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Review

Stereoselective binding of chiral drugs to plasma proteins

Qi SHEN, Lu WANG, Hui ZHOU, Hui-di JIANG, Lu-shan YU*, Su ZENG*

Laboratory of Pharmaceutical Analysis and Drug Metabolism, College of Pharmaceutical Sciences, Zhejiang University, Hangzhou 310058, China

Chiral drugs show distinct biochemical and pharmacological behaviors in the human body. The binding of chiral drugs to plasma proteins usually exhibits stereoselectivity, which has a far-reaching influence on their pharmacological activities and pharmacokinetic profiles. In this review, the stereoselective binding of chiral drugs to human serum albumin (HSA), α1-acid glycoprotein (AGP) and lipoprotein, three most important proteins in human plasma, are detailed. Furthermore, the application of AGP variants and recombinant fragments of HSA for studying enantiomer binding properties is also discussed. Apart from the stereoselectivity of enantiomer-protein binding, enantiomer-enantiomer interactions that may induce allosteric effects are also described. Additionally, the techniques and methods used to determine drug-protein binding parameters are briefly reviewed.

Keywords: chiral drug; plasma protein; drug binding; stereoselectivity; human serum albumin; α1-acid glycoprotein; lipoprotein

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Introduction

Chiral drugs contain at least one chiral center, resulting in 2^{n-1} pairs of enantiomers. Widely used chiral drugs, such as rosuvastatin, duloxetine and salbutamol, play an important role in treating human diseases[1–3]. In the environment of living systems, specific binding between molecules (*eg*, enzymes, receptors, transporters, and DNA) is required for their medicinal effect. Thus, the physiochemical and biochemical properties of racemic mixtures and individual stereoisomers can differ significantly[4]. In some cases, one enantiomer is active, while the other may produce deleterious side-effects, including toxicity[5].

Numerous studies have reported the stereoselectivity of chiral drug metabolism and pharmacokinetic profiles[6–11]. Enantiomers commonly display pharmacokinetic processes (*eg* absorption, distribution, metabolism, and excretion) in a stereoselective manner[5, 12–14]. Moreover, the plasma protein binding and tissue distribution of some chiral drugs also exhibit stereoselectivity[15]. To limit the scope of this review, the binding of drugs to tissue proteins is not covered. Drugs bind to plasma proteins with varying degrees, and these bindings are commonly reversible. A binding equilibrium exists between the bound and free molecules, but only the unbound drug exerts efficacy[16]. Moreover, stereoselective binding can sometimes significantly affect the amount of free drug present in the plasma, and in many cases, this can be species-dependent[17]. Consequently, the characterization of drug binding to plasma proteins is an important factor for determining the overall pharmacological activity of a drug[18].

Blood is separated into the blood cells and plasma. Plasma contains various proteins and several function as carriers, including human serum albumin (HSA), α1-acid glycoprotein (AGP) and lipoproteins[19, 20]. Among plasma proteins, HSA and AGP play predominant roles by binding to most drugs[21]. As a result, the structure, function, and pharmaceutical properties of HSA and AGP have been extensively investigated[22, 23]. Fully characterizing the mechanism by which drugs bind to proteins such as HAS and AGP has become essential to interpret the pharmacokinetic, pharmacodynamic, and toxicological profiles of chiral drugs. *In vivo* binding studies using plasma samples and *in vitro* binding studies using plasma proteins, including natural proteins, recombinant fragments and variants, are helpful for understanding plasma protein binding properties. In addition, recent advances in determining the concentrations of enantiomers and fundamental analytical techniques are introduced. Here, we focused our attention on the enantioselective binding of chiral drugs to plasma proteins and the methods used to evaluate stereoselective binding.

* To whom correspondence should be addressed.
E-mail yuls@zju.edu.cn (Lu-shan YU);
zengsu@zju.edu.cn (Su ZENG)
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Methods and models

Because the pharmacological activity of one enantiomer may differ from its antipode, it is particularly important to know the extent of binding for each enantiomer. Some methods have been proposed to assess protein-binding capabilities based on diverse analytical tools[16, 24, 25]. Gilabert *et al*[26] established 3 steps to study the stereoselective binding between enantiomers and proteins: 1) equilibration of racemic mixture and proteins, 2) separation of the unbound fraction, and 3) determination of the concentration of the enantiomers from either the free fraction or drug-protein complexes.

