UNIT

VI

DRUG-PROTEIN BINDING

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INTRODUCTION

When a drug interacts with multiple tissue components (such as blood and extravascular tissues), it might create a complex in the body and if the drug interacts with protein, the phenomenon is known as drug-protein binding.

Mechanisms of Drug-Protein Binding

i. **Reversible Drug Binding:** Usually drug-protein binding is a reversible process, due to presence of weak chemical interactions, such as hydrogen bonds, hydrophobic bonds, ionic bonds, or Van der Waal's forces.

ii. **Irreversible Drug Binding:** This uncommon binding happens as a result of covalent bonding and is frequently a cause of the drug's carcinogenicity or tissue toxicity; for example, hepatotoxicity is caused by covalent binding of chloroform and paracetamol metabolites to the liver.

Classification of Drug-Protein binding

i. **Intracellular binding:** When a drug binds to a cell protein that may or may not be the drug receptor, causes some pharmacological phenomenon. So, primary receptors are those with which a drug interacts to produce a response.

ii. **Extracellular binding:** When a drug binds to an extracellular protein, it usually does not cause any pharmacological response. So, these kinds of receptors are known as secondary or quiet receptors with which no response occurs. Extracellular binding of drugs can be of two types:

   a. Either the drug binds to blood components like plasma protein and blood cells

   b. Or drug binds to extravascular tissue proteins, fats, bones, etc.

Out of these, plasma protein-drug binding is of utmost importance.

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Binding of Drugs to Blood Components

Binding of Drugs to Plasma Protein

The binding of drugs to plasma proteins is a reversible phenomenon. The extent or order of binding of drugs to various plasma proteins is:

Albumin > a1-Acid Glycoprotein > Lipoproteins > Globulins

a) Binding of Drugs to Human Serum Albumin (HSA)

HSA is the most abundant plasma protein (59% of total plasma) with a high drug binding ability. It binds to a wide range of pharmaceutical drugs (from weak acids to neuronal molecules to weak bases) and endogenous substances (such as fatty acids, bilirubin). There are four different sites identified on HSA for drug-binding. They are:

- **Site I (Warfarin binding site):** It binds with large number of drugs, e.g. several NSAIDs, phenytoin, sulphonamides and bilirubin.
- **Site II (Diazepam binding site)**: Drugs which bind to this site include benzodiazepines, medium chain fatty acids, etc.
- **Site III (Digitoxin binding site)**
- **Site IV (Tamoxifen binding site):** Usually, Site I and site II are responsible for binding of most of the drugs.

b) Binding of Drugs to α1-Acid Glycoprotein (α1-AGP)

α1-AGP is also known as orosomucoid is one of the acute-phase protein, that has a plasma concentration range of 0.6-1.2 mg/mL (1-3% of total plasma). This glycoprotein binds to various basic drugs like propranolol, imipramine, amitriptyline, quinidine etc.

c) Binding of Drugs to Lipoproteins

A drug that binds to lipoproteins does so by dissolving (or partitioning) in the lipid core of the protein and thus its capacity to bind depends upon its lipid content. Based on the density of lipoproteins, these are divided into four types:

- Chylomicrons (least dense and largest in size) [99% by weight lipid content]

- Very-low density lipoproteins (VLDL) [91% by weight lipid content]
- Low-density lipoproteins (LDL) (predominant in humans) [80%]
- High-density lipoproteins (HDL) (most dense and smallest in size) [44%]

Binding of drugs to lipoproteins is non-competitive i.e. there are non-specific binding sites and binding is not dependent on the drug concentration. Various types of drugs bind to lipoproteins such as diclofenac (acidic drug), cyclosporin-A (neutral drug) and chlorpromazine (basic drug).

d) Binding of Drugs to Globulins

The following plasma globulins have been identified:

- *a1-globulin:* Also known as transcortin or CBG (corticosteroid binding globulin), which binds to many steroidal drugs, thyroxine and cyanocobalamin.
- *a2-globulin:* Also known as ceruloplasmin, which binds many vitamins like Vit. A, D, E and K and cupric ions.
- *β1-globulin:* Also known as transferrin, which binds to ferrous ions.
- *β2-globulin:* Binds to carotenoids.
- *γ-globulin:* Binds specifically to antigens.

