Mechanistic Studies on Surfactant-Induced Membrane Permeability Enhancement

Wei J. Xia[1,2] and Hayat Onyuksel[1,3]

Received February 8, 2000; accepted February 15, 2000

Purpose. To gain some mechanistic understanding of surfactant-induced membrane permeabilization and identify a surfactant physical property that can be used as a predictor for intestinal membrane permeability enhancement.

Methods. The maximum surface pressures (π_{CMC}) of series of anionic and non-ionic surfactants as indicators of surface activity were determined using a bubble surface tensiometer, and related to *in vivo* intestinal membrane permeability and acute damage data of the same surfactants from a previous work. Phospholipid bilayers with constant surface pressures and monolayers with different surface pressures were used as model membranes to systematically study membrane permeability enhancement and membrane penetration of surfactants at different concentrations.

Results. Surfactants that did not permeabilize or acutely damage the intestinal wall generally exhibited a $\pi_{CMC} < 25$ dyne/cm. Permeability enhancement and acute damage increased as π_{CMC} increased beyond 25 dyne/cm. This critical threshold value at around 25 dynes/cm was also observed with *in vitro* experiments using phospholipid vesicles and monolayers. Data support the hypothesis that the threshold phenomenon originates from the interfacial tension at the membrane/water interface, which controls the surface adsorption process of surfactant molecules onto the membrane.

Conclusions. For a surfactant to permeabilize and acutely damage the intestinal wall, it must exhibit a surface pressure of greater than 25 dynes/cm. This threshold value is related to an intrinsic property, surface pressure, of the phospholipid membranes. Since the surfactant surface pressure is a property of the surfactant monomer, partition of the surfactant monomer, not the micelle, into the membrane is an obligate step in membrane permeabilization. Above the surfactant critical micelle concentration, CMC, micelles may act as a depot to continuously replace aqueous surfactant monomers taken up by the membrane. For some surfactants above CMC, sufficient number of monomers can partition into the membrane to cause solubilization of membrane lipids in surfactant micelles.

KEY WORDS: membrane permeability; surface pressure; liposome; surface tension; phospholipid monolayer; bile salts; nonylphenoxy polyoxyethylene; model membrane.

INTRODUCTION

The use of surface-active agents to increase the permeability of intestinal wall to drugs has been widely investigated, although general use in human therapeutics has not occurred yet (1–3). Swenson *et al.* have demonstrated that surfactant-induced intestinal permeability enhancement is correlated with acute damage of the intestinal epithelium, when surfactants are used at the concentrations above the critical micellar concentration (4,5). For the surfactants taurodeoxycholate (TDC) and nonylphenoxy-polyoxyethylene-10.5(NPPOE-10.5), this epithelial damage undergoes restitution within a few hours or less (4). Thus the detection of the presence or absence of epithelial damage may depend upon the time of observation relative to the last dosing of the permeability enhancer. The safety of chronic dosing of permeability-enhancing surfactants is not well understood (6).

The physical chemical aspects of surfactant-induced membrane permeability enhancement and acute toxicity have not been extensively investigated in detail. It is not clear, for example, why some surfactants are permeability enhancers, while others are not. In general, non-ionic surfactants must possess a hydrophile/lipophile balance (HLB) which is lower than some threshold, with the specific threshold value dependent upon surfactant structure (7). For example, nonylphenoxy-polyoxyethylenes have been shown to require an HLB less than 17 to exhibit intestinal permeability enhancement and acute mucosal damage (5). Florence and his co workers have demonstrated that, for the non-ionic surfactants, an intermediate HLB value was optimal for permeability enhancement, therefore permeability enhancement did not appear to be related to surfactant properties such as HLB or ethylene oxide chain length over a broad range (1,8). Similarly, Swenson *et al.* (4,5) using a group of ionic and non-ionic surfactants and isolated rat intestine have shown that some surfactants were effective permeability enhancers and some were not, even though all the surfactants were used above their CMC. These studies clearly indicate that permeability enhancement cannot solely be explained by the surfactant's hydrophobic property as presented by HLB or CMC values. In this study we used a physical parameter, surface pressure, π, to relate the surface activities of the surfactants to their *in vivo* and *in vitro* membrane permeability enhancement abilities as determined previously by Swenson *et al.* (5). Our data showed that ineffective surfactants had maximum surface pressures below 25 dynes/cm where as, the maximum surface pressures of effective permeability enhancers were always above 25 dynes/cm. The higher the surfactant surface activity the more effective was the surfactant as permeability enhancer. The meaning of the threshold value and the relationship between the surface activity and the permeability enhancement property of a surfactant was explained by studying the effects of the surfactants on model membranes, phospholipid bilayers and monolayers, under well-controlled *in vitro* conditions.

Our goals in this work were to: (a) gain some mechanistic understanding of surfactant-induced membrane permeabilization, and (b) identify a simple physical parameter to be used as a predictor for intestinal permeability enhancement and acute damage.

