Review Article

Role and application of protein binding in drug distribution process

Lavisha Jindal, Gurmeet Singh

Department of Pharmacy, ISF College of Pharmacy, Moga, Punjab, India

Correspondence:
Ms. Lavisha Jindal, Department of Pharmacy, ISF College of Pharmacy, Moga - 142 001, Punjab, India.
Phone: +91-9814675200.
E-mail: lavishajindal121@gmail.com

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ABSTRACT

The amount of drug which binds to proteins is considered protein-drug binding. The binding of drugs to protein is reversible and irreversible. Reversible generally involves a weak chemical bond. Irreversible arises due to covalent bonding. Irreversible binding can also occur because of carcinogenicity or tissue toxicity of the drug. The plasma protein binding to a drug has a significant role in the pharmacokinetics and pharmacodynamics of a drug. The amount of free active fraction of drug is determined by how much drug is bound to plasma protein. Protein binding to the drug can enhance or detract the drug's efficiency. Protein binding can enhance the distribution of drugs throughout the body. It may also alter the duration of the action of the drug. Protein binding to drug alters therapeutic effect by forming a drug-protein complex which is itself biologically active. This review focuses on what is protein dug binding, how the protein binds to a drug. A description of the different methods used for determining protein-drug binding is also given. Protein-drug binding influences biological barriers, and how they cross them is also discussed. A discussion is also included to help readers to know the different factors affecting protein-drug binding.

Keywords: Protein, plasma, DNA, metabolism, drug distribution

INTRODUCTION

The drug is a substance which can be used to modify or explore physiological systems or pathological states for the benefits of the recipients.[1] Plasma, tissue, proteins, or other macromolecules, such as α_1 acid glycoprotein, human serum albumin, and DNA, binds with various drugs to form a drug macromolecule complex. When drug interacts with protein molecule and gets bind to it, there is formation of complex molecule. The complex formed is called as protein-drug complex. Protein participates in transmembrane signaling and regulates intracellular ionic composition when they act as ion channels. They become a common target of drug action. Drug binding depends on the bioactivation. Many drugs exhibit physicochemical affinity for plasma proteins and get reversibly bound to them.[2] Plasma proteins such as albumin, globulin, and α1-acid glycoprotein, or lipoproteins present in the body binds numerous drugs molecules. α1-acid glycoprotein bind to basic drugs and plasma albumin binds to acidic drugs. Binding to albumin is quantitatively more important.[3] These proteins binding changes the biological properties of the drug molecules as free drug concentration is reduced. Protein binding is a rapid process. Many individual factors can modify the degree of proteins binding to a drug and therefore should be considered when establishing for protein binding of different drugs. The protein binds drug at different rates, reducing the rate at which drug compounds are absorbed by cells.[4] Many compounds, like adrenaline, would not reach all the targets in the body and would be absorbed to fast in the absence of proteins. Protein-drug binding may inactivate a drug by binding, so firmly that sufficient concentration is not available at the receptor site. It also brings configuration changes in the protein which may become capable of binding to other agents.[5]

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BINDING (DRUG DISTRIBUTION)

There are two forms of the drug in the blood

- Bound
- Unbound.

The protein-bound drug is neither metabolized nor excreted. Due to pharmacokinetics and pharmacodynamics inertness of drug, it is pharmacologically inactive.[6] The unbound fraction of the drug undergoes metabolism in the liver and the other tissues. A drug will more efficiently traverse cell membranes or can diffuse if, it is less bound. Pharmacologic effects are given by unbound fraction.[7] The unbound fraction can be metabolized and/or excreted. The protein-bound drug does not cross membranes except large paracellular spaces, such as capillaries as a result, the drug which is highly bound to plasma protein is restricted to vascular compartments.[8]The bound fraction is not for action. Despite that, it is in equilibrium with the unbound fraction in plasma. It dissociates when the concentration of the unbound fraction is reduced due to elimination. Thus, plasma protein binding is tantamount of temporary storage of the drug.[9]

One drug can bind to many sites on the protein molecules. Furthermore, more than one drug can bind to the same site. It arises displacement interactions between drugs bound to the same site. Drug binding with high affinity will displace that bound with lower affinity. If the drug bound is displaced, then the concentration of free-form will be doubled. However, this is transient because the displaced drug will diffuse into tissues and also gets metabolized or excreted. The two different highly bound drugs will not displace each other, their binding sites may also not overlap, for example, probenecid and indomethacin are highly bound to albumin, and they do not displace each other. Basic drugs do not generally displace acidic drugs and vice versa.[10]

Protein binding is a reversible and rapid equilibrium process. It is also considered as an absorption process because it obeys the law of mass of action. The interaction between a protein and a drug molecule for a simple case of 1:1 protein-drug complex can be represented as:[4]

$$P+D \approx PD$$

P is the concentration of the unbound protein in terms of free binding sites. D is the concentration of the unbound drug. PD is the concentration of a protein-drug complex.

