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Review Article

Plasma Protein Binding of Drug and Its Therapeutic Effect

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ABSTRACT

Drug absorption, distribution, metabolism, and elimination (ADME) are all included in the broad topic of plasma protein binding (PPB). Alpha-1-glycoprotein, albumin, globulin, and lipoprotein are among the proteins found in the human body that serve as drug binding sites. Drug-protein interaction now depends on a number of variables, including pathophysiological, environmental, drug-related, binding site-related, chemical, etc. Additionally, one must research the pharmacokinetics and pharmacodynamics of proteins and medications, including volume of distribution, clearance, half-life, and other factors. A variety of bonds, including hydrophobic interactions and Van der Waals forces, are examined in the mechanism of drug-protein interactions. Liquid chromatography, spectroscopy, and other separative and non-separative methods are available for studying mechanisms. This article covers every aspect of plasma protein binding, including free and bound drug concentration, case studies of various medications, such as ibuprofen and warfarin, and case studies of various populations with varying ages, genders, cultures, and medical conditions that explain PPB. In addition to avoiding any risk associated with drug concentration or drug-to-drug interactions, the article seeks to understand and improve PPB's implications on drug efficacy and its clinical significance

INTRODUCTION

Plasma protein binding definition: -

"Plasma binding capability of drugs not only provides a strength but also weakness for the distribution of the drug. Drug can be distributed farther from the site of administration with protein binding and blood flow. But due to its inability to come out from the capillary system, the concentration gradient developed by the concentration of the free un-ionized drugs becomes the deciding factor in determining the rate of distribution of the drug in the body tissues. Pharmacological and toxicological effects are based on the concentration of the free un-ionized drugs. Protein binding influences drug action." [1]

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Importance In pharmacokinetics and Pharmacodynamics: -

Absorption, distribution, metabolism, and excretion (ADME) are the four stages of drug metabolism that the body goes through to determine how quickly and effectively a drug reaches its target site, how long it remains active, and how it is eventually eliminated from the body. Pharmacokinetics is a branch of pharmacology that studies these stages of drug metabolism [2]. Pharmacokinetics is a crucial field of study since it directly affects a medicine's therapeutic efficacy and potential for adverse drug reactions [3].

Objectives: -

The main purpose of plasma protein binding (PPB) of drugs is to control the concentration of the free, active drug that is accessible to the tissues, functioning like a reversible reservoir.[4]

Types of Protein Involved in Drug Binding

Sr. No.	Plasma Protein	Drug Bound	Binding Nature	Normal Concentration	Key Character
	Albumin	Warfarin Aspirin	Acidic Drug	3.5-5 g/dl	High Capacity, Low Affinity
2	a1-Acid Glycoprotein	Proponolol, Lidocaine	Basic drugs	0.5-1 g/dl	Acute-phase protein
3	Lipoproteins	Cyclosporine	Lipophilic Drugs	Variable	Highly Lipid Soluble Drugs
4	Globulins	Steroids, Thyroxine	Specific Binding	2-3 g/dl	Include Transport Protein Like Transferrin

Table No. 1

Mechanism of plasma protein binding:

Plasma protein binding refers to the scenario where a drug interacts with a blood protein as it moves through the blood plasma. It alters the concentration of the unbound drug as well as the plasma constituents. [5]

•Types of Binding Interactions:

1. Electrostatic interactions (ionic bonds) -

There are many instances of electrostatic bonding in anaesthesia and intensive care. Ionic (electrostatic) bonds are a type of bonds that are formed easily and are reversible. Such bonds are for humed between some ionized species and some anionic or cationic sites on a protein. Such a bond is formed as a result of the transfer of electrons between atoms. Notably, some atoms are positively charged. It occurs in neutral molecules, which means there are no net electric charges, and the molecules are in a state of equilibrium. Positively charged, neutral species are drawn to an anionic moiety. For example, the positively charged tertiary and quaternary amines, namely acetylcholine, physostigmine, and neostigmine, are attracted to an anionic glutamate residue in acetylcholinesterase and facilitate their interaction with the enzyme. Similarly, the anticoagulant heparin with an anionic pentasaccharide moiety is attracted to positively charged arginine residues in antithrombin III resulting in the formation of a stable complex.[7]

2) Hydrogen Bonding

Hydrogen bonding has an important role as a type of dipole-dipole interaction in biological

processes. There are numerous examples of hydrogen bonds in biology, and they all have different intrinsic energies. Typically, hydrogen bonds can be formed between a positively charged hydrogen of a hydroxyl (-OH) or a secondary amine (-NH) group and an adjacent electronegative atom with lone pairs, such as oxygen, nitrogen, or fluorine. Hydrogen bonds are a type of rapid chemical bond that are formed and broken (although, relative to other dipole-dipole interactions, they have greater bond strength and are more difficult to break).[7]

3) Van der Waals Forces

These are also referred to as dipole-dipole interactions. These occur between partially positive and partially negative charges which can attract or in some cases where a charge is neutral, positive charges repel other positive charges. The most important distance where this intermolecular magnetism occurs is called the Van der Waals distance. These forces decay rapidly with distance, therefore they're strong in solids and liquids but very weak in gases. [7]

4) Hydrophobic Interactions

These are essentially the opposite of polar bonds. When atoms are covalently bonded and have an equal (or nearly equal) affinity toward the shared electrons, the bond is non-polar. Symmetrical molecules like hydrogen and nitrogen, as well as carbon-hydrogen bond, are non-polar bonds. Non-polar and polar covalent bonds.

