Solubilization of Cationic Drugs in Lung Surfactant

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Purpose. The association of hydrophobic, cationic drugs with lung surfactant was determined to assess the pharmacokinetic implications on drug disposition and retention in the lung.

Methods. The distribution coefficients, K, were determined at 25 and 37° in normal saline solution buffered at pH 7.4 for a series of structurally related, cationic drugs. Drugs were dispersed into lung surfactant, equilibrated, and then centrifuged to separate the aqueous phase from the surfactant pellet. Drug concentrations in the supernatant and pellet were determined following dilution using spectrophotometric assays. In addition, the apparent acid dissociation constant of quinacrine in the presence and absence of surfactant was determined by measuring the pH-dependent absorption spectra. The effect of stereochemistry on the distribution of drugs into surfactant was examined with *(R)*- and *(S)*-propranolol.

Results. The mole fraction distribution coefficients for amitriptyline, promethazine, promazine, ethopropazine, imipramine, R-propranolol, and S-propranolol at 25°C were 6,560 ± 500, 28,400 ± 1,500, 12,100 ± 840, 5,480 ± 330, 4,490 ± 250, 8,680 ± 260, 8,190 ± 530, respectively. At 37°C, the distribution coefficients were generally smaller indicating a significant exothermic heat of transfer for these solutes from aqueous solution to the lung surfactant. The pKa of quinacrine was 7.43 ± 0.04 in aqueous solution and was shifted to 7.62 ± 0.06 in the presence of lung surfactant. From this shift, the double layer potential for quinacrine-lung surfactant was estimated to be –0.012 V assuming a dielectric constant equivalent to that of water.

Conclusions. Cationic drugs have very favorable distributions from an aqueous solution to the lipid phase of lung surfactant. The transfer process generally has both a large entropic and enthalpic contribution. The latter thermodynamic aspect may be related to the charge interaction between the solute and the negatively charged surfactant. Finally, no significant effect of stereochemistry was evident with the distribution of *(R)*- and *(S)*-propranolol.

KEY WORDS: lung surfactant; phenothiazines; pKa; partition coefficient; solubilization.

INTRODUCTION

Local drug delivery, such as inhalation, offers two distinct advantages over oral and intravenous administration methods. First, drug is directly delivered to the site of action achieving more rapid and efficacious activity. Second, since the amount of drug required for a therapeutic outcome is smaller, the drug concentration in the blood stream is reduced thereby limiting the occurrence and severity of systemic side effects.

Unique to the lung is the presence of lung surfactant both at the air/water interface as well as in lamellar bodies of alveolar type II cells (1,2). Lung surfactant has been shown to affect the solubilization of neutral steroids as well as the dissolution rate of aerosol particles *in vitro* (3,4). Moreover, lung surfactant has been postulated to affect the pharmacokinetic disposition and clearance of drugs administered directly to the lung and following systemic administration (5).

Lung surfactant is a lipid rich material that exists largely in a liquid crystalline, bilayer state. Although water soluble proteins are necessary for the intriguing superstructures present in the lung, the lipid bilayers are believed to provide the solubilization milieu for hydrophobic drugs (3,4). In addition, the negatively charged phosphatidylglycerols result in a material that can attract positively charged species. This hydrophobic environment coupled with a negative surface charge provides the basis for the postulate concerning the pharmacokinetic distribution of cationic drugs (6). A recent study has suggested that 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), the major metabolite of a tobacco-specific nitrosamine, NNK, has a stereoselective retention in the lung (7). Association of solutes within the acidic environment of lamellar bodies may be responsible for this sequestration. Thus, lung surfactant may have a role in the distinct pharmacokinetic distribution of enantiomers.

Taken together, solute affinity to lung surfactant appears to be related to the solute's hydrophobicity, cationic character, and stereochemistry. Therefore, this study was undertaken to (i) define the distribution coefficients of several structurally related cationic drugs in lung surfactant, (ii) examine the effect of surface charge, and (iii) assess the stereoselective affinity of lung surfactant lipids.

Experimental Section

Materials

Amitriptyline, ethopropazine, imipramine, promazine, promethazine, quinacrine, *(R)*- and *(S)*-propranolol were purchased from Sigma Chemical Co. (St. Louis, MO) and used as received. Survanta™ lung surfactant was purchased from Ross Laboratories (Columbus, OH) and used without further purification. All other chemicals were reagent grade or better.

