Protein binding of drugs

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Introduction

- Bound Drug is Pharmacodynamically inert.
- Binding: Half life of drug.
- Irreversible bonding: Covalent bonding: responsible for the Carcinogenicity or Tissue toxicity.
Binding Of Drug

1. Plasma Proteins
2. Blood cells

To Extra vascular Tissues
1. Proteins
2. Fats
3. Bones, etc.
A) Binding to Blood Components

I) Plasma Protein Binding of drug:

- Reversible
- Order: Albumin > $\alpha_1$ AG > Lipoproteins > Globulins.
- *Involves binding to:*

1. Human serum
2. Albumin.
3. $\alpha_1$ Acid Glycoprotein,
4. Lipoproteins,
5. Globulins
1. Binding to HSA

- Primary site:
- Secondary site:
- Bond: Hydrophobic bond

I. Warfarine & Azapropazone site.
II. Diazepam site.
III. Digoxine site.
IV. Tamoxifen site.
2. Binding to Lipoproteins

- Mol wt : 2-3 lacks Dalton.
- Bound drug dissolves in **Lipid core.**
- Lipid Core composed of:
  - Inside: Triglycerides, cholesterol esters,
  - Outside: Appoprotein.
- E.g:
  - Acidic: Diclofenac.
  - Neutral: Cyclosporine A
  - Basic: Chlorpromazine.
3. Binding to $\alpha_1$ Acid Glycoprotein

- Binding by: Hydrophobic bonds.
- E.g.: Basic Drugs: Imipramine, Amytriptyline, Lidocaine.

4. Binding to Globulin

<table>
<thead>
<tr>
<th>Globulin</th>
<th>Synonym</th>
<th>Binds to:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. $\alpha_1$ Globulin</td>
<td>Transcortine /Corticosteroid Binding globulin</td>
<td>Steroidal drugs, Thyroxin &amp; Cyanocobalamine.</td>
</tr>
<tr>
<td>2. $\alpha_2$ Globulin</td>
<td>Ceruloplasmine</td>
<td>Vitamin A,D,E,K.</td>
</tr>
<tr>
<td>3. $\beta_1$ Globulin</td>
<td>Transferine</td>
<td>Ferrous ions</td>
</tr>
<tr>
<td>4. $\beta_2$ Globulin</td>
<td>---</td>
<td>Carotinoids</td>
</tr>
<tr>
<td>5. $\gamma$ Globulin</td>
<td>---</td>
<td>Antigens</td>
</tr>
</tbody>
</table>
II) Binding To Blood Cells

- 40% of Blood comprises of blood cells
- Majority is RBCs: 500 times more diameter as Albumin.
- RBC Components that binds to drug:
  - Hb
    - 7-8 times conc. Of albumin
    - Binds to: Phenytoins, Pentobarbital, Phenothiazine.
  - Carbonic Anhydrase
    - Binds to: Acetazolamide, Chlorthalidone,
  - Cell membrane
    - Binds to: Imipramine, Chlorpromazine.
B) Tissue Binding Of Drug:

• **Importance**:
  1. Apparent Vd.
  2. Localization of drug at specific site: biological half life.

• **Factors affecting**:
  Lipophilicity, structural feature of drug, Perfusion rate, pH difference.

• **Binding Order**:
  Liver > Kidney > Lungs > Muscle.

<table>
<thead>
<tr>
<th>Organ</th>
<th>Binding of:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.Liver</td>
<td>Irreversible binding of Epoxides of Halogenated Hydrocarbon &amp; Paracetamol.</td>
</tr>
<tr>
<td>2.Lungs</td>
<td>Basic drugs: Imipramine, Chlorpromazine, &amp; AntiHistaminics.</td>
</tr>
<tr>
<td>3.Kidney</td>
<td>Metallothione protein binds to Heavy metals &amp; results in Renal accumulation 7 toxicity.</td>
</tr>
<tr>
<td>4.Skin</td>
<td>Chloroquine &amp; Phenothiazine binds to Melanin.</td>
</tr>
<tr>
<td>Organ</td>
<td>Binding of:</td>
</tr>
<tr>
<td>------------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>5.Eye</td>
<td>Chloroquine &amp; Phenothiazine also binds to Eye Melanin &amp; results in Retinopathy.</td>
</tr>
<tr>
<td>7.Bones</td>
<td>Tetracycline(yellow discoloration of teeth), Lead(replaces Ca &amp; cause brittleness)</td>
</tr>
<tr>
<td>8.Fats</td>
<td>Lipophilic drugs (thiopental), Pesticides (DDT)</td>
</tr>
</tbody>
</table>
Factors Affecting Protein binding:

A) Drug Related:

1. Physicochemical Characteristics:
   - Lipophilicity α binding.
   - Anionic/Acidic binds : HAS
   - Cationic/Basic binds : AAG

2. Concentration of Drug:

3. Drug protein/tissue affinity: Digoxine Affinity to cardiac muscle.

B) Protein/Tissue Related:

1. Physicochemical Characteristics:
   Lipophilicity α binding.

2. Concentration
3. Number of Binding Sites

- Alb. Has more.
- Tamoxifen & Dicumarol binds to $1^0$ & $2^0$ sites of alb.
- Indomethacine binds to 3 site.

