Advanced Drug Delivery Reviews xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Advanced Drug Delivery Reviews

journal homepage: www.elsevier.com/locate/addr

In vitro, in silico and integrated strategies for the estimation of plasma protein binding. A review ☆

George Lambrinidis, Theodosia Vallianatou, Anna Tsantili-Kakoulidou *

Department of Pharmaceutical Chemistry, School of Pharmacy, University of Athens, Panepistimiopolis, Zografou, Athens 15771, Greece

ARTICLE INFO

Available online xxxx

Keywords:
Plasma protein binding
Human serum albumin
α_1-Acid glycoprotein
In silico predictions
Ligand based modeling
Structure based modeling

ABSTRACT

Plasma protein binding (PPB) strongly affects drug distribution and pharmacokinetic behavior with consequences in overall pharmacological action. Extended plasma protein binding may be associated with drug safety issues and several adverse effects, like low clearance, low brain penetration, drug–drug interactions, loss of efficacy, while influencing the fate of enantiomers and diastereoisomers by stereoselective binding within the body. Therefore in holistic drug design approaches, where ADME(T) properties are considered in parallel with target affinity, considerable efforts are focused in early estimation of PPB mainly in regard to human serum albumin (HSA), which is the most abundant and most important plasma protein. The second critical serum protein α_1-acid glycoprotein (AGP), although often underscored, plays also an important and complicated role in clinical therapy and thus the last years it has been studied thoroughly too.

In the present review, after an overview of the principles of HSA and AGP binding as well as the structure topology of the proteins, the current trends and perspectives in the field of PPB predictions are presented and discussed considering both HSA and AGP binding. Since however for the latter protein systematic studies have started only the last years, the review focuses mainly to HSA. One part of the review highlights the challenge to develop rapid techniques for HSA and AGP binding simulation and their performance in assessment of PPB. The second part focuses on in silico approaches to predict HSA and AGP binding, analyzing and evaluating structure-based and ligand-based methods, as well as combination of both methods in the aim to exploit the different information and overcome the limitations of each individual approach. Ligand-based methods use the Quantitative Structure–Activity Relationships (QSAR) methodology to establish quantitate models for the prediction of binding constants from molecular descriptors, while they provide only indirect information on binding mechanism. Efforts for the establishment of global models, automated workflows and web-based platforms for PPB predictions are presented and discussed. Structure-based methods relying on the crystal structures of drug–protein complexes provide detailed information on the underlying mechanism but are usually restricted to specific compounds. They are useful to identify the specific binding site while they may be important in investigating drug–drug interactions, related to PPB. Moreover, chemometrics or structure-based modeling may be supported by experimental data a promising integrated alternative strategy for ADME(T) properties optimization. In the case of PPB the use of molecular modeling combined with bioanalytical techniques is frequently used for the investigation of AGP binding.

Contents

☆ This review is part of the *Advanced Drug Delivery Reviews* theme issue on "In silico ADMET predictions in pharmaceutical research".

* Corresponding author.
E-mail address: tsantili@pharm.uoa.gr (A. Tsantili-Kakoulidou).

