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Adipose tissue storage of drugs as a function of binding competition. In-vitro studies with distribution dialysis

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Abstract—Distribution dialysis was used to study binding competition between homogenates of adipose tissue and of lean tissues. The concentration ratios adipose/X (X = blood, muscle, lung, liver) of eight lipophilic drugs were determined in the absence and in the presence of a competing binding system X. With drugs which do not undergo storage in adipose tissue in-vivo, yet have a high volume of distribution, such as imipramine or desipramine, there was strong binding competition, and the balance of distribution was shifted from adipose to lean tissues. In the case of indomethacin with a low volume of distribution this shift was from adipose tissue to blood. With diazepam there was a marked binding competition which was not, however, sufficient to shift the balance of distribution away from adipose tissue. Binding competition was negligible with thiopentone. In contrast, with the equally lipophilic hexethal a moderate binding competition was observed. This is consistent with a decreased adipose tissue storage of the latter barbiturate. It is concluded that binding competition exists not only between blood and tissues but also among individual tissues. It is suggested that occurrence and extent of adipose tissue storage of drugs are determined by binding competition between lean and adipose tissues and, more generally, that distribution of lipophilic drugs is largely a function of binding competition.

Non-specific binding to both blood components and tissues has been recognized as a major determinant in the distribution of drugs in the organism (Øie 1986; Tillement et al 1988; Fichtl et al 1991). Recent studies of the particular aspect of accumulation and storage of drugs in adipose tissue have shown that such storage cannot be explained as a mere partition process since it is not a function of lipophilicity (Bickel 1984). Whereas many lipophilic xenobiotics are stored in adipose tissues, many others, for instance most basic drugs (Betschart et al 1988), are not. On the other hand, uptake of drugs into adipose tissue preparations in-vitro in the absence of other tissues is clearly a function of lipophilicity. This brought about the hypothesis that in-vivo the presence of other (lean) tissues, including blood, may diminish or prevent adipose tissue storage by exerting a binding competition between lean and adipose tissues (Bickel 1984). Binding competition can be simulated and quantified in-vitro with the technique of distribution dialysis. In this modification of equilibrium dialysis both dialysis chambers contain a binder, and drug is allowed to distribute between the two different binding systems. Using this technique the distribution in-vitro of a variety of drugs between tissue homogenates and blood has been studied (Bickel & Gerny 1980; Bickel et al 1987; Clausen & Bickel 1993). The latter work demonstrated that distribution could be predicted from binding values. Thus, distribution in this in-vitro system is the result of a binding competition between tissue and blood.

In this study, distribution dialysis was used to determine the binding competition between adipose tissue and lean tissues including blood. Eight lipophilic model drugs were selected so as to include four which are stored in adipose tissue in-vivo and four which are not. The results suggest the correctness of the hypothesis that the occurrence and extent of adipose tissue storage of a particular drug is determined by binding competition between adipose and lean tissues.

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Materials and methods

Drugs. The following radioactively labelled drugs were used: [10,11-^{3}H]desipramine (NEN, Boston, MA, USA), [methyl-^{3}H]diazepam, [2-^{14}C]indomethacin, [4-^{14}C]phenytoin (NEN Research Products, Du Pont, UK), [ring-U^{14}C]phenylbutazone (Ciba-Geigy, Basel, Switzerland). Unlabelled hexethal (5-ethyl-5-*n*-hexylbarbituric acid) was given by Professor M. Rowland, Manchester, UK.

Distribution dialysis. The technique of two-chamber distribution dialysis with a Dianorm apparatus was used as previously described (Bickel et al 1987; Clausen & Bickel 1993). The two Teflon chambers (1 mL each) were separated by a cellulose dialysis membrane (Union Carbide) with a 12 000 Da cutoff and 25 μm thickness. In this study, model drugs were allowed to distribute between adipose tissue homogenate in the one chamber and blood, plasma, or homogenates of skeletal muscle, lung, or liver in the other. In control experiments, the distribution of the drugs was measured between adipose tissue and buffer as well as between buffer and buffer. Tissue homogenates were prepared from male Sprague-Dawley rats (SIV U strain, Tierzucht-Institut, University of Zurich), 200–300 g. Epididymal and mesenteric adipose tissue was sonicated (2 × 30 s at 20 kHz) before homogenization with a glass homogenizer. Homogenates (100 μg mL^{-1}) were used. Human or rat whole blood (or plasma thereof), stabilized with sodium citrate, was used at a dilution of 100 μL mL^{-1}. Identical results were obtained with human and rat blood. Phosphate buffer (0·01 M, pH 7·4, containing 0·9% NaCl) served for all operations. Unless otherwise stated the drug was added to the chamber not containing adipose tissue. Drug concentrations were within the range of therapeutic plasma concentrations. All experiments were carried out at 37°C. The time to reach distribution equilibrium was less than 4 h. After a distribution time of 4 h the drug concentrations in the two chambers were determined and distribution expressed as the concentration ratio adipose tissue/·opposing phase X (X = buffer, blood, or homogenate of a lean tissue).