C) Drug Interaction:

1. Competition between Drugs for binding site.


E.g. Adm. Of Phenylbutazone to Warfarine therapy patient, result in Hemorrhagic reaction.
2. Competition Between Drug & Normal Constituents:

- FFA competes with HAS.
- Free FFA level increased during conditions:
  - Physiological C. (Fasting)
  - Pathological C. (Diabetes, M.I)
  - Pharmacological (Heparin & Caffeine adm.).
- Acidic Drug displaces: Bilurubine from Alb. & results in Kernictarus.

3. Allosteric Changes In Protein Molecule:

- By drug or its Metabolite.
- Allosteric Modulators: are agents responsible.
- E.g. Aspirins acetylating of Lysine of Alb. So modifying capacity of NSAIDS binding.
D) Patient Related:

1. Age:
   - **Neonates:** Low Alb. content: more free drug.
   - **Young Infants:** High dose of Digoxine due to large renal clearance.
   - **Elderly:** Low Alb.: so more free drug.

2. Intersubject Variability: Due to Genetic & Environmental Factors.

3. Disease State:

<table>
<thead>
<tr>
<th>Disease</th>
<th>Influence On Plasma Proteins</th>
<th>Acidic</th>
<th>Binding to Basic</th>
<th>Neutral</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Renal failure</td>
<td>↓ Alb. contents</td>
<td>↓</td>
<td>No Effect</td>
<td>No Effect</td>
</tr>
<tr>
<td>2. Hepatic failure</td>
<td>↓ Alb. Synthesis</td>
<td>↓</td>
<td>Normal or</td>
<td>↓ No Effect</td>
</tr>
<tr>
<td>3. Inflammatory states</td>
<td>↑ AAG level</td>
<td>No Effect</td>
<td>↑ No Effect</td>
<td></td>
</tr>
</tbody>
</table>
Volume Of Distribution

- At distribution Equilibrium: Conc. of drug in body is determined By: Vol. of Tissue in which drug is present.
- Different tissue have diff. conc. So Vd cannot have a true physiologic meaning.
- (Amount of drug in body) $\alpha$ (Conc. Of drug in plasma)
  \[ X\alpha C \]
  \[ X = Vd. C \]

Def: Hypothetical Vol. of body fluid into which drug is dissolved or distributed.

It is Apparent Vd: Because: All parts of body equilibrated with drug do not have equal conc.
• Real Vd: has direct physiological meaning, is related to body water.

<table>
<thead>
<tr>
<th>Body fluid</th>
<th>Volume (lit.)</th>
<th>% of Body wt.</th>
<th>% of TWB</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Vascular fluid (plasma)</td>
<td>6 (3)</td>
<td>9 (4.5)</td>
<td>15 (7.5)</td>
</tr>
<tr>
<td>2. Extracellular fluid (excl plasma)</td>
<td>12</td>
<td>14</td>
<td>28</td>
</tr>
<tr>
<td>3. Intracellular fluid (excl blood cells)</td>
<td>24</td>
<td>34</td>
<td>57</td>
</tr>
<tr>
<td><strong>Total Body</strong></td>
<td><strong>42</strong></td>
<td><strong>60</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>
Markers used to measure Real Vd

<table>
<thead>
<tr>
<th>Physiological fluid compartment</th>
<th>Markers used</th>
<th>Approximate vol.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Plasma</td>
<td>Evans Blue, Indocyanine Green, I-131 alb.</td>
<td>3</td>
</tr>
<tr>
<td>2. Erythrocytes</td>
<td>Cr-51</td>
<td>2</td>
</tr>
<tr>
<td>3. Extracellular fluids</td>
<td>Non metabolizable saccharides like Raffinose, Inuline, Mannitol, &amp; Radio isotopes of selected ions: Na⁺, Cl⁻, Br⁻, So₄²⁻.</td>
<td>15</td>
</tr>
<tr>
<td>Total body water</td>
<td>D₂O, HTO, Antipyrine</td>
<td>42</td>
</tr>
</tbody>
</table>
References

- D.M.Brahmankar & Sunil B.Jaiswal’s Biopharmaceutics & Pharmacokinetics A Treatise, Vallabh Prakashan, New Delhi, pg. no. 86-102.
- Presentation at ISPA Educational Workshop, Copenhagen.
- www.hucmlrc.howard.edu/pharmacology