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

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\cite{1,2,3}. In the environment of living systems, specific binding between molecules (e.g., enzymes, receptors, transporters, and DNA) is required for their medicinal effect. Thus, the physicochemical and biochemical properties of racemic mixtures and individual stereoisomers can differ significantly\cite{4}. In some cases, one enantiomer is active, while the other may produce deleterious side-effects, including toxicity\cite{5}.

Numerous studies have reported the stereoselectivity of chiral drug metabolism and pharmacokinetic profiles\cite{6,7,8,9,10,11}. Enantiomers commonly display pharmacokinetic processes (e.g. absorption, distribution, metabolism, and excretion) in a stereoselective manner\cite{12,13,14}. Moreover, the plasma protein binding and tissue distribution of some chiral drugs also exhibit stereoselectivity\cite{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\cite{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\cite{17}. Consequently, the characterization of drug binding to plasma proteins is an important factor for determining the overall pharmacological activity of a drug\cite{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\cite{19,20}. Among plasma proteins, HSA and AGP play predominant roles by binding to most drugs\cite{21}. As a result, the structure, function, and pharmaceutical properties of HSA and AGP have been extensively investigated\cite{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.
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\cite{16, 24, 25}. Gilbert et al\cite{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\cite{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 (e.g., 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\cite{16} and Howard et al\cite{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\cite{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\cite{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\cite{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 Arg\cite{90}.

Despite the techniques and computational models mentioned above, important mathematical drawbacks of parameter estimation [e.g., protein binding percentage (PB), number of binding sites (n), affinity constants (K), and enantioselectivity to the protein (ES)] have been ignored. Sandblad et al\cite{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\cite{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\cite{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\cite{25}. 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\cite{54}.

