Influence of platelet-activating factor, lyso-platelet-activating factor and edelfosine on Langmuir monolayers imitating plasma membranes of cell lines differing in susceptibility to anti-cancer treatment: the effect of plasmalogen level

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Three structurally related but differing in biological activities single-chained ether phospholipids (PAF (platelet-activating factor) and lyso-PAF) and an anti-cancer drug (edelfosine (ED)) were investigated in Langmuir monolayers imitating natural membranes. The aim of the undertaken experiments was to study the influence of these lipids on monolayers mimicking plasma membranes of cell lines differing in susceptibility to the anti-cancer activity of ED, i.e. promyelocytic leukaemia cells (HL-60) and promyeloblastic leukaemia cells (K-562). As these cells differ essentially in the cholesterol/phospholipid ratio and plasmalogen concentration in the membrane, we have carried out systematic investigations in artificial systems of various compositions. The results for model leukaemia cell membrane were compared with data acquired for systems imitating normal leucocytes. Our results show that the level of plasmalogens significantly modulates the influence of the single-chained phospholipids on the investigated systems. The experiments confirmed also that the interactions of ether lipids with a model membrane of HL-60 cells (in biological tests sensitive to ED) have opposite character when compared with K-562, being resistant to ED. Moreover, the values of the parameters characterizing monolayers serving as membrane models (strength of interactions, monolayers fluidity and morphology) proved both sensitivity of these cells to ED and lack of their susceptibility towards PAF. Interestingly, it has been found that lyso-PAF, which is usually described as an inactive de-acetylated precursor of PAF, displays a stronger effect on HL-60 model membranes than ED.

1. Introduction

Ether phospholipids comprise a broad family of biologically active compounds, which are very important owing to their important functions in living organisms [1,2]. Among this group, a special place belongs to single-chained lipids, which are naturally synthesized as a result of enzymatic activity of phospholipase A2, which hydrolyses the sn-2 bond in the glycerol backbone of a phospholipid molecule [3,4]. In this reaction, a molecule of free fatty acid and the single-chained ether phospholipid, lyso-PAF is released [5], which is known to be a biologically inactive de-acetylated precursor of platelet-activating factor (PAF) [6]. Interestingly, there is strong evidence that ether lyso-lipids disclose their activities either by binding with a specific receptor localized in cellular membrane [7–9] or by direct incorporation into the lipid bilayer [10–12]. The second mechanism is possible due to the amphiphatic character of PAF and its lyso-derivative possessing...
predominantly long saturated C16 or C18 hydrocarbon chains [13]. Besides the above-mentioned natural single-chained ether phospholipids, their synthetic analogues of anti-cancer properties, called alkyl-lysophospholipids, have been synthesized. A representative drug of this type is edelfosine (ED), which was proved to be highly effective against various tumour cell lines, such as leukaemia [14–16], breast [17] and lung [18] cancers. Interestingly, despite very similar chemical structures (differences concern only small substituent at C2 of glycerol, scheme 1) as well as comparable sites of action (cellular membrane), ED, PAF and lyso-PAF reveal significantly different or even antagonistic activities in living cells.

For example, in contrast to ED, PAF does not disclose antineoplastic activity, and moreover it is identified as a potential factor inducing tumour cell growth and proliferation [19–23]. Contrary to PAF, its lyso-derivative is described by low membrane activity or even antagonistic effect to PAF, which was observed in the activation of neutrophils and blood platelets [24].

