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In vitro enantioselective displacement of propranolol from protein binding sites by acetyl salicylic acid and salicylic acid

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Abstract

The influences of acetyl salicylic acid (ASA) and salicylic acid (SA) on the enantioselective binding of propranolol (PL) and its enantiomers to plasma proteins and human serum albumin (HSA) were investigated. The equilibrium dialysis was employed for protein binding studies. We observed statistically significant displacement of racemic-PL, (+)-(*R*)-PL, and (−)-(*S*)-PL (0.1–10 μM) from their protein binding sites by ASA (200 μg/ml) and SA (100 μg/ml). ASA and SA displaced PL stereoselectivly from its binding sites. We concluded that ASA and its metabolite SA could change *R*/*S* ratio of PL unbound fractions and they might affect pharmacokinetic properties of PL.

Keywords: Propranolol; Streoselective; Protein Binding; Interaction; Salicylic Acid

1. Introduction

The binding of drugs by plasma proteins is an important phenomena, because it influences the size of the free fraction of the drugs in plasma.

Many basic drugs have chiral centers and are available commercially as racemic mixtures. Some drugs show stereoselective pharmacokinetics and pharmacodynamics, including differences in metabolism, tissue distribution and excretion. Many of these stereospecific characteristics may be due to the stereoselctive protein binding in plasma and tissue; however, few studies have examined the individual enantiomers of drugs. Propranolol (PL) available as the racemic mixture is a nonselective beta adrenergic blocking agent used in the treatment of hypertension, angina pectoris and cardiac arrhythmias as well as other diseases. PL is administrated in racemic form, but only (−)-(*S*)-PL is the active form of the drug. This enantiomer of PL appears to be cleared more slowly from the body than is the inactive enantiomer (Hoffman, 2001).

Stereoselctivity in the plasma protein binding of PL was described by several groups (Walle et al., 1983; Murai-Kushiya et al., 1993; Albani et al., 1984; Ding et al., 1999; Mehvar and Brocks, 2001). The mean percentage of unbound (+)-(*R*)-PL is greater than the (−)-(*S*)-PL, which is compatible with the larger volume of distribution of the *S* isomer (Lenard et al., 1990). It has also been reported that the dissociation constant of (−)-(*S*)-PL is two times lower than that of (+)-(*R*)-PL for AGP (Hanada et al., 2000).

PL is widely used in clinical practice and is frequently administered along with other drugs. The absorption, protein binding and metabolism of PL may all be affected by the co-administration of other drugs (Wood and Feely, 1983). The use of ASA in the cardiovascular diseases has been established for many years. Hydrolysis of ASA to salicylic acid (SA) by non-specific esterase occurs in the liver and to a lesser extent, in the stomach that only 68% of the dose reaches to the systemic circulation as ASA (Needs and Brooks, 1985). Both ASA and SA are bound to serum albumin and many of reported drug interactions involve displacement of the co-administered drug from plasma proteins (Miners, 1989). Because ASA in many conditions like myocardial infraction or migraine co-administrates with PL, it may lead to changes in the pharmacokinetic of PL, ASA or both. It has been reported that PL can displace ASA from its binding

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Table 1
Mean percentage of unbound fraction (C_u) of propranolol (PL) enantiomers to human plasma proteins in the absence and presence of acetyl salicylic acid (ASA) and salicylic acid (SA) to human plasma proteins

PL (μM)	C_u of PL enantiomers (±S.D.)			C_u of PL enantiomers in the presence of ASA200 μg/ml (±S.D.)			C_u of PL enantiomers in the presence of SA100 μg/ml (±S.D.)		
	R	*S*	1 (*R*/*S*)	*R*	*S*	2 (*R*/*S*)	*R*	*S*	3 (*R*/*S*)
0.1	28.5 (2.3)	8.2 (1.5)	3.40 (0.22)	48.8 (1.8)	9.6 (2.5)	5.08 (0.48)**	40.7 (2.9)	8.8 (1.1)	4.62 (0.37)*
0.5	39.1 (3.1)	27.2 (1.7)	1.40 (0.25)	65.2 (2.3)	29.3 (1.7)	2.22 (0.13)*	59.6 (3.5)	27.5 (3.4)	2.16 (0.41)*
1	60.1 (2.4)	47.7 (3.1)	1.25 (3.10)	75.5 (1.7)	35.5 (4.1)	2.12 (0.24)*	69.7 (1.4)	32.8 (1.1)	2.12 (0.65)**
5	62.0 (3.2)	51.2 (2.2)	1.26 (0.30)	81.7 (2.3)	37.7 (3.8)	2.16 (0.17)**	78.4 (2.6)	35.4 (2.1)	2.21 (0.19)**
10	69.2 (4.3)	48.7 (3.6)	1.42 (0.21)	84.8 (3.7)	41.2 (4.7)	2.10 (0.14)*	80.7 (4.6)	38.6 (1.8)	2.09 (0.21)*