Experimental procedures

All experiments were carried out in pH = 7.4 phosphate buffered saline solution (PBS). In the solubilization experiments, 50 μL of diluted lung surfactant was added to 1 mL drug (1×10^{-3} M) in the buffer solution contained in a microcentrifuge tube and equilibrated at 37 ± 1°C for at least 12 h. After equilibration, the mixture was centrifuged at 13,000 rpm for 1 h to separate the aqueous and surfactant phase. Then, a 0.2 mL aliquot of supernatant was transferred into a 10 mL volumetric flask and diluted with buffer. The remaining supernatant was carefully removed. The pellet was quantitatively transferred with ethanol to a 25 mL volumetric flask and brought to volume with ethanol. The drug concentration in the diluted supernatants and pellets were determined spectrophotometrically using appropriate standard curves (Beckman, IL). Measurements were conducted at five different concentrations, and the distribution coefficients were calculated based on the five samples. Error analysis indicated that variability in the measurement of a distribution coefficient would be less than 10%.

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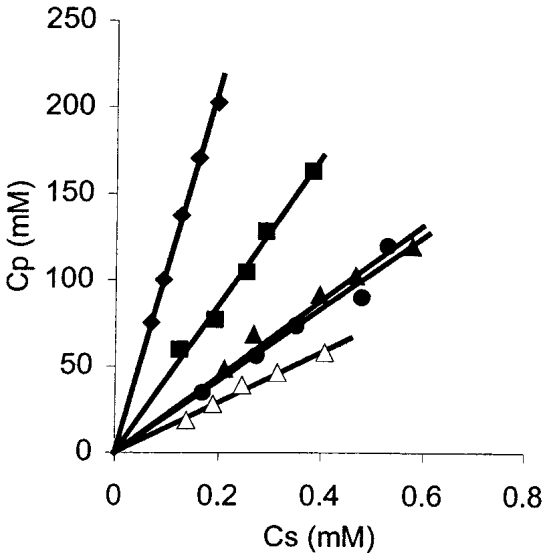

Fig. 1. The concentration of drug in pellet (surfactant phase) as a function of the concentration of drug in supernatant (aqueous phase) at 25°C for for (♦—-) Promethazine, (■—-) Promazine, (▲—-) Ethopropazine, (●—-) Amitriptyline, (Δ—-) Imipramine.

The mole fraction distribution coefficient, K, and associated 95% confidence limits were obtained by plotting X_p against X_s, where X_p and X_s are the mole fractions of drug in the pellet and supernatant. The mole fraction pellet concentration is based on the phospholipid content only, that is, $X_p = n_p/(n_p + n_l)$ where n_p is the moles of drug in the pellet, and n_l is the moles of surfactant phospholipid in the pellet. The moles of phospholipid in surfactant were measured by a spectrophotometric assay for the total number of moles of elemental phosphorus (8). Statistical analysis was carried using an Excel spreadsheet.

A titration experiment was performed with quinacrine to provide an estimate of the surface charge of the surfactant lipids. For the titration experiment, quinacrine was initially placed in solution at pH = 4.0, and then titrated with 0.15 M NaOH solution to pH = 10.0. The volume change caused by addition of NaOH was negligible, because the initial volume of the quinacrine solution was about 60 ml. The absorbance of the solution was measured as a function of pH at 444 nm. The experimental data were fit by KaleidaGraph using the following equation:

$$\log\frac{\alpha}{1-\alpha} = pH - pKa \quad (1)$$

where

$$\alpha = \frac{A - A_{\min}}{A_{\max} - A} \quad (2)$$

and α is the fraction ionized, and A is the absorbance.

RESULTS AND DISCUSSION

The purpose of this work is to investigate the general aspects of drug solubilization in lung surfactant. An earlier effort had focused on the distribution of neutral steroids and their partitioning behavior into surfactant (3). In this study, several phenothiazines were examined to expand the analysis to a second series of hydrophobic compounds. In addition, these compounds carry a net positive charge, which would allow assessment of the electrostatic interaction. To investigate the contribution of the electrostatic interaction, the surface charge was investigated through the measurement of the acid dissociation constant of quinacrine. Finally, the possibility of stereoselective affinity was investigated with propranolol enantiomers. The findings provide a quantitative assessment of the influence of hydrophobicity, charge and stereochemistry in the partitioning process, which is useful for exploring the pharmacokinetic implications of drug retention in the lung.

