Protein Binding as a Predictor of Drug Bioavailability and Distribution

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***Abstract*—The degree to which a drug binds to plasma proteins, as assessed by evaluating the free active fraction, plays a crucial role in influencing the pharmacokinetics and pharmacodynamics of the drug. This review emphasizes the key methodologies employed to analyze drug–protein interactions. There is a table that will help to understand different drugs that binds to proteins to form drug protein complex. This review also discusses various pros and cons of Drug protein binding. Protein binding plays a crucial role in determining the bioavailability and distribution of active compounds. It serves as a limiting factor in the ability of drugs to traverse biological membranes and barriers. Frequently, drugs encounter difficulties in crossing these membranes primarily because of the substantial molecular weight of the drug-protein complex. This leads to the accumulation of active compounds and a notable decrease in their pharmacological efficacy.**

***Index Terms*—Drug protein Binding, Pharmacokinetics, Pharmacodynamics, Pharmacological efficacy, Bioavailability.**

I. INTODUCTION

The binding of drugs to plasma proteins plays a crucial role in pharmacokinetics, which encompasses absorption, distribution, metabolism, and elimination, as well as pharmacodynamics, which refers to the pharmacological effects of the drugs. Drugs, once distributed in the bloodstream, attach to plasma proteins to varying extents. Typically, this binding is reversible, establishing an equilibrium between the bound and unbound molecular forms [1]. So, only the unbounded drugs are able to show their pharmacological or toxicological effects. A simple figure is also shown in Figure 1 for understanding. Binding to plasma proteins serves as both an advantage and a limitation in the distribution of drugs throughout the body. The association with albumin facilitates the transport of drugs in the bloodstream, enabling them to access areas far from the initial site of administration [2]. The proportion of unbound drug may also impact the rate of drug elimination. Consequently, binding influences both the duration and intensity of the drug's effects. The binding and transportation of both endogenous and exogenous substances represent one of the key functions of plasma proteins [2,12].

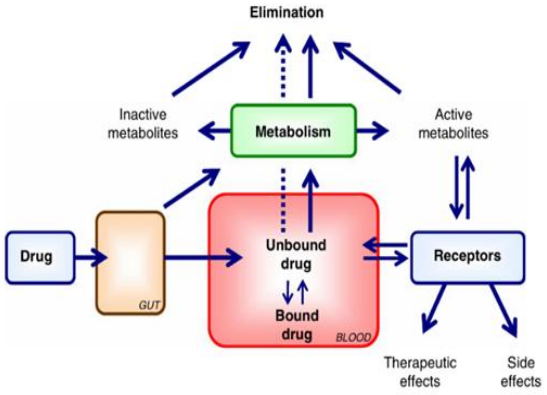
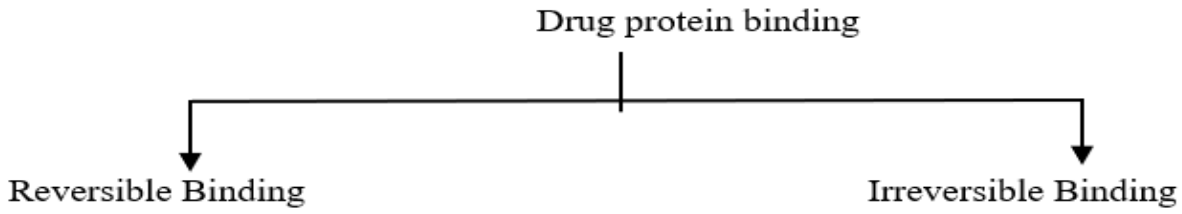


Fig.1: Drug distribution and protein binding [1]

Endogenous substances encompass bilirubin, fatty acids, L-tryptophan, vitamins, and a variety of hormones. Additionally, most drugs exhibit some degree of binding to plasma proteins. Albumin serves as the primary binding protein for acidic and neutral drugs in significant quantities. In contrast, bases are bound to a lesser degree by albumin, and emerging evidence suggests that globulins play a predominant role as binding proteins for basic drugs. The regulatory frameworks in numerous countries require pharmaceutical companies to conduct a range of studies for new medications, which includes a comprehensive in vivo pharmacokinetic analysis.