Chiral drugs with different pharmacological activities have been extensively explored due to their stereoselective pharmacokinetics\cite{55, 56}. To characterize the stereoselective pharmacokinetics involved in protein binding and/or metabolism, several studies have been conducted in vivo and in vitro\cite{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\cite{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\cite{59}. Likewise, the unbound fraction of R-oxymethyl (OXY) in human plasma was approximately two-fold higher than that of S-OXY, and the metabolic kinetics were slightly different for the enantiomers\cite{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 | Enantioselectivity (ES) | Reference |
|-------------------|---------------|-----------------|--------------------|----------------------------|------------------------|-----------|
| Phenindamine E1   | Whole plasma  | UF/AEKC         |                    |                            |                        | [30]      |
| Phenindamine E2   |               |                 |                    |                            |                        |           |
| Trimeprazine E1   |               |                 |                    |                            |                        |           |
| Trimeprazine E2   |               |                 |                    |                            |                        |           |
| Promethazine E1   |               |                 |                    |                            |                        |           |
| Promethazine E2   |               |                 |                    |                            |                        |           |
| Rac-zopiclone     | Total plasma  | EKC             | 47±4               | (9.39±0.10)×10^2 (mol/L)^1 | 2.5                    | [31]      |
| R-zopiclone       |               |                 | 49±6               | (2.60±0.17)×10^3 (mol/L)^1 | 1.5                    |           |
| S-zopiclone       |               |                 | 45±3               | (9.20±0.20)×10^3 (mol/L)^1 | 1.8                    |           |
| Nomifensine E1    | Total plasma  | UF/EKC          | 58±7               | (1.69±0.17)×10^4 (mol/L)^1 | 1.4                    | [32]      |
| Nomifensine E2    |               |                 | 64±4               | (5.30±0.5)×10^4 (mol/L)^1  |                        |           |
| Brompheniramine E1| HSA           | UF/AEKC         |                    | (6.30±0.4)×10^4 (mol/L)^1  |                        |           |
| Brompheniramine E2|               |                 |                    |                            |                        |           |
| Chlorpheniramine E1|              |                 |                    |                            |                        |           |
| Chlorpheniramine E2|              |                 |                    |                            |                        |           |
| Hydroxyzine E1    |               |                 |                    |                            |                        |           |
| Hydroxyzine E2    |               |                 |                    |                            |                        |           |
| Orphenadrine E1   |               |                 |                    |                            |                        |           |
| Orphenadrine E2   |               |                 |                    |                            |                        |           |
| R-Amlodipine      | HSA           | ED/Flow         |                    |                            |                        | [33]      |
| S-Amlodipine      |               | Injection-CE    |                    |                            |                        |           |
| S-Propafenone      | HSA           | UF/chiral HPLC  |                    |                            |                        | [34]      |
| S-Propafenone      |               | Partial filling |                    |                            |                        |           |
| S-Rotigotine      | HSA           | EKC             |                    |                            |                        |           |
| R-Naproxen        | HSA           | EKC             |                    |                            |                        |           |
| N-Naproxen-NHBu   |               |                 |                    |                            |                        |           |
| N-Naproxen-NHBu   |               |                 |                    |                            |                        |           |
| N-Naproxen-cRGD   |               |                 |                    |                            |                        |           |
| R-(-)-Tetrahydropalmatine | HSA     | ED/HPLC-UV   | 1.80×10^6 (mol/L)^1 |                            | 1.8                    | [35]      |
| S-(+)-Tetrahydropalmatine |       |                | 2.20×10^6 (mol/L)^1 |                            |                        |           |
| R-zopiclone       | HSA           | EKC             | 36±8               | 3.09±0.19 (logK)           | 1.95                   | [36]      |
| S-zopiclone       |               |                 | 47±6               | 3.38±0.19 (logK)           |                        |           |
| S-etodolac        | HSA           | UF/chiral HPLC  | 5.30×10^6 (mol/L)^1 |                            | 6.06                   |           |
| R-etodolac        | (-)-Catechin  | UF/CD-EKC      | 64                  | 8.37×10^6 (mol/L)^1        |                        |           |
| S-Catechin        |               | 53              | 3.28±0.16 (logK)    |                            |                        |           |
| Propanocaine E1   | HSA           | EKC             | 48.7               | 3.40±0.14 (logK)           |                        |           |
| Propanocaine E2   |               |                 | 60.1               |                            |                        |           |
| R-Rotigotine      | BSA           | Partial filling |                    |                            |                        | [37]      |
| S-Rotigotine      |               | ACE             |                    |                            |                        |           |
| R-Propranolol     | AGP           | Fluorescence    |                    |                            |                        | [38]      |
| S-Propranolol     |               | Spectrophotometry|                     |                            |                        |           |
| R-Propafenone      | AGP           | UF/chiral HPLC  |                    |                            |                        | [39]      |
| S-Propafenone      |               |                 |                    |                            |                        |           |
| S-Propafenone- (+)-Tetrahydropalmatine | AGP | ED/HPLC-UV | 9.61×10^6 (mol/L)^1 |                            | 1.8                    |           |
| S-Propafenone- (-)-Tetrahydropalmatine |       |                | 14.6×10^6 (mol/L)^1 |                            |                        |           |
| R-Mexiletine      | AGP           | UF/chiral HPLC  | 31±2.8             | 7.65×10^6 (mol/L)^1        |                        | [40]      |
| S-Mexiletine      |               |                 | 22±3.2             | 9.95×10^6 (mol/L)^1        |                        |           |
| R-Propranolol      | LDL           | HPLC            |                    |                            |                        | [41]      |
| S-Propranolol      |               |                 |                    |                            |                        |           |

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)\[35\]. 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\[69\]. 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\[73\]. 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*\[74\]. 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\[75, 76\]. 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\[77\]. 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[85-86].

