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Protein binding of disopyramide—displacement by mono-*N*-dealkyldisopyramide and variation with source of α-1-acid glycoprotein

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The binding of disopyramide to human serum proteins and human α-1-acid glycoprotein (AAG) was determined over a wide drug concentration range. Addition of $3{\cdot}7 \times 10^{-6}$ mol litre^{-1} mono-*N*-dealkyldisopyramide caused a 20–100% increase in disopyramide free fraction. The disopyramide free fraction in AAG solutions prepared from various commercially available sources of α-1-acid glycoprotein varied up to 2·5 fold at corresponding disopyramide concentrations. Pronounced differences in the calculated binding constants (affinity and capacity) were observed among the commercially available AAG preparations. These findings suggest that binding studies should be performed in appropriately harvested human serum or plasma to avoid possible artifacts associated with the use of commercial preparations of α-1-acid glycoprotein for binding studies.

The free (unbound) fraction of disopyramide in human serum is concentration-dependent at concentrations achieved clinically (Meffin et al 1979; Lima et al 1981; Giacomini et al 1982). Moreover, the free fraction values of disopyramide vary several fold at any given concentration of the drug in human serum and tend to decrease as serum concentrations of α-1-acid glycoprotein (AAG) increase (Haughey & Kraft 1984). A recent study (Bredesen et al 1982) demonstrates that the free fraction of disopyramide in human serum increases almost 2-fold following the addition of 14·4 μmol litre^{-1} of mono-*N*-dealkyldisopyramide. These studies and others (Hinderling et al 1974; Chen-Sharps et al 1981) prompted the present investigation to characterize the protein binding of disopyramide in human serum and solutions of AAG in the presence of mono-*N*-dealkyldisopyramide (a major metabolite of disopyramide) and to determine the value of using AAG for drug protein binding displacement studies. Because many basic and acidic compounds are known to bind to AAG (Urien et al 1982), we sought to determine whether commercially available AAG would provide a convenient protein source for such binding studies.

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Methods

Whole blood (50 ml) was obtained from four normal volunteers by venepuncture and allowed to clot in glass centrifuge tubes at room temperature (20 °C). The resulting serum was harvested and adjusted to pH 7·4 with 5–15 μl concentrated H_2SO_4 (Mallinckrodt Inc., Paris, Kentucky, USA). Solutions of AAG were prepared in phosphate buffer (Lima et al 1981) at pH 7·4 using commercially available material (Sigma Chemical Co., St. Louis, MO, USA, Lot 32F-9330). Solutions of ^{14}C-labelled disopyramide phosphate (specific activity 9·73 μCi mg^{-1}, G. D. Searle Co., Chicago, IL, USA), disopyramide phosphate (G. D. Searle Co., Chicago, IL, USA, Lot 296) and mono-*N*-dealkyldisopyramide phosphate (G. D. Searle Co., Chicago, IL, USA, Lot CD3-185A) were prepared in methanol and were added to 12 × 70 mm polypropylene test tubes and evaporated to dryness under a gentle stream of nitrogen at 50 °C. The resulting residue was brought to a final volume of 1·2 ml with either human serum or solutions of AAG. Serum and AAG solutions contained 10^{-7} to 10^{-3} M disopyramide and either 0, $1{\cdot}34 \times 10^{-6}$, or $3{\cdot}70 \times 10^{-6}$ M mono-*N*-dealkyldisopyramide. The free (unbound) fraction of disopyramide in serum or AAG solutions was determined by ultrafiltration. Serum or protein solutions (1·0 ml) were placed in an ultrafiltration device (MPS-1 Micropartition System, Amicon Corporation, Danvers, Massachusetts, Lot MA 0064) equipped with a low molecular weight cutoff membrane (MPS-1 YMT, Lot MF0039, Amicon Corporation) and centrifuged at 37 °C (1500 *g*) for 2–10 min to obtain ultrafiltrates. Samples of serum and AAG solutions were obtained before ultra filtration and samples of the ultrafiltrate (0·2 ml) were saved for liquid scintillation counting. Correction for quenching was performed using [^{14}C]toluene as an internal standard. The disopyramide-free fraction (FF) was calculated from the quotient of the d min^{-1} ml^{-1} ultrafiltrate and the d min^{-1} ml^{-1} protein solution. Disopyramide bound fraction was calculated as 1-FF.

