Enantioselective Plasma Protein Binding of Propafenone: Mechanism, Drug Interaction, and Species Difference

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ABSTRACT The interaction of propafenone (PPF) enantiomers with human plasma, human serum albumin (HSA), α_1-acid glycoprotein (AGP), as well as with plasma from rat, rabbit, and cow was investigated using indirect chiral high performance liquid chromatography (HPLC) and ultrafiltration techniques. The stronger binding of the *S*-PPF found in human plasma was due to AGP. Two classes of binding sites in AGP were identified: one with high-affinity and small binding capacity ($K_{1(S)} = 7.65 \times 10^6$ M^{-1}, $n_{1(S)} = 0.50$; $K_{1(R)} = 2.81 \times 10^6$ M^{-1}, $n_{1(R)} = 0.46$), which revealed stereoselectivity; the other with low-affinity and high-binding capacity ($n_{2(S)}K_{2(S)} = 9.95 \times 10^3$ M^{-1}; $n_{2(R)}K_{2(R)} = 9.74 \times 10^3$ M^{-1}). The binding to HSA was found to be weak and not enantioselective ($nK_S = 2.08 \times 10^3$ M^{-1}, $nK_R = 2.05 \times 10^3$ M^{-1}). The interaction between enantiomers observed in human plasma was confirmed as a competitive type interacting at the high-affinity site in AGP. The binding mode of both enantiomers with AGP was mainly hydrophobic bond. PPF enantiomers had higher-binding affinity for the F-S variant of human AGP. Drug-drug binding interaction studies showed that verapamil, diazepam, nifedipine, furosemide, nitrendipine, and nimodipine did not affect the binding of PPF enantiomers except quinidine and aprindine at the therapeutic concentration. Comparative studies indicated considerable species-dependent binding stereoselectivity between plasma of the four species investigated. *Chirality 21:692–698, 2009.* © 2008 Wiley-Liss, Inc.

KEY WORDS: propafenone; stereoselectivity; plasma protein binding; AGP; HSA

INTRODUCTION

PPF (Fig. 1) is a class Ic antiarrhythmic agent used clinically as a racemic mixture. However, the two enantiomers of PPF show differences in drug efficacy and pharmacokinetic profiles. Although both enantiomers are equally potent in their activity as sodium channel blockers, the (*S*)-enantiomer exhibits β-blocking activity ~100 times higher.[1] Besides, (*R*)-PPF is cleared faster than (*S*)-PPF after administration of racemic PPF to healthy volunteers.[2] It has been thought that besides stereoselective metabolism, stereoselective protein binding might be also responsible for the differences in pharmacokinetics between PPF enantiomers.

Some work has been reported about the binding of PPF in serum[3] and AGP,[4–6] as well as the species differences.[7] However, there are no reports about the binding of PPF with HSA and the binding mode with AGP. The mechanism of stereoselective protein binding is still not very clear. And also we do not know whether there is interaction between enantiomers or with other drugs, which is very important for high protein binding drugs. Besides, the species difference study and the interaction with genetic variants of AGP were mainly focused on PPF racemic mixture. The binding differences between PPF enantiomers in these two aspects are not clear.

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HPLC with precolumn derivatization has been a useful, low-cost and convenient method for the assays of drug enantiomers in biological fluids.[8–12] Ultrafiltration was chosen for the study because of its speed, simplicity, and accuracy.[13] Previously, our lab has studied the enantioselective binding of esmolol, mexiletine, ketoprofen, flurbiprofen, and etodolac to plasma proteins.[14,15]

In this study, the interaction of PPF enantiomers with human plasma, HSA, and AGP was investigated using indirect chiral HPLC [precolumn derivatization with 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate (GITC)] and ultrafiltration techniques. The stereoselective binding mechanism between PPF enantiomers and AGP variants was revealed by competitive inhibition test. We also studied the species-dependent binding stereoselectivity, the

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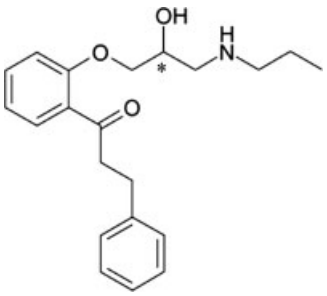

Fig. 1. Structure of PPF (the chiral center is indicated by *).

interaction of different drugs with PPF enantiomers as well as the interaction between two enentimers in PPF racemate.