It was previously reported that the simultaneous binding of S-warfarin and S-benzodiazepines demonstrated mutual and exceedingly enhanced bindings[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[90]. 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}·s^{-1} for R-FBP and 2.5×10^{-7} (mol/L)^{-1}·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)^{3}) 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^{9} (mol/L)^{3}] 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 displacable[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-alprrenolol, 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[96] 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 FIS variant preferred binding to (-)-mefloquine, whereas the stereoselectivity was reversed for the A variant\[111]\). Warfarin enantiomers had a higher binding affinity for the FIS variant compared to the A variant, and the dissociation constants for the FIS 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 FIS variants of AGP proved to be the key variants for (-)- and (+)-THP binding\[30]\). 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 FIS 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±2.3)×10^8 (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 vivo 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.

**Acknowledgements**

This project was supported by the Natural Key Basic Research and Development Program of China (No 973 Program) (No 2011CB710800) and the National Major Special Project for Science and Technology Development of the Ministry of Science and Technology of China (No 2012ZX09506001-004).

**References**

1. Glynn RJ, Koenig W, Nordestgaard BG, Shepherd J, Ridker PM. Rosuvastatin for primary prevention in older persons with elevated C-reactive protein and low to average low-density lipoprotein cholesterol levels: exploratory analysis of a randomized trial. Ann Intern Med 2010; 152: 488–96.
2. Nagashima W, Kimura H, Ito M, Tokura T, Arai M, Aleksić B, et al. Effectiveness of duloxetine for the treatment of chronic nonorganic orofacial pain. Clin Neuropharmacol 2012; 35: 273–7.
3. Patel M, Thomson NC. Levosalbutamol for chronic obstructive pulmonary disease: a treatment evaluation. Expert Opin Pharmacother 2012; 13: 1069–75.
4. Smith SW. Chiral toxicology: it’s the same thing...only different. Toxicol Sci 2009; 110: 4–30.
5. Wang Y, Cao J, Wang X, Zeng S. Stereoselective transport and uptake of propranolol across human intestinal Caco-2 cell monolayers. Chirality 2010; 22: 361–8.
6. Sun SY, Wang YQ, Li LP, Wang L, Zeng S, Zhou H, et al. Stereoselective interaction between tetrahydropalmatine enantiomers and CYP enzymes in human liver microsomes. Chirality 2013; 25: 43–7.
7. Niwa T, Murayama N, Yamazaki H. Stereoselectivity of human cytochrome p450 in metabolic and inhibitory activities. Curr Drug Metab 2011; 12: 549–69.
8. Campo VL, Bernardes LS, Carvalho I. Stereoselectivity in drug metabolism: molecular mechanisms and analytical methods. Curr Drug Metab 2009; 10: 188–205.
9. Sayre CL, Takemoto JK, Martinez SE, Davies NM. Chiral analytical method development and application to pre-clinical pharmacokinetics of pinocembrin. Biomed Chromatogr 2012; 27: 681–4.
10. Zhao M, Li LP, Sun DL, Sun SY, Huang SD, Zeng S, et al. Stereoselective metabolism of tetrahydropalmatine enantiomers in rat liver microsomes. Chirality 2012; 24: 368–73.
11. Liu M, Zhang D, Yang M, Zhao T, Wang X, Zhang Y, et al. Pharmacokinetics of terazosin enantiomers in healthy Chinese male subjects. Chirality 2012; 24: 1047–50.
12. Wang QX, Qiu J, Wang P, Jia GF, Li JL, Zhou ZQ. Stereoselective kinetic study of hexaconazole enantiomers in the rabbit. Chirality 2005; 17: 186–92.
13. Wu CY, Benet LZ. Predicting drug disposition via application of BCS: Transport/absorption/elimination interplay and development of a biopharmaceutics drug disposition classification system. Pharm Res 2005; 22: 11–23.
14. Scordo MG, Spina E, Dahl ML, Gatti G, Perucca E. Influence of CYP2C9, 2C19, and 2D6 genetic polymorphisms on the steady-
Acta Pharmacologica Sinica

state plasma concentrations of the enantiomers of fluoxetine and norfluoxetine. Basic Clin Pharmacol Toxicol 2005; 97: 296–301.

15 Brocks DR. Drug disposition in three dimensions: an update on stereoselectivity in pharmacokinetics. Biopharm Drug Dispos 2006; 27: 387–406.