Bearing in mind that the main site of action of the above-mentioned ether lipids is the cellular membrane, we have undertaken systematic studies focused on examining their interactions with the main components of natural membrane lipids. In our investigations, the Langmuir monolayer technique complemented with a modern physico-chemical method, such as Brewster angle microscopy (BAM) and techniques based on synchrotron radiation scattering, grazing incidence X-ray diffraction and X-ray reflectivity, have been applied. At the initial stage of our studies, we have confirmed that similar to ED, both PAF and lyso-PAF form stable monolayers of a low degree of condensation [25]. These results show that molecules of ether lipids possessing relatively large polar head-groups, when compared with their hydrophobic parts, are strongly penetrated by water molecules. In the characteristics of both lipid films, we found differences that can be attributed to the hydroxyl group presence and its lack in the molecule of lyso-PAF and PAF, respectively. Our data confirmed that this free OH group capable of hydrogen bond formation with the adjacent lipid molecule in the monolayer or with water molecules of sub-phase determines different film properties of lyso-PAF when compared with PAF. Comparison of the interactions of single-chained ether lipids with phosphatidylcholines (PCs) revealed that PAF, lyso-PAF and ED mix favourably with saturated-monounsaturated PCs (e.g. SOPC), while their interactions with fully saturated PCs (DSPC, DPPC) are thermodynamically unfavourable, which may lead to phase separation in monolayers [26,27]. Similar trends were observed in the case of other classes of membrane phospholipids, namely the investigated ether lipids pack favourably in more fluid films formed by SOPE and DOPE [28] than fully saturated lipid molecules (DSPC, C18:0-Sphingomyelin) [29]. The miscibility and interactions of ether lipids were also investigated with cholesterol and GM3 ganglioside [27]; the latter is thought to be a marker in cancer progression of various tumour cells [30,31]. It was found that PAF, lyso-PAF and ED mix favourably with both these lipids; however, the interactions are thermodynamically more favourable with cholesterol. Moreover, our experiments on cholesterol/PC/ether lipid systems evidenced that the level of cholesterol significantly affects the influence of a particular ether lipid on model membranes (the higher the level of sterol, the stronger the effect of the ether lipid). Thorough analysis of the results for binary and multi-component films allowed us to draw an interesting conclusion that the concentration of cholesterol—rather than condensation of the monolayer—may be responsible for the different effects of the studied ether lipids on model membranes [27]. Moreover, ED was found to be the most effective agent modifying membrane organization. Although this is in accordance with the observed stronger cytotoxicity of ED compared with PAF and lyso-PAF, these results do not explain dissimilarities in bioactivity of PAF and lyso-PAF.

In this work, we focus our attention on the influence of ED, PAF and lyso-PAF on multi-component systems mimicking cellular membranes differing in sensitivity to the anti-cancer effect of ED. Monolayers studied herein imitate the membrane of normal leucocytes (insensitive to ED) as well as two lines of leukaemic cells differing in resistivity towards ED treatment, i.e. highly sensitive to the cytotoxic effect of ED human promyelocytic leukaemia cells (HL-60), and resistant to ED immature human promyeloblastic leukaemia cells (K-562) [14,32–35]. Important differences between these cell lines concern mainly the fluidity of membranes and the sterol content, namely K-562 and normal leucocyte cells contain a higher sterol proportion than HL-60 cells. Therefore, it has been suggested that the level of cholesterol influences the mentioned sensitivity of these cells to ED and by decreasing sterol concentration in the membrane of K-562 cells, its sensitivity towards ED can be augmented [34,35]. On the other hand, HL-60 and K-562 cells membranes differ not only in the proportion of cholesterol to phospholipids but also in the concentration of other membrane components, which may potentially differentiate the effect of ether lipids on these systems (e.g. sphingomyelin, PCs) [32,33]. In this context, special attention was paid to differences in the concentration of plasmalogens being a class of double-chained sn-1 ether/sn-2 ester lipids that appear in the above-mentioned cells. In general, the expression of plasmalogens is characteristic for tumorigenicity, and an abnormal level of these glycerolipids was identified in various cancerous membranes. Interestingly, the level of choline plasmalogens in insensitive to the effect of ED normal lymphocytes and K-562 cells is lower when compared with HL-60 cells [33,36]. Therefore, it seems that the hypothesis of the role of plasmalogen in cytotoxicity of the ether lipids is worth verifying. Thus, in this work the interactions of ED, PAF and lyso-PAF with choline and ethanolamine plasmalogens were investigated in binary films, then the effect of these

Scheme 1. Chemical structures of the investigated ether phospholipids.
single-chained ether lipids on model membranes imitating normal leucocytes, HL-60 and K-562 cell membranes was compared. Finally, to verify the potential correlation between the effect of ED, PAF and lyso-PAF on these model systems and the level of plasmalogens, we have investigated an artificial membrane of the same composition as normal leucocytes, in which choline plasmalogen made up 30% of total PCs. This allowed us to evaluate the effect of cholesterol level versus choline plasmalogen content on the effect of ether lipids on model membranes.

