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A film balance study of the monolayer-forming properties of dietary phospholipids and the interaction with NSAIDs on the monolayers

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Abstract

The monolayer-forming properties of milk and egg phospholipids and a synthetic model of human gastric mucosal phospholipids were studied by using a Langmuir film balance. Compression isotherms were constructed at pH 5.6 – the pH of unbuffered water – and at room temperature. Furthermore, the pH was reduced to 2 and the temperature raised to 37°C in order to mimic the normal stomach environment. Milk, egg and synthetic human gastric phospholipids formed liquid-expanded monolayers at the air-water interface. The increase in temperature to 37°C and the reduction of the pH to 2 caused only minor changes in monolayer formation. In addition, we investigated the interaction between a number of anti-inflammatory drugs (NSAIDs) and a monolayer at the air/water interface. Milk phospholipids were chosen as a representative example of monolayer-forming phospholipids. NSAIDs adsorbed from the subphase to the monolayer of milk phospholipids and increased the surface area of the monolayer.

Key words: Phospholipid monolayer; Langmuir film; NSAID; Dietary surfactant; Gastroprotection

1. Introduction

The human gastric mucosa is well protected against luminal acid as well as other extrinsic and intrinsic ulcerogenic agents. The gastric mucosal barrier against acid back-diffusion is a complex and dynamic defense system, consisting physically of mucus and bicarbonate and phospholipid secretion (Fig. 1). Furthermore, epithelial cell restitution and maintenance of mucosal blood flow are responsible for the defense system. The hydrophobicity of the protective barrier is due to surface-active phospholipids, which can also be administered exogenously to enhance the protective effect (Hills et al., 1983; Kiviluoto et al., 1991; Slomiany and Slomiany, 1991). Our previous animal and human studies with milk phospholipids support this finding (Kivinen et al., 1992a,b). This surface-active phospholipid layer, covering the gastric epithelial cell membranes,

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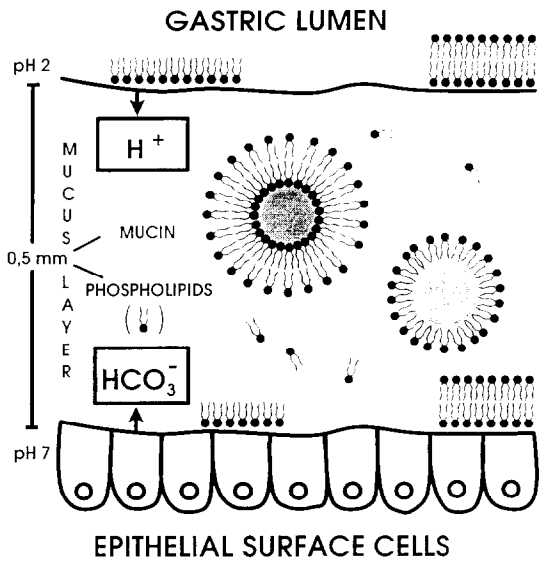

Fig. 1. Phospholipids in gastric mucosal defense: a dynamic continuum and surface-active layers.

acts physically as a biological Langmuir film with a total surface area of 800 cm^2.

Non-steroidal anti-inflammatory drugs (NSAIDs) have been used satisfactorily for decades in the treatment of inflammatory or rheumatic disorders and as common pain-killers. All the NSAIDs inhibit cyclo-oxygenase, reducing the endogenous production of mucosal prostaglandins that are inflammatory mediators (Campbell, 1991). The inhibition of prostaglandin synthesis cannot explain all the aspects of NSAID toxicity: leukotrienes and oxygen radicals may be involved through the lipoxygenase pathway (Rainsford, 1987). Unfortunately, gastrointestinal side-effects are associated continuously with NSAID therapy, especially with chronic use of these drugs (Silvoso et al., 1979). In addition, even the most recently developed formulations have failed to resolve this problem. NSAIDs result in gastric mucosal damage by affecting the gastric mucosal barrier due to altered production of inflammatory mediators (Wallace and Granger, 1992). As weak organic acids, NSAIDs can directly damage gastric mucosa with or without gastric acid. Commonly used NSAIDs, aspirin and indomethacin increase gastric acid secretion, which may contribute to NSAID-induced damage (Gerkens et al., 1977; Levine and Schwartzel, 1984). NSAIDs also inhibit mucus secretion, modify its structure and viscosity, and reduce surface hydrophobicity by altering the phospholipids (Lichtenberger et al., 1983; Sarosiek et al., 1986). An earlier investigation reported controversial results for a fenamate, tolfenamic acid, which was shown to protect rat gastric mucosa against ethanol and acetylsalicylic acid (Kivinen, 1990).