To illustrate enantioselective drug-protein binding, classical methods, such as equilibrium dialysis (ED), ultrafiltration (UF) and ultracentrifugation (UC), are commonly combined with chiral separation techniques[27–29]. ED is an apparatus with two compartments separated by a semipermeable membrane, and only unbound drug molecules can permeate through the membrane. ED is carried out in solution, and true equilibrium is maintained during the whole process. Although ED has several disadvantages (*eg*, time-consuming, solubility, and non-specific adsorption), it is still considered the reference method for binding measurements. UF is a more rapid and simple alternative that depends on centrifugation forces and a sieve-like membrane to separate drug-protein complexes and the free drug. UC, another type of technique that avoids membrane effects, is based on the sedimentation coefficient differences of substances. For extensive reviews of these three approaches that discuss their advantages and pitfalls, refer to Vuignier[16] and Howard *et al*[21]. Table 1 briefly summarizes the progress made in recent years regarding the methods used to study the enantiodifferentiation of chiral drugs with plasma proteins.

With the development of computational models for the prediction of drug pharmacokinetics, it is important to generate models that predict drug binding affinities and stereoselectivity to plasma proteins for virtual screening. In the last decade, several models have been developed to study the binding between HSA and restricted drug families[45–47], and a few global models have been developed based on different approaches, such as genetic function approximation, multiple linear regression, heuristic regression procedures and ant colony systems[48–50]. Monti *et al* combined molecular mechanisms (MM) and molecular dynamics (MD) with circular dichroism (CD) to identify the main interactions between ketoprofen enantiomers and the surrounding amino acids at short distances in bovine serum albumin[51]. Similarly, Yu *et al* took advantage of MM and MD to identify several key residues that are involved in the enantioselectivity for the binding of AGP to mexiletine enantiomers, such as Arg90[43].

Despite the techniques and computational models mentioned above, important mathematical drawbacks of parameter estimation [*eg*, protein binding percentage (PB), number of binding sites (n), affinity constants (K_a), and enantioselectivity to the protein (ES)] have been ignored. Sandblad *et al*[52] calculated the adsorption energy distribution (AED) to provide a narrower selection of probable models from the surface plasmon resonance (SPR) raw data. Using this method, both the *R*/*S*-propranolol-AGP and *R*/*S*-warfarin-HSA systems were heterogeneous, comprising both high-affinity chiral sites and weak nonselective sites. Recently, the novel direct equations extracted from the classical interaction model allowed for advantageous univariate mathematical data treatment, providing the first evidence of quantitative (±)-catechin-HSA stereoselectivity[40]. Therefore, the integration of robust *in vitro* information with molecular docking estimates could provide a synergistic approach for the understanding of stereoselective binding.

Stereoselectivity of plasma protein binding to chiral drugs

Plasma

Human plasma contains HSA, AGP, lipoproteins, and globulins, which are responsible for the plasma protein binding of drugs[53]. All of these proteins can simultaneously bind to a drug, and the overall plasma protein binding is the sum of each binding. It is known that HSA accounts for 60% of plasma protein, while the amount of AGP is only 3% of plasma protein[23]. Plasma protein binding (PPB) limits free drug motion and reduces the volume of distribution, renal extraction, liver metabolism and tissue penetration. In contrast, drug absorption and half-life increase with PPB[54].

Chiral drugs with different pharmacological activities have been extensively explored due to their stereoselective pharmacokinetics[55, 56]. To characterize the stereoselective pharmacokinetics involved in protein binding and/or metabolism, several studies have been conducted *in vivo* and *in vitro*[57]. Herein, we enumerate the cases intending to evaluate the contribution of protein binding and metabolism to stereoselective pharmacokinetics *in vivo*. Lansoprazole, which is extensively metabolized in the liver, is frequently prescribed for the treatment of acid-related disorders. The disposition of lansoprazole differed in extensive and poor metabolizers of CYP2C19, which showed genetic polymorphisms[58]. Interestingly, Kim *et al* also investigated that enantioselective disposition of lansoprazole in 6 extensive metabolizers and 6 poor metabolizers, but the enantioselective protein binding was more important than the effect of CYP2C19 genetic polymorphisms[59]. Likewise, the unbound fraction of *R*-oxybutynin (OXY) in human plasma was approximately two-fold higher than that of *S*-OXY, and the metabolic kinetics were slightly different for the enantiomers[60]. Therefore, the enantioselective binding to plasma proteins was a major factor that was responsible for the stereoselective pharmacokinetics of OXY.