Applying the law of mass action, the expression becomes:

$$K = \frac{[PD]}{[P][D]}$$

K is the association constant.

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

If total protein concentration is given by $[P_t]$, then,

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

Total protein concentration is the sum of unbound protein and protein present in the complex.

Or,

$$[P]=[P_t]-[PD] \quad (3)$$

Substituting the value of [P] in equation (1), then

$$[PD]=K[D]([P_t]-[PD])$$

$$[PD]=K[D][P_t]-K[D][PD]$$

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

$$[PD](1+K[D])=K[D][P_t]$$

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

Where $[PD]/[P_t]$ represents an average number of drug molecules bound per mole of protein $[P_t]$.

Substituting $[PD]/[P_t]$ by r,

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

If there are n number of independent binding sites, then:

$$r = n\frac{K[D]}{1+K[D]} \quad (4)$$

$$r(1+K[D])=nK[d]\ r+rK[D]=nK[D]$$

$$r= nK[D]–rK[D]$$

$$r=[D]\ (nK–rK)$$

$$\frac{r}{[D]} = nK - rK \quad (5)$$

This is known as a Scatchard plot.

Equation (5) is not used for analyzing data if in the experimental system the amount and nature of protein are unknown. Then, a different equation is used,

$$\frac{[Db]}{[D]} = -K[Db] + nK[Pt]$$

Where D_b gives the concentration of the bound drug. The ratio of $[D_b]/[D]$ is plotted against $[D_b]$. K is obtained from the slope and $nK[P_t]$ is determined from the intercept.

ANALYTICAL TOOLS USED FOR DETERMINING PROTEIN BINDING

Various methods are used for determining protein-drug binding. These methods can be used to study the solubility nature of drug in presence and absence of protein. It is used to study the influence of protein on the drug molecule in the aqueous and organic solvent.[4]

Techniques used for investigating protein-drug binding are divided into two techniques.

1. The separative method
2. Non-separative method.

Separative method

In this method, the investigating of the free ligand is separated from the bound species and is directly used for determining either the bound drug concentration or unbound drug concentration. In separative methods, different types of techniques are used for determining protein-drug binding.[7]

Equilibrium dialysis

Equilibrium dialysis is the method which is most widely used for investigating protein-drug binding. This method is performed in a solution, in which equilibrium is maintained throughout the whole process. Thus, it is regarded as the reference method for determining protein-drug binding. In this method, there are two compartments separated by a semipermeable membrane (cellophane) which acts as a molecular sieve which is permeable for small drug molecules and not to macromolecules. One compartment contains the protein sample and other contains the drug molecules. The solution is stirred until equilibrium has been achieved.[7] Samples from both sides of the membrane are withdrawn and analyzed. If the concentration of the drug within and outside the membrane is the same, it indicates that no protein binding has occurred. If the concentration inside the membrane is more, it indicates that protein binding has occurred because both bound and unbound drug is present within the membrane.[4]

Ultrafiltration

Ultrafiltration has been a similar method to equilibrium dialysis method.[4] In this method, the solution is also separated by a semipermeable membrane as the equilibrium dialysis method, except the analyzing speed, is increased by applying pressure to force the solution through the membrane.[7] However, hydraulic pressure or centrifugation is used in ultrafiltration to force the solvent and the free drug across the membrane while preventing the passage of protein-bound drug across the membrane. It is more convenient than other methods for routine determination since this method is less time- consuming.[4]

Ultracentrifugation

Ultracentrifugation is a method by which compounds with different molecular weights are separated with the help of centrifugal force. Centrifugation is done, until the protein and the drug-protein complex gets sediment at the bottom. The free drug remains in the supernatant, and its quantity can be analyzed.[7]