2. Experimental design

2.1. Material and methods

1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), egg sphingomyelin (SM), 1-(1Z-octadecenyl)-2-oleoyl-sn-glycero-3-phosphocholine (choline plasmalogen (PC-plasm)), 1-(1Z-octadecenyl)-2-oleoyl-sn-glycero-3-phosphoethanolamine (ethanolamine plasmalogen (PE-plasm)) were products of high purity (more than or equal to 99%) purchased from Avanti Polar Lipids, while Cholesterol (Chol) (99% or more) was supplied by Sigma. The investigated single-chained ether lipids 1-O-octadecyl-2-acetyl-sn-glycero-3-phosphocholine (PAF) and 1-O-octadecyl-sn-glycero-3-phosphocholine (lyso-PAF) of high purity (more than 99%) were purchased from Bachem AG Switzerland, whereas ED (1-O-octadecyl-2-O-methylrac-glycero-3-phosphocholine), of purity 99.1% or more, was provided by Biaffin GmbH&Co KG, Germany. Spreading solutions of these lipids were prepared in chloroform : methanol 9:1v/v mixture, apart from cholesterol, which was dissolved in chloroform. The solvents were purchased from Aldrich (HPLC grade, more than or equal to 99.9%). Mixtures were prepared from the respective stock solutions and deposited onto a water sub-phase with the Hamilton micro syringe (±2.0 μl). After spreading, the films were left for 10 min before compression was initiated (barrier speed of 20 cm²min⁻¹).

2.2. Methods

In the experiments, a NIMA (UK) Langmuir trough (total area = 300 cm²) placed on an anti-vibration table was used. Surface pressure was measured (± 0.1 mN m⁻¹) using a Wilhelmy plate made of filter paper (ashless Whatman Chr1) connected to an electrobalance. The sub-phase temperature (20°C) was controlled thermostatically (±0.1°C) by a circulating water system. For the experiments, Ultrapure Milli-Q Water was used. BAM experiments were performed with an UltraBAM instrument (Accurion GmbH, Goettingen, Germany) equipped with a 50 mW laser emitting p-polarized light at a wavelength of 688 nm, a 10× magnification objective, polarizer, analyser and a CCD camera. The spatial resolution of BAM was 2 μm.

2.3. Monolayers composition

In the first step of our studies, binary mixtures composed of particular single-chained ether lipids (30% of ED, PAF or lyso-PAF in mixed film) and choline or ethanolamine plasmalogens (PC-plasm and PE-plasm, respectively) were investigated. Then, based on the data published in the literature concerning the lipid profiles for normal leucocytes and two leukaemic cells (HL-60 and K562), multi-component systems imitating membranes of the above-mentioned cells were prepared. These mixtures were composed of cholesterol and the lipids prevailing in the outer membrane of natural bilayer: sphingomyelin, POPC (as representatives of major phospholipids in these cells) and PC-plasm. As regards normal leucocytes, the ratio of cholesterol to phospholipids was reported to be 0.68, while SM/PC = 0.23 [36,37]. Although in these cells ethanolamine plasmalogens were identified, they lacked choline plasmalogens [36]. Therefore, our membrane mimicking system of normal leucocytes was composed of 40.5 mol% of Chol, 11.1 mol% of SM and 48.4 mol% of POPC.

