A POSSIBLE CONTRIBUTION OF PHOSPHOLIPIDS IN TISSUE DISTRIBUTION OF QUINIDINE IN RATS

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The tissue distribution of quinidine was investigated at three different steady-state plasma concentrations of quinidine in rats. The tissue distribution of quinidine (tissue-to-plasma concentration ratio, \(C_t/C_p\)) was studied in the liver, lung, kidney and heart and the highest distribution was found in the lung. Tissue binding characteristics of quinidine was determined in normal tissue homogenates and lipid-depleted tissue homogenates in vitro. No correlation was observed between the tissue bindings (product of association constant \((K_i)\) and number of binding sites \((n)\) \(nK_i\) estimated in each normal tissue homogenate and the values of \(C_t/C_p\) in vivo. However, a marked decrease in the tissue binding of quinidine was observed in all lipid-depleted tissue homogenates, and the largest decrease was observed in the lung tissue. This result suggested that lipid may have an important role in the tissue binding of quinidine. However, no good relationship was observed between the values of \(C_t/C_p\) and the phospholipid contents in each tissue. In order to investigate the role of lipid in the tissue binding of quinidine, phospholipids extracted from each tissue were used for binding study. The phospholipids binding of quinidine \((nK_i)\) increased in the following order; heart < liver < kidney < lung, and the plots of the values of \(C_t/C_p\) obtained in vivo against the binding ability of phospholipids (product of \(nK_i\) and the content of phospholipid in each tissue) gave a good linear relationship.

Based on these observations, it was concluded that some species of phospholipids had an important and determining role in the tissue distribution of quinidine in vivo.

Keywords — quinidine; tissue distribution; lung-selective tissue distribution; tissue binding; lipid-depleted homogenate; phospholipid; phospholipid binding

INTRODUCTION

Quinidine, a weak basic drug, has been used for the treatment of cardiac arrhythmias and the disposition kinetics of the drug have been studied in man and animals by many workers.\(^1,2\) Some investigators have also reported that quinidine was preferentially taken up in the lung tissue in vivo \(^3,4\) and in isolated perfused lung preparations.\(^5\)

The lung is known to perform important non-respiratory functions such as accumulation and metabolism of drugs, in addition to its role in gas exchange. Several reviews concerning the pulmonary accumulation of drugs have been reported.\(^6,7\) Some endogenous substances, such as 5-hydroxytryptamine (5-HT), norepinephrine, bradykinine and prostaglandines are rapidly removed from the circulation and consequently deactivated by the lung. Some exogenous amines such as imipramine, chlorpromazine and amphetamine are also reported to be concentrated preferentially in the lung. However, the mechanism of the selective pulmonary distribution of basic compounds still remained unclear.

Recently, the binding of drugs to tissue constituents has been stressed as an important pharmacokinetic determinant.\(^8,9\) Terasaki, et al. reported that the nuclear binding is a determinant of the extensive tissue distribution of adriamycin and that the marked variation in the tissue concentration of adriamycin is due mainly to the difference in the tissue desoxyribonucleic acid (DNA) concentration.\(^10\)

In the present study, the distribution characteristics of quinidine in lung, liver, kidney and heart were investigated in rats at three different steady state concentrations of quinidine in plasma. The determinant factor for the tissue distribution of quinidine was investigated from a viewpoint of binding characteristics to tissues in vitro, using whole homogenates and lipid-depleted homogenates. The binding study of quinidine was also performed using phospholipids extracted from each tissue homogenate, since Sastrasinh, et al. reported that acidic phospholipids were identified as the renal brush
border membrane binding sites of aminoglycoside antibiotics. In the present phospholipid binding study, the importance of phospholipids in tissue distribution of quinidine is discussed.

MATERIALS AND METHODS

Materials — Quinidine sulfate of analytical grade was purchased from Wako Pure Chemical Ind., Ltd. (Osaka, Japan) and used without further purification. All other reagents were of the finest grade available.