Binding of Drugs to Red Blood Cells

There are three components in RBCs through which a drug can bind. These are:

a. Hemoglobin (Hb)

Drugs like pentobarbital, phenytoin, and phenothiazines bind to hemoglobin.

b. Carbonic Anhydrase

Drugs like carbonic anhydrase inhibitors such as acetazolamide and chlorthalidone can bind with the carbonic anhydrase enzyme.

c. Cell Membrane

Imipramine and chlorpromazine are reported to bind with the RBC membrane.

Binding of Drugs to Tissue Components (Tissue Localization of Drugs)

A drug has the capacity to bind with the several tissue components. Drug binding to tissues is important for the below mentioned viewpoints:

- It causes the increase in the apparent volume of distribution (V_d) of drugs in contrast to plasma protein binding which decreases it.
- Tissue-drug binding results in localization of a drug at a specific site in the body (with a subsequent increase in biological half-life).
- In general, the order of binding of majority of drugs that bind to extravascular tissues is Liver > Kidney > Lung > Muscles. Various examples of the binding of drugs to tissues is given below:
- **Liver:** Epoxides of halogenated hydrocarbons and paracetamol bind irreversibly to liver tissues resulting in hepatotoxicity.
- **Lungs:** Basic drugs like imipramine, chlorpromazine and antihistamines accumulate in lungs.
- **Kidneys:** Metallothionin, a protein present in kidneys, binds to heavy metals such as lead, mercury, and cadmium which results in their renal accumulation and toxicity.
- **Skin:** Chloroquine and phenothiazines accumulate in skin by interacting with melanin.
- **Eyes:** The retinal pigments of the eye also contain melanin. Binding of chloroquine and phenothiazines to it leads to retinopathy.
- **Hairs:** Arsenicals, chloroquine and phenothiazines are reported to deposit in hair shafts.
- **Bones:** Tetracycline is a well-known example of a drug that binds to bones and teeth. Administration of this antibiotic to infants or children during odontogenesis results in permanent brown-yellow discoloration of teeth.

- **Fats:** Lipophilic drugs such as thiopental and the pesticide DDT accumulate in adipose tissues by partitioning into it.

Comparison between plasma protein-drug binding and tissue-drug binding

	Plasma Protein-Drug Binding	Tissue-Drug Binding
1.	Involves weak interactions	Involves strong covalent bonds
2.	Reversible binding	Irreversible binding
3.	Does not cause toxicity	Responsible for tissue toxicity
4.	Drugs that bind to plasma proteins have small apparent volume of distribution	Drugs that bind to extravascular tissues have large apparent volume of distribution
5.	Half-life of plasma protein bound drug is relatively short	Half-life of extravascular tissue bound drug is relatively long
6.	Other drugs can cause the displacement from the binding sites	Displacement by other drugs generally does not occur
7.	Competitive phenomenon	Non-competitive binding

Factors Affecting Protein-Drug Binding

Factors affecting protein-drug binding can be broadly categorized as:

Drug Related Factors

1. **Physicochemical characteristics**

Cloxacillin absorbs slower than ampicillin after an I.M. injection due to its higher lipophilicity and larger (95 percent) protein binding, whilst the latter is less lipophilic and only 20 percent bound with proteins.

2. **Concentration of drug in the body**

The therapeutic concentration of any drug is inadequate to saturate HSA, hence the concentration of pharmaceuticals that bind to it has minimal impact.

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3. **Drug-Protein/Tissue Affinity**

Digoxin has a stronger affinity for cardiac muscle proteins than skeletal muscle proteins or plasma proteins. Lidocaine has a strong affinity for AAG than HSA.

Protein/Tissue Related Factors

1. **Physicochemical properties**

Lipophilic drugs tend to bind to lipoproteins and adipose tissue by dissolving them in their lipid core.

2. **Concentration of Protein/Tissue binding component**

During diseased condition, the concentration of protein and tissue components available for binding fluctuates such as in renal failure, the concentration of albumin drops, which further affects binding of acidic drugs.

3. **Number of binding sites on the protein**

Albumin has significant number of binding sites compared to other proteins, whereas, α-1 acid glycoprotein has poor binding capacity due to its low concentration and small molecular size.

DRUG INTERACTIONS

1. **Drug-drug competition for binding sites (Displacement Interactions)**

If one of the drug (say, drug A) is bound to target site, then another drug (say, drug B) with more affinity for the same site causes drug A to be displaced from its binding site. Thus, displacement interaction refers to a drug-drug interaction that involves a shared binding site. Drug A is referred to as the displaced drug, whereas drug B is referred to as the displacer. Warfarin (displaced drug) and phenylbutazone (displacer) is another example. Displacement interactions can result in an unanticipated increase in the displaced drug's free concentration, which can improve therapeutic response or on the other side can cause toxicity.