MATERIALS AND METHODS

Materials

Non-ionic surfactants, nonylphenoxy polyoxyethylene, NPPOE −9, −10.5, −20, −30, −50 and 100 were obtained from Rhone-Poulenc Inc. (Princeton, NJ). These correspond to the manufacturer's designations Igepal CO-630, 710, 850, 880,

[1] College of Pharmacy, University of Illinois at Chicago, Chicago, Illinois 60612.

[2] Present address: Dow Pharmaceutical Sciences, Petaluma, California 94954.

[3] To whom correspondence should be addressed. (e-mail: hayat@uic.edu)

0724-8741/00/0500-0612$18.00/0 © 2000 Plenum Publishing Corporation

970, and 990, respectively. These surfactants are polydisperse and the numerical designations - 9, −10.5, etc. represent the average number of POE groups per molecule. Non-ionic surfactant, polysorbate-80 was from Ruger Chem. Co. (Irving NJ). Anionic surfactants, sodium taurocholate (TC), sodium taurodeoxycholate (TDC), sodium cholate (C), sodium deoxycholate (DC) and sodium lauryl sulfate (SDS) were purchased from Sigma Chem. Co. (St. Louis, MO). Egg-phosphatidylcholine (EPC) and cholesterol (Chol) were also obtained from Sigma Chem. Co (St. Louis, MO). 1,2-dipalmitoyl-sn-glycerol-3-phosphocholine (DPPC), and sodium 1,2-dipalmitoyl-sn-glycerol-3-phospho glycerol (DPPG) were purchased from Sygenal Ltd. (Eichenwegl, Switzerland). 5(6)-carboxyfluorescein (CF) was from Eastman Chem. Co. (Rochester, NY).

Surface Tension and Surface Pressure Determinations

Surface tensions of surfactant solutions were determined using Sensadyne 6000 Surface Tensiometer (Chem-Dyne Research Corp., Milwaukee, WI), as described before (9). This method measures the pressure difference (ΔP) between nitrogen bubbles formed at the end of two immersed probes of different diameters (R_1 and R_2). The relationship between surface tension (γ) and bubble pressure is described by the Young-Laplace equation: $\Delta P = \gamma\ (1/R_1 + 1/R_2)$. The nitrogen gas pressure was maintained at 60 psi. The bubble frequency was set at 1 sec^{-1} in reference to phosphate buffer solution, which was slow enough for the system to reach equilibrium for the surfactants used. Each surface tension value was the mean of twenty readings. Standard deviation varied from 0.1 to 0.2 dyne/cm. Surfactants were dissolved in a 66 mM phosphate buffer (pH 6.5) containing 72 mM Na_2SO_4 and measurements were made at 37°C. These conditions were comparable to the *in vivo* data of Swenson *et al.* (4,5). Surface tensions of the same surfactant solutions were also measured at 20 ± 0.5°C in 10mM isotonic phosphate buffer (pH 7.5) to correlate *in vitro* experiments with phospholipid surface monolayers performed at room temperature.

The maximum surface pressure of a surfactant was determined by the difference between the surface tension measurements of buffer in the absence and presence of the surfactant above CMC. Even though surface pressure, π, changed insignificantly after CMC, in this study we used π_{CMC} and $\pi_{1\%}$ to represents the maximum surface activity effect of a particular surfactant at the air/water interface just after CMC and at 1% concentration, respectively.

Preparation of Carboxyfluorescein (CF) Containing Phospholipid Vesicles

Liposomes were prepared by the reverse phase evaporation (REV) method as described (10). The lipids, EPC:Chol: DPPG, 4:3.3:1 in molar ratios, were dissolved in chloroform and mixed with isotonic CF aqueous solution (2.72% w/v, pH 7.4) at an organic to aqueous volume ratio of 3:1. Gentle sonication (Bransonic Ultrasonic Corp. Goleta, CT) led to the formation of a W/O type emulsion. The sample was moved to a rotary evaporator (Labconco, Kansas City, MI) to evaporate the organic solvent under argon gas at reduced pressure and 55°C. Unilamellar liposomes were formed after careful evaporation of the organic solvent. Unentrapped CF was removed from the liposomal suspension by washing with buffer and centrifuging. The mean size and size distribution of liposomes were measured by quasielastic light-scattering (Nicomp, Model 270, Pacific Scientic Corp.) Liposomes were sealed in a vial under nitrogen gas and stored at 4°C condition. The same batch of liposomes, mean size 700 nm, was used for all the studied surfactants in order to eliminate any batch to batch variation in size and morphology of liposomes.