Animal Studies — Male Wistar rats, 250—300 g, were used. Rats were anesthetized by intraperitoneal injection of sodium pentobarbital (Nembutal sodium solution®, Abbott Laboratories, U.S.A.) at a dose of 30 mg/kg and kept supine on a surface controlled at 37 °C to maintain their body temperature above 36 °C.

Intravenous Bolus Injection: Quinidine sulfate was administered intravenously in the rat tail vein at a dose of 12.5 mg/kg as quinidine base. Blood samples were collected by heparinized-syringe from a jugular vein at appropriate time intervals. Plasma was separated by centrifugation at 3000 rpm for 10 min and stored at -30 °C until analyzed. Some kinetic parameters were estimated from the plasma concentration-time curve using a two compartment model.

Tissue Distribution: The anesthetized rats were cannulated with polyethylene tubing (PE-50, Clay Adams, U.S.A.), both in the right femoral artery and in the left femoral vein. Bolus intravenous injection of quinidine sulfate was made at a dose of 8.94, 3.58 or 1.79 mg/kg as the quinidine base via a femoral vein cannula, immediately followed by a constant rate infusion for 120 min at a dose of 53.95, 21.58 or 10.79 μg/min/kg as the quinidine base, respectively. The steady-state concentration of quinidine in plasma (approximately 1, 0.4 and 0.2 μg/ml, respectively) was obtained within 1 h after the initiation of constant rate infusion. A micro infusion pump (Type IM-1, Narishige Scientific Instrument Lab., Tokyo, Japan) was used for constant rate infusion. Blood samples were collected via the cannula in a femoral artery at appropriate time intervals. After centrifugation, plasma sample was separated for the analysis of quinidine. Rats were exsanguinated after infusion of ice cold-0.9% NaCl solution from the portal vein 2 h after initiation of infusion of the drug solution. Then, the liver, heart, lung and kidney were removed. The removed tissues were frozen by a dry ice-acetone mixture and stored at -30 °C until analyzed. The tissues, except the liver, were homogenized with 5-fold volume of 1.25 M sucrose-0.05 M Tris-HCl buffer (pH 7.4) at 4 °C using a glass homogenizer. The liver was homogenized with 9-fold volume of buffer using a homogenizer with a teflon pestle.

In Vitro Tissue Binding Studies — Freshly removed tissues were homogenized with 0.25 M sucrose-0.05 M Tris-HCl buffer (pH 7.4) to make 20% (w/v) homogenates and used for the binding study of quinidine. The lipid-depleted tissue homogenates were prepared according to the method of Goldberg, et al. as follows12: Freshly removed tissues were homogenized with 19-fold volume of a mixture of chloroform-methanol (2 : 1 v/v) and were centrifuged at 8000 × g for 15 min. The lipid-depleted pellets, obtained after centrifugation, were washed with 10 ml of buffer and used for the binding study after resuspending in 10 ml of 0.25 M sucrose-0.05 M Tris-HCl buffer (pH 7.4). Each normal tissue homogenate suspension or lipid-depleted tissue homogenate suspension was placed in a bag of cellulose membrane (Seamless cellulose tubing, Visking Company) and was dialyzed against a sufficient amount of buffer for 48 h at 4 °C. Concentrated undialyzed fractions of the homogenate suspensions were used for the binding study. Quinidine solution was mixed with each homogenate suspension to make a final concentration of quinidine from 5 to 500 μM. Two ml of the homogenate suspension containing various concentrations of quinidine were introduced into a bag of cellulose membrane and placed in a tube containing 2 ml of the buffer. Incubation was performed at 4 °C for 60 h. The unbound fraction of quinidine in dialysate was determined fluorometrically by the method of Yokosuka, et al. 13

