

PROTEIN BINDING

Dr. Muhammad Usman Minhas



**PROTEIN BINDING**

**Definition:**

 Many drugs interact with plasma or tissue proteins or with other macromolecules to form a drug-macromolecule complex. This process is known as protein binding or more specifically drug protein binding.

 When drugs interact with plasma and proteins or with other macromolecules, such as melanin and DNA, to form a drug macromolecule complex. This formation of drug protein complex is often named as drug protein binding.

 Or

The protein binding process is defined as a phenomenon of complex formation following the interaction of drug moiety and the protein molecule.

**Types of Drug Protein Binding**:

There are two types of drug protein binding

**Reversible protein binding**

Drug binds the protein with weaker chemical bonds such as:

>Hydrogen bonds

>Vander Vaals forces

Amino acid complex composed of protein chains have hydroxyl, carboxyl or other sites available for drug interaction.

**Irreversible protein binding**

It is usually result of chemical activation of drug which then attaches strongly to protein or macromolecule by covalent or chemical bonding. It accounts for certain types of drug toxicity that may occur for over a prolong period of time. For example hepatotoxicity of high dose of acetaminophen is due to formation of reaction metabolite intermediate N-acetylbenzoiminoquinone, which interact with liver proteins.

Protein bound drug is a large complex that cannot easily cross cell membrane. Therefore, has

>Restricted distribution

>Pharmacologically inactive.

Free and bound drug crosses cell membrane and is therapeutically active.

**Fate of Bound/Unbound Drug:**

The bound drug is kept in the blood stream while unbound component may be metabolized or excreted making it the active part. So, if a drug is 95% bound and 5% is free it means that 5% is active in the system and causing pharmacological effects.

A bound drug is pharmacodynamically inert. Binding increases half-life of drugs.

**Types of Protein Binding According to Site of Binding:**

1. **To blood components:**
* **Plasma proteins**:

|  |  |  |
| --- | --- | --- |
| **Protein** | **Molecular Weight (Da)** | **Normal Range of concentrations** |
| **(g/L )** | **(mol/L)** |
| Albumin | 65000 | 35-50 | 5-7.5×104 |
| α 1 Acid glycoprotein | 44000 | 0.4-1.0 | 0.9-2.2 ×105 |
| Lipoprotein | 200000-3400000 | variable |  |

* **Erythrocytes:**

RBCs specifically consist of 3 major constituents that eventually may get bound to drug substances namely:

1-Haemoglobin

2- Carbonic Anhydrase

3- Cell Membrane

1. **Hemoglobin:**

It is referred to as the O2 carrying pigment of RBCs. It has 4 identified variants, namely

-HbA1

-HbA1e

-HbA2

-HbF

Example of drugs that bind to RBCs: Phenytoin, pentobarbital and amobarbital

1. **Carbonic anhydrase:**

 It designates the specific metabolic enzymes that predominantly catalyzes the combining of CO2 and H2O to give rise to the formation of carbonic acid in various body processes.

Example of drugs that binds to carbonic anhydrase: Acetazolamide, methazolamide, ethoxzolamide, disulphamide etc.

1. **Cell Membrane:**

It refers to membrane pertaining to any of the protoplasmic masses making up organized tissues, consisting of a nucleus surrounded by cytoplasm enclosed in a cell or plasma membrane.

Example of drugs that binds to RBC membrane: imipramine, chlorpromazine etc.

1. **To extravascular tissues:**

Studies have revealed that a large proportion of drug no matter acidic, basic or neutral undergo reversible binding to tissues as well.

The order of binding with extravascular tissues is in following order:

Liver > Kidney > Lungs > Skin > Eyes > Bones

|  |  |
| --- | --- |
| **TISSUES** | **EFFECTS** |
| Liver | Irreversible binding of drugs like paracetamol& their epoxide metabolites to liver tissues result in hepatotoxicity |
| Kidney | The protein called as metallothion binds with heavy metals such as lead, mercury and cadmium resulting in major renal failures or renal toxicity. |
| Lungs | Drugs like imipramine, desipramine or other drugs in lungs eventually leads to congestion in heart or may even produce severe lung cancer |
| Skin  | Many drugs accumulate in skin with reaction with melanin. E.g. chloroquine |
| eyes | Retinal pigment of eye contain melanin. Drugs like chloroquine are responsible for retinopathy |
| bones | Drugs like tetracyclines binds with calcium in bones and results in irragularities in bone growth in children. |

**Effects of Protein Binding:**

1. **Effect of protein binding on pharmacokinetics of drug:**
2. **On volume of distribution**: Extent of drug protein binding in the plasma/tissue effects Vd. Drugs that are highly bound to plasma protein have low fraction of free drug.