Monitoring the Release of Carboxyfluorescein from Phospholipid Vesicles

The amount of CF released from liposomes was determined by the fluorescence self-quenching method. The method was based on the loss of fluorescence efficiency due to self-quenching at high concentration inside liposomes and increased efficiency if CF is released and diluted into the surrounding medium. Experiments were carried out at room temperature (20 ± 1.0°C). A 0.01 ml aliquot of liposome suspension (~0.03 μmole total phospholipids) was introduced into 1.99 ml surfactant solution, either below or slightly above CMC, in a 2 ml semi-micro spectrophotometer cuvette. The concentration of CF in the dispersion medium was determined by a fluorophotometer (FM-2 Ocular Fluorophotometer, Coherent, Palo Alto CA), at given time intervals. The amount of CF released was calculated according to the equation:

$$\%\ \text{CF released} = 100 \times (C_t - C_c)/(C_T - C_c)$$

where C_T is the total concentration of CF after all liposomes were dissolved by Triton X_{100} (final concentration 0.2%). C_c and C_t are the concentrations of CF in buffer alone (10 mM Phosphate pH 7.4) and in surfactant solution respectively, at a given time. Each data point represents the mean of triplicates and an average of duplicate experiments.

Phospholipid Monolayer Film Experiments

Monolayer studies were performed using an apparatus developed in our laboratory based on previously published studies (11). This consisted of a 6 × 8 × 4 cm glass container with a volume of 150 ml and a movable plastic bar, which can compress the surface film. A du Nouy Ring Surface Tensiometer (Fisher Sci. Model 20. Pittsburgh, PA) was employed to measure the surface pressures of the monolayer film. The subphase consisted of isotonic phosphate buffer (10 mM, pH 7.4) with or without the presence of a surfactant. The surface pressure of the phospholipid (DPPC) monolayer film was determined by surface tension measurements at room temperature. DPPC was spread from a solution (1.0 mg/ml) of n-hexane:isopropyl ether:ethyl alcohol (3.5:0.9:0.1 v/v/v) by means of a microsyringe in an amount (~10–15μ L) sufficient to produce the desired initial surface pressure so that only a slight adjustment, if necessary, with the movable bar was made. After evaporation of the organic solvent (~15 min), the initial surface pressure of the surface monolayer film was adjusted to the desired initial surface pressure by slightly condensing or expanding the surface film with the movable bar. Surfactant solutions (10 ml) to give concentrations either below (0.067% for non-ionic, and 0.5 mM for ionic surfactants) or just above CMC (0.2% for nonionic, and 2 mM for SDS, DC, TDC and 8 mM for TC, 10 mM for C) were carefully introduced into the subphase (140 ml) with

a glass pipette without disturbing the surface film. After equilibration (5 min) the surface pressure of the film was measured. Each point is the mean of three experiments.

RESULTS

Surfactant Surface Pressure

Surface pressures determined just above CMC at 20°C ($\pi_{CMC-20°C}$) and at 1% (w/v) at 37°C ($\pi_{1\%-37°C}$) were almost identical for ionic surfactants and only slightly higher surface pressure values were obtained for non-ionic surfactants at 1% concentrations. This indicated that the physical parameter used in this study to represent surface activity of a surfactant, was a property of the surfactant monomers at the interface and was not dependent on the bulk concentration after CMC.

Surface pressure decreased as the number of polyoxyethylene groups per molecule increased for NPPOE's. This decreasing affinity of the surfactant to air-water interface is consistent with increasing surfactant polarity as the POE/molecule increases, and is typical for POE-containing non-ionic surfactant series (12). The surface pressure values of the dihydroxy bile acids DC and TDC were larger than for the trihydroxy bile acids C and TC, respectively, consistent with the lower polarity of the former.

Relationship Between Biological Data and Surface Pressure

The biological data reported in this study and the experimental details are described in Swenson *et al.* (4,5). The in vivo penetration enhancement effect of a surfactant is represented by the systemic plasma phenol red (penetrant) concentration after 1 hr perfusion. Similarly, acute mucosal damage is demonstrated by the total lactate dehydrogenase (LDH) released into the intestinal lumen during a one-hour perfusion. LDH is a biological marker which was shown to correlate with intestinal wall damage assessed histologically (4).

Surface Pressure Versus Penetration Enhancement

Figure 1 presents the relationship between $\pi_{1\%-37°C}$ and plasma phenol red concentration observed at the end of one hour rat intestinal coperfusion of surfactant and phenol red. This figure demonstrates that a surface pressure threshold exists at around 25 dyne/cm, below which the plasma phenol red concentration remains approximately equal to the control value. Generally, there appears to be an approximately linear relationship between surface pressure and plasma phenol red concentration above this critical surface pressure. However, NPPOE-9 (with the highest surface pressure of the series, 38.4 dyne/cm) gives a lower plasma phenol red concentration level than the next more hydrophilic non-ionic surfactant, NPPOE-10.5. Being the most hydrophobic surfactant studied, NPPOE-9 is likely to form aggregates more stable than micelles at the concentration used. This was also indicated by the slightly turbid appearence of NPPOE-9 solution. All the other surfactant solutions used in this study were clear to the eye. The non-ionic surfactants polysorbate-80 (TW-80), NPPOE-100,-50 and -30, and the anionic surfactant TC exhibited similar and low surface pressures, and they were not effective permeability enhancers.

