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Comparison between in-vivo and in-vitro tissue-to-plasma unbound concentration ratios ($K_{p,f}$) of quinidine in rats

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The comparison between in-vivo and in-vitro tissue-to-plasma concentration ratio of drug unbound ($K_{p,f}$) has been made using quinidine as a model for weak basic drugs. In-vitro $K_{p,f}$-values were calculated from the binding data to tissue homogenates determined by equilibrium dialysis. In-vivo $K_{p,f}$-values were calculated from the tissue distribution data after intravenous administration of quinidine, by considering the difference in the unbound concentration between plasma and the tissues produced by the pH difference across the cell membrane. It was concluded that the extensive tissue distribution of quinidine observed in-vivo may be explained by tissue binding and the pH-difference across the cell membrane in most tissues.

The recent development of physiological pharmacokinetic models has made it possible to predict quantitatively the distribution and elimination of drugs in various species (Himmelstein & Lutz 1979). One of the parameters required to develop these models is the tissue-to-plasma partition coefficient (K_p), which is defined as the ratio of the drug concentration in the tissue to that in the venous plasma. We have succeeded in estimating K_p-values of ethoxybenzamide from in-vitro binding studies using plasma and tissue homogenates, suggesting that in-vitro binding data can be used to estimate the in-vivo tissue distribution (Lin et al 1982).

Weak basic drugs such as quinidine, imipramine and propranolol show characteristic tissue distributions with respect to their extensive tissue distribution and marked differences in K_p-values among tissues (Bickel et al 1975; Biarchetti et al 1980; Harashima et al 1983). In our previous study, we determined the tissue distribution of quinidine after its intravenous administration to rats and found a good correlation of the K_p-values for quinidine and propranolol or imipramine in various tissues suggesting that there exists a common mechanism for tissue distribution among these basic drugs (Harashima et al 1983).

For the present study, we selected quinidine as a model drug for weak basic drugs and compared the tissue binding determined in in-vitro studies with those obtained in in-vivo (Harashima et al 1983).

Methods

Adult Wistar (Nihon Seibutsu Zairyo, Tokyo, Japan) male rats, 250–300 g were used. Five rats were decapitated and each tissue excised, pooled and frozen at −20 °C until study. 17% tissue homogenates were prepared in 0·01 M phosphate buffer containing 0·15 M KCl (pH 7·0) and were predialysed for 2 days at 4 °C against the same phosphate buffer to remove the substances which affect the fluorometric determination of quinidine. The tissue binding was determined by equilibrium dialysis at 37 °C for 4 h using semimicrocells and a semipermeable membrane (Spectrapor membrane, Spectrum Medical Industries Inc., CA) against 0·01 M phosphate buffer containing 0·15 M KCl (pH 7·0). The initial concentration of quinidine in each homogenate was 2·0 μg ml^{-1}. Heat-treated homogenates were prepared placing them in boiling water (100 °C) for 15 min. n-Butanol-treated homogenates were prepared by the method of Ishitani et al (1977). Briefly, 2 ml of 17% liver homogenate was extracted with water-saturated n-butanol by shaking for 30 min. Treated homogenates were predialysed against the phosphate buffer for 2 days at 4 °C. Subcellular distribution of quinidine in the liver was determined by the method of Schneck et al (1977). 17% liver homogenate was prepared in 0·05 M Tris buffer containing 0·25 M sucrose (pH 7·0) at 4 °C. Quinidine was spiked into the homogenate at the initial concentration of 1·74 μg ml^{-1} and then was incubated for 10 min at 4 °C. Four subcellular fractions, nuclei and cell debris, mitochondria, microsomes, and cytosol were obtained by the

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method of Schneck et al (1977). The concentration of quinidine in each fraction was determined by the double extraction method of Cramer (1963).

The in-vivo tissue-to-plasma partition coefficient (K_p) was determined as follows. Quinidine sulphate dissolved in 0·9% NaCl (saline) was infused at 30 mg kg^{-1} through the femoral vein cannula for 30 s into adult male Wistar rats. Blood samples (0·25 ml) were obtained through the femoral artery cannula at 5, 15, 45, 60, 90, 120 and 150 min after drug administration. In a different experiment, animals were killed 30, 60, 90, 120 and 150 min after drug administration and tissues quickly excised, rinsed and blotted. A combination thin-layer chromatography-fluorometric method reported by Ueda et al (1977) was used to separate quinidine from its metabolites.