Parallel artificial membrane assay

This method is used to predict the drug passive permeability through biological membranes. This technique is based on the formation of an artificial membrane using the hydrophobic filter material as support upon to produce an organic solvent is placed upon to produce an artificial lipid.[11,12] The artificial membrane is used to separate two compartments, one consisting buffer solution to be tested and second consisting only fresh buffer. However, the liquid membrane must be chosen to allow the free drug to permeate through it and the protein remains in the donor compartment. The use of PAMA for protein-drug binding can finish the equilibrium binding constants.[7]

Liquid chromatographic techniques

Liquid chromatographic technique is used to determine drug-protein interaction. It is divided into two approaches. These approaches depend that both interacting species are free in solution or whether one component, that is, protein is immobilized on chromatographic support.[13]

Capillary electrophoretic techniques

This technique is used in chemical analysis to separate molecules in an electric field according to the size and charge. It is performed in a sub-millimeter diameter tube, called a capillary, which contains a flowing electrolyte solution.[7]

Among the techniques used for investigating protein-drug interaction/binding, this method provides a favorable possibility of evaluating interactions in free solution.[14] There are many advantages of this method such as high efficiency and separation selectivity, low sample and reagent consumption, and high-speed of analysis. It also has some disadvantages such as the protein may get absorbed on the capillary walls and the low detection limits of commonly used UV detectors. One protein sample can be used for screening.[15] The protein preparation does not need to be purified. Protein binding of racemic drugs can be performed by injecting the racemic mixture directly. The precise value of drug concentration is not required.[16]

Non-separative methods

This group of techniques for determining the protein-drug binding relies on the detection of a charge in a physicochemical property of either the ligand or the protein because of the binding. There are two different techniques used in this group.[7]

Spectroscopic techniques

This technique is performed in solution, which enables true equilibrium measurements.[7] This method gives a better understanding of the protein binding mechanism. It also facilitates insight into three-dimensional protein structure.[17] Fluorescence spectroscopy helps in the identification of the binding site of a drug and can also be used to calculate the binding distance between the fluorophore on the protein and the drug.[18]

Calorimetric techniques

For investigating protein-drug binding two types of calorimetric approaches are used: Isothermal titration calorimetry and differential scanning calorimetry. ITC is mostly used to investigate biomolecular interaction. ITC can be used for molecules of arbitrary size and spectroscopically silent compounds.[19,20] DSC was developed to characterize protein stability and folding. The same instruments are in both ITC and DSC.[19] The reaction cell has a mixture of drug and protein is heated at a controlled rate. When a small molecule binds to

the protein, the drug stabilizes the protein and the transition midpoint of the drug stabilizes the protein and the transition midpoint of the protein-ligand complex, thus occurs at a higher temperature than the midpoint in the absence of ligand.[21]

BIOLOGICAL BARRIERS AND INFLUENCE OF PROTEIN BINDING ON PASSAGE OF DRUG ACROSS THEM

Protein binding is easy to study *in vitro*, but its effect on crossing biological barriers in a living organism could be difficult to study. This is due to the complexity of the entire barrier-crossing process and additional side effects that simply cannot be obtained in the laboratory.[8] Crossing biological barriers is very difficult. The most important, and the most difficult to pass, is the blood-barrier which separates crucial organs from the environment. Drugs can pass through the BBB by transmembrane diffusion, especially those which are lightweight or with high lipophilicity or carried by transports, as in the case of glucose. Log BB and log PS are two parameters that describe the amount of a drug that is passed into CNS.[8] Log BB gives a ratio of drug concentration in the CNS and plasma and log PS shows the permeability of certain surface. Albumin, like other proteins, does not pass through the barrier and its drug-macromolecules complex, cannot cross. Benzodiazepines, steroids, and a few hormones are some drugs that cross the BBB without difficulty, demonstrate higher concentrations in the CNS than their unbound plasma fraction would indicate.[8] Protein binding has a significant role in penetrating BBB; it can decrease the passage, or it can also affect the other way with mechanisms still to be discovered. Passage through the BBB is more complicated by the mechanism which protects the CNS, such as active efflux and the use of strong protein binding mechanisms.[8]

