A STUDY OF THE INTERACTION BETWEEN PROPRANOLOL AND NSAIDs IN PROTEIN BINDING BY GEL FILTRATION METHOD

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ABSTRACT

Drug protein binding phenomena can lead to some interesting drug - drug interactions when one drug displaces another in the binding site. Studies of protein binding are conducted by several methods including equilibrium dialysis, ultra-filtration and chromatographic methods. Gel filtration is a simple chromatographic method in protein binding studies. Propranolol binds to plasma proteins by 90% -95% in circulation system and other drugs with high protein binding may displace it. In this study protein binding of propranolol has been studied using gel filtration to Bovine Serum Albumin (BSA) alone and in the presence of Acetyl salicylic acid (ASA), Indomethacin and mefenamic acid has been studied using gel filtration method. The results indicated that ASA decreased protein binding of propranolol by 20% to BSA and other drugs did not displace propranolol from the binding site. Therefore, ASA may alter pharmacological effects of propranolol.

KEY WORDS

Propranolol, NSAIDs, BSA, Protein Binding, Gel Filtration

INTRODUCTION

The binding of drugs to plasma and tissue proteins is an important factor affecting their distribution and rate of metabolism. Pharmacological effect is closely related to the free concentration of drug at its site of action.

There are examples of many drug-drug interactions which have been reported to present displacement of the bound drug by a second therapeutic agent.

Acidic drugs commonly bind to plasma albumin and concomitantly administered drugs may displace one another from their binding site. Basic drugs may bind to either albumin or α -acid glycoprotein.

Propranolol is the most widely used adrenergic α -receptor blocking agent that binds to plasma proteins by 90%-95%.

Evans *et al.* reported that propranolol mainly bound to albumin (1) but Sager and co- workers reported that propranolol binds mainly to α -acid glycoprotein (2). Certainly, propranolol binds to albumin as well as to α -acid glycoprotein (3, 4).

It has been reported that binding of propranolol to albumin or α -acid glycoprotein is related to the animals' species (5). Serum binding of some basic drugs such as propranolol is increased with inflammation or stress and it is confirmed that this is due to an increase of the plasma proteins (5).

The absorption, protein binding and metabolism of propranolol may all be affected by the co-administration of other drugs (6). It has been reported that propranolol can displace ASA from its binding sites (7) and also the antihypertensive effect of propranolol may be influenced by concomitant therapy with NSAIDs (8, 9, 10). Indomethacin also magnified the effect of propranolol on total peripheral resistance (11).

NSAIDs have high protein binding that may represent displacement of bound drugs especially those bound to albumin (12). Mefenamic acid can displace warfarin from its binding sites (internet). Therefore, NSAIDs may change protein binding of propranolol to albumin.

Protein binding studies are carried out with several

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methods among which equilibrium dialysis is a routine method. Ultracentrifugation may generate erroneous results due to sieve effect to compounds with molecular weights >300. Ultracentrifugation is not suitable for measuring salicylate binding to BSA at low salicylate concentrations (13).

The application of gel filtration to the study of protein binding of small molecules has been reported three decades ago (14, 15). Sephadex gel filtration provided greater precision and accuracy in the determination of binding parameters of salicylate than did equilibrium dialysis (13).

In this study we used gel filtration method for determining the interaction and displacement in protein binding studies. Chromatographic methods, which now take advantage of the technology of high performance liquid chromatography, are generally faster (16). In chromatographic methods such as gel filtration for quantification of drug - protein complex studies, Hummel and Dryer method is applied because a very small amount of protein is added to the mobile phase containing the drugs.

While there are many reports about interaction between propranolol and NSAIDs, there is no any evidence about displacement of propranolol from its binding sites by NSAIDs.

So in this study there is an attempt to investigate propranolol displacement by Acetyl Salicylic Acid, Mefenamic Acid and Indomethacin from its binding sites on BSA. Another objective of this study is to verify whether gel filtration is a simple and appropriate method for this purpose or not.

MATERIAL AND METHODS

Reagents : Bovine Serum Albumin and Sephadex G-25 were purchased from Sigma.

