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EFFECTS OF DIHYDROQUINIDINE ON *IN VITRO* AND *IN VIVO* QUINIDINE DISPOSITION*

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Abstract—The effects of dihydroquinidine on the metabolism of quinidine by 10,000 *g* supernatant fractions of rabbit and rat liver homogenates and on the disposition of quinidine in the rabbit were investigated. From Lineweaver–Burk plots, the following mean ± S.E.M. values for V_{max} and K_m for quinidine and K_i for dihydroquinidine for the rabbit liver preparations were obtained: 240 ± 50 nmoles/min/g liver, 0.55 ± 0.14 mM, and 0.56 ± 0.09 mM respectively. The corresponding values for the rat liver homogenates were 74 ± 6 nmoles/min/g liver, 0.12 ± 0.02 mM and 0.14 ± 0.06 mM. From Dixon plots, the following values for K_i for dihydroquinidine were obtained: 0.54 ± 0.09 mM for the rabbit liver preparations and 0.14 ± 0.04 mM for the rat liver homogenates. In rabbits pretreated with dihydroquinidine for an average of 26 days, no changes in the distribution and elimination half-life values for quinidine, total body clearance, or apparent volume of drug distribution were observed when compared to the control quinidine disposition constants obtained in the same animals. As their structures suggested, the data showed that the interactions of dihydroquinidine and quinidine during drug metabolism by rabbit and rat liver 10,000 *g* supernatant fractions were competitive. Additionally, the affinities of dihydroquinidine and quinidine for the drug-metabolizing enzymes in these preparations were the same. The data also suggested that the small amounts of dihydroquinidine normally found in quinidine preparations probably have no significant effect on the disposition of quinidine in the body when therapeutic doses of the drug are used.

Quinidine, a widely used cardiac antiarrhythmic agent, is known to contain varying quantities of dihydroquinidine and other congeneric alkaloids as impurities [1, 2]. While the amounts of the latter alkaloids in quinidine preparations are relatively small, the levels of dihydroquinidine have been reported to be as high as 24 per cent of the total alkaloid content [1, 2]. Structurally, quinidine and dihydroquinidine are virtually identical, dihydroquinidine differing from quinidine only by saturation of the vinyl side chain of the quinuclidine ring system. They possess similar physicochemical properties [3–8], pharmacologic activities [1, 9–12], binding characteristics to plasma proteins [13], and disposition kinetics after intravenous administration [14].

In man, drug metabolism is the principal mechanism for the removal of quinidine from the body. Only 10–20 per cent of a given quinidine dose is excreted in the urine [15–17]. Quinidine metabolites that have been identified to date include 3-hydroxyquinidine [18], 2′-oxoquinidinone [18] and *o*-desmethylquinidine [19]. Additionally, Palmer *et al.* [20] have suggested that quinidine and some of its metabolites are eliminated as conjugated drug species. Presumably, the metabolism and excretion patterns of dihydroquinidine are similar to quinidine, based on present information [14].

From these observations coupled with the report that quinidine and quinine inhibited the metabolism of pentobarbital by hepatic microsomal enzymes [21], it was of interest to investigate the potential effects of dihydroquinidine on the biotransformation of quinidine. A change in the rate of quinidine metabolism could be quite significant clinically, since this drug is effective only within a narrow drug concentration range of 2–5 mg/l in the plasma [22].

This paper describes the effects of dihydroquinidine on the *in vitro* metabolism of quinidine by the rabbit and rat liver homogenates and *in vivo* quinidine disposition in the rabbit.

MATERIALS AND METHODS

Drugs. Quinidine, free of the dihydro analog, was prepared from quinidine sulfate, USP powder, according to the method of Thron and Dirscherl [23]. The separated dihydroquinidine was used after purification, as described previously [13]. Aqueous solutions of quinidine and dihydroquinidine were prepared from their sulfate salt forms. All drug concentrations and doses are expressed in terms of the free base.

Enzyme preparation. Male New Zealand white rabbits (3–4 kg) and male Sprague–Dawley rats (180–250 g) were used. All studies were initiated between 9:00 and 10:00 a.m. of each experimental day after overnight fasting. Following placement in a restraining cage, the rabbits were killed by injecting a 10–20 cm^3 bolus of air into a marginal ear vein. Rats were killed by cervical dislocation.

