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Technical Note

Stereoselective Binding of Disopyramide to Plasma Proteins

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INTRODUCTION

Disopyramide, a class I antiarrhythmic agent, is marketed as a racemic mixture of two optical isomers, S(+)- and R(−)-disopyramide. Studies from our laboratories have suggested that S(+)-disopyramide is a more potent compound in terms of its anticholinergic (1) and antiarrhythmic effects (2). Previously, we (3) and others (4,5) have reported that racemic disopyramide exhibits concentration-dependent binding to plasma proteins in the therapeutic plasma concentration range. Several studies have also demonstrated that, in the therapeutic plasma concentration range, the binding of both enantiomers of disopyramide is dependent upon the plasma concentration (6–10). The S(+)-enantiomer is more avidly bound than the R(−)-enantiomer, suggesting the possibility of some interesting and important interactions when the enantiomers are administered together. Accordingly, in a recent study, we determined the pharmacokinetics of S(+)-disopyramide and R(−)-disopyramide in humans following administration of the enantiomers separately and as a pseudoracemic mixture (10). The data demonstrated that there were significant differences in the pharmacokinetics of the R(−)- and S(+)-enantiomers, with the R(−)-enantiomer having a higher clearance (CL) and larger apparent volume of distribution (V) than the S(+)-enantiomer. We also observed that the CL of R(−)-disopyramide was higher when the pseudoracemate was administered than when the enantiomer was administered alone, whereas the CL of S(+)-disopyramide was lower when the pseudoracemate was administered than when the S(+)-enantiomer was administered alone. A possible explanation for these observations is that there is an *in vivo* interaction between the two enantiomers with respect to plasma protein binding.

In this study, we examined the interaction of R(−)- and S(+)-disopyramide in human plasma. Although the binding of the individual enantiomers has been studied previously, there have been no studies of the interaction between the enantiomers. In addition, the binding of R(−)- and S(+)-disopyramide to human serum albumin and α_1-acid glycoprotein was studied to ascertain the stereoselectivity of the interaction at each plasma protein.

EXPERIMENTAL

The R(−)- and S(+)-disopyramide were resolved from racemic disopyramide by the method of Burke *et al.* (11). Purity (greater than 98%) was ascertained by measuring optical rotation and determining melting points. After resolution, each enantiomer was tritiated commercially (New England Nuclear, Boston, Mass.) [specific activity was 125 mCi/mmol for ^{3}H-S(+)-disopyramide and 111 mCi/mmol for ^{3}H-R(−)-disopyramide] and purified by high-performance liquid chromatography.

To study the interaction between R(−)- and S(+)-disopyramide, we collected blood from a single healthy volunteer. Plasma was obtained by centrifugation and stored frozen (−20°C). One-milliliter plasma samples were dialyzed at 37°C against 1 ml of isotonic Sorensen's phosphate buffer (pH 7.4) containing 0.018 μCi of ^{3}H-S(+)-disopyramide or ^{3}H-R(−)-disopyramide and various amounts of unlabeled S(+)- or R(−)-disopyramide to produce concentrations that encompassed the therapeutic plasma concentration range of disopyramide. The fraction unbound of R(−)- and S(+)-disopyramide was determined in the presence of various ratios of the S(+)- to the R(−)-enantiomer. The ratios of S(+)- to R(−)- and R(−)- to S(+)-disopyramide ranged between 0.25 and infinity. Infinity implies that only one enantiomer was present. Dialysis was carried out at 37°C as previously described (10). Preliminary experiments documented that tritium exchange did not occur during the procedure. The fraction unbound (fu) was calculated as the concentration of radioactivity in the buffer divided by the concentration of radioactivity in the plasma. A small volume shift was observed in most samples, and therefore the equilibrium concentration of unlabeled drug in plasma was cal-

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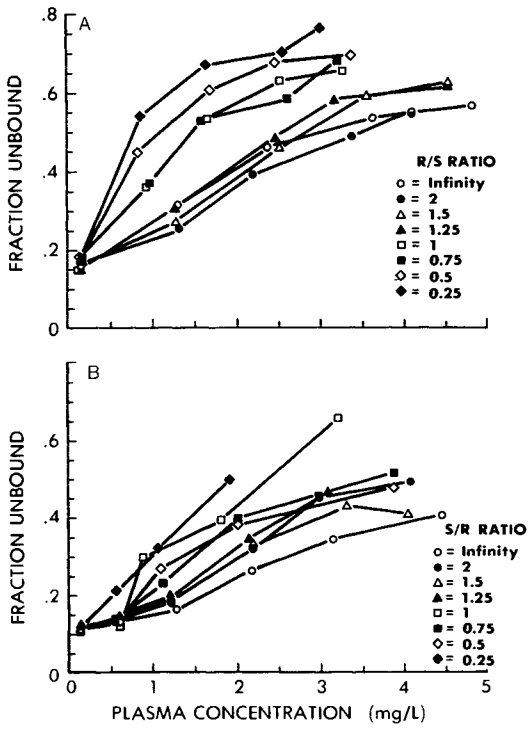

