FACTORS AFFECTING PROTEIN BINDING OF DRUGS.

DRUG RELATED FACTORS

a. Physiochemical characteristics of the drug – Protein binding is directly related to the lopophilicity of drug. An increase in lipophilicity increases the extent of binding. Acidity/ anionic drugs bind to HSA; basic/ cationic drugs to AAG; neutral/ unionized drugs to lipoproteins.
b. Concentration of the drug in the body – Alteration in the concentration of drug substance as well as the protein molecules or surfaces subsequently brings alteration in the protein binding process. At low concentrations, most drugs may be bound to proteins. At high concentrations, more free drugs may be present owing to saturation of binding sites on proteins.
c. Drug's affinity towards protein/ tissue. – This factor entirely depends upon the degree of attraction or affinity the protein molecule or tissues have towards drug moieties. For Digoxin has more affinity for cardiac muscles proteins as compared to that of proteins of skeletal muscles or those in the plasma like HSA. Digoxin has more affinity for proteins of cardiac muscles that nose of skeletal muscles or plasma. Iophenoxic acid, a radio-opaque medium, has half life of 2 and a half years due to its high affinity to plasma proteins.

PROTEIN RELATED FACTORS

a. Physiochemical characteristics of the protein or binding agent – Lipoproteins and adipose tissue tend to bind lipophilic drugs by dissolving them in their lipid core. The physiologic pH determines the presence of active anionic and cationic groups on the albumin molecules to bind a variety of drugs.
b. Concentration of protein/ binding component – Among the plasma protein, binding predominantly occurs withalbumin, as it is present in high concentration in comparision to other plasma protein. The amount of several proteins and tissue components available for binding, change during disease state.
c. Number of binding sites on the protein – Albumin has a large number of binding sites as compared to other proteins. Indomethacin binds to 3 sites on albumin. AAG is a

protein with limited binding capacity due to low concentration and molecular size. Lidocaine binds to 2 sites on AAG in presence of HSA.

DRUG INTERACTIONS

a. Competition between drugs for the binding site (displacement interactions) - Competition between drugs for the binding sites e.g., warfarin and phenyl butazone. Interactions will result when:
    i. The displaced drug (e.g., warfarin) is more than 95% bound having small volume of distribution, rapid onset of action and also has a narrow therapeutic index
    ii. The displacer drug (e.g., phenyl butazone) has a high degree of affinity and competes for the same binding sites and the drug/ protein concentration ratio is high.
b. Competition between drug and normal body constituents – interactions with free fatty acid levels are increased during fasting, diabetes, MI, etc.
c. Allosteric changes in protein molecule – The process Involves alterations of the protein structure by the drug or its metabolite modifying the binding capacity. E.g., Aspirin acetylation of albumin; modify the binding capacity of NSAIDs (increased affinity)

PATIENT RELATED FACTORS

a. Age – neonates: low albumin content, more free drug. Young infants: high dose of digoxine due to large renal clearance. Elderly: low albumin content, so more free drug
b. Inter-subject variations – Due to genetic and environmental factors
c. Disease states

Disease	*Influence On Plasma Proteins*	*Binding to Acidic*	*Basic*	*Neutral*
1.Renal failure	↓ Alb. contents	↓	No Effect	No Effect
2.Hepatic failure	↓ Alb. Synthesis	↓	Normal or ↓	No Effect
3.Inflammatory states	↑ AAG level	No Effect	↑	No Effect

PROTEIN BINDING OF DRUGS

The interacting molecules are generally the macromolecules such as protein, DNA or adipose. The protein is particularly responsible for such an interaction. The phenomenon of complex formation of drug with protein is called as protein binding of drug. As a protein bound drug is neither metabolized nor excreted hence it is pharmacologically inactive due to its pharmacokinetic and Pharmacodynamic inertness.

- Protein + drug ⇌ Protein - drug complex
- Protein binding may be divided into:
  1. Intracellular binding.
  2. Extracellular binding.

MECHANISMS OF PROTEIN DRUG BINDING

• Binding of drugs to proteins is generally of reversible & irreversible.

• Reversible generally involves weak chemical bond such as:

1. Hydrogen bonds
2. Hydrophobic bonds
3. Ionic bonds
4. Van der Waal's forces.

• Irreversible drug binding, though rare, arises as a result of covalent binding and is often a reason for the carcinogenicity or tissue toxicity of the drug.

A. BINDING OF DRUG TO BLOOD COMPONENTS

Plasma protein-drug binding: -

- The binding of drugs to plasma proteins is reversible.
- The extent or order of binding of drug to plasma proteins is: Albumin ›à1-Acid glycoprotein ›Lipoproteins ›Globulins.