Uptake into adipose tissue slices. Diazepam (25 μM, 900 μL) and slices of rat epididymal adipose tissue (100 mg) were incubated at 37°C for 3 or 6 h in closed borosilicate vessels in a shaker according to Di Francesco & Bickel (1985). Controls contained no tissue slices. After incubation the slices were removed and the filtrate of the medium used for analysis of the remaining drug.

Analytical procedures. After distribution dialysis, tissue homogenates (200 μL) were diluted with 800 μL distilled water. Ten millilitres scintillator (Instagel-II, Packard, Warrenville, IL, USA) was added and the radioactivity determined by liquid scintillation counting after correction for counting efficiency. Blood-containing phases (200 μL) were mixed with 5 mL each of Soluene-350 (Packard), isopropanol, and H_2O_2 30%. After an incubation of about 20 min, 10 mL scintillator (9 mL Instagel-II + 1 mL 0·5 M HCl) was added. To decrease chemiluminescence, liquid scintillation counting was performed after 2–3 h.

The unlabelled drug, hexethal, was extracted from tissue homogenates or blood and subsequently determined by HPLC as described by Steiner et al (1991). Buffer phases containing

Table 1. Distribution between homogenates of adipose tissue and tissues of drugs not stored in adipose tissue in-vivo.

Drug (μM)	Buffer	Blood	Muscle	Lung	Liver
Imipramine (5)	10·6±0·9	6·3±0·8	1·1±0·04	0·76±0·03	0·26±0·06
Desipramine (10)	7·9±0·8	6·6±1·1	0·80±0·06	0·53±0·15	0·21±0·05
Indomethacin (25)	1·1±0·03	0·17±0·02	0·59±0·03	—	0·49±0·06
Phenylbutazone (400)	1·9±0·3	1·0±0·05	1·2±0·05	1·3±0·1	1·2±0·1

Concentration ratios adipose/buffer and adipose/tissue ± s.d. of drugs in distribution dialysis. With indomethacin, blood was used instead of plasma.

Table 2. Distribution between homogenates of adipose tissue and tissues of drugs stored in adipose tissue in-vivo.

Drug (μM)	Buffer	Blood	Muscle	Lung	Liver
Diazepam (25)	17·8±2·8	9·1±2·1	6·4±1·1	—	3·5±1·3
Phenytoin (50)	1·8±0·3	1·3±0·2	1·0±0·1	—	0·74±0·14
Thiopentone (100)	4·4±0·4	3·6±0·6	4·1±0·5	3·1±0·4	2·9±0·2
Hexethal (100)	4·0±0·3	1·9±0·4	1·5±0·1	1·6±0·3	1·3±0·2

Concentration ratios adipose/buffer and adipose/tissue ± s.d. of drugs in distribution dialysis. With diazepam and phenytoin blood was used instead of plasma.

Table 3. Physicochemical and distribution characteristics of the model drugs used.

Drug	log P	pK_a	Vd (L kg^{-1})	ASI	Distribution in-vivo
Imipramine	4·6	9·5	23	0·3	Bickel et al (1983)
Desipramine	4·0	10·2	20	0·3	Moor et al (1992)
Indomethacin	3·1	4·5	0·2	0·4	Hucker et al (1966)
Phenylbutazone	3·3	4·5	0·1	< 1·0	Burns et al (1953), Bickel & Gerny (1980)
Diazepam	2·8	3·3	1·1	4·6	Igari et al (1982)
Phenytoin	2·5	8·3	0·6	5·0	Noach et al (1958)
Thiopentone	2·8	7·5	8	5·0	Mühlebach et al (1985), Ebling et al (1989)
Hexethal	2·9	7·7		2·3	Steiner et al (1991)

P, Partition coefficient octanol/water, pK_a, −log ionization constant for the acids, phenytoin, thiopentone, hexethal, indomethacin, phenylbutazone, and the bases, diazepam, imipramine, desipramine. Vd, Volume of distribution. ASI, Adipose storage index (Bickel 1984) for distribution in-vivo.

hexethal were acidified with 0·5 M HCl and underwent direct HPLC analysis.

Results

Binding competition between adipose and other tissues. The distribution of eight model drugs was determined in-vitro using the two-chamber distribution dialysis. After equilibrium of the drugs between adipose tissue homogenate in one chamber and buffer, blood, or homogenates of muscle, lung, or liver in the other, distribution was expressed as $K_p = c_{Ad}/c_X$. The results are given in Tables 1 and 2. Whereas the adipose/buffer experiments show the distribution into the adipose tissue preparation in the absence of any binding competition, such competition may occur in the adipose/blood or adipose/tissue experiments, recognizable by diminished K_p values compared with adipose/buffer.