In vitro plasma protein binding experiments can provide valuable data, but an optimized experimental design is required to reach physiological relevance. As stated previously, whole plasma proteins studies and the contribution of individual proteins to total plasma enantioselective protein binding warrant further study. The stereoselectivity of the binding of propranolol (PL) enantiomers to plasma proteins is

Table 1. Binding parameters and techniques for the enantiodifferentiative study of chiral drugs with plasma protein.

Chiral drugs	Proteins	Techniques	Protein binding (%)	Protein binding estimations Binding constants (K or logK or nK)	Enantioselectivity (ES)	Reference
Phenindamine E1	Whole plasma	UF/AEKC			2.5	[30]
Phenindamine E2						
Trimeprazine E1					1.5	
Trimeprazine E2						
Promethazine E1					1.4	
Promethazine E2						
Rac-zopiclone	Total plasma	EKC	47±4			[31]
R-zopiclone			49±6			
S-zopiclone			45±3			
Nomifensine E1	Total plasma	UF/EKC	58±7			[32]
Nomifensine E2			64±4			
Brompheniramine E1	HSA	UF/AEKC		$(9.39\pm0.10)\times10^{2}$ $(mol/L)^{-1}$	2.8	[33]
Brompheniramine E2				$(2.60\pm0.17)\times10^{3}$ $(mol/L)^{-1}$		
Chlorpheniramine E1				$(9.20\pm0.20)\times10^{2}$ $(mol/L)^{-1}$	1.8	
Chlorpheniramine E2				$(1.69\pm0.17)\times10^{3}$ $(mol/L)^{-1}$		
Hydroxyzine E1				$(5.30\pm0.5)\times10^{3}$ $(mol/L)^{-1}$	1.2	
Hydroxyzine E2				$(6.30\pm0.4)\times10^{3}$ $(mol/L)^{-1}$		
Orphenadrine E1				$(1.26\pm0.13)\times10^{3}$ $(mol/L)^{-1}$	13.3	
Orphenadrine E2				$(1.67\pm0.13)\times10^{3}$ $(mol/L)^{-1}$		
R-Amlodipine	HSA	ED/Flow		$(9.91–11.2)\times10^{4}$ $(mol/L)^{-1}$		[34]
S-Amlodipine		Injection-CE		$(9.02–10.4)\times10^{4}$ $(mol/L)^{-1}$		
R-Propafenone	HSA	UF/chiral HPLC		2.05×10^{3} $(mol/L)^{-1}$		[35]
S-Propafenone				2.08×10^{3} $(mol/L)^{-1}$		
R-Rotigotine	HSA	Partial filling		$(17.6\pm0.6)\times10^{3}$ $(mol/L)^{-1}$		[36]
S-Rotigotine		-ACE		$(8.90\pm0.3)\times10^{3}$ $(mol/L)^{-1}$		
R-Naproxen	HSA	EKC		$(4.80\pm0.40)\times10^{5}$ $(mol/L)^{-1}$		[37]
S-Naproxen				$(9.20\pm1.20)\times10^{5}$ $(mol/L)^{-1}$		
R-Naproxen-NHBu				$(0.90\pm0.12)\times10^{5}$ $(mol/L)^{-1}$		
S-Naproxen-NHBu				$(1.02\pm0.10)\times10^{5}$ $(mol/L)^{-1}$		
R-Naproxen-cRGD				$(0.23\pm0.12)\times10^{5}$ $(mol/L)^{-1}$		
S-Naproxen-cRGD				$(0.24\pm0.10)\times10^{5}$ $(mol/L)^{-1}$		
(–)-Tetrahydropalmatine	HSA	ED/HPLC-UV		1.80×10^{4} $(mol/L)^{-1}$		[38]
(+)-Tetrahydropalmatine				2.20×10^{4} $(mol/L)^{-1}$		
R-zopiclone	HSA	EKC	36±8	3.09±0.19 (logK)	1.95	[31]
S-zopiclone			47±6	3.38±0.19 (logK)		
S-etodolac	HSA	UF/chiral HPLC		5.30×10^{5} $(mol/L)^{-1}$	6.06	[39]
R-etodolac				0.87×10^{5} $(mol/L)^{-1}$		
(–)Catechin	HSA	UF/CD-EKC	64	3.47±0.06 (logK)	1.5	[40]
(+)Catechin			53	3.28±0.16 (logK)		
Propanocaine E1	HSA	UF/EKC	48.7	3.20±0.16 (logK)	1.5	[41]
Propanocaine E2			60.1	3.40±0.14 (logK)		
R-Rotigotine	BSA	Partial filling		$(9.40\pm0.4)\times10^{3}$ $(mol/L)^{-1}$		[36]
S-Rotigotine		-ACE		$(7.30\pm0.2)\times10^{3}$ $(mol/L)^{-1}$		
R-Propranolol	AGP	Fluorescence		2.62×10^{5} $(mol/L)^{-1}$		[42]
S-Propranolol		Spectrophotometry		8.57×10^{5} $(mol/L)^{-1}$		
R-Propafenone	AGP	UF/chiral HPLC		2.81×10^{6} $(mol/L)^{-1}$		[35]
				9.74×10^{3} $(mol/L)^{-1}$ (n_2K_2)		
S-Propafenone				7.65×10^{6} $(mol/L)^{-1}$		
				9.95×10^{3} $(mol/L)^{-1}$ (n_2K_2)		
(–)-Tetrahydropalmatine	AGP	ED/HPLC-UV		9.61×10^{4} $(mol/L)^{-1}$		[38]
(+)-Tetrahydropalmatine				14.6×10^{4} $(mol/L)^{-1}$		
R-Mexiletine	AGP	UF/chiral HPLC	31±2.8			[43]
S-Mexiletine			22±3.2			
R-Propranolol	LDL	HPLC		$(5.20\pm2.3)\times10^{5}$ $(mol/L)^{-1}$		[44]
				$(1.90\pm0.1)\times10^{5}$ $(mol/L)^{-1}$ (n_1K_1)		
S-Propranolol				$(2.70\pm0.2)\times10^{5}$ $(mol/L)^{-1}$ (n_1K_1)		