Fig. 1. The binding of each enantiomer in the presence of the opposite enantiomer at the absolute concentrations and ratios are shown. Data from plasma obtained from a single subject are shown. (A) R(−)-Disopyramide binding in the presence of S(+)-disopyramide; S(+)-disopyramide binding in the presence of R(−)-disopyramide. The concentration indicates the postdialysis plasma concentration of the R(−)-enantiomer (a) and the S(+)-enantiomer (B). A ratio of infinity implies that only one enantiomer is present.

culated using the specific activity of the total drug in each sample as previously described (12).

We also carried out experiments to examine the interaction of each enantiomer with albumin and α_1-acid glycoprotein. The studies were carried out as described for the plasma protein binding experiments with the following differences. Human α_1-acid glycoprotein (human orosomucoid, Sigma Chemical Company, St. Louis, Mo.) at a concentration of 110 mg/dl or human serum albumin (crystallized and lyophilized, Sigma Chemical Company, St. Louis, Mo.) at a concentration of 4 g/dl was substituted for plasma in the dialysis experiments. Equilibrium concentrations of S(+)- and R(−)-disopyramide in the protein solutions were low, ranging between 0.001 and 0.025 μg/ml.

RESULTS AND DISCUSSION

Figures 1a and b depict the fraction unbound of R(−)-disopyramide (Fig. 1a) and S(+)-disopyramide (Fig. 1b) as a function of the total postdialysis concentration of each enantiomer, respectively, in the plasma of a single volunteer. Each curve represents a different initial ratio of R(−)- to S(+)-enantiomer. At any given total R(−)-disopyramide concentration a higher ratio of S(+) to R(−) was associated with a larger fraction unbound of R(−)-dispyramide, suggesting that the enantiomers are interacting at the same site and that S(+)-disopyramide can displace R(−)-disopyramide. Similarly, R(−)-disopyramide could displace S(+)-disopyramide although to a lesser extent (Fig. 1b).

The binding of disopyramide to both human serum albumin and α_1-acid glycoprotein was stereoselective. In the human serum albumin solution the fraction unbound (mean ± SD) of S(+)- and R(−)-disopyramide was 0.712 ± 0.012 and 0.883 ± 0.036 ($P < 0.001$), respectively. In α_1-acid glycoprotein the fraction unbound of S(+)- and R(−)-disopyramide was 0.319 ± 0.085 and 0.496 ± 0.088 ($P < 0.001$), respectively.

In this study, we examined the interaction of the enantiomers and observed that each enantiomer could displace the other, suggesting that the two enantiomers interact at the same site. Consistent with its greater apparent affinity constant, S(+)-disopyramide (8) was a more potent displacing agent than R(−)-disopyramide (Fig. 1).

These studies may provide an explanation for the observation in our *in vivo* study (10) that there was a higher CL and larger V of the R(−)-enantiomer when the S(+)-enantiomer was present. The fu of the R(−)-enantiomer is greater in the presence of the S(+)-enantiomer, resulting in a higher CL and a larger V. A similar explanation can be put forth to explain the higher CL and larger V of the S(+)-enantiomer when administered alone. The R(−)-enantiomer weakly displaces the S(+)-enantiomer and the fu of the S(+)-enantiomer is actually greater at a given total disopyramide concentration when only the S(+)-enantiomer is present.

Previous studies have demonstrated that disopyramide binds primarly to α_1-acid glycoprotein (13,14). Binding to albumin has also been described, but it has been suggested that this binding is primarily to small amounts of α_1-acid glycoprotein present in albumin solutions (14). Our data demonstrated that the binding to both proteins was stereoselective; however, the fraction bound to human serum albumin was small. These results are consistent with contamination of human serum albumin with small quantities of α_1-acid glycoprotein. The data are in agreement with results obtained by Lima *et al.* (6) demonstrating stereoselective binding of disopyramide to α_1-acid glycoprotein but do not agree with results of Huang and Øie (15), who observed that the binding of R(−)- and S(+)-disopyramide to human α_1-acid glycoprotein was not stereoselective (15).

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