Biochimica et Biophysica Acta 1828 (2013) 1384–1389

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamem

# Disrupting membrane raft domains by alkylphospholipids

A.B. Gomide [a], C.H. Thomé [b], G.A. dos Santos [b,e], G.A. Ferreira [b,c], V.M. Faça [d], E.M. Rego [b,e], L.J. Greene [b,c], R.G. Stabeli [f], P. Ciancaglini [g], R. Itri [a,*]

[a] Institute of Physics, University of São Paulo, SP, Brazil
[b] National Center for Cell Based Therapy — Hemotherapy Center of Ribeirão Preto, Brazil
[c] Dept. of Cell and Molecular Biology and Pathogenic Bioagents — FMRP-University of São Paulo, Ribeirão Preto, SP, Brazil
[d] Dept. of Biochemistry and Immunology — FMRP-University of São Paulo, Ribeirão Preto, SP, Brazil
[e] Dept. of Clinical Medicine — FMRP-University of São Paulo, Ribeirão Preto, SP, Brazil
[f] Federal University of Rondonia (UNIR) and Oswaldo Cruz Foundation — Oswaldo Cruz Foundation (Fiocruz-Rondonia), Porto Velho, RO, Brazil
[g] Dept. of Chemistry — FFCLRP-USP, University of São Paulo, Ribeirão Preto, SP, Brazil

ARTICLE INFO

*Article history:*
Received 17 December 2012
Received in revised form 17 January 2013
Accepted 22 January 2013
Available online 1 February 2013

*Keywords:*
Alkylphospholipid
Giant vesicles
Model membranes
Ld–Lo lipid domains
Lipid rafts

ABSTRACT

Using phase contrast and fluorescence microscopy we study the influence of the alkylphospholipid, ALP, 10-(octyloxy) decyl-2-(trimethylammonium) ethyl phosphate, ODPC, in giant unilamellar vesicles, GUVs, composed of DOPC (1,2-dioleoyl-sn-glycero-3-phosphocholine), brain sphingomyelin (SM) and cholesterol (Chol). The results show that adding 100 μM ODPC (below CMC) to the outer solution of GUVs promotes DOPC membrane disruption over a period of 1 h of continuous observation. On the other hand, the presence of SM and Chol in homogeneous fluid lipid bilayers protects the membrane from disruption. Interestingly, by adding 100 μM ODPC to GUVs containing DOPC:SM:Chol (1:1:1), which display liquid ordered (Lo)–liquid disordered (Ld) phase coexistence, the domains rapidly disappear in less than 1 min of ODPC contact with the membrane. The lipids are subsequently redistributed to liquid domains within a time course of 14–18 min, reflecting that the homogenous phase was not thermodynamically stable, followed by rupture of the GUVs. A similar mechanism of action is also observed for perifosine, although to a larger extent. Therefore, the initial stage of lipid raft disruption by both ODPC and perifosine, and maybe other ALPS, by promoting lipid mixing, may be correlated with their toxicity upon neoplastic cells, since selective (dis)association of essential proteins within lipid raft microdomains must take place in the plasma membrane.



## 1. Introduction

Alkylphospholipids (ALPs) are structurally related to a naturally occurring phospholipid, 2-lysophosphatidylcholine (lysolecithin, LPC), and present promising cytotoxic effects on different neoplastic cell lines. ALPs act preferably on the cell membrane rather than DNA [1]. There is increased interest in their biological activity because they are able to induce apoptosis in tumor cells and may serve to complement existing DNA directed anticancer chemotherapies [2]. Among the most well-known ALPs are edelfosine [3], miltefosine [4], and perifosine [5].

