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TISSUE BINDING OF DRUGS

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Studies on drug-protein binding in pharmacokinetics were restricted to plasma and its proteins for several decades. Only the past ten years have seen an increasing interest in tissue binding. In a sense, this is not a logical development, because "if plasma protein binding of a drug is considered significant, it is difficult to believe that tissue binding of a drug would not also be significant" (Benet 1978). Indeed, the importance of tissue binding had long been suggested by the fact that 98 percent of all protein is located extravascularly and that these and other tissue macromolecules are likely to contain binding sites for drugs. Furthermore, it has long been known that volumes of distribution in excess of the total water content of the body must represent tissue binding. There is no doubt that the discrepancy between importance of and interest in tissue binding has been correctly explained in what is considered as the first review on drug-protein binding: "Indeed, the difficulties inherent in studying interactions with the proteins of fixed tissues present a sharp contrast to the readiness with which those of the plasma lend themselves to investigation" (Goldstein 1949).

In recent years, an increasing number of tissue binding studies have been published. Most of them used a selection of physicochemical binding techniques that had been developed in the study of plasma protein binding. These techniques were then extended to characterize drug binding in vivo, in perfused organs, tissue slices, homogenates, subcellular fractions, or on the level of molecular binders such as proteins, DNA, mucopolysaccharides, melanin, phospholipids. Results were reported on the mere fact of tissue binding or its localization, but also on its quantitation or the binding forces involved. The development can be followed in a number of

reviews (Gillette 1973a, 1973b; Jusko and Gretch 1976; Wilkinson 1983; Kurz and Fichtl 1983). The latter review not only quotes more than 100 individual studies and their major results, but also discusses the particular technical problems involved in tissue binding studies as pointed out above. Wilkinson's review provides an in-depth discussion of the pharmacokinetic consequences of both plasma and tissue binding.

PHARMACOKINETIC CONSEQUENCES OF TISSUE BINDING

Tissue binding is a major determinant of drug distribution in terms of both volume of distribution (as a measure of gross overall distribution) and site-specific localization. However, recent work has demonstrated that the consequences of binding on distribution and elimination are not simple and depend on the particular pharmacokinetic characteristics of the drug in question. For instance, the view that only the free concentration of a drug is available and functional was based on findings in vitro and is only partially correct. If only free drug is available for excretion, metabolism, receptor interaction, or other cellular processes, then the transport processes are called restrictive. This is the case when free drug is transported across a permeability barrier together with its osmotic share of plasma water, so that the remaining free concentration is unchanged. In contrast, a non-restrictive transport is increasingly emphasized. This type is characterized by the free drug being transported selectively through a membrane—i.e., out of the plasma water rather than with it. Non-restrictive transports occur wherever binding or disappearance of the drug creates a concentration gradient or a so-called "sink effect." In such a case, the free concentration before the permeability barrier decreases and the disturbed binding equilibrium will be restored by dissociation of drug-protein complex. Thus, in this type of transport process, total drug concentration (free and bound) becomes available—i.e., the availability is not restricted to free drug. The oral first-pass effect of a drug is not only a "sink effect" due to rapid elimination but also, at least initially, due to reversible tissue binding in the first-pass-organ (Stegmann and Bickel 1977; Wedlund and Wilkinson 1984). If displacement of tissue-bound drug were to occur, then this would result in a decrease of the apparent volume of distribution (Houin, Barre, and Tillement 1984). This contrasts with displacement of plasma-bound drug which results in increased volumes of distribution.

The simplest expression which relates volume of distribution to tissue binding is given by

$$V_{D(tot)} = V_{pl} + V_{tiss} \cdot \frac{f_{f(pl)}}{f_{f(tiss)}}$$

where

V_{pl} = plasma volume
V_{tiss} = volume of the other body tissue water
f_f = free (unbound) fractions in plasma and tissue, respectively.

The tissue term of this equation may be broken down into more specific binding components. The general form of the equation shows that the distribution of a drug across the physiological compartments ($V_{D(tot)}$) varying from 5 to more than 10,000 l in an adult person) is the resultant of plasma and tissue binding. The volume of distribution increases with increasing tissue binding.

In contrast to distribution, clearance as irreversible removal of drug is not influenced by tissue binding. On the other hand, elimination half-life is a function of both clearance and volume of distribution and hence can be influenced by tissue binding. Nonlinear or concentration-dependent tissue binding is likely to occur at high drug concentrations and high volumes of distribution. The pharmacokinetic consequences of this type of tissue binding have been little investigated.