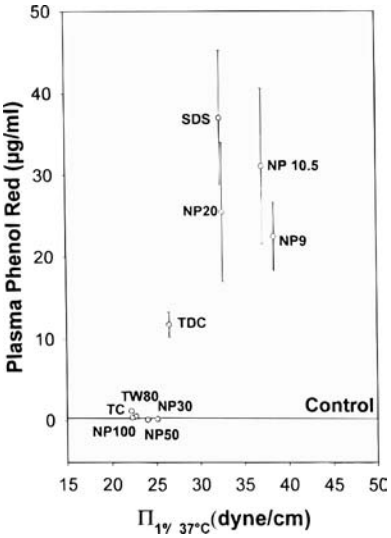

Fig. 1. Relationship between the surface pressures of 1% surfactant solutions and plasma phenol red concentrations after one-hour intestinal perfusion (Control, 66 mM phosphate buffer + 72 mM Na_2SO_4, pH 6.5, 37°C).

Surface Pressure Versus Acute Membrane Damage

Figure 2 presents the relationship between surface pressure and LDH released during perfusions with surfactants. For non-ionic surfactants, it is clear that a threshold surface pressure of ~25 dynes/cm is again observed, with TC as an apparent exception. In this case, the units of LDH released were not significantly different between NPPOE-10.5 and NPPOE-9. The bile salt TC and TDC appeared to cause higher amounts of LDH release even though surface pressures were relatively low. However, the proportionality between LDH release and surface pressure is consistent with the more hydrophobic nature of TDC

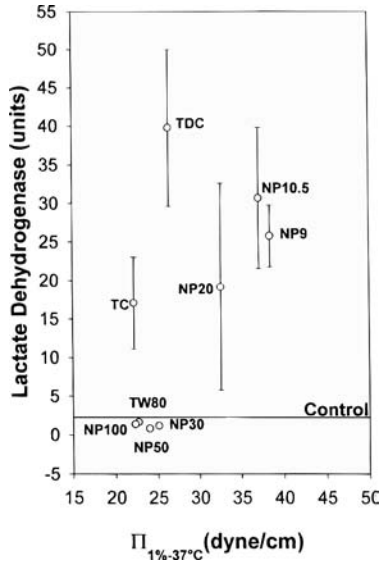

Fig. 2. Relationship between the surface pressures of 1% surfactant solutions and total amounts of lactate dehydrogenase appeared in perfusate after one-hour intestinal perfusion (Control, 66 mM phosphate buffer + 72 mM Na_2SO_4, pH 6.5, 37°C).

relative to TC. Similar results were obtained when another acute toxicity marker, lipid phosphate release was related to surface pressures of the surfactants (data not shown).

An important observation is that a threshold value at around 25 dyne/cm was also indicated with the acute toxicity experiments.

Surfactant Effect on Phospholipid Vesicle Bilayers

In order to study the effects of surfactants on membranes in a more controlled fashion we prepared liposomes encapsulating a marker, carboxyfluorescein (CF), and followed the release of the marker through phospholipid bilayers due to the effect of surfactant molecules which were added into the external aqueous phase of the liposome dispersion.

Phospholipid bilayers have been previously used as model membranes for cell lysis studies (13). Carboxyfluorescein is a trivalent polar compound at neutral pH and poorly permeable through phospholipid bilayers. An increase of CF released from the inside aqueous media of vesicles to the outside surfactant solution indicates a change in the permeability of phospholipid bilayers.

Figure 3 shows the profiles of %CF released from liposomes with time after CF containing liposomes were introduced into surfactant solutions. The concentration of surfactants, 0.5 mM, was below CMC for all cases. The CF released from liposomes either instantly or gradually depending on the surfactant type, and reached a plateau for most cases after 30 minutes. These release profiles are in good agreement with previously observed results by others using similar technique and surfactants (14).

Figure 4 is the plot of %CF released from liposomes after one hour versus the corresponding surface pressures of the surfactants obtained under similar *in vitro* experimental conditions. Similar to the results of *in vivo* experiments, surfactants with surface pressures below 25 dyne/cm showed no significant influence on the CF permeability of phospholipid bilayer. Over the threshold, permeation enhancement activity increased

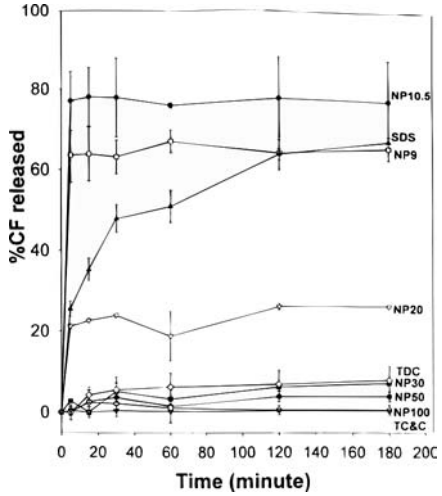

Fig. 3. Percentage of carboxyfluorescein released from liposomes with time in the media of 0.5 mM, below CMC surfactant solutions at room temperature. (Control:10 mM phosphate buffer + 140 mM NaCl, pH 7.4).