According to the method of Chen & Gross (1979), the K_p-value was calculated from the apparent K_p-value, which was defined as tissue concentration divided by arterial plasma concentration at 120 min after drug administration. The in-vivo tissue-to-plasma unbound concentration ratio ($K_{p,f(in\text{-}vivo)}$) is defined by

$$K_{p,f(in\text{-}vivo)} = \frac{C_t}{C_{p,f}} = \frac{K_p}{f_p} \quad (1)$$

where C_t is the total tissue concentration, $C_{p,f}$ is the plasma unbound concentration and f_p is the plasma unbound fraction. Considering the difference in unbound concentrations between plasma and the tissue, which is produced by the pH difference across the cell membrane, the $K'_{p,f}$ defined as the ratio of the total-to-unbound concentration in the tissue can be expressed by

$$K'_{p,f(in\text{-}vivo)} = \frac{C_t}{C_{t,f}} = \frac{1 + 10^{pK_a\text{-}pH_p}}{1 + 10^{pK_a\text{-}pH_t}} \times K_{p,f(in\text{-}vivo)} \quad (2)$$

where $C_{t,f}$ is the tissue unbound concentration, and pH_t and pH_p are the tissue and plasma pH, respectively. The values of 0·3 for f_p (Harashima et al 1983), 8·6 for pK_a, 7·0 for pH_t, and 7·4 for pH_p (Roos 1981) were used for the calculation.

The tissue-to-plasma unbound concentration ratio ($K_{p,f}$) calculated from the in-vitro tissue bindings was defined as $K_{p,f(in\text{-}vitro)}$. The tissue binding was determined using diluted tissue homogenate and if the tissue binding of quinidine did not depend on the homogenate concentration, $K_{p,f(in\text{-}vitro)}$ is expressed by (Yu et al 1981; Lin et al 1982)

$$K_{p,f(in\text{-}vitro)} = \frac{C_t}{C_f} = \frac{C_b + C_f}{C_f} = 1 + \frac{d \cdot C_{b,dil}}{C_f} \quad (3)$$

where d is the dilution factor (d = 6 in this study) and $C_{b,dil}$ is the bound concentration in the diluted tissue homogenate, and C_f is the unbound concentration.

Results and discussion

Dilution of the liver homogenate affects quinidine binding ($C_{b,dil}/C_f$) linearly. The binding was proportional to the concentration of homogenate, and similar proportionalities were also observed in the lung, muscle and brain homogenates. These results suggest the validity of the calculation of $K_{p,f(in\text{-}vitro)}$ by equation 3 from the in-vitro tissue binding data. The $K_{p,f(in\text{-}vitro)}$-values thus obtained were compared with $K'_{p,f(in\text{-}vivo)}$-values and relatively good agreements were observed in the kidney, liver, spleen and heart (Table 1). In the lung, the extensive tissue binding, which can account for the large values of $K'_{p,f(in\text{-}vivo)}$ was not observed in in-vitro experiments. Eiling et al (1975) reported that there existed a non-effluxable pool of imipramine in the rabbit isolated perfused lung. Vestal et al (1980) proposed the contribution of alveolar macrophages in the uptake of propranolol into the rabbit isolated perfused lung. The discrepancy shown between $K'_{p,f(in\text{-}vivo)}$ and $K_{p,f(in\text{-}vitro)}$ in the present study (Table 1) may be explained by such special transport mechanisms. In the brain, the value of $K_{p,f(in\text{-}vitro)}$ was overestimated by more than 7 times that of $K'_{p,f(in\text{-}vivo)}$ (Table 1). This large discrepancy may be explained by an active efflux mechanism for quinidine as reported by Ochs et al (1980). On the other hand, in the muscle and gut the values of $K'_{p,f(in\text{-}vitro)}$ were three to four times larger than those of $K_{p,f(in\text{-}vivo)}$, but were close to those of $K_{p,f(in\text{-}vivo)}$. These discrepancies may be due to the inappropriate intracellular pH used for calculation by equation 2 or the inapplicability of the extrapolation of tissue binding using diluted homogenates to 100% homogenates by equation 3. We examined the linearity of the ratio, C_b/C_f against the concentration of tissue homogenates as far as 30%. This is mainly due to experimental difficulty in preparing higher concentrations of tissue homogenates and that in doing equilibrium dialysis by use of such homogenates. In addition, the Donnan effect will have to be considered when high concentrations of tissue homogenate are used for equilibrium dialysis experiments (Keen 1966) and therefore it will be difficult to interpret the binding data. Mintun et al (1980) explained the extensive tissue

