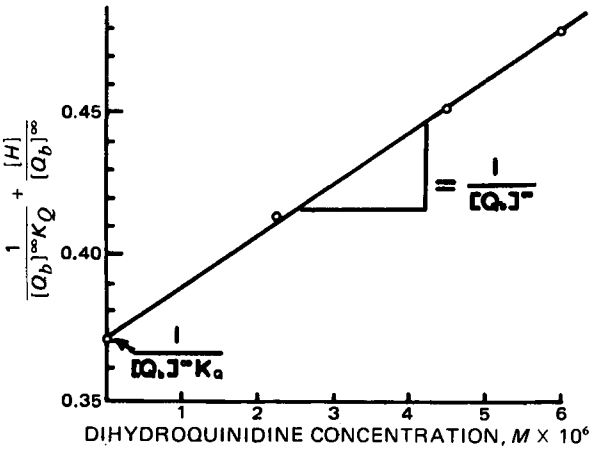

Figure 4—*Relationship between the slope of the individual $1/D_b$ versus $1/D_f$ curves in Fig. 3, obtained by linear regression analysis, and the corresponding dihydroquinidine concentration. See text for discussion.*

the differences were not significant. At the 20% level of the impurity, however, the free quinidine concentrations were increased ($p < 0.05$). Moreover, these increases were approximately those that would have been predicted assuming equivalent affinities and competitive interactions. For example, at a concentration of 23 mg/liter and in the absence of dihydroquinidine, approximately 60% of the total plasma quinidine concentration was present in the bound form. The presence of 20% dihydroquinidine produced an additional 6% (0.5 × 20% × 60%) increase in the free quinidine fraction (from 0.42 to 0.48).

The results of the second series of experiments are summarized in Figs. 3 and 4. In the presence of constant levels of dihydroquinidine of 2.25×10^{-6}, 4.5×10^{-6}, and 6.0×10^{-6} M, the quinidine fraction bound to plasma proteins decreased with increasing dihydroquinidine concentrations. Furthermore, all double reciprocal graphs of the binding data for quinidine at each impurity level were shown (Fig. 3) to intercept the $1/D_b$ coordinate at essentially the same point (approximately 1.75×10^4 M^{-1}). These observations support the conclusion that dihydroquinidine and quinidine interactions for human plasma protein are competitive (11, 18, 19).

Additional evidence was obtained in the following manner. If the interactions of dihydroquinidine and quinidine for binding to human plasma proteins are competitive, the process can be described by:

$$[Q_b] = \frac{[Q_b]^\infty K_Q [Q_f]}{1 + K_Q[Q_f] + K_H[H]} \qquad \text{(Eq. 2)}$$

Rewritten in its reciprocal form, Eq. 2 becomes:

$$\frac{1}{[Q_b]} = \frac{1}{[Q_b]^\infty K_Q [Q_f]} + \frac{K_H[H]}{[Q_b]^\infty K_Q [Q_f]} + \frac{1}{[Q_b]^\infty} \qquad \text{(Eq. 3)}$$

where K_Q and K_H are the affinity constants for plasma proteins of quinidine and dihydroquinidine, respectively; $[Q_b]$ and $[Q_f]$ are the bound and free plasma quinidine concentrations, respectively; $[Q_b]^\infty$ is the maximum concentration of bound quinidine at saturation; and $[H]$ is the plasma concentration of dihydroquinidine.

If it is assumed that dihydroquinidine and quinidine have equivalent affinities for the plasma proteins as the data suggest, then $K_H = K_Q$ and Eq. 3 reduces to:

$$\frac{1}{[Q_b]} = \left[\frac{1}{[Q_b]^\infty K_Q} + \frac{[H]}{[Q_b]^\infty}\right]\frac{1}{[Q_f]} + \frac{1}{[Q_b]^\infty} \qquad \text{(Eq. 4)}$$

It can be seen from Eq. 4 that the slope of the line for each quinidine-binding curve in the presence of dihydroquinidine is a linear function of the competitor concentration. Therefore, a plot of

$$\frac{1}{[Q_b]^\infty K_Q} + \frac{[H]}{[Q_b]^\infty}$$

the slope of each regression line obtained by a given concentration of dihydroquinidine, *versus* $[H]$ should be a straight line with a slope of $1/[Q_b]^\infty$ and an intercept of $1/[Q_b]^\infty K_Q$ if the interactions are competitive.

A plot of this relationship is shown in Fig. 4; a linear relationship, indicative of competitive binding interactions between dihydroquinidine and quinidine, was obtained. Furthermore, by using the slope (1.82×10^4 M^{-1}) and intercept (0.37) values obtained by regression analysis of this curve, a value of 4.92×10^4 M^{-1} was computed for K_Q, which is in excellent agreement with the previous studies using quinidine alone ($K_Q = 4.78 \pm 1.00 \times 10^4$ M^{-1}).

Although the binding of quinidine and dihydroquinidine in human plasma might have been suspected to be similar and their interactions competitive because of their structural similarities, this study presents definitive evidence to support these conclusions. The observed competitive effects of dihydroquinidine on quinidine binding suggest that they are directly related to the levels of impurity in the initial quinidine sample. The therapeutic importance of these observations, however, is presently not known. *A priori*, it appears that they are of little or no clinical significance in view of the generally low levels (<7%) of dihydroquinidine in commercial quinidine preparations.

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