The term 'non polar compounds' can refer to a variety of different non polar compounds, which are, in turn, able to be categorized in multiple ways based on their structure. [7]

• Formation of Drug- Protein Complex: -

1) The interacting molecules are mostly macromolecules, examples of which are proteins, DNA, or even adipose tissue. Proteins are the main players in these interactions.

2) The term used to describe the process by which a drug forms a complex with a protein is referred to as protein binding of the drug.

3) A drug that is complexed with a protein is not metabolized or excreted. Thus, it becomes pharmacologically inactive because it has no pharmacokinetic and pharmacodynamic activity.

4) The interaction can be expressed as Protein + drug ⇌ Protein-drug complex.

5) Protein binding can be classified as either intracellular binding or extracellular binding.

6) The drug's binding to the protein can be classified as reversible and irreversible.

7) Reversible binding is associated with weak interactions such as chemical interactions: 1. Hydrogen bonds 2. Hydrophobic interactions 3. Ionic interactions 4. Van der Waals interactions.

8) Irreversible drug binding rarely occurs but is a result of a covalent bond.[8]

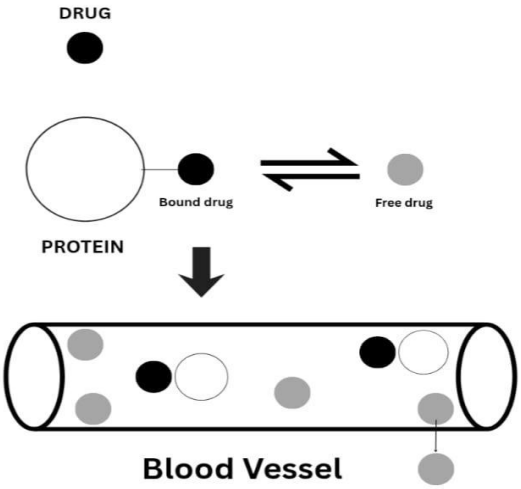

Diagram No.1

The drug is encarporated in the body through iv and then the drug gets absorbed into the cell through the blood circulation. After incorporating the drug binds with the protein present in the body and form a bond with the protein it may be reversible or irreversible the drug protein complex is formed. if the drug protein complex is reversible then the drug breaks the bond with the protein and become free drug and shows the activity of the drug at its particular site but if it is in the bound state then it becomes inactive and cannot show its desired activity at its particular site. The free drug is in the active state they can bound to thier particular site and shows its desired activity on the body.

• Binding site of Drug: -

•Albumin protein binding → Albumin is considered to be of major binding protein or drug binding protein in quantitative dominance in plasma and makes up ~80% of human plasma. It also helps in the transport of many hydrophobic molecules including bilirubin, fatty acids, lipophilic drugs and metal ions. Albumin contains two types of binding sites referred to as Sudlow site 1 and Sudlow site 2. Human Serum Albumin is made up of three homologous domains, the three domains of albumin protein form six binding sites per unit.[6]

•Alpha-1 acid glycoprotein binding → It binds to basic and also steroid drugs. It has only one binding site and is considered as the second major binding site also referred as' oromacoid'.[6]

•Globulin: - is another binding site which act on certain drugs like fat soluble vitamins (A, D, E, K).[6]

•Haemoglobin: - It can also bind drugs to some extent.[6]

• Free Drug and bound Drug: -

• Free Drug: -

In essence, the free drug refers to an unbound drug which is also the pharmacologically active component, hence free drugs are the only ones that can penetrate the plasma membranes. When the free drug concentration surpasses the total drug concentration, even keeping the drug concentration within the therapeutic range may lead to patient toxicity. Uremia, liver disease, and hypoalbuminemia increase free drug

concentration. Drug-drug interaction may also increase free drug concentration. [9]

• Bound Drug: -

Bound drug is the drug that binds to protein or tissue in blood. This is inactive because it does not cross the cell membrane. The drug acts as a reservoir. The bounded drug slowly releases and acts as an active component and exerts a physiological effect. [10]

• Drug protein binding equilibrium:

Drug-protein binding equilibrium is a reversible equilibrium involving a free (unbound) drug, a protein-bound drug, and blood proteins, usually tubular proteins like albumin. The free drug is pharmacologically active, crosses membranes, and is a bound drug.[14]

Many (n) drugs show attraction or affinity for sites on nonreceptor proteins, resulting in possible reversible