To determine whether there were any differences in disopyramide binding to AAG purchased from various commercial sources, solutions containing $2{\cdot}27 \times 10^{-5}$ M AAG were prepared in isotonic phosphate buffer (pH 7·4) using human AAG from Sigma Chemical (St. Louis, MO, USA, Lot 32F-9330), Calbiochem Behring

Corp (La Jolla, CA 92037, Lot 112150) and human orosomucoid from Calbiochem Behring Corporation (La Jolla, CA, Lot 101624). The disopyramide-free fraction (FF) was determined over a wide concentration range by ultrafiltration as described above. Binding constants for serum and protein solutions were determined using a non-linear regression computer program (MACMOL) described by Priore & Rosenthal (1976) as discussed by Lima et al (1981). To verify the ultrafiltration technique for measuring the unbound fraction of disopyramide in human serum, 50 blood specimens were obtained from cardiac patients receiving chronic disopyramide therapy. Serum (1·0 ml) was placed into a two-chambered dialysis apparatus equipped with a 6000–8000 molecular weight cutoff membrane (Spectrapor No. 1, Spectrum Medical Industries, Los Angeles, CA, USA, 90054) and dialysed against isotonic phosphate buffer (pH 7·4) at 37 °C for 30–35 h. After equilibration the serum raffinate was removed and placed into an ultrafiltration device equipped with a YMT membrane (Amicon Corp, Danvers, Mass, USA) and processed as described above. Post-equilibrium disopyramide concentrations in the buffer side of the dialysis apparatus and corresponding concentrations in the ultrafiltrate were determined by an enzyme immunoassay technique (EMIT, Syva Company, Palo Alto, CA) which was modified to measure disopyramide concentrations as low as 0·25 μg ml^{-1}.

Results

Post-equilibrium disopyramide concentrations measured in the dialysis buffer by enzyme immunoassay (independent variable) were in good agreement with the disopyramide concentrations measured in the ultrafiltrates obtained from corresponding serum raffinates (dependent variable, r = 0·96). Post-equilibrium disopyramide buffer concentrations were below the lower limits of detection for the EMIT assay in nine specimens and these values were excluded from linear regression analysis. The slope (0·98) and y-intercept (0·05 mg litre^{-1}) values obtained by linear regression were not significantly different from one and zero, respectively. In human serum, disopyramide free fraction increased 20–104% after the addition of 3·70 × 10^{-6} M mono-*N*-dealkyldisopyramide (MND) to serum specimens containing from 1·05 × 10^{-5} to 4·85 × 10^{-7} M disopyramide, $P < 0·05$. No statistically significant increases in disopyramide free fraction were observed in the presence of 1·34 × 10^{-6} M MND. Double reciprocal plots of disopyramide binding in serum from each volunteer subject are shown in Fig. 1. The apparent affinity (K_1) and capacity (n P_T) constants for the high affinity low capacity binding site in serum averaged 1·89 ± 0·62 × 10^6 litre mol^{-1} and 7·27 ± 2·05 × 10^{-6} nmol litre^{-1} in the absence of MND, 1·38 ± 0·26 × 10^6 litre mol^{-1} (n.s.) and 7·27 ± 1·84 × 10^6 nmol litre^{-1} (n.s.) in the presence of 1·34 × 10^{-6} M MND, and 0·94 ± 0·36 × 10^6 litre mol^{-1} ($P < 0·05$ vs no MND) and 7·15 ± 2·12 × 10^6 nmol litre^{-1} (n.s.) in the presence of 3·70 × 10^{-6} M MND. Double reciprocal plots of disopyramide binding to AAG in solutions ranging in protein concentration from 3 × 10^{-5} to 3 × 10^{-6} M are shown Fig. 2. No statistically significant differences were observed in the affinity constants (K_1) describing the drug-protein interaction when MND was added to the protein solutions (Table 1). However, there was a consistent

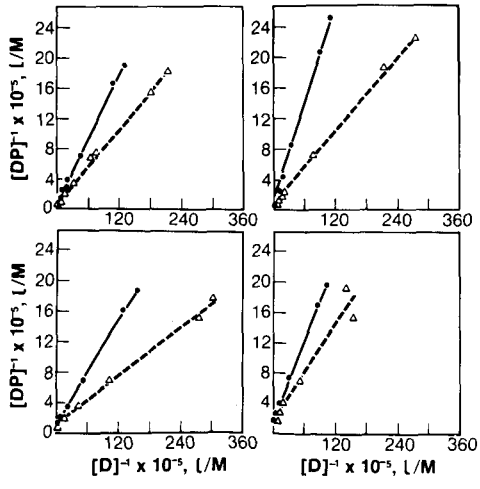

FIG. 1. Double reciprocal plots of disopyramide binding in human serum (n = 4) in the presence (●) and absence (△) of 3·70 × 10^{-6} M, mono-*N*-dealkyldisopyramide.