Following a midline incision, the liver was rapidly excised and placed in a beaker on ice. Approximately 60 g of liver was accurately weighed, minced with a stainless-steel razor blade, and homogenized in a cold room with 2 vol. of ice-cold 1.15% KCl in a

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chilled, Potter–Elvehjem homogenizer with a motor-driven polyteflon pestle. In studies involving rats, the livers of several animals were pooled. The homogenates were centrifuged for 30 min at 4° in a Sorvall model RC2-B centrifuge to obtain the 10,000 *g* supernatant fraction.

In vitro *quinidine metabolism.* Incubation mixtures contained 10,000 *g* supernatant fraction equivalent to 667 mg liver, NADP (1 μmole), glucose 6-phosphate (20 μmoles), $MgCl_2$ (25 μmoles), nicotinamide (20 μmoles), varying quantities of quinidine and dihydroquinidine, and 0.1 M potassium phosphate buffer (pH 7.4) added to produce a final volume of 5 ml. Quinidine concentrations of 12.5, 25, 50 and 100×10^{-2} μmoles per 5 ml of incubation mixture and dihydroquinidine concentrations of 3, 6, 12 and 50×10^{-2} μmoles per 5 ml of incubation mixture were used. The samples were incubated in a Dubnoff metabolic incubator in the presence of air at 37° for 15 min at a shaking rate of approximately 100 oscillations/min. Reactions were terminated by the addition of 0.5 ml of 5 N NaOH, and the disappearance of quinidine was measured. All determinations were performed in duplicate and the results averaged. The analysis for quinidine was done as described previously [16].

The values of V_{max} and K_m for quinidine and K_i for dihydroquinidine were determined by linear regression analyses of Lineweaver–Burk plots. Dixon plots were also constructed and used to evaluate the effects of dihydroquinidine on the metabolism of quinidine.

In vivo *quinidine disposition.* Male New Zealand white rabbits weighing 4–5 kg were used. Each animal was given a quinidine dose of 4–5 mg/kg intravenously before and after treatment with dihydroquinidine. In this manner, each animal served as its own control. While in a restraining cage, a vein infusion set with winged adapter (no. 2COO75, Travenol Laboratories, Deerfield, IL) was inserted into a marginal ear vein and used to give doses and collect blood samples (0.5 ml) for drug analysis [16]. To insure that the total quinidine dose was given, the contents of the infusion set were flushed with heparinized normal saline (5 units/ml). Blood specimens were collected in heparinized tubes at 0, 5, 10, 15 and 30 min and at 0.5-hr intervals for up to 4–5 hr after the dose. Patency of the sampling set was maintained by periodic flushing with heparinized saline.

To evaluate the effects of dihydroquinidine on the *in vivo* disposition of quinidine, 1 day after receiving the control quinidine, each animal was treated with dihydroquinidine, according to the following schedule, for an average of 26 days (range: 11–41): 50 mg dihydroquinidine daily by subcutaneous injection and 25 mg dihydroquinidine base in oil given subcutaneously every 5 days. At the end of this treatment period, a second i.v. quinidine injection was given in the manner described above. This dose was the same as the control.

In all studies, after administration of the quinidine i.v. doses, blood quinidine concentrations declined biexponentially when the data were plotted on semilogarithmic graph paper. The disposition characteristics of quinidine before and after treatment with dihydroquinidine were, therefore, evaluated by fitting the blood drug concentration–time data to the following two-term exponential equation, using the nonlinear least squares regression program NONLIN [24].

$$Cp = Ae^{-\alpha t} + Be^{-\beta t}. \quad (1)$$

Cp is the blood drug concentration at time t, α and β are first-order rates constants for the fast and slow disposition processes, respectively, and A and B are the ordinate axis intercepts for the two processes. Total body clearance (Cl) was computed by:

$$Cl = \frac{\text{Dose}}{(A/\alpha + B/\beta)}. \quad (2)$$

Apparent volume of distribution (Vd) was determined by:

$$Vd = Cl/\beta. \quad (3)$$

Half-life values for drug distribution ($T_{\frac{1}{2}\alpha}$) and elimination ($T_{\frac{1}{2}\beta}$) were calculated according to:

$$T_{\frac{1}{2}\alpha} = 0.693/\alpha \text{ and } T_{\frac{1}{2}\beta} = 0.693/\beta. \quad (4)$$

Student's paired *t*-test was used to assess the significance of the observed differences.