Drug + Protein = Drug Protein Complex

A similar situation exists with plasma proteins, most commonly with albumin, which with many acidic or steroidal medicines, and α1-acid glycoprotein, which binds many basic or neutral drugs. A drug may also reversibly bind with intracellular proteins. The drug-protein interaction resembles the drug-receptor interaction since it's rapid, reversible, and saturable, and different ligands can compete for the same site. It lacks an essential pharmacological effect but decreases the free concentration of the drug available to act at the receptors, in the quantity of the drug remaining available may be only a fraction of the total body cargo. Proteins such as albumin can thus act as protein depots, liberating the bound drug when the free drug is distributed to other compartments or eliminated. Competition for binding to proteins either in the plasma or intracellular can occur between different drugs, and between drugs and endogenous ligands. A highly protein-bound drug like aspirin can displace other drugs such as warfarin from their binding sites on plasma proteins; the increase in unbound drug concentration can increase the therapeutic effect of the displaced drug. Most similar relationships have significant clinical impacts, although one example of drug interactions with an endogenous ligand is the displacement of bilirubin from the albumin by sulphanilamide drugs, which decreases the bilirubin concentration in the tube, causing an increased risk of kernicterus. [15]

• The Concept of Free Fraction (Fu):

The unbound part of a drug in the body is referred to as the free fraction. This unbound form has both harmful and therapeutic effects due to its pharmacological activity. It is the part of a medication that is not attached to blood proteins and has the potential to improve therapeutic effects and bioavailability. Because free fraction directly affects a drug's mechanism of action and possible effects on the body, it is crucial to comprehend it.[12] The fraction unbound (fu) is the term used to describe the drug fraction that is not bound to protein. The free concentration (Cfree) divided by the total concentration yields the fu.[11]

(C tot = C free + C bound), equation … (1),

$$fu = Cfree(Cfree + Cbound) \ldots (1)$$

fu is dictated by the maximum protein binding capacity (Bmax), which is proportional to the concentration of the binding protein, considering the presence of single or multiple binding sites on the protein with same and uniform binding affinity, equilibrium dissociation constant (Kd, the drug's affinity for the protein), and the drug free concentration, Cfree (equation (2)).

$$fu = Kd + Cfree\ /(Bmax + Kd + Cfree) \ldots (2)$$

When Cfree is significantly lower than Kd, which is the case most of the time, fu is determined only

by Bmax and Kd, and hence remains constant (i.e. linear binding), as represented by equation (3).

$fu=Kd$ / $(Bmax+Kd)$ .... (3)

In linear binding case, fu is independent of both Cfree and Ctot. It is the pharmacological factors (Kd and Bmax) that "determine" fu that dictate Cfree and Ctot. The steady-state effects that fu has on Ctot and Cfree depends on whether a protein binding impacts drug clearance. When protein binding impacts total drug clearance, it is case when the rate of dissociation of drug from plasma protein is low compared to the (intrinsic) elimination rate constants of the organs responsible for elimination. When the rate of dissociation of drug from plasma proteins is high compared to the intrinsic clearance of the drug, then protein binding does not stand to limit total drug clearance. When drug clearance is limited by protein binding, changes in fu tend to influence Ctot but not Cfree. In situations where protein binding is not a limiting factor to drug clearance, changes in fu tend to influence Cfree but not Ctot. Most of the medications that have strong binding to plasma proteins, protein binding tends to limit their elimination. [13]

Factors Affecting Plasma Protein Binding

1. Things that are related to drugs:

• **Drug Concentration and Affinity**: In general, higher drug concentrations lead to better binding. But at high concentrations, protein binding sites may become full, which raises the percentage of free drugs. There are also differences in how much drugs like plasma proteins. Acetaminophen, for example, has a much lower affinity for albumin than warfarin does. Digoxin binds to cardiac muscle proteins more strongly than to skeletal muscle or plasma-like HAS proteins. = [18]

•**Lipophilicity**: Drugs that are lipophilic (fat-soluble) tend to stick to plasma proteins better than drugs that are hydrophilic (water-soluble). This is because lipophilic drugs easily interact with the hydrophobic pockets of plasma proteins, such as albumin. Medications that are very lipophilic, like warfarin and diazepam, bind strongly to proteins. This slows down their release into the bloodstream and makes them work longer. Hydrophilic drugs, such as acetaminophen, exhibit reduced protein binding and are more accessible in their unbound state. [16] Cloxacillin given through an intramuscular injection has a 95% protein binding rate, which is thought to be due to its higher lipophilicity.[17]

• **Molecular Size:** Bigger drug molecules stick to plasma proteins better than smaller ones. Larger drugs can interact with proteins in many ways, which can help keep the drug-protein combination stable. This binding makes less free medicine available for excretion or pharmacological action. [16]

•**Ionization**: Drugs that bind to plasma proteins differently are often ionized, or charged, at physiological pH. Acidic drugs such as phenytoin usually attach to albumin, while important drugs such as propranolol usually attach to alpha-1 acid glycoprotein. So, the level of ionization, which depends on the pH of the blood and the pKa of the drug, may affect how well proteins bind. [16]

2. Plasma Protein Related:

Most plasma proteins are human serum plasma proteins, so many drugs bind to them more than they do to other proteins molecules. [19]

