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*Research Article*

# Role of Phosphatidylserine in the Cellular and Subcellular Lung Distribution of Quinidine in Rats

**Akio Nishiura,[1] Teruo Murakami,[1] Yutaka Higashi,[1] and Noboru Yata[1,2]**

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The role of phosphatidylserine in the cellular and subcellular lung distribution of quinidine was investigated in rats, since quinidine was found to bind preferentially to phosphatidylserine. The concentration of phosphatidylserine in the cellular and subcellular fractions was determined after separation by two-dimensional thin-layer chromatography. Selective accumulation of quinidine *in vivo* was observed in the alveolar macrophage at the cellular level and in the plasma membrane at the subcellular level. Both alveolar macrophages and plasma membranes were rich in phosphatidylserine compared to other fractions. When plasma levels were kept at steady state by i.v. infusion, the distribution of quinidine in the lung cellular and subcellular fractions was linearly correlated with the concentration of phosphatidylserine ($r = 0.906$). These results suggest that the concentration of phosphatidylserine is a dominant determinant of the cellular and subcellular lung distribution of quinidine.

KEY WORDS: quinidine; lung; alveolar macrophage; subcellular fractions; plasma membrane; phosphatidylserine.

## INTRODUCTION

Basic drugs such as quinidine are known to distribute preferentially to the lung *in vivo* (1,2). The lung is also a major site of sequestration of numerous basic amines within the body (3). Intracellular binding in the alveolar macrophage of basic compound has been reported to be a major component of the slow effluxable pool (4). Phospholipidosis induced by chronic treatment with basic drugs is also known to increase the accumulation of basic drugs in the macrophage compartment (5,6).

In a previous report, we demonstrated that the tissue distribution of quinidine *in vivo* depends on the composition of phospholipids in tissues and that a determinant of interorgan variation in the tissue distribution of quinidine is the concentration of phosphatidylserine in the tissues. Quinidine was found to bind tightly to phosphatidylserine *in vitro*, and a good correlation between the concentration of phosphatidylserine in each tissue and the tissue-to-plasma concentration ratios of quinidine at steady-state plasma conditions was obtained (7,8).

Therefore, in the present study, the role of phosphatidylserine in quinidine distribution was investigated at the lung cellular level such as alveolar macrophages and lung surfactant and subcellular levels at a steady-state plasma concentration in rats.

[1] Department of Parmaceutics and Therapeutics, Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine, 1-2-3 Kasumi, Minami-ku, Hiroshima 734, Japan.

[2] To whom correspondence should be addressed.

## 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.

### Animals

Male Wistar rats, 200–300 g, were used. Rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (Nembutal 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.

Quinidine sulfate (8.97 mg as base/kg) was administered rapidly via a femoral vein cannula, immediately followed by a constant-rate infusion at a dose of 53.95 μg as base/min/kg in the same manner as described in the previous report (7). A steady-state plasma concentration of quinidine (approximately 1 μg/ml) was obtained within 1 hr after the initiation of constant-rate infusion. Rats were exsanguinated via the abdominal aorta by the infusion of an ice-cold 0.9% NaCl solution from the portal vein 2 hr after the initiation of drug infusion.

Collection of the lung surfactant and the alveolar macrophages was made according to the method of Reasor and Heyneman (9) with a slight modification. Briefly, the lungs of the rat at a steady-state plasma concentration of quinidine (plasma level, approximately 1 μg/ml) were lavaged with 5-ml aliquots of ice-cold isotonic 0.05 *M* Tris–HCl buffer so-



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lution containing 0.25 *M* sucrose until 80 ml of lavage fluid had been collected. The lavage fluid was combined and centrifuged at 500*g* for 10 min to pellet the alveolar macrophage. The alveolar macrophage was resuspended in a 2-ml cold 0.9% NaCl solution ($8-14 \times 10^6$ cells/ml/lung). The supernatant of the lavage fluid was centrifuged at 105,000*g* for 60 min to pellet the lung surfactant. The lung surfactant was resuspended in 1 ml cold 0.9% NaCl. After being lavaged the lung tissue was also used to determine the phospholipid composition and the concentration of quinidine in the lung parenchyma.

