UNIT-IV

COMPLEXATION AND PROTEIN BINDING:

Protein binding:

***Introduction*:**

- The interacting molecules are generally the macromolecules such as protein, DNA or adipose. The proteins are 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 Pharmacodynamics inertness.
- Protein + drug ⇌ Protein-drug complex.
- Protein binding may be divided into –
  - ✓ Intracellular binding.
  - ✓ Extracellular binding.

Mechanisms of protein drug binding:

- Binding of drugs to proteins is generally of reversible and 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.

- *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.
- *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.
- *Metabolism* - Protein binding decreases the metabolism of drugs and enhances the biological half life. Only unbound fractions get metabolized.

  Example: Phenylbutazone and Sulfonamide.
- *Elimination* – Only the unbound drug is capable of being eliminated. Protein binding prevent the entry of drug to the metabolizing organ (liver) and to glomerulus filtration.

  Example: Tetracycline is eliminated mainly by glomerular filtration.
- *Systemic solubility of drug* – Lipoprotein act as vehicle for hydrophobic drugs like steroids, heparin, oil soluble vitamin.
- *Drug action* - Protein binding inactivates the drugs because sufficient concentration of drug cannot be build up in the receptor site for action.

  Example: Naphthoquinone.
- *Sustain release* – The complex of drug protein in the blood act as a reservoir and continuously supply the free drug.

  Example: Suramin sodium-protein binding for antitrypanosomal action.
- *Diagnosis* – The chlorine atom of chloroquine replaced with radiolabeled I-131 can be used to visualize-melanomas of eye and disorders of thyroid gland.

Factors affecting protein binding:

- Drug related Factor.
  - ✓ Physicochemical properties of drug Increase in lipophilicity increases the drug binding with the protein.

- ✓ Total concentration of drug – Alternation in drug and protein concentration alter the drug protein binding.

❖ Protein related Factors.

- ✓ Physicochemical properties of protein – Lipoprotein bind with lipophilic drugs.
- ✓ Quantity of protein – Disease state affect the concentration of protein in blood.
- ✓ Number of binding sites – Albumin has more no of binding sites.

❖ Affinity and Magnitude of association constant.

❖ Drug Interaction.

- ✓ Displacement reaction
- ✓ Composition of drugs and normal body constituents.
- ✓ Allosteric changes in protein molecules.

❖ Patient related factors.

- ✓ Age – Noenata have low albumin content, thus less drug binding.
- ✓ Disease state – Disease sate alter the drug binding.

Binding of drug to blood plasma proteins –

- The binding of drugs to plasma proteins is reversible.
- The extent or order of binding of drug to plasma proteins is: Albumin › ά1-Acid glycoprotein › Lipoproteins › Globulins.

❖ ***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 bind to HSA.
- Four different sites on HSA for drug binding.
- Site I: warfarin and azapropazone binding site.
- Site II: diazepam binding site.

- Site III: digitoxin binding site.
- Site IV: tamoxifen binding site.

❖ **Binding of drug to α1-Acid glycoprotein –**

- It is called as orosomucoid. It has a M.W. 44,000.
- Its plasma conc. range of 0.04 to 0.1 g %.
- It binds to no. of basic drugs like imipramine, lidocaine, propranolol, and quinidine.

❖ **Binding of drug to Lipoproteins –**

- Binding by Hydrophobic Bonds, Non-competative.
- Mol wt: 2-34 Lacks dalton.
- Lipid core composed of: Inside: triglyceride & cholesteryl esters. Outside: Apoprotein. e.g. Acidic: Diclofenac. Neutral: Cyclosporin A. Basic: Chlorpromazine.
- Its types are LDL, HDL, VLDLand Chylomicrons.

❖ **Binding of drug to Globulins** –

- α1 Globulin (Transcortine /Corticosteroid Binding globulin) - Steroidal drugs, Thyroxin & Cyanocobalamine (Vit B12).
- α2 Globulin (Ceruloplasmine) - Vitamin A, D, E, K.
- β1 Globulin (Transferin) - Ferrous ions.
- β2 Globulin – Carotinoids.
- γ Globulin – Antigens.

Kinetics of Protein Binding:

❖ An equation relating reaction velocity to Drug concentration (Mol/L) for a system where a Drug D binds reversibly to an Protein P of to form an Protein-Drug complex .

❖ This system can be represented schematically as follows:

$$P + D_F ======= PD$$

- Applying the law of mass action, the equilibrium or association constant (K) is;

$$K = [PD]/ [P] [D_F]$$

- The [PD], [P] and [D] are the concentration of protein-drug complex, protein and drug in Mol/L.

$$K[P][D_F] = [PD]$$

Free protein concentration can obtain as;

$$[P_T] = + [PD]$$

$$[P] = [P_T] \_ [PD]$$

[PT] is the total protein.