RESULTS

In vitro *quinidine metabolism.* The following mean ± S.E.M. values for V_{max} and K_m were obtained for the metabolism of quinidine by the 10,000 *g* rabbit liver supernatant fraction (N = 8): 240 ± 50 nmoles/min/g liver and 0.55 ± 0.14 mM. The corresponding values observed for the rat liver homogenates (N = 8) were 74 ± 6 nmoles/min/g liver and 0.12 ± 0.02 mM respectively.

Using Lineweaver–Burk plots, the effects of 0.1 mM dihydroquinidine on the metabolism of quinidine by the 10,000 *g* rabbit and rat liver supernatant fractions are presented in Figs. 1 and 2. From the slopes of these graphs, the computed values for the constant, K_i, were 0.56 ± 0.09 mM for the rabbit homogenates and 0.14 ± 0.06 mM for the rat liver preparation.

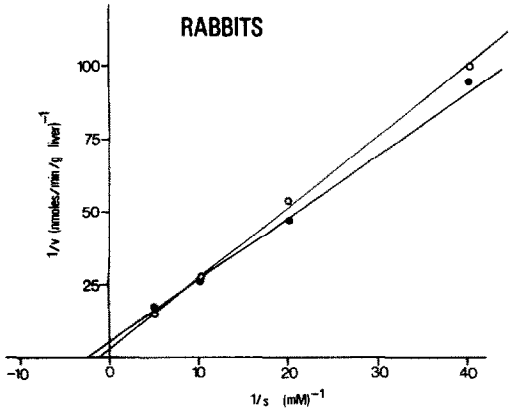

Fig. 1. Lineweaver–Burk plots of the metabolism of quinidine (closed circles, N = 8) and its interaction with 0.1 mM dihydroquinidine (open circles, N = 7) for the 10,000 *g* rabbit liver supernatant fraction.

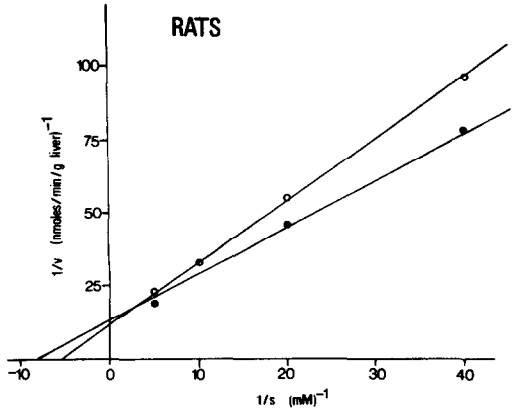

Fig. 2. Lineweaver–Burk plots of the metabolism of quinidine (closed circles, N = 8) and its interaction with 0.1 mM dihydroquinidine (open circles, N = 8) for the 10,000 *g* rat liver supernatant fraction.

With 3, 6 and 12 × 10^{-2} μmoles of dihydroquinidine in the starting incubation mixture, linear regression analysis of Dixon plots resulted in the following mean ± S.E.M. values for K_i in the rabbit and rat liver homogenate preparations, respectively: 0.54 ± 0.09 (N = 6) and 0.14 ± 0.04 mM (N = 5). These values of K_i for dihydroquinidne were similar to the K_m constants observed for the quinidine in the respective liver homogenate preparations.

In vivo *quinidine disposition.* The effects of dihydroquinidine on the *in vivo* disposition of quinidine in rabbits are summarized in Table 1. When compared to the pretreatment control values, no significant differences were observed in the distribution (1.4 vs 1.5 min) and elimination (119.1 vs 124.9 min) half-life values for quinidine, total body clearance rate (86.4 vs 105.2 ml/min), or apparent volume of drug distribution (13.2 vs 17.3 l.) following treatment with dihydroquinidine for an average of 26 days. In these studies, using the assay method previously reported from this laboratory [14], the plasma dihydroquinidine concentrations were above 1 mg/l throughout each experiment.