### Quinidine Binding to the Subcellular Fraction of the Lung *in Vitro*

Five to six lungs of untreated rats were used for one set of experiments. The freshly removed lungs were minced with scissors and homogenized in a 0.3 *M* sucrose–4 m*M* $CaCl_2$ solution to make a 10% homogenate. The homogenate was filtered through double layers of gauze. Subcellular fractionation was carried out using the discontinuous sucrose gradient technique according to the method of Schneck *et al.* (10) to obtain pure nuclear, mitochondrial, microsomal, and cytosol fractions. Each pellet, obtained after centrifugation, was resuspended to make a 10% suspension in a 0.25 *M* sucrose–0.05 *M* Tris–HCl buffer (pH 7.4). The suspension was dialyzed against a sufficient amount of buffer for 48 hr at 4°C. The concentrated undialyzed fraction of each lung subcellular fraction was then mixed with an equal volume of quinidine solution to make a final concentration of quinidine of from 5 to 500 μ*M*. The binding characteristics of quinidine to each subcellular fraction were investigated at 4°C by an equilibrium dialysis for 60 hr in the same manner as described in the previous report (7). Data were analyzed by Scatchard analysis. Measurement of quinidine binding to cytosol proteins (supernatant at 100,000*g* for 1 hr) was also performed using a Sephadex G-75 column according to the method of Schneck *et al.* (10). The protein content in each fraction was determined spectrophotometrically at 280 nm and the concentration of quinidine was determined by high-performance liquid chromatography (HPLC) in the same manner as described in the previous report (7).

### Distribution of Quinidine in Lung Subcellular Fractions *in Vivo*

The lung of rats at a steady-state plasma concentration of quinidine (plasma level, approximately 1 μg/ml) was freshly removed after exsanguination. Subcellular fractionation was carried out by ultracentrifugation according to the method of Josepovitz *et al.* (11) and purified nuclear, lysosomal, mitochondrial, plasma membrane, and microsomal fractions were obtained. Then the concentrations of quinidine and individual phospholipid components in each subsellular fraction were determined.

### Analytical Method

The concentration of quinidine in the plasma, lung tissue, lung surfactant, alveolar macrophages, and lung subcellular fraction was determined by high-performance liquid chromatography in the same manner as described in the previous report (7). Individual phospholipid components were isolated using two-dimensional thin-layer chromatography (TLC) according to the method of Poorthuis *et al.* (12) with a slight modification as described in the previous report (8) and assayed according to the method of Chen *et al.* (13). The concentration of protein in the homogenate of the lung tissue and the subcellular fraction was determined by the method of Lowry *et al.* (14) using bovine serum albumin as a standard reference.

## RESULTS AND DISCUSSION

### Role of Phosphatidylserine in the Quinidine Distribution into Lung Parenchyma, Alveolar Macrophages, and Lung Surfactant

Lung parenchyma, alveolar macrophages, and lung surfactant were isolated from the rat at a steady-state plasma concentration of quinidine (plasma concentration, 1.04 ± 0.03 μg/ml). And the amount of quinidine distributed and concentration of individual phospholipid component in each fraction were determined (Fig. 1 and Table I). Approximately 30% of the total quinidine in the lung distributed into alveolar macrophages, and only 2% was found in the lung surfactant fraction (Fig. 1). Lung surfactant is considered to be released into the airspaces from type II epithelial cells and to be cleared by alveolar macrophages, indicating that the basic amines distributed in the lung surfactant fraction are removed by the alveolar macrophage, resulting in the accumulation of the drugs in the alveolar macrophage (15). As shown in Fig. 2, a marked accumulation was observed in the alveolar macrophages when the distribution of quinidine was evaluated on the basis of the protein content in each fraction. The magnitude of the cellular distribution of quini-