http://dx.doi.org/10.1016/j.addr.2015.03.011
0169-409X/

1. Introduction

The concept of drug-likeness and property—based drug design were introduced at the end of 90 s to phase the challenge of pharmacokinetics which next to toxicity were the main reasons for the failure of drug candidates during clinical phases [1,2]. Since then, the development of rapid in vitro permeability assays and in silico techniques, as well as their combined use, resulted in considerable improvement of compound quality in terms of bioavailability [3–6]. In the beginning of the 21st century, statistics showed that the attrition rate rooted less on poor absorption but rather on drug efficacy and drug safety issues [7], both involving plasma protein binding (PPB). Apart from metabolic stability, drug efficacy is related to distribution, half life and clearance, processes that are strongly influenced by PPB. Extended PPB is responsible for low clearance and low brain penetration, while it may be the cause for drug–drug interactions through the displacement of one drug by the other [8–11]. The free drug principle dictates that in fact it is the free (unbound) drug concentration that permeates biological membranes and exerts the biological action at the site of action. However, depending on the drug and the target, the high affinity of drugs for plasma proteins may be also beneficial for efficacy and toxicity [12]. The function of plasma proteins as drug carriers may facilitate access to the site of action, while reducing side effects. In particular, application of plasma proteins on drug delivery may be important in anticancer therapy [13]. More to the point, since the free drug principle applies also to drug interactions with specific off-target proteins, plasma protein binding may be related to drug safety issues and should be taken into account in estimating therapeutic margins [12]. Nevertheless, despite the well-established role of PPB in understanding pharmacokinetics and pharmacodynamics, there is a challenging debate about its real influence on in vivo efficacy and its clinical relevance [12,14–18]. Such arguments are based on the fact that knowing plasma protein binding provides information on the free drug fraction, not the free drug concentration at the therapeutic target, which is after all crucial for in vivo activity. Considering the complexity of biological systems, such viewpoints are valuable warnings in the process of drug development against over interpretation of individual processes independently, although they should not themselves be misinterpreted [12,15,16]. The knowledge of plasma protein binding, remains very important throughout a drug discovery and development project, but should be integrated to pharmacokinetic data in order to support optimization and prioritization of drug candidates. However, there are no concrete guidelines how to exploit and interpret such combined information [19]. On the other hand, it is not unusual that lead compounds or even whole drug discovery projects had to be abandoned due to very strong binding to plasma proteins, or because of short half life and poor distribution due to weak binding [20]. Therefore, considerable research efforts are oriented towards the development of new technologies for rapid assessment of plasma protein binding, as well of computational models, enabling in silico prediction of PPB.

2. Plasma protein binding: an overview of the role of the principal partners

Plasma, accounting for 55% of the human blood's composition, is an aqueous solution which contains 92% water, 7% proteins, 1% other solutes such as inorganic ions. The most important proteins for drug binding are albumin and α_1-acid glycoprotein, followed by lipoproteins [21]. Human serum albumin (HSA) is the most abundant plasma protein, present at concentration ~7×10^{-4} M and accounting for 55% of the total plasma protein content. Its main physiological role is to transport fatty acids. It is also involved in the maintenance of colloidal osmotic blood pressure, the fluid distribution between body compartments [22–24], the protection of the organism by binding toxic metabolites and the storage of nitric oxide [25]. It accounts for most of the antioxidant capacity of human plasma, and displays (pseudo-)enzymatic properties. HSA is a highly soluble, monomeric, positively charged protein. It displays an extraordinary ligand-binding capacity and binds reversibly acidic as well as neutral compounds at different sites. Basic drugs are also bound by HSA although to a lesser extent. Due to its primary importance, the term plasma protein binding is often associated with this protein. Generally HSA is considered as a non-specific 'ligand promiscuous' binder and carrier [26], where entropy driven hydrophobic interactions are the dominant recognition forces [27]. In fact, analysis of HSA binding data has served in early lipophilicity studies for the establishment of octanol–water as the reference system to measure partition coefficients [28]. The protein is a 66-kD monomeric polypeptide of 585 amino acids, folded into three similar helical domains (I–III) [29]. The three domains of HSA assemble asymmetrically forming a heart shape, with approximate dimensions of 80–80–30 Å [30]. Each domain includes 10 α-helices and is further split into two sub-domains, the six helix subdomain A and the four helix subdomain B, connected by a long extended loop. A conserved set of 17 disulfide bridges connects the individual helices and retains the protein structure which is maintained in all mammalian species, [29]. The disulfide bridges contribute also to HSA's increased thermostability [31]. Subdomains I and II are almost perpendicular to each other, with subdomain IIA being connected to the interface region between subdomain IA and IB by hydrophobic and hydrogen bonds interactions. Domain III interacts only with subdomain IIB, forming a Y-shaped assembly with domains II and III. A big channel created by subdomains IB, IIIA, and IIIB separates domains I and III which are connected only by few contacts. This topology is shown in Fig. 1.

The absence of β-sheets gives to the protein a high degree of conformational flexibility, as indicated also by molecular dynamic simulations [32,33]. This high flexibility may be responsible for the astonishing binding capacity of HSA which is reflected in multiple binding sites, as supported also by crystallographic data [31,34,35]. However two overlapping stereoselective sites appear to predominate for binding of drugs or drug like molecules. They are known as Sudlow's sites 1 and 2 [36,37]. Site 1, termed the warfarin site lies in subdomain IIA and binds warfarin and azapropazone. Site 2, termed the benzodiazepine

Please cite this article as: G. Lambrinidis, et al., In vitro, in silico and integrated strategies for the estimation of plasma protein binding. A review, Adv. Drug Deliv. Rev. (2015), http://dx.doi.org/10.1016/j.addr.2015.03.011