



***In-Vitro* Study of Dipyridamole–Propranolol Interaction in Protein Binding in the Absence and Presence of Nicotine**

Zahra Rezaei*, Soghra Khabnadideh, Elham Afiatjou

Faculty of Pharmacy, Pharmaceutical Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

Abstract

The binding of drugs by plasma proteins is an important phenomenon, because it influences the size of the free fraction of the drugs in plasma. In this study the influence of dipyridamole (DP) on the protein binding of propranolol (PL) and PL on the protein binding of DP were studied alone and in the presence of nicotine (NC). The equilibrium dialysis was employed for protein binding studies. Results showed that DP (10 µg/ml) increased the protein binding of PL (1-40 µg/ml) by 4.1%, and PL (30 µg/ml) decreased the protein binding of DP (1-30 µg/ml) by 3.7%. NC (2 µg/ml) did not have any influence on the protein binding of PL and DP. Therefore, co-administration of PL and DP may cause an alteration in their pharmacological effects, but cigarette smoking possibly does not change protein binding of PL and DP.

Key words: Dipyridamole; Interaction; Nicotine; Propranolol; Protein binding.

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1. Introduction

The binding of drugs to plasma and tissue proteins is an important factor affecting the drugs, 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. The acidic drugs commonly bind to plasma albumin and concomitantly administered drugs may displace each other from their binding site. The

basic drugs may bind to either albumin or α -acid glycoprotein (AGP).

Propranolol (PL) is the most widely used adrenergic β -receptor blocking agent that binds to plasma proteins by 90%-95%. Evans reported that PL mainly binds to albumin [1], but Sager et al. reported that PL binds mainly to α -acid glycoprotein [2]. Certainly, PL binds to albumin and α -acid glycoprotein [3]. It has been reported that binding of PL to albumin or α -acid glycoprotein is related to the species of animals [4]. Serum binding of some basic drugs such as PL is increased with inflammation or stress, and it is accepted that this is due to an increase of the plasma proteins [4]. The absorption, protein binding and metabolism of PL may all be affected by the co-administration of other drugs [5]. It has

*Corresponding Author: Zahra Rezaei, Department of Medicinal Chemistry, Faculty of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran, P.O. Box 71345-1583.
Tel (+98)711-2425305, Fax (+98)711-2426070
E-mail: rezaeiza@sums.ac.ir

been reported that PL can displace acetylsalicylic acid from its binding sites [6].

Dipyridamole (DP) is commonly used as a coronary vasodilator and antiplatelet drug that binds to plasma protein by 99% [7]. AGP and albumin are responsible for the high protein binding of DP in plasma [8]. It has been reported that DP and its analogues enhance the *in vitro* activity of antimetabolite anticancer drugs with reduced AGP binding; therefore, nucleoside transport inhibition can be maintained in the absence of AGP binding [9].

Cigarette smoking can affect drug therapy by both pharmacokinetic and pharmacodynamic mechanisms by polyaromatic hydrocarbons and nicotine [10]. It has been reported that smoking displaces lidocaine from protein binding sites [11]. It has also been reported that cigarette smoking results in faster clearance of heparin, possibly related to smoking-related activation of thrombolysis with enhanced heparin binding to antithrombin III [10]. The protein binding of DP and PL which have high protein binding capacities may be altered in smoking which could be of clinical importance; therefore, in the present study, we investigated the displacement of PL and DP from protein binding sites by each other and in the absence

and presence of nicotine (NC).

2. Materials and methods

2.1. Materials

Dipyridamole was obtained from Boehringer; propranolol was obtained from Sigma; and nicotine was purchased from Merck. Normal human pooled plasma was obtained from the Shiraz Blood Bank.

2.2. Protein binding experiments

In vitro protein binding of the drugs was measured by equilibrium dialysis. Dialysis was performed in duplicate at 25 °C for 10 h in Perspex half-cell separated by a cellophane membrane. One compartment contained phosphate buffer (1.2 ml; pH 7.4; ionic strength 0.17) in which the drug was dissolved, and the other contained plasma (1.2 ml). The concentrations of the drugs was determined by spectrophotometric method [12, 13].