Standard drugs vials of Propranolol, Indomethacin, Mefenamic Acid and Acetyl Salicylic Acid were purchased from Merck Co.

Methods

Protein binding study : The method of Hummel and Dryer was followed for protein binding study. Solutions of propranolol at concentrations 1, 2, 5, 7.5 and 10 M were prepared in phosphate buffer pH = 7.4. The propranolol solutions were applied to a Sephadex G-25 column (50 × 5 cm) and 2ml fractions were collected. Concentration of propranolol in the total fractions was determined by spectrofluorimetric method (17).

The fluorescence of fractions was obtained and when it became constant and equal to fluorescence of the original sample, then 1 ml of BSA 4% with each propranolol sample was separately added to the column and the column was eluted with propranolol. The fractions were collected until the fractions were free of protein. Identification of proteins was carried out using Lowry method (18).

Drug interaction study : The above steps were repeated with:

Propranolol 1-10 M + ASA 1.6 mM + BSA 4%

Propranolol 1-10 M + Mefenamic Acid 20 M + BSA 4%

Propranolol 1-10 M + Indomethacin 7 M + BSA 4%

Statistical Analysis : Statistical analyses of the experimental data were performed using a student's t-test. Differences in mean values were deemed significant if $p < 0.01$.

Table 1. AUC of binding of propranolol alone and in the presence of second drug to BSA as Hummel and Dryer method for fractions 1-12 (n=3)

Concentration of propranolol (M)	AUC of propranolol Mean $\pm$ SD	AUC of propranolol + ASA 1.6 (mM) Mean $\pm$ SD	AUC of propranolol + Ind 7 (M) Mean $\pm$ SD	AUC of propranolol + Mef. A 20 (M) Mean $\pm$ SD
1	24.58 $\pm$ 1.53	19.52 $\pm$ 1.49*	23.84 $\pm$ 3.75	26.39 $\pm$ 1.48
2	47.35 $\pm$ 3.45	37.5 $\pm$ 1.93*	46.9 $\pm$ 2.24	49.6 $\pm$ 11.43
5	109.24 $\pm$ 1.80	89.5 $\pm$ 1.97*	114.24 $\pm$ 2.44	114.24 $\pm$ 3.1
7.5	161.35 $\pm$ 3.1	129.96 $\pm$ 3.86*	178.34 $\pm$ 3.63	184.21 $\pm$ 4.75
10	212.87 $\pm$ 3.56	171.24 $\pm$ 4.78*	208.36 $\pm$ 5.1	218.1 $\pm$ 4.1

BSA = Bovine Serum Albumin; ASA = Acetyl Salicylic Acid; Ind = Indomethacin; Mef.A = Mefenamic Acid
 $P < 0.01$

RESULTS

The results were obtained using Hummel and Dryer method. In this method concentration of propranolol was obtained in each fraction. Then a curve of concentration of propranolol was plotted versus fraction numbers. Fig.1 shows the curve for 1 M propranolol.

The results revealed that concentration of propranolol in the initial fractions was constant. After adding 1ml BSA 4% to the column, concentration of propranolol increased and then decreased. AUC was calculated in the ascending (fractions 1-12) and descending part of the curve (fractions 13-39) for each concentration of propranolol (Table 1).

The curve was plotted and AUC was calculated for 1 M propranolol alone (Fig. 1) and in the presence of ASA 1.6 mM, Mefenamic Acid 20 mM and Indomethacin 7 mM (Fig. 1).

Variations in AUC show that only ASA 1.6mM can displace propranolol 1 M significantly from its binding sites on BSA (Fig . 1) ($p < 0.01$).

Fig. 1. Protein binding of Propranolol (1 M) to 1 ml BSA 4% in the presence of ASA 1.6 M, Indomethacin 7 M and Mefenamic acid 20 M by gel filtration method (n=3)

AUC of Fractions 1-12 of Propranolol = 24.58 ± 1.53

AUC of Fractions 1-12 of Propranolol in the presence of ASA = 19.82 ± 1.49

AUC of Fractions 1-12 of Propranolol in the presence of Indomethacin = 23.84 ± 3.75

AUC of Fractions 1-12 of Propranolol in the presence of Mefenamic Acid = 26.39 ± 1.48

As shown in Table 1, ASA 1.6 mM could displace propranolol from its binding sites on BSA significantly about 20% for each propranolol concentration.