• The number of places where the protein can bind:

It is also important to think about how many protein molecules are in the protein molecule. Albumin has a lot of binding sites and is also more likely to finish the binding process. Flucloxacillin,

ketoprofen, and indomethacin are just a few of the drugs that can bind to albumin molecules in plasma at more than one site. [20,21]

• The physicochemical properties of the protein binding agent:

Adipose tissue and lipoproteins usually bind lipophilic drugs by breaking them down in the lipid core. The physiological pH determines whether the drug has active anionic and cationic groups that bind to albumin. [22]

3.Patho-physiological Consideration:

Various medical conditions that change plasma protein levels or the ability of drugs to bind to proteins can cause big changes in protein binding. Drug toxicity has been observed in connection with renal diseases. The main reason for this is that proteins are not evenly spread out in the body and the body can't get rid of harmful substances.
It has also been observed that inadequate albumin production in hepatic diseases leads to improper drug protein binding. In these cases, the patients' dosages of the medications theophylline and diazepam are changed

4.Environmental Aspects:

Drugs' lipid solubility, protein binding, and association constant with their receptor are all impacted by pH. The quantity of available binding sites can be impacted by endogenous ligand excess (or deficit, for that matter) through pure competition, as in the case of hyperbilirubinemia. Protein binding is clearly influenced by protein level since it affects the total number of binding sites that are available. It's important to discuss temperature, but let's be honest: in most situations, the pertinent temperature will be either 1-2 degrees above or below normal body temperature. [23,24]

•Different case studies explaining these factors;

• Warfarin in Senior Citizens:
o Due to hypoalbuminemia, a 35% increase in the free fraction was noted, requiring dose reductions to avoid bleeding complications.
• Phenytoin in Infants:
o There was a 50% increase in free drug levels, which led to a smaller therapeutic window and more difficulties controlling seizures.
• Ceftriaxone in Patients with Renal Impairment:
o Higher free drug levels due to decreased protein binding increased both the risk of nephrotoxicity and efficacy.
• Ibuprofen in Expectant Mothers:
o the unbound drug fraction increased by 20% due to decreased albumin levels, which may have an impact on pregnancy dosage modifications. [29,30,31,32,33,34,35,36,37]

Impact on pharmacokinetics:

The drug that is attached to proteins is not broken down or removed. The drug is not pharmacologically active because it is pharmacokinetically and pharmacodynamically inert. [25] The part of the drug that isn't bound is broken down in the liver and other tissues. If a drug is less bound, it can move through cell membranes more easily. The unbound fraction has pharmacologic effects. [26] The unbound fraction can be removed or broken down by the body.
The process of protein binding reaching equilibrium is quick and can be undone. It is also thought of as an absorption process because it follows the law of mass of action. A simple example of a 1:1 protein-drug complex is the interaction between a protein and a drug molecule, which can be written as: [27]
$P+D \rightarrow PD$
P represents the unbound protein's concentration in terms of free
binding locations. D is the unbound drug's concentration. PD is a protein-drug complex's concentration.

When the law of mass action is applied, the expression becomes:

K=[PD]/[P][D]

The association constant is denoted by K.

[PD]=K[P][D] …. (1)

If [Pt] represents the total protein concentration, then

[Pt]=[P]+[PD]…. (2)

The sum of unbound protein and protein is the total protein concentration.

existing within the complex.

Or,

[P]=[Pt]-[PD]….. (3)

After changing the value of [P] in equation (1),

[PD]=K[D]([Pt]-[PD])

[PD]=K[D][Pt]-K[D][PD]

[PD]+K[D][PD]=K[D][Pt]

[PD](1+K[D]) =K[D][Pt]

[PD]/[Pt] =K[D]/1+K[D]

where the average number of drug molecules is represented by [PD]/[Pt].

bound per protein mole [Pt].

Using r to replace [PD]/[Pt],

r=K[D]/1+K[D]

If n independent binding sites exist, then:

r=n{K[D]/1+k[d]}

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

r = nK[D]–rK[D]

r=[D] (nK–rK)

r/[D]=nK-rK … (5)

We call this a Scatchard plot.

For data analysis, equation (5) is not utilized if the experimental

system, both the type and quantity of protein are unknown. Next, a

A different formula is applied.

[Db]/[D]= -K[Db]+nK[Pt]

Where Db

provides the bound drug's concentration. The proportion of

Plotted against [Db] is [Db]/[D]. K is derived from the slope and

The intercept is used to calculate nK[Pt].