16 Vuignier K, Schappler J, Vuithey JL, Carrupt PA, Martel S. Drug-protein binding: a critical review of analytical tools. Anal Bioanal Chem 2010; 398: 53–66.

17 Fitos I, Vay J, Simony M. Species-dependency in chiral-drug recognition of serum albumin studied by chromatographic methods. J Biochem Biophys Meth 2002; 54: 71–84.

18 Ascoli GA, Domenici E, Bertucci C. Drug binding to human serum albumin: Abridged review of results obtained with high-performance liquid chromatography and circular dichroism. Chirality 2006; 18: 667–79.

19 Tozer TN, Rowland M. Introduction to pharmacokinetics and pharmacodynamics: the quantitative basis of drug therapy. Philadelphia: Lippincott William & Wikins Publishers; 2006.

20 Kwong TC. Free drug measurements: methodology and clinical significance. Clin Chim Acta 1985; 151: 193–216.

21 Howard ML, Hill JJ, Galluppi GR, McLean MA. Plasma protein binding in drug discovery and development. Comb Chem High T Scr 2010; 13: 170–87.

22 Otagiri M. Study on binding of drug to serum protein. Yakugaku Zasshi 2009; 129: 413–25.

23 Chuang VTG, Otagiri M. Stereoselective binding of human serum albumin. Chirality 2006; 18: 159–66.

24 Jin YX, Tang YH, Zeng S. Analysis of flurbiprofen, ketoprofen and etodolac enantiomers by pre-column derivatization RP-HPLC and application to drug-protein binding in human plasma. J Pharm Biomed Anal 2008; 46: 953–8.

25 Monti S, Manet I, Manoli F, Sortino S. Binding and photochemistry of enantioisomeric 2-(3-benzoylpheny)propionic acid (ketoprofen) in the human serum albumin environment. Photochem Photobiol Sci 2007; 6: 462–70.

26 Escuder-Gilabert L, Martinez-Gomez MA, Villanueva-Camamas RM, Sagrado S, Medina-Hernandez MJ. Microseparation techniques for the study of the enantioselectivity of drug-plasma protein binding. Biomed Chromatogr 2009; 23: 225–38.

27 Lammers I, Lhiaubet-Vallet V, Jimenez MC, Ariese F, Miranda MA, Gooijer C. Stereoselective binding of flurbiprofen enantiomers and their methyl esters to human serum albumin studied by time-resolved phosphorescence. Chirality 2012; 24: 840–6.

28 Pistolozzi M, Bertucci C. Species-dependent stereoselective drug binding to albumin: A circular dichroism study. Chirality 2008; 20: 552–8.

29 Maddi S, Yamsani MR, Seeling A, Scriba GKE. Stereoselective plasma protein binding of amiodipine. Chirality 2010; 22: 262–6.

30 Martinez-Gomez MA, Villanueva-Camamas RM, Sagrado S, Medina-Hernandez MJ. Evaluation of enantioselective binding of basic drugs to plasma by ACE. Electrophoresis 2007; 28: 3056–63.

31 Asensi-Bernardi L, Martin-Biosca Y, Medina-Hernandez MJ, Sagrado S. On the zopiclone enantioselective binding to human albumin and plasma proteins. An electrokinetic chromatography approach. J Chromatogr A 2011; 1218: 3111–7.

32 Asensi-Bernardi L, Martin-Biosca Y, Sagrado S, Medina-Hernandez MJ. Electrokinetic chromatographic estimation of the enantioselective binding of nomifensine to human serum albumin and total plasma proteins. Biomed Chromatogr 2012; 26: 1357–63.

33 Martinez-Gomez MA, Villanueva-Camamas RM, Sagrado S, Medina-Hernandez MJ. Evaluation of enantioselective binding of antihistamines to human serum albumin by ACE. Electrophoresis 2007; 28: 2635–43.