It has been shown that alkylphospholipids accumulate in cell membranes, resist cell lipases, interfere with lipid-based signal transduction as well as with phospholipid biosynthesis [6], which leads to apoptosis, inhibit neovascularization, prevent invasion and induce tumor cell differentiation [7]. Edelfosine may exert its pro-apoptotic effect by activation of Fas/CD95 [8] while perifosine may induce cell cycle arrest and apoptosis in human carcinoma cell lines by blocking the AKT phosphorylation [9]. It has also been proposed that edelfosine affects the protein composition of lipid rafts in yeast [10] and leukemic cells [8,11], in which the amount of cholesterol plays an important role. ALPs may use lipid rafts for cellular internalization and to inhibit phosphatidylcholine synthesis to varying degrees, acting as gateways to induce apoptosis in lymphoma and leukemic cells [12,13].

Lipid rafts are highly ordered membrane domains enriched in cholesterol, sphingolipids and proteins, which participate in diverse cellular processes such as signal transduction and lipid trafficking [14]. Lipid rafts display lateral liquid ordered (Lo)–liquid disordered (Ld) phase separation in the membrane plane on the order of hundreds of nanometers in living cells [15]. They can also be observed as microscopic domains in isolated membrane systems [16,17]. Certainly, molecules that affect lipid phase separation may have important effects on physiological responses.

The alkylphospholipid 10-(octyloxy) decyl-2-(trimethylammonium) ethyl phosphate, ODPC (Fig. 1), originally synthesized by Agresta et al. [18], showed cytotoxic effects on some cancer cell lines comparable to others ALPs, but with lower hemolytic activity and cytotoxicity. In particular, we demonstrated that ODPC (10–50 μM) leads to apoptosis and inhibits the proliferation of leukemia cells, sparing normal hematopoietic

* Corresponding author. Tel.: +55 11 30917012; fax: +55 11 30916749.
*E-mail address:* rosangela.itri@gmail.com (R. Itri).

0005-2736/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbamem.2013.01.017

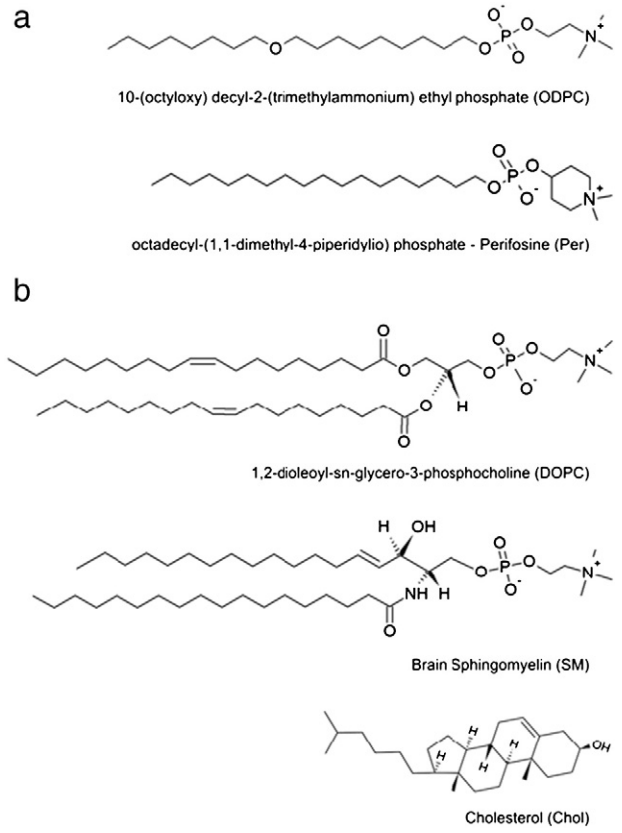


**Fig. 1.** Chemical structures of (a) ALPs: 10-(octyloxy) decyl-2-(trimethylammonium) ethyl phosphate (ODPC) and Perifosine (Per), and (b) lipids: 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), brain sphingomyelin (SM) and cholesterol (Chol).

and epithelial cells [19]. Furthermore, an early loss of LAT2, an adaptor protein associated with lipid rafts in its palmitoylated form, takes place in the lipid raft enriched fraction of leukemia cells within 3 h after treatment with 25 μM ODPC [20].

