약 제 학 회 지
J. Korean Pharm. Sci.
15(1) 1~7 (1985)

Protein Binding of Disopyramide

Displacement by Mono-N-Dealkyl-Disopyramide and Variation with Commerial Source of Alpha-1 -Acid Glycoprotein

David B. Haughey, Irving Steinberg and Min Hwa Lee*

College of Pharmacy, University of Minnesota

(Received Feburary 3, 1985)

This study was performed to determine whether commercially available alpha-1-acid glyocpyotein, and it was recommended that binding studies be perform in human serum that has been appropriately collected to best characterize the drug-protein interaction.

Previous studies show that the free (unbound) fraction of disopyramide in human serum is drug concentration dependent at corresponding serum disopyramide concentrations that are achieved clinically.[1~3] Moreover, disopyramide free fraction values vary several fold at any given drug concentration in human serum and tend to decrease as serum cocentrations of alpha-1-acid glycoprotein(AAG) incrase.[4] A recent study[5] demonstrates that the free fraction of disopyramide in human serum increases almost 2-fold following the addition of 14.4μM/L mono-N-dealkyldisopyramide. These studies and others.[6],[7] 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 utility of using commercially available alpha-1-acid glycoprotein for drug protein binding displacement studies. Because many basic and acidic compounds are known to bind to alpha-1-acid glycoprotein[8] the present study was performed to determine whteher commercially available AAG would provide a convenient protein source for such binding studies.

Experimental

Whole blood (50ml) was obtained from 4 normal volunteers by venipuncture and allowed to clot in glass centrifuge tubes at room temperature. The resulting serum

College of Pharmacy, Seoul National University.

was harvested and adjusted to pH7.4 with 5~15μl concetnrated sulfuric acid (Mallinckrodt Inc., Paris, Kentucky, USA). Solutions of alpha-1-acid glycoprotein were prepared in phosphate buffer[9] at pH 7.4 using commercially available AAG (Sigma Chemical Co., St. Louis, MO, USA, Lot 32F—9330). Solution of ^{14}C labelled disopyramide phosphate (specific activity=9.73 μCi/mg, 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 mm by 70mm 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 1.2ml with either human serum or solutions of AAG. Serum and AAG solutions contained 10^{-7} to 10^{-3} M/L disopyramide and either 0, 1.34×10^{-6}, or 3.70×10^{-6}M/L mono-N-dealkyldisopyramide. The free (unbound) fraction of disopyramide in serum or AAG solutions was determined by ultrafiltration. Serum of protein solutions (1.0ml) were placed into an ultrafiltration device (MPS-1 Micropartition System, Amicon Corporation, Danvers, Massachusettes, Lot MA 0064) equiped with a low molecular weight cutoff membrane (MPS-1 YMT, Lot MF 0039, Amicon Corporation) and centrifuged at 37°C(1500×G) for 2~10 minutes to obtain ultrafiltrates. Aliquots of serum and AAG solutions were obtained prior to ultrafiltration and aliquots of the ultrafiltrate (0.2ml) were saved for liquid scintillation counting. Correction for quenching was performed using ^{14}C toluene as an internal standard. Disopyramide free fraction (FF) was calculated from the quotient of the disintegrations per minute (DPM) per milliliter ultrafiltrate and the DPM per milliliter 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 2.27×10^{-5} M/L AAG were prepared in isotonic phosphate buffer (pH 7.4) using human alpha-1-acid glycorprotein obtained from Sigma Chemical (St. Louis, MO, USA, Lot 32F-9330), Calbiochem Behring Corp (La Jolla, California 92037, Lot 112150) and human orosomucoid obtained from Calbiochem Behring Corporation (La Jolla, CA, Lot 101624 Disopyramide free fraction (FF) was determined over a wide drug 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 Prior & Rosenthal[10] as discussed previously by Lima et al.[11] To verify the ultrafiltration technique for measuring the unbound fraction of disopyramide in

human serum, fifty blood specimens were obtained from cardiac patients receiving chronic disopyramide therapy. Serum (1.0m*l*) was placed into a two-chambered dialysis apparatus equipped with a 6,000~8,000 molecular weight cutoff membrane (Spectrapor No.1, Spectrum Medical Industries, Los Angeles, CA, USA, 90054) and dialyzed against isotonic phosphate buffer (pH 7.4) at 37°C for 30~35 hours. After equilibration the serum raffinate was removed and placed into an ultrafiltration device equipped with a YMT^R 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^R, Syva Company, Palo Alto, CA) which was modified to measure disopyramide concentrations as low as 0.25μg/m*l*.