MATERIALS AND METHODS

Materials

The drugs were obtained as follows: PPF from Yatai Pharmaceutical Factory (Zhejiang, China); diazepam from Changshu Pharmaceutical Factory (Jiangsu, China); furosemide, nifedipine, nitrendipine and nimodipine from Tailison Pharmaceutical Company (Zhejiang, China); aprindine from Zhenjiang NO.3 Pharmaceutical Factory (China); mifepristone from Department of Pharmacology, Zhejiang University. S-propafenon, R-propafenon, S-propranolol, verapamil, disopyramide, GITC, imipramine, dipyridamole, quinidine, HSA and Human AGP from Sigma (St. Louis, MO); Healthy human plasma was supplied by Ningbo Blood Center (Zhejiang, China); Healthy Cattle plasma was provided by Hangzhou Meat Processing Factory (Zhejiang, China); Sprague-dawley rats and New Zealan white rabbit were from Laboratory Animal Research Center of Zhejiang University. Double-distilled water was used. Methanol and acetonitrile were HPLC grade and all other reagents were of analytical grade. Phosphate buffered solution (PBS) was a mixture of 1.78 g KH_2PO_4, 7.61 g NaH_2PO_4, 9 g NaCl, and 1 l double-distilled water, with the pH 7.4 adjusted with 3 M NaOH.

HPLC Analysis

HPLC was performed on Agilent1100 system consisting of G1311A pump, G1315A (DAD) UV detector, manual injector and Chem-Stations software. An Aglient Zorbax C18 (250 × 4.6 mm, 5 μm) column was utilized. The mobile phase consisted of acetonitrile and 0.01 M phosphate buffer (pH 4.5) (3:2, v/v) for analyzing PPF enantiomers or (1:1, v/v) for rac -PPF at a flow rate of 0.80 ml/min. Aliquot of 20 μl sample was injected and detected at 248 nm (or 220 nm) at room temperature.

Sample Preparation

HSA and AGP were dissolved in PBS to give a concentration of 40 and 1.0 mg/ml, respectively. The stock solutions of PPF racemate and enantiomers were prepared by dissolving the respective compound in methanol to yield a concentration of 1 mg/ml. Appropriate amounts of each stock solution were taken, evaporated to dryness under air, and reconstituted in PBS and blank plasma, respectively.

To 150 μl of protein sample, 20 μl of the internal standard solution (I.S., 100 μg/ml S-propranolol in acetonitrile), 20 μl of concentrated ammonia water and 2 ml of dichlormethane were added. The mixture was vortexed for 3 min and then centrifuged at 3000 g for 10 min. The organic layer was transferred to another tube and evaporated to dryness under a gentle stream of air at room temperature. A 40 μl aliquot of GITC (1.02 mg/ml in acetonitrile) was added to the residue and the chiral derivatization was allowed to react at 30°C for 30 min. The reaction mixture was evaporated to dryness under a gentle air stream. The residue was reconstituted with 100 μl of the mobile phase and subjected to HPLC analysis.

Ultrafiltration

Ultrafiltration experiments were performed using a Microcon centrifugal system (America, Millipore) with the filter membrane of 30 kDa cutoff. Both PBS and plasma samples were equilibrated at 37°C for 15 min. A 500 μl aliquot of each plasma sample was centrifuged at 9000 g for 15 min at 37°C. For HSA and AGP samples, the centrifugation was performed for 5 min at 9000 and 3000 g, respectively. Aliquot of 150 μl ultrafiltrate and 150 μl plasma samples (unfiltered) were collected and prepared as discussed earlier.

Nonspecific filter membrane binding was performed in protein-free PBS buffer under the concentrations of 0.5, 3.0, and 15.0 μg/ml. The mixture was transferred to the ultrafiltration device without incubation, and centrifuged at 2000 g for 5 min at 37°C. Both the PBS samples (unfiltered) and the ultrafiltrate were directly injected on to the HPLC system.

All the PBS solutions and blank plasma were preincubated for 10 min at 37°C before mixed with PPF.