Figure 1 is a plot of the concentration of drug in the surfactant pellet as a function of the aqueous supernatant at 25°C. For the phenothiazines, the pellet concentration increased with an increase in concentration of the supernatant in a linear manner. In addition, linear fits yielded intercepts that were not significantly different from zero at 95% confidence. Because these results are consistent with concentration independent partitioning of drugs with lung surfactant, the data were pooled and the distribution coefficient was calculated from the slope of the line.

From the graph, it is evident that the order for the distribution coefficients is: promethazine > promazine > amitriptyline > ethopropazine > imipramine. In Table I, the calculated distribution coefficients and associated 95% confidence limits are given. From the distribution coefficients, the moles of drugs solubilized per mole of phospholipid in Survanta™ at 25°C can be determined. With these solutes, the solubilization

Table I. Distribution Coefficients, K, of Drugs in Lung Surfactant at 25°C and 37°C Along with Octanol/Water Partition Coefficients, Acid Dissociation Constants, and Change in Molar Enthalpy and Entropy for the Transfer of Drug from the Aqueous Phase to the Surfactant Phase

Name	K (×10^{-3}) at 25°C	K (×10^{-3}) 37°C	LogP[a,c]	pKa[b,c]	ΔH (kJ/mol)	ΔS (J/mol°K)
Amitriptyline	6.56 ± 0.50	4.55 ± 0.15	6.14 ± 0.33	9.24	–23.4	5.47
Imipramine	4.49 ± 0.25	4.70 ± 0.28	4.47 ± 0.38	9.49	–54.5	97.8
Promazine	12.1 ± 0.84	6.06 ± 0.42	4.63 ± 0.25	9.43	–44.2	70.3
Promethazine	26.1 ± 1.5	12.1 ± 0.51	4.69 ± 0.26	8.98	–24.5	10.8
Ethopropazine	5.48 ± 0.33	3.73 ± 0.23	5.75 ± 0.27	9.88	2.92	–79.7
R-Propranolol	8.68 ± 0.26	4.61 ± 0.15	3.10 ± 0.19		–40.5	60.4
S-Propranolol	8.19 ± 0.53	5.45 ± 0.60	3.10 ± 0.19		–26.0	12.3

[a] Octanol/water partition coefficient (P)
[b] Negative of the logarithm of the acid dissociation constant
[c] Values obtained from SciFinder Scholar Database, which can be electronically updated and therefore as subject to change

ratios ranged from 0.018 to 0.203. The ratios can be compared with those determined for a series of steroids (3). The results in the present study of cationic phenothiazines are generally much higher than that of the neutral steroids, which had solubilization ratios ranging from 0.019–0.026. From the reciprocal of the values of the solubilization ratio, it can be deduced that between 5 and 55 lipid molecules are needed to solubilize each drug molecule.

The distribution coefficients were also determined at 37°C. As with the 25°C data, good linear relationships were obtained when the pellet drug concentration was plotted as a function of the supernatant concentration (Fig. 2). The data for promethazine, however, was distinct in that the pellet concentration seemed to increase at higher supernatant concentrations. The rank order of the distribution coefficients was similar to that obtained at 25°C except that the K for amitriptyline was now larger than that of amitriptyline (Table I). The range for drug solubilization per mole of phospholipid in Survanta™ 37°C did not extend to such large values as seen at 25°C, as the maximum solubilization ratio was only 0.16.

Before analyzing the results, it is worthwhile to note the composition of lung surfactant. In this study, the organic extract of native surfactant was used as a model system. The composition of Survanta™ is as the follows: 11.0–15.5 mg/ml phospholipids, 0.5–1.75 mg/ml triglycerides, 1.4–3.5 mg/ml free fatty acids, 0.1–1.0 mg/ml protein B/C, and 7.65–10.35 mg/ml sodium chloride. The results provided above are based on the mole fraction of phospholipid, which has a number of implications. First, the moles of phospholipid present in our surfactant preparation have been independently measured and therefore may be readily compared to other studies. Second, in the sample of Survanta™, the concentration of phospholipids was fixed at 2.0 mM. In reporting the mole fraction solubilization, only this value was considered in the calculation. Finally, the high content of lipid is expected to provide a favorable environment for the solubilization of hydrophobic drugs.

