Protein binding
Chapter 10

PROTEIN BINDING OF DRUGS

Many drugs interact with plasma or tissue proteins or with other macromolecules, such as melanin and DNA, to form a ***drug-macromolecule complex***. The formation of a drug protein complex is often named ***drug-protein binding***. Drug-protein binding may be a reversible or an irreversible process. ***Irreversible*** **drug-protein binding is usually a result of chemical activation of the drug, which then attaches strongly to the protein or macromolecule by covalent chemical bonding**. Irreversible drug binding accounts for certain types of drug toxicity that may occur over a long time period, as in the case of chemical carcinogenesis, or within a relatively short time period, as in the case of drugs that form reactive chemical intermediates**. For example, the hepatotoxicity of high doses of acetaminophen is due to the formation of reactive metabolite intermediates that interact with liver proteins.**

Most drugs bind or complex with proteins by a reversible process. ***Reversible drug protein binding*** implies that the drug binds the protein with weaker chemical bonds, such as **hydrogen bonds or van der Waals forces.**

The amino acids that compose the protein chain have hydroxyl, carboxyl, or other sites available for reversible drug interactions.

Reversible drug -protein binding is of major interest in pharmacokinetics. The protein-bound drug is a 1-**large complex** that cannot easily transverse cell or possibly even capillary membranes and therefore has a restricted distribution. Moreover, the protein-bound drug is usually 2-**pharmacologically inactive**.

In contrast, the free or unbound drug crosses cell membranes and is therapeutically active. Studies that critically evaluate drug-protein binding are usually performed ***in vitro*** using a purified protein such as albumin. Methods for studying protein binding, including equilibrium dialysis and ultrafiltration, make use of a semipermeable membrane that separates the protein and protein-bound drug from the free or unbound drug .

By these *in vitro* methods, the concentrations of bound drug, free drug, and total protein may be determined. Each method for the investigation of drug-protein binding *in vitro* has advantages and disadvantages in terms of cost, ease of measurement, time, instrumentation, and other considerations.

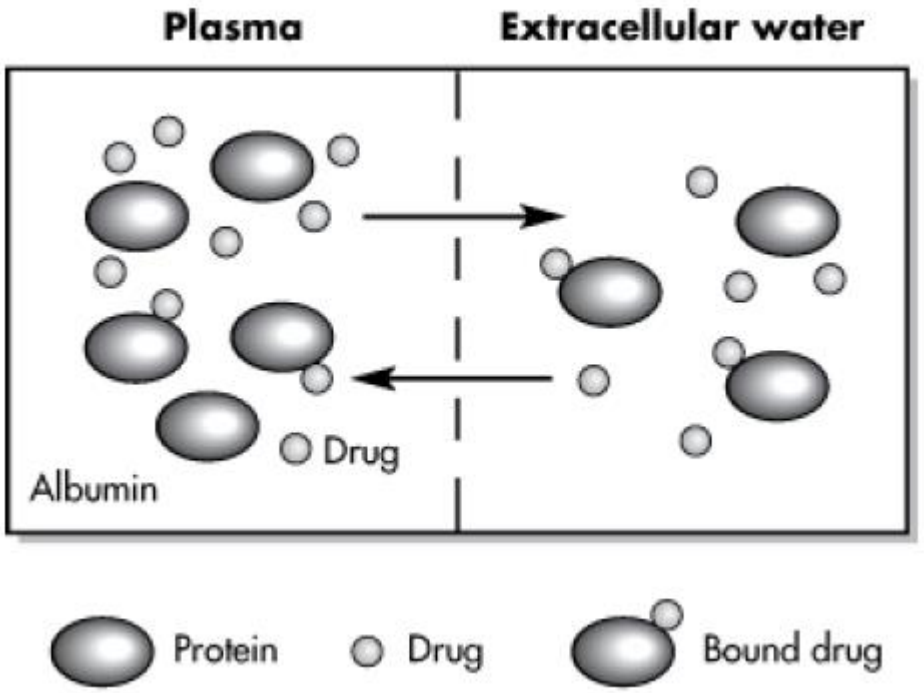

Diagram showing that bound drugs will not diffuse across membrane but free drug will diffuse freely between the plasma and extracellular water.

