Drug comparison and categorizing regarded with human serum albumin from years 2006 to 2012

Minoo Shahani[1,*], Padina Vaseghi Maghvan[2], Roya Tadayon[1], Amin Rostami[1], Marzie Ramandi[3], Taher Mohammadian[4]

[1]Proteomics Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
[2]Department of Basic Science, Science and Research Branch, Islamic Azad University, Tehran, Iran
[3]Department of surgery, Faculty of medicines, Shahid Beheshti University of medical sciences, Tehran, Iran.
[4]Department of Microbiology School of Basic Sciences Isamic Azad University Shahr-e-Qods Branch Qods City, Iran

*Corresponding Author: email address: minoo.shahani@gmail.com (M.Shahani)

ABSTRACT

Human serum albumin (HSA) is the most abundant protein constituent of blood plasma. This protein consists of a single polypeptide chain of 585 amino acid residues, which has many important physiological functions. HSA can bind and carry many drugs, including anticoagulants, tranquilizers, and general Anesthetics. Some technique such as: fluorescence spectroscopy, three-dimensional fluorescence**,** UV–visible, FT-IR, circular dichroism (CD), X-ray scattering (SAXS) technique and molecular modeling was utilized to investigate the effects of acceptor on conformation of the donor (HSA). The binding site number *n* and apparent binding constant K_A drugs corresponding thermodynamic parameters, the free energy change (ΔG), enthalpy change (ΔH), and entropy change (ΔS) were calculated. The hydrophobic effect, van der Waals forces, hydrogen bond and electrostatic interactions play a major role in stabilizing the complex. More investigation also revealed that these drugs bind to the amino acids on the hydrophobic pocket of HSA and induce changes to the secondary structure of the HSA. In this study for better understanding of HSA-drug interactions, we categorized drugs into ten groups from years 2006 to 2012 and are suggested that two important parameters such as ΔG^0_{H20} and $[D]_{1/2}$ can be calculated for each groups and refer to ten categories to finally indicate that fine structural change in human serum albumin.

Keywords: Human serum albumin; Drugs; hydrogen bond; electrostatic interactions.

INTRODUCTION

Investigating drugs-plasma proteins interactions is an interesting research field in biochemistry and clinical medicine. Human Serum Albumin (HSA), an important globular protein, is considered as carrier, responsible for blood osmotic pressure and several other important functions and also has three high affinity specific binding sites (I, II and III) for drugs. X-ray crystallography analyses have revealed that the drug binding sites are located in sub domains IIA and IIIA [1,2]. The three-dimensional structure of human serum albumin has been determined through X-ray crystallography measurements [3]. This globular protein has three domains that including two sub domains (A and B) which are stabilized by 17 disulfide bridges [4]. The drug-protein binding affinity is an important factor for regulation of drug concentration in blood. So, this binding property can change the activity and half life of the drug in the body [5]. HSA involves in distribution, transportation and metabolism of a wide variety of ligands including different metabolites and pharmaceutical reagents and also maintenance of the blood osmotic pressure [6]. Due to its importance and characteristics, researchers usually use HSA as a protein model for study the complexes of proteins and drugs [7]. HSA binds to many poorly water soluble drugs and carry them through the blood stream [8, 9]. Following this binding, metabolism distribution, free concentration and elimination rate of various drugs can be significantly regulated [10].various techniques are used for investigating plasma protein-drug interaction including fluorescence,

pH-metery, circular dichorism (CD) and calorimetery techniques[3]. In this review, HSA-drug interaction is investigated and classified articles published from years 2006 to 2012. Ten Categories of drugs that can bind and transport by HSA are as follows: Anti-Tumor, Antibiotics, Antivirus, Non-Steroid, Pain killer, Antifungal, Nerve agent, Chinese medicine, Anti-insect and the group of Others. Here their interactions are discussed in detail and finally give an insight on HSA ability for drug management in body is represented.