Data Analysis

Binding parameters were calculated according to a Langmuir equation (eq. 1) using a nonlinear least-squares method[16]:

$$r = \sum_{i=1}^{m} \frac{n_i K_i C_f}{1 + K_i C_f} \quad (1)$$

where, r is the number of bound drug per albumin molecule, n_i is the number of binding sites for the ith site, K_i is the binding constant for the ith site, C_f is the unbound drug concentration.

Plasma protein binding at different concentrations was corrected for the average filter membrane binding, according to the following equations,

$$B = 1 - (C_f/C_t)(1 + \bar{P}) \quad (2)$$

$$P = 1 - A_{ultrafiltrate}/A_{PBS} \quad (3)$$

where, B and P ($\bar{P}$) are percent protein binding and (average) nonspecific binding. C_f and C_t represent filtered and unfiltered drug concentrations in plasma and protein solutions. $A_{ultrafiltrate}$ and A_{PBS} are filtered and unfiltered drug peak areas in PBS.

Statistical analysis is conducted using paired t-test.

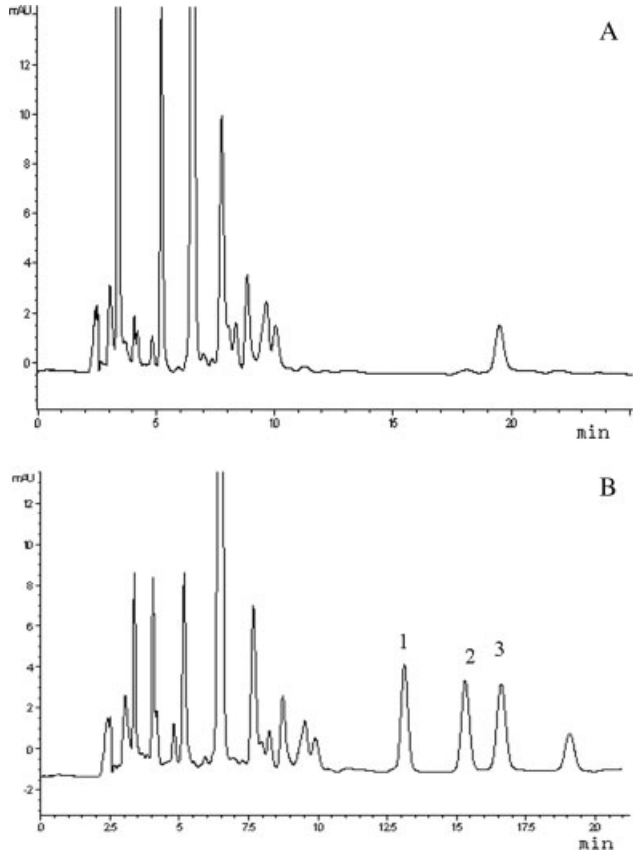

Fig. 2. Representative HPLC chromatograms of PPF enantiomers in human plasma. (**A**): blank plasma (**B**): blank plasma spiked with PPF and *S*-propanolol. Peaks: 1 *S*-propanolol, 2 *R*-PPF, 3 *S*-PPF.

RESULTS AND DISCUSSION

Method Validation

Figure 2 shows the representative chromatograms of blank human plasma and spiked samples. The two enantiomers and I.S. were well-resolved from each other and from the matrix components under the chromatographic conditions employed. Calibration curves were linear for HSA, AGP, and Sorenson PBS over the concentration range between 0.01 and 30 μg/ml and for Plasma between 0.5 and 30 μg/ml, with the correlation coefficients being greater than 0.998 for both R- and *S*-PPF.

The extraction recoveries of PPF enantiomers in different protein solutions were more than 89%, while the intra and interday precision were less than 12%. The lower limit of quantitation (LLOQ) was 0.01 μg/ml for each enantiomer.

The average nonspecific binding of PPF to the filter membrane was as low as 3.81%.

According to the aforementioned results, the method established was selective, reproducible, and sensitive enough to be applied to this experiment.