Drugs may bind to various macromolecular components in the blood, including **albumin, α1-acid glycoprotein, lipoproteins, immunoglobulins (IgG), and erythrocytes (RBC).**

Albumin is a protein with **a molecular weight of 65,000-69,000 Da** that is synthesized in the **liver** and is the **major component** of plasma proteins responsible for reversible drug binding. In the body, albumin is **distributed in the plasma and in the extracellular fluids of skin, muscle, and various other tissues. I**nterstitial fluid albumin concentration is about 60% of that in the plasma. **The elimination half-life of albumin is 17-18 days**. Normally, albumin concentration is maintained at a relatively constant level of 3.5-5.5% (weight per volume) or 4.5 mg/dL. **Albumin is responsible for maintaining the osmotic pressure of the blood and for the transport of endogenous and exogenous substances**. As a transport protein for endogenous substances, albumin complexes with free fatty acids (FFAs), bilirubin, various hormones (such as cortisone, aldosterone, and thyroxine), tryptophan, and other compounds. **Many weak acidic (anionic) drugs bind to albumin** by electrostatic and hydrophobic bonds. Weak acidic drugs such as salicylates, phenylbutazone, and penicillins are highly bound to albumin. **However, the strength of the drug binding is different for each drug.**

α1-*Acid glycoprotein* is a globulin with a molecular weight of about **44,000 Da**. The plasma concentration of α1-acid glycoprotein is low (0.4-1%) and **binds primarily basic (cationic) drugs** such as propranolol, imipramine, and lidocaine.

Globulins (α -,β -,γ globulins) may be responsible for the plasma transport of certain endogenous substances such as **corticosteroids**. These globulins have a low capacity but high affinity for the binding of these endogenous substance.

Lipoproteins are macromolecular **complexes of lipids and proteins and are classified according to their density and separation in the ultracentrifuge**. The terms VLDL, LDL, and HDL are abbreviations for very-low-density, low-density, and high-density lipoproteins, respectively. **Lipoproteins are responsible for the transport of plasma lipids to the liver and may be responsible for the binding of drugs if the albumin sites become saturated.**

Erythrocytes**, or red blood cells (RBCs),** may bind both endogenous and exogenous compounds. RBCs consist of about **45% of the volume of the blood**. **Phenytoin, pentobarbital, and amobarbital are known to have a RBC/plasma water ratio of 4 to 2, indicating preferential binding of drug to the erythrocytes over plasma water**. **Penetration into RBC is dependent on the free concentration of the drug.** In the case of phenytoin, RBC drug level increases linearly with an increase in the plasma-free drug concentration. Increased drug binding to plasma albumin reduces RBC drug concentration. With most drugs, however, binding of drug to RBC generally does not significantly affect the volume of distribution, because the drug is often bound to albumin in the plasma water. **Even though phenytoin has a great affinity for RBC, only about 25% of the blood drug concentration is present in the blood cells, and 75% is present in the plasma because the drug is also strongly bound to albumin**. For drugs with strong erythrocyte binding, the hematocrit will influence the total amount of drug in the blood. For these drugs, **the total whole-blood drug concentration should be measured.**

Effect of Changing Plasma Protein: An Example

The effect of increasing the plasma α1-acid glycoprotein (AAG) level on drug penetration into tissues may be verified with **cloned transgenic animals that have 8.6 times the normal α1-acid glycoprotein levels.** In an experiment investigating the activity of the tricyclic antidepressant drug imipramine, equal drug doses were administered to both normal and transgenic mouse. **Since imipramine is highly bound to AAG**, **the steadystate imipramine serum level was greatly increased in the blood due to protein binding.**

Imipramine serum level (transgenic mouse): 859 ng/mL
Imipramine serum level (normal mouse): 319.9 ng/mL
Imipramine brain level (transgenic mouse): 3862.6 ng/mL
Imipramine brain level (normal mouse): 7307.7 ng/mL

However, the imipramine concentration was greatly reduced in the brain tissue because of higher degree of binding to AAG in the serum, resulting in reduced drug penetration into the brain tissue. **The volume of distribution of the drug was reported to be reduced. The antidepressant effect was observed to be lower in the transgenic mouse due to lower brain imipramine level**. **This experiment illustrates that high drug protein binding in the serum can reduce drug penetration to tissue receptors for some drugs** .

