Protein Binding of Drugs

Many drugs interact with plasma proteins to form a drug protein complex which 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 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. Reversible drug protein binding is of major interest in pharmacokinetics.

The protein bound drug is a 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 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 drug. By these in vitro methods the concentrations of bound drug, free drug, and total protein may be determined.

Drugs may bind to various macromolecular components in the blood, including albumin, α1 acid glycoprotein, lipoproteins, immunoglobulins and erythrocytes.

Albumin is a protein with a molecular weight of 65000 Da that is synthesized in the liver and is the major component of plasma proteins responsible for reversible drug binding. The elimination half-life of albumin is 18 days. Normally, albumin concentration is maintained at a relatively constant level of 4.5%. Albumin is responsible for maintaining the osmotic pressure of the blood and for the transport of endogenous and exogenous substances. Many weak acidic (anionic) drugs such as salicylates, and penicillin are highly bound to albumin by electrostatic and hydrophobic bonds.

The α1 Acid glycoprotein is a globulin with a molecular weight of about 44000 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.

Lipoproteins are macromolecular complexes of lipids and proteins and are classified according to their density. 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 may bind both endogenous and exogenous compounds. RBCs consist of about 45% of the volume of the blood. Phenytoin, and pentobarbital are known to have a RBC/plasma water ratio of 4 to 2 indicating preferential binding of drug to the erythrocytes over plasma water. 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.

Effect of Protein Binding on the Apparent Volume of Distribution

The extent of drug protein binding in the plasma or tissue affects volume of distribution. Drugs that are highly bound to plasma proteins have a low fraction

of free drug in the plasma. The protein bound drug does not diffuse easily and is therefore less extensively distributed to tissues. Drugs with low plasma protein binding have larger free drug level, generally diffuse more easily into tissues, and have a greater volume of distributions.

Relationship of Plasma Drug Protein Binding to Distribution and Elimination

In general, drugs that are highly bound to plasma protein have reduced overall drug clearance. For a drug that is metabolized mainly by the liver, binding to plasma proteins prevents the drug from entering the hepatocytes resulting in reduced drug metabolism by the liver. In addition, molecularly bound drugs may not be available as substrates for liver enzymes, thereby further reducing the rate of metabolism.

Protein bound drugs act as larger molecules that cannot diffuse easily through the capillary membranes in the glomeruli. The elimination half-lives of some drugs, such as the cephalosporin which are excreted mainly by renal excretion, are generally increased when the percent of drug bound to plasma proteins increases.

On the other hand, drug that is both extensively bound and actively secreted by the kidneys, such as penicillin, has a short elimination half-life, because active secretion takes preference in removing or stripping the drug from the proteins as the blood flows through the kidney.

KINETICS OF PROTEIN BINDING

The kinetics of reversible drug protein binding for a protein with one simple binding site can be described by the law of mass action, as

$$\text{Protein} + \text{drug} \Leftrightarrow \text{drug–protein–complex}$$

$$[P] + [D] \Leftrightarrow [PD] \qquad (10.15)$$

According to the law of mass action, an association constant, Ka can be expressed as the ratio of the molar concentration of the products and the molar

concentration of the reactants

This equation assumes only one binding site per protein molecule

$$K_a = \frac{[PD]}{[P][D]} \qquad (10.16)$$

The extent of the drug protein complex formed is dependent on the association binding constant Ka. The magnitude of Ka yields information on the degree of drug protein binding. Drugs strongly bound to protein have a very large Ka and exist mostly as the drug protein complex. With such drugs, a large dose may be needed to obtain a reasonable therapeutic concentration of free drug

To study the binding behavior of drugs, a determinable ratio r is defined, as follows

$$r = \frac{\text{moles of drug bound}}{\text{total moles of protein}}$$

Because moles of drug bound is [PD] and the total moles of protein is [P] + [PD], this equation becomes

$$r = \frac{[PD]}{[PD] + [P]} \qquad (10.17)$$

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

$$r = \frac{K_a[D]}{1 + K_a[D]} \qquad (10.18)$$

This equation describes the simplest situation, in which 1 mole of drug binds to 1 mole of protein in a 1:1 complex. This case assumes only one independent binding site for each molecule of drug. If there are n identical independent binding sites per protein molecule, then the following is used

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

In reality, drug protein binding sometimes exhibits a phenomenon of cooperativity. For these drugs, the binding of the first drug molecule at one site on the protein molecule influences the successive binding of other drug molecules.