The composition of HL-60 and K-562 membranes was estimated based on the results of the comparative studies on the lipid profile of these cells. As was reported, the cholesterol to phospholipid ratio was equal to 0.5 and 0.65, while the SM/PC ratio was 0.23 and 0.1 for the HL-60 and K-562 membranes, respectively [32]. The content of choline plasmalogens in these systems was estimated based on the findings that within choline glycerophospholipids in HL-60 cells, a ca two times larger fraction is composed of ether lipids than that in K-562 cells [33]. Thus, the composition of our model system was estimated as follows: 33.3 mol% of Chol, 12.5 mol% of SM, 37.9 mol% of POPC and 16.3 mol% of PC-plasm for HL-60 cellular membrane, and 39.4 mol% of Chol, 5.5 mol% of SM, 46.8 mol% of POPC and 8.3 mol% of PC-plasm for K-562 cellular membrane. Into these monolayers ED, PAF and lyso-PAF were introduced. Finally, to verify the relationship between the content of plasmalogens in the mixture and the effect of the studied single-chained ether lipids, the influence of ED, PAF and lyso-PAF on the mixture mimicking normal leucocytes enriched in choline plasmalogen, in which 30 mol% of POPC fraction was substituted by PC-plasm was studied (40.5 mol% of Chol, 11.1 mol% of SM, 33.9 mol% of POPC and 14.5 mol% of PC-plasm).

2.4 Data analysis

In order to analyse the condensation, miscibility and interactions between molecules in the studied monolayers, from the surface pressure–area (π–A) curves recorded during compression of the monolayers, the following parameters were calculated.

Areas for ideal mixing of the monolayer components (Aid) were calculated based on the following equation [38]:

\[ A_{id} = \sum A_i X_i \]  

(2.1)

where \( A_i \) is the mean area per molecule for the respective one-component films, \( X_i \) is the mole fraction of the respective component in the mixed monolayer. \( A_{id} \) calculated from the above equation means a linear combination of the areas of all the respective single components and their molar fractions in the mixtures. Then, to compare \( A_{id} \) values with the values estimated from the isotherms at the same surface pressures (A) (5, 15 and 30 mN m⁻¹), the excess areas of mixing (AExc) were calculated from the following equation [38]:

\[ A_{Exc} = A - A_{id} \]  

(2.2)

For binary films of plasmalogens and the studied ether lipids, the excess free energy of mixing (ΔGExc) values were also calculated according to the following equation [38]:

\[ \Delta G_{Exc} = N \int_0^\sigma A_{Exc} \, d\sigma = N \int_0^\sigma (A - A_{id}) \, d\sigma \]  

(2.3)

where \( A_{id} \) values were calculated from equation (2.1).
The compression modulus values, which allow one to compare the state of the monolayer, were calculated from the following equation [39]:

$$C_S^{-1} = -\frac{d\pi}{dA},$$ (2.4)

wherein \( A \) is the mean area per molecule value at a given surface pressure \( \pi \).

The morphology of the studied monolayers was investigated on the basis of BAM images taken at various stages of the films compression.

3. Results

3.1. Interactions of edelfosine, platelet-activating factor and lyso-platelet-activating factor with plasmalogens

The \( \pi - A \) isotherms for the monolayers formed by choline and ethanolamine plasmalogens, single-chained ether lipids (ED, PAF and lyso-PAF) and their mixtures containing 30 mol% of ED, PAF and lyso-PAF, respectively, are shown in figures 1 and 2.

As was shown previously [25,40], ED, PAF and lyso-PAF form stable monolayers of liquid-like state and uniform texture within a wide range of surface pressures. The course and shape of \( \pi - A \) curves as well as BAM images (not presented) for choline plasmalogen also proves its liquid character and film homogeneity. However, comparing the maximal values of the compression modulus for the foregoing monolayers (\( C_S^{-1} \) does not exceed 68 mN m\(^{-1}\) for single-chained ether lipids [25,40] and 140 mN m\(^{-1}\) for PC-plasm), it is evident that the choline plasmalogen film is more ordered when compared with the remaining monolayers. On the other hand, in the course of the isotherm for ethanolamine plasmalogen monolayer, a phase transition at ca 28 mN m\(^{-1}\) is observed. The maximal values of the compression modulus below and above this region (135 and 225 mN m\(^{-1}\)) indicate that with compression, the state of the monolayer becomes more condensed. As regards mixed systems (figures 1 and 2), the addition of ED, PAF or lyso-PAF makes both plasmalogen films more fluid (e.g. ca 30% decrease of \( C_S^{-1} \) values was observed for plasmalogen-containing systems after incorporation of ether lipids) and in the case of PE-plasm-containing systems, the phase transition vanishes (figure 2).