1) Anti-Tumor

Anti tumors are among the most important drugs, so many investigations carried out on binding properties of this group of drugs. Nucleosides and their derivatives exhibit significant antitumor, antiviral, and antibacterial activities. Nobiletin is regarding as a promising anti-inflammatory and anti-tumor promoting agent. Using fluorescence, UV–vis, FT-IR, CD techniques and molecular modeling, the binding of Nobiletin to HSA was studied. It is shown that the CD and FT-IR spectra were changed after its binding to the ligand. According to some Computational mapping, it is suggested that Nobiletin is binding to the large hydrophobic cavity of subdomain IIA [4]. Due to the presence and importance of metal ions in plasma, the impact of Cu^{2+} on the interaction of Anthracenedione mitoxantrone as an antitumor agent and HSA is investigated using fluorescence spectroscopy, ultraviolet–visible absorption spectroscopy and circular dichroism spectroscopy. It is reported that the presence of Cu^{2+} significantly increases the quenching efficiency of MTO to HSA. Cu2+ also induces changes on the secondary structure of HSA with an increase in the α-helix content [7]. In another study, researchers investigated the binding of 30-azido-30-deamino daunorubicin (ADNR) to HSA at different temperatures and pH 7.4. Fluorescence spectroscopy showed that the hydrophobic forces play an important role in the interaction between ADNR and HSA [11].In addition; the effects of common ions on the binding constants were also studied at room temperature [11, 12].

Hydrogen bond has been shown to be the major intermolecular force in stabilizing the docetaxel–HSA complex. CD, FT-IR and UV–visible spectroscopy supported the occurrence of changes in the secondary structure of HSA following the interaction between docetaxel (an anti neoplastic agent of the taxoid family) and HSA [13].By the combination of fluorescence spectroscopy, UV absorption and molecular modeling it is revealed that the hydrophobic interaction was the predominant intermolecular force stabilizing the complex of HSA and daunorubicin (an anticancer drug) by calculating the Vant'Hoff equation [11]. Paclitaxel (Taxol, a potent anticancer drug) is shown to interact with HSA at the binding sites located at the interface of subdomains IIA and IIIA, and in the cleft between either domains of I and III of the protein, respectively. It is also claimed that the binding mode is regulated by the C13 side chain [14].

The interaction of JDC-108 (1, 11-Didechloro-6-methyl-40-O-demethyl rebeccamycin) and HSA is also showed that hydrophobic forces are very important in the reaction and the binding reaction is mainly entropy-driven [15]. It is reported that following the interaction of fluoxymesterone (FLU) and cyclophosphamide (CYC) with HSA, α-helix content of HSA decreased as drug concentration increased. Both drugs also cause some conformational changes in HSA [16]. Based on the quantitative information provided by calorimetric and spectroscopic techniques, It is reported that the binding of mitoxantrone with HSA is dominated by electrostatic interactions [17].

2) Antibiotics

Antibiotics are widely used in treatment of different infectious and daily life [18]; so many researches are done to study the features of antibiotics interactions with HSA. *Xin-Xiang Zhang* et al. reported that hydrogen bonds and van der Waals interactions are dominated in the binding of fluoroquinolones to HSA [19]. Mechanism of interaction and detailed physico-chemical characterization of the binding of four fluoroquinolones:levofloxacin, sparfloxacin, ciprofloxacin HCl and enrofloxacin with human serum albumin has been studied. It is shown that under the physiological pH, levofloxacin, ciprofloxacin, hydrochloride, enrofloxacin and sparfloxacin interaction with HSA is dominated

by hydrogen bonding, Van der Waal's interactions, hydrophobic interactions, hydrogen bonding, and hydrophobic and electrostatic interactions respectively. No changes in the secondary structure of HSA are seen following the presence of these drugs as it reported by the authors [20].The same as sparfloxacin , hydrophobic and electrostatic interactions play an important role in interaction of chloramphenicol with HSA as it studied by UV/vis, circular dichroism (CD) and three-dimensional fluorescence [21]. The interaction between HSA and sulfamethazine (SMZ) is also mainly occurring via Electrostatic interaction and hydrogen bond .This binding can unfold the polypeptides of HSA and transfer the HSA secondary conformation [18]. Interactions of paeonol with human serum albumin (HSA) have been investigated by calorimetry and circular dichroism. Paeonol has been used as a tranquillizer and anti-bacterial properties .The binding constant, changes of enthalpy, entropy, and Gibbs free energy were obtained. Circular dichroism (CD) spectra showed that paeonol changes the secondary structure of HSA [22].