1. Binding of drug to human serum Albumin.

   •It is the most abundant plasma protein (59%), having M.W. of 65,000 with large drug binding capacity

   •Both endogenous compounds such as fatty acid, bilirubin as well as drug binds to HSA.

•Four diff. sites on HSA for drug binding. Site I: warfarin & azapropazone binding site.

Site II: diazepam binding site.

Site III: digitoxin binding site.

Site IV: tamoxifen binding site.

2. Binding of drug to 1-Acid glycoprotein:(orosomucoid) It has a M.W. 44,000 and plasma conc. range of 0.04 to 0.1 g%. It binds to no. of basic drugs like imipramine, lidocaine, propranolol, quinidine.
3. Binding of drug to Lipoproteins: Binding by: Hydrophobic Bonds, Non-competitive. Lipid core composed of: Inside: triglyceride & cholesteryl esters. Outside: Apoprotein. e.g. Acidic: Diclofenac. Neutral: Cyclosporin A. Basic: Chlorpromazine
4. Binding of drug to Globulins

Globulin	Synonym	Binds to
1. α1 Globulin	Transcortine /Corticosteroid Binding globulin	Steroidal drugs, Thyroxin & Cyanocobalamine.
2. α2 Globulin	Ceruloplasmine	Vitamin A,D,E,K.
3. β1Globulin	Transferin	Ferrous ions
4. β2Globulin	---	Carotinoids
5. γ Globulin	---	Antigens

B. BINDING OF DRUG TO BLOOD CELLS

• In blood 40% of blood cells of which major component is RBC(95%). The RBC is 500 times in diameter as the albumin. The rate & extent of entry into RBC is more for lipophilic drugs.

The RBC comprises of 3 components.

a) Haemoglobin: It has a M.W. of 64,500 Dal. Drugs like phenytoin, pentobarbital bind to haemoglobin.

b) Carbonic anhydrase: Carbonic anhydrase inhibitors drugs are bind to it like acetazolamide & chlorthalidone.

c) Cell membrane: Imipramine & chlorpromazine are reported to bind with the RBC membrane.

C. BINDING OF DRUG TO EXTRAVASCULAR TISSUE PROTEIN

• Importance:

1. It increases apparent volume of distribution of drug.
2. localization of a drug at a specific site in body.

• Factor affecting: lipophilicity, structural feature of drug, perfusion rate, pH differences.

Binding order: Liver › Kidney › Lung › Muscles

Tissue	Binding of
1.Liver	Irreversible binding of Epoxides of Halogenated Hydrocarbon & Paracetamol.
2.Lungs	Basic drugs: Imipramine, Chlorpromazine, & AntiHistaminics.

Tissue	Binding of
3.Kidney	Metallothionin protein binds to Heavy metals & results in Renal accumulation and toxicity.
4.Skin	Chloroquine & Phenothiazine binds to Melanin.
5.Eye	Chloroquine & Phenothiazine also binds to Eye Melanin & results in Retinopathy.
6.Hairs	Arsenicals, Chloroquine, & Phenothiazine.
7.Bones	Tetracycline(yellow discoloration of teeth), Lead(replaces Ca & cause brittleness)
8.Fats	Lipophilic drugs (thiopental), Pesticides (DDT)
9.Nucleic Acid	Chloroquine & Quinacrine.

SIGNIFICANCE OF PROTEIN/TISSUE BINDING OF DRUG.

a. Absorption-

   • As we know the conventional dosage form follow first order kinetics. So, when there is more protein binding then it disturbs the absorption equilibrium.

b. Distribution-

   • A protein bound drug in particular does not cross the BBB, the placental barrier, the glomerulus, thus, protein binding decreases the distribution of drugs.

c. Metabolism-

   • Protein binding de creases the metabolism of drugs & enhances the biological half life.

   • Only unbound fraction gets metabolized e.g. Phenylbutazone & Sulfonamide.

d. Elimination-

   • Only the unbound drug is capable of being eliminated. Protein binding prevent the entry of drug to the metabolizing organ (liver) & to glomerulus filtration e.g., Tetracycline is eliminated mainly by glomerular filtration.

e. Systemic solubility of drug –

   • Lipoprotein act as vehicle for hydrophobic drugs like steroids, heparin, etc.

f. Drug action-

   • Protein binding inactivates the drugs because sufficient concentration of drug can not be build up in the receptor site for action e.g., Naphthoquinone

g. Sustain release-

   • The complex of drug protein in the blood act as a reservoir & continuously supply the free drug e.g., Suramin sodium-protein binding for antitrypanosomal action.

h. Diagnosis-

•The chlorine atom of chloroquine replaced with radiolabelled I- 131 can be used to visualize-melanomas of eye & disorders of thyroid gland.

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