The analysis of \( A^{\text{Exc}} \) values for the mixed films indicates that at the studied surface pressures, the systems behave non-ideally (\( A^{\text{Exc}} \neq 0 \)); however, the observed deviations from ideality depend on the kind of plasmalogen (choline or ethanolamine) and single-chained ether lipid (ED, PAF or lyso-PAF) present in the system (figure 3). As regards PC-plasm-containing films, the most negative deviations from ideality, indicating the most favourable mixing between molecules, exist in the system with ED, while for monolayer-containing lyso-PAF these deviations are positive. The values of the excess Gibbs energy of mixing (\( \Delta G^{\text{Exc}} \)) at \( \pi = 30 \text{ mN m}^{-1} \) were found to be \(-1850\), \(-1050\) and \(+1320 \text{ J mol}^{-1}\) for mixtures of PC-plasm with ED, PAF and lyso-PAF, respectively. In the case of the mixed systems containing PE-plasm and the studied herein single-chained ether lipids, the deviations from ideality are positive, which means that forces between molecules in the respective mixed films are less favourable than those existing between the lipids in their one-component monolayers. It turns out that this unfavourable effect is the weakest for the PE-plasm/lyso-PAF monolayer. Interestingly, a very similar trend was found in studies on the interactions of ED, PAF and lyso-PAF with phosphatidylethanolamines [28].

3.2. The influence of edelfosine, platelet-activating factor and lyso-platelet-activating factor on multi-component systems

In figure 4, the isotherms for monolayers imitating HL-60, K-562 and normal leucocyte membranes as well as normal leucocyte membranes enriched by choline plasmalogen are
shown together with the isotherms for the same films containing additionally ED, PAF and lyso-PAF. To be able to compare the properties of these films based on the $\pi$–$A$ curves, the compression modulus and excess area of mixing values were calculated (figure 5) and BAM images were recorded (figure 6).

The isotherms registered for the model systems deprived of single-chained ether lipids confirm that these films are of liquid state and in the course of the curves characteristic phase transitions appear. This is especially pronounced in the compression modulus versus the surface pressure dependences plotted for these systems (figure 4, bottom). The above-mentioned transition appears at $\pi \approx 20 \text{ mN m}^{-1}$ for the HL-60 model membrane and at $\approx 25 \text{ mN m}^{-1}$ for the remaining systems. The maximal values of $C_{S}^{-1}$ below the minimum in the plots shown in figure 4 are similar for all the studied systems ($C_{S}^{-1} \approx 140 \text{ mN m}^{-1}$), while above this region $C_{S}^{-1}$ values increase to $\approx 250 \text{ mN m}^{-1}$ for HL-60 model membranes, $205 \text{ mN m}^{-1}$ for normal leucocytes and K-562 model systems and to $170 \text{ mN m}^{-1}$ for the mixture imitating normal leucocytes enriched in choline plasmalogen.

The values of the excess area of mixing $A_{\text{Exc}}$ (figure 5) for all of the investigated mixtures are negative, which indicates non-ideal behaviour of the system, favourable mixing of monolayer components as well as more favourable interactions in the mixed film when compared with those existing in the respective one-component monolayers. Moreover, in the whole range of surface pressures, the $A_{\text{Exc}}$ values are the lowest (most negative) for model membranes of HL-60 cells. For example, at $\pi = 30 \text{ mN m}^{-1}$ the $A_{\text{Exc}} = -5.81 \text{ Å}^2$/molecule for HL-60; $-4.72 \text{ Å}^2$/molecule for K-562; $-4.55 \text{ Å}^2$/molecule for normal leucocytes; and $-4.35 \text{ Å}^2$/molecule for normal leucocytes model membranes enriched in choline plasmalogen.

The most negative values found for the HL-60 mixture suggest that the proportion of lipids in this film guarantees the most favourable packing of molecules and beneficial interactions between them. Moreover, comparing the results obtained for normal leucocytes and normal + PC-plasm monolayers (differing only in the composition of choline monolayer fraction), it is evident that replacement of a part of PC lipid by choline plasmalogen (30%) weakens the interactions between molecules in the mixed film.