Flavonoid drugs are often have high affinity for binding to the HSA. The previous reports on these [8–11] involve quercetin, kaempferol, delphinidin, scutellarin, alpinetin, formononetin and Farrerol have been shown a spectrum of physiological activities such as anti-bacterial and the conformational changes of HSA was observed caused by the interaction with farrerol [23].

3) Antivirus

In category of anti viral drugs, researches have shown that the HSA is partially unfolded in the complex of HSA- Betulinic acid (BA, effective against cancerous and HIV-infected cells). BA can bind to free HSA at nano molar concentrations [24].

HSA is shown to be stabilized at low concentrations of the Oseltamivir phosphate (OP;tamiflu), an antiviral pro-drug, while at the high concentrations of the drug, a partial destabilization is observed in drug-HSA complex[25]. UV–Vis and fluorescence quenching methods in combination with Fourier transformed infrared (FT-IR) and circular dichroism (CD) spectroscopy revealed that silibinin, a naturally occurring compound for treating a range of hepatitic diseases, interacts HSA and form a complex mainly via electrostatic interactions, it also induces some changes to the secondary structure of HSA [26].

4) Non-Steroid

In this class of drugs, it is reported that the complex of HSA-rofecoxib is mainly stabilizing by van der Waals interaction and hydrogen bonds under physiological condition. Rofecoxib is a non-steroidal anti-inflammatory drug. More investigation also revealed that this drug binds to the amino acids on the hydrophobic pocket of HSA and induce changes to the secondary structure of the H

SA [27]. The interaction between indomethacin and human serum albumin (HSA) was investigated by fluorescence quenching technique and UV–vis absorption spectroscopy. Indomethacin is a member of the arylalkanoic acid class of non-steroidal anti-inflammatory drugs (NSAIDs), The distance *r* between donor (HSA) and acceptor (indomethacin) was obtained according to fluorescence resonance energy transfer (FRET).

The study suggests that the donor and the acceptor are bound at different locations but within the quenching distance [28]. Meloxicam is a non-steroidal anti-inflammatory drug. The interaction of meloxicam with HSA in aqueous solution and at physiological pH is shown to greatly decrease the affinity of albumin for bilirubin (40% reduction in the CD Cotton effect intensity and 15% reduction of the fluorescence intensity) [29]. The binding of ketoprofen with human serum albumin (HSA) was studied by fluorescence and absorption spectroscopic methods. Ketoprofen is a non-steroidal anti-inflammatory drug. The thermodynamic parameters, ΔH, ΔG and ΔS were calculated and the main sort of acting force between ketoprofen and HSA was studied [30].

5) Antifungal

Thiophanate methyl (MT), as widely used fungicides, is claimed to bind to the HSA mainly through hydrophobic forces and also formation of hydrogen bonds between the pesticides and the residues of HSA [31]. Another fungicide, methyl thiophanate (MT), is reported to bind to HSA primarily at sub-domain IIA [32].