It is less likely for the drug-protein complex to cross the placental barrier. Some drugs can accumulate in placental tissue by binding with proteins in these regions, and upon release, enter the fetus or infant in an uncontrolled way.[8] Due to high permeability, the placenta act as a filter than an actual barrier. Macromolecules, such as insulin or heparin and immunoglobulins, are some bacterial cells that retain within the placenta. Drugs such as barbiturates, antibiotics, sulfonamides, and alcohols can pass through the placenta barrier. Small molecules cross the barrier by simple diffusion, while drugs also cross by facilitated diffusion or active transport or by endocytosis.[8] It is estimated that the penetration of drugs through the placenta is limited mainly by protein binding instead of lipophilicity.[22] Protein binding can take place in maternal and fetal tissues; drug molecules can also form a repository in the placenta, from which it can be released in an uncontrolled way into the maternal or fetal plasma.[8] The placenta is considered a very weak barrier in the case of xenobiotics and most of the administered drugs can easily cross it. Plasma protein binding can affect this process because it significantly limits placental transit, but α-fetoprotein concentration increases which can enhance the passage.[23]

As a barrier, skin also prevents the penetration of many chemical compounds.[23] Earlier, it was assumed that most administered drug particles get absorbed into skin circulation, thus allowing them to pass into the bloodstream and this process is regulated by the skin structure.[23,24] Later, it was suggested that the most important factor which determines skin penetration are the structure and properties of the drug.[23] Administered drugs can bind with the proteins within the skin layer, which can be desirable if only local action is intended: The drug will accumulate at its site of activity, and it will not cause any adverse systemic effects. In transdermal drugs, such skin protein binding will disturb their flow into the circulation.[8] Benzocaine can accumulate in the skin through non-specific binding. Tacrolimus and pimecrolimus are most likely to bind non-specifically with various skin proteins.[25] Skin penetration is very important for transdermal drugs because they have a strong effect on bioavailability and protein-binding interacts with this process.[8]

SIGNIFICANCE

The significance of protein binding of drugs is that as the protein-bound drug is neither metabolized nor excreted; hence, it is pharmacologically inactive due to its pharmacokinetics and pharmacodynamics inertness.[4] Abound drug always with a specific tissue or to a particular site to which it is bound and with that site it possesses a greater affinity. The major benefit is the prolonged duration of action of the drug because the protein-bound-enormous size of the drug complex cannot experience membrane transport.[7] Usually weak chemical bonds such as ionic bonds, hydrophobic bonds, a hydrogen bond or wander wall forces are involved in protein-drug binding and thus, form this process a reversible process. The covalent bond formation is very rare to form, and it may result in permanent irreversible binding with a great potential of portending adverse effects such as carcinogenicity, teratogenicity, tissue, or organ toxicity.[8]

Absorption

The binding of the absorbed drug to plasma proteins decrease free dug concentration. The conventional dosage form follows first-order kinetics. Hence, more protein binding can disturb the absorption equilibrium. Thus, sink condition and concentration gradient are established which now acts as the driving force for further absorption.[26]

Systemic solubility of the drug

Neutral endogenous macromolecules, a water-insoluble drug such as steroids and heparin are circulated and distributed in tissue by binding to lipoprotein which acts as a barrier hydrophobic compound. After binding to the protein, the metabolism of drugs decreases and also it enhances the biological half-life of the drug. Only the unbound fraction gets metabolized.[26]

Distribution

The plasma protein-drug binding thus favors uniform distribution of drug throughout the body by its buffer function. A protein-bound drug, in particular, does not cross the blood-brain barrier, placental barrier, and glomerulus. Thus, protein binding decreases the distribution of drugs.[26]

Elimination

Only the unbound drug is capable of being eliminated. Protein binding prevents the entry of drugs to the metabolizing organ and glomerulus filtration. Tetracycline is eliminated mainly by glomerular filtration.[26]

- Influence way of drug distribution into tissues in the body[26]
- Limits the amount of free drug available to access sites of action in the cell[27]
- Reduces or eliminates the pharmacological activity of the drug since the bound drug is not available for binding to the receptor site[26]
- The protein-drug complex itself has biological activity[8]
- Co-administration of a special drug that also binds to plasma proteins may cause displacement of the bound drug, leading to significant toxicity because the free drug interacts with the receptor to produce a pharmacological response[28]
- Prolongs the duration of action of a drug[29]
- Retards the excretion of a drug and increases its accumulation.[26]

FACTOR AFFECTING PROTEIN-DRUG BINDING

Drug-related factors

Lipophilicity

It is the foremost desirable physicochemical factor that's prerequisite for protein binding to occur. Protein binding is directly related to lipophilicity and stereoselectivity of the drug. An increase within the lipid content of drug moiety, enhances the speed also extends the process of protein binding. In intramuscular injection of cloxacillin as attributed to greater lipophilicity displays 95% protein binding.[30]

The concentration of drug within the body

Alteration within the concentration of drug substance also as protein molecules or surfaces subsequently brings alteration within the protein binding process. The extent of protein-drug binding can change with both drug changes also as protein concentration.