Information regarding parameters such as drug clearance, apparent volume of distribution (V_d), and protein binding is utilized to formulate dosing regimens designed to consistently attain target drug concentrations in patients. The objective of this procedure is to guarantee maximum clinical effectiveness while minimizing toxicity for the patient [3, 16].

II. DRUG–PROTEIN BINDING: A THEORETICAL OVERVIEW



Reversible Binding Irreversible Binding

Reversible Protein Binding: In a protein-drug complex, reversible binding occurs when a drug interacts with its target protein through non-covalent interactions like hydrogen bonds, ionic bonds, or van der waal's forces. This binding is temporary, allowing the drug to exert its therapeutic effect without permanently altering the protein. The equilibrium between the bound and unbound states determines the drug's efficacy and duration of action.

Irreversible drug-protein binding: occurs when a drug forms a stable covalent bond with a protein, creating a permanent complex. This binding is non-reversible under physiological conditions and persists until the protein is degraded. It can have therapeutic effects, as in enzyme inhibitors, or cause toxicity if unintended proteins are affected. Such interactions influence drug efficacy and safety.

In 1894, Fischer proposed one of the initial models of protein-ligand binding, utilizing the metaphor of a lock to represent a rigid protein binding pocket and a key to symbolize a specific and rigid ligand. This analogy was intended to elucidate the interaction between an enzyme and its substrate. However, the constraints of this model became evident with the advent of protein crystal structures, which revealed a range of pocket shapes for the same receptor when co-crystallized with various ligands [15].

In case of reversible binding:

[Free drug] + [free protein] ⇌ [Drug Protein Complex]

P + D ⇌ PD

Let's denote free protein molecule be 'P' and free drug molecule be 'D' and the protein drug complex be 'PD'

P + D PD

Then, $K_{eqm} = \frac{[PD]}{[P][D]}$ where, K is the equilibrium stability constant.

$K[P][D] = [PD]$ (1)

$[P_t] = [P] + [PD]$ (2)

i.e. $[P] = [P_t] - [PD]$. (3)

From (1) and (3)

$K\{[P_t] - [PD]\}[D] = [PD]$

i.e. $K[D][P_t] - K[D][PD] = [PD]$

i.e. $K[D][P_t] = [PD] + K[D][PD]$

i.e. $K[D][P_t] = [PD]\{1+K[D]\}$

$$[PD] = \frac{K[D][P_t]}{1+K[D]}$$

$[PD]/[P_t] = K[D][P_t]/1+K[D]$

Let $r = [PD]/[P_t]$

So,

$r = K[D]/\ 1+K[D]$ (4), where, r = number of moles of drug bound per mole of protein.

And Equation (4) is nothing but Langmuir adsorption isotherm. Figure 2 helps in representing reversible binding.

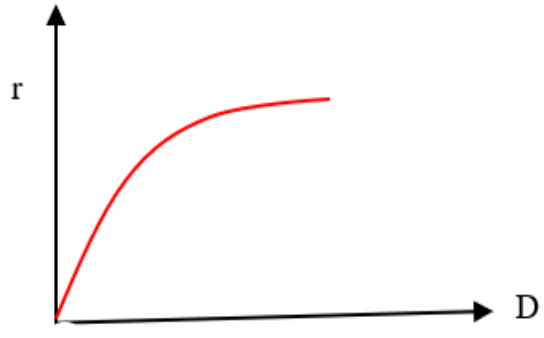
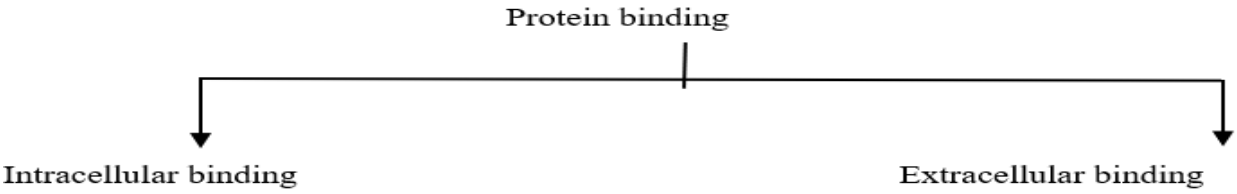