34 Liu X, Song Y, Yue Y, Zhang J, Chen X. Study of interaction between drug enantiomers and human serum albumin by flow injection-capillary electrophoresis frontal analysis. Electrophoresis 2008; 29: 2876–83.

35 Hong Y, Tang Y, Zeng S. Enantioselective plasma protein binding of propafenone: mechanism, drug interaction, and species difference. Chirality 2009; 21: 692–8.

36 Chu BL, Lin JM, Wang Z, Guo B. Enantiospecific binding of rosiglitazone and its antipode to serum albumins: investigation of binding constants and binding sites by partial-filling ACE. Electrophoresis 2009; 30: 2845–52.

37 Gonzalez-Bejar M, Alarcon E, Poblete H, Scaiano JC, Perez-Prieto J. Stereoselective interaction of epimeric naproxen-RGD peptides with human serum albumin. Biomacromolecules 2010; 11: 2255–60.

38 Sun DL, Huang SD, Wu PS, Li J, Ye YJ, Jiang HD. Stereoselective protein binding of tetrahydropalmatine enantiomers in human plasma, HSA, and AGP, but not in rat plasma. Chirality 2010; 22: 618–23.

39 Guo CC, Hu HH, Yu LS, Jiang HD, Zeng S. Analysis of chiral non-steroidal anti-inflammatory drugs flurbiprofen, ketoprofen and etodolac binding with HSA. J Pharm Anal 2011; 1: 26–32.

40 Sabela MI, Gurnede NJ, Escuder-Gilabert L, Martin-Biosca Y, Bisetty K, Medina-Hernandez MJ, et al. Connecting simulated, bioanalytical, and molecular docking data on the stereoselective binding of (+)-(−)-catechin to human serum albumin. Anal Bioanal Chem 2012; 402: 1899–909.

41 Martinez-Gomez MA, Escuder-Gilabert L, Villanueva-Camamas RM, Sagrado S, Medina-Hernandez MJ. Evaluation of enantioselective binding of propanoicine to human serum albumin by ultrafiltration and electrokinetic chromatography under intermediate precision conditions. J Chromatogr B Analyt Technol Biomed Life Sci 2012; 889–909: 87–94.

42 Zhang F, Du Y, Ye B, Li P. Study on the interaction between the chiral drug of proparanolol and alpha1-acid glycoprotein by fluorescence spectrophotometry. J Photochem Photobiol B 2007; 86: 246–51.

43 Yu LS, Hong YJ, Li L, Jin YX, Zheng MY, Jiang HL, et al. Enantioselective drug-protein interaction between mexiletine and plasma protein. J Pharm Pharmacol 2012; 64: 792–801.

44 Sobanski MR, Hage DS. Identification and analysis of stereoselective drug interactions with low-density lipoprotein by high-performance affinity chromatography. Anal Bioanal Chem 2012; 403: 563–71.

45 Markuszewski M, Kaliszaz R. Quantitative structure-retention relationships in affinity high-performance liquid chromatography. J Chromatogr B Analyt Technol Biomed Life Sci 2002; 768: 55–66.

46 Andrisano V, Gotti R, Recanatini M, Cavalli A, Varoli L, Bertucci C. Stereoselective binding of 2-(4-biphenyl)-3-substituted-3-hydroxypropionic acids on an immobilised human serum albumin chiral stationary phase. J Chromatogr B Analyt Technol Biomed Life Sci 2002; 768: 137–45.

47 Hanai TK, Koseki A, Yoshikawa R, Ueno M, Kinoshita T, Homma H. Prediction of human serum albumin-drug binding affinity without ACE. Electrophoresis 2007; 28: 4878–81.

48 Gunther SB, Narayanran R, Khandelwal A. In silico ADME modelling 2: Computational models to predict human serum albumin binding affinity using ant colony systems. Bioorgan Med Chem 2006; 14: 4118–29.

49 Colmenarejo G. In silico prediction of drug-binding strengths to human serum albumin. Med Res Rev 2003; 23: 275–301.
for the prediction of binding affinities to human serum albumin using the heuristic method and a support vector machine. J Chem Inf Comp Sci 2004; 44: 1693–700.