Drug affinity toward protein

This factor entirely depends upon the degree of attraction or affinity the molecule or tissues have toward drug moieties. Drugs having their high specific protein binding site. For digoxin has more affinity for cardiac muscle proteins as compared to that of proteins of skeletal muscles or those in the plasma-like HAS.[31]

Protein-related factors

The concentration of protein component

This is the most important parameter to be given consideration. As the human serum plasma proteins constitute the major portion of the plasma protein, a large number of drugs undergo extensive binding with them as compared to the concentration of other protein molecules.[32]

Number of binding sites on the protein

In association with the concentration of protein molecules available in the protein molecule is also significant. Albumin not only possesses the sizable amount of binding sites but also has a greater potential of completing the binding process. Numerous drugs exhibit multiple site binding with albumin molecules in plasma such as flucloxacillin, ketoprofen, and indomethacin.[33,34]

Physicochemical properties of protein binding agent

Lipoproteins and adipose tissue tend to bind lipophilic drugs by dissolving them in their lipid core. The physiological pH determines the presence of active anionic and cationic groups of the drug to bind on the albumin.[9]

Drug interaction

Displacement interactions are predominant ones among reactions between drug and protein. If two or more drugs have the same or identical affinity for the same site then they struggle with one another to bind at the same site. Consider a drug A is bound to a specific site on the molecule and if a second drug B is administered, then the drug moiety having a greater affinity toward the bound site would effectively displace the previous drug. This phenomenon is said to be a displacement reaction. The drug which is been faraway from its binding site is claimed to be a displaced drug while the one that does the displacement is called as a displacer. The best example for such interactions is the competitive protein binding that happens between warfarin and phenylbutazone for HAS, where phenylbutazone is displacer while warfarin is displaced. Clinically such a reaction is important when the displaced drug is quite 95% bound to plasma protein occupy a small volume of distribution even less than that of 0.15l/kg. This is also important when active drugs or administered pharmacological agent possess narrow therapeutic index.[33,35]

Patient-related factors

Patient-related factors have their importance as the drug has to generate its response to the administered patient. In these numerous parameters are considered such as age and disease state.[36]

Age-related factors

1. Neonates – low albumin content; more free drug[37]
2. Young infants – high dose of digoxin because of large renal clearance[31]
3. Elderly – low albumin content; then more free drug[37]
4. Total body water (both ICF and ECF) greater in infants.[38]
5. Fat content – higher in infants and elderly
6. Skeletal muscle – lesser in infants and elderly[39]
7. Organ composition – BBB is poorly developed in infants and myelin content is low and cerebral blood flow is high, hence greater penetration of drug in brain plasma.[8]

Intersubject variability

Due to genetic and environmental factors.[40]

Disease state

A decrease in the protein binding is monitored in the case of pathological states.[41] Hypoalbuminemia, conditions leading to change of albumin compartment volume and presence on albumin binding

sites of pathological inhibitors of drug binding are most important of the disease state factors.[42] Some other reasons for disease state affecting protein-drug binding are:

1. Altered albumin and other drug-binding protein concentrations[43]
2. Alteration or reduce perfusion to organs or tissue[44]
3. Altered tissue pH
4. Alteration of permeability of physiological barrier. Ex-BBB (in meningitis and encephalitis) becomes more permeable thus polar antibiotics ampicillin, penicillin G which do not normally cross gain access to the brain, and patients suffering from CCF perfusion rate to the entire body decrease its effect the distribution.[45]

CONCLUSION

Protein molecules present in the body bind to drugs administered in the body. This protein-drug binding can lead to various factors which can affect the drug release in the body. Many proteins such as albumin, human serum albumin, DNA, and globulin get binds to a drug molecule. The only unbound fraction of the drug undergoes the metabolism. It is a reversible and rapid equilibrium process. Many methods are used for determining protein-drug binding in the body. Separative and non-separative methods are the two methods used for determining protein-drug binding. Crossing biological barriers are very difficult for protein-drug complexes. It is very difficult for protein-drug complex to cross the placenta barrier. It is pharmacologically inactive due to its pharmacokinetics and pharmacodynamic internees. The binding of the protein-drug molecule can also be affected by various factors.

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