Extraction of Phospholipids — A tissue sample (about 10 g) was homogenized with 40 ml of chloroform-methanol (1 : 1 v/v) mixture to extract phospholipids according to the method of Folch, et al. 14 The homogenate was filtered through a filter paper (No. 131, Toyo Roshi, Co., Ltd., Japan). The residue was then repeatedly extracted three times with the same volume of chloroform-methanol mixture (2 : 1 v/v). All extracts were combined together. The combined extract was washed twice with 24 ml of 0.9%
NaCl aqueous solution. After centrifugation, the organic layer was separated and evaporated to dryness under nitrogen gas. The residue was dissolved in 0.5 ml of chloroform and 10 ml of acetone and 0.5 ml of 10% MgCl₂ in methanol were mixed with the chloroform solution. The solution was left on ice for 1 h, and then centrifuged at 2500 rpm for 5 min. The precipitate was washed twice with a sufficient amount of acetone and dried under nitrogen gas.

Phospholipid Binding of Quinidine — Binding of quinidine to phospholipids was determined by utilizing an organic solvent-water partition system according to a slightly modified method of Sastrasinh, et al. ²

Extracted phospholipids from each tissue were dissolved in chloroform at a concentration greater than 280 mg as inorganic phosphorus/ml. Then, the phospholipid solution was diluted with heptane to make a final concentration of 40 mg as inorganic phosphorus/ml. One hundred µl of the resultant solution were diluted with an additional 100 ml of heptane and used as an organic layer (heptane layer) in the solvent-water partition system. Two ml of buffer solution (0.25 M sucrose-0.1 M Tris-HCl buffer) containing various concentrations of quinidine (1-100 µM) were mixed with 2 ml of the heptane solution containing phospholipids (40 µg as an inorganic phosphorus/ml) in a L-shaped tube at 37 °C. After shaking for 2 h (equilibrium was achieved), the emulsified mixture was centrifuged at 2000 rpm for 10 min to separate the aqueous phase. The aqueous phase was used for the analysis of quinidine. In this system, the transfer of quinidine into the organic phase in the presence of phospholipids was attributed to the binding to the tested phospholipids, since the transfer of quinidine into the heptane layer without any phospholipids was negligible. The binding parameter of quinidine to phospholipids was calculated from Scatchard plot as described by Weber, et al. ¹⁵ and Sastrasinh, et al. ¹¹

Analytical Method — The concentration of quinidine in plasma and tissues (homogenates) was determined by high performance liquid chromatography (HPLC) as follows: a plasma sample (50 µl) was made alkaline with 1 ml of 0.1 N NaOH and extracted with 6 ml of benzene. Four ml of the organic layer were evaporated to dryness under reduced pressure. A homogenate sample (0.5 ml) was acidified with 0.5 ml of 1 N HCl and the mixture was extracted with 6 ml of a mixture of dichloromethane and isopropanol (4:1 v/v). After centrifugation, the organic layer was discarded and 0.5 ml of the aqueous layer was collected. To the aqueous layer (0.5 ml), 0.9 ml of 3 N NaOH was added and the mixture was extracted with 6 ml of the mixture of dichloromethane and isopropanol. Five ml of organic layer was evaporated to dryness. Each residue was dissolved in 100 µl of methanol containing propranolol (1-10 µg/ml) as an internal standard and 10 µl of the solution were injected into a HPLC column. The HPLC was carried out with a LC-5A (Shimadzu, Kyoto, Japan) apparatus equipped with a fluorometric detector (Model RF-530, Shimadzu) and a TSK-Gel (Toso Soda, ODS-120T) reverse phase column. Elution was done with acetonitrile-methanol-0.05 M phosphate buffer (pH 3.0) (2:2:6 v/v) at ambient temperature and the flow rate was 0.8 ml/min. The eluted quinidine and propranolol were detected by using wavelengths for excitation at 310 nm and emission at 380 nm. The retention time of quinidine and propranolol were 6.8 min and 16.8 min, respectively.