The present report examines firstly the monolayer-forming properties of milk and egg phospholipid mixtures and a synthetic model of human gastric mucosal phospholipids. Secondly, we discuss the effects of some NSAIDs on the milk phospholipid monolayer investigated by using the Langmuir film balance. Milk phospholipids were chosen as a representative example of a dietary phospholipid mixture by virtue of the similarity in the monolayer-forming properties of all phospholipids studied.

2. Experimental

Milk phospholipids were extracted from dairy whey powder with non-chlorinated solvents and purified by HPLC. The phospholipids were lyophilized and packed under argon. Egg phospholipids were isolated from fresh egg yolks by extraction with methanol-chloroform after removing less polar lipids and pigments with acetone. The phospholipid fraction was analysed and characterized by TLC. A synthetic model of human gastric mucosal phospholipids was prepared by using commercial phospholipids purchased from Sigma. The phospholipid composition of the preparates is listed in Table 1.

A 2200 KSV Langmuir Blodgett (LB) balance was used to evaluate the monolayer-forming properties of the phospholipids. All phospholipid mixtures were dissolved in chloroform at a concentration of 1 mg/ml and spread onto the air/water interface. Milli-Q water at pH of 5.6 – the pH of unbuffered water – and room temperature (21°C) was used to examine the behaviour of phospholipids under normal, intact circumstances. Furthermore, the pH of the Milli-Q wa-

Table 1
Composition of phospholipids in milk, egg and human gastric mucosa (wt%) analysed by supplier or researchers

Phospholipid	Dietary phospholipids		
	Milk [a]	Egg [b]	Human gastric mucosa [c]
Phosphatidylcholine	36.0	69.1	44.8
Phosphatidylethanolamine	34.7	23.9	31.5
Phosphatidylinositol	–	–	10.8
Phosphatidylserine	1.8	2.7	–
Sphingomyelin	21.6	1.0	6.9
Cardiolipin/unknown	–	–	4.1
Lysophosphatidylcholine	–	–	1.9

[a] LipidTeknik AB (1991).
[b] Wells and Belyavin (1987).
[c] Schmitz and Renooj (1990).

ter was reduced to 2 using HCl and the temperature raised to 37°C in order to reflect simply the normal human stomach environment for both parameters. The solvent was allowed to evaporate for about 15 min, after which the surface pressure-area isotherms were recorded at a compression speed of 10 mm/min. The measurements were repeated several times in order to ensure reproducibility of the isotherms.

Milk phospholipids were spread on subphases containing NSAIDs at a concentration of 10 mM (Fig. 2). Naproxen ((*S*)-6-methoxy-α-methyl-2-naphthaleneacetic acid) was purchased from Sigma, and aspirin (*o*-acetylsalicylic acid) was obtained from Fluka. Diclofenac (2-[(2,6-dichlorophenyl)amino]benzeneacetic acid), ibuprofen (α-methyl-4-(2-methylpropyl)benzeneacetic acid) and tolfenamic acid (2-(3-chloro-2-methylphenyl)aminobenzoic acid) were kindly donated by Leiras Pharmaceuticals, Tampere, Finland. All NSAIDs except aspirin were used as sodium salts and were dissolved in Milli-Q water (pH 5.6) at a temperature of 21°C. The pH values of the drug solutions were subsequently measured (Fig. 4). The monolayer and subphase were allowed to attain equilibrium, as demonstrated by the surface pressure values remaining constant, before the compression isotherms were recorded. The surface activities of the anti-inflammatory drugs were also assessed without spreading the phospholipids by compressing the barrier to observe any increase in surface pressure.