51 Monti S, Ottani S, Manoli F, Manet I, Scagnolari F, Zambelli B, et al. Chiral recognition of 2-(3-benzoylphenyl)propionic acid (ketoprofen) by serum albumin: an investigation with microcalorimetry, circular dichroism and molecular modelling. Phys Chem Chem Phys 2009; 11: 9104–13.

52 Sandblad P, Arnell R, Samuelsson J, Forstedt T. Approach for reliable evaluation of drug proteins interactions using surface plasmon resonance technology. Anal Chem 2009; 81: 3551–9.

53 Tillement JP, Duché JC, Barré J. Drug binding to blood proteins: characteristics, roles and pathophysiological changes. Bull Acad Natl Med 2006; 190: 935–46.

54 Kerns EH, Kleintop T, Little D, Tobien T, Mallis L, Di L, et al. Integrated high capacity solid phase extraction-MS/MS system for pharmacoeutical profiling in drug discovery. J Pharm Biomed 2004; 34: 1–9.

55 Hao HP, Wang GJ, Sun JG. Enantioselective pharmacokinetics of ibuprofen and involved mechanisms. Drug Metab Rev 2005; 37: 215–34.

56 Hamdy DA, Brocks DR. Nonlinear stereoselective pharmacokinetics of ketonazole in rat after administration of racemate. Chirality 2009; 21: 704–12.

57 Shibukawa A, Ishizawa N, Kimura T, Sakamoto Y, Ogita K, Matsuo Y, et al. Plasma protein binding study of oxybutynin by high-performance frontal analysis. J Chromatogr B Analyt Technol Biomed Life Sci 2002; 768: 177–88.

58 Katsuki H, Nakamura C, Arimori K, Fujiyama S, Nakano M. Genetic polymorphism of CYP2C19 and lansoprazole pharmacokinetics in Japanese subjects. Eur J Clin Pharmacol 1997; 52: 391–6.

59 Kim KA, Shon JH, Park JY, Yoon YR, Kim MJ, Yun DH, et al. Enantioselective disposition of lansoprazole in extensive and poor metabolizers of CYP2C19. Clin Pharmacol Ther 2002; 72: 90–9.

60 Mizushima H, Takanaka A, Abe K, Fukazawa I, Ishizuka H. Stereoselective pharmacokinetics of oxybutynin and N-desethyloxybutynin in vitro and in vivo. Xenobiota 2007; 37: 59–73.

61 Rezaei Z, Khabnadideh S, Hemmateenejad B, Dehghani Z. Enantioselective binding sites on human serum albumin. Mol Pharmacol 1975; 11: 824–32.

62 Kragh-Hansen U, Huang CT, Otagiri M. Practical aspects of the ligand-binding and enzymic properties of human serum albumin. Biopharm Bull 2002; 25: 695–704.

63 Zhu CJ, Zhang JT. Stereoselective plasma protein binding and target tissue distribution of clausenamide enantiomers in rats. Chirality 2009; 21: 402–6.

64 Fortuna A, Alves G, Falcao A, Soares-da-Silva P. Binding of licarbazepine enantiomers to mouse and human plasma proteins. Biopharm Drug Dispos 2010; 31: 362–6.

65 Shen Z, Bakhtiar R, Komuro M, Awano K, Taga F, Colletti A, et al. Enantiomer ratio of MK-0767 in humans and nonclinical species. Rapid Commun Mass Spectrom 2005; 19: 1125–9.

66 Theodore P. All about albumin:biochemistry, genetics, and medical applications. London: Academic Press Publishers; 1995.

67 Carter DC, He XM. Structure of human serum albumin. Science 1990; 249: 302–3.

68 Kragh-Hansen U. Molecular aspects of ligand binding to serum albumin. Pharmacol Rev 1981; 33: 17–53.

69 Carter DC, Ho JX. Structure of serum albumin. Adv Protein Chem 1994; 45: 153–203.

70 Sudlow G, Birkett DJ, Wade DN. The characterization of two specific drug binding sites on human serum albumin. Mol Pharmacol 1975; 11: 824–32.