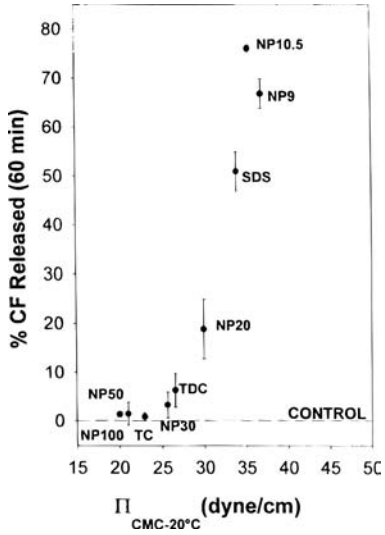

Fig. 4. Relationship between the maximum surface pressure of surfactants and percentage of carboxyfluorescein released from liposomes with the effect of surfactants (0.5 mM) after 1 hour. (Control: 10 mM phosphate buffer + 140 mM NaCl, pH 7.4, room temperature).

almost linearly with the surface pressure. The NPPOE-9 did not deviate from the line as before, most probably due to its lower concentration used in this case.

Interaction of Surfactants with Phospholipid Monolayers

In order to be able to vary the membrane rigidity or flexibility, which can not easily be done with liposome bilayers or biological membranes, we prepared phospholipid monolayers at the air/water interface and adjusted the membrane pressure by controlling the number of phospholipid molecules per surface area. According to studies that relate pressure to molecular area, DPPC monolayers at 16–26 dyne/cm pressures was suggested to be in a "condensed liquid" to "close packed solid" state, and the surface area occupied by each DPPC molecule is roughly 50−35 Å (15). This type of film is considered to be a two-dimensional crystalline phase which was studied first by Langmuir and later used by others as a model for biological membrane (11,15).

Figure 5 presents the equilibrium surface pressure of DPPC surface monolayers after surfactant introduction into the sub phase. The initial surface pressures of the pure DPPC films were 16 dyne/cm and 26 dyne/cm. At low initial surface film pressure each of the studied surfactants were effective to increase the surface pressure of DPPC film. However, surfactants with surface pressures less than 25 dyne/cm, such as TC, C, NP-100 and NP-50, had no effect on the 26 dynes/cm film, whereas the other surfactants further increased the surface pressure. Figure 6 presents the relationship between the equilibrium surface pressure (π_E) of the DPPC/surfactant mixed surface monolayer and the maximum surface pressure (π_{CMC}) of the corresponding surfactant itself. There appears to be a close relationship between π_E and π_{CMC} beyond 25 dynes/cm.

DISCUSSION

Surface Pressure of Permeability Enhancers

Membrane penetration enhancers are generally surface active agents and have the tendency to adsorb at interfaces. In

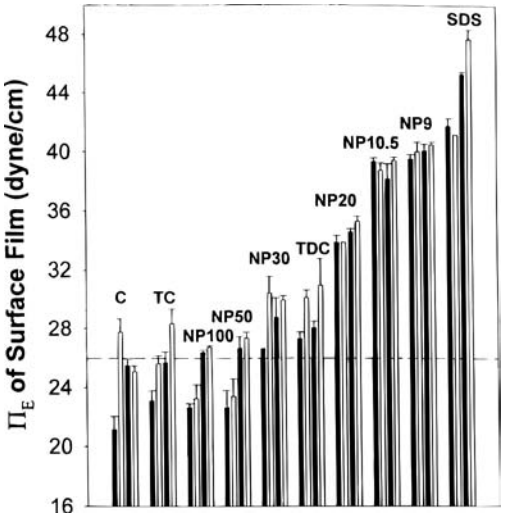

Fig. 5. The equilibrium surface pressures of surface films in the presence of surfactant molecules in the sub-phase. First two columns from left for each surfactant are for 16 dynes/cm initial surface pressure, last two columns are for 26 dynes/cm initial surface pressure. Darker shades are for surfactant concentrations below CMC, lighter shades are for concentrations above CMC.