Table 1. Classification of drugs on human serum Albumin

Categories of drug	**Subgroup**	**Techniques used**	**Force**	**Molecular Alteration**
Anti-Tumors	1-Nobiletin	fluorescence, UV–visible FT-IR, CD	hydrophobic cavity	the CD and FT-IR spectra were changed after its binding to hsa
	2-Anthracenedione mitoxantrone (MTO) / Cu2+	fluorescence, UV–visible CD	the presence of Cu2+ significantly increases the quenching efficiency of MTO to HSA	increases the quenching efficiency of MTO to HSA ,changes secondary structure of HSA (α-helix)
	3-deamino daunorubicin	fluorescence	hydrophobic forces	
	4- docetaxel	CD, FT-IR , UV–visible	Hydrogen bond	change in the secondary structure of HSA
	5- daunorubicin	Fluorescence, UV–visible	hydrophobic interaction	
	6- Paclitaxel	binding mode is regulated by the C13 side chain		
	7- JDC-108		hydrophobic forces	
	8- fluoxymesterone/ cyclophosphamide			conformational changes to HAS(α-helix content of HSA decreased)
	9- mitoxantrone	calorimetric,spectroscopic	electrostatic interactions	
Antibiotics	1- Fluoroquinolones		hydrogen bonds and van der Waals interaction	
	2- levofloxacin, sparfloxacin, ciprofloxacinHCl ,enrofloxacin		hydrogen bonding ,Van der Waal's interactions, hydrophobic , electrostatic	No changes in the secondary structure of HSA are seen
	3-chloramphenicol	UV/vis, circular dichroism (CD) and three-dimensional fluorescence	hydrophobic and electrostatic interactions	
	4-sulfamethazine (SMZ)		Electrostatic interaction and hydrogen bond	unfold the polypeptides of HSA and transfer the HSA secondary conformation
	5-paeonol	calorimetry , circular dichroism		changes the secondary structure of HSA

	6- Flavonoid drugs such as: quercetin, kaempferol, delphinidin, scutellarin, alpinetin, formononetin, Farrerol			the conformational change of HSA
Antivirus	1-Betulinic acid(BA)			HSA is unfolded in the complex of HSA- Betulinic acid
	2-Oseltamivir phosphate(OP)			Effective on stabilization and destabilization of HSA at nano molar and high concentrations
	3-silibinin	UV–Vis , fluorescence quenching methods in combination with Fourier transformed infrared (FT-IR) and circular dichroism (CD)	electrostatic interactions	changes to the secondary structure of HSA
Non-Steroid	1-Rofecoxib		van der Waals interaction , hydrogen bonds	changes to the secondary structure of the HSA
	2-Indomethacin (NSAIDs)	fluorescence quenching technique and UV–vis absorption spectroscopy		HSA and the NS are bound at different locations but within the quenching distance
	3-Meloxicam	fluorescence intensity, CD		The interaction of meloxicam with HSA decrease the affinity of albumin for bilirubin
	4-ketoprofen	fluorescence and absorption spectroscopic methods		acting force was studied
Antifungal	1-Thiophanate methyl (MT)		hydrophobic force	formation of hydrogen bonds between the pesticide and the residues of HSA
	2-methyl thiophanate (MT)			bind to the site IIA of HAS
Nerve agent	1-Fluoxetine hydrochloride (FLX)			bind to the site I of HSA and induces conformational changes

	2-clozapine		hydrophobic effect, van der Waals forces and hydrogen bond	binds to the subdomain IIA has and does not change the secondary structure of the HAS
	3-theophylline, and theobromin		hydrophobic interaction	stabilizing in the complex with HAS
	4-acetylcholinesterase inhibitors such as: •galanthamine and neostigmine •huperzine	biochromatographic approach circular dichroism and FT-IR spectroscopy	van der Waals interactions and hydrogen bondin hydrophobic interactions	change the secondary structure of the HSA
	5-Carbamazepine (CBZ)	circular dichroism and FT-IR spectroscopy		change the secondary structure of the HSA
Chinese medicine	1-Sinomenine	fluorescence		urea cause HSA-sinomenine complex become more stable Compared to the free HAS
	2-Salvianolic acid B(Sal B)	fluorescence and circular dichroism (CD)		the secondary structure of HSA is not changed in the presence of Sal B
	3-Daphnin	Fourier transform infrared (FT-IR)	hydrophobic force	
	4-Icariin 5-Puerarin 6-Honokiol 7-flavonol drugs 8-tetramethylpyrazine (TMPZ)			
Pain killer	1-ibuprofen	X-ray scattering (SAXS) technique		
Anti-insect	1- amodiaquine (AQ)	fluorescence spectroscopy	hydrogen bond and van der Waals forces ,molecular docking and molecular dynamic	sub-domain IIA of HSA
Others	1-Sulfonylureas	performance affinity chromatography		studying the change in binding
	2-Colchicines	resonance light scattering analysis (RLS) and FT-IR		studying the change in binding