Phospholipids were determined as inorganic phosphorus. Phospholipids extracted from the tissue were converted to inorganic phosphorus by the method of Ames and Dubin,¹⁶ and the inorganic phosphorus was assayed according to the procedure of Chen, et al. ¹⁷

Tissue DNA was precipitated by adding 10% perchloric acid to the tissue homogenate and the concentration of DNA in the precipitate was determined spectrophotometrically (UV-190, Shimadzu, Japan) according to the method of Burton.¹⁸

The concentration of protein in the homogenate was determined by the method of Lowry, et al. ¹⁹ using bovine serum albumin as a standard reference.

RESULTS AND DISCUSSION

Tissue Distribution Study

The plasma concentration of quinidine is shown in Fig. 1 as a function of time after intravenous administration of quinidine sulfate at a dose of 12.5 mg/kg as a quinidine base. Four pharmacokinetic parameters, calculated from the biexponential curves according to a two compartment model, are also listed in the figure. The distribution of quinidine in the body was considered to be completed within 30 min after
parameters of quinidine obtained after intravenous administration in rats were similar to the values reported by Fremstad, et al. 20) These parameters were used to calculate the rate of infusion and bolus injection required to obtain a steady-state as early as possible after infusion. The tissue-to-plasma concentration ratio was determined at three different steady-state levels of quinidine, since Chen and Gross reported that the tissue-to-plasma concentration ratios were not equivalent between after constant-rate infusion and after intravenous bolus injection. 21) Quinidine plasma concentration reached a steady-state approximately 60 min following the initiation of the constant rate infusion. Tissue to plasma concentration ratios of quinidine were determined 120 min after initiation of constant infusion to ensure the steady-state condition. The results are summarized in Table I. The observed plasma concentrations did not differ greatly from that predicted from the pharmacokinetic parameters. The greatest $C_t/C_p$ value was observed in the lung at the three different steady-states among the tissues studied. In the lung, the kidney and the heart, the values of $C_t/C_p$ ratio were almost constant at the three different steady-states. However, the liver was not. The liver/plasma ratio increased with an increase in the plasma concentration at a steady state, probably due to its metabolizing activity. With respect to metabolism of quinidine in the liver, Yu, et al. reported that the formation of O-desmethyl quinidine was apparently saturat-

![Graph](image)

FIG. 1. Plasma Concentration—Time Curve of Quinidine after Intravenous Administration at a Dose of 12.5 mg/kg in Rats

Each point represent the mean ± S.E. of 10 rats. Pharmacokinetics parameters in the figure were calculated from the exponential curve according to the two-compartment model.

$A = 1.55$ (μg/ml), $B = 1.30$ (μg/ml), $\alpha = 0.0989$ (min$^{-1}$), $\beta = 0.00603$ (min$^{-1}$).

intravenous administration. The biological half-life (postdistribution phase) was 1.91 h and the apparent distribution volume in postdistribution phase was 8.94 l/kg. The pharmacokinetic

<table>
<thead>
<tr>
<th></th>
<th>$C_p$(μg/ml) or $C_t$(μg/ml)$^a)$</th>
<th>$C_t/C_p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma calculated observed</td>
<td>0.2</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>(0.196)</td>
<td>(0.398)</td>
</tr>
<tr>
<td>Lung</td>
<td>11.2</td>
<td>20.9</td>
</tr>
<tr>
<td></td>
<td>(1.8)</td>
<td>(3.3)</td>
</tr>
<tr>
<td>Kidney</td>
<td>4.09</td>
<td>7.72</td>
</tr>
<tr>
<td></td>
<td>(0.34)</td>
<td>(0.47)</td>
</tr>
<tr>
<td>Heart</td>
<td>1.18</td>
<td>3.86</td>
</tr>
<tr>
<td></td>
<td>(0.11)</td>
<td>(0.31)</td>
</tr>
<tr>
<td>Liver</td>
<td>0.286</td>
<td>1.70</td>
</tr>
<tr>
<td></td>
<td>(0.029)</td>
<td>(0.64)</td>
</tr>
</tbody>
</table>

$^a)$ The concentrations of quinidine in plasma ($C_p$) and tissue ($C_t$) were determined 2 h after bolus injection followed by a constant rate infusion. Each value represents the mean (S.E.) of six to ten rats.
ed although the formation of 3-hydroxyquinidine remained relatively linear in perfused rat liver preparation.\textsuperscript{22} Thus, the increase in $C_q/C_p$ ratio in the liver with an increase in the plasma quinidine concentration was considered to be derived from the saturation of metabolic activity in the formation of O-desmethyl quinidine.