E1 the first elute; E2 the second elute

the opposite of HSA, but acetyl salicylic acid (ASA) and salicylic acid (SA) significantly displaced the binding of *R*-PL to a greater extent than *S*-PL from both plasma protein and HSA binding sites[61]. These data suggest that ASA and SA do not affect the binding of PL to AGP because of the different acid-base properties of these drugs. For nomifensine enantiomer E1 (the first elute, protein binding to HSA 40%±5%), other plasma proteins were expected to contribute according to the plasma protein binding (58%±7%), but not for E2 (the second elute, PB 63%±4% and 64%±4% for HSA and plasma, respectively)[32]. Thus, the relative importance of HSA for binding nomifensine enantiomers was confirmed. The binding of bimoclomol to human plasma was stereoselective, and AGP was mainly responsible for the preference toward *S*-bimoclomol, whereas HSA did not play a role[62]. Another study showed that HSA and human plasma binding tended to be stereospecific in regard to *S*-amlodipine, whereas the opposite binding of its enantiomer was observed for AGP[29].

As the amino acid sequence differs among animals, interspecies plasma proteins exhibit different binding abilities. It has been recognized that preclinical data from animals cannot be extrapolated to humans[63]. Further studies also showed that stereoselective differences are dependent on species[28, 29]. Nevertheless, mammalian results are occasionally consistent with human disposition[64]. For example, the *in vitro* concentration ratios of *R*-MK0767 to *S*-enantiomer were similar in dog and human plasma (1.5–1.7), but the stereoselectivities in rat and rabbit plasma were inverted[65]. Although the results of enantioselective binding between animal and human are complicated, *in vitro* experiments may help explain some of these unusual discrepancies.