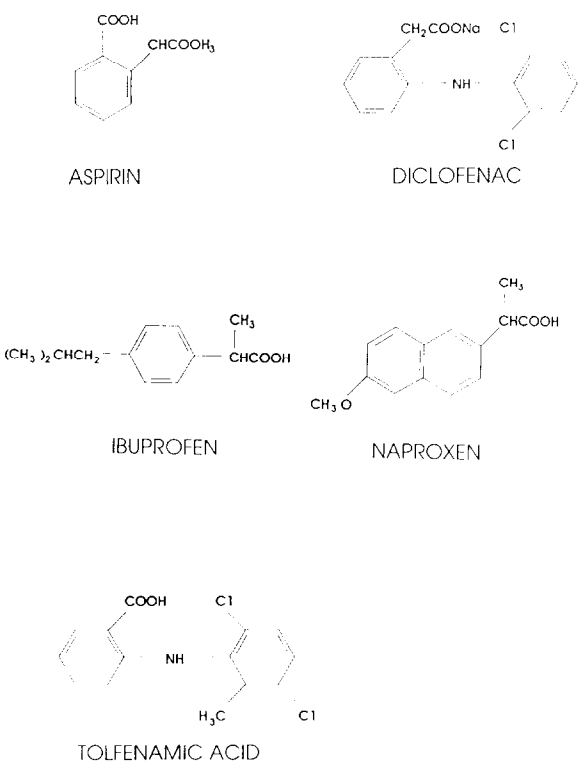

Fig. 2. Molecular structures of NSAIDs.

Table 2
Fatty acid composition of phosphatidylcholine from milk, egg and human gastric mucosa analysed by supplier or researchers

Fatty acid	Milk (wt%) [a]	Egg (wt%) [b]	Human gastric mucosa (mol%) [c]
Myristic acid	2.5	–	–
Palmitic acid	13.8	33.7	30.0
Palmitoleic acid	–	1.0	2.5
Stearic acid	15.2	15.8	8.1
Oleic acid	42.5	27.7	15.9
Linoleic acid	9.1	14.1	37.1
Linolenic acid	1.4	< 0.5	–
Arachidonic acid	0.4	4.4	6.4

[a] LipidTeknik AB (1991).
[b] Wells and Belyavin (1987).
[c] Schmitz and Renooj (1990).

3. Results and discussion

3.1. Dietary phospholipids

Fig. 3 demonstrates the spreading isotherms determined in this study for the phospholipid mixtures. All compression isotherms are of the liquid-expanded type. As shown in Fig. 3, the monolayers of milk and human surfactants are slightly more expanded than that of egg phospholipids. This is probably due to the difference in lipid composition. The main phospholipids in the human gastric mucosal surface and milk are phosphatidylcholine and phosphatidylethanolamine, as is the case in most animal cell membranes. Milk phospholipids could thus replace naturally the endogenous phospholipids destroyed by gastritis, ulcer disease or NSAID therapy. These phospholipids are zwitterionic compounds without an electrical charge over a wide range of pH, i.e., pH 3–10. Under strongly acidic conditions (such as in the stomach; pH 1–2), ionization of phosphate is suppressed and the surfactant becomes effectively cationic, resulting in stronger adsorption (Butler et al., 1983). Compression isotherms of these phospholipids have been reported by others (Yoshikawa et al., 1987).

The mean molecular area of the surfactants decreases on raising the temperature to 37°C and reducing the pH to 2.

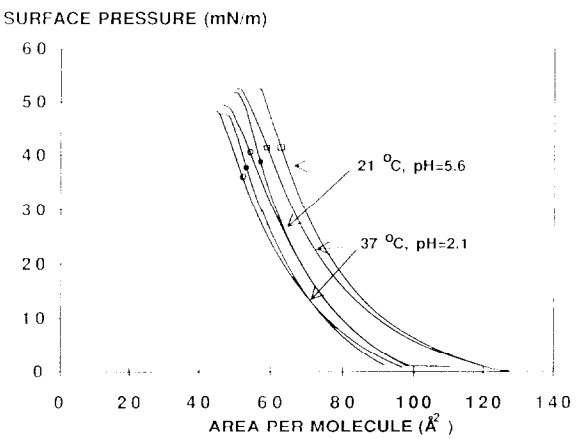

Fig. 3. Surface pressure-area isotherms of egg (●), milk (○) and synthetic gastric model (□) phospholipids.