From BAM images taken for these model membranes, it can be seen that the trend of morphological changes observed during compression of the respective monolayers is similar for all the model systems. Namely, at low surface pressures...
(i.e. approx. 3–8 mN m⁻¹, depending on the monolayer), the domains of the condensed phase are formed, and afterwards in a wide range of surface pressures the film is homogeneous. Finally, at higher surface pressures, small points of condensed domains appear in the pictures. The only difference in the morphology of particular model systems concerns the surface pressure region at which the mentioned structures can be observed. For example, for HL-60 model membranes the domains of condensed phase observed at the higher surface pressure region are formed at lower \( p \) when compared with the remaining films. This in turn is in agreement with the phase transition region observed in the isotherms. Moreover, comparing the pictures for normal leucocytes and those enriched with PC-plasm, it can be seen that in the presence of choline plasmalogen, the domains below the transition region are larger.

**Figure 4.** The surface pressure \( (\pi) \) versus mean molecular area isotherms for model membranes together with the curves for the model membrane containing ED, PAF and lyso-PAF, respectively. Last picture—the compression modulus \( (C_S^2) \) versus the surface pressure \( (\pi) \) plots for model systems. (Online version in colour.)

**Figure 5.** The excess area of mixing \( (A_{Exc}) \) values for the investigated monolayers calculated at surface pressures of 5, 15 and 30 mN m⁻¹, respectively. The presented error bars for the excess areas of mixing represent maximum values calculated with the exact differential method. (Online version in colour.)
The addition of ED, PAF and lyso-PAF into these systems affects the position of the phase transition observed in the isotherms and causes a decrease in \( C_{S2} \) values, indicating a fluidizing effect of single-chained ether lipids. The decrease in \( C_{S2} \) at the concentration of single-chained ether lipids equal to 15 mol% is the strongest for HL-60 membranes and does not exceed 40%. The images taken for the selected monolayer containing additionally single-chained ether lipids do not show radical morphological differences between the films (figure 7). On the other hand, the effect of the presence of single-chained ether lipids reflects strongly in the changes of \( A^{Exc} \) values; however, the values of this parameter remain negative for all the systems investigated. A change in direction of \( A^{Exc} \) values depends on the system studied and kind of ether lipid added into the monolayer. In general, \( A^{Exc} \) increases (becomes less negative) for HL-60 and normal + PC-plasm membranes, while for K-562 and normal leucocytes they become more negative (these results will be compared in the Discussion).

### 4. Discussion

The investigated single-chained ether lipids are membrane active molecules having different or even opposite biological effects, including different toxicity to cells, evidenced in biological studies. In short, ED is highly toxic to e.g. leukaemic cells, but spares normal leucocytes. On the other hand, PAF molecules do not provoke apoptosis in ED sensitive cells [41]. The ability of ED, PAF and lyso-PAF to be incorporated in cellular membranes suggests that the physiological activity of these molecules may be membrane-related. Therefore, a lot of investigations have been focused on comparing the influence of these ether lipids on membrane systems, both natural and artificial. The results of our recent experiments performed on model systems prove that the level of cholesterol in membranes (but not membrane condensation) strongly modifies the effect exerted by ED, PAF and lyso-PAF on the studied system [27]. This finding correlates well with reports of other authors claiming that cholesterol...
concentration is crucial from the viewpoint of the effect of ED on HL-60 (ED-sensitive) and K-562 (ED-insensitive) leukaemic cells [34,35]. Nonetheless, thorough analysis of the lipid profiles for the foregoing cell lines as well as for those insensitive to ED normal leucocytes, proved that cholesterol is not the only lipid differing among membranes of the foregoing cells. Namely, HL-60 cells contain a higher level of ether lipids when compared with K-562 cells and normal leucocytes. Therefore, to verify our previous hypothesis on the role of cholesterol as a major factor regulating the effect of ED, PAF and lyso-PAF on model membranes, we have undertaken experiments, with the results discussed below.