Binding with Human Plasma

Results showed that PPF displayed extensive and concentration dependent binding to plasma proteins (as shown in Fig. 3.) which agree with previous reports for PPF racemate.[3,6] In this study, the *S*-PPF exhibited higher protein binding than the R-PPF either incubated with the single enantiomer or with the racemate. From the Scatchard curve, each of PPF enantiomer interacted with at least two classes of binding sites: one with high-affinity and small binding capacity, the other with low-affinity and high-binding capacity. PPF mainly interacted with the former (which is the main cause for the stereoselective binding) at low concentrations. Binding was saturated at the high-affinity binding site as the concentration increased, and PPF tended to bind with the low-affinity binding site, which usually has little stereoselectivity.

Chiral drugs with high protein binding may have competitive interaction between enantiomers. Figure 3B shows the protein binding of the enantiomers that measured with the racemate. Both of the *R*- and *S*- enantiomers in racemate decreased in protein binding more or less, when compared with the data from incubation with the single

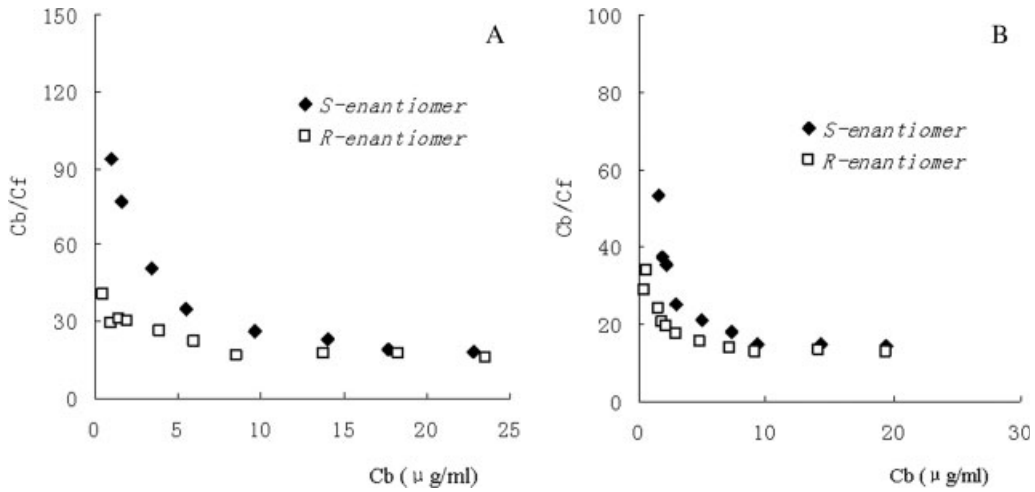

Fig. 3. Scatchard plots for the binding of PPF enantiomers in plasma. (**A**) Incubated with the single enantiomer separately ($n = 3$). (**B**) Incubated with the racemate ($n = 3$).

TABLE 1. Binding parameters of PPF enantiomers in 0.1% AGP

Reference		n_1	K_1 (M^{-1})	n_2K_2 (M^{-1})
Present study	*S*-PPF	0.50	7.65×10^6	9.95×10^3
	R-PPF	0.46	2.81×10^6	9.74×10^3
Gills et al.[4]	*rac*-PPF	0.79	1.31×10^5	$n_2 = 0.20$ $K_2 = 1.67 \times 10^7$
Oravcová et al.[5]	*S*-PPF	0.99 ± 0.08	$(8.93 \pm 1.82) \times 10^5$	$(1.06 \pm 0.09) \times 10^4$
	R-PPF	1.34 ± 0.09	$(6.18 \pm 0.93) \times 10^5$	$(6.87 \pm 0.72) \times 10^3$
Šoltés et al.[6]	*S*-PPF	0.98 ± 0.08	$(9 \pm 1.88) \times 10^5$	$(1.07 \pm 0.09) \times 10^4$
	R-PPF	1.33 ± 0.09	$(6.2 \pm 0.94) \times 10^5$	$(6.9 \pm 0.72) \times 10^3$

enantiomer separately, indicating that *S*- and *R*-PPF may have competitive interaction with the same binding site.

To investigate the mechanism involved in the stereoselectivity and competitive interaction, binding studies of PPF enantiomers with HSA and AGP were conducted.