Results

Post-equilibrium disopyramide concentrations measured in the dialysis buffer by enzyme immunoassay were in good agreement with the disopyramide concentrations measured in the ultrafiltrates obtained from corresponding serum raffinates (Fig. 1). Post-equilibrium disopyramide buffer concentrations were below the lower limits of detection for the EMIT^R assay in nine specimens and these values were excluded from linear regression analysis. The slope and y-intercept values obtained by linear regression were not significantly different from one and zero, respectively (Fig. 1). In human serum, disopyramide free fraction increased 20~104% after the addition of 3.70×10^{-6} M/L mono-N-dealkyldisopyramide to serum specimens containing from 1.05×10^{-5} M/L to 4.85×10^{-7} M/L disopyramide, $p<0.05$. No statistically significant increases in disopyramide free fraction were observed in the presence of 1.34×10^{-6} M/LMND. Double reciprocal plots of disopyramide binding in serum from each volunteer subject are shown in Figure 2. The apparent affinity (K_1) and capacity (n P_T) constants for the high affinity low capacity binding site in serum averaged $1.89\pm0.62\times10^6$L/M and $7.27\pm2.05\times10^{-6}$M/L in the absence of MND, $1.38\pm0.26\times10^6$L/M (N.S.) and $7.27\pm1.84\times10^{-6}$M/L(N.S.) in the presence of 1.34×10^{-6}M/L MND, and $0.94\pm0.36\times10^6$L/M ($p<0.05$ vs no MND) and $7.15\pm2.12\times10^6$M/L (N.S.) in the presence of 3.70×10^{-6}M/L MND. Double reciprocal plots of disopyramide binding to AAG in solutions ranging in protein concentration from 3×10^{-5} to 3×10^{-6}M/L are shown Fig.3. No statistically significant differences were observed in the affinity constants (K_1) describing the dr-

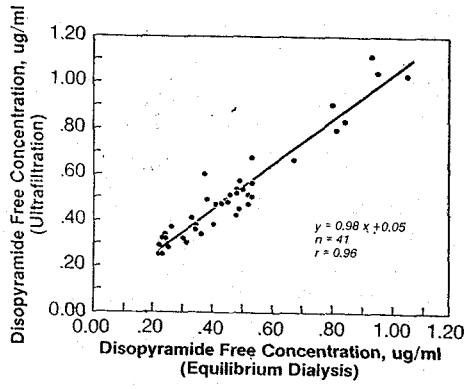

Figure 1—Comparison of disopyramide unbound concentrations determined in 41 human serum specimens by ultrafiltration and equilibrium dialysis.

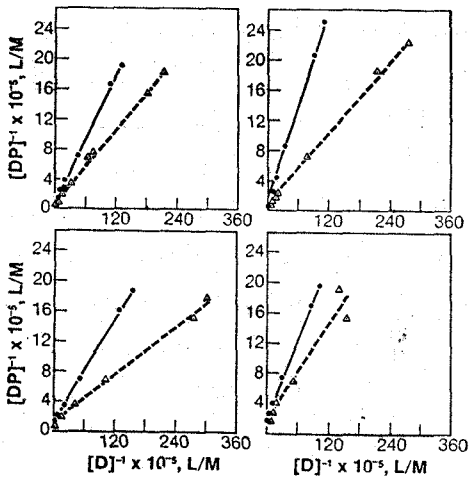

Figure 2—Double reciprocal plots of disopyramide binding in human serum (n=4) in the presence (•) and absence (△) of 3.70×10^{-6}M /L mono-N-dealkyldisopyramide

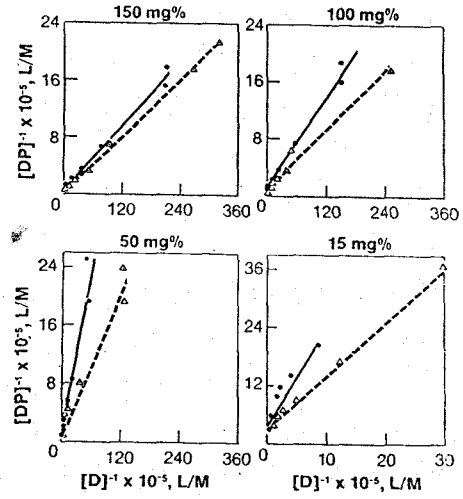

Figure 3—Reciprocal plots of disopyramide binding to alpha 1-acid glycoprotein at various protein concentrations in the presence (•) and absence (△) of 3.70×10^{-6}M/L mono-N-dealkyldisopyramide.

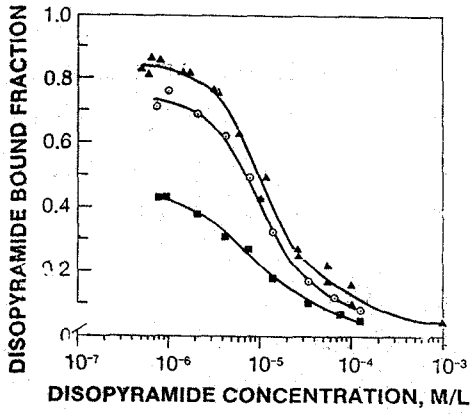

Figure 4—Disopyramide binding to various commercial sources of AAG in isotonic phosphate buffer(2.27 $\times10^{-5}$M/L AAG, pH 7.4). =Sigma AAG, =Cabliochem AAG, =Calbiochem Orosomucoid.