Table 1. Comparison of tissue-to-plasma unbound concentration ratios.

Tissue	K_p[a]	$K_{p,f(in\text{-}vivo)}$[b]	$K'_{p,f(in\text{-}vivo)}$[c]	$K_{p,f(in\text{-}vitro)}$[d]
Lung	45·2	151·0	58·1	11·1
Kidney	18·4	61·3	23·6	14·0
Liver	26·2	87·3	33·5	22·6
Spleen	27·0	90·0	34·6	29·1
Heart	13·7	45·8	17·6	21·6
Muscle	3·4	11·3	4·3	14·0
G.I.	20·6	68·7	26·4	104·0
Brain	1·5	4·9	1·9	14·5

[a] Calculated using the plasma and tissue concentrations at 120 min after intravenous administration of 30 mg kg^{-1} quinidine as reported previously (see text).
[b] Calculated by equation 1 using the value of 0·3 for f_p.
[c] Calculated by equation 2 using the values of 7·4 for pH_p, 7·0 for pH_t and 8·6 for pK_a.
[d] Calculated by equation 3 using in-vitro tissue binding data.

distribution of tetraethylammonium ion (TEA) which had a large steady state distribution volume (Vd_{ss}; 1·6 litre kg^{-1}), by an active transport mechanism, since the tissue binding of TEA was negligible. On the other hand, quinidine showed extensive binding to most of tissue homogenates studied (Table 1). These results suggest that the mechanisms for the extensive tissue distribution of TEA (cation) and quinidine (weak base) are completely different, namely the tissue distribution of TEA can be explained mainly by an active transport mechanism but that of quinidine can be explained by extensive tissue binding.

We reported that adriamycin, an anthracyclic antitumour agent, bound extensively to tissue DNA and that the tissue variation in K_p-values was determined by the tissue DNA concentrations (Terasaki et al 1982). Francesco & Bickel (1977) reported that the tissue macromolecules which bind weak basic drugs such as chlorpromazine and imipramine were phospholipid. Fichtl et al (1980), however, reported that the tissue distribution of these drugs could not be explained only by the partition to phospholipids. Therefore, to elucidate these controversial findings, we attempted preliminary experiments to identify the macromolecule that binds quinidine, using heat and organic solvent-treated liver homogenates. As shown in Table 2, binding of quinidine to the heat-treated liver homogenate was little changed compared with the non-treated homogenate (control), whereas that to the n-butanol-treated liver homogenate was decreased to less than half of the control. The intracellular localization of quinidine in the liver was also determined and the percentages of quinidine distributed to the nuclear, mitochondria, microsomal and cytosol fractions were 29, 39, 19 and 13% of the initial amount of quinidine added, respectively. These findings suggest the possibility that the main macromolecules binding quinidine are phospholipids, since the binding was decreased greatly after their extraction; also quinidine was localized in the subcellular fractions which are rich in phospholipids.

Table 2. Binding ratio (C_b/C_f) of quinidine to 17% liver homogenates.[a]

Experiments[b]	Control[c]	Heat-treated[d]	n-butanol treated[e]
1st	3·78	4·85 (128)[f]	1·20 (31·7)[f]
2nd	3·22	3·09 (96·0)[f]	1·59 (49·4)[f]

[a] Determined by equilibrium dialysis (see text).
[b] Two independent experiments.
[c] Untreated homogenate.
[d] Heated on boiling water (100 °C) for 15 min.
[e] Extracted by n-butanol.
[f] Per cent of the control.

Thus, it is suggested that the extensive tissue distribution of quinidine observed in in-vivo may be explained by extensive tissue binding and the pH difference across the cell membrane in most tissues.

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