Binding with HSA

The binding percentages of *S*- and *R*-enantiomer at different concentrations in 4% HSA were 55% and 54%, respectively, indicating lack of stereoselectivity. The free fraction and the bound fraction of *S*-/*R*- PPF had a good positive correlation with a coefficient of 0.997, indicating a nonsaturation binding with HSA. The binding parameters ($nK_S = 2.08 \times 10^3\ M^{-1}$, $nK_R = 2.05 \times 10^3\ M^{-1}$) for HSA were calculated by Henry's equation. No interactions tended to happen between the PPF enantiomers or with other drugs due to the nonsaturation binding with HSA. The binding constants measured with the enantiomer from racemate ($nK_S = 2.03 \times 10^3\ M^{-1}$, $nK_R = 2.02 \times 10^3\ M^{-1}$) were similar to the data measured with the single enantiomer. It was proved that HSA was not responsible for the stereoselectivity and competitive interaction of PPF enantiomers in human plasma.

Binding with AGP

The binding of PPF enantiomers to AGP decreased as the drug concentration increased, indicating concentration-dependent binding. The binding percentages of *S*- and *R*-PPF at concentrations studied were over the range of 98.1–32.6% and 96.7–24.9%, respectively. The free *S*-PPF/free *R*-PPF concentration ratio was more than 2.0 at lower concentration, and the stereoselectivity weakened as the drug concentration increased (more than 10 μg/ml).

From the Scatchard curve, each of PPF enantiomer interacted with at least two classes of binding sites in AGP. By assuming that only two classes of binding sites were involved, eq. 1 could be expressed as three-parameter model (eq. 4) or four-parameter model (eq. 5). The two models were fitted by nonlinear least square method and the three-parameter model was found better to simulate the binding characteristics of PPF. Binding parameters obtained from eq. 4 measured with single enantiomer are listed in Table 1. The results showed that the primary contribution in stereoselective binding arose from the high-affinity binding site in AGP.

With regard to the binding parameters, several reported values are available (Table 1). Gillis et al.[4] reported that PPF was strongly bond to AGP including two classes of binding sites both with high affinity and low capacity. However, they used racemate rather than enantiomers. Therefore, the results may not be so indicative for enantiomers. Both of Oravcová et al.[5] and Šoltés et al.[6] measured the binding of PPF enantiomers with AGP by using a drug solution saturating a nonchiral high-performance size exclusion column. They observed two classes of binding sites: one with high affinity, the other with low-affinity and high capacity, which is the same to our results. The differences in binding parameters may due to the different methods we use. However, none of them were able to study the binding of PPF enantiomers in racemic sample. The interaction between two enantiomers is still unclear. Therefore, our results are more indicative and will extend the knowledge of PPF protein binding.

According to the results, one of the enantiomers could increase the free fraction of the other, indicating that binding competition between enantiomers may occur at the same site, which can be confirmed by a competition model (eqs. 6 and 7). Based on this model, *S*-PPF and *R*-PPF may have competition at the high-affinity binding site, while be independent from each other at the low-affinity binding site. From Figure 4, the competition model was validated by the good agreement between the calculated and observed Scatchard plots for the result measured with racemate. It should be noted that the slopes of the Scatchard plots for both enantiomers measured in racemate were quite different from those with *R*- or *S*-PPF alone. The observations indicated that competitive interactions between PPF enantiomers occurred at the high-affinity binding site.

$$r = \frac{n_1 K_1 C_f}{1 + K_1 C_f} + n_2 K_2 C_f \tag{4}$$

$$r = \frac{n_1 K_1 C_f}{1 + K_1 C_f} + \frac{n_2 K_2 C_f}{1 + K_2 C_f} \tag{5}$$

$$r(S) = \frac{n_1(S) K_1(S) C_f(S)}{1 + K_1(S) C_f(S) + K_1(R) C_f(R)} + n_2(S) K_2(S) C_f(S) \tag{6}$$

$$r(R) = \frac{n_1(R) K_1(R) C_f}{1 + K_1(S) C_f(S) + K_1(R) C_f(R)} + n_2(R) K_2(R) C_f(R) \tag{7}$$