In order to determine PL concentration, 1 ml NaOH 0.1 N was added to 3 ml of the sample, and the resulted solution was extracted using 20 ml heptane containing 1% v/v methanol, shaken for 5 min, and after that centrifuged at 3000 rpm for 10 min. The organic phase was separated from the aqueous

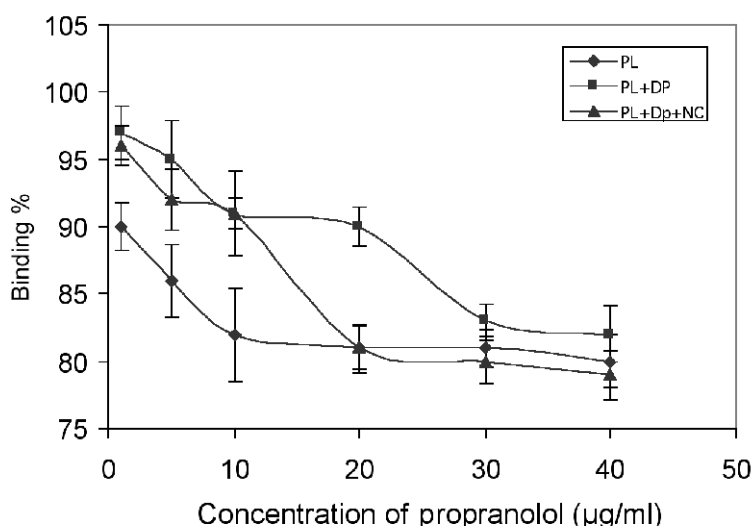


Figure 1. The binding of propranolol (1-40 µg/ml) to plasma proteins in the presence of dipyridamole (10 µg/ml) and nicotine (2 µg/ml).

phase, and the solvent was evaporated under a stream of nitrogen gas. The residue was dissolved in 2 ml HCl 0.1 N and PL was extracted as hydrochloride salt. After adjusting the pH to 7.4, the absorbance was measured at 289 nm.

For determination of DP concentration, 1 ml NaOH 1 N was added to 0.5 ml of the sample, and the resulted solution was extracted using 4 ml n-hexane/isoamyl alcohol (with the ratio of 75/25), shaken for 5 min, and then was centrifuged at 3000 rpm for 15 min. The organic phase was separated, and the solvent was evaporated. The residue was dissolved in phosphate buffer, pH 7.4, and its absorbance was measured at 413 nm.

The binding data for plasma proteins was analyzed and the molar concentration of the unbound (C_u) and the bound drugs (C_b) were determined for DP and PL.

2.3. Binding interaction study

As drug-plasma protein binding is usually studied over concentration ranges which are representative of the therapeutic drug levels, therefore, concentrations similar to the usual plasma level of the drugs (or higher) were chosen in our experiments.

We obtained the binding parameters of PL to the plasma proteins at the concentrations

between 1-40 $\mu\text{g/ml}$, in the presence of DP (10 $\mu\text{g/ml}$). Displacement of PL from protein binding sites by DP, was also measured in the presence of nicotine (2 $\mu\text{g/ml}$). In the second step, binding parameters of DP at the concentrations between 1-30 $\mu\text{g/ml}$ were obtained in the presence of PL (30 $\mu\text{g/ml}$), and also PL (30 $\mu\text{g/ml}$) + NC (2 $\mu\text{g/ml}$) to plasma proteins.

2.4. Statistical analysis

The statistical comparison was performed using one way ANOVA with post hoc Tucky-Kramer test ($p < 0.05$, $n = 3$).

3. Results

The binding of PL (1-40 $\mu\text{g/ml}$) to plasma proteins and its interaction with DP (10 $\mu\text{g/ml}$) was analyzed with Scatchard plots. DP altered the protein binding of PL to plasma proteins by 4.1%, but this was not associated with any significant alteration in the number of binding sites (Figure 1). As shown in Table 1, DP (10 $\mu\text{g/ml}$) could significantly increase C_u of PL (1-20 $\mu\text{g/ml}$), but NC (2 $\mu\text{g/ml}$) had no effect on the protein binding of PL in the presence of DP.

The protein binding of DP (1-30 $\mu\text{g/ml}$) to plasma proteins was also analyzed in the absence and presence of PL (30 $\mu\text{g/ml}$) and

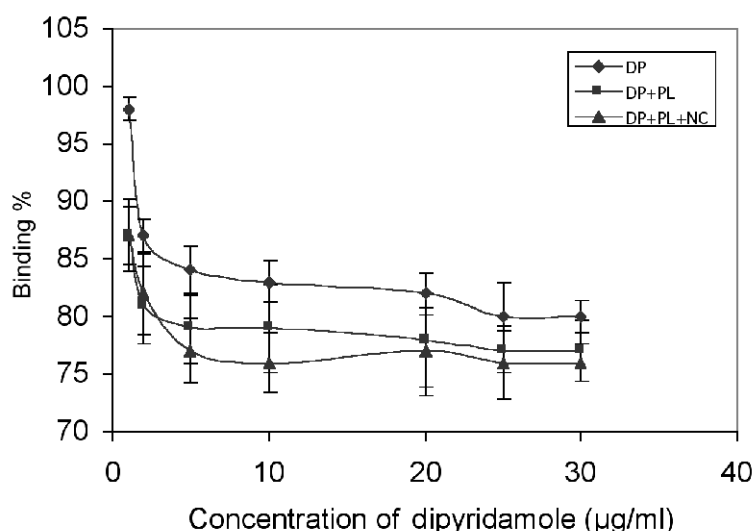


Figure 2. The Binding of dipyridamole (1-30 $\mu\text{g/ml}$) to plasma proteins in the presence of propranolol (30 $\mu\text{g/ml}$) and nicotine (2 $\mu\text{g/ml}$).