DISCUSSION

The structural similarities of dihydroquinidine and quinidine suggested *a priori* that the two compounds were substrates for the same drug-metabolizing enzymes of the liver. Therefore, drug metabolism studies were performed to characterize the nature of the interaction between the two agents. The control and dihydroquinidine-treated Lineweaver–Burk plots shown in Figs. 1 and 2 for the metabolism of quinidine by the 10,000 *g* liver supernatant fractions of the rabbit and rat, respectively, intercepted the 1/*v* axis at essentially the same point ($P > 0.05$). Additionally, from Lineweaver–Burk and Dixon plots, no differences were seen between the computed values of K_m for quinidine and K_i for dihydroquinidine with the respective enzyme preparations. These observations demonstrate that the interaction between dihydroquinidine and quinidine for drug metabolism in the study was competitive. Furthermore, they reveal that the two compounds possessed similar affinities for the quinidine-metabolizing enzymes of the rabbit and rat liver. On the basis of these findings, it is reasonable to assume that dihydroquinidine was capable of altering the rate of quinidine removal by the intact liver.

With the present data, it is possible to estimate that saturation of the quinidine-metabolizing enzymes would occur at a liver concentration of about 1.1 mM in the rabbit and 0.24 mM in the rat, or approximately 360 and 80 mg/l respectively. It can be seen that these values are many times greater than the plasma quinidine concentrations of approximately 2–5 mg/l [22] that are reportedly required for antiarrhythmic activity with the drug. In view of a previously observed [25] liver/plasma ratio for quinidine in dogs of 17 to 39, these findings would suggest that it would be very unlikely that the capacity of the drug-metabolizing enzymes would be exceeded with the doses of quinidine used clinically.

In order to evaluate the effects of dihydroquinidine on quinidine disposition *in vivo*, rabbits were pretreated with dihydroquinidine for an average of 26 days and subsequently given a dose of quinidine intravenously. The plasma dihydroquinidine concentrations in all studies were always higher than 1 mg/l, which is considerably greater than the levels that might be seen in man on a standard quinidine dosage regimen. In these studies, pretreatment with relatively high doses of dihydroquinidine had no effect on the distribution and elimination behaviour of quinidine (Table 1). The absence of any effects by dihydroquinidine on the elimination half-life and clearance rate of quinidine is consistent with the *in vitro* findings which suggest that the capacity of the quinidine-metabolizing enzymes of the rabbit liver is much greater than the concentrations that could be produced by therapeutic doses of quinidine.

In this study, a species difference in the metabolism of quinidine by the 10,000 *g* liver supernatant fractions was observed. The capacity of the drug-metabolizing enzymes was observed to be greater in the rabbit liver preparation (240 vs 74 nmoles/min/g liver). Drug-metabolizing enzymes of rat liver homogenates, on the other hand, appeared to have a greater affinity for quinidine than the enzymes of the

Table 1. Quinidine disposition constants for control and dihydroquinidine-treated rabbits*

Treatment	$T_{\frac{1}{2}\alpha}$ (min)	$T_{\frac{1}{2}\beta}$ (min)	*Cl* (ml/min)	*Vd* (l.)
Control	1.4 ± 0.5	119.1 ± 66.3	86.4 ± 40.1	13.2 ± 5.5
Dihydroquinidine	1.5 ± 0.2†	124.9 ± 56.8†	105.2 ± 32.1†	17.3 ± 6.3†

* Mean ± S.D., N = 5.
† No significant difference at the 0.05 level.

rabbit preparation (0.12 vs 0.55 mM). Furthermore, the intrinsic clearance of quinidine, calculated according to the method of Rane *et al.* [26] was also greater for the 10,000 *g* rat liver fraction (0.62 vs 0.44 ml/min/g liver).

No attempt was made in this study to determine if the competition for hepatic quinidine metabolism by dihydroquinidine occurred equally for all biotransformation pathways or if the process was selective. Additionally it is not known whether dihydroquinidine is biotransformed into metabolites which are similar to those reported [18–20] for quinidine. Huynh-Ngoc and Sirois [27] have suggested that dihydroquinidine and quinidine might possess different disposition characteristics in the body by virtue of their differing physicochemical properties. Thus, it is possible that the effects of dihydroquinidine seen here might be acting selectively. Nevertheless, the findings of this study suggest that the small amounts of dihydroquinidine that are presently seen in commercial quinidine preparations will probably have little effect on the elimination of quinidine in man.

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