* $p<0.05$ groups 2 and 3 vs. group 1 ($n=3$).
** $p<0.01$ groups 2 and 3 vs. group 1 ($n=3$).

sites (Tekur et al., 1987) but there is not any report about the effect of ASA on protein binding of PL and its enantiomers.

In this study, we investigated the enantioselective displacement of PL by ASA and its metabolite, SA from its binding sites on the plasma proteins. Because ASA mainly binds to albumin, we also studied enantioselective displacement of PL by ASA and SA from HSA.

2. Methods

2.1. Materials

Human serum albumin was obtained from Bohringer, PL enantiomers from Sigma, ASA and SA from Merck. Normal human pooled plasma was obtained from Shiraz Blood Bank.

2.2. Binding experiments

In vitro protein binding of the drugs was studied 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 and ionic strength 0.17) in which the drug was dissolved (final drug concentrations: 0.1–10 μM) and the other contained human serum or HSA (580 μM) in the same buffer (1.2 ml). The concentration of PL was determined by fluorometric method (Iwamoto and Watanab, 1985). The validation study shows this method can be used in the determination of PL in applied concentrations.

The binding data for plasma proteins and HSA were analyzed and molar concentrations of unbound (C_u) and bound drug (C_b) were separately determined for racemic and PL enantiomers.

2.3. Binding interaction study

We studied protein binding of PL and its enantiomers at the concentrations of 0.1–10 μM to plasma proteins and also HSA in the absence and presence of ASA 200 and SA 100 μg/ml. The binding parameters of PL and its enantiomers were obtained at the concentrations (0.1–10 μM) to HSA. Then K_d (dissociation constant), n (number of binding sites) and α (fraction of nonspecific binding) for PL and its enantiomers were obtained alone and in the presence of ASA and SA as described by Hanada and co-workers (Hanada et al., 2000) using this equation:

$$C_b = \left(\frac{C_u n P}{K_d + C_u}\right) + \alpha C_u$$

where C_b and C_u are the concentrations of bound and unbound drug and P is the HSA concentration.

2.4. Statistical analysis

The statistical comparison was performed using one way ANOVA with post hoc Tucky–Kramer test ($p<0.05$).

3. Results

In order to analyze the effect of ASA and SA on the binding of PL and its enantiomers to human plasma proteins, we studied protein binding of PL and its enantiomers to plasma proteins. As shown in Table 1, PL binds stereoselectively to human plasma. ASA 200 μg/ml and SA 100 μg/ml, when used with racemic, (+)-(*R*)-PL, and (−)-(*S*)-PL showed decrease in the binding of PL to human plasma proteins stereoselctively. These results show that ASA and SA displace (+)-(*R*)-PL more than (−)-(*S*)-PL on the plasma proteins and as a result *R*/*S* ratio is changed (Table 1).

The binding of PL and its enantiomers at the concentrations of 0.1–10 μM to HSA and interaction between them and ASA and

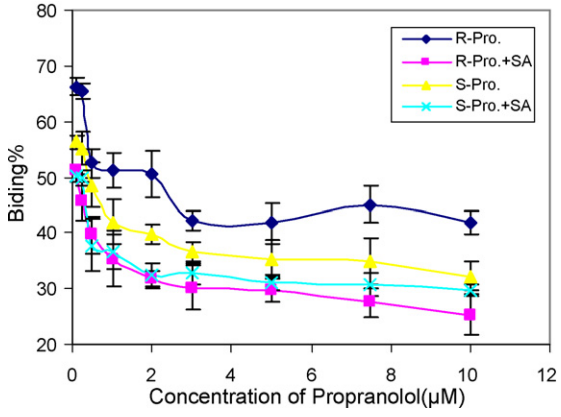

Fig. 1. The binding of propranolol enantiomers to HAS 4% in the absence and presence of SA 100 μg/ml.