Recently, Harashima \textit{et al.} reported that the nonlinear tissue distribution of quinidine was observed in the muscle, skin, liver, lung, and gastrointestinal tract in rats by a physiologically based pharmacokinetic model.\textsuperscript{23} However, in the present study, the tissue distribution of quinidine to the lung was linear as shown in Table I. The discrepancy is attributable to the difference in the plasma level of the quinidine used.

\textbf{Tissue Binding Study}

In recent years, it has become known that tissue binding exerts a pronounced influence on the distribution of many drugs in the body, especially drugs having a large apparent volume of distribution. Lin, \textit{et al.} reported that the \textit{in vivo} tissue distribution of ethoxybenzamide could be estimated from the \textit{in vitro} binding studies using plasma and tissue homogenates.\textsuperscript{24} Fremstad and Jacobsen also reported that the heart to serum concentration ratio of quinidine in normal and anuric rats could be explained by the binding properties in heart homogenates and serum samples and from a viewpoint of the pH-dependent distribution of unbound quinidine between intra- and extracellular space.\textsuperscript{20} However, this was not the case for lung. Harashima, \textit{et al.} reported that the tissue distribution of quinidine observed \textit{in vivo} may be explained by the tissue binding and the pH-difference across the cell membrane in most tissues, such as kidney, liver, spleen and heart.\textsuperscript{25} However, they also reported that, in the lung, the extensive tissue distribution was not observed \textit{in vitro} tissue binding experiments.\textsuperscript{26}

In order to account for the extensive distribution of quinidine \textit{in vivo}, binding characteristics of quinidine to tissue homogenates, normal or lipid-depleted, were determined by equilibrium dialysis method. The reason why lipid-depleted homogenate was used in the binding study is based on the reports in which several investigators predicted the role of phospholipids as major intracellular binders of drugs.\textsuperscript{26,27} Scatchard

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Scatchard_plot.png}
\caption{\textit{Scatchard Plot of the Binding of Quinidine to Rat Normal Homogenate (Open Symbol) and Lipid-Depleted Homogenate (Closed Symbol)}
\textit{Each point represents the mean of 4 trials. The binding of quinidine was determined by equilibrium dialysis method at 4°C.}}
\end{figure}
### Table II. Binding Parameters of Quinidine to Normal and Lipid-Depleted Tissue Homogenates

<table>
<thead>
<tr>
<th>Tissue</th>
<th>$K_a$ (µM$^{-1}$)</th>
<th>$n_b$ (nmol/mg protein)</th>
<th>$n_K$ (ml/mg protein)</th>
<th>Decrease in $nK$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>homogenate</td>
<td>Liver</td>
<td>2.13 × 10$^{-2}$</td>
<td>61.2</td>
<td>1.31</td>
</tr>
<tr>
<td></td>
<td>Lung</td>
<td>2.73 × 10$^{-2}$</td>
<td>45.7</td>
<td>1.25</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>1.03 × 10$^{-2}$</td>
<td>62.0</td>
<td>0.640</td>
</tr>
<tr>
<td></td>
<td>Heart</td>
<td>2.24 × 10$^{-2}$</td>
<td>27.3</td>
<td>0.613</td>
</tr>
<tr>
<td>Lipid-depleted</td>
<td>Liver</td>
<td>1.14 × 10$^{-2}$</td>
<td>89.3</td>
<td>1.02</td>
</tr>
<tr>
<td>homogenate</td>
<td>Lung</td>
<td>0.995 × 10$^{-2}$</td>
<td>51.5</td>
<td>0.512</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>0.227 × 10$^{-2}$</td>
<td>141</td>
<td>0.319</td>
</tr>
<tr>
<td></td>
<td>Heart</td>
<td>1.38 × 10$^{-2}$</td>
<td>24.6</td>
<td>0.338</td>
</tr>
</tbody>
</table>