Another drug that is highly bound to AAG, **saquinavir,** is highly bound to AAG and has reduced free drug concentrations in transgenic mice that express elevated AAG. In this study, the drug was **bound to both albumin and AAG** (2.1% to AAG versus 11.5% to albumin). Elevated AAG caused saquinavir's volume of distribution to be reduced in this study.

For a drug that **distributes into the plasma and a given tissue in the body**, the amount of drug bound may be found by Equation 10.7. Because drug may bind to both plasma and tissue proteins, the bound and unbound drug concentrations must be considered. **At steady state, unbound drug in plasma and tissue are in equilibration.**

$$D_B = V_p C_p + V_t C_t \qquad (10.7)$$

$$C_u = C_{ut}$$

Alternatively,

$$C_p f_u = C_t f_{ut} \qquad (10.8)$$

or

$$C_t = C_p \frac{f_u}{f_{ut}} \qquad (10.9)$$

where all terms refer to steady-state condition: *f*u is the unbound (free) drug fraction in the plasma, *f*ut is the unbound drug fraction in the tissue, *C*u is the unbound drug concentration in the plasma, and *C*ut is the unbound drug concentration in the tissues. Substituting for *C*t in Equation 10.7 using Equation 10.9 results in

$$D_B = V_p C_p + V_t \left[C_p \left(\frac{f_u}{f_{ut}} \right) \right] \qquad (10.10)$$

Rearranging,

$$\frac{D_B}{C_p} = V_p + V_t\left(\frac{f_u}{f_{ut}}\right) \qquad (10.11)$$

$$D_B = V_P C_P + V_t\left[C_P\left(\frac{f_u}{f_{ut}}\right)\right]$$

Because $D_B/C_p = V_{app}$, by substitution into Equation 10.11, V_{app} may be estimated by Equation 10.12:

$$V_{app} = V_p + V_t\left(\frac{f_u}{f_{ut}}\right) \qquad (10.12)$$

Equation 10.12 relates the amount of drug in the body to plasma volume, tissue volume, and fraction of free plasma and tissue drug in the body. Equation 10.12 may be expanded to include several tissue organs with *V*ti each with unbound tissue fraction *f*uti.

$$V_{app} = V_p + \sum V_{ti}\left(\frac{f_u}{f_{uti}}\right)$$

where *V* ti = tissue volume of the *i*th organ and *f*uti = unbound fraction of the *i*th organ. The following are important considerations in the calculation of *V*app.

1. The volume of distribution is a constant only when the drug concentrations are in equilibrium between the plasma and tissue.

2. Values of *f*u and *f*ut are concentration dependent and must also be determined at equilibrium conditions.

3. Equation 10.12 shows that *V*app is an **indirect measure of drug binding in the tissues** rather than a measurement of a true anatomic volume.

$$V_{app} = V_p + V_t\left(\frac{f_u}{f_{ut}}\right) \qquad (10.12)$$

4. When *f*u and *f*ut are unity, Equation 10.12 is simplified to

$$\frac{D_B}{C_P} = V_P + V_t$$

When no drug binding occurs in tissue and plasma, the volume of distribution will not exceed the real anatomic volume. **Only at steady state are the unbound plasma drug concentration, *C*u, and the tissue drug concentration, *C*ut, equal**. At any other time, *C*u may not equal to *C*ut. **The amount of drug in the body, *D*B, cannot be calculated easily from *V*app and *C*p under nonequilibrium conditions**. **For simplicity, some models assume that the drug distributed to a tissue is approximated by the drug present in the fluid of that tissue.**