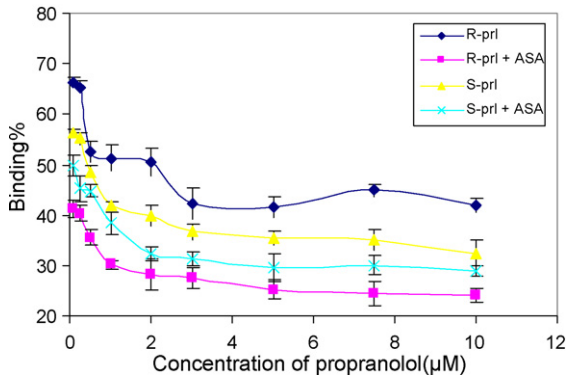

Fig. 2. The binding of propranolol enantiomers to HAS 4% in the absence and presence of ASA 200 μg/ml.

SA were also analyzed with Scatchard plots. ASA 200 μg/ml caused an alteration in the binding of (+)-(*R*)-PL by 39.39% and (−)-(*S*)-PL by 13.31% to HAS, respectively (Fig. 1). SA 100 μg/ml also decreased the binding of (+)-(*R*)-PL by 30.73% and (−)-(*S*)-PL by 14% to HAS, respectively (Fig. 2).

Tables 2 and 3 summarize the binding characteristics of PL and its enantiomers to HSA. The dissociation constant (K_d) of PL enantiomers was obtained in the absence and presence of ASA and SA. The Kd of PL enantiomers reduced significantly by ASA and also SA, and the reduction of K_d for (+)-(*R*)-PL was more than (−)-(*S*)-PL.

Although ASA changed significantly the number of binding sites (*n*) of PL enantiomers to HSA stereoselectively, it was not shown by SA.

The α parameter of PL enantiomers reduced stereoselectively to HSA by ASA and SA.

These results show that ASA and SA displace (+)-(*R*)-PL more than (−)-(*S*)-PL from its binding sites on HSA significantly.

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 (Tekur et al., 1987; Amitava and Timothy, 1996). Tekur and co-workers reported that PL could displace ASA from its active site on the plasma proteins (Tekur et al., 1987). It has also been reported that PL binds to plasma proteins stereoselectively (Walle et al., 1983; Murai-Kushiya et al., 1993; Albani et al., 1984; Ding et al., 1999; Mehvar and Brocks, 2001). In this study we showed that ASA and its metabolite, SA could displace PL from its binding site stereoselctively.

As shown in Table 1, ASA and also SA displaced PL enantiomers from human plasma proteins binding site stereoselectively, so the *R*/*S* ratio was changed. It has been reported that each enantiomer is competitively displaced from AGP by another enantiomer of the same drug, suggesting that they bind to the same site in plasma (Hanada et al., 2000). Therefore, the ratio of these enantiomers in plasma affects their protein bindings.

Although PL binds mainly to AGP, it also binds to albumin (Frans et al., 1984) and ASA may displace PL from albumin binding site, because ASA is an acidic drug and binds mainly to albumin.

ASA and SA also significantly displaced the binding of (+)-(*R*)-PL more than (−)-(*S*)-PL from HSA binding sites (Figs. 1 and 2) therefore the ratio of (+)-(*R*)-PL to (−)-(*S*)-PL will be changed. As it has been reported that PL has stereospecific pharmacokinetic and pharmacodynamic (Mehvar and Brocks, 2001), its pharmacological properties affected by its enantiomers ratio.

Tables 2 and 3 present the effect of ASA and SA on the K_d, *n* and α parameter of PL enantiomers to HAS. Although the number of binding (*n*) of PL enantiomers to HSA is small, ASA and SA changed it significantly. Therefore, competitive antagonism between the two drugs is possible.