For analysis of the distribution coefficients of drugs, Table I provides the octanol/water partition coefficients and pKa. In examining the table, there is no apparent correlation between K and logP. This was true for both the 25°C and 37°C data. Neither was any correlation apparent between K and the pKa.

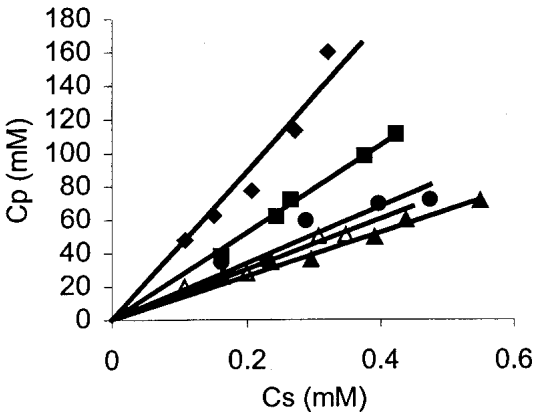

Fig. 2. The concentration of drug in pellet (surfactant phase) as a function of the concentration of drug in supernatant (aqueous phase) at 37°C for (♦—-) Promethazine, (■—-) Promazine, (▲—-) Ethopropazine, (●—-) Amitriptyline, (Δ—-) Imipramine.

The rank order of the distribution coefficients can be rationalized from their chemical structures (Fig. 3). It is expected that the phenothiazines are preferentially oriented in the lipid bilayers such that the hydrophobic ring resides in the palisade region and the positively charged amino group remains at the surface to interact with the negatively charged phosphate and carboxylic acid moieties. Comparing the individual phenothiazines, the difference in the chemical structure of amitriptyline relative to imipramine is the substitution of nitrogen for a carbon atom. Because the nitrogen has the capability to hydrogen bond, it may be more favorable to interact in the head group region of the bilayer. This may account for the larger K.

In comparison to imipramine, promazine has a sulfur in place of the two carbon atoms on the central ring. The sulfur atom is a better electron-withdrawing group than carbon, which makes the nitrogen atom more positively charged. This may account for the more favorable association of promazine. The sole difference between promethazine and promazine is the additional secondary methyl group on the side chain of promethazine. This subtle difference resulted in distribution coefficients that differed by a factor of two. Finally, the two ethyl groups attached to the nitrogen atom of ethopropazine were unfavorable resulting in a relatively low distribution coefficient. The bulky nature of this modification would make insertion in the head group region of the bilayer difficult. Although specific aspects of the chemical structures have implications on the quantitative distribution, it is evident that hydrophobicity has a dominant in the observed favorable distribution of phenothiazines into lung surfactant.

Turning the discussion toward a consideration of the role of stereochemistry in the surfactant distribution, it has been shown that NNAL enantiomers have stereospecific retention within the lung (7). The work with propranolol was carried out to determine the effect of lung surfactant on the distribution of enantiomers. In Fig. 4, the concentration of drug in the surfactant pellet is plotted as a function of the supernatant concentration. From the slopes, the distribution coefficients at 25°C were found to be 10,360 ± 310 and 9,780 ± 630 (Table I) for the R and S enantiomers, respectively. At 37°C, distribution coefficient for S-propranolol is a little higher than R-propranolol but not statistically different. Like the other cationic drugs, propranolol has a very favorable distribution that can lead to extended lung retention; however, no stereospecificity was evident in this study.

Although it was the hypothesis of this study that lung surfactant lipids gives rise to stereospecific retention of propranolol, no evidence was obtained to support this premise. This finding indicates that the nature of the lipid aggregates does not support stereoselective retention. The lipids are known to exist in a bilayer state, and whereas each is a unique stereoisomer, the presence of many lipids within the same plane may allow the solute molecules to interact simultaneously with multiple lipids. The fact that many lipids are available to accommodate each drug also argues against the presence of stereoselective affinity. Thus, the explanation for the stereoselective affinity previously observed may lie in the unique structural features of lung surfactant as it is found in the lung. Alternatively, other proteins, such as beta adrenergic receptors, may be the site for this phenomenon. Additional study is required to explore these questions.