DISCUSSION

Protein binding of propranolol is affected by many factors such as the kind of protein, buffer, pH, temperature and the species of animal (5). Therefore co-administration of propranolol and drugs with high protein binding may change free or bound concentrations of propranolol.

Gel filtration is a simple method in the studies of protein binding interactions of drugs.

Albumin - phenylbutazone and albumin - warfarin interactions are studied by different methods in protein binding and it is reported that chromatographic methods have validity as a tool to study drug - protein interactions (16).

The chromatographic procedure curve shows that concentration of propranolol in the initial fractions is equal to eluent or free propranolol concentration. After adding BSA, concentration of propranolol increases because these fractions contain free and bound drug. When total proteins are extracted from the column in the final fractions concentration of propranolol is reduced (Fig. 1). The amount of bound drug can be obtained from the AUC for each concentration.

Although protein binding parameters are used for determining the displacement of a drug from its binding sites, in this method we could determine displacement of propranolol from binding sites by a second drug using variation in AUC, because this variation shows differences in concentrations of the free and bound drug.

Fig.1 shows that ASA 1.6 M decreased protein binding of propranolol 1M to BSA about 20% and other drugs did not affect the binding of propranolol to BSA which is observed in all of the samples (Table 1). However, there are some reports about propranolol - NSAIDs interactions (8, 9, 10, 11) but our results show that these interactions are not due to alteration in protein binding, else ASA- propranolol interaction.

ASA decreased protein binding of propranolol only about 20% at different concentrations of propranolol (1-10 mM), because the concentration of ASA was so much higher than propranolol in all of the experiments that there were no significant alterations in displacement of propranolol at 1-10 M concentration by ASA.

ASA can cause alteration in the protein binding of some acidic and basic drugs. It has been reported that SA displaces warfarin from protein binding (19).

Miners and coworkers have reported that salicylic acid displaces protein binding of ibuprofen, tolmetin and sodium diclofenac (20). Also displacement of valproic acid from protein binding by salicylates and several NSAIDs like tolmetin and ibuprofen have been described (12). ASA can displace imipramine from protein binding site (21), while antidepressant agents such as imipramine are basic (cationic) drugs that are known to bind to α -Acid glycoprotein (22).

Uma and co-workers reported that propranolol increased protein binding of ASA to plasma proteins by 10%-50% and it is suggested that ASA and Propranolol bound to two different sites on plasma proteins (7). However, we suggest that a competitive antagonism between the two drugs for binding to BSA is possible.

Even ASA displaces propranolol from its binding sites only about 20%, but it probably significantly increases free concentration of propranolol, because propranolol has a high protein binding. If a drug reduces binding from 99% to 95%, it will thereby increase the unbound concentration of free and active drug from 1% to 4% (a fourfold increase). Therefore ASA may change pharmacokinetic of propranolol, but clinical studies (in-vivo) should be employed. Propranolol is a basic drug and has binding sites within the plasma that are different from those occupied by acidic drugs (alpha-acid glycoprotein rather than albumin). Moreover, propranolol has a large volume of distribution, so perhaps is actually no clinically important displacement interaction.