The α parameter is also reduced by ASA and SA. This indicates that ASA and SA displace PL enantiomers from non specific site on HSA. It has been reported that ASA can displace imipramine from protein binding site. However, imipramine is a basic drug that is known to bind to AGP like PL (Ferry et al., 1986; Jurez et al., 2002). It has also been reported that SA displaces protein binding of ibuprofen, tolmetin, sodium diclofenac, valproic acid and warfarin from protein binding sites (Amitava and Timothy, 1996; Chkrabarti, 1978).

The stereoselctivity in the binding of PL enantiomers to plasma proteins is the opposite of HSA, but ASA and SA displace (+)-(*R*)-PL more than (−)-(*S*)-PL from both plasma proteins and HSA. It can be suggested that ASA and SA do not affect the binding of PL to AGP. Because PL is a basic drugs, but ASA is an acidic drug.

In conclusion, as (−)-(*S*)-PL is the active form of the drug and its in vivo protein binding is higher than (+)-(*R*)-PL, displacement of this enantiomer from its binding site is more important and it can cause alteration in pharmacological properties. On the other hand, since this enantiomer is excreted from the body slower, the increase in its free concentration in plasma can affect the drug's clearance.

Table 2
Binding characteristics of propranolol (PL) enantiomers in the absence and presence of salicylic acid (SA) to human serum albumin (HSA)

	A—(+)-(*R*)-PL (±S.D.)	B—(−)-(*S*)-PL (±S.D.)	C—(+)-(*R*)-PL + SA (±S.D.)	D—(−)-(*S*)-PL + SA (±S.D.)
n	0.0322 (0.0016)	0.0277 (0.001)	0.0196 (0.0007)	0.0181 (0.0008)
K_d (μM)	26.71 (0.963)	24.20 (0.945)	21.29 (1.001)	26.002 (1.003)
α	0.15 (0.009)	0.03 (0.001)	0.00 (0.001)	0.10 (0.001)

The HSA concentration, 580 μM; the PL concentration, 0.1–10 μM; the SA concentration, 100 μg/ml. α, fraction of non specific binding; K_d, dissociation constant; *n*, number of binding sites. All of groups have difference significant with $p < 0.01$ except "*n*" for D vs. A and "K_d" for D vs. A ($n = 9$).

Table 3
Binding characteristics of propranolol (PL) enantiomers in the absence and presence of acetyl salicylic acid (ASA) to human serum albumin (HSA)

	A—(+)-(*R*)-PL (±S.D.)	B—(−)-(*S*)-PL (±S.D.)	C—(+)-(*R*)-PL + ASA (±S.D.)	D—(−)-(*S*)-PL + ASA (±S.D.)
n	0.0322 (0.0016)	0.027 (0.001)	0.017 (0.0008)	0.0169 (0.0007)
K_d (μM)	26.71 (0.963)	24.20 (0.945)	20.99 (0.878)	25.99 (1.0001)
α	0.15 (0.0091)	0.03 (0.0011)	0.05 (0.0012)	0.108 (0.0014)

The HSA concentration, 580 μM; the PL concentration, 0.1–10 μM; the ASA concentration, 200 μg/ml. α, fraction of non specific binding; K_d, dissociation constant; *n*, number of binding sites. All of groups have difference significant with $p < 0.05$ except "K_d" for D vs. A ($n = 9$).

Although, ASA and SA displace (−)-(*S*)-PL from its binding sites only about 14%, but it probably significantly increases the free concentration of (−)-(*S*)-PL, because PL 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 form 1% to 4% (a four-fold increase). Also, ASA and SA displace (+)-(*R*)-PL more than (−)-(*S*)-PL, so the ratio of the free form of these enantiomers will be changed. Because, PL is administered as racemic form increasing the free form of (+)-(*R*)-PL in plasma can competitively displace the (−)-(*S*)-PL (Hanada et al., 2000). This effect can be added to alteration of (−)-(*S*)-PL by ASA and SA so, there is more increasing in the concentration of active form (−)-(*S*)-PL. Therefore, ASA and SA may change pharmacokinetic of (−)-(*S*)-PL, but clinical studies should be employed. Moreover, PL has a large volume of distribution, so perhaps is actually no clinically important displacement interaction.

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