71 Kragh-Hansen U, Chuang VT, Otagiri M. Practical aspects of the ligand-binding and enzymic properties of human serum albumin. Biopharm Bull 2002; 25: 695–704.

72 Sjolholm I, Ekman B, Kober A, Ljungstedt-Pahlman L, Seving B, Sjodin T. Binding of drugs to human serum albumin: XI. The specificity of three binding sites as studied with albumin immobilized in microparticles. Mol Pharmacol 1979; 16: 767–77.

73 Ascenzi P, Bocedi A, Notari S, Fanali G, Tesse R, Fasanò M. AllostERIC modulation of drug binding to human serum albumin. Mini Rev Med Chem 2006; 6: 483–9.

74 Valette H, Dolle F, Guenther I, Hinnen F, Fuseau C, Coulon C, et al. Myocardic kinetics of the (11)C-labeled enantiomers of the Ca²⁺ channel inhibitor S11568: an in vivo study. J Nucl Med 2001; 42: 932–7.

75 Yoshida H, Kohno Y, Endo H, Ohmi N, Fukushima K, Suwa T, et al. Stereoselective disposition and chiral inversion of KE-298, a new antirheumatic drug, in rats. Chirality 1997; 9: 22–8.

76 Glowiak FK, Caldwell J. Protein binding of indobufen enantiomers: pharmacokinetics of free fraction-studies after single or multiple doses of rac-indobufen. Chirality 2002; 14: 736–41.

77 Liu X, Du Y. Study on the binding of chiral drug duloxetine hydrochloride to human serum albumin. Eur J Med Chem 2010; 45: 4043–9.

78 Zsila F, Bikazi Z, Lockwood SF. In vitro binding of leukotriene B₄ (LTB₄) to human serum albumin: evidence from spectroscopic, molecular modeling, and competitive displacement studies. Bioorg Med Chem Lett 2005; 15: 3725–31.

79 Lhiaubet-Vaillet V, Bosca F, Miranda MA. Stereo-differentiating drug-biomolecule interactions in the triplet excited state: studies on supramolecular carporfen/protein systems and on carporfen-dichroism and molecular modelling. Phys Chem Chem Phys 2009; 11: 9104–13.

80 Ding Y, Zhu X, Lin B. Study of interaction between drug enantiomers and serum albumin by capillary electrophoresis. Electrophoresis 1999; 20: 1890–4.

81 Kragh-Hansen U. Structure and ligand binding properties of human serum albumin. Dan Med Bull 1990; 37: 57–84.

82 Loun B, Hage DS. Chiral separation mechanisms in protein-based HPLC columns. 1. Thermodynamic studies of (R)- and (S)-warfarin binding to immobilized human serum albumin. Anal Chem 1994; 66: 3814–22.

83 Mallik R, Yoo MJ, Chen S, Hage DS. Studies of verapamil binding to human serum albumin by high-performance affinity chromatography. J Chromatogr B Analyt Technol Biomed Life Sci 2008; 876: 69–75.

84 Bocedi A, Notari S, Menegatti E, Fanali G, Fasano M, Ascenzi P. AllostERIC modulation of anti-HIV drug and ferric heme binding to human serum albumin. FEBS J 2005; 272: 6287–96.

85 Chen J, Hage DS. Quantitative studies of allostERIC effects by biointeraction chromatography: analysis of protein binding for low-solubility drugs. Anal Chem 2006; 78: 2672–83.

86 Kim HS, Hage DS. Chromatographic analysis of carbamazepine binding to human serum albumin. J Chromatogr B Analyt Technol Biomed Life Sci 2008; 816: 57–66.