Substituting the [P] in last equation,

$$K[P][D_F] = [PD]$$

$$K ([P_T] – [PD]) [D_F] = [PD]$$

Where,

DF is the free drug.

$$K [P_T] [D_F] – K [PD] [D_F] = [PD]$$

$$K [P_T] [D_F] = [PD] + K [PD] [D_F]$$

$$K [P_T] [DF] = [PD] (1+ K [D_F])$$

$$[PD] = (K [P_T] [D_F])/ (1+ K [D_F])$$

$$[PD]/ [P_T] = K [D_F]/ 1+ K [D_F]$$

- Let R be expressed as moles of drug bound [PD] per mole of total protein

$$[P_T] R = [PD]/ [P_T] = K [D_F]/ 1+ K [D_F]$$

- If V is the number of independent binding sites available then R,

$$R = V (K [D_F]/ 1+ K [D_F])$$

$$1/R = 1/VK[D_F] + 1/V$$

- The graph is plotted between 1/R versus 1/[DF], called Klotz reciprocal plot, gives a straight line whose slope is 1/VK and intercept is V.

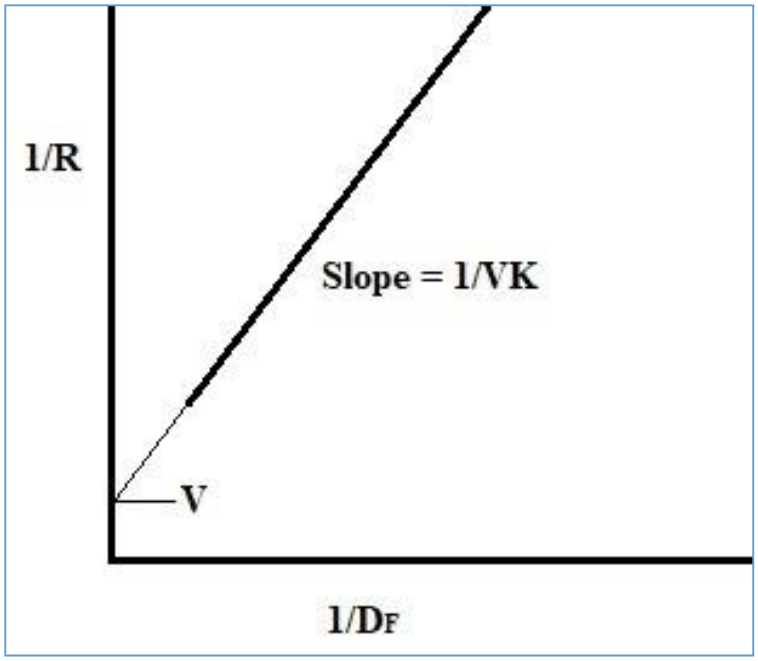

Figure: Klotz reciprocal plot.

$$R + R\,K\,[D_F] = V\,K\,[D_F]$$

$$R/[D_F] = VK - RK$$

Complexation and drug action:

- Protein binding inactivates the drugs because sufficient concentration of drug cannot be build up in the receptor site for action.
  Example: Naphthoquinone.
- Only free drug participate in drug action.
- Complexation can alter the pharmacological action of drug by interfering interaction with receptor.
- The action of drug to remove the toxic effect of metal ion from the human bodies is through the complexation reaction.
- It has been seen that in some instance complexation can also lead to poor solubility or decreased absorption of drug in the body, which decreases the bioavailability of drug in the blood. Thus the drug action gets altered.
- Drug complex with hydrophilic drug also enhance the drug elimination, thus helps in drug action termination and reduction in drug toxic action.
- Examples :

- Tetracycline and Calcium – Poor absorbed complex.
- Polar drug and complexing agent – Well absorbed lipid soluble complex.
- Carboxy methyl cellulose and amphetamine – Poor absorbed complex.
- PVP and I_2 – Better absorption.

Crystalline structures of complexes:

Complex or co-ordination compounds cover the range from quite simple inorganic salts to elaborate metal-organic hybrid materials and intricate bioactive metalloproteins. Their present uses and their potential applications are diverse due to their compositions, their molecular and crystal structures and their chemical and physical properties. Besides their use as chemical reactants, complex compounds are considered for extraction processes and as active agent in remedies and for drug delivery.

Thermodynamic treatment of stability constants Complexes:

- The relationship between the standard free energy change of complexation and the over all stability constant K is related as;

$$\Delta G = -2.303RT \text{ Log } K$$

- The Standard Enthalpy Change ΔH may be obtained from the slope of a plot of Log K Versus 1/T, thus the equation will be;

$$\text{Log } K = -(\Delta H/2.303R) \times (1/T) + \text{Constant}$$

- When the value of K at two temperatures are known, the following equation can be written as;

$$\text{Log } (K2/K1) = -(\Delta H/2.303R) \times (T2\text{-}T1/T1T2)$$

- The Standard entropy change may be obtained from the expression;

$$\Delta G = \Delta H - T\,\Delta S$$

- As the stability constant for molecular complexation increases, ΔH and ΔS becomes more negative.

- As binding between the donor and receptor becomes stronger, ΔH becomes more negative.
- Since the specificity of interacting sites becomes negative, ΔS also become more negative.
- But the extent of change in ΔH is large enough to overcome the unfavourable entropy change resulting in negative ΔG value and hence complexation.

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