Table 1. Apparent affinity constants (K_1 litre mol^{-1}) and number of binding sites per mole of protein (n_1) associated with the high affinity low capacity disopyramide binding site on α-1-acid glycoprotein determined from binding studies performed in the presence of 3·70 × 10^{-6} M MND and in the absence of MND.

AAG concn		MND = 0		MND = 3·7 × 10^{-6} M	
M	(mg %)	K_1	n_1	K_1	n_1
3·41 × 10^{-5}	150	1·06 × 10^6	0·416	1·24 × 10^6	0·317
2·27 × 10^{-5}	100	0·76 × 10^6	0·357	1·16 × 10^6	0·332
1·14 × 10^{-5}	50	1·12 × 10^6	0·399	5·31 × 10^5	0·368
5·68 × 10^{-6}	25	0·96 × 10^6	0·318	2·00 × 10^5	0·612
3·41 × 10^{-6}	15	1·21 × 10^6	0·267	3·31 × 10^5	0·365
$\bar{x}$		1·06 × 10^6	0·351	0·69 × 10^6	0·399
sd		0·17 × 10^6	0·061	0·48 × 10^6	0·121

Table 2. Apparent affinity constants (K_1) and number of binding sites per mole of protein (n_1) associated with the high affinity low capacity disopyramide binding site on α-1-acid glycoprotein from three different commercial sources.

	K_1, litre mol^{-1}	n_1
Sigma AAG	1·24 × 10^6	0·291
Calbiochem AAG	0·64 × 10^6	0·221
Calbiochem Orosomucoid	0·32 × 10^6	0·249

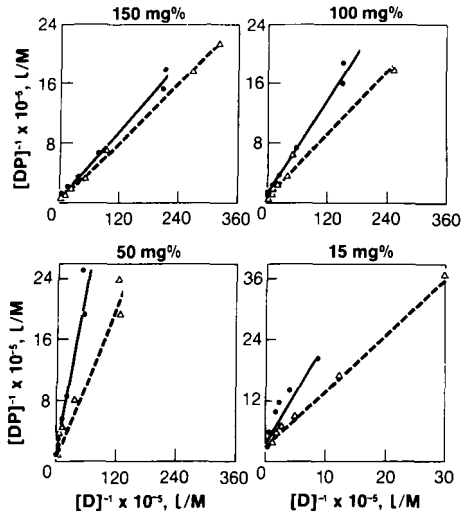

FIG. 2. Reciprocal plots of disopyramide binding to α-1-acid glycoprotein at various protein concentrations in the presence (●) and absence (△) of $3{\cdot}70 \times 10^{-6}$ M mono-*N*-dealkyl-disopyramide.

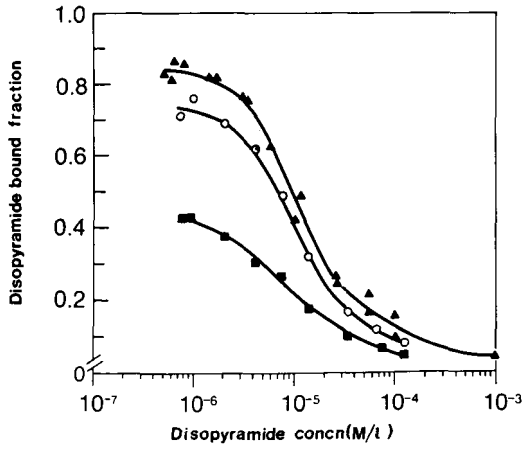

FIG. 3. Disopyramide binding to various commercial sources of AAG in isotonic phosphate buffer ($2{\cdot}27 \times 10^{-5}$ M AAG, pH 7·4).

decrease in K_1 from $1{\cdot}24 \times 10^6$ to $3{\cdot}31 \times 10^5$ litre mol^{-1} in solutions of AAG ranging in protein concentration from 3×10^{-5} to 3×10^{-6} M after the addition of $3{\cdot}70 \times 10^{-6}$ M MND. Pronounced differences in disopyramide bound fraction were noted when the binding of disopyramide was compared in AAG solutions prepared from three different commercial sources (Fig. 3). The apparent affinity (K_1) constants varied almost 4-fold (Table 2). Moreover, the maximum fraction of disopyramide bound to AAG ranged from 0·40 to 0·85 in solutions prepared from the three products.