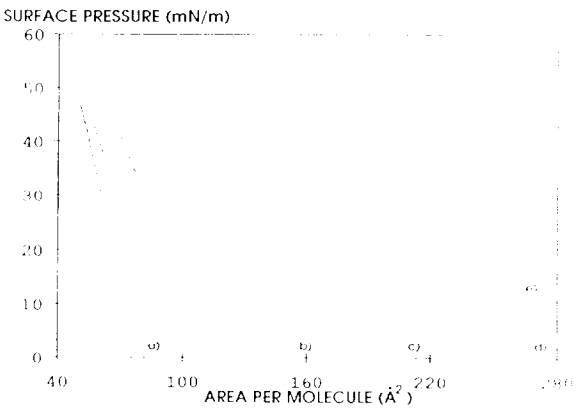

Fig. 4. Isotherms of milk phospholipids on subphases (at 21 °C) containing 10 mM NSAIDs: (a) pure water as control (pH 5.6); (b) naproxen (pH 6.7); (c) aspirin (pH 5.7); (d) ibuprofen (pH 6.7); (e) diclofenac (pH 6.9).

The same behaviour is observed for the collapse pressure for egg and milk phospholipids although the influence of the temperature and subphase acidity is very small. This leads to the simplified conclusion that, in the acidic environment of the human stomach, the endogenous hydrophobic layer also retains its protective nature despite the continuous attack of the acidic pH of 1–2.

3.2. Interaction with anti-inflammatory drugs

Milk phospholipid monolayers were spread on a water subphase containing NSAIDs at a concentration of 10 mM. The pH of each NSAID-subphase was measured. All the NSAIDs used in the study, except acetylsalicylic acid, are insoluble or slightly soluble in water, therefore, sodium salts were used. The NSAIDs used are weak acids with pK_a values as pure compounds varying from 3.2 to 4.6. As expected, the pH values in their aqueous solutions as sodium salts were greater than the pK values, ranging from 5.7 to 8.1. The NSAID molecules are thus considered to be at least 50% ionized, most probably being closer to 90%. The possibility of formation of ion complexes is then likely to occur in the monolayer. The idea was to allow the drug to reach the phospholipid monolayer at its own pH value, this being the reason why buffer solutions were not

used to maintain a constant subphase pH of 2. After the attainment of spreading equilibrium, the compression isotherms were recorded.

It was found that NSAIDs adsorbed from the subphase to the monolayer of milk phospholipids and increased the mean molecular area of the layer (Fig. 4). No increase in surface pressure could be observed on a monolayer-free surface; obviously, the NSAIDs used in this study are alone unable to form a monolayer and thus do not possess surface-active properties. The isotherms formed on naproxen and aspirin subphases are very similar, however, the increase in molecular area caused by aspirin is greater. This similarity is expected, since the molecular structures of the drugs are similar. These NSAIDs probably adopt the same configuration at a air/water interface. Ibuprofen and diclofenac, on the other hand, interact with milk phospholipids somewhat differently, resulting in a greater increase in surface area.

It appears likely that NSAID molecules penetrate into the lipid layer and form different isotherms depending on the molecular structure of the NSAID. The collapse pressure of milk phospholipids decreases on NSAID subphases,