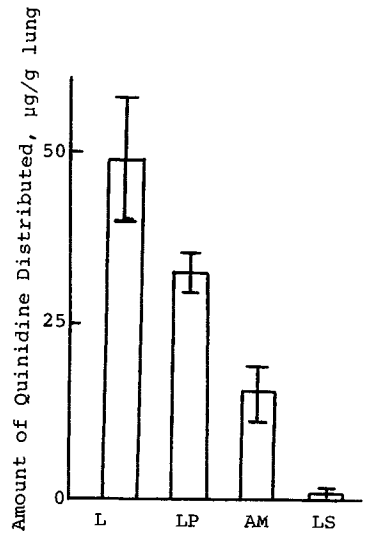


Fig. 1. Distribution of quinidine in lung parenchyma, alveolar macrophages, and lung surfactant. L, lung; LP, lung parenchyma; AM, alveolar macrophage; LS, lung surfactant. Each value represents the mean ± SE of four trials.

Table I. Concentration of Phospholipid Components in Lung Parenchyma, Alveolar Macrophages, and Lung Surfactant[a]

| | Lung parenchyma | Alveolar macrophage | Lung surfactant |
|---|---|---|---|
| PhS | 2.30 ± 1.70 | 22.4 ± 2.8 | 1.98 ± 1.80 |
| PhG | 1.50 ± 0.53 | 27.9 ± 5.3 | 23.5 ± 3.5 |
| PhI | 0.69 ± 0.32 | 13.8 ± 2.4 | 0.68 ± 0.25 |
| PhC | 7.37 ± 2.52 | 30.5 ± 3.5 | 73.0 ± 10.0 |
| PhE | 2.50 ± 1.53 | 21.9 ± 5.2 | 20.9 ± 4.7 |
| Total | 24.5 ± 0.6 | 117 ± 2.5 | 134 ± 5.4 |

[a] Micrograms per milligram of protein. Each value represents the mean ± SE of three or four experiments. The total value represents the total phospholipid concentration in each fraction. PhS, phosphatidylserine; PhG, phosphatidylglycerol; PhI, phosphatidylinositol; PhC, phosphatidylcholine; PhE, phosphatidylethanolamine.

dine was found to depend on the concentration of phosphatidylserine in the cellular fraction (Fig. 2). As reported previously (8), quinidine binds to phosphatidylserine more tightly than to phosphatidyl glycerol or phosphatidyl inositol (acidic phospholipids) (>50-fold) and to phosphatidylserine or phosphatidyl ethanolamine (neutral phospholipids) (>300-fold) *in vitro*. The lower distribution of quinidine to the lung surfactant may be attributable to the lower concentration of phosphatidylserine, whereas the concentration of total phospholipid is the highest. A major phospholipid in the lung surfactant is known to be a disaturated phosphatidylcholine (9). However, quinidine binding to disaturated phosphatidylcholine was low (8). The low distribution of quinidine to the lung surfactant was similar to the results obtained with imipramine (4). These findings are in good agreement with the previous results (8) that the higher concentration of phosphatidylserine in the tissues resulted in the higher distribution of quinidine. The present results may also imply that the slow effluxable pool in the lung is related to high concentrations of phosphatidylserine in the alveolar macrophage, since Eling *et al.* (16) showed the existence of a slowly effluxable pool in the lung, which accounts for the persistence of the basic amines in the lung and the induction of phospholipidosis.