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2. Drugs and normal body constituents are in competition

NSAIDs and sulphonamides cause bilirubin to be displaced from the bilirubin-albumin complex, resulting in an increase in the concentration of free bilirubin in the blood. Kernicterus disease is caused by free bilirubin in neonates with insufficient BBB and bilirubin metabolizing capacity.

3. Allosteric modifications in protein molecules

Drug can cause change in the protein structure, hence changing its binding ability. e.g. aspirin acetylates the lysine fraction of albumin, and decreasing its affinity for flufenamic acid.

PATIENT RELATED FACTORS

Age is the most important factor between the patients which affect the protein binding.

- **Neonates (< 28 days):** In newborns, albumin content is low which leads to the increase in the unbound concentration of phenytoin and diazepam.
- **Young infants (28 days to 1 year):** Young infants are administered with high digoxin dose (4-6 times the adult dose on body weight basis) in case of congestive heart failure, because of its high binding capacity and large renal clearance in infants.
- **Elderly:** In old people, the albumin content is lowered, whereas AAG levels got increased which results in increase and decrease in free drug concentration respectively.

Significance of Protein/Tissue Binding of Drugs

- **Absorption-** The binding of pharmaceutical drugs to plasma proteins reduces free drug concentration, which maintains the sink condition, and aids in drug absorption.
- **Drug systemic solubility-** Water-insoluble drugs, neutral endogenous macromolecules like heparin and many hormones, and lipid-soluble vitamins are circulated and transported to tissues through binding to lipoproteins, which act as a carrier for such hydrophobic substances.

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- **Distribution-** Drug entry at affinity sites is limited by plasma protein binding, which limits the accumulation of a large fraction of the drug in these tissues and leads to hazardous effects. As a result of plasma protein-drug binding, drugs are distributed uniformly throughout the body. Protein-bound proteins do not penetrate the blood-brain and blood-placental barriers, protecting the brain and placenta from harmful substances.
- **Tissue binding and apparent volume of distribution-** A drug that is heavily linked to blood components stays in the bloodstream. The dissemination of such a drug is limited. A drug with extravascular tissue binding has a large distribution volume.
- **Elimination-** Only the unbound or free drug may be removed from the body, because the drug-protein complex cannot pass through the metabolizing organ (liver). Also, the large molecular size of complex prevents it from being filtered by the glomerulus.
- **Displacement-Interactions and toxicity-** Displacement interactions are important in those drugs which are more than 95% bound (warfarin- 99% plasma protein binding). In this case, a displacement of just 1% from 99% bound drug results in doubling of the free drug concentration i.e. a 100% rise, which may causes toxicity.
- **Diagnosis-** Since chloroquine tends to interact with the melanin of eyes, thus the chlorine atom of chloroquine is replaced with radiolabeled I-131 which can be used to visualize melanomas of the eye.
- **Therapy and drug targeting-** Tumor cells have greater affinity for LDL (low density lipoprotein) than normal tissues. Thus, binding of a suitable antineoplastic drug to it can be used as a therapeutic tool and targeting of tumor cells.

Kinetics of Drug-Protein Binding

The Protein (P) and drug (D) binding may be an adsorption process obeying the law of mass action, and can be represented as:

$$P + D \rightleftharpoons PD$$

Applying the law of mass action, the expression becomes:

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

$$[PD] = K_a[P][D]$$

where, K_a = association constant

[P] = concentration of unbound protein

[D] = concentration of unbound drug

[PD] = concentration of protein-drug complex.

If the total protein concentration in the body is designated as $[P_t]$, we can write,

$$[P_t] = [P] + [PD]$$

If r is the number of moles of drug bound to total moles of protein, then,

$$r = \frac{[PD]}{[P_t]} = \frac{[PD]}{[P] + [PD]}$$

Substituting the value of [PD] in above equation:

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

Above equation holds when there is only one binding site on the protein and the protein-drug complex is a 1:1 complex. If more than one or N number of binding sites is available per mole of the protein, then:

$$\boldsymbol{r} = \frac{NK_a[D]}{1 + K_a[D]} \qquad (1)$$

The value of **association constant, K_a** and the **number of binding sites N** can be obtained by plotting equation (1) in four different ways as shown below:

1. **Direct Plot** is made by plotting r versus [D]. When all the binding sites are occupied by the drug, then the protein is saturated, and plateau is reached.