• Value of distribution:

In this case, the volume of distribution of a drug is mostly based on how much of it is bound to plasma proteins instead of being bound to tissue. Ibuprofen is one of the examples above. It has a volume of distribution of only 0.1L/kg because it binds strongly to plasma albumin, which keeps it in the circulating volume like albumin usually does. Amiodarone is also highly protein-bound, but it prefers to spread throughout the tissues, with only a small amount of amiodarone bound to plasma proteins. [23,24]

• Clearance:

Albumin binding can lower drug clearance by making the molecules bigger and the drug's free fraction smaller. As the unbound fraction goes down, CL goes down too. A drug with a low CL usually stays in the plasma longer and needs a lower dose to reach a certain plasma concentration. The free fraction of drugs is lowered by albumin binding, which changes the drug's CL and VD. These two values set the mean residence time or terminal half-life. When the drug moves from the vascular space to the tissue or is removed from the body, a new balance will be reached to keep the free drug concentration stable, which will lead to a steady release of the drug. Macromolecules that are bigger than 20 kDa or 10–100 nm in diameter are moved from interstitial fluid into the first lymphatic vessels. Most medications and molecules, on the other hand, are taken out of tissues and administration sites by draining blood capillaries. The lymphatic system moves albumin from the interstitial fluid back into the blood. We anticipate that albumin will travel with the drug's bound fraction as it moves from tissues back into the bloodstream via the lymphatic system. Future studies should test this theory. Also, binding to

albumin to make the molecule bigger may make it harder for the kidneys to clear it out, since the kidneys usually only filter out molecules that are smaller than 60 kDa.

• Half-life:

Let's use diazepam as an example. Drugs that mostly bind to proteins, like diazepam, tend to have longer half-lives because they bind strongly to albumin. The drug's bound part works like a reservoir, slowly releasing the free drug into the bloodstream. This could lead to a longer-lasting therapeutic effect and fewer doses needed. The long half-life of diazepam is mostly due to how slowly it leaves protein binding sites. [28]

•Impact On Therapeutic Efficacy:

The amount of pharmacologically active drug that is available in its free form is directly affected by the amount of protein that binds to it. This also affects how well the drug works. The free drug fraction eventually has therapeutic effects after it interacts with target receptors and passes through biological membranes. So, changes in how proteins bind to the drug could have a big effect on how well it works. [28]

•Decreased Amount of Protein Bound to Enhancing Effects, Example:

When people have lower levels of protein (for instance, low albumin), the amount of P.B. will decrease. Therefore, the amount of drug remaining in the free form will increase. An example would be someone who has some form of liver disease or malnourished from drug use and their albumin levels will be lower. Consequently, the concentration of free drug for protein bound medications such as phenytoin will be higher due to either of these two states. This increase may increase the potential for the therapeutic effect of the drug but also increase the risk of toxicity and/or side effects. Risks that have been increased as a result are likely due, in part, to the amount that is free. For some drugs, even a slight increase in free concentration may have a very significant impact on the efficacy and/or safety of the drug.

• [TDM] Therapeutic Drug Monitoring:

TDM is performed routinely by health care professionals for the measurement of both total and unbound (free) drug concentrations from drugs with protein binding as an important component in maintaining their efficacy. TDM ensures the unbound fraction of the drug stays within the defined therapeutic range, which is especially important for those patients whose protein levels vary significantly and/or take numerous medications that may interfere with their protein binding. TDM is also particularly important for drugs with a narrow therapeutic index since very small changes in unbound drug concentration can result in adverse and/or subtherapeutic effects.[28]

•METHOD FOR DETERMINING PLASMA PROTEIN BINDING:

Various methodologies are utilized to determine the binding affinity of proteins to pharmaceuticals. These methods can check how well a drug dissolves when protein is present and when it is not. They are also used to see how protein changes the drug molecule in both water and organic solvents.

•Two different methods are used to study how drugs bind to proteins.

1. The process of separation.
2. A method that doesn't separate things.

•Separative approach:

This method separates the free ligand from the bound species and uses it right away to find out how much of the drug is bound or unbound.

Different techniques are used in separative methods to find out how drugs and proteins bind to each other. [38]

Methods	Principle
Equilibrium Dialysis	Semipermeable membrane allows free drug diffusion until equilibrium
Ultrafiltration	Pressure/centrifugation forces free drug through membrane
Ultracentrifugation	Separation by molecular weight using high centrifugal force
PAMPA (Parallel Artificial Membrane Assay)	Artificial lipid membrane separates free drug. Separation. via chromatographic interaction Capillary Electrophoresis
Liquid Chromatography	Separation via chromatographic interaction Capillary Electrophoresis.
Capillary Electrophoresis	Electrophoresis Separation based on size & charge in electric field

Table no.1 reference -[38,43,44,45,46,47,48]

•Non-Separative Approach-

This collection of methods for figuring out protein-drug binding depends on finding a charge in a physicochemical characteristic of either the protein or the ligand as a result of the binding. There are two various methods employed by this group. [38]

Method	Principle
Spectroscopic Techniques (e.g., Fluorescence spectroscopy)	Detects changes (forty-three chemical–physical properties) in (fluorescence/absorbance); due to binding of drug
Calorimetric Techniques (ITC, DSC)	Measurement of the heat change associated with the drug-protein interaction.
Subtypes: -	
ITC (Isothermal Titration Calorimetry)	Measures heat released/absorbed during binding at constant temperature.
. DSC (Differential Scanning Calorimetry)	Measures change in protein stability with temperature

Table no.2 reference - [38,50,51,52,53,54]