According to our studies on binary monolayers, ED and PAF, in contrast to lyso-PAF, interact favourably with choline plasmalogen. Moreover, all the studied single-chained ether lipids interact less favourably with PE-plasm than with PC-plasm. Therefore, it is interesting to compare the results obtained herein for mixtures containing ED, PAF or lyso-PAF and plasmalogens with the results collected previously for the system containing single-chained ether lipids and SOPC or SOPE, respectively (SOPC and SOPE differ from choline and ethanolamine plasmalogen only as regards the linkage at the sn-1 position). Based on the values of the excess area of mixing obtained for the respective mixtures, it can be concluded that ED and PAF interact stronger with choline plasmalogen when compared with SOPC [27]. On the other hand, miscibility of lyso-PAF is more unfavourable with plasmalogen when compared with SOPC. This allows one to conclude that the differences in the linkage at the sn-1 position of SOPC versus PC-plasm molecule influence the interactions with single-chained ether lipids. In the case of the mixtures containing ethanolamines, ether lipid/PE-plasm and ether lipid/SOPE, the monolayers behave very similarly. In this case, the miscibility and interactions are the most favourable for the mixtures containing lyso-PAF. It is worth mentioning here, that in contrast to both ED and PAF, lyso-PAF possesses at the sn-2 position of its molecule a hydroxyl group, which in some aspects determines different behaviour of this lyso-lipid [25].

As was evidenced in our previous experiments involving various classes of membrane lipids, the investigated single-chained ether lipids interact preferentially with cholesterol and GM3 ganglioside [27]. The data collected in the present report indicate that choline plasmalogen is also included in the foregoing group of membrane lipids favourably mixing with single-chained ether lipids. Moreover, ED, PAF and lyso-PAF interact comparably with choline plasmalogen and GM3 ganglioside. The role of choline plasmalogen content on the effect of particular single-chained ether lipids on membrane

Figure 7. BAM images for model membranes containing selected single-chained ether lipids. BAM images presented in the first column from left correspond to the surface pressure of 0 mN m⁻¹ and the molecular area of 75 Å²/molecule, whereas for other images surface pressure values are indicated in the photo.
verify the results obtained herein for multi-component monolayers imitating normal leucocytes and normal leucocytes + PC-plasm differing only in the composition of the PC lipid fraction. The analysis was focused on the region of higher surface pressures \( (\pi = 30 \text{ mN m}^{-1}) \), which is of biological relevance [42]. The results obtained for above-mentioned systems proved that the replacement of part of POPC by choline plasmalogen changes the influence of the single-chained ether lipids on these systems. Namely, the addition of ether lipids into the model membrane of normal leucocytes strengthens the interactions in the mixed film and stabilizes the system (for all the ether lipids except for lyso-PAF), while in the system also containing plasmalogen, in the presence of single-chained ether lipids the interactions become less favourable. Moreover, choline plasmalogen alters not only the observed trend of changes in monolayer properties (manifested in the decrease or increase in \( A_{\text{Exc}} \) values) caused by single-chained ether lipids but also the magnitude of the observed effects. The changes in \( A_{\text{Exc}} \) (its absolute values) owing to ether lipids incorporation are larger in the case of the monolayer enriched in choline plasmalogen. For example, the addition of ED and PAF into the model membrane of normal leucocytes causes a decrease in \( A_{\text{Exc}} \) by \( ca \ 15\% \) and \( 20\% \), respectively, while in the case of the monolayer imitating normal leucocytes enriched in PC-plasm, \( A_{\text{Exc}} \) values become less negative by \( ca \ 35\% \) and \( 50\% \) in the presence of ED and PAF, respectively.