Human serum albumin (HSA)

HSA, a single non-glycosylated stranded protein consisting of 585 amino acids, is the most abundant protein in plasma, reaching high concentrations of approximately 0.5 to 0.7 mmol/L[66]. He and Carter[67] characterized the atomic structure of HSA using X-ray crystallography and described it as a heart-shaped protein with three homologous domains (labeled I, II, and III), each containing two subdomains (A and B) with similar structure[68–70].

As a carrier for endogenous ligands such as fatty acid, bilirubin and peptides[71], HSA solubilizes hydrophobic compounds. It also assists in providing a homogeneous and buffered drug distribution through the body and increases the biological lifetime of a drug by preventing its metabolism[49]. According to recent reports, two principal hydrophobic binding sites for aromatic and heterocyclic molecules were identified in the native conformation of HSA[70]. Site I (warfarin-azapropazone site) and Site II (indole–benzodiazepine site) are located in subdomains IIA and IIIA, which correspond to Sudlow's Sites I and II, respectively. Another high-affinity binding site (Site III) was shown to specifically bind to digitoxin on albumin[72] .

In particular, HSA exhibits the highest potential stereoselectivity among all plasma proteins, and it plays a key role in the distribution, metabolism and elimination of enantiomers[23]. The protein binding properties of a chiral drug, including the specific binding sites and affinity constants, could differ among enantiomers, resulting in different biological properties. Therefore, it is important to study the nature of the interaction between chiral drugs and HSA. Cooperative and allosteric equilibria between different binding sites and competition between multiple drugs or between drugs and endogenous ligands make it difficult to interpret HSA binding properties *in vivo*[73]. However, alternative methodologies have been proposed extensively *in vitro*.

Competition interaction

Competitive binding was exploited to determine protein binding sites using drug displacement assays. The displacement of equilibrium between a racemic drug can weaken the efficacy for the more active enantiomer and take the place of their antipodes[74, 75]. One example of this is the competitive binding between indobufen enantiomers[76]. *R*-indobufen displaced its antipode, thus increasing the steady state concentration of free *S*-enantiomer in patients. Consequently, the quicker elimination of the *S*-enantiomer is associated with its weaker binding to HSA.

Usually, displacement experiments using classical markers or probes are performed to elucidate specific binding sites *in vitro*[77]. Additionally, in the case of enantiomers sharing the same binding site, the binding mechanism may be different.

Using the well-characterized HSA ligand, rac-ibuprofen, Zsila *et al* suggested that leukotriene B4 bound to site II in subdomain IIIA using CD displacement experiments[78]. The naproxen (NPX) moiety of *S*-NPX bound to a cyclopentapeptide with an arginine-glycine-aspartate sequence (cRGD) bioconjugate that is farther from Trp than the *R*-epimer (approximately 16 and 6 Å, respectively). This finding highlights the critical role that the absolute configuration of epimeric macromolecular systems plays because the chirality of its different stereocenter can affect its binding mode to HSA, although both bioconjugates bound preferentially to site I[37]. In contrast, there is an increasing trend toward investigating chiral recognition in excited states. The interaction between excited carprofen (CP) and HSA shows remarkable stereodifferentiation, which is reflected by the markedly different triplet lifetimes of the two CP enantiomers in both binding sites (site I and site II), especially Trp-containing site I[79].

Cooperativity and allosteric interaction

Because the conformational adaptability of HSA extends well beyond the immediate vicinity of the binding site(s), cooperativity and allosteric modulation occur among binding sites[73]. Cooperativity represents a synergistic effect between two ligands that are sequentially bound to the different receptor sites[80]. Allosteric modulation occurs when the interaction between one ligand and HSA changes the interaction of a second ligand with the same protein at a separate site. This interaction implies that the simultaneous binding of two ligands induces conformational changes of the protein, in addition to other factors such as pH, temperature and ionic strength[81, 82].

To date, allosteric interactions have been reported to affect the binding equilibrium between HSA and endogenous or exogenous compounds, such as fatty acids, heme, carbamazepine and verapamil[83–86].