When a highly protein bound drug is displaced from binding by a second drug or agent, a sharp increase in the free drug concentration in the plasma may occur, leading to toxicity. For example, an increase in free warfarin level was responsible for an increase in bleeding when warfarin was co administered with phenylbutazone which competes for the same protein binding site.

Albumin has two known binding sites that share the binding of many drugs. Binding site I is shared by phenylbutazone, sulfonamides, phenytoin, and valproic acid. Binding site II is shared by the semisynthetic penicillins, probenecid, medium chain fatty acids, and the benzodiazepines. Some drugs bind to both sites. Displacement occurs when a second drug is taken that competes for the same binding site in the protein as the initial drug.

Drug Distribution and Pharmacodynamics

A decrease in protein binding that results in increased free drug concentration will allow more drug to cross cell membranes and distribute into all tissues, more drug will be available to interact at a receptor site to produce a more intense pharmacologic effect. Clinically the pharmacodynamic response is influenced by both the distribution of the drug and the concentration of the unbound drug fraction. The drug dose and the dosage form must be chosen to provide sufficiently high unbound drug concentrations so that an adequate amount of drug reaches the site of drug action (receptor). The onset of drug action depends on the rate of the free drug that reaches the receptor.

The intensity of a drug action depends on the total drug concentration at the receptor site and the number of receptors occupied by drug.

Drug Excretion & Clearance

Drug excretion is the removal of the intact drug. Nonvolatile drugs are excreted mainly by renal excretion, a process in which the drug passes through the kidney to the bladder and ultimately into the urine. Other pathways for drug excretion may include the excretion of drug into bile, sweat, saliva, milk (via lactation), or other body fluids. Volatile drugs, such as gaseous anesthetics or drugs with high volatility, are excreted via the lungs into expired air.

The renal blood flow (RBF) is the volume of blood flowing through the renal vasculature per unit time. Renal blood flow exceeds 1.2 L/min or 1700 L/day. Renal plasma flow (RPF) is the renal blood flow minus the volume of red blood cells present. Renal plasma flow is an important factor in the rate of drug filtration at the glomerulus.

$$RPF = RBF(1 - Hct)$$

Where Hct is hematocrit. It is the fraction of blood cells, about 45% of the total blood volume.

Assuming a hematocrit of 0.45 and a RBF of 1.2 L/min, using the above equation, RPF = 1.2 (1- 0.45) =0.66 L/min or 660 mL/min, approximately 950 L/day. A normal adult male subject has a GFR of approximately 125 mL/min. About 180 L of fluid per day are filtered through the kidneys. The ratio GFR/RPF is the filtration fraction.

Up to 99% of the fluid volume filtered at the glomerulus is reabsorbed. Besides fluid regulation, the kidney also regulates the retention or excretion of various solutes and electrolytes. With the exception of proteins and protein-bound substances, most small molecules are filtered through the glomerulus from the plasma. The filtrate contains some ions, glucose, and essential nutrients as well as waste products, such as urea. The essential nutrients and water are reabsorbed at various sites, including the proximal tubule, loops of Henle, and distal tubules.

RENAL DRUG EXCRETION

Renal excretion is a major route of elimination for many drugs. Drugs that are 1-nonvolatile, 2-water soluble, 3-have a low molecular weight, or 4-slowly biotransformed by the liver are eliminated by renal excretion. The processes by which a drug is excreted via the kidneys may include any combination of the following: Glomerular filtration, Active tubular secretion, Tubular reabsorption.