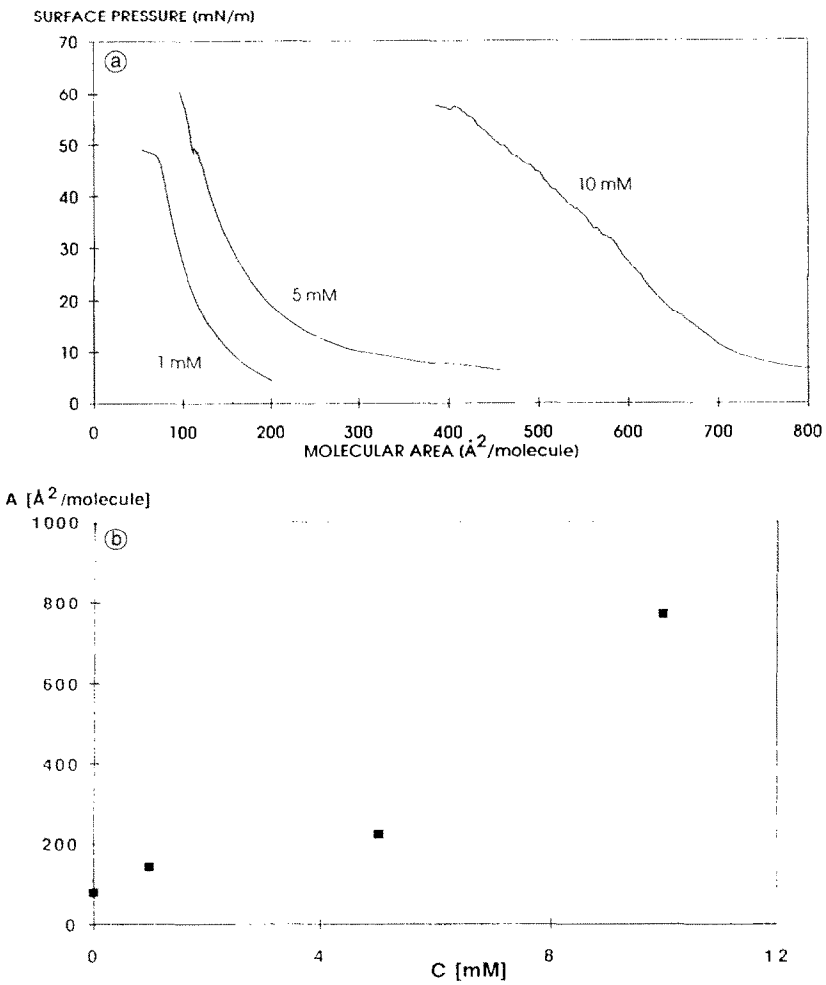

Fig. 5. (a) Isotherms of milk phospholipids on subphases containing 10, 5 and 1 mM tolfenamic acid (pH 8.1; 21°C). (b) Extrapolated values of mean molecular areas of tolfenamic acid in subphase concentrations of 1, 5 and 10 mM.

the collapse pressure of ibuprofen being lower than that of the others, probably as a result of the different position of the molecules of ibuprofen with respect to those of phospholipid.

Our findings confirm data reported in previous studies employing DSC, suggesting that the main action of indomethacin, diflunisal and sulindac on phospholipid liposome membranes is the reduction of the co-operative interaction between the phospholipid molecules (Hwang and Shen, 1981) and that phenylbutazone molecules destroy the specific structural arrangement of the polar head-group region of the phospholipid bilayers (Sainz et al., 1993a,b). On adsorption to the phosphatidylcholine layer, the NSAIDs weaken the interaction between the molecules, rendering the layer more fluid. This observation may lead to the conclusion that, for mixed layers of this type, the hydrophobicity and therefore also the ability to form a protective layer are reduced.

Tolfenamic acid differs widely from the other NSAIDs in its much greater degree of interaction with the phospholipids. The increase in molecular area was 4-fold that of diclofenac (Fig. 5a). Tolfenamic acid alone is insufficiently amphiphilic in nature for monolayer formation. However, the presence of phospholipids promotes the adsorption of tolfenamic acid at the interface. A mixed monolayer is formed, the molecular surface area increasing with tolfenamic acid concentration, as can be seen from the extrapolated values of the mean molecular area (Fig. 5b). It seems likely that tolfenamic acid is positioned perpendicularly at the interface, forming a mixed monolayer with the phospholipid. Further studies with other fenamates should help to clarify the possible explanation of this phenomenon. Our results provide support for and possibly help to elucidate earlier findings on the gastroprotective effect of tolfenamic acid against ulcerogenic agents (Kivinen et al., 1990).

3.3. Conclusion

Milk, egg and synthetic human gastric phospholipids form liquid-expanded monolayers at the air-water interface. The increase in temperature to 37°C and the reduction of the pH to 2 resulted in only minor changes in monolayer formation. NSAIDs adsorb to the milk phospholipid layer and render it more fluid. Tolfenamic acid interacts with the milk phospholipid layer to a greater extent than the other NSAIDs. Further studies employing different concentrations of NSAIDs in the subphase should help in clarifying the distribution of the NSAIDs in the layer and mechanism of action of other fenamates. These results may elucidate the earlier finding of the capacity of tolfenamic acid to 'protect' the rat gastric mucosa against ethanol and acetylsalicylic acid.

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