*a) Association constants. b) Number of binding sites. c) Binding. d) The percent represents the ratio of $nK$ in lipid-depleted homogenate against $nK$ in normal homogenate.*

Plots of the binding are shown in Fig. 2. Association constant ($K$) and number of binding sites ($n$) were calculated by the non-linear least squares method and summarized in Table II. In both homogenates (normal and lipid-depleted), the Scatchard plots showed one class binding site within the concentration range of quinidine employed in the present study. To evaluate the binding characteristics of quinidine to the homogenates, the product of association constant and number of binding sites, $nK$, were used.

Total concentration of quinidine ($C$) in the homogenates is expressed as the sum of the concentration of unbound form ($C_b$) and the concentration of bound form ($C_b$). $C_b$ is generally expressed as $n (P) C_f / (K_d + C_f)$ because the binding is considered to be a saturated phenomenon, where $n$ represents the number of binding sites; ($P$), the concentration of total protein; $K_d$, the dissociation constant.

Assuming $K_d > C_f$, the following equation is derived.

$$C = (1 + n(P)/K_d) C_f$$

Then, when using association constant $K$,

$$C = C_f + nK(P) C_f$$

This equation means that the binding characteristics of quinidine to the tissues will be linear and that the value of $nK$ or $nK(P)$ will be used as the value evaluating the binding characteristics in a limited experimental condition.

In the case of normal homogenates, the binding characteristics expressed by the value of $nK$ for the liver and lung were similar and were about twice greater than those for the kidney and heart. In the present study, the values of $C_f/C_p$ in vivo showed a linear phenomena except for the liver. The linear phenomena in $C_f/C_p$ ratio were subjected to large values in the dissociation constant compared to the concentration of unbound quinidine in plasma. In the in vitro binding experiments, the association constants obtained in normal tissue homogenates were around $1-3 \times 10^4$/M (Table II). Therefore, dissociation constants were around $30-100$ µM. On the other hand, assuming that plasma protein binding is about 75%, the concentration of unbound quinidine in plasma at the three different steady-state conditions was considered to be less than 1 µM, which is much lower than the value of dissociation constant. These findings indicate that the binding of quinidine to tissues in vivo also is linear in the concentration range used in the present study.

Based on this consideration, correlation between $C_f/C_p$ ratio and the value of $nK$ was examined. However, less correlation between the value of $nK$ obtained in normal homogenates and the value of $C_f/C_p$ obtained in vivo (Table I) at three different steady-state concentrations of quinidine in plasma was observed (correlation coefficient: 0.388). This result suggests that the in vitro tissue binding of quinidine determined with normal homogenate does not reflect the extent of tissue distribution of quinidine in vivo.
TABLE III. The Contents of Total Protein, Total Phospholipid and DNA in Each Tissue

<table>
<thead>
<tr>
<th></th>
<th>Total protein&lt;sup&gt;a&lt;/sup&gt; (mg/g tissue)</th>
<th>Total phospholipid&lt;sup&gt;a&lt;/sup&gt; (mg/g tissue)</th>
<th>Total phospholipid&lt;sup&gt;b&lt;/sup&gt; (mg/g tissue)</th>
<th>DNA&lt;sup&gt;a&lt;/sup&gt; (mg/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>80.0 ± 3.5</td>
<td>77.5 ± 1.7</td>
<td>1.44 ± 0.14</td>
<td>1.45 ± 0.14</td>
</tr>
<tr>
<td>Lung</td>
<td>50.8 ± 2.0</td>
<td>63.2 ± 2.1</td>
<td>1.40 ± 0.29</td>
<td>6.64 ± 0.40</td>
</tr>
<tr>
<td>Kidney</td>
<td>79.0 ± 2.5</td>
<td>73.0 ± 3.3</td>
<td>0.96 ± 0.29</td>
<td>2.05 ± 0.15</td>
</tr>
<tr>
<td>Heart</td>
<td>67.0 ± 2.5</td>
<td>64.2 ± 2.2</td>
<td>1.03 ± 0.08</td>
<td>1.11 ± 0.01</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E. of five rats. a) The contents in normal tissue. b) The contents in lipid-depleted tissue.