As regards the results for HL-60 versus K-562 model membranes, the obtained data indicate that the addition of single-chained ether lipids destabilizes the HL-60 model system and weakens the interactions between molecules as well as visibly decreases its fluidity, while for K-562 imitating monolayer this influence is opposite. These results seem to correlate with the finding that ED affects both these leukaemic cells in different ways, namely HL-60 cells are sensitive to ED, while K-562 cells are resistant to this drug. Moreover, it is evident that the presence of ED in the model HL-60 membrane changes more strongly \( A_{\text{Exc}} \) values than the presence of PAF, which is in accordance with the finding that HL-60 cells are sensitive to ED, but not to PAF molecules. Interestingly, the effect of lyso-PAF on lipid interactions in HL-60 model membranes is even greater than the effect of ED. However, there is no report of biological experiments on the cytotoxic effect of this ether lipid on the mentioned leukaemic cells. Furthermore, the effect of ED and PAF on K-562 model membranes is of the same magnitude as the effect on normal leucocytes, both being insensitive to ED. The effect of lyso-PAF on HL-60 model membranes is similar to that exerted on normal leucocyte model systems. Thus, assuming that the increase in \( A_{\text{Exc}} \) values in monolayers correlates with the toxic effect of single-chained ether lipids it can be concluded that lyso-PAF might be toxic to both leukaemic and normal cells. Such considerations lead to the question concerning the origin of the observed interesting differences in the activity of ED versus lyso-PAF. What makes lyso-PAF the most effective in the destabilization of HL-60 model membrane? First of all, we should refer to the molecular properties of both single-chained phospholipids. As mentioned before, the only difference in the chemical structure of these lipids is the presence of a free hydroxyl group in lyso-PAF. Our earlier studies proved that as far as the film-forming properties of the mentioned ether lipids are concerned, this molecular motif is of great importance, as it enables the formation of hydrogen bonding with other molecules in the monolayer as well as water [25]. This capability influences both the specific properties of the lyso-PAF monolayer at higher surface pressures (formation of ordered phase) as well as interactions with other lipids. The latter was found to be important, e.g. in the case of lyso-PAF/SM mixed films, as we reported in one of our previous papers [29]. In that study, we showed that from the thermodynamic point of view lyso-PAF interacts with sphingomyelin, the most unfavourable among all three investigated ether phospholipids. These interactions may be of importance in the case of the systems studied herein, as both HL-60 and normal leucocyte model membranes contain comparable levels of SM, which is at two times higher than that of K-562. This fact together with the above-mentioned characteristics of lyso-PAF–SM interactions may be the reason for the interesting trend observed here. On the other hand, it is also worth mentioning here that the reason for the unfavourable interactions between lyso-PAF and SM may result from the fact that in contrast to PAF and ED, both these compounds possess groups that are primarily hydrogen bond donors. However, this hypothesis needs in vivo verification. The data collected herein indicate that independently of the concentration of cholesterol in the monolayer, the addition of single-chained ether lipids destabilizes the films of higher choline plasmalogen content (HL-60 and normal + PC-plasma model membranes) and strengthens the interactions in systems lacking choline plasmalogen (normal leucocytes model membrane) or of lower choline plasmalogen level (K-562). It should be pointed out that cellular membranes sensitive to ED are of a lower level of cholesterol and are enriched by choline plasmalogens (HL-60), while insensitive species have a higher sterol level and simultaneously lack plasmalogens (normal erythrocytes) or are of decreased choline plasmalogen level than the sensitive cells (e.g. K-562 cells). Therefore, the results published previously [27] together with the data obtained herein allow one to postulate that the proportion of cholesterol to choline plasmalogen in membrane may regulate the toxicity of single-chained ether lipids on cells.

5. Conclusion

All of the studies performed so far allowed us to better understand the behaviour of ED, PAF and lyso-PAF in an environment of model membranes and revealed subtle differences in the membrane activity of these compounds. The results collected herein are evidence that the observed differences in the effect of these compounds on particular artificial membrane systems correlate with the findings obtained from biological studies. Namely, the influence of these ether lipids on monolayers imitating HL-60 is opposite to the effect on K-562 cellular membranes. This is in agreement with sensitivity/insensitivity of these cells to ED and PAF. Additionally, the effect of ED on the HL-60 model system is more pronounced than that of PAF, which in biological studies has been shown to be inactive in these cells. It was also proved that the level of plasmalogens in the model system modifies the influence of single-chained ether lipids on the studied monolayers. As evidenced from the results of the experiments on binary monolayers, ether lipids strongly interact with choline plasmalogen; thus cholesterol should not be considered as the unique lipid influencing single-chained ether lipids membrane properties, and in this aspect the ratio of cholesterol to plasmalogens may
be of importance. Moreover, our results lead to the conclusion that more attention should be paid to the biological effect of lyso-PAF, which in our experiments reveals a larger effect on HL-60 model membranes than ED, the latter being highly toxic to these cells.

References


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