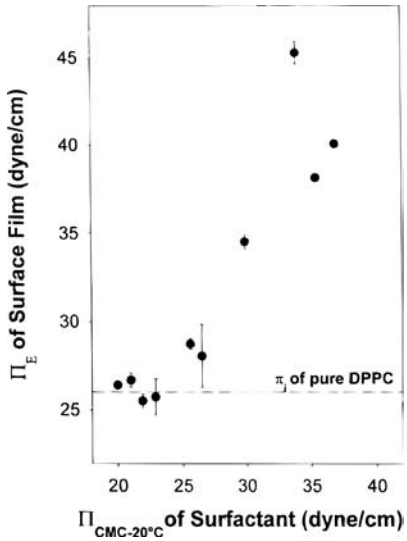

Fig. 6. Relationship between the equilibrium surface pressures of surfactant treated DPPC surface films (π_E) and the surface pressure (π_{CMC}) of the pure surfactants. Initial surface pressure of pure DPPC monolayer is 26 dyne/cm. (Control: 10 mM phosphate buffer + 140 mM NaCl, pH 7.4, room temperature).

order to show the penetration enhancement effect, the surfactant first must go to the lipid membrane/aqueous medium interface. In this study we determined the adsorption characteristics of a group of surfactants at the air/water interface by determining their surface pressures from simple surface tension measurements, and related these values to their effects on membrane permeability or acute damage. The extent of interaction of a surfactant with a membrane is a complex phenomenon and both the properties of the membrane and the surfactant are involved. The physical and chemical properties of a biomembrane are somewhat constant and defined for a physiological condition. Factors related to the surfactant include its affinity for the membrane (or its tendency to escape from water), its free monomer concentration in the aqueous environment adjacent to the membrane, and its structure to accumulate at a given membrane surface area. The independent physical property of a surfactant, surface pressure, used in this study encompasses all of these factors.

Critical Surface Pressure for Membrane Permeability Enhancement and Acute Damage

An important observation from this study is that a critical surface pressure of around 25 dyne/cm must be exceeded to cause membrane permeability enhancement or acute membrane damage (Figs. 1, 2). The reason for this critical threshold is believed to be related to the ability of the surfactant to partition and accumulate on the membrane surface. It is likely that a surfactant with a low surface pressure may not accumulate on the membrane at sufficient quantities to disrupt the membrane. We believe that a critical molar ratio of membrane lipids/surfactant monomers needs to be locally reached in order to disrupt membrane structure, as also suggested by Small (16) for dissolution of lecithin bilayers by bile salts. This critical ratio is different for different surfactants. A recent study using alkyl sulfate surfactants has shown that the surfactant chain length has a profound influence on the amount of surfactant needed for solubilization of the lipid bilayer (17). LeCluyse *et al.* (18) have demonstrated that, in order to promote drug absorption, the enhancer (acylcarnitines) must partition to the membrane at a sufficient amount that disorders more than 15 to 20% of the membrane lipids. In another study we have shown that, at the same concentration, the surfactant with higher surface pressure showed more adsorption on a lipid surface (19).

It is generally believed that surfactant micelles are required in order to cause membrane permeability enhancement and/or solubilization of membrane lipids. However, permeability enhancement does not necessarily involve solubilization of the membrane lipid molecules by surfactant micelles. Our data with model membranes have demonstrated that even at surfactant concentration below CMC the permeability enhancing effect was observed (Fig. 3). Therefore, penetration enhancement effect is due to monomers, not micelles. The role of micelles in this process is to act as a "depot" rather than as an "invader". Micelles continuously supply additional monomers to the aqueous environment in order to reestablish the intermicellar concentration after the association of initial monomers with the lipid membrane. Further more, for surfactants with low surface pressures such as, NPPOE-50, NPPOE-100, TC and C we did not observe significant penetration enhancement effect even at concentrations above CMC (data not shown). These results suggest that micelle presence is not the primary requirement for penetration enhancement. This situation can be utilized for the safe delivery of insoluble drugs when solubilization in micelles is desired without causing any damage to the membrane; i.e. by using a surfactant with a surface pressure less than 25 dynes/cm.

The rate at which the surfactant monomers escape from its aggregates and the energy required for the aggregate to break down are also important for penetration enhancement by surfactants. In fact, this study clearly showed that for the surfactant, NPPOE-9, which most probably formed metastable

liquid crystalline aggregates at high concentrations, the permeability enhancement was not as high as it would be predicted from its surface pressure. Israelachvili *et al.* (20) have estimated that the life-time of a surface active agent in a micelle is $\sim 10^{-4}$ seconds, while the life-time in a bilayer which is a more stable aggregate, is $\sim 10^{4}$ seconds. A recent study demonstrated that surfactants in a free form caused high toxicity to the cells however in niosomes, bilayer structure, had no effect on the cell viability (21). Thus surfactants that form more orderly structures like liquid crystalline phases may provide slower penetration effect due to low monomer replenishment rates, which are orders of magnitudes slower than those for surfactants which form simple micellar phases.