Determination of the total phospholipid in the homogenate after treatment by organic solvent mixture showed the removal of phospholipid from the tissues by 96.4%, 95.2%, 94.8% and 96.87% in the liver, the lung, the kidney, and the heart, respectively. Also a marked decrease in binding was observed in each lipid-depleted homogenate when evaluated by the value of $nK$ as shown in Table II. The largest decrease in binding due to the removal of lipid was observed in lung homogenate, which suggested that guanidinium may have higher affinity to lipids in the lung.

**Phospholipids Binding of Quinidine**

Further investigation was carried out to confirm the role of lipids in tissue binding of quinidine in vitro. Ohmiya et al. reported that the removal of chlorpromazine and imipramine from the perfusate were accelerated in the perfused lung isolated from chlorphentermine-treateed rats. Since chlorphentermine is known to be highly accumulated by the lung and to cause pulmonary phospholipidosis, they suggested that phospholipids play an important role as the storage site of chlorpromazine and imipramine in the lung tissue. However, no direct evidence is reported in the binding of drug to phospholipids.

In the present study, we also examined a possible contribution of phospholipids in the tissue distribution of quinidine. At first, the contribution of the contents of total phospholipids in tissues was studied. The contents of total phospholipids in each tissue are listed in Table III with the contents of total protein and DNA. However, no good relationship was observed be-

![FIG. 3. Scharchard Plot of the Binding of Quinidine to Phospholipids Extracted from Each Tissue of Rats](Image URI)

Each point represents the mean of 4 trials. The binding of quinidine was determined in a heptane–pH 7.4, Tris–HCl buffer partition system at 37°C.
TABLE IV.  Binding Parameters of Quinidine to Phospholipid Extracted from Each Tissue

<table>
<thead>
<tr>
<th></th>
<th>Lung</th>
<th>Kidney</th>
<th>Liver</th>
<th>Heart</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K^{a)}$ ($\mu M^{-1}$)</td>
<td>5.24 x 10^{-2}</td>
<td>4.34 x 10^{-2}</td>
<td>3.24 x 10^{-2}</td>
<td>2.86 x 10^{-2}</td>
</tr>
<tr>
<td>$n^{b)}$ (nmol/mg lipid)</td>
<td>66.6</td>
<td>41.7</td>
<td>43.6</td>
<td>45.1</td>
</tr>
<tr>
<td>$nK^{c)}$ (ml/mg lipid)</td>
<td>3.49</td>
<td>1.87</td>
<td>1.41</td>
<td>1.29</td>
</tr>
</tbody>
</table>

a) Association constants.  b) Number of binding sites.  c) Binding.

tween the values of $C_d/C_p$ and the phospholipids contents in each tissue (correlation coefficient: 0.505). This result indicates that the contents of total phospholipids in each tissue does not reflect the extent of tissue distribution of quinidine. Next, the phospholipid was extracted from each tissue homogenate and used for the binding study of quinidine in vitro. The Scatchard plots are shown in Fig. 3, and binding parameters are listed in Table IV. Plotting the average values of $C_d/C_p$ obtained at three different steady-state condition of quinidine in each tissue except liver against the product of $nK$ obtained in phospholipids binding in vitro and content of total phospholipids in each tissue is shown Fig. 4. As already discussed, the use of the product of $nK$ and total phospholipids contents is sufficient to express the tissue distribution of quinidine, since the unbound concentration of quinidine used in the present study is lower than that of dissociation constants in phospholipids binding, 20—30 $\mu M$.

