Biochemical Pharmacology, Vol. 30, No. 18, pp. 2633-2636, 1981.
Printed in Great Britain.

0006-2952/81/182633-04$02.00/0

PRELIMINARY COMMUNICATION

CONTAMINATION OF ALBUMIN BY ALPHA 1-ACID GLYCOPROTEIN

John J. LIMA and Lisa B. SALZER

Division of Clinical Practice, College of Pharmacy,
and Division of Cardiology, College of Medicine,
Ohio State University, Columbus, Ohio 43210

(Received 21 April 1981; accepted 30 June 1981)

Disopyramide is a basic antiarrhythmic drug which is bound to protein in human plasma (1). It is an unusual compound because its bound fraction is concentration dependent at therapeutic plasma concentrations (1-3). It has been reported in early studies that albumin is the major protein which binds the drug in human plasma (4). More recently we reported that disopyramide binds to two independent sites on albumin, and that as much as 35% of the drug is bound to albumin at a concentration of 1×10^{-7}M (5). In the present study, we show that: (i) disopyramide at most, is 5% - 10% bound to albumin at drug concentrations ranging between 10^{-7} and 10^{-3}M; and (ii) sources of human serum albumin which bind more than 5% - 10% of the drug are likely to be contaminated by α 1-acid glycoprotein (AAG).

Materials and Methods.

Sources of Protein. Crystalline Human Serum Albumin (HSA) Cohn's fraction V, fatty acid free, was purchased from the following manufacturers: Sigma Chemical Co., St. Louis, MO, Lot #19C-7080, designated H_1 in this study; United States Biochemical Corporation, Cleveland, OH, Lot numbers 19024, 23211 and 19132 designated H_2, H_3, H_4 respectively. The crystals were dissolved in phosphate buffer, pH 7.4, to a concentration of 4%. A commercial solution of normal HSA, USP, 5% (American National Red Cross, processed by Hyland Division Travenol Laboratories, Inc., Costa Mesa, CA), Lot #2745M012AA was designated H_5; and normal HSA, USP, 25% (American Red Cross Blood Services, processed by Armour Pharmaceutical Co., Phoenix, Arizona), Lot #T71604, diluted to 5% with normal saline was designated H_6. Glycoprotein, Human, fraction VI was purchased from Sigma Chemical Co., and designated AAG. Crystals were dissolved in phosphate buffer to a concentration of 60 g/liter.

Binding Studies. Each protein solution was dialyzed against an equal volume of phosphate buffer containing ^{14}C-labelled disopyramide (specific activity = 18.7 mCi/mmole) and unlabelled disopyramide ranging in concentration between 1×10^{-7} and 1×10^{-3}M. Equilibrium dialysis was performed in a plexi-glass two-chambered apparatus of 1 ml capacity (Technilab Instrument Corp., Pequannock, NJ), separated by a cellulose dialysis membrane (Spectrapor, Spectrum Medical Industries, Los Angeles, CA). After 16 hours of mild agitation at 37°C, 0.75 ml of buffer and protein were pipetted into 10 ml of liquid scintillation reagent (PCS, Amersham, Arlington Heights, IL), and counted on a Beckman Model 8100 liquid scintillation counter. Previous studies in our lab indicated that equilibration between protein and buffer occurs in 12-16 hours under these conditions. Sample quenching was corrected by internal standardization using ^{14}C-labelled toluene (Amersham, Arlington Heights, IL).

Analysis of Binding. The free fraction (FF) of disopyramide was determined by calculating the ratio of disintegrations/minute (dpm) in the buffer (B) and protein (P) solutions respectively; the bound fraction (BF) is 1-FF. The ratio of bound and free disopyramide concentrations was plotted against the bound concentration, and the association constant, K, and capacity constant, N (product of the number of sites per mole of protein and the molar concentration of protein) were estimated for each class of binding sites (6). These were used as initial estimates in the computer program MACMOL (7), and the percent of disopyramide bound (BF x 100) and corresponding post-equilibrium total (free + bound) drug concentrations were entered. The program provides the least-square estimates of the binding constants for each class of binding sites.

Immunologic Studies. The presence of AAG in each albumin solution except sample H_2 (discarded prior to immunological studies) was analyzed by the Ouchterlony-double diffusion technique (8). Twenty µl of rabbit antiserum to human AAG (Nephelometric Grade Rabbit antiserum to Human α_1-acid glycoprotein, Behring Diagnostic, American Hoechst Corp., Sommerville, NJ) was applied to a center well punched in agarose gel in a rosette pattern (Immuno-TEC® II OT Agarose Plate Kit, Calbiochem-Behring Corp., La Jolla, CA). Opposite wells were filled with the protein solutions (antigen) and appropriate controls. The plates were placed in a water bath and incubated for 24 hours at 25°C. Antibodies and any antigens present in the samples diffuse toward each other and form precipitin lines upon confluence.