To provide the thermodynamic foundation for the ob-

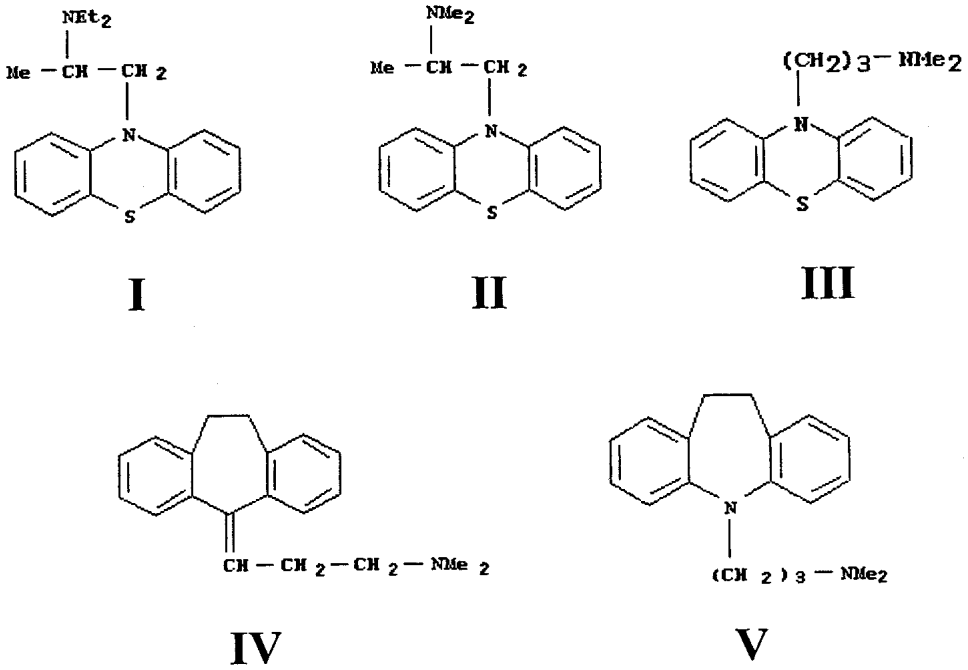

Fig. 3. Structures of the compounds used in this study. Ethopropazine (I), Promethazine (II), Promazine (III), Amitriptyline (IV), and Imipramine (V).

served distribution coefficients, the change in the molar free energy for the transfer of drugs from the aqueous phase to the lung surfactant was calculated taking 37°C as the standard temperature. The change in molar free energy for the transfer was large and negative in each case. The values for the phenothiazines and the enantiomers of propranolol ranged from –21.1 kJ/mol to –24.2 kJ/mol. In addition, the changes in molar enthalpy and molar entropy were estimated from the distribution coefficients determined at the two temperatures 25°C and 37°C and are given in Table I.

The contribution to the free energy arising from the hydrophobicity, ΔG_{hc}, is often related to the octanol/water partition coefficient.

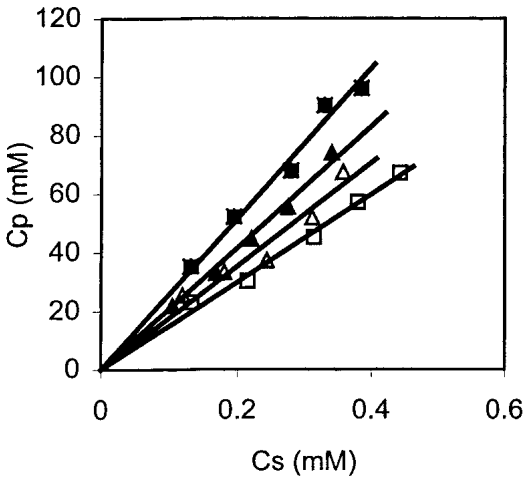

Fig. 4. The concentration of drug in pellet (surfactant phase) as a function of the concentration of drug in supernatant (aqueous phase) at 25°C and 37°C for (■—-) R-propranolol at 25°C, (□—-) R-propranolol at 37°C, (▲—-) S-propranolol at 25°C, (Δ—-) S-propranolol at 37°C.

$$\Delta G_{hc} = -2.303RT\log P \quad (3)$$

For amitriptyline, the free energy for transfer that was calculated from the partition coefficient is –36 kJ/mol. The latter has been corrected to the unitary free energy of transfer. The free energy for transfer calculated from the experimentally observed distribution coefficients is –22.2 kJ/mol, which is smaller in absolute magnitude. The discrepancy likely arises from the fact that the partition coefficient is for the nonionized form of the compounds. Similar findings were calculated for the other compounds.