Fig. 2: Reversible binding

III. THE UNBOUND FRACTION

The pharmacological effect of all medications is attributed to the fraction that remains unbound to plasma proteins, commonly referred to as the free fraction. Although understanding the proportion of unbound drug is of academic significance, it is the variations in unbound concentrations throughout a dosing interval that must be characterized to effectively forecast the drug's impact [4,11]. The unbound fraction is typically presented as a singular value associated with any measured concentration of a specific drug. However, in practice, numerous drugs exhibit varying unbound fractions that are influenced by the concentration of the drug and several other factors [3]. It can be concluded that data representing the unbound fraction as a standalone value is somewhat limited in utility without an accompanying drug concentration. Both elements should be analyzed in conjunction to accurately interpret the potential effects of the drug.

IV. BINDING



Generally, Reversible binding is due to:

1. Hydrogen bonds
2. Hydrophobic bonds
3. Ionic bonds
4. Van der waal's force

And irreversible binding is due to Covalent Bond.

This binding is both help and hurdle in drug distribution. It influences both the extent and intensity of drug action [5,13].

The proteins that bind:

Table1: Protein and drugs that bind.

Protein	Drugs that bind
Human Serum Albumin	Large variety of all types of drugs specifically acid drugs such as Barbiturates, Benzodiazepines, Phenytoin.
1-Acid Glycoprotein	Basic drugs such as Imipramine, Lidocaine, Quinidine, etc.
Lipoproteins	Basic, Lipophilic drugs like Chlorpromazine.
α 1-Globulin	Steroids like Corticosterone and Thyroxine and Cyanocobalamine
α 2-Globulin	Vitamins A, D, E and K and Cupric ions

Minor variations in protein binding typically do not influence the average unbound concentrations, particularly for medications characterized by a low extraction ratio [6].

Binding to α1-acid glycoprotein (AAG):

AAG is found in plasma at levels that are typically 100 times less than those of albumin. While its specific function within the body remains unclear, it is possible that it contributes to normal coagulation, immune responses, and tissue repair mechanisms. Certain acidic medications, including warfarin, may compete with basic drugs for what seems to be a singular binding site, potentially located within the protein component of the glycoprotein molecule [11]. Pharmacokinetic investigations utilizing radio labelled

AAG suggest that 60% of the protein resides in the central compartment, likely the plasma, while the remaining portion is found in a peripheral compartment, presumably the extravascular space. It is uncommon for basic drugs to exclusively bind to AAG, although disopyramide and erythromycin may exhibit such binding at therapeutic concentrations [20].

Binding to albumin:

Albumin is the primary protein found in plasma, and its concentration typically fluctuates by less than two-fold in healthy individuals. It plays a crucial role in the overall binding of basic drugs within the plasma [11]. Albumin typically exhibits low affinity but high capacity for binding basic drugs, making it uncommon for fluctuations in albumin concentrations to lead to significant alterations in their plasma protein binding. However, substantial variations in albumin levels can indeed affect the binding of basic drugs [19].

Binding to other proteins:

Lipoproteins have been reported to interact with certain basic medications, including amitriptyline and nortriptyline. Pharmaceutical compounds have the ability to attach to lipoproteins, a category of biological nanoparticles responsible for lipid transport within the body. This interaction can facilitate the delivery of drugs to various tissues and influence their metabolic processes [18]. Table 1 helps in clear understanding.