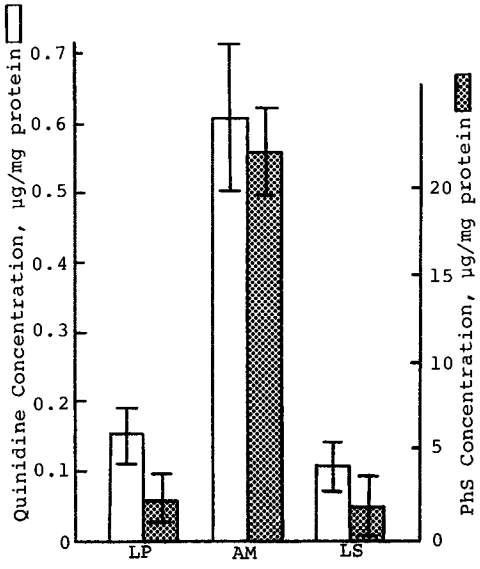


Fig. 2. Concentrations of quinidine and phosphatidylserine in lung parenchyma, alveolar macrophages, and lung surfactant. LP, lung parenchyma; AM, alveolar macrophage; LS, lung surfactant. The bar represents the SE of three or four trials.

### Binding of Quinidine to Subcellular Fractions *in Vitro*

Scatchard plots of quinidine binding are shown in Fig. 3. In mitochondrial and microsomal fractions, two classes of binding sites were observed, whereas one class of binding site was observed in nuclear and cytosol fractions within the concentration range of quinidine employed in the present study. These phenomena are consistent with the results for chlorpromazine and imipramine binding to the liver subcellular fractions reported by Bickel and Steele (17). The association constant ($K$) and number of binding sites ($n$) were calculated by the nonlinear least-squares method and are summarized in Table II. Upon assessment of binding extent by multiplying the association constant and number of binding sites, $nK$, greater binding was observed in the microsomes and mitochondria.

On the other hand, some binding of quinidine to the cytosol fraction was also observed irrespective of the low phospholipid concentration (0.4 μg total phospholipid/mg protein). A possible contribution of protein to the quinidine binding in the cytosol fraction was examined using a Sephadex G-75 column. As shown in Fig. 4, a similar elution pattern of quinidine and soluble protein was obtained, indicating binding to cytosol protein.

Thus phospholipids, especially phosphatidylserine, and proteins were found to participate in quinidine binding in subcellular fractions. However, the contribution of proteins to quinidine binding was considered to be small compared to that of phospholipid binding, since the binding of quinidine

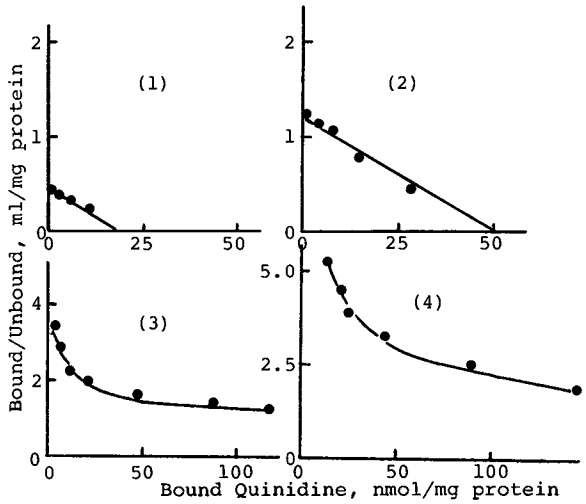


Fig. 3. Scatchard plot of the binding of quinidine to subcellular fractions of rat lung. (1) Nucleus; (2) cytosol; (3) mitochondrion; (4) microsome. Each point represents the mean of four trials.

Table II. Binding Parameters of Quinidine to Subcellular Fractions of Rat Lung[a]

| | $K \times 10^2$ (l/μM)[b] | | $n$ (nmol/mg protein)[c] | | $nK$ (ml/mg protein)[d] | |
|---|---|---|---|---|---|---|
| | $K_1$ | $K_2$ | $n_1$ | $n_2$ | $n_1K_1$ | $n_2K_2$ |
| Nucleus | 2.86 | | 17.0 | | 0.49 | |
| Cytosol | 2.24 | | 52.4 | | 1.17 | |
| Mitochondrion | 14.0 | 0.63 | 5.13 | 579 | 7.20 | 3.62 |
| Microsome | 35.9 | 0.58 | 23.20 | 1061 | 8.33 | 6.15 |

[a] Each value represents the mean of four trials.
[b] Association constants.
[c] Number of binding sites.
[d] Binding.

