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Pharmacokinetics made easy 8

DRUG PROTEIN BINDING

D. J. Birkett, Professor of Clinical Pharmacology, Flinders University of South Australia, Adelaide

1. What is protein binding?

Drug protein binding is the reversible interaction of drugs with proteins in plasma. It also includes the reversible interaction of drugs with red blood cell and tissue membranes and other blood constituents. It can be represented as:

free drug + free protein \rightleftharpoons drug-protein complex equation 1

It is important to remember that the process is reversible and that the rates of drug binding and release are very fast, occurring in the millisecond range. This means that if, for example, liver cells very efficiently extract free drug from the blood, the drug-protein complex can rapidly dissociate and drug initially bound to protein can be extracted in one pass through the liver (see Article 4 'How drugs are cleared by the liver', *Aust Prescr* 1990; 13:88-9).

2. What are the binding proteins in plasma?

The major drug binding proteins in plasma are:

- albumin
- α_1 -acid glycoprotein
- lipoproteins.

Albumin and α_1 -acid glycoprotein have structurally selective binding sites for drugs, in the same way that the active sites of enzymes are structurally selective for substrates.

Each albumin molecule has at least 6 distinct binding sites for drugs and endogenous compounds. Two of these very tightly and specifically bind long chain fatty acids. There is another site which selectively binds bilirubin. There are two major drug binding sites called site I and site II which mainly bind acidic drugs. Site I binds drugs such as warfarin and phenylbutazone, whereas site II binds drugs such as diazepam and ibuprofen. Drugs which bind at the same site can be predicted to displace each other competitively when administered together.

α_1 -acid glycoprotein is an acute phase reactant which has one binding site selective for basic drugs such as disopyramide and lignocaine.

'Binding' of drugs to lipoproteins and red cell and other membranes is more a dissolving of the drugs in the lipids of the membrane rather than a true binding reaction. Very lipid soluble drugs partition preferentially into the membrane lipids rather than the plasma water. Some drugs bind strongly to particular tissue components such as DNA (e.g. some anticancer drugs and quinacrine) and melanin-rich tissues (e.g. chloroquine, amiodarone).

3. What determines extent of binding to plasma proteins?

The binding of a drug to a protein binding site is a saturable process governed by the same mass action expression that describes the interaction of a substrate with an enzyme binding site. The extent to which a drug is bound in plasma or blood is usually expressed as the fraction unbound (f_u).

$$\text{fraction unbound } (f_u) = \frac{\text{unbound drug concentration}}{\text{total drug concentration}} \quad \text{equation 2}$$

The tighter the binding, the lower is the fraction unbound. The distinction between *fraction* unbound and unbound concentration is important as we shall see below. The fraction unbound of a drug is determined by:

- the affinity of the drug for the protein
- the concentration of the binding protein
- the concentration of drug relative to that of the binding protein.

In most cases, drug concentrations at therapeutic doses are well below those of the binding protein and the fraction unbound is constant across the therapeutic range of drug concentration. However, the concentration of α_1 -acid glycoprotein is relatively low, and saturation of the binding sites can occur in the therapeutic range. An example is disopyramide where the unbound concentration increases linearly with dose, but there is a less than proportionate increase in total concentration as saturation occurs causing fraction unbound to increase. Albumin concentrations are high, and saturation rarely occurs with drugs binding to this protein. An exception is salicylate which has high therapeutic concentrations.

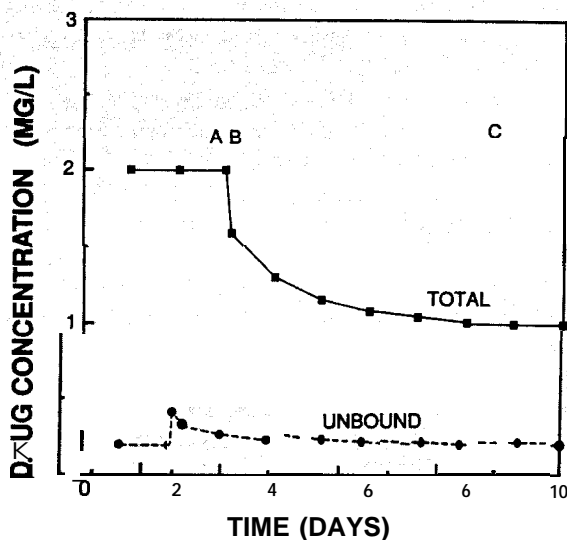
Fig. 1

Sequence of events following displacement of a highly protein bound drug.