Glomerular filtration is a process that occurs for most small molecules (MW < 500), including nonionized and ionized drugs. Protein-bound drugs behave as large molecules and do not get filtered at the glomerulus. The major driving force for glomerular filtration is the hydrostatic pressure within the glomerular capillaries.

Glomerular filtration rate (GFR) is measured by using a drug that is eliminated by filtration only (ie, the drug is neither reabsorbed nor secreted). Examples of such drugs are inulin and creatinine. Therefore, the clearance of inulin is equal to the GFR, which is equal to 125 mL/min. The value for the GFR correlates fairly well with body surface area. Glomerular filtration of drugs is directly related to the free drug concentration in the plasma. As the free drug concentration in the plasma increases, the glomerular filtration for the drug increases proportionately, thus increasing renal drug clearance for some drugs.

Active tubular secretion is an active transport process. As such, active renal secretion is a carrier-mediated system that requires energy input, because the drug is transported against a concentration gradient. The carrier system is capacity limited and may be saturated. Drugs with similar structures may compete for the same carrier system.

Two active renal secretion systems have been identified, for (1) weak acids and (2) weak bases. For example, probenecid competes with penicillin for the same carrier system (weak acids). Active tubular secretion rate is dependent on renal plasma flow.

Drugs commonly used to measure active tubular secretion include p -amino-hippuric acid (PAH) and iodopyracet. These substances are both filtered by the

glomeruli and secreted by the tubular cells.

For a drug that is excreted solely by glomerular filtration, the elimination half-life may change markedly in accordance with the binding affinity of the drug for plasma proteins. In contrast, drug protein binding has very little effect on the elimination half-life of the drug excreted mostly by active secretion. Because drug protein binding is reversible, drug bound to plasma protein rapidly dissociates as free drug is secreted by the kidneys. For example, some of the penicillins are extensively protein bound, but their elimination half-lives are short due to rapid elimination by active secretion.

Tubular reabsorption occurs after the drug is filtered through the glomerulus and can be an active or a passive process. If a drug is completely reabsorbed (eg, glucose), then the value for the clearance of the drug is approximately zero. For drugs that are partially reabsorbed, clearance values are less than the GFR.

Generally, the unionized species is more lipid soluble (less water soluble) and has greater membrane permeability and it is easily reabsorbed from the renal tubule back into the body.

The process of drug reabsorption can significantly reduce the amount of drug excreted, depending on the pH of the urinary fluid and the pKa of the drug. The pKa of the drug is a constant, but the normal urinary pH may vary from 4.5 to 8.0, depending on diet, pathophysiology, and drug intake. Vegetable and fruit diets or diets rich in carbohydrates result in higher urinary pH, whereas diets rich in protein result in lower urinary pH. Drugs such as ascorbic acid and antacids such as sodium carbonate may decrease (acidify) or increase (alkalinize) the urinary pH, respectively, when administered in large quantities. The most important changes in urinary pH are caused by fluids administered intravenously. Intravenous fluids, such as solutions of bicarbonate or ammonium chloride, are used in acid-base therapy. Excretion of these solutions may drastically change urinary pH and alter drug reabsorption and drug excretion by the kidney.

The percentage of ionized weak acid drug corresponding to a given pH can be obtained from the Henderson-Hasselbalch equation

$$pH = pK_a + \log \frac{[ionized]}{[nonionized]} \qquad (6.3)$$

The extent of dissociation is more greatly affected by changes in urinary pH for drugs with a pKa of 5 than with a pKa of 3. Weak acids with pKa values of less than 2 are highly ionized at all urinary pH values and are only slightly affected by pH variations.

TABLE 6.2 Effect of Urinary pH and pK_a on the Ionization of Drugs

pH OF URINE	PERCENT OF DRUG IONIZED: pK_a=3	PERCENT OF DRUG IONIZED: pK_a=5
7.4	100	99.6
5	99	50.0
4	91	9.1
3	50	0.99

For a weak base drug, the Henderson-Hasselbalch equation is given as

$$pH = pK_a + \log + \frac{[nonionized]}{[ionized]}$$

The greatest effect of urinary pH on reabsorption occurs with weak base drugs with pKa values of 7.5-10.5.