87 Fitos I, Visy J, Magyar A, Kajtar J, Simoníy M. Stereoselective effect of warfarin and bilirubin on the binding of 5-(o-chlorophenyl)-1,3-dihydro-3-methyl-7-nitro-2H-1,4-benzodiazepin-2-one enantiomers to human serum albumin. Chirality 1990; 2: 161–6.

88 Fitos I, Visy J, Kardos J. Stereoselective kinetics of warfarin binding to human serum albumin: effect of an allostERIC interaction. Chirality 2002; 14: 442–8.

89 Chen J, Hage DS. Quantitative analysis of allostERIC drug-protein
binding by biointeraction chromatography. Nat Biotechnol 2004; 22: 1445–8.

90 Lammers I, Lhiaubet-Vallet V, Consuelo Jimenez M, Ariese F, Miranda MA, Gooijer C. Stereoselective binding of flurbiprofen enantiomers and their methyl esters to human serum albumin studied by time-resolved phosphorescence. Chirality 2012; 24: 840–6.

91 Kaneko K, Chuang VT, Minomo A, Yamasaki K, Bhagavan NV, Lammers I, Lhiaubet-Vallet V, Consuelo Jimenez M, Ariese F, Miranda Hochepied T, Berger FG, Baumann H, Libert C. Alpha(1)-acid glycoprotein. In Huang Z. Effect of alpha-1-acid glycoprotein binding on pharmacokinetics and disposition in the rat: cardiac and plasma concentrations and plasma protein binding. Biopharm Drug Dispos 2002; 23: 9–15.

92 Bos OJ, Fischer MJ, Wilting J, Janssen LH. Drug-binding and other physicochemical properties of a large tryptic and a large peptic fragment of human serum albumin. Biochim Biophys Acta 1988; 953: 37–47.

93 Peters T Jr. Serum albumin. Adv Protein Chem 1985; 37: 161–245.

94 Dockal M, Carter DC, Ruker F. The three recombinant domains of human serum albumin. Structural characterization and ligand binding properties. J Biol Chem 1999; 274: 29303–10.

95 Shi D, Jin YX, Tang YH, Hu HH, Xu SY, Yu LS, et al. Stereoselective binding of mexiletine and ketoprofen enantiomers with human serum albumin domains. Acta Pharmacol Sin 2012; 33: 710–6.

96 Il’ichev YV, Perry JL, Ruker F, Dockal M, Simon JD. Interaction of ochratoxin A with human serum albumin. Binding sites localized by competitive interactions with the native protein and its recombinant fragments. Chem Biol Interact 2002; 141: 275–93.

97 Dockal M, Chang M, Carter DC, Ruker F. Five recombinant fragments of human serum albumin-tools for the characterization of the warfarin binding site. Protein Sci 2000; 9: 1455–65.

98 Bos OJ, Fischer MJ, Wilting J, Janssen LH. Mechanism by which warfarin binds to human serum albumin. Stopped-flow kinetic experiments with two large fragments of albumin. Chem Biochem Interact 1989; 38: 1979–84.

99 Twine SM, Gore MG, Morton P, Fish BC, Lee AG, East JM. Mechanism of binding of warfarin enantiomers to recombinant domains of human albumin. Arch Biochem Biophys 2003; 414: 83–90.

100 Huang JX, Azad MA, Yuriev E, Baker MA, Nation RL, Li J, et al. Molecular characterization of lipopolysaccharide binding to human alpha-1-acid glycoprotein. J Lipids 2012; 2012: 1–15.

101 Huang Z. Effect of alpha-1-acid glycoprotein binding on pharmacokinetics and pharmacodynamics. Curr Drug Metab 2013; 14: 226–38.

102 Allen PC HE, Hill EA, Stokes AM. Alpha-1-acid glycoprotein. In plasma proteins: analytical and preparative techniques. Oxford (UK): Blackwell Scientific; 1977.

103 Hocheplied T, Berger FG, Baumann H, Libert C. Alpha(1)-acid glycoprotein: an acute phase protein with inflammatory and immunomodulating properties. Cytokine Growth Factor Rev 2003; 14: 25–34.