Table 1. Effect of dipyrindamole on the protein binding of propranolol in the presence and absence of nicotine.

Concentration of PL ($\mu\text{g/ml}$)	Concentration of unbound PL ($\mu\text{g/ml}$)		
	PL	PL + DP (10 $\mu\text{g/ml}$)	PL + DP (10 $\mu\text{g/ml}$) + NC (2 $\mu\text{g/ml}$)
1	0.10 \pm 0.01	0.03 \pm 0.02**	0.04 \pm 0.01**
5	0.71 \pm 0.10	0.25 \pm 0.15**	0.41 \pm 0.08*
10	1.80 \pm 0.32	0.91 \pm 0.24**	0.90 \pm 0.11**
20	3.81 \pm 0.45	2.00 \pm 0.54*	3.61 \pm 0.95

PL: propranolol; DP: dipyrindamole; NC: nicotine.

*Significantly different from PL alone, $p < 0.05$, (n=3).

**Significantly different from PL alone, $p < 0.01$, (n=3).

NC (2 $\mu\text{g/ml}$). The results show that DP displaced PL from plasma protein binding sites. The decrease in the binding of DP to plasma proteins with 30 $\mu\text{g/ml}$ of PL was only around 3.7% at the above mentioned concentration of DP (Figure 2).

Table 2 shows the effect of PL (30 $\mu\text{g/ml}$) on the C_u of DP (1-20 $\mu\text{g/ml}$) in the absence and presence of NC (2 $\mu\text{g/ml}$). PL could significantly increase C_u of DP but NC did not alter this displacement.

4. Discussion

The clinical importance of the plasma protein binding of drugs has been indicated in several reports that attribute the exaggerated effects of certain drugs and endogenous hormones to their inadvertent displacement from binding to plasma proteins by the administration of other agents [6, 14]. This kind of interaction leads to an alteration in the free levels of the pharmacologically active component of the drug and influences both the therapeutic and toxic effects of the agent.

A significant decrease in the free level of PL, when used in combination with DP, is probably due to an increase in the binding of PL to non specific or specific sites of the plasma proteins. The binding of PL, at the

above-mentioned concentrations, increased significantly ($p < 0.025$) when used concurrently with DP (10 $\mu\text{g/ml}$) by 4.1%. Such interactions are not unknown. To recognize a competitive antagonism between the two drugs, we studied the effect of PL on protein binding of DP. The results showed a significant decrease ($p < 0.05$) in the binding of DP (1-30 $\mu\text{g/ml}$) when used with PL (30 $\mu\text{g/ml}$) by 3.7%, but this was not associated with any significant alteration in the number of binding sites. These results indicate that a competitive antagonism between the two drugs is possible, because they bind to AGP and albumin [1, 8]. Even the alterations in the protein binding of drugs are only around 4%, but it probably causes a significant variation in the free concentration of the two drugs; for example, if a drug reduces binding from 99% to 95%, it will thereby increase the concentration of the free and active form of the drug from 1% to 4% (a four-fold increase). However, it has been reported that NC can alter the protein binding of drugs [11], but we found that NC with a concentration of 2 $\mu\text{g/ml}$ did not have a significant effect on these interactions. Therefore, cigarette smoking possibly does not change the protein binding of PL and DP.

Table 2. Effect of propranolol on the protein binding of dipyrindamole in the presence and absence of nicotine.

Concentration of DP ($\mu\text{g/ml}$)	Concentration of unbound DP ($\mu\text{g/ml}$)		
	DP	DP + PL (30 $\mu\text{g/ml}$)	DP + PL (30 $\mu\text{g/ml}$) + NC (2 $\mu\text{g/ml}$)
1	0.02 \pm 0.01	0.13 \pm 0.02*	0.13 \pm 0.05*
5	0.81 \pm 0.12	1.35 \pm 0.09**	1.05 \pm 0.10
10	1.75 \pm 0.21	5.63 \pm 0.15**	4.21 \pm 0.15**
20	3.61 \pm 0.31	5.75 \pm 0.54*	6.00 \pm 0.88**

PL: propranolol; DP: dipyrindamole; NC: nicotine.

*Significantly different from PL alone, $p < 0.05$, (n=3).

**Significantly different from PL alone, $p < 0.01$, (n=3).

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