6) Nerve agent

Fluoxetine hydrochloride (FLX), a well known psychotropic drug, is mainly bind to the site I of HSA and induces some conformational changes to HSA after interaction [33]. clozapine as a drug used for treatment of schizophrenia, binds to the subdomain IIA human serum albumin, through hydrophobic effect, van der Waals forces and hydrogen bonding and does not change the secondary structure of the HSA after complexation[34]. In another study, is claimed that caffeine, theophylline, and theobromine are stabilizing in the complex with HSA by hydrophobic interaction according to the calculation of free energy changes (ΔG), enthalpy change (ΔH), and entropy change (ΔS) at different

temperatures [35]. The interaction of a series of acetylcholinesterase inhibitors (AChEIs; donepezil, galanthamine, huperzine and neostigmine) with human serum albumin (HSA) was studied using a biochromatographic approach. A comparative thermodynamic study with benzodiazepine molecules was also done to determine the potential binding site of these drugs on HSA. The ΔH° and ΔS° values for donepezil, galanthamine and neostigmine, were negative due to van der Waals interactions and hydrogen bonding which govern this association with albumin where as the positive values of ΔH° and ΔS° of huperzine binding on HSA indicated a predominance of hydrophobic interactions [36]. A highly lipophilic neutral tricyclic compound, Carbamazepine (CBZ), can also change the secondary structure of the HSA following interaction with the protein under the simulative physiological conditions confirmed by circular dichroism and FT-IR spectroscopy [37].

7) Chinese medicine

The interaction of sinomenine with human serum albumin (HSA) in the absence and presence of urea has been studied by fluorescence. Sinomenine is one of alkaloids extracted from Chinese medical plant, [11–13]. The results revealed a static quenching mechanism play a role in the complexes. However, the binding ability of sinomenine to denatured HSA is weaker than that of sinomenine to native HSA. Urea causes HSA-sinomenine complex become more stable compared to the free HSA [38]. The interaction of Salvianolic acid B (Sal B) with human serum albumin (HSA) was investigated by fluorescence and circular dichroism (CD). Salvia miltiorrhiza (Danshen) is a popular traditional Chinese herb widely used in treatment of coronary heart disease [14]. The CD spectroscopy indicated that the secondary structure of HSA is not changed in the presence of Sal B. Furthermore, the effect of metal ions on Sal B-HSA complex was also studied [39]. The interaction between Daphnin with human serum albumin has been studied by spectroscopic methods such as: Fourier transform infrared (FT-IR). Daphnin (Chinese name: ruixiang) is a folk medicine in China that is used for reliefing of fever. The thermodynamic parameters (ΔH^0 and ΔS^0) and the molecular modeling study indicated that hydrophobic forces play an important role to stabilize the Daphnin–HSA complex [40]. In addition, the effects of other Chinese medicines such as: icariin [41], Puerarin[42], honokiol [43], three iridoid glycosides extracted from Cornus officinalis Sieb (ET Zucc). (CIG) [44], flavonol drugs, kaempferol , galangin [45] and tetramethylpyrazine (TMPZ) [46] on human serum albumin (HSA) were studied.