BINDING COMPETITION

Most drugs are bound to both tissue components and plasma proteins. This fact strongly suggests that overall distribution (volume of distribution V_D) results from a binding competition between tissue and plasma. This is also expressed in the above equation. Theoretically, increasing the plasma-bound fraction of a drug from 0 to 1 in the absence of tissue binding would lead to a decrease of V_D from total body water volume to plasma volume. In contrast, increasing the tissue-bound fraction from 0 to 0.99 would lead to a dramatic increase of apparent V_D up to the order of 10^3 to 10^4 liters in an adult human.

Binding competition can be simulated and studied by a simple in vitro technique named distribution dialysis (Bischoff and Stauffer 1957; Kallee, Lohss, and Oppermann 1957; Bickel and Gerny 1980). When equilibrium dialysis is used for the purpose of binding studies, then a drug or other ligand is dialyzed against plasma, an individual

protein, or other binders. Once diffusion equilibrium is reached, the free and bound drug concentrations can be determined. By contrast, in the distribution dialysis technique, both dialysis chambers contain a binder, and a drug is allowed to distribute between the two different binding systems. Distribution is then expressed as drug concentration ratio of the two chambers—e.g., concentration ratio intracellular/extracellular preparation if a tissue preparation and plasma are used. The technique has been tested with respect to various drugs and binders, and the influences of their concentrations (Bickel and Gerny 1980).

We have tested a number of drugs with the distribution dialysis technique. The drugs were dissolved in diluted whole blood (10 percent) contained in one dialysis chamber and dialyzed until equilibrium against tissue homogenates (10 percent) in the other chamber. Distribution was expressed as the concentration ratio c_i/c_e (intracellular/extracellular preparation). In addition, binding of the drugs to the individual binders was determined by conventional equilibrium dialysis. Kurz and Fichtl (1983) have subdivided drugs into types I, II, and III according to the relation between their tissue and plasma binding. We have added a type 0 for drugs not bound by either tissues or plasma. The results of this investigation are given in Table 9.1 for one or two drugs per type. These results strongly suggest that

TABLE 9.1
Intracellular (i)/Extracellular (e) Binding Competition

Type	Binding Ratio	Prototypes	Binding i	Binding e	Concentration Ratios: Distribution Dialysis i/e*	Concentration Ratios: In Vivo tiss/bl
0		Phenazone	0	0	1.0	1.0
I	tiss < pl	Salicylic acid	0	+	0.9	0.4
		Phenylbutazone	+	++	0.8	0.5
II	tiss ≈ pl	Pentobarbital	+	+	1.2	1.5
		Thiopental			2.0	2.0
III	tiss > pl	Imipramine	++	+	3-13†	4-30†

*Tissue homogenates/whole blood.
†Depending on tissue used.
tiss = tissue, bl = blood, pl = plasma.

distribution between the two compartments is determined by the binding of the drugs to the competing binders present in each compartment. The ratio c_i/c_e, as determined by distribution dialysis, was 1.0 for the type 0 drug, phenazone (Antipyrine), which is not bound to blood or tissue components. As expected, the c_i/c_e values of the type I drugs, salicylic acid and phenylbutazone, are below unity, showing the predominance of plasma binding. Type II drugs are expected to have values around unity, which is the case for pentobarbital. The value of 2.0 for thiopental is due to the high concentrations in adipose tissue, a typical storage tissue for this drug. Thus, thiopental is partly a type III drug. Finally, the typical type III drug, imipramine, yields c_i/c_e values far above unity, indeed for most tissues around 10. If distribution in the much more complex system of an organism were also determined by binding competition, then the tissue/blood concentration ratios obtained in vivo should be comparable to the c_i/c_e values from distribution dialysis. The last column of Table 9.1 shows that this is indeed the case for the drugs studied so far.

ADIPOSE TISSUE STORAGE OF DRUGS

Accumulation and storage of drugs in adipose tissue can be considered as a particular form of tissue binding. Studies on the distribution of thiopental and polychlorinated hydrocarbons (e.g., DDT) in the 1950s had led to the notion, still widely accepted today, that lipophilic drugs accumulate and are stored in adipose tissue. This is believed to be a simple partition between the aqueous and lipid phases of the body. Recent studies (Bickel 1984) have shown that the expected correlation between adipose tissue storage and lipophilicity (octanol/water partition coefficient P) does not exist. In fact, many highly lipophilic compounds, such as basic drugs, are not stored and often have their lowest concentrations in adipose tissue. These and other findings suggest that the lipophilicity concept of adipose tissue storage needs to be re-evaluated. Table 9.2 contains a selection of drugs and other xenobiotics of widely differing lipophilicities (log P from 0.75 to 6.5). Clearly, there is no correlation between adipose tissue storage (in vivo) and lipophilicity. Several drugs with log P values much higher than thiopental show negligible adipose tissue storage. Structural features of the drugs may therefore be considered as an additional factor influencing adipose tissue storage. Thiopental is known to undergo a redistribution from blood to highly perfused viscera, to poorly perfused tissues like muscle and skin, and ultimately, to adipose tissue where maximum concentrations are reached about one hour after administration.