**Vd= plasma protein – bound drug**

Plasma protein bound drugs does not diffuse easily and is therefore less extensively distributed to tissues. Drugs with low plasma protein binding have larger unbound/free drug fraction (fu), generally diffuse more easily in tissues and have a greater volume of distribution. Since apparent Vd is influenced by

-lipid solubility

-protein binding

 **Plasma protein binding α 1/ apparent Vd**

**Example**: fu α Vd

Vdof 4 cephalosporin antibiotics in humans & mice demonstrates that the difference in Vd of Cefazolin, Cefotetan, Moxalactam&Cefoperazine are due mostly to differences in the degree of protein binding like:

* fu&Vd in plasma is the highest for Cefoperazine while Cefazolin has lowest fu&Vd in plasma.
* Drugs such as Furosemide, Sulfisoxazole, Tolbutamide& Warfarin are bound greater than 90% to plasma proteins & had a Vd value ranging from 7.7-11.2L/70kg of body weight.
* Basic drugs such as Imipramine, nortryptylene&Propanolol are extensively bound to both tissues & plasma protein and have a larger Vd values.

Displacement of drug from plasma protein can affect the pharmacokinetic of a drug in several ways:

* Directly increase the free drug concentration & as a result reduce binding in blood.
* Increase the fu that reaches the receptor sites directly causing the toxic response.
* Increase fu causing transient increase in Vd& decrease plasma concentration.
* Increase furesulting more diffusion into tissues of eliminating organs (liver & kidney).
1. **On drug distribution**: The drug bound to plasma protein is not available for distribution, hepatic metabolism, renal elimination & pharmacological action. The high molecular weight of drug-protein complex restricts the passage across the blood capillaries and its low lipid-solubility prevents the passage across cell membrane. Only free drug circulating in blood can cross capillaries and cell membranes and hence, is available for distribution, glomerular filtration & hepatic metabolism.

Drug-protein binding interaction is a reversible process. As free drug concentration in blood decreases, the drug-protein complex dissociates to liberate the free drug and maintain equilibrium. Therefore, a drug bound to protein is considered to be in temporary storage. Because of reversible binding of a drug to the proteins, free drug levels of a drug are maintained for a longer time in the blood. Accordingly, the biological half-life of highly protein bound drug is longer than that of a drug having a negligible or no protein binding.

1. **On drug elimination:** The driving force for drug excretion in urine is free drug concentration in plasma. The glomerular capillaries permit the passage of most of drug molecules but restrict the passage of plasma proteins & the drug-protein complex. Therefore, only free drug is filtered. The elimination half-lives of drugs which are excreted mainly by glomerular filtration, are generally increased when the % of a drug bound to the plasma proteins is increased. If a drug is neither secreted nor reabsorbed by the tubules and is not protein bound, its renal clearance is a measure of the glomerular filtration rate (GFR). If a drug is protein bound, the renal clearance of the total drug in the plasma is less than GFR but the renal clearance of a free drug in the plasma is equal to GFR.

 A protein bound drug is unable to enter the hepatocytes resulting in a reduced drug metabolism by the liver. In addition, the bound drug is not available as a substrate for liver enzymes or other enzymes thereby further reducing the rate of metabolism. In general drugs that are highly bound to plasma protein have reduced overall drug clearance.



1. **Effect of protein binding on pharmacodynamics of drug:**

The pharmacodynamics response is influenced by both the distribution of drug and the concentration of free drug fraction. The drug dose & dosage form must be chosen to provide sufficiently high free drug concentration so that an adequate amount of drug reaches site of drug action (receptor). The onset of drug action depends on the rate of free drug that reaches the receptor & produce a minimum effective concentration (MEC) to produce pharmacodynamics response. The intensity of drug action depends on total drug concentration of receptor site & total number of receptors occupied by drug. To achieve a pharmacodynamics response with initial dose, the amount (mass) of drug when dissolved in the volume of distribution must give a drug concentration ≥ MEC at the receptor site. Subsequent drug doses maintain the pharmacodynamics effect by sustaining the drug concentration at the receptor site.