The tissue distribution of drug is often expressed as a tissue-to-plasma partition coefficient ($K_p$ value) in physiological pharmacokinetics. In this study, the values of $C_d/C_p$ obtained from the lung, kidney and heart are equal to the values of $K_p$, since quinidine is not metabolized in these organs. However, the value of $C_d/C_p$ in the liver does not represent a true $K_p$ value. Therefore, we estimated the value of $K_p$ in the liver from the reported data. The value of $K_p$ in a metabolizing tissue such as the liver can be calculated from the following equation.

$$K_p = \frac{(Q + Cl_{int} \cdot f_b)}{Q} \frac{C_l}{C_b}$$

Where, $Q$ represents the blood flow rate in the liver; $Cl_{int}$, hepatic intrinsic clearance; $f_b$, free fraction of quinidine in plasma. The value of $Cl_{int}$, 160 ml/min/kg was cited from the data reported by Harashima, et al. who demonstrated a best fitting curve of plasma concentration-time over a plasma concentration range of quinidine from 0.5 to 10 $\mu g/ml$. The free fraction of quinidine in plasma at 1 $\mu g/ml$ was also estimated from their data as 0.25. Using 58.8 ml/min/kg for the blood flow rate and the value of $C_d/C_p$ obtained at 1 $\mu g/ml$ in the present study, the $K_p$ value of quinidine in the liver was calculated as 16.8. Therefore, this value was used as the $C_d/C_p$ value for the liver in Fig. 4.

An excellent correlation was observed (correlation coefficient: 0.992) between the values of $C_d/C_p$ and the phospholipid binding ability ($nK \times$ phospholipids content). This finding suggests that the phospholipid binding ability ($nK \times$ phospholipid content) in each tissue may be a determinant in the tissue distribution of quinidine and that variation in tissue distribution ($C_d/C_p$) of quinidine may be due mainly to the difference in the phospholipid binding ability of each tissue.

![FIG. 4. Relationship between Tissue-to-Plasma Concentration Ratio of Quinidine at Steady-State in Vivo and Phospholipid Binding Ability (nK \times Phospholipid Content) in Each Tissue](image)

Experimental values for the lung, kidney and heart were used as $C_d/C_p$ values and calculated values were used for the liver. The correlation coefficient is 0.992.
Recently, Terasaki, et al. reported that adriamycin bound extensively to tissue DNA and that the extensive tissue distribution of adriamycin in vivo could be predicted by the tissue DNA concentrations. In this study, a good linear relationship was also observed between the values of \( C_d/C_p \) obtained in vivo and the DNA concentrations of each tissue (correlation coefficient: 0.975). However, only a small amount of quinidine was found in the cell nuclear fraction of each tissue (data are not shown). Based on these results, the DNA concentrations were not considered to participate in the tissue distribution of quinidine in vivo.

Tissue distribution of quinidine could not be explained from the phospholipid contents alone in tissues but from the binding ability by phospholipids. This finding suggests that concentrations of some types of phospholipids in the tissues are important in the tissue distribution of quinidine. Okumura, et al. reported that the cationic moiety as well as the lipophilic moiety of drugs were required for lung accumulation of drugs. Quinidine having a \( pK_a \) value of 8.6 is a basic compound, with a positive charge at a physiological pH. Therefore, quinidine is expected to be distributed in tissues rich in anionic phospholipids such as phosphatidic acid, phosphatidyl inositol and phosphatidyl serine due to its binding to anionic phospholipids in tissues. Furthermore, the contribution by other components, in addition to lipids, in tissues such as protein binding of quinidine should also be taken into consideration because quinidine was found to bind lipid-depleted homogenated by 80%, 50%, 56% and 40% in the liver, the heart, the kidney and the lung, respectively as shown in Table II.

To clarify the determinant of the tissue distribution of quinidine, a study of subcellular distribution of quinidine and the contribution of anionic phospholipids to the tissue binding of quinidine is now underway.

REFERENCES