Uptake of diazepam into adipose tissue slices. The K_p value of the moderately lipophilic drug, diazepam, in the adipose/buffer experiment was unexpectedly high (17·8). Therefore, distribution of diazepam was also determined in the system adipose tissue slices in buffer, which lacks a potentially disturbing dialysis membrane. The tissue/buffer ratio was maintained. After an equilibration time of 3–6 h the resulting K_p values were within the range of 12–13.

Discussion

Experiments aimed at a quality control of the equilibrium dialysis system were carried out with phenytoin, indomethacin, and diazepam. With all three drugs equilibrium between two buffer phases was reached within 4 h and resulted in concentration ratios very close to 1·00. This confirms results with other drugs (Bickel et al 1987). The analytical and inter-experiment reproducibility of the three drugs was within reasonable limits.

The distribution of a drug in the system adipose/buffer represents binding of the drug to adipose tissue homogenate in the absence of binding competition. It has been shown that binding to adipose tissue homogenate (Bickel et al 1987; Clausen & Bickel 1993) as well as uptake in-vitro into other adipose tissue preparations (Di Francesco & Bickel 1985; Betschart et al 1988) is correlated with log P in a linear fashion. The same tendency is apparent with the adipose/buffer ratios in Tables 1 and 2. The one exception is diazepam with a K_p value of 17·8, i.e. far in excess of that of even the more lipophilic drugs, imipramine and desipramine; diazepam, in contrast to the other drugs used, is un-ionized at pH 7·4.

In contrast to the system adipose/buffer the distribution of a drug in the system adipose/tissue shows the influence of binding competition by blood and lean tissues. It has been demonstrated that drug distribution obtained in the distribution dialysis can be calculated from binding values and thus is clearly a function of the binding competition in the system (Clausen & Bickel 1993). In Tables 1 and 2 these binding competitions are represented by the differences of the concentration ratios K_p of adipose/buffer and adipose/tissue. Table 1 demonstrates the results of drugs which are not stored in adipose tissues in-vivo (adipose storage index, ASI < 1) and Table 2 drugs which are stored in this tissue

(ASI > 1). Table 3 shows the ASI values of the eight drugs used and the distribution studies they are based upon, as well as other parameters of pharmacokinetic importance.

Among the drugs not stored in adipose tissue (Table 1) there is strong binding competition which shifts the balance of distribution from adipose to lean tissues. With imipramine and desipramine these binding competitions increase in the sequence blood, muscle, lung, liver; the K_p values are diminished by a factor of 30–40 along this sequence. Whereas binding to blood components has little consequence, binding to lean tissues is dominant and is the reason for the very high volumes of distribution of these drugs. Lung and liver binding may even have been underestimated due to the abolished lysosomotropism in the in-vitro system used (Clausen & Bickel 1993). With indomethacin and, to a lesser degree, phenylbutazone the dominating factor is binding to blood components (serum albumin). This prevents the latter drugs reaching high concentrations in tissues including adipose tissue and explains the very low volume of distribution of these drugs.

With diazepam (Table 2) there is still considerable binding competition of lean tissues against adipose tissue; however, the concentration ratios adipose/lean tissues (K_p) are well above unity, i.e. in favour of adipose tissue. The case of phenytoin is similar, if less pronounced. With thiopentone, a classical fat seeker, binding competition is almost negligible. Of particular interest is the comparison of thiopentone and hexethal. The two barbiturates have comparable log P-values, yet the ASI of the latter compound is much smaller (Table 3). In accordance with equal lipophilicity of the two compounds, the distribution dialysis experiments adipose/buffer yielded equal K_p values; however, in contrast to thiopentone there was a marked binding competition of lean tissues including blood in the case of hexethal. This binding competition is in accordance with the diminished adipose tissue storage of hexethal as compared with thiopentone.

Plasma binding of a drug may restrict its volume of distribution (Vd), whereas tissue binding invariably increases Vd. Hence, distribution largely results from these opposite effects. However, binding competition exists not only between blood and tissues but also among individual tissues. In this work binding competition between adipose and lean tissues was studied. It is concluded that binding competition was weak with drugs stored in adipose tissue in-vivo, thus allowing lipophilic drugs to invade adipose tissue. In contrast, drugs not stored in adipose tissue exhibited strong binding competition which prevents these drugs from reaching adipose tissue. These findings confirm the hypothesis that uptake into adipose tissue, being a function of log P in-vitro but not in-vivo, is due to the presence in-vivo of additional tissues which may inhibit or prevent a drug from reaching the less-perfused adipose tissues (Bickel 1984). In conclusion, binding competition determines whether or to what extent a lipophilic drug will accumulate in adipose tissue in-vivo. In a more general sense, binding competition is a factor determining the volume of distribution, distribution kinetics, and distribution patterns.

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