Methods for Assessing the Extent of Protein Binding:

The methods presently employed for measuring free fractions encompass equilibrium dialysis, ultrafiltration, micro dialysis, ultracentrifugation, fluorescence spectroscopy, chromatography, and capillary electrophoresis [1,6]. Equilibrium dialysis (ED) and similar methodologies rely on variations in molecular size and/or weight. Two compartments are divided by a semipermeable membrane that functions as a molecular sieve, permitting only molecules below a specific molecular weight to pass through. Ideally, this membrane is fully permeable to the drug while remaining impermeable to both the protein and the drug–protein complex. One compartment holds the protein sample, while the other contains the drug under investigation. Following a predetermined incubation period, equilibrium is established, allowing for the measurement of the free drug fraction in the second compartment [1,7,9,10]. Ultrafiltration (UF) has been suggested as a swift alternative to electrodialysis (ED). This technique closely resembles ED, with the primary distinction being the enhanced analysis speed achieved by applying pressure to drive the solution through the membrane. However, challenges similar to those faced in ED, including the possibility of nonspecific binding of compounds to the filter membrane, the Donnan effect, and protein leakage, may also occur [7,9]. In ultracentrifugation (UC), a technique closely related to centrifugation, a mixture of drug and protein is subjected to a centrifugal field. The process continues until both the protein and the drug–protein complex settles at the bottom of the tube. Due to the typically low sedimentation coefficient of the drug in comparison to that of the protein, the unbound drug remains in the supernatant, allowing for its quantification. One of the key benefits of UC is its ability to mitigate issues related to membrane effects [1,10]. Liquid chromatographic techniques employed to evaluate drug–protein interactions can be categorized into two primary methodologies, depending on whether both interacting entities are present in a free solution (size-exclusion chromatography) or if one component, typically the protein, is fixed onto the chromatographic medium (affinity chromatography). Both methodologies can utilize zonal elution (small-plug injection) or frontal analysis (large-plug injection). In zonal elution (ZE), the retention time or peak area is utilized to derive the association constants, while frontal analysis (FA) quantification relies on the measurement of plateau height [1,8]. High-performance affinity chromatography (HPAC) relies on the immobilization of a protein onto a support medium, followed by the introduction of an interacting solute into the column. Compounds exhibiting a strong affinity will engage with the immobilized protein and will elute later than those with little or no affinity [7,9].

Promiscuous Drugs: A Comparison of Hydrophobicity and Molecular Weight.

Over the last decade, researchers have primarily concentrated on drug characteristics, including hydrophobicity and molecular weight, as factors contributing to promiscuity [14]. Bayesian classification conducted on 3,138 compounds and 79 targets reveals a correlation with molecular weight. Specifically, drugs characterized by high promiscuity

tend to possess a greater molecular weight, whereas those with low molecular weight exhibit weak promiscuity. Furthermore, the analysis indicates that promiscuous drugs generally have higher hydrophobicity and a greater number of nitrogen atoms, while the count of oxygen atoms is comparatively lower. Additionally, it was observed that marketed drugs demonstrate a higher degree of selectivity, resulting in reduced promiscuity.

V. DISCUSSION

- Future research should focus in incorporating real time monitoring technologies, computational modelling and artificial intelligence to better predict and manage drug protein binding interactions in clinical settings.
- Strategies should be proposed to minimize adverse effects and optimize drug combinations.
- Challenges should be emphasized in managing highly protein-bound drug in patients with renal or hepatic dysfunction.
- To assess the clinical significance of altered protein binding in different patient populations.
- Challenges should be addressed to design drugs with optimal binding profiles to balance efficacy and safety.

VI. CONCLUSION

- Alterations in the protein binding of medications are frequently observed in certain patient populations, which may lead to fluctuations in unbound drug concentrations, consequently influencing pharmacological efficacy.
- Only the unbound drug fraction is pharmacologically active.
- Advances in the analytical methods and the integration of pharmacogenomics are essential for improving the understanding of drug-protein interactions and supporting personalized medicines.
- At high drug concentration protein binding sites may become saturated increasing the free drug fraction. This non-linear pharmacokinetics can lead to enhanced drug effects or toxicity.

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