The values of the change in entropy of transfer were also all positive except for ethopropazine. This is expected because the long alkyl chains of the surfactant lipid can provide a favorable hydrophobic domain for the solubilization of the nonpolar portions of the phenothiazines. The increase in entropy caused by the disordering of the water structure that occurs with removal of the solute from the water and incorporation into the lipid bilayer.

The enthalpy changes associated with the drug transfer ranged from a small endothermic value for ethopropazine to large exothermic values for amitriptyline and promethazine, and even larger values for promazine and imipramine. For the classic hydrophobic interaction, the enthalpy change usually is small and exothermic. However, the electrostatic interactions must be considered in conjunction with the hydrophobic interaction. The association of the cationic drug with the negatively charged surface would lead to charge neutralization that is typically exothermic. Moreover, the electrostatic interactions seems to be important the overall interaction as evident by the large enthalpy changes.

While reasonable, there are a number of caveats in these interpretations. First, the thermodynamic characterization is based on measurements at only two temperatures, which have an extremely limited range. Of even greater concern is the presence of the gel to liquid crystalline phase transition in

lung surfactant. Because of the complexity of the mixture, the phase transition of lung surfactant has been found to be fairly broad and extends from about 20 to almost 40°C (9). As such, the solutes have the potential to distribute into two different phases of the lipid bilayer. With an increase in temperature, there will be a decrease in the fraction of lipid in the gel state in favor of that in the liquid crystalline state. This shift in the fraction of lipid, in turn, will alter the observed distribution coefficient and the calculated entropy and enthalpy changes. Thus, the somewhat unexpected thermodynamic parameters observed with imipramine may be related to specific favorable interactions of this drug with the gel state of the lung surfactant.

In an effort to estimate the surface charge of the surfactant, an ionizable solute was titrated in the presence and absence of lung surfactant (Fig. 5). The lower and upper plateaus correspond to the absorbance of the neutral (Amin) and the cationic form (Amax) of quinacrine. In aqueous solution, the data were well fit by the Henderson–Hasselbach equation. From a fit of the data, the pKa of quinacrine in aqueous solution was found to be 7.43 ± 0.04. In the presence of lung surfactant, a fit to the classic Henderson–Hasselbach expression as given in the experimental section led to a plot of the residuals that was not randomly distributed (10,11). Therefore, the data were fit to "stretched" expression where the pKa is essentially assumed to be pH dependent (11). That is,

$$b \log\frac{\alpha}{1-\alpha} = pH - pKa \tag{4}$$

where b is a constant. From the fit, the pH independent pKa was found to be 7.62 ± 0.06, which was significantly greater than that determined in the absence of lung surfactant. The value of b was found to be 0.586 ± 0.048.

This has two important implications. The first issue is the direction of the pKa shift. The negatively charged bilayers of the lung surfactant will preferentially attract positive ions as observed with all charged surfaces in the presence of mobile counterions (12,13). As such, the concentration of hydrogen ion (H^+) at the surface of surfactant will be higher than that in the bulk solution. Therefore, the pH at the surface is lower than that in the bulk, where it is measured during the titration. The result is that the inferred pKa is higher for a drug solubilized at the surface of lung surfactant when compared to the pKa measured in a simple aqueous solution. Moreover,

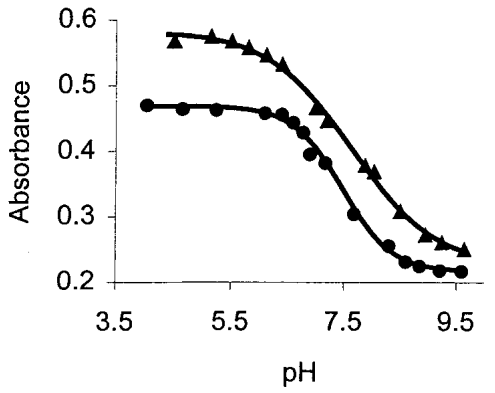

Fig. 5. Absorbance of quinacrine as a function of pH in the (▲) absence and (●) presence of lung surfactant.

from the shift in the pKa, an estimate of the surface potential can be obtained with the assumption that the effective dielectric constant at the site of solubilization is equal to that of water (14).