**Determinants of Protein Binding/ Factors Affecting Protein Binding:**

Drug protein binding is influenced by following factors:

1. **Drug:**
2. **Physicochemical properties of drug:**
* Phenobarbital (weakly acidic drug) having albumin protein binding 20%-70%
* Valproic acid, aspirin (acidic drug) which is mostly ionized with moderate lipophilicity having albumin protein binding of 80%-95%
* Lidocaine, methadone (basic drugs) which is mostly ionized (pKa>7) with moderate lipophilicity having α-acid glycoprotein protein binding of 50%-95%
1. **Total concentration of drug in the body**: Plasma drug concentration is generally reported as:

 Plasma Drug (total) = Protein bound+ Free drug

Increase the concentration of drug in the body, greater will be the free drug in the body.

1. **The proteins:**
2. **Quantity protein available for drug protein binding**
3. **Quality/physiochemical nature of protein synthesized**

With a constant concentration of proteins only a certain number of binding sites are available for drug. At low drug concentration, most of the drug may be bound to the protein. At high drug concentration, the protein binding sites may become saturated, with the consequent rapid increase in the free concentration.

1. **Affinity between drug and protein**: As plasma proteins have different binding sites but drugs have specific affinity for specific binding site. More than 2 drugs may have affinity for same binding sites. So, in such cases drug having greater affinity may occupy the receptor site & toxicity occur due to displaced drug. In reality, drug-protein binding sometimes exhibit the phenomenon of co-operativity (binding of 1st drug at one site on protein influence the successive binding of other drug molecule)
2. **Drug interaction:**
3. **Competition for the drug by the other substances at a protein binding site:**It depends on the affinity of drug to the plasma proteins. The most likely bound drug is capable of displacing others. Free drug increase due to displacement by other drug with high affinity. For e.g. Phenytoin is highly bound to plasma proteins (90%), Tolbutamide (96%) and Warfarin (99%). Drugs that displace these drugs are Aspirin, Sulphonamides&Phenylbutazone.
4. **Alteration of a protein by a substance that modifies the affinity of drug for protein:** Fore.g. Aspirin acetylates lysine residues of albumin.
5. **Pathophysiological condition of patients**:
* Drug-protein binding may reduce in uremic patients, hepatic patients, burns & pregnancy for albumins.
* Drug-protein binding may increase in myocardial infarcts, surgery, trauma & rheumatoid arthritis for α-acid glycoprotein.

**Kinetics of Protein Binding:**

The kinetics of reversible drug-protein binding for a protein with 1 simple binding site can be described by the Law of Mass Action as follows:

**Protein + Drug ↔Protein-drug complex**

 **[P] + [D] ↔ [PD]**

At a dynamic equilibrium, rates of forward reaction & backward reaction are equal. If number of binding sites on the protein is 1, then the molar concentrations of bound drugs are equal i.e.

**[PD] = [PB] [DB]**

K1&k2 are the rate constants for association & dissociation respectively. According to law of mass action, equilibrium association constant (kas)can be expressed as the ratio of molar concentration of the products and molar concentration of the reactants.

**Kas = k1 / k2 = [PD] / [PF] [DF] = [DB] / [PF] [DF]**

Extent of drug-protein complex formed is dependent on the association binding constant (ka). The magnitude of kas yields information on the degree of drug-protein binding. Very large kas indicates that the drug binds strongly to the protein & hence free drug concentration of such drug is low. Therefore, a very large dose of drug is required to achieve a reasonable therapeutic concentration of free drug. Total drug concentration in plasma Dtis the sum of both the bound & unbound drug concentrations, so

**Dt= DF + DB**

**Pt= PF + PB**

 **PF = Pt – PB**

Then**, kas = [DB] / [DF] ([Pt] – [PB])**because, [PB] =[DB]

 **= [DB] / [DF] ([Pt] – [DB])**

However, if protein contains “n” number of binding sites, then,

**Kas= [DB] / [DF] ([n [Pt] – [DB])**

Degree of binding is frequently expressed as the bound-to-total concentration ratio. This ratio has limiting values of 0 & 1.0. Drugs with values greater than 0.9 are said to be highly bound. Fraction of the drug in the plasma unbound (fu) is of greater utility than the fraction of the bound**. Fu = [Du] / [Dt] = [DF] / [DF] + [DB]**