•Plasma Protein Binding's Clinical Importance:

The binding of drug molecules to protein molecules in order to create a drug-protein complex is an important step in determining how the drug is metabolized or eliminated. In addition,

drug-protein complexes produce non-metabolic (or inactive) pharmacokinetic and inert pharmacodynamic properties. Drugs are typically targeted to certain tissues or sites of action, with each target tissue or site having a greater affinity for the drug than for other tissues/sites. For this reason, the binding of the drug to protein will increase the duration of the drug's effect. The size and weight of the drug-protein complex restrict the ability of the complex to pass through cellular membranes, prolonging the length of time that the drug will remain in circulation. [38] The protein-drug binding process is typically reversible, and involves non-covalent (or weak) chemical bonds (such as hydrogen, ionic, and hydrophobic), and very rarely, covalent bonds. Covalent bond formation will create a permanent (or irreversible) bond, and therefore, it may be possible to anticipate possible negative outcomes, such as organ or tissue toxicity, teratogenesis, or carcinogenesis. [39]

1. Absorption: -

The concentration of free drug in circulation decreases because the drug is being absorbed and bound to plasma proteins. Most traditional dosage forms are said to follow first-order kinetics, and an increase in protein binding can affect how the drug is absorbed (e.g., change in the rate of absorption). [40]

2. Distribution: -

By serving as a buffer, protein binding of plasma drugs ensures equitable distribution within the body. In some instances, drug-bound medications are not physiologically able to traverse the glomerulus (where urine is produced), the placental barrier, or the blood-brain barrier; Therefore, protein binding decreases drug distribution. [40]

3. Elimination: -

Removal (the only drugs that can be removed are those that are unbound). Protein binding prevents drugs from entering the metabolizing organ or passing through the glomerulus. Primary mode for removing tetracycline is glomerular filtration. [40]

Distribution of drugs within the tissue of the body

• Quantity of free drug that can access the cell's action site is limited [41]

• The drug loses its ability to produce a pharmacological effect because a protein-bound drug cannot bond to its receptor • There is pharmacological activity from the protein-drug complex [39]

• Co-administration of a special type of drug that also binds to plasma proteins causes the bound drug to be "displaced" and can lead to severe toxicity because the recipient drug is unable to bond with the receptor to elicit the desired pharmacological effect [42]

• Therefore, the effect of the drug can extend its duration of action [43]

• The accumulation of the drug is increased, and the excretion of the drug is delayed.[40]

•Drug-Drug Interaction in Plasma Protein Binding:

If More Than One Drug have the Same Affinity to Bind to the Same Site. If one or more of those drugs has the same affinity for a site, then they will compete for that binding. If a second drug (Drug B) is administered when Drug A had been previously bound, then the higher affinity drug will displace Drug A from that particular site of action. This is called Displacement Reaction.

For Example: - When Phenylbutazone displaces Warfarin from their binding sites on HSA [55,56]. Narrow Therapeutic Index (NTI) Drugs: Drugs such as Warfarin, Phenytoin and Digoxin are all NTI medications; thus, their effects can be easily

altered by small changes in free concentrations and have large effects in toxicity or therapeutic failure.

For example: -If a patient is taking Warfarin, it is a highly bound drug to albumin, and when that patient takes an aspirin-like drug (i.e. Ibuprofen) then there is a risk for Warfarin to be displaced with the possible side effect being Severe Bleeding and High amounts of Free Warfarin [57]

CONCLUSION

Plasma protein binding (PPB) is one of the vital determinants of the pharmacokinetics and pharmacodynamics of a medication, controlling the distribution of active and inactive drug fractions. The free drug form penetrates cellular membranes and exerts pharmacological effects, whereas the bound drug fraction functions as a reservoir, increasing its duration. Drug-protein interactions are reversible in nature and occur due to weak intermolecular forces such as hydrophobic, hydrogen, ionic, and Van der Waals forces. Numerous factors including those related to drugs, proteins, physiological, or pathological states influence PPB extensively directly influences pharmacokinetics of medications and, therefore, their safety and efficacy. Changes in protein binding capacity, induced by disease conditions and drug interactions, may result in a substantial increase in the free drug fraction and toxicity or lack of medication effectiveness. Modern techniques facilitate the determination of plasma protein binding, providing an opportunity for therapeutic drug monitoring and adjustment of dosage regimens for narrow therapeutic window medications. In general, a comprehensive knowledge of plasma protein binding mechanisms is necessary for achieving positive therapeutic results and avoiding negative outcomes.