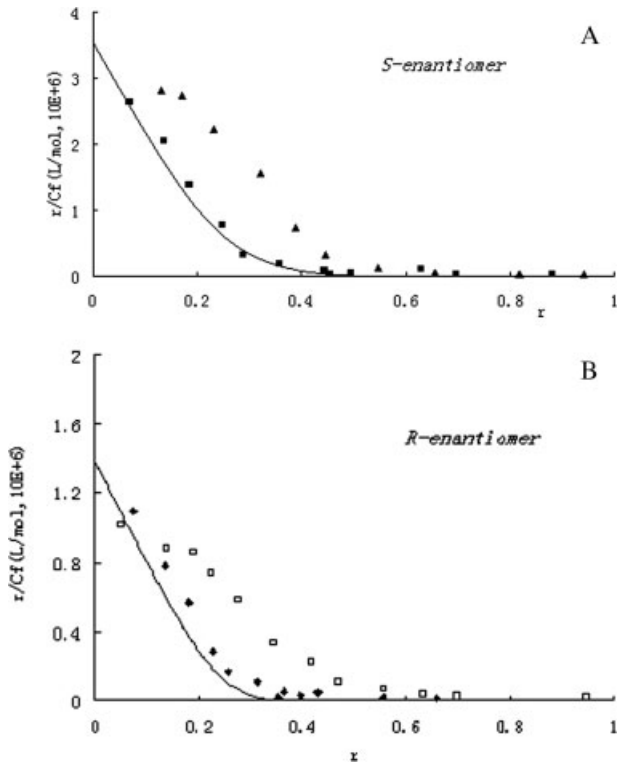

Fig. 4. Simulated and observed Scatchard plots for the binding of PPF enantiomers from the racemate in 0.1% AGP (n = 3). ▲: S-enantiomer measured with *S*-enantiomer alone. ■: *S*-enantiomer measured with the racemate. □: *R*-enantiomer measured with *R*-enantiomer alone. ♦: *R*-enantiomer measured with the racemate ——: Simulated curves.

There are several types of interactions between small ligand and macromolecule, including hydrophobic bond, hydrogen bond, van der waals force, electrostatic action, and so on. Different drugs or different enantiomers of chiral drugs may have dissimilar types of interaction with proteins. For example, the total changes in free energy for (*R*)- and (*S*)-warfarin binding were similar, but the contributions due to entropy were different. (*R*)-warfarin interacted mainly with the binding site interior, while (*S*)-warfarin interacted more with the site's outer surface.[17]

From the thermodynamics standpoint, the binding interaction between drug and protein is due to the change in free-energy (ΔG), while decrease in enthalpy (ΔH) or/and increase in entropy may also promote the interaction. When the temperature changes are minimal, the enthalpy changes (ΔH) can be regarded as a constant. From the eqs. 8–10, the values of ΔG, ΔH, andΔS can be calculated:

$$\Delta G = -RT \ln K \tag{8}$$

$$\Delta G = \Delta H - T\Delta S \tag{9}$$

$$\ln K_2/K_1 = (\Delta H/R) \cdot (1/T_1 - 1/T_2) \tag{10}$$

Ross et al.[18] summarized the thermodynamic rule to characterize self-association and ligand binding of biomacromolecule: if $\Delta H > 0$ and $\Delta S > 0$, the main force is hydrophobic interaction; $\Delta H < 0$ and $\Delta S < 0$ implies hydrogen bond or van der waals force; $\Delta H \approx 0$ and $\Delta S > 0$ suggests an electrostatic action. The binding constants of PPF enantiomers and thermodynamic parameters in 0.1%AGP at different temperatures are shown in Table 2. Based on the aforementioned principle ($\Delta H > 0$, $\Delta S > 0$), the interaction of both enantiomers with AGP was mainly hydrophobic bond.

Because both AGP and PPF were solvation in water solution, hydrone and other coexistence ions may affect the final results. Therefore, it may appear being regulated by several types of bonds, as well as the environment.