**Determination of Binding Sites and Binding Constants:**

Most kinetic studies invitro use purified albumin as a standard protein source because this protein is responsible for the major portion of plasma drug-protein binding. Both the free drug [D] and the protein bound [PD] as well as total protein concentration ([P] + [PD]) may be determined. Binding behavior of drug may be determined by determinable ratio,

**r = moles of drug bound / total moles of protein**

 **= [PD] / [PD] + [P] (As: [PD] = Ka [P] [D])**

 **= ka[P] [D] / ka [P] [D] + [P]**

 **= ~~[P]~~ (ka [D]) / ~~[P]~~ (1 + ka[D])**

 **= ka [D] / 1 + ka [D]**

This shows only 1 binding site on the protein. Drug bind to protein in 1:1 complex. If there are “n” number binding sites are available per mole of protein, then:

**r = n ka [D]/ 1 + ka [D]**

Protein molecule may contain more than 1 type of binding sites on which the drug molecule can bind with different association constants. For e.g. 4 different sites on human albumin has been identified for drug binding namely, warfarin binding site (site 1), diazepam binding site (site 2), digitoxin binding site (site 3) &tamoxifen binding site (site 4). Then,

**r = n1 k1 [P] / 1 + k1 [D] + n2 k2 [P] / 1+ k2 [D]**

Assumptions made in developing these equations are:

* Drug molecules bind to protein at independent binding sites.
* Affinity of drug for 1 binding site does not influence binding to other sites.
* Number & association constants are different for different types of binding sites.

**Methods for Studying Drug-Protein Binding:**

* Equilibrium dialysis
* Dynamic dialysis
* Diafiltration
* Ultrafiltration
* Gel chromatography
* Spectrophotometry
* Electrophoresis
* Optical rotatory dispersion and circulatory dichroism

**Considerations in the Study of Drug-Protein Binding:**

* Equilibrium between bound & free drug must be maintained
* The method must be valid over a wide range of drug & protein concentration
* Extraneous drug binding or drug adsorption onto the apparatus walls, membranes or other components must be avoided or considered in the method
* Denaturation of protein or contamination of protein must be prevented
* The method must consider pH & ionic concentration of the media
* The method should be capable of detecting both reversible & irreversible drug binding including fast & slow phase association & dissociation of proteins.
* The method should not introduce interfering substances such as organic solvents

**Clinical Significance of Protein Binding:**

Most drugs bind reversibly to plasma proteins to some extent. When clinical significance of fraction of drug bound is considered, pharmacologic or therapeutic plasma drug concentration must be considered. Fraction of drug bound can change with:

* Plasma drug concentration
* Dose of drug administered
* Patient’s plasma protein concentration

**Condition:** If patient has low plasma protein concentration, then for any given of drug, concentration of free bioactive drug may be higher than anticipated.

**Variables affecting**: Plasma protein concentration is controlled by a number of variables, including

* Protein synthesis
* Protein catabolism
* Distribution of albumin between intravascular & extravascular space
* Excessive elimination of plasma protein, particularly albumin.

**Pathologic conditions altering protein concentration in plasma:**

1. Conditions in which plasma protein concentration decreases:
2. Conditions in which plasma protein concentration increases:

**Examples:**

1. **Liver disease**: liver disease results in plasma albumin concentration due to decrease in protein synthesis
2. **Nephrotic syndrome**: In nephrotic syndrome, an accumulation of waste metabolites such as urea & uric acid, as well as accumulation of drug metabolites may alter the protein binding of drug.
3. **Severe burns**: It may cause an increase in distribution of albumin into the extracellular fluid resulting in smaller plasma albumin concentration.
4. **Genetic disease**: Quality of protein that is synthesized in plasma may be altered due to change in amino acid sequence. Both chronic liver disease & renal disease such as uremia may cause an alteration in the quality of plasma protein synthesized.

**REFERENCES:**

Applied Pharmacokinetics and Biopharmaceutics by Leon Shargel

Essentials of Biopharmaceutics and Pharmacokinetics by AshutoushKar

Biopharmaceutics and Pharmacokinrticsm by V. Venkateswarlu