For example, amphetamine, a weak base, will be reabsorbed if the urine pH is made alkaline and more lipid soluble nonionized species are formed. In contrast, acidification of the urine will cause the amphetamine to become more ionized (form a salt). The salt form is more water soluble and less likely to be reabsorbed and has a tendency to be excreted into the urine more quickly. In the case of weak acids (such as salicylic acid), acidification of the urine causes greater reabsorption of the drug and alkalinization of the urine causes more rapid excretion of the drug.

DRUG CLEARANCE

Drug clearance is a pharmacokinetic term for describing drug elimination from the body without identifying the mechanism of the process. Drug clearance is described in terms of volume of fluid clear of drug per time unit (eg, mL/min). For example, if the ClT of penicillin is 15 mL/min in a patient and penicillin has a VD of 12 L, then from the clearance definition, 15 mL of the 12 L will be cleared of drug per minute. Alternatively, ClT may be defined as the rate of drug elimination divided by the plasma drug concentration. This definition is a practical way to calculate clearance based on plasma drug concentration data.

$$Cl_T = \frac{\text{elimination rate}}{\text{plasma concentration}(C_p)}$$

$$\text{Elimination rate} = \frac{dD_E}{dt} = C_p Cl_T$$

As a first-order elimination rate, is equal to k Cp VD, so

$$Cl_T = \frac{kC_p V_D}{C_p} = kV_D$$

As the plasma drug concentration decreases during elimination, the rate of drug elimination decreases accordingly, but clearance remains constant. Clearance is constant as long as the rate of drug elimination is a first-order process.

Drug clearance (total body clearance) considers the entire body as a single drug-eliminating system. Just as the elimination rate constant (k) represents the sum total of all the rate constants for drug elimination, including excretion and biotransformation, Cl T is the sum total of all the clearance processes in the body, including clearance through the kidney (renal clearance), lung, and liver (hepatic clearance).

$$\text{Renal clearance} = k_e V_D$$
$$\text{Lung clearance} = k_l V_D$$
$$\text{Hepatic clearance} = k_m V_D$$
$$\text{Body clearance} = k_e V_D + k_l V_D + k_m V_D$$
$$= (k_e + k_l + k_n) V_D = kV_D$$

A body clearance of a drug reflects all the distribution and elimination processes of the drug in the body. The volume of distribution and elimination rate constant are affected by blood flow.

For any organ, clearance may be defined as the fraction of blood volume containing drug that flows through the organ and is eliminated of drug per unit time. From this definition, clearance is the product of the blood flow (Q) to the organ, and the extraction ratio (ER). The ER is the fraction of drug extracted by the organ as drug passes through.

$$\text{Clearance} = Q(\text{ER})$$

If the drug concentration in the blood (Ca) entering the organ is greater than the drug concentration of blood (Cv) leaving the organ, then some of the drug has been extracted by the organ. The ER is Ca - Cv divided by the entering drug concentration (Ca)

$$\text{ER} = \frac{C_a - C_v}{C_a}$$

ER is a ratio with no units. The value of ER may range from 0 (no drug removed by the organ) to 1 (100% of the drug is removed by the organ). An ER of 0.25 indicates that 25% of the incoming drug concentration is removed by the organ as the drug passes through.

Substituting for ER into last equation yields

$$Cl = Q\left(\frac{C_a - C_v}{C_a}\right)$$

The physiologic approach to clearance shows that clearance depends on the blood flow rate and the ability of the organ to eliminate drug, whereas the classical definitions of clearance is that a constant or static fraction of the volume in which the drug is contained is removed per unit time by the organ. However, clearance measurements using the physiologic approach require invasive techniques to obtain measurements of blood flow and extraction ratio.