In order to better understand the role of lipid rafts in the mechanism of action of ODPC, we used model membranes constituted by giant unilamellar vesicles, GUVs, and investigated how the lipid membrane responds to the addition of ODPC in the outer GUVs solution. We show that the main effect of ODPC, at concentrations below *cmc*, is to promote membrane disruption with time on binary systems composed of an unsaturated lipid DOPC and Cholesterol (Chol) and DOPC and Sphingomyelin (SM) to a lesser or to a greater extent depending on the membrane composition. On the other hand, ODPC does not affect the membrane integrity of GUVs composed of a homogeneous mixture of DOPC:Chol:SM, but is indeed able to disrupt the lipid domains by favoring lipid mixing until the complete abolishment of the phase separation. Further, this striking effect is compared with that promoted by perifosine on Ld–Lo phase coexistence.

## 2. Material and methods

### 2.1. Materials

The lipids DOPC (1,2-dioleoyl-*sn*-glycero-3-phosphocholine), brain sphingomyelin (SM), cholesterol (Chol), and the fluorescent label Rhodamine-DOPE [1,2-dioleoyl-*sn*-glycerol-3phosphoethanolamine-N-(lissamine rhodamine B sulfonyl)] (Rh-DOPE) were purchased from Avanti Polar Lipids (Alabaster, AL). The respective chemical structures are given in Fig. 1. The ALP ODPC, 10-(octyloxy) decyl-2-(trimethylammonium) ethyl phosphate, was synthesized as previously described [18,19] whereas perifosine (Per) was purchased from Selleck Chemicals (Houston, TX, USA). Their chemical structures are also represented in Fig. 1.

### 2.2. Methods

#### 2.2.1. Giant unilamellar vesicles preparation

Giant unilamellar vesicles (GUVs) were prepared by the electroformation procedure [21] and were composed of pure DOPC and mixtures of DOPC:Chol (8:2), DOPC:SM (8:2) and DOPC:SM:Chol (6:1:3) and (1:1:1). The latter composition is known to exhibit Lo–Ld phase separation at 22 °C [22]. Briefly, ~20 μl of 2 mg/ml lipid chloroform solution of desired composition was spread on the surfaces of two conductive glass slides coated with indium tin oxide. We investigated the lipid compositions of DOPC:SM:Chol containing up to 0.2 mol% of the fluorescent Rhodamine-DOPE (Rh-DOPE), which is known to preferentially concentrate in the liquid disordered phase (Ld) [23]. The glass slides were placed with their conductive sides facing each other and separated by a 2 mm thick Teflon® frame. The chamber was filled with 0.2 M sucrose solution and placed inside an oven at ~55 °C. The glass plates were connected to a function generator and an alternating current of 2 V with a 10 Hz frequency was applied for 2 h. The GUVs were observed the following day, allowing the vesicles to reach their thermodynamic equilibrium completing the phase separation process. The vesicles were then diluted in 0.2 M glucose containing the desired amount of ODPC and immediately observed in the optical microscopy. The osmolarities of the sucrose and glucose solutions were measured with a cryoscopic osmometer Osmomat 030 (Gonotec, Germany) and carefully matched to avoid osmotic pressure effects. All measurements were done at room temperature (22–25 °C).

#### 2.2.2. Optical microscopy observation

Vesicles were observed in the phase contrast mode by means of an inverted microscope Axiovert 200 (Carl Zeiss; Jena, Germany) equipped with a Plan Neo-Fluar 63× Ph2 objective (NA 0.75) and A-plan 10× Ph1 (NA 0.25). Images were recorded with an AxioCam HSm digital camera (Carl Zeiss). A filter with excitation at 538–563 nm and emission at 570–640 nm (Zeiss filter set 43 HE) and a mercury lamp HXP-R 120 W were used for observing vesicles labeled with Rh-DOPE in the fluorescence mode.

## 3. Results and discussion

In order to understand how ODPC interacts with lipid membranes, giant unilamellar vesicles, GUVs, containing DOPC, SM and Chol were used to mimic the outer hemilayer of the plