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Review

Albumin–drug interaction and its clinical implication ☆

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ABSTRACT

Background: Human serum albumin acts as a reservoir and transport protein for endogenous (e.g. fatty acids or bilirubin) and exogenous compounds (e.g. drugs or nutrients) in the blood. The binding of a drug to albumin is a major determinant of its pharmacokinetic and pharmacodynamic profile.
Scope of review: The present review discusses recent findings regarding the nature of drug binding sites, drug-albumin binding in certain diseased states or in the presence of coadministered drugs, and the potential of utilizing albumin–drug interactions in clinical applications.
Major conclusions: Drug–albumin interactions appear to predominantly occur at one or two specific binding sites. The nature of these drug binding sites has been fundamentally investigated as to location, size, charge, hydrophobicity or changes that can occur under conditions such as the content of the endogenous substances in question. Such findings can be useful tools for the analysis of drug–drug interactions or protein binding in diseased states. A change in protein binding is not always a problem in terms of drug therapy, but it can be used to enhance the efficacy of therapeutic agents or to enhance the accumulation of radiopharmaceuticals to targets for diagnostic purposes. Furthermore, several extracorporeal dialysis procedures using albumin-containing dialysates have proven to be an effective tool for removing endogenous toxins or overdosed drugs from patients.
General significance: Recent findings related to albumin–drug interactions as described in this review are useful for providing safer and efficient therapies and diagnoses in clinical settings. This article is part of a Special Issue entitled Serum Albumin.

1. Introduction

Human serum albumin (HSA) is a major protein component of blood plasma and plays an important role in the regulation of colloidal osmotic pressure and the transport of numerous endogenous compounds such as fatty acids, hormones, toxic metabolites (e.g. bilirubin), bile acids, amino acids, and metals [1–4]. HSA also binds a wide variety of drug molecules [1,3,5,6]. Drug molecules in the general circulation are either bound to plasma proteins or exist in unbound (free) form. Depending on the chemical properties of the drug molecules, unbound drugs can passively diffuse through the barriers constituted by endothelial cells and tissue cells into organ tissue and undergo metabolism, biliary excretion or glomerular filtration in kidney [7,8]. The unbound drugs can also be distributed intracellularly via specific transport systems (e.g. receptor-mediated endocytosis, protein-mediated transport). Only free drug molecules interact with therapeutic targets (e.g. receptors) to produce therapeutic effects [9]. In most cases, the tissue unbound concentration is in proportion to the plasma unbound concentration of drug. Thus, serum albumin–drug binding is an important factor in our understanding of the pharmacokinetics and pharmacological effects of drugs [5,10–12].

Drugs that bind to HSA with high affinities usually interact with one or two specific sites on the protein. The nature of these sites has been a subject of investigation [1,3,5,6,13]. Recent X-ray crystallographic data clearly demonstrated the location of these sites on HSA [6,14]. Changes in protein binding are often discussed based on this binding site concept. Competitive displacement between coadministered drugs which share the same binding site is a typical example [3,5]. Such knowledge of drug binding sites is one of the important issues for analyzing the mechanism of altered pharmacological effect accompanied with altered protein binding.

The binding of many drugs to HSA in patients can be changed by diseased states (e.g. renal and liver diseases) [2,5,15]. Understanding the pharmacokinetics and pharmacological activity of a drug in a diseased state is important and useful in terms of providing patients with effective medications. In addition, diagnoses and novel therapy

Abbreviations: HSA, human serum albumin; 6-MNA, 6-methoxy naphthalene acetic acid; CYP, cytochrome P450

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using drug–albumin interactions or drug–drug interactions at protein binding level are expected to produce a more effective treatment for patients. Thus, understanding drug–albumin interactions has become important for optimal therapy.

In this review, the nature of binding sites on HSA and mechanism of drug binding to HSA will be described. Furthermore, drug-HSA binding in certain diseased states or in the presence of coadministered drugs, and the possible utility of HSA in clinical applications will be described.

2. Drug binding to human serum albumin

HSA is a single-chain, non-glycosylated polypeptide that contains 585 amino acids with a molecular weight of 66,500 Da. Crystallographic data show that HSA contains three structurally similar α-helical domains, i.e., I–III, which can be further divided into subdomains A and B (Fig. 1) [1,14,16]. The polypeptide chain forms a heart-shaped structure with an approximate dimension of 80 × 80 × 30 Å [17]. HSA contains 35 cysteine residues, and all of these except one, Cys34 (in domain I), are involved in disulfide bond formation that serves to stabilize HSA. Crystallographic data also show that interdomain and intersubdomain interactions contribute significantly to the stability of the HSA molecule.

Pioneering studies by Sudlow using a fluorescent probe displacement method in 1975 and 1976 showed the presence of two specific drug binding sites, namely, site I (also called the warfarin binding site) and site II (the benzodiazepine binding site) on HSA through screening [18,19]. These excellent studies accelerated the topology analysis and mapping of the drug binding sites on HSA. Bos et al. proposed that sites I and II are located in domains II and III, respectively, using albumin fragments derived from pepsin and trypsin digestions [20,21]. At present, through crystallographic studies, the locations of sites I and II are assigned to subdomains IIA and IIIA, respectively [1,14,16]. The entrance to site I in subdomain IIA faces subdomain IIIA [6,13,22]. Furthermore, the site has an extended binding region owing to the residues from subdomain IIB and IIIA. In contrast to site I with an entrance with contribution from different neighboring subdomains, the involvement of other subdomains in the drug-binding capacity of site II in subdomain IIIA is relatively modest. The entrance to site II is completely open to solvent, where salt-bridges from Glu450 and Arg485 (in subdomain IIIA) to Arg348 and Glu383 (in subdomain IIB), respectively, stabilize the flanking wall of the pocket close to the entrance.

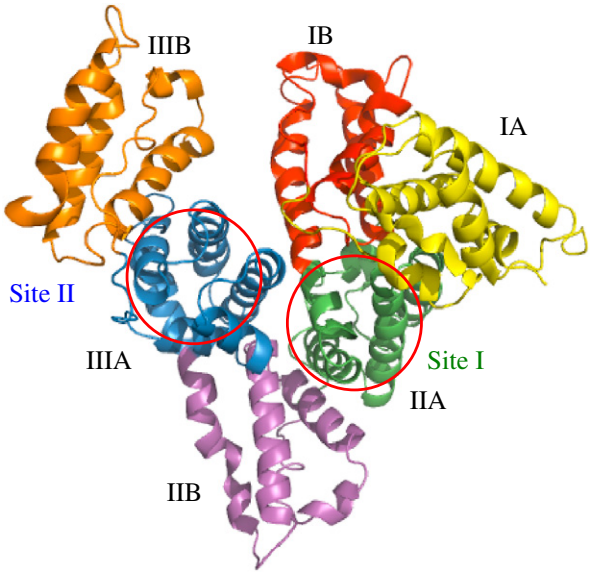

Fig. 1. Crystal structure of rHSA. The subdivision of rHSA into domains (I-III) and subdomains (A and B) is indicated, and the approximate locations of site I and site II are also shown. Atomic coordinates were taken from the PDB entry 1UOR. The illustration was made with PyMOL.

2.1. Site I

After the seminal work by Sudlow [18,19], a number of studies were directed toward characterizing the environment of site I. Initially, Fehske et al. reported that site I contains a warfarin–azapropazone binding area, consisting of two overlapping binding sites for warfarin and azapropazone [23–25]. They also showed the existence of a lone tryptophan residue, Trp214, in the non-overlapping part of the warfarin site. Similarly, Yamasaki et al. proposed three binding regions, subsite Ia, Ib and Ic within site I [26]. Results from other studies also indicated the presence of two independent binding regions within this site [27–30]. Ligands that strongly bound to site I are generally believed to be dicarboxylic acids and/or bulky heterocyclic molecules with a negative charge localized in the middle of the molecule (Table 1) [3,5,22]. Kragh-Hansen described this site as "a large and flexible region" based on the diversity of interaction ligands and the apparent ability to accommodate more than one of them at a time [29]. Crystallographic studies directed at HSA-site I drug complexes demonstrated that site I is larger than site II and that site I drugs occupy different parts of the binding pocket of subdomain IIA, even to the part adjacent to the interface with subdomain IB [6,31]. Furthermore, this site was confirmed to be a pocket comprised of two largely apolar clusters with a pair of centrally-located polar features (formed by the side-chains of Tyr150, His242, Arg257 located at the bottom of the pocket and Lys195, Lys199, Arg218 and Arg222 on an outer cluster at the pocket entrance) (Fig. 2a)[6,22]. The preference for flat aromatic compounds (as CMPF; 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid) arises because they are able to fit snugly between the side-chains of Leu238 and Ala291 in the center of the cleft [6,22]. The enantiomers of warfarin bind in the same position, both involved in three hydrogen bonding interactions with Tyr150, His242, and either Lys199 or Arg222 [6,32]. Thus, site I shows poor stereoselectivity, which might also be due to the flexibility of this site.

2.2. Site II

In addition to site I, detailed investigations have also been conducted to better understand the environment within site II. Wanwimorluck, through a quantitative structure-activity relationship (QSAR) study, suggested that site II comprised a hydrophobic cleft of about 16 Å deep and 8 Å wide with a cationic group located near the surface [33]. According to the crystal structure of HSA, site II is a largely apolar cavity with a single dominant polar patch near the pocket entrance, centered on Tyr411 and Arg410 (Fig. 2b) [13]. This arrangement of polar and apolar features is consistent with the typical structures of site II drugs, which are aromatic carboxylic acids with a negatively charged acid group at one end of the molecule that is separated by a hydrophobic center (Table 2) [3,5]. Diazepam, diflunisal, and ibuprofen, the site II drugs, interact with the hydroxyl group of Tyr411 [6]. Arg410 and Ser489 also contribute to salt-bridge formation and hydrogen-bond interactions with diflunisal and ibuprofen. Site II was proposed to be a smaller or narrower site than site I because large molecules rarely bind to site II [3,5,6,13]. Indeed, to date no one has succeeded in dividing this site in to different sub-binding regions as in the case of site I. Site II also appears to be less flexible, since ligand binding to this site often shows stereoselectivity and is strongly affected by structural modification of ligand with a relatively small group [3,5]. (*R*)-ibuprofen binds to site II with an affinity that is 2.3 times higher than the (*S*)-enantiomer [34]. Furthermore, diazepam but not its fluorinated analog flunitrazepam binds to site II [35]. Thus, although the site can bind a variety of ligands, it appears to be more restricted than site I. Meanwhile, recent crystallographic data suggest that site II can adapt to ligands