It was previously reported that the simultaneous binding of *S*-warfarin and *S*-benzodiazepines demonstrated mutual and exceedingly enhanced binding[87]. Due to the different kinetic features of warfarin enantiomers binding to HSA, Fitos *et al*[88] explained that *S*-lorazepam acetate allosterically enhanced the binding of *S*-warfarin by accelerating the relaxation kinetics of *S*-warfarin. A majority of the studies regarding allosteric interactions involved only qualitative observations. Chen and Hage[89] used a biointeraction chromatography technique to provide quantitative information on both directions of an allosteric effect, especially for a multisite binding agent. Aside from the effects of secondary interactions or the nonspecific binding of phenytoin, the coupling constant for the effect of L-tryptophan on phenytoin was accurately determined to be a negative allosteric interaction. This result is quite different from the results of direct competition on the effects of phenytoin toward L-tryptophan. Therefore, examining the interaction between two ligands in both directions on a multisite binding agent is necessary. They also applied the previously described chromatography techniques to study the allosteric effects of *R*- and *S*-ibuprofen on the binding of benzodiazepines to HSA[85]. Additionally, detailed information on the identity of the ligand binding pocket(s) and specific amino acid(s) of HSA that are responsible for this allosteric effect is needed. Lammers *et al*[90] showed the stereoselective binding of flurbiprofen (FBP) enantiomers and their methyl esters to HSA using time-resolved phosphorescence. Based on the phosphorescence lifetimes, *R*-flurbiprofen quenched Trp more effectively than *S*-flurbiprofen, in contrast to its methyl esters. However, the quenching constants of 3×10^{-7} $(mol/L)^{-1}\cdot s^{-1}$ for *R*-FBP and 2.5×10^{-7} $(mol/L)^{-1}\cdot s^{-1}$ for its antipode were not influenced by methylation, suggesting that stereoselectivity existed in the accessibility of HSA Trp-214. Recently, single amino acid mutants and HSA conformational rearrangements were reported to elucidate their governing role in allosteric ligand binding[91].

Recombinant domains of HSA

Recombinant HSA domains are a useful tool for characterizing the stereoselective binding properties of chiral drugs, and they also represent a suitable platform for the characterization of ligand binding. Based on the quasi-independence of the three HSA domains, proteolytic and chemical cleavage have been used to produce fragments of HSA to define binding sites[92, 93]. Considering the folding of the domains and their viability as "stand alone" proteins, the cloning and expression of independent recombinant domains of human serum albumin was introduced[94].

To study the accurate localization of ketoprofen and mexiletine binding sites on HSA, Shi *et al*[95] produced three highly purified recombinant HSA domains, each of which had a specific ligand binding site. They found that HSA DOM III possessed the chiral recognition ability for the ketoprofen enantiomers, whereas HSA DOM II recognized the mexiletine enantiomers. Recombinant fragments of native proteins provide an indispensable contribution, but we doubt that they could completely displace the native functional protein. For example, measurements of ochratoxin A (OTA) complexes with recombinant proteins using fluorescence spectroscopy revealed that it bound to all domains, but the binding constants decreased in the series as follows: DOM II>>DOM III>DOM I[96]. Interestingly, the OTA binding constant for DOM II (7.9×10^{5} $(mol/L)^{-1}$) was smaller than the largest constant for HSA by nearly a factor of 7, whereas the binding constant for OTA with DOM III [1.1×10^{5} $(mol/L)^{-1}$] was similar to that of the secondary binding site for HSA.

Additionally, the essential structural elements required for the formation of functional ligand binding sites on HSA remain unclear. A defined set of five recombinant proteins comprising combinations of domains and/or subdomains of the N-terminus were prepared to investigate the binding mechanism of warfarin to the stand-alone protein fragments[97]. The primary warfarin binding site was centered in subdomain IIA and received indispensable structural contributions from subdomain IIB and domain I, but domain III was not involved in this binding site. Aside from the characterization of the warfarin binding site, the kinetic step(s) in the binding mechanism between enantiomers and albumin may also be responsible for chiral discrimination. The binding of warfarin to albumin occurred in at least two steps — a rapid diffusion-controlled step and a slower rate-limiting step[98]. Domain fragments of recombinant human albumin that possessed a functional warfarin binding site, corresponding to domains 1 and 2 (D12) and domains 2 and 3 (D23), were produced to demonstrate that the preference for the *R*-enantiomer can largely be explained by these domains, particularly the observation that D12 had a faster rate for *R*-warfarin binding in the second step[99].