Table Ⅰ—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 Alpha-l-Acid Glycoprotein Determined from Binding

AAG Concentration		MND=0		MND=3.7×10^{-6}M/L	
M/L	(mg%)	K_1, L/M	n_1	K_1, L/M	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 Ⅱ-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 Alpha-l-Acid Glycoprotein from Three Different Commercial Sources

	K_1, L/M	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

ug-protein interaction when MND was added to the protein solutions(Table 1). However, there was a consistent decrease in K_1 from 1.24×10^{6}L/M to 3.31×10^{5}L/M in solutions of AAG ranging in protein concentration from 3×10^{-5} to 3×10^{-6}M/L after the addition of 3.70×10^{-6}M/L MND. Pronounced differences in disopyramide bound fraction were noted when the binding of disopyramide was compared in alpha-l-acid glycoprotein solutions prepared from three different commercial sources of AAG (Fig. 4). The apparent affinity (K) constants varied almost 4-fold (Table Ⅱ). Moreover, the maximum fraction of disopyramide bound to AAG ranged from 0.40 to 0.85 in solutions prepared from the three different 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 (R and S) isomers of disopyamide, and the binding constants must therefore be considered "apparent" binding constants. The present study showed that the unbound fraticon of disopyramide increased to a variable extent when mono-N-dealkyldisopyramide was added to human serum at a concentration of 3.70×10^{-6}M/L. These findings are in agreement with a previous study by Bredesen[5] which demonstrated an increase in disopyramide free fraction 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[6]. Moreover, displacement of disopyramide by MND occurred through an interaction between MND and disopyramide competing for binding sites located on alpha-l-acid glycoprotein, and the magnitude of disopyramide displacement by MND varied with AAG concentration. Consequently, the bound fraction of disopyramide in human serum is quite variable, and is related to the 1) serum concentration of disopyramide, 2) serum concentration of alpha-l-acid glycoprotein and 3) extent of accumulation of mono-N-dealkyl-disopyramide during therapy. Serum concentrations of MND that result in displacement of disopyramide from alpha-l-acid glycoprotein 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[5,10,11] Previous studies have determined the binding of basic compounds to commercially available human alpha-l-acid glycoprotein[7,8,12]. Pronounced differences in disopyramide binding were observed among three different commercial AAG preparations studied in the present investigation. These findings suggest that methods used to isolate and purify AAG on a commercial scale may be 1) associated with changes in protein structure or 2) 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 commmercial sources of alpha-l-acid glycoprotein should be interpreted with caution. For example, switching from a more expensive source of alpha-l-acid glycoprotein to a less expensive supplier could result in markedly different drug binding (Fig. 4). At present we recommend that binding studies be performed in human serum that has been appropriately collected to best characterize the drug-protein interaction.

References

1) Lima, J.J., Boudoulas, H., Blanford, M., *J. Pharmacol. Exp. Ther.*, **219**, 741—747 (1981)
2) Meffin, P.J., Robert, E.W., Winkle, R.A., Harapat, S., Peters, F.A., Harrison, D.C., *J. Pharmacokinet. Biopharm.*, **7**, 29—46 (1979)
3) Giacomini, K.M., Swezey, S.E., Turner-Tamiyasu, K., Blaschke, T.F., *J. Pharmacokin. Biopharm.*, **10**, 1—14 (1982)
4) Haughey, D.B., Kraft, C.J., *Clin. Pharmacokinet.*, **9**, Suppl. 97—98 (1984)
5) Bredesen J., Pike, E., Lunde, P.K.M., *Br. J. Clin. Pharmacol.*, **14**, 673—676 (1982)
6) Hinderling, P.H., Bres, J., Garrett, E.R., *J. Pharm. Sci.*, **63**, 1684—1689 (1974)
7) Chen-Sharps, J., Lima, J., Haughey, D.B., *Drug Intel. Clin. Pharm.*, **15**, 478 (1981)
8) Urien, S., Albengres, E., Zini, E., Tillement, J.P., *Biochem. Pharmacol.*, **31**, 3687—3689 (1982)
9) Priore, R.L., Rosenthal, H.E., *Anal. Biochem.*, **70**, 231—240 (1976)
10) Aitio, M., Vuorenmaa T., *Br. J. Clin. Pharmacol.*, **9**, 149—152 (1980)
11) Aitio, M., Mansury, L., Tala, E., Haataja, M., Aitio, A., *Br. J. Clin. Pharmacol.* **11**, 279—285 (1981)
12) Abramson, F.P., *Clin. Pharmacol. Ther.*, **32**, 652—658 (1982)