Protein Binding of Drugs

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ABSTRACT

Basic considerations involved in the interaction of drugs with proteins are reviewed. The important role of the concentration of unbound drug in determining therapeutic and toxicologic effects is emphasized. Ultrafiltration is suggested to be the method of choice for the determination of concentrations of unbound drug in plasma in clinical practice.

Introduction

The interaction of drugs with plasma proteins has been extensively studied. A review article by Meyer and Guttman in 1968 cited almost 600 references. Other reviews have focused on particular classes of drugs, especially antibiotics. Despite this vast amount of information, there has often been some confusion about the therapeutic significance of drug-protein interactions. It is the purpose of this paper to offer some basic principles that may explain the pharmacologic and toxicologic activities of drugs, as they are affected by drug protein interactions. To maintain clarity, the treatment will be kept as simple as possible, and several complicating factors will not be discussed.

Basic Considerations

Consider a molecule of drug A in solution in plasma, as shown in figure 1. In this state, it will encounter molecules of protein in solution. Because drug molecules are much smaller than protein molecules, they may interact with localized regions of the protein. The drug may or may not experience a physical interaction with such a region. Albumin seems to be especially well-provided with such interacting sites, although certain drugs may also interact with globulins. It should be emphasized that the drug-protein interaction is usually reversible, in the sense that there is no covalent bonding between the two molecules. Most commonly, there is an interaction between a non-ionic region of the drug and a hydrophobic region of the protein. Basic and acidic drugs may interact with acidic and basic sites, respectively.

Since no chemical bond has been formed, the drug molecule is not permanently attached to the protein molecule. In fact, any particular molecule will spend a fraction of time interacting with a particular type of binding site, other fractions of time interacting with other types of binding sites and the remaining fractions of time free in solution. The term "protein binding" is, therefore, misleading, since the drug molecules are, in general, not permanently bound to the protein molecules, but are simply removed from the
aqueous environment for a fraction of each time period in a dynamic process.

At any time, the amount of drug bound to a particular interacting site on a protein is given by the law of mass action:

\[ [D_i S_i] = k_a [D_f] [S_f] \quad \text{(Eq. 1)} \]

where \([D_i S_i]\) is the molal concentration of drug-site interactions, \(k_a\) is the association constant, \([D_f]\) is the molal concentration of free drug, and \([S_f]\) is the molal concentration of available sites not currently interacting. The term \([D_i S_i]\) may be replaced by \(r [P]\), where \(r\) is the number of occupied sites, each site containing one molecule of drug, and \([P]\) is the molal protein concentration. Similarly, \([S_f]\) may be replaced by \((n-r) [P]\), where \(n\) is the total number of sites available for binding per mole of protein. Equation 1 then becomes:

\[ r = k_a (n-r) [D_f] \quad \text{(Eq. 2)} \]

which may be rearranged to yield equation 3:

\[ r = \frac{n k_a [D_f]}{1 + k_a [D_f]} \quad \text{(Eq. 3)} \]

This equation may be used to calculate \(r\) for different values of \([D_f]\), when \(n\) and \(k_a\) are known. The molal concentration of bound drug is then equal to \(r [P]\). In another rearrangement, equation 2 gives the Scatchard equation:

\[ \frac{r}{[D_f]} = k_a n - k_a r \quad \text{(Eq. 4)} \]

When \(\frac{r}{[D_f]}\) is plotted as a function of \(r\), the slope of the line is \(-k_a\) and the intercept on the abscissa is equal to \(n\). Inversion of equation 3 gives the reciprocal relationship:

\[ \frac{1}{r} = \frac{1}{n} + \frac{1}{n k_a [D_f]} \quad \text{(Eq. 5)} \]

When \(\frac{1}{r}\) is plotted as a function of \(\frac{1}{[D_f]}\), the slope of the line is \(\frac{1}{n k_a}\), the intercept on the ordinate is \(\frac{1}{n}\). This equation is similar to the Lineweaver and Burk equation for enzyme kinetics and demonstrates the analogy between drug-protein and substrate-enzyme interactions. To calculate \(r\), the molal concentration of bound drug is divided by \([P]\), which must be known. Invariably, however, more than one type of interacting site is involved, so that equation 2 becomes:

\[ r = [D_f] ((n_1-r_1) k_{a1} + (n_2-r_2) k_{a2} + \ldots) \quad \text{(Eq. 6)} \]

The plot based on equations 4 and 5 will not be linear, but will show curvature indicating more than one class of binding sites. It has been assumed in the preceding discussion that binding sites of one class are too distant to react with each other. This is not always the case. The presence of a maximum in the Scatchard plot is indica-
tive of cooperativity between binding sites.4

Therapeutic Considerations

The drug molecule on its path between plasma and its site of action must pass through one or more biological membranes, usually by passive diffusion. Under equilibrium conditions, the concentrations of free drug molecules on either side of the membrane will be equal, as shown in figure 1. If the drug and protein have net ionic charges at physiological pH, the equilibrium will be modified by the Donnan effect. Only those drug molecules that are actually in solution may pass through the membrane. The protein molecules are normally too large to pass the membrane. There is some albumin in interstitial fluid, and tissues contain other proteins that may interact with drug molecules. In particular, the receptors that are involved in the pharmacologic or toxic effect of the drug may have large association constants. It is hazardous to assume that the drug that is bound in plasma is "unbound" in tissues. However, it is often the case that the activity of a drug is dependent upon its unbound concentration in plasma. This follows from equation 2, which may be restated as:

\[ [D_f] = \frac{r_1}{k_{a1}(n_1-r_1)} = \frac{r_2}{k_{a2}(n_2-r_2)} = ... \]

(Eq. 7)

This equation suggests that the concentration of free drug in extracellular fluids should be the same in tissue water as in plasma water, irrespective of the relative binding strengths of plasma and tissue proteins. In reality, equality of concentration across the membrane may not be found, because of such factors as active transport and the Donnan effect. In addition, this relationship does not take account of the fact that a finite time is required for equilibration between body compart-

ments. However, when conditions approach a steady state (after repeated administration of a drug at intervals not much longer than the \( t_4 \) for the elimination of the drug), it would be expected that concentrations of unbound drug would be similar in tissue water and plasma water. There is considerable scientific evidence, especially for antibiotics\(^2\,5\,6\,17\) but also for other drugs\(^1\,10\,12\) that there may be a better correlation of pharmacologic response with the concentration of unbound drug in plasma than with the total plasma concentration.

It has been stated\(^6\) that the effect of protein binding on drug distribution is important only when the drug is highly bound (e.g., >80 percent bound). Several authors\(^3\,9\,15\) have prepared curves demonstrating the pharmacokinetic consequences of plasma protein binding of drugs, which support this statement.

Toxicologic Considerations

Another example of the misleading nature of the term "protein binding" is illustrated in figure 2. If plasma proteins contained an infinite number of interacting sites, the percentage of drug molecules interacting with protein would be constant for all concentrations of the drug, since it would depend only on \( k_a \). For many drugs,
this percentage is approximately constant over the range of therapeutic concentrations. However, when the amount of drug in plasma is increased and the capacity of available sites is exceeded, there will be a dramatic increase in concentration of free drug, as shown in figure 2. This may be accompanied by only a slight decrease in the percentage of bound drug, but may have a profound effect upon the pharmacologic and toxicologic effects of the drug. It must be emphasized that this is a simplified treatment, since figure 2 represents an isolated in vitro system. In a mammalian system, the unbound drug would tend to migrate into the tissues, thereby reducing its concentration in plasma. The net result would be to increase the amount of drug in the tissues.

Methodology

The four common methods, equilibrium dialysis, ultrafiltration, ultracentrifugation and gel filtration, have recently been evaluated by Kurz et al. and will not be discussed here in detail. Essentially, in equilibrium dialysis and ultrafiltration, the aim is to recreate the conditions shown in figure 1 for glomerular filtration. In equilibrium dialysis, a buffer solution is provided into which the drug molecules migrate. This disturbs the equilibrium of drug-protein interaction. Eventually, a new equilibrium is reached, at a lower plasma concentration. This method, therefore, cannot be used to determine the concentration of free drug in the plasma of a patient and, in addition, it is relatively time-consuming.

In ultrafiltration, aqueous phase containing dissolved free drug passes through the membrane. The total concentration of protein in the plasma sample is thereby increased so that volumes of ultrafiltrate should be small, less than 10 percent of the original sample. This method has the advantage of providing the investigator with a good approximation to the concentration of unbound drug in the patient’s plasma and can be performed rapidly. Ultrafiltration has recently been evaluated by the present authors. The aim in ultracentrifugation and gel filtration is to separate the drug-protein complex from the plasma water containing free drug. These methods are technically more difficult and appear to offer no advantages over ultrafiltration and equilibrium dialysis.

Conclusions

That the concentration of drug which is free in plasma is often a more useful indicator of therapeutic activity than the total drug concentration has long been conjectured. In this article, the theoretical basis for this conjecture has been established. Several recent publications provide direct supportive evidence. The relationship of concentrations of free drug to total drug may vary as a function of drug concentration, total protein concentration and albumin concentration. It is suggested that concentrations of free drug in plasma be determined whenever a patient is receiving a drug that is subject to extensive interaction with plasma protein or is being administered with other drugs that may compete for binding sites. Such determinations may readily be made by utilizing the technique of ultrafiltration.

References

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