It has been demonstrated that AGP is encoded by two closely linked gene locus: ORM1 and ORM2 located on chromosome 9q31→34. Human AGP displays genetic polymorphism and has three main genetic variants: A, F1, and S variant. The ORM1 is encoded by the alleles of the same gene, while the ORM2 is the product of the other gene.[19] Native AGP isolated from plasma is not homogeneous, but exists as a mixture of two or three main genetic variants (i.e. A variant and F1 and/or S variants) in most individuals. The relative occurrence of the three main phenotypes in the population was found to be about 50% for F1 + S + A, 35% for F1 + A, and 15% for S + A. Pooled commercial AGP contains three variants in the same proportions.[20]

Some drug molecules have different selectivities in binding affinities for the genetic variants.[21,22] In the binding competition experiments, we investigated the binding of PPF enantiomers with different genetic variants of AGP and determined which one accounts for the binding stereoselectivity (as shown in Table 3). Disopyramide and imipramine were chose as specific ligands of the A variant, while dipyridamole and mifepristone were of the F1-S variant.[23] The results showed that dipyridamole and mifepristone strongly displaced PPF, with the relative displacement for *S*-PPF and *R*-PPF being 10 and 5, respectively. It also indicated that PPF mainly bound with F1-S variants, which was responsible for the binding stereoselectivity.

Hervé et al.[24] also investigated the binding affinities of PPF to different variants of AGP by competitive binding experiments. However, they used racemate rather than enantiomers. And the experiment was conducted using equilibrium dialysis at 4°C. They got different results with higher-binding affinity with A variant. It can not be determined whether the difference is attributed to the different temperature/methods or it does appear different between racemate and enantiomers. Further research needs to be done to find the reason.

TABLE 2. Binding constants of PPF enantiomers and thermodynamic parameters in 0.1%AGP

	T (°C)	$K \times 10^6$ (M^{-1})	ΔH (kJ/mol)	ΔS (J/K)	ΔG (kJ/mol)
S	37	7.65	9.90	163.71	−40.85
	27	6.73		163.70	−39.21
R	37	2.81	10.28	156.61	−38.27
	27	2.46		156.60	−36.70

TABLE 3. Displacement of PPF enantiomers (5.3 μM) in human AGP solution (20 μM) in the presence of different drugs

Additive		Free fraction (%)		Relative displacement	
		S-PPF	*R*-PPF	*S*-PPF	*R*-PPF
Blank		7.61	13.92	1	1
Disopyramide (μM)	10	17.01	31.66	2.24	2.27
	20	19.34	33.12	2.54	2.41
Imipramine (μM)	10	13.21	21.85	1.73	1.56
	20	18.45	29.64	2.42	2.13
Dipyridamole (μM)	10	78.05	76.74	10.25	5.51
	20	81.73	79.60	10.73	5.72
Mifepristone (μM)	10	69.13	67.10	9.08	4.82
	20	73.17	71.85	9.61	5.16

Binding Interactions with Some Commonly Used Drugs

The binding interactions (mainly for competitive displacement) between drugs may strongly affect their distribution and metabolism. Usually, only competitive interactions between high protein-binding drugs have clinical significance.[25,26]

For chiral drugs, interactions may happen between enantiomers or with other drugs. Based on the former study, competitive interactions existed between PPF enantiomers, which lead to the increase of their free fractions. The present investigation examined the effect of coadministered drugs on the stereoselective binding of PPF enantiomers to plasma proteins. The following drugs with high protein binding ratio were studied, including antiarrhythic drugs: aprindine, quinidine; antihypertensive, and antianginal drugs: verapamil, nifedipine, nitrendipine and nimodipine; emictory: furosemide; sedativehypnotics: diazepam. As shown in Figure 5, within therapeutic concentration, aprindine and quinidine could displace PPF from protein and increase its free fraction, which indicated competitive interactions. Furthermore, *R*-PPF was displaced more than *S*-PPF, which agrees with the former result that *S*-PPF had higher protein binding ratio. Other drugs did not show binding displacement (data not shown), suggesting that only aprindine and quinidine shared the same binding site as PPF. Therefore, the caution should be taken for PPF coadministration with aprindine or quinidine.

However, it has been demonstrated that plasma binding displacement rarely leads to obvious side effect for most drugs, though it is very common and sometimes can be predicted. Only drugs with special pharmacokinetics characteristic and low therapeutic index may result in side effect. Drug combination should be performed extremely carefully when plasma binding displacement may affect elimination process such as metabolism and tubular excretion, which make side effect more possible.