REFERENCES

1. Evans, G.H., Nies, S.A. and Shand, D.G. (1973). Plasma Binding of Propranolol. *J. Pharmac. Exp. Ther.* 186, 114-122.
2. Sager, G., Odd, G., Jacobsen, N. and Jacobsen, S. (1979). Variable Binding of Propranolol In Human Serum, *Biochemical. Pharmacol.* 28, 905-911.
3. Krishnia, R., William, G.T. and Richard, E.M. (1985). Assay of total and Free Propranolol in Plasma by Liquid Chromatography With Fluorometric Detection. *Clin. Chem.* 31, 131-134.
4. Sophiunapoulos, J.A. (1987). Ultrafiltration is Theoretically Equivalent to Equilibrium Dialysis But Much Simpler to Carry out. *Arch. Biochem. and Biophys.* 187, 132-133.
5. Frans, M., Bel, P., Rene, A., Breackman, G. and Marc, G.B. (1984). Binding of Oxprenolol and Propranolol to Serum Albumin and α -Acid glycoprotein in Man And Other Species. *Biochem. Pharmacol.* 33, 2065-2069.
6. Wood, A.J. and Feely, J. (1983). Pharmacokinetic Drug Interaction With Propranolol. *Clin. Pharmacol.* 8 (3), 253-262.
7. Uma, T., Manju, B., Sen, P. and Mahjan, P. (1987). *In vitro* interaction between aspirin and propranolol at the plasma protein binding sites. *Indian J. Med. Res.* 86, 95-100.
8. Houston, M.C. (1991). Nonsteroidal Anti-inflammatory Drugs And Antihypertensives. *Amer. J. Med.* 90, Suppl. 5A, 42S-47S.
9. Johnson, A.G., Nguyen, T.V. and Day, R.O. (1994). Do Nonsteroidal Anti-inflammatory Drugs Affect Blood Pressure? A Meta-analysis, *Ann. Intern. Med.* 121, 289-300.
10. Pope, J.E., Anderson, J.J. and Felson, D. (1993). A Meta-analysis Of The Effects Of Nonsteroidal Anti-inflammatory Drugs On Blood Pressure. *Arch. Intern. Med.* 153, 477-484.
11. Kasznicki, J. and Owczarek, A.W. (2001). Effects of Indomethacin on Hemodynamic Parameters After Intravenous Administration of Propranolol and Enalaprilate in Rabbits. *Polish. J. Pharmacol.* 53, 487-493.
12. Amitava, D. and Timothy, G.T. (1996). *In vitro* Displacement of Phenytoin From Protein Binding by Nonsteroidal Antiinflammatory Drugs Tolmetin, Ibuprofen and Naproxen in Normal and Uremic Sera. *T.D.M.* 18 (1), 97-99.
13. Kamesvar, R.K., Mahmou, S.A., Thomas, P.J. and Ashim, K.M. (2001). Binding of Cosalane A Novel Highly Lipophilic Anti-HIV Agent - to Albumin and Glycoprotein, *J. of Pharmaceu. Scien.* 90 (5), 659-666.
14. Cooper, P.F. and Wood, G.C. (1968). Protein Binding of Small Molecules : New Gel Filtration Method. *Pharm. Pharmacol.* 20, 150s-156s.
15. Wood, G.C. and Cooper, P.F. (1970). The Application of Gel Filtration to the Study of Protein Binding of Small Molecules. *Chromatog. Rev.* 12, 88-107.
16. Seville, B., Zini, R., Madjar, C.V., Thuaud, N. and Tillemen, J.P. (1990). Separation Procedures Used to Reveal and Follow Drug - Protein binding. *J. Chromatog.* 12 (531), 51-77.
17. Iwamoto, K. and Watanab, J. (1985). Avoidance of First - Pass Metabolism of Propranolol After Rectal Administration as a Function of the Absorption Site. *Pharmace. Res.* 53-54.

18. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randell, R. (1951). Protein measurment with the Folin Phenol reagent. J. Biol. Chem. 243, 1331-1332.
19. Chkrabarti, S.K. (1978). Cooperativity of Warfarin Binding with SA Induced by Free Fatty Acid Anions. Biochem. Pharmacol. 627, 739-743.
20. Miners, J.O. (1989). Drug Interaction Involving ASA and SA. Clin. Pharmacoki. 6, 372-344.
21. Jurez, O.J., Jung, C.H., Flores, P.J. and Asseff, I.L. (2002). Clinical evidence of an Interaction Between Imipramine and Acetyl Salisylic Acid on Protein Binding in Depress Patients. Clin. Neuropharmacol. 25 (1), 32.
22. Ferry, D.G., Caplan, N.B. and Cubeddu, L.X. (1986). Interaction Between Antidepressants and α 1-Adrenergic Receptor Antagonists on the Binding to α 1-acid Glycoprotein. J. Pharmaceu. Scein. 75 (2), 146-149.