Table III. Concentration of Quinidine at Steady-State Plasma Conditions and Phospholipid Components in Each Lung Subcellular Fraction[a]

| | Nucleus | Lysosome | Mitochondrion | Plasma membrane | Microsome |
|---|---|---|---|---|---|
| Quinidine | 0.15 ± 0.01 | 0.21 ± 0.04 | 0.19 ± 0.02 | 0.55 ± 0.04 | 0.37 ± 0.01 |
| PhS | 3.62 ± 0.52 | 7.78 ± 1.50 | 4.93 ± 0.75 | 21.6 ± 1.7 | 7.81 ± 0.82 |
| PhG | 2.82 ± 0.53 | 9.44 ± 4.61 | 7.90 ± 2.43 | 11.9 ± 2.1 | 2.64 ± 0.86 |
| PhI | 2.42 ± 1.51 | 11.1 ± 5.2 | 4.05 ± 2.22 | 10.9 ± 2.6 | 2.85 ± 0.89 |
| PhC | 34.8 ± 4.2 | 42.3 ± 3.2 | 61.7 ± 5.5 | 76.1 ± 6.8 | 37.1 ± 7.3 |
| PhE | 13.5 ± 4.3 | 13.5 ± 1.5 | 23.8 ± 4.2 | 25.2 ± 6.3 | 9.11 ± 2.10 |
| Total | 59.7 ± 6.2 | 91.6 ± 8.7 | 191 ± 10 | 190 ± 5.5 | 145 ± 12 |

[a] Micrograms per milligram of protein. Each value represents the mean ± SE of three or four experiments. The total value represents the total phospholipid concentration in each fraction. PhS, phosphatidylserine; PhG, phosphatidylglycerol; PhI, phosphatidylinositol; PhC, phosphatidylcholine; PhE, phosphatidylethanolamine.

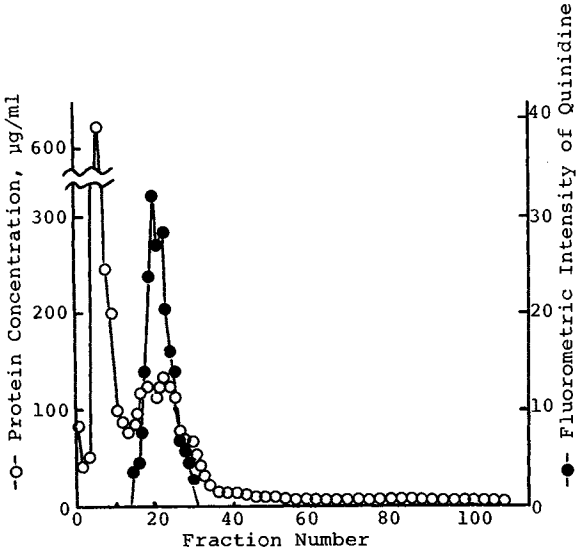


Fig. 4. Binding of quinidine to 100,000$g$ supernatant derived from rat lung. The supernatant containing quinidine was put on a Sephadex G-75 column (2.3 × 35 cm) and eluted with 0.05 $M$ Tris–HCl (pH 7.4) at a flow rate of 26 ml/hr at 4°C. The eluent was collected at 5 ml/tube.