Species Differences

Comparative binding studies were performed in human, rat, rabbit, and cow plasma. The binding was as high as 90% and exhibited concentration dependent in all species. However, the stereoselectivity showed significant differences. And all the stereoselectivities disappeared at concentrations above 15 μg/ml bound ligand since the AGP, which was responsible for the stereoselectivity, was saturated. Puigdemont et al.[7] also reported high plasma protein binding (86–99%) of PPF in these four species. But they did not study the enantioselectivity differences.

The binding was a little more pronounced in human plasma than in other species. In case of the lowest ligand concentration, the binding stereoselectivity was about two with free fractions of *S*-PPF and *R*-PPF being 1.10% ± 0.01% and 2.27% ± 0.05%, respectively.

However, in rat plasma, the binding stereoselectivity was about five in favor of the *R*-PPF, with free fractions of *S*-PPF and *R*-PPF being 5.26% ± 0.25% and 1.12% ± 0.01%, respectively.

In cow plasma, the binding was also in favor of the *R*-PPF. The stereoselectivity was about two with free fraction of 2.22% ± 0.05% for *S*-PPF and 1.35% ± 0.02% for *R*-PPF.

In rabbit plasma, however, there was no stereoselectivity observed with free fractions of *S*-PPF and *R*-PPF being 1.89% ± 0.04% and 1.85% ± 0.04%, respectively.

The plasma protein binding of PPF enantiomers showed significant species dependency in stereoselectivity. It also

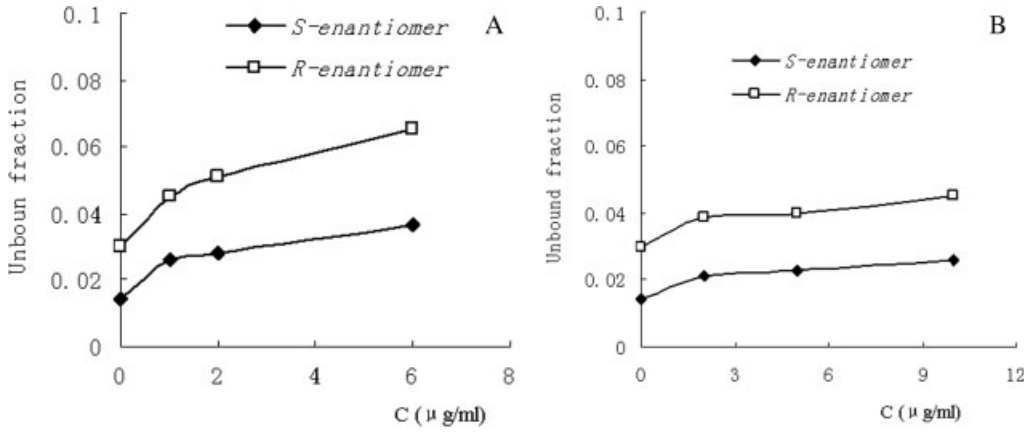

Fig. 5. The binding interaction of PPF enantiomers with (**A**) aprindine and (**B**) quinidine.

reminds us that we should take species differences into consideration when estimating human pharmacokinetics of chiral drugs based on stereoselectivity pharmacokinetics results from animals.

CONCLUSION

These results showed that the binding of PPF enantiomers was concentration dependent and stereoselective in human plasma, with *S*-PPF bound stronger than *R*-PPF. Both the stereoselectivity and the comparative interaction between enantiomers in human plasma were due to the high-affinity binding site in AGP. The binding mode of both enantiomers with AGP was mainly hydrophobic bond. PPF enantiomers had higher binding affinity for the F-S variant of human AGP. Drug-drug binding interaction studies showed that verapamil, diazepam, nifedipine, furosemide, nitrendipine, and nimodipine did not affect the binding of PPF enantiomers except quinidine and aprindine at therapeutic concentration. Therefore, caution should be taken for PPF coadministraion with quinidine or aprindine. The species-dependent binding stereoselectivity was also found in the PPF-protein binding. Human plasma binding was in favor of the *S*-PPF, whereas rat and cow plasma binding were in favor of the *R*-PPF. Rabbit plasma binding to PPF did not demonstrate stereoselectivity.

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