8) Pain killer

The interaction between ibuprofen whit human serum albumin (HSA) has been studied by Small angle X-ray scattering (SAXS) technique. The work represents a type of analysis which could be exploited in future investigations on proteins in solution, in the binding of drugs or endogenous compounds and in the pharmacokinetic properties as well as in the study of allosteric effects, cooperation or anti cooperation mechanisms[47].

9) Anti-insect

The interaction of amodiaquine (AQ) (drug for Malaria) with human serum albumin (HSA) has been studied by fluorescence spectroscopy. Based on the sign and magnitude of the enthalpy and entropy changes, hydrogen bond and van der Waals forces were suggested as the main interacting forces .The binding of AQ to HSA was modeled by molecular docking and molecular dynamic simulation methods. Az a result AQ binds mainly to the sub-domain IIA of HSA [48].

10) Others

Sulfonylureas are drugs commonly used to treat type II diabetes .The interaction between sulfonylurea drugs and the human serum albumin has been studied by performance affinity chromatography for studying the changes induced after the binding [49].Colchicines as a drug used for treatment of GOUT, binds to human serum albumin and was studied by resonance light scattering analysis (RLS) and FT-IR [50].

DISCUSSION

In the present work, the binding of varieties of drugs to HSA is studied by employing some different optical techniques and molecular modeling. Not all drugs cause changes in the secondary structure of HSA, since some results showed no alteration or at least no major changes in the secondary structure of HSA after binding to special drugs [20, 34, 39].However this stability

in secondary structure is not related to how hydrophobic effects, van der wals forces and hydrogen- bonds are modified[20,34,39].On the other hand, forces and interactions between drugs and HSA can increase the steadiness of drugs-HSA complex. Regardly Thermodynamic parameters $\Delta G^0<0$, $\Delta H^0<0$ and $\Delta S^0>0$ at different temperatures, it is indicated that hydrogen binding interactions and van der wals forces played a major role during the interactions among most of the studied drugs and Albumin [13, 19, 24, 48]. Whereas in some cases just hydrophobic bonds [4, 7, 14, 15, 35, 40] or only just electrostatic interactions [26]. But in most cases, protein-drug complex, are stabilizing by collections of different forces and bonds [20, 34, 36].

In addition binding of different drugs to the hydrophobic cavity of sub domain ‖A, which is a suitable binding site for many drugs [4, 34, 32, 48], dose not cause the same switches in HSA after binding to different drugs for example Nobiletin (anti-tumor) and Clozapine (never agent) both are set up on sub domain ‖A, but one of them induce major conformational changer at secondary structure whereas another do just little alter in this structure [4, 34].

Although in low drug concentrations, no major conformational changes occur in protein structure, a significant increase in protein α-helix and a significant decrease in β-sheet structures was observed at high drugs contents. This is indicative of a partial stabilization of protein secondary structure at high drugs concentrations. Whereas with increasing other drugs concentration, a decrease in protein α-helical content and an increase in amounts of β-sheets and random coil structures can be observed[24,25]. Thus drugs concentration is not main factor for suggesting how they have impact on secondary structure.

The reports indicated that the probable mechanism of some drug's interaction with HSA is a static quenching process. The binding process was exothermic, enthalpy driven and spontaneous, as indicated by the thermodynamic parameters analyzed, and the major part of the action force is van der wals, H-bonds and hydrophobic interactions [27]. It seems that if drugs are attached to the hydrophobic amino acids, they would affect the molecular quenching [14, 27] even so they bind to the others site where have no quenching effects [28]. Moreover the presence of metal ions (e.g. Zn^{2+}, Cu^{2+}, Co^{2+}, Ni^{2+}, Fe^{3+}) increases quenching impacts [7, 39], decreases the binding constant of the drug –HSA complex and increases the free concentration of unbound drugs, this declines the storage time of the drugs in blood plasma as well as an enhancement of the maximum effectiveness of the drugs, and these effects are significant in vivo. Studying properties of the drugs binding to physiologically important protein HSA is greatly important in pharmacy, pharmacology and biochemistry, which may provide some references for the rational use of drugs in the clinic [39].

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