The second issue is the added complication from the pH dependence of the pKa. The most reasonable explanation for this observation is that during titration, quinacrine is protonated and thereby reduces the magnitude of the negative surface potential. Thus, the pH dependence of the surface charge is in effect a function of the extent of ionization of quinacrine.

To analyze the data, quinacrine is assumed to be absorbed at the negatively charged surface of lung surfactant. For the dissociation of a weak base, $BH^+ \leftrightarrow H^+ + B$, the following equation can be used to describe the relationship between the pKa shift and the double layer potential (12,13),

$$\mathrm{pKa} - \mathrm{pKs} = \frac{\Delta G_a^0 - \Delta G_s^0}{2.303RT} + \frac{F\varphi_w^0}{2.303RT} \tag{5}$$

where the pKa and pKs are the dissociation constants of quinacrine in the absence and presence of lung surfactant, respectively, F is Faraday's constant, and ϕ_w° is the surface potential. ΔG_a^0 and ΔG_s^0 are the corresponding changes in Gibbs free energy of the dissociation process at 37°C in the presence and absence of surfactant. The standard change in Gibbs free energy in the case of dissociation is largely determined by the dielectric constant of the medium. Thus, if the solute resides in a similar dielectric medium, whether in aqueous solution or at the bilayer surface, ΔG_a^0 will be equal to ΔG_s^0. Using the assumption, Eq. 1 can be rewritten as equation 2.

$$pKa - pKs = \frac{F\varphi_w^0}{2.303RT} \tag{6}$$

According to Eq. 2, the double layer potential was found to be –0.012 V in the absence of quinacrine. Given the composition of lung surfactant, which is in the presence of 150 mM sodium chloride, this is a reasonable value for the surface potential. However, the assumption of constant dielectric typically fails for drug solubilized in micelles, and therefore this result may be fortuitous. Finally, with an estimate of the surface potential, the contribution to the Gibbs free molar energy for transfer from water to the surfactant surface can be estimated from the following:

$$\Delta G = -zF\phi_w^\circ \tag{7}$$

The magnitude is about 1 kJ/mol, which is somewhat small to account for the discrepancy between the observed and predicted free energy of transfers as discussed earlier. Thus, the phenothiazines may be affecting the lipid bilayer to a greater extent, which may explain the large exothermic energies of transfer.

In this work, favorable association has been demonstrated for hydrophobic species that carry a net negative charge. In addition, previous studies have shown that hydrophobic steroids preferentially associate with lung surfactant (2). These results are consistent with the suggestion by Upton and Doolette (5), who reviewed the pharmacokinetic distribution of cationic drugs and concluded there is significantly more drug delivered to the lung relative to other organs when

normalized to organ mass. For oral or parenteral delivery, the impact is modest, since the lung has little mass in comparison to the other organs in the body. However, a profound effect may be realized for drugs specifically delivered to the lung (15,16). In this case, higher drug levels may be obtained for longer periods because of the association with lung surfactant. The importance of this work is that the contribution of hydrophobicity and charge has been quantitatively addressed, and these results can be used to estimate the relative retention of drugs in lung surfactant. Moreover, there are potentially interesting applications for enhancing the lung retention of delivery systems composed polymers or other excipients (2,17,18).

In conclusion, hydrophobicity has been shown to have a significant impact on the solubilization of the drugs in lung surfactant. For the phenothiazines, electrostatic interactions play an important role in addition to hydrophobic interactions. Specific chemical moieties modulate the overall distribution coefficient. There is no observable difference in distribution coefficients between R- and S- propranolol, which suggests other factors influence the retention time of enantiomers. Finally, the surface charge of the lung surfactant has been estimated and has been found to increase the dissociation constant of a weak base.

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