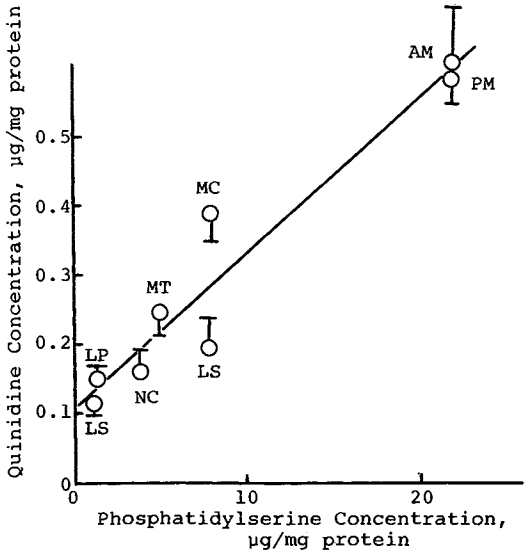


Fig. 5. Relationship between the concentration of quinidine and the concentration of phosphatidylserine in lung cellular and subcellular fractions of rats. LP, lung parenchyma; AM, alveolar macrophage; LS, lung surfactant; NC, nucleus; MT, mitochondrion; LS, lysosome; PM, plasma membrane; MC, microsome. The bar represents the SE of three trials.

($nK$) to cytosol fractions was much lower than to phosphatidylserine rich fractions such as mitochondria and microsomes.

### Role of Phosphatidylserine in the Subcellular Distribution of Quinidine *in Vivo*

Lung subcellular fractions, such as the nucleus, lysosome, mitochondrion, plasma membrane, and microsome, were prepared from the rats at a steady-state plasma concentration of quinidine (plasma concentration, 1.04 ± 0.03 μg/ml). The concentration of quinidine and individual phospholipid components in each fraction is shown in Table III. The order of the quinidine concentration normalized to the protein content was nucleus < lysosome < mitochondrion < microsome < plasma membrane. Thus, quinidine was significantly concentrated in the plasma membrane and microsome. The subcellular distribution of quinidine is roughly explained by the concentration of phosphatidylserine (Table III).

Recently, Yoshida *et al.* (18) reported that basic drugs such as imipramine, quinine, and metoclopramide are selectively accumulated in the rat lung mitochondrial fraction in an isolated lung perfusion method. They also considered that those basic drugs may bind not to lipid but to other lung components such as protein. However, in the present *in vivo* study, quinidine preferentially distributed in the plasma membrane fraction rich in the concentration of phosphatidylserine. The discrepancy between their findings and ours may be attributable to the difference in the experimental conditions.

The importance of lysosomal fractions in the subcellular distributions of basic compounds has been discussed by Hostetler (19). The lysosome is reported to be an intracellular site of accumulation for exogenous amines (20) and for phospholipids (5). Therefore, we examined the lysosomal localization of quinidine in lung, kidney, spleen, muscle, liver, heart, and brain. This experiment was performed using digitonin according to the method of Horadagodard and Batt (21), since digitonin selectively disrupts lysosomes and releases their contents into the soluble fraction. The lysosomal distribution was calculated from the difference in the quinidine concentration in the supernatant obtained from homogenate treated with digitonin (cytosol and lysosome) and untreated (cytosol). Twenty percent of the total quinidine in each tissue studied was found in the lysosomal fraction, and there was no significant difference among tissues. The above finding indicates that lysosomal uptake and accumulation of quinidine are not a determining factor in the interorgan variations in the tissue distribution of quinidine, although lysosomes may be one of the intracellular accumulation sites of quinidine.

To examine the role of phosphatidylserine in the intrapulmonary distribution of quinidine, quinidine concentrations were plotted against the concentration of phosphatidylserine in the cellular and subcellular fractions (Fig. 5). The linear correlation between them ($r$ = 0.906) suggests that the concentration of phosphatidylserine accounts for the extent of the cellular and subcellular distributions of quinidine *in vivo*. Accordingly, a higher concentration of phosphatidylserine in the fractions such as the alveolar macrophage and plasma membrane resulted in a higher distribution of quinidine. On the other hand, the value of the intercept of the line represents binding independent of phosphatidylserine. Proteins also contribute to quinidine binding. Thus, these results indicate that the content of quinidine localized in protein components is constant among various cellular and subcellular fractions, and a dominant determining factor in the variation of quinidine distribution among various fractions is the phosphatidylserine concentration.

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