α1-acid glycoprotein (AGP)

AGP, also called orosomucoid, is a major binding protein for basic drugs and a diversity array of ligands[42, 57, 100]. Because AGP has only one drug-binding site, its binding to each molecule differs from albumin. The drug-binding properties of AGP are saturable and displaceable[101]. Human AGP is present in the plasma of healthy subjects at concentrations between 0.6 and 1.2 mg/mL, accounting for approximately 1% to 3% of the total protein[102].

AGP is an acute phase reactant protein, and its serum concentration may increase up to three- or four-fold as a result of inflammation or immunological response[103, 104]. The serum level of AGP significantly increases in renal disease patients, and the concomitant reduction in the free concentration of the *S*-alprenolol, with a large binding constant, was higher than that of the *R*-isomer, which has a small binding constant[105]. To study the impact of plasma protein binding on pharmacodynamics, Steeg *et al*[106] indicated that the plasma protein binding of *S*-propranolol was restricting its effects on heart

rate due to the elevated AGP concentration.

The AGP molecule consists of a single polypeptide chain of 183 amino acids with up to five asparaginyl linked glycans[107]. In addition to the high heterogeneity of glycans, polymorphisms have also been identified in the protein portion or AGP[108]. F1S variants are encoded by the AGP A gene, and the A variant is encoded by the AGP B gene[109]. Although the binding activities of many racemic drugs to AGP are known to be stereoselective, the effect of the AGP subfractions has not been fully elucidated.

The stereoselective binding of coumarin-type anticoagulants to AGP F1S variants was the same as native AGP binding with the *S*-enantiomers of warfarin and acenocoumarol[110]. Similarly, Zsila *et al* reported that both AGP and the F1S variant preferred binding to (–)-mefloquine, whereas the stereoselectivity was reversed for the A variant[111]. Warfarin enantiomers had a higher binding affinity for the F1S variant compared to the A variant, and the dissociation constants for the F1S and A variants differed by 12.6-fold for the *S*-enantiomer and 8.3-fold for the *R*-enantiomer[109]. The affinity of AGP for (+)-tetrahydropalmatine (THP) was notably higher than (–)-THP, and the F1S variants of AGP proved to be the key variants for (–)- and (+)-THP binding[38]. Deramciclane effectively displaced acridine orange-10-dodecyl bromide, a high-affinity fluorescent probe of native AGP, binding to variant A, whereas it was less effective displacing the same probes bound to the F1S variant; this phenomenon could not be caused by its enantiomer[112]. Apart from the different enantioselective binding ability of AGP genetic variants, their relative concentrations also influenced the stereoselective binding results[113]. These data prompted us to further study the AGP phenotype that affects drug disposition in humans and its possible influence on pharmacologically relevant variables.

Lipoprotein

Plasma lipoproteins are a group of binding agents that are known to interact with solutes in serum, and they can bind several basic and neutral hydrophobic drugs[114]. Because apolipoprotein and lipid constituents of lipoprotein are chiral compounds, their enantioselective binding should be considered.

Recently, the interactions between *R*/*S*-propranolol and low-density lipoprotein (LDL) were studied by using HPAC[44]. Two types of interactions occurred between *R*-propranolol and LDL, whereas only the second interaction was observed for *R*-propranolol, which involved saturable binding with an association equilibrium constant (K_a) of $(5.2\pm2.3)\times10^5$ $(mol/L)^{-1}$ at 37 °C. This study was the to provide information regarding LDL chiral selectivity. In another study, halofantrine enantiomers showed some stereoselectivity for lipoprotein binding *in vitro*, but they did not show stereoselectivity for plasma protein binding[115].

Perspectives

Given the importance of stereoselective binding to plasma proteins and that approximately 50% of marketed drugs are racemates, it would be extremely useful to develop *in vitro* models to evaluate and predict binding affinities and relevant sites. Recently, the recombinant domains of HSA and variants of AGP have become powerful tools to elucidate the stereoselective binding properties of chiral drugs *in vitro*. However, further studies are needed to determine whether the binding results are consistent *in vivo*.

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