At the plateau, r = N; When r = N/2, [D] = $1/K_a$.

2. **Scatchard Plot** is made by transforming equation (1) into a linear form. Thus,

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

$$r + rK_a[D] = NK_a[D]$$

$$r = NK_a[D] - rK_a[D]$$

$$\frac{r}{[D]} = NK_a - rK_a$$

A plot of r/[D] versus r yields a straight line.

Slope of the line = – K_a, y-intercept = NK_a and x-intercept = N.

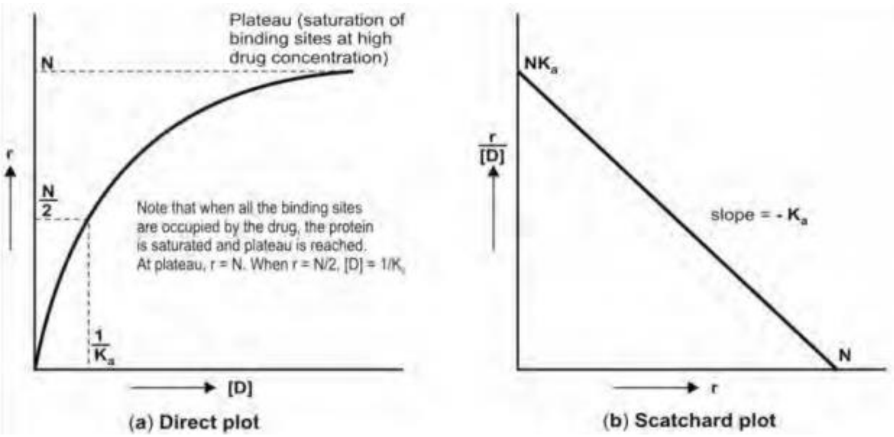

Figure 6.1: (a) Direct plot and (b) Scatchard plot for the determination of association constant, K_a and number of binding sites, N.

3. **Klotz Plot/Lineweaver-Burke Plot (Double Reciprocal Plot)-** The reciprocal of equation (1) yields:

$$\frac{1}{r} = \frac{1}{NK_aD} + \frac{1}{N}$$

A plot of 1/r versus 1/[D] yields a straight line with slope 1/NK_a and y-intercept 1/N.

4. **Hitchcock Plot** is made by reciprocating equation (1) as –

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

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

Multiplying with $NK_a[D]$ $on\ both\ sides\ -$

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

Dividing both sides by NK_a gives –

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

A plot of [D]/r versus [D] yields a straight line with slope 1/N and y-intercept 1/ NK_a

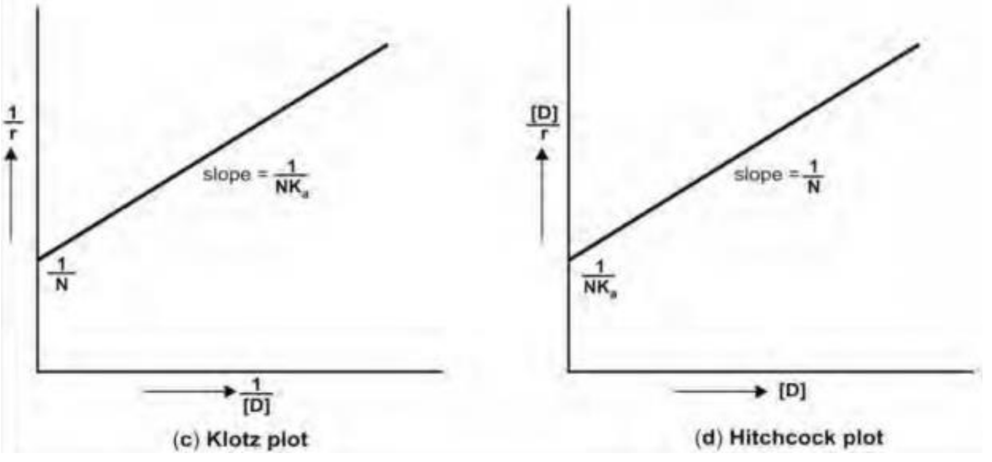

Figure 6.2: (a) Klotz plot and (b) Hitchcock plot for the determination of association constant, K_a and number of binding sites, N.