REFERENCES

1. Koch-Weser J, Sellers EM. Binding of drugs to serum albumin (two parts). NEnglJ_7Med
2. J. E. Riviere, "Absorption, distribution, metabolism, and elimination," Veterinary Pharmacology and Therapeutics, 9th ed., 2009.
3. K. Tsaioun, B. J. Blaauboer, and T. Hartung, "Evidence-based absorption, distribution, metabolism, excretion (ADME) and its interplay with alternative toxicity methods," ALTEX-Alternatives to Animal Experimentation, vol. 33, no. 4, pp. 343-358, Nov. 201
4. https://doi.org/10.1016/B978-1-4160-4252-5.50228-8
5. https://www.sciencedirect.com/
6. Sudlow, G. D. J. B., D. J. Birkett, and D. N. Wade. "The characterization of two specific drug binding sites on human serum albumin." Molecular pharmacology 11.6 (1975): 824-832.
7. https://anaesthetics.ukzn.ac.za/wp-content/uploads/2024/03/Bonding-binding-and-isomerism-AICM-2011.pdf
8. https://www.iptsalipur.org/wp-content/uploads/2020/08/BP302T_PYP_UNIT_IV.pdf
9. https://pubmed.ncbi.nlm.nih.gov/12476936/
10. Plasma protein binding - Wikipedia https://share.google/TrURLUD1aGVXsKiWA
11. https://pubmed.ncbi.nlm.nih.gov/11811421/#:~:text=Traditionally%2C%20equilibrium%20values%20(e.g.%2C,the%20rationalization%20of%20drug%20pharmacokinetics.
12. https://www.wisdomlib.org/concept/free-fraction#:~:text=Free%20fraction%20describ

es%20the%20unbound,From:%20South%20African%20Family%20Practice
13. https://pmc.ncbi.nlm.nih.gov/articles/PMC5698572/#:~:text=As%20mentioned%20in%20the%20opening,)%2C%20equation%20(1).
14. https://en.wikipedia.org/wiki/Plasma_protein_binding#:~:text=A%20drug%20in%20blood%20exists,and%20unbound%20states%2C%20such%20that:
15. https://www.sciencedirect.com/topics/medicine-and-dentistry/drug-protein-binding#:~:text=Drug%20protein%20binding%20refers%20to,of%20highly%20protein%2Dbound%20drugs.
16. H. Wen, H. Jung, and X. Li, "Drug delivery approaches in addressing clinical pharmacology-related issues: opportunities and challenges," The AAPS Journal, vol. 17, pp. 1327-1340, Nov. 2015.
17. Bohnert T, Liang-Shang G. Plasma protein binding: From discovery to development. J Pharm Sci 2013; 102:2953-94.
18. Masaki O. Study on binding of drug to serum protein.Yakugaku Zasshi 2009;129:413-25
19. Christine T, Anastasia A. The role of protein binding on the mode of drug action as well the interactions withother drugs. Eur J Drug Pharmacokinet 2008; 33:225-30.
20. Caterina P, Di Paolo A, Luca G. Pharmacokinetics drug-drug interaction and their implication inclinical management. J Res Med Sci 2013; 18:601-10.
21. David RT. Drug excretion. In: Pharmacology. ScienceDirect; 2009.
22. Wright JD, Boudinot FD, Ujhelyi MR. Measurement and analysis of unbound drug concentrations. Clin Pharmacokinet 1996; 30:445-62
23. Ehrnebo, M. "Drug binding to blood cells." Drug-protein binding, Praeger Pub., New York (1986): 128-137.
24. Olson, Richard E., and David D. Christ. "Plasma protein binding of drugs." Annual reports in medicinal chemistry 31 (1996): 327-336.
25. Schefie RT. Protein binding: What does it mean? DICP 1989; 23:27-31.
26. Karine V, Julie S, Jean-Luc V, Pierre-Alain C, Sophie M. Drug-protein Binding: A Critical Review of Analytical Tolls. Berlin, Germany: Springer-Verlag; 2010.
27. Agarwal SP, Rajesh K. Physical pharmacy. In: Complexation and Protein Binding. Ch. 7. New Delhi: CBS Publishers and Distributors; 2006. p. 208-14.
28. K. Pathak and S. Raghuvanshi, "Oral bioavailability: issues and solutions via nanoformulations," Clinical Pharmacokinetics, vol. 54, pp. 325-357, Apr. 2015.
29. Bertucci, C., & Domenici, E. (2002). Reversible and covalent binding of drugs to human serum albumin: methodological approaches and physiological relevance. Current Medicinal Chemistry, 9(14), 1463–1481. https://doi.org/10.2174/0929867023369467
30. Peters, T. Jr. (1996). All About Albumin: Biochemistry, Genetics, and Medical Applications. Academic Press.
31. Park, K. (1996). Protein binding and its role in drug disposition and action. Clinical Pharmacokinetics, 30(3), 187–191. https://doi.org/10.2165/00003088-199630030-00002
32. Benet, L. Z., & Hoener, B. A. (2002). Changes in plasma protein binding have little clinical relevance. Clinical Pharmacology & Therapeutics, 71(3), 115–121. https://doi.org/10.1067/mcp.2002.121829
33. Schmid, K., & Wang, W. (1973). The role of alpha-1-acid glycoprotein in drug binding. Biochimica et Biophysica Acta (BBA) -