Results and Discussion. Figure 1 shows the percent binding of disopyramide at various post-equilibrium drug concentrations in the protein solutions. The binding of disopyramide to protein in the samples of HSA varied between 5 and 33% at total drug concentrations of 1×10^{-8}M. Modified Scatchard plots indicated that the ratio of bound and free disopyramide concentrations at various bound concentrations was linear in samples H_1, H_4 and AAG indicating the presence of one independent binding site. Modified Scatchard plots of

Table 1. Comparison of binding constants characterizing the interaction between disopyramide and protein(s)

	FIRST BINDING SITE		SECOND BINDING SITE	
Protein(s)	K, M^{-1}	N, M	K, M^{-1}	N, M
H_1	1.9×10^{5}	3.4×10^{-7}	----------	----------
H_2	1.75×10^{6}	2.5×10^{-7}	5.4×10^{3}	1.6×10^{-5}
H_3	2.7×10^{6}	8.4×10^{-8}	2.9×10^{2}	1.8×10^{-4}
H_4	1.4×10^{5}	7.64×10^{-7}	----------	----------
H_5	1.7×10^{6}	2.3×10^{-7}	1×10^{5}	9.7×10^{-7}
H_6	3.3×10^{6}	3×10^{-8}	2.2×10^{5}	4×10^{-7}
AAG (60g/l)	9.5×10^{5}	3.3×10^{-6}	----------	----------

samples H_2, H_3, H_5 and H_6 were biphasic indicating the presence of at least two binding sites. The affinity and capacity constants characterizing each class of binding sites are included in Table 1. The association constant for AAG and the first binding site on protein in samples H_2, H_3, H_5 and H_6 were similar, suggesting that the first binding site on these samples of albumin was the same as that on AAG. The binding of disopyramide was less than 10% in the protein samples characterized by one binding site (H_1 and H_4). The percent binding was higher in the HSA samples characterized by the presence of two binding sites, and was proportional to the capacity constant, and unrelated to the association constant for the first binding site (Table 1, Figure 1). These data suggest that disopyramide is

only 5% - 10% bound to one binding site on HSA (e.g. samples H_1, H_4), and further, samples of HSA which bind disopyramide higher than 5% - 10%, and which indicate the presence of two or more binding sites are contaminated by AAG.

Figure 2 confirmed our hypothesis. No precipitin lines were evident in samples H_1 and H_4, the two samples of HSA in which disopyramide was bound less than 10%. However, precipitin lines were evident in samples H_3, H_5 and H_6. Furthermore, the heaviest precipitin lines were evident in sample H_5, the HSA sample in which both the percent binding and the capacity constant for the first binding site were the highest (Table 1, Figure 1).

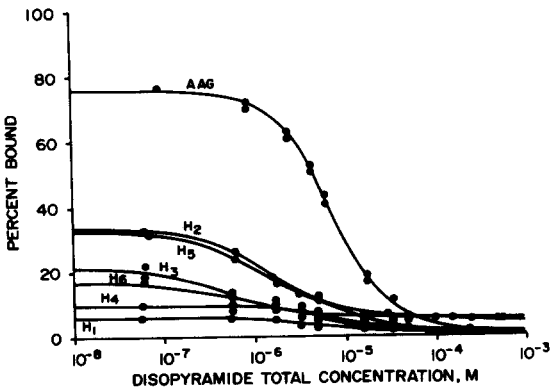

Figure 1. Percent binding of disopyramide in protein solutions at various post-equilibrium concentrations. Symbols are measured, lines are computer fitted.

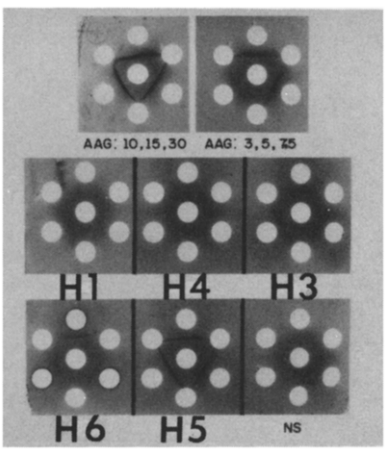

Figure 2. Agarose plates showing interaction between antisera and sample tested. Numbers following AAG refer to concentrations of AAG, g/l, and NS refers to normal saline.

Alpha 1-acid glycoprotein has a high affinity for many basic drugs (9), and is the major binding protein for disopyramide in human plasma (3). In contrast, albumin is the major binding protein in human plasma for many acidic drugs (9,10). The results of our study demonstrate that many commercial sources of albumin are contaminated by AAG. Consequently, the purity of commercially available albumin must be assessed prior to use particularly in binding studies of basic drugs.

Supported by Grant GM-28424-01 from the National Institutes of General Medical Sciences, National Institutes of Health.

REFERENCES

1. P.H. Hinderling and E.R. Garrette. J. Pharm. Sci. 63:1684, 1974.
2. P.J. Meffin, E.W. Robert, R.A. Winkle, S. Harapat, F.A. Peters and D.C. Harrison. J. Pharmacol. Biopharm. 7:29, 1979.
3. J.J. Lima, H. Boudoulas and M. Blanford. Submitted for publication, 1981.
4. Y.W. Chien, H.J. Lambert and A. Karim. J. Pharm. Sci. 63:1877, 1974.
5. J.J. Lima and S. Kisslinger. Drug Intel. Clin. Pharm. 14:635, 1980.
6. H.E. Rosenthal. Anal. Biochem. 20:525, 1967.
7. R.L. Priore and H.E. Rosenthal. Anal. Biochem. 70:231, 1976.
8. O. Ouchterlony. Prog. Allergy 5:1, 1958.
9. O. Borga, K.M. Piafsky and O.G. Nilsen. Clin. Pharmacol. Ther. 22:539, 1977.
10. K.M. Piafsky and O. Borga. Clin. Pharmacol. Ther. 22:545, 1977.