Phospholipid Monolayer Studies

The complexity of the biological system complicates the identification of the forces involved at the biological membrane surface. Phospholipid monolayers can be used to simplify this situation. One attractive aspect of phospholipid monolayers is that their pressure can be adjusted to the desired value. McDonald and Simon (22) have suggested that the molecular packing and lateral pressures of lipid bilayers and monolayers can be closely related. Therefore, phospholipid monolayers with pressures simulating biological membranes have been successfully used to increase our understanding on the interactions of chemical compounds with phospholipid membranes (11,23).

If an insoluble monolayer is spread on an aqueous subphase, and a solution of a water soluble surfactant is then injected into the sub-phase, while the surface area and temperature kept constant, then the interaction of surfactant molecules with surface monolayer will be reflected by an increase in surface pressure. In most cases, the increase of surface pressure results from nonspecific reduction of the free energy of activity of the surfactant molecules by their incorporation into the phospholipid monolayer (24). Data obtained from this study indicated that the penetration was apparently controlled by a pressure difference between the initial pressure of the surface film (π_i) and the maximum surface pressure of the surfactant (π_{CMC}). Therefore the surface pressure of the membrane is also critical for surfactant/membrane interaction. A surfactant showing interaction with one type of lipid membrane may become inactive in the other. As can be seen from Fig. 5, ineffective surfactants became effective when the initial pressure of surface layer was lowered.

Our data with phospholipid membranes strongly suggest that the physico-chemical basis of the threshold pressure value of $\sim$25 dyne/cm is closely related to the surface pressure of the phospholipid membranes (Fig. 6). This value is believed to be at the same level as the surface pressure of the intestinal membrane. It is also close to the estimated surface pressure value, 30 dyne/cm, reported for erythrocyte membrane (25). From the thermodynamic point of view, the movement of a surfactant molecule in a system is driven by its chemical potential. The process is spontaneous and in favor of minimizing the chemical potential of the molecule as well as the total free energy of the system. The surface pressure at an interface is directly related to the level of interfacial free energy. If a surfactant has higher surface activity than the surface pressure at the interphase, it partitions into the membrane. Otherwise, the surfactant is not likely to accumulate in the membrane and exhibit any penetration enhancement or acute damage effect.

The accumulation of surfactants on the membrane may consequently cause a change in the membrane function. Depending on the magnitude of interaction, they may cause a change in membrane permeability by alteration the lipid order, orientation and fluidity, they may also cause an acute membrane damage involving solubilization of membrane lipids. Other studies using phospholipid monolayers as model membranes have also shown the range of 25 to 30 dyne/cm to be critical. For example, it was found that amphiphilic polypeptides with surface pressures above 30 dyne/cm, bound to lipid membranes (26) or interacted with lipid membranes to cause increased membrane permeability to ions (27) and cell lysis (28).

CONCLUSIONS

The current work provides information on the surfactant induced membrane permeability at a molecular level. The results showed that a surfactant must exhibit a maximum surface pressure of 25 dyne/cm or higher to exceed the surface pressure of the biological membrane and cause permeability enhancement and acute damage on a biological membrane. Subsequent steps in permeation enhancement are not clear, however we suggest that accumulation of surfactant monomers in the brush border membrane is the first and necessary step to disrupt the membrane. This study for the first time demonstrated by both *in vivo* and *in vitro* studies that surface pressure, which is an independent physical property of a surface active agent, correlated with the ability of the surfactant to enhance the membrane permeability and acutely damage the membrane.

It should be kept in mind that orally dosed surfactant will mix with amphiphiles from bile and the diet. These may have profound effect on the ability of the dosed surfactant to enhance intestinal permeability (29). Also, since biological membranes usually carry a net negative charge, it is thus likely that the interaction of cationic surfactants with membranes may be more complicated due to additional ionic forces.

ACKNOWLEDGEMENTS

We would like to thank Dr. Curatolo for very helpful discussions and critical comments on the draft of the manuscript. This work was supported by a research grant from Pfizer Inc.

REFERENCES

1. K. A. Walters, P. H. Duagard, and A. T. Florence Non-ionic surfactants and gastric mucosal transport of paraquat. *J. Pharm. Pharmacol.* **33**:207–213 (1980).
2. E. J. van Hoogdalem, A. G. de Boer, and D. D. Breimer. Intestinal drug absorption enhancement: An overview. *Pharmacol. Ther.* **44**:407–443 (1989).
3. S. Muranishi. Absorption enhancers. *Crit. Rev. Ther. Drug Carrier Syst.* **7**:1–33 (1990).
4. E. Swenson, W. B. Milisen, and W. Curatolo. Intestinal permeability enhancement: efficacy, acute local toxicity, and reversibility. *Pharm. Res.* **11**:1132–1142 (1994).
5. E. Swenson, W. B. Milisen, and W. Curatolo. Intestinal permeability enhancement: Structure-activity and structure-toxicity relationships for nonyl-phenoxypolyoxyehylene surfactant permeability enhancers. *Pharm. Res.* **11**:1501–1504 (1994).
6. E. Swenson, and W. Curatolo. Intestinal permeability enhancement for proteins, peptides, and other polar drugs: mechanisms and potential toxicity. *Adv. Drug Del. Rev.* **8**:39–92 (1992).