TABLE 9.2
Adipose Tissue Storage of Drugs

Compound	Lipophilicity log P*	Storage in Vivo ASI†	Uptake in Vitro percent‡
Morphine	0.75	0.17	8
Glutethimide	1.9	2.0	48
Pentobarbital	2.1	1.2	29
Thiopental	2.8	4.1	71
Phenylbutazone	3.25	0.55	55
Imipramine	4.6	0.56	87
Chlorpromazine	5.3	0.43	85
DDE	5.8	3.7	>95
6-CB	6.5	10.0	>95

Notes:

*Partition coefficient octanol/water.

†Adipose Storage Index (Bickel 1984). Values > 1 indicate adipose tissue storage, < 1 no storage.

‡Relative uptake into adipose tissue slices in percent of total amount of drug in tissue at equilibrium.

DDE = lit-dichloro-2,2-bis(p-chlorophenyl)ethene.

6-CB = 2,4,5,2',4'5'-hexachlorobiphenyl.

This redistribution pattern is clearly determined by the perfusion rates of the individual tissues. Our studies have shown that DDE and the virtually unmetabolizable 2,4,5,2',4',5'-hexachlorobiphenyl (6-CB) undergo a qualitatively identical redistribution pattern. However, the time to reach maximum concentrations in adipose tissue is so much longer (35-42 days) than with thiopental that this cannot be attributed to the slow perfusion rate of adipose tissue. As an explanation, one could assume a permeability barrier between blood and adipose tissue which would slow down the uptake of 6-CB and prevent that of imipramine or chlorpromazine. However, as is also shown in Table 9.2, in vitro uptake of drugs into adipose tissue (slices) does parallel log P. This fact speaks against the existence of a local permeability barrier. On the other hand, the very difference between uptake into adipose tissue in vitro and in vivo yields a

clue for better understanding. The difference of the two systems is the presence of non-adipose (lean) tissues in the in vivo situation, and hence the possibility of a binding competition between adipose and lean tissues. In addition, lean tissues as potential competitors (viscera, muscle, skin), are provided with more than 90 percent of the drug-containing cardiac output, as compared with a mere 5 percent for adipose tissue. Thus, lean tissue binding of a drug will strongly influence its appearance or non-appearance in adipose tissue.

Based on these findings, a unifying concept of adipose tissue storage has been proposed (Bickel 1984). It consists of a physiologic pharmacokinetic model based on tissue sizes and perfusion rates as the constant framework, and on tissue binding and elimination rate as the variable parameters. While elimination is a modifying factor, tissue binding is the chief determinant of adipose tissue storage. Competition between binding to lean tissues and to adipose tissue determines adipose invasion rate which, in turn, determines whether and to what extent adipose tissue storage occurs. Structural elements of the drugs influence binding to lean tissues, whereas the partition coefficient influences affinity to adipose tissue. This flow-limited binding competition model explains the different types of kinetic behavior of prototype drugs (e.g., thiopental, basic lipophilic drugs, 6-CB) and seems to be applicable to any drug.

SUMMARY

Only in recent years was tissue binding studied on a large scale. There can be no doubt that tissue binding is a major determinant of the distribution of a drug in the body. However, the precise influence of tissue binding on distribution and elimination is rather complex. It is dependent on a variety of factors like the presence of various binders, the particular pharmacokinetic characteristics of the drug, and the possibility of non-linear binding.

Since most drugs bound to tissues are also plasma protein-bound, a binding competition is likely to occur. This can be simulated and studied by the in vitro technique of distribution dialysis. The use of this technique has shown that drugs distribute according to their binding to individual extracellular and intracellular binders. Since the distribution values obtained with this in vitro system are comparable with those obtained in vivo, it is concluded that overall distribution of a drug in the body is indeed a function of binding competition. Recent studies on adipose tissue storage of drugs have shown that this process is the result of a binding competition between adipose

and non-adipose tissues rather than a partition between the aqueous and lipid phases of the body.

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