Discussion

Disopyramide is administered as a racemic mixture and the protein binding of each stereoisomer has not been fully characterized in human serum. The analytical methods employed in the present investigation do not distinguish the individual isomers of disopyramide, and the binding constants must therefore be considered 'apparent' binding constants. The present study showed that the unbound fraction of disopyramide increased to a variable extent when MND was added to human serum at a concentration of $3{\cdot}70 \times 10^{-6}$ M. These findings are in agreement with a previous study by Bredesen et al (1982) which demonstrated an increase in the free fraction of disopyramide in human serum after the addition of MND. The present results also demonstrate that MND can displace disopyramide at serum concentrations much lower than those reported previously to cause significant displacement of disopyramide from plasma proteins (Hinderling et al 1974). Moreover, displacement of disopyramide by MND occurred through an interaction between MND and disopyramide on AAG, and the magnitude of disopyramide displacement by MND varied with AAG concentration. Consequently, the bound fraction of disopyramide in human serum is variable, and is related to (i) the serum concentration of disopyramide, (ii) the serum concentrations of AAG and (iii) the extent of accumulation of MND during therapy. Serum concentrations of MND that result in displacement of disopyramide from AAG are generally not encountered among patients with normal renal function, but may occur in patients with renal impairment, or patients who are receiving other drugs which induce *N*-dealkylation of disopyramide (Aitio & Vuorenmaa 1980; Aitio et al 1981; Bredesen et al 1982). Previous studies have determined the binding of basic compounds to commercially available human AAG (Chen-Sharps et al 1981; Abramson 1982; Urien et al 1982). Pronounced differences in disopyramide binding were observed among three different commercial AAG preparations examined herein. These findings suggest that methods used to isolate and purify AAG on a commercial scale may be associated with changes in protein structure or introduce exogenous substances which can impair the binding of disopyramide to AAG. As such, the binding data obtained from binding studies which use solutions prepared from commercial sources of AAG should be interpreted with caution. For example, switching from a more to a less expensive source of AAG could result in markedly different drug binding (Fig. 3). At present we recommend that binding studies be performed in human serum that has been appropriately collected to best characterize the drug-protein interaction.

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Letter to the Editor

The metabolic chiral inversion of 2-arylpropionic acids—A novel route with pharmacological consequences—a correction

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In our recent review (Hutt & Caldwell 1983) on the metabolic chiral inversion of the 2-aryl propionic acids, Fig. 1 presented flying wedge diagrams to depict the three-dimensional structures of the enantiomers, assigned according to Shen (1979). Close examination of the Figure, in the light of model building, reveals that the absolute configurational designation of these structures is incorrect and should be reversed, i.e. the enantiomer labelled by us as *R*(−) is in fact *S*(+) and vice versa. We apologise for this error, and would also point out that the diagram of Shen (1979) upon which our assignments were based is similarly incorrect.

Since the appearance of our review, two additional examples of the metabolic chiral inversion of 2-arylpropionates have been brought to our attention. Both 2-[3-(2-chlorophenoxyphenyl)]-propionic acid (Tamegai et al 1979) and loxoprofen (Nagashima et al 1984) have been shown to undergo this reaction in the rat. In addition, it is of interest to note that indoprofen apparently does not exhibit metabolic inversion in man, since the plasma level-time curves of the *S*-(+)-enantiomer were identified when the enantiomer was given as such or as the racemic mixture (Tamassia et al 1984). Two general methods for the HPLC resolution of enantiomers of the 2-arylpropionates have been reported, one (Maitre et al 1984) involving the formation of diastereoisomeric amides with a chiral amine, and the other (Wainer & Doyle 1984) using a chiral stationary phase.

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