7. W. C. Griffen. Classification of surface-active agents by "HLB". *J. Soc. Cosmet. Chem.* **1**:311–326 (1947).
8. A. T. Florence. Surfactant interactions with biomembranes and drug absorption. *Pure Appl. Chem.* **53**:2057–2068 (1981).
9. W. J. Xia, and H. Alkan-Onyuksel. A novel approach to study the interaction between bile salt monomers and phospholipid bilayers. *Proc. Intern. Symp. Cont. Rel Bioact. Mat.* **21**:794–795 (1994).
10. R. R. C. New. *Liposomes a Practical Approach*, Oxford University Press, New York, 1990.
11. N. A. Williams, and N. D. Weiner. Interaction of recombinant leukocyte A interferon with lipid monolayers. *Int. J. Pharm* **51**:241–244 (1989).
12. H. Lange, and P. Jeschke. Surface monolayers. In M. J. Schick, ed.: *Surfactant Sciences Series*, Vol. 23; Marcel Dekker Inc.; N.Y. and Basel, 1987, pp 1–44.
13. K. Edwards, and M. Almgren. Surfactant-induced leakage and structural change of lecithin vesicles: Effect of surfactant head group size. *Langmuir* **8**:824–832 (1992).
14. Y. Nagawa and S. Regen. Surfactant-induced release from phosphatidylcholine vesicles. Regulation of rupture and leakage pathways by membrane packing. *J. Am. Chem. Soc.* **114**:1668–1672 (1992).
15. A. Khaiat and I. R. Miller: Adsorption of ribonuclease at the air-water interface and on phospholipid monolayers, *Biochim. Biophys. Acta*. **183**:309–319 (1969).
16. D. M. Small: The formation of gallstones. *Adv. Int. Med.* **16**:243–264 (1970).
17. M. Silvander, G. Karlsson, and K. Edwards. Vesicle solubilization by alkyl sulfate surfactants- A cryo-TEM study of the vesicle to micelle transition. *J. Coll. Int. Sci.* **179**:104–113 (1996).
18. E. L. LeCluyse, L. E. Appel, and S. C. Sutton: Relationship between drug absorption enhancing activity and membrane perturbing effects of acylcarnitines. *Pharm. Res.* **8**: 84–87 (1991).
19. W. J. Xia. Mechanistic Studies of Surfactant-Induced Membrane Permeability Enhancement. *Doctoral Thesis*. The University of Illinois at Chicago. 1995.
20. J. N. Israelachvili, S. Marcelja, and R. G. Horn. Physical principles of membrane organization. *Quart. Rev. Biophys.* **13**:121–200 (1980).
21. D. Dimitrijevic, C. Lamandin, I. F. Uchegbu, A. J. Shaw, and A. T. Florence. The effect of monolayers and of micellar vesicular forms of non-ionic surfactants (solulan C24 and solulan 16) on Caco-2 cell monolayers. *J. Pharm. Pharmacol.* **49**:611–616 (1997).
22. R. C. MacDonald and S. A. Simon: Lipid monolayer states and their relationships to bilayers. *Proc. Natl. Acad. Sci. USA.* **84**:4089–4093 (1987).
23. M. Yazdanian, H. Yu, and G. Zografi. Ionic interaction of fatty acid monolayers at the air/water interface. *Langmuir* **6**:1093–1098 (1990).
24. G. L. Gaines. *Insoluble monolayers at liquid-gas interfaces*, Intersci. Publ., New York, 1966.
25. R. A. Demel, W. S. M. Geurts van Kessel, R. F. A. Zwaal, B. Roelofsen, and L. L. M. Van Deenen. Relation between various phospholipase actions on human red cell membrane and the interfacial phospholipid pressure in monolayers. *Biochim. Biophys. Acta.* **406**:97–107 (1975).
26. K. A. Shiffer, J. Goerke, N. Duzgunes, J. Fedor, and S. B. Shohet: Interaction of erythrocyte protein 4.1 with phospholipids. A monolayer and liposome study. *Biochim. Biophys. Acta* **937**:269–280 (1988).
27. H. K. Kimelberg and D. Papahadjopoulos: Phospholipid-protein interactions: membrane permeability correlated with monolayer "penentration". *Biochim. Biophys. Acta* **233**:805–809 (1971).
28. P. Bougis, H. Rochat, G. Pieroni, and R. Verger: Penetration of phospholipid monolayers by cardiotoxins. *Biochemistry* **20**:4915–4920 (1981).
29. W. Curatolo. Intestinal fluids and permeability enhancers. *Proc. Intl. Symp. Control. Rel. Bioact. Mater.* **21**:575–576 (1994).