Protein Structure, 295(1), 165–175. https://doi.org/10.1016/0005-2795(73)90018-7

34. Gandhi, M., Aweeka, F., Greenblatt, R. M., & Blaschke, T. F. (2004). Sex differences in pharmacokinetics and pharmacodynamics. Annual Review of Pharmacology and Toxicology, 44, 499–523.https://doi.org/10.1146/annurev.pharmtox.44.101802.121453 7. Anderson, G. D. (2005). Pharmacokinetics and pharmacodynamics of anti-epileptic drugs in special populations. Acta Neurologica Scandinavica, 112(s181), 23–32. https://doi.org10.1111j.1600-0404.2005.00520.x
35. Dickinson, P. A., et al. (2007). Clinical relevance of physiological changes in the gastrointestinal tract in disease and special populations. Drug Metabolism and Disposition, 35(4), 501–511.https://doi.org/10.1124/dmd.106.012617
36. Andy, Adinarayana. "The Role of Protein Binding in Drug Pharmacokinetics Implications for Drug Efficacy and Safety."
37. Andy, A. & Weatherwax Family Pharmacies Inc. (2019b). The Role of Protein Binding in Drug Pharmacokinetics Implications for Drug Efficacy and Safety. In IJIRMPS (Vols. 7–7, Issue 5, pp. 1–2) [Journal-article].
38. Agarwal SP, Rajesh K. Physical pharmacy. In: Complexation and Protein Binding. Ch. 7. New Delhi: CBS Publishers and Distributors; 2006. p. 208-14
39. Karine V, Julie S, Jean-Luc V, Pierre-Alain C, Sophie M. Drug-protein Binding: A Critical Review of Analytical Tolls. Berlin, Germany: Springer-Verlag; 2010.
40. Karolina W. Biological Barriers, and the Influence of Protein Binding on the Passage of Drug Across them. Molecular Biology Reports; 2020.
41. Le J. Drug Distribution to Tissues. MSD Manual; 2020.
42. Lakshmana SP, Suriyaprakash TN, Ruckmani K, Thirumurugan R. Biopharmaceutics and pharmacokinetics. In: Ahmed TA, editor. Basic Pharmacokinetics Concepts and Some Clinical Applications. Vol. 10. London: IntechOpen; 2015. p. 5772-61160.
43. Hoffman A, Danenberg HD, Katzhendler I, Shuval R, Gilhar D, Friedman M. Pharmacodynamic and pharmacokinetics rationales for the development of an oral controlled-release amoxicillin dosage form. J Control Release 1998; 54:29-37.
44. Stempel E. Prolonged drug action. J Am Pharm Assoc 1959; 20:0095-9561.
45. Lazaro E, Lowe PJ, Briand X, Faller B. New approach to measure protein binding based on a parallel artificial membrane assay and human serum albumin. J Med Chem 2008; 51:2009-17.
46. Kansy M, Senner F, Gubernator K. Physicochemical high throughput screening: Parallel artificial membrane permeation assay in the description assay in the description of passive absorption processes. J Med Chem 1998; 41:1007-10.
47. Hage DS, Tweed SA. Recent advances in chromatographic and electrophoretic methods for the study of drug-protein intracyion. J Chromatogr B 1997; 699:499-525.
48. Heegaard NH, Nilsson S, Guzman NA. Affinity capillary electrophoresis: Important applications areas and some recent developments. J Chromatogr B 1998; 715:29-54.
49. Gullo VP, Hughes DE. Exploring new approaches for natural product drug discovery in the biotechnology industry. Drug Discover Today Technool 2005; 2:281-6.
50. Bush MH, Kraak JC, Poppe H. Principles and limitations of methods available for the

determination of binding constants with affinity capillary electrophoresis. J Chromatogr A 1997; 777:329-53.
51. Oravcova J, Bohs B, Linder W. Drug-protein binding studies-new trends in analytical and experimental metholodgy. J Chromatogr B 1983; 677:1-28.
52. Liu YC, Yang ZY, Du J, Yao XJ, Lei RX, Zheng XD, et al. Study on the interactions of kaempferol and quercetin with intravenous immunoglobulin by fluorescence quenching. Fourier transformation infrared spectroscopy and circular dichrosim spectroscopy. Chem Pharm Bull 2008; 56:443-51.
53. Velazquez-Campoy A, Ohtaka H, Nezami A, Muzammil S, Velazquez-CampoyA, Leavitt SA, et al. Characterization of protein-protein interactions by isothermal titration calorimetry. In: Protein-Protein Interactions. 2004. p. 35-54.
54. Freire E. Isothermal titration calorimetry. Curr Protoc Cell Biology 2004; 17:1-24.
55. Plotnikov V, Rochalski A, Brandts M, Brandts JF, Williston S, Frasca V, et al. An autosampling differential scanning calorimeter instrument for studying molecular interactions. Assay Drug Dev Technol 2002; 1:83-90
56. Caterina P, Di Paolo A, Luca G. Pharmacokinetics drug-drug interaction and their implication inclinical management. J Res Med Sci 2013; 18:601-10.
57. Laznicek M, Laznicokva A. The effect of lipophilicity on the protein binding and blood cell uptake of some acidic drugs. J Pharm Biomed Anal 1995; 13:823-8.

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