Initially, the total concentration is 2 mg/L and the unbound concentration 0.2 mg/L giving a fraction unbound of 0.1. Displacement occurs at A with addition of a displacing drug which is then continuously present. The fraction unbound increases to 0.2 and the unbound concentration doubles to 0.4 mg/L with no change in total concentration.

At B, redistribution occurs over minutes to hours resulting in decreases in both total and unbound concentrations. The clearance of unbound drug is not changed so the unbound concentration ($C_{uss} = \text{dose rate}/CL_u$) falls back to the initial value over 3-5 half-lives (haM-life is 24 hours in this case).

At C, the final situation in the presence of displacing drug, total concentration is reduced by 50% to 1 mg/L, unbound concentration is the same as initially at 0.2 mg/L and fraction unbound is increased from 0.1 to 0.2. There is no change in drug effect. The example is based on warfarin displacement interactions except that the fraction unbound has been increased from 0.02 to 0.2 for graphical clarity.



The concentration of albumin is decreased in liver disease and renal disease resulting in decreased drug binding. Alpha I-acid glycoprotein is an acute phase reactant and concentrations increase in rheumatoid arthritis and post-myocardial infarction resulting in increased drug binding in these situations,

The binding affinity can be changed due to competition from endogenous compounds such as fatty acids, or from other drugs competing for the same protein binding sites.

4. Are protein binding drug interactions important?

Much has been made of the significance of protein binding interactions based on 'test-tube' experiments. For example, warfarin is about 98% bound and 2% unbound ($f_u = 0.02$). If a competing drug reduces the binding from 98% to 96%, the *in vitro* unbound fraction rises from 0.02 to 0.04, a twofold increase. If nothing else happened, this would represent a twofold increase in the active, unbound concentration.

In vivo, however, two compensating mechanisms operate as shown in Fig. 1. As described in Article 2 (Aust Prescr 1988;11:36-7), the volume of distribution (V_D) is dependent on the ratio of fraction unbound in blood and tissues (f_u/f_{ut}).

Table 1

Protein binding and therapeutic range of phenytoin

Phenytoin fraction unbound average 0.1 in normal patients, but increases up to twofold in renal failure patients (GFR < 20 mL/min) due to low albumin concentration and accumulation of competing endogenous compounds.

Patient	f_u	Therapeutic range based on total drug mg/L	Therapeutic range based on unbound drug mg/L
Normal	0.1	1.0 - 2.0	1-2
Renal failure	0.2	5-10	1-2

If the fraction unbound in blood increases because of competitive displacement without a change in tissue binding, the volume of distribution increases as the displaced drug 'spreads out' and is bound in the tissues. This happens quickly within minutes to hours. Secondly, as described in Article 4, fraction unbound is a factor in the clearance of total drug (clearance = fraction unbound X intrinsic clearance), but clearance of unbound drug is determined only by intrinsic clearance and does not depend on protein binding. Therefore, when fraction unbound increases due to displacement, drug is eliminated more rapidly until the unbound (active) steady state concentration returns to the starting point. The end result is an increase in unbound fraction, a decrease in total drug concentration, but no change in the steady state unbound concentration (see equation 2). Total concentration at steady state is reduced in proportion to the increase in fraction unbound. This takes 3-5 half-lives to occur. In general, the only situation where unbound drug concentration at steady state is dependent on the degree of protein binding is that of high hepatic clearance drugs given intravenously (e.g. lignocaine, see Article 4).

In nearly all cases where a clinically important protein binding interaction has been postulated, other mechanisms, such as concurrent inhibition of drug metabolism, have been shown to be the *in vivo* cause of the increase in drug effect. This is why it is so important in studies of drug interactions to measure both total and unbound drug in determining the mechanism(s). Except in very rare circumstances, protein binding displacement *in vivo* does not result in increased drug effect.

5. Protein binding and therapeutic drug monitoring

Drug assays for therapeutic drug monitoring nearly always measure total drug. As it is the unbound drug which is active, a false impression can be gained if the fraction unbound is changed substantially. This is illustrated in Table 1 for phenytoin. Phenytoin binding to albumin is reduced particularly in renal failure patients, but also in some other situations such as liver disease or the presence of competing drugs. In such cases, total concentration measurements are misleading and control of therapy needs to be based on measurement of unbound phenytoin concentration. Such measurements are available in specialised centres, but are expensive and currently are carried out only in special circumstances such as those mentioned above.

6. Conclusions

Protein binding is important in determining the cause of changes in total clearance due to drug interactions and in interpreting results of therapeutic monitoring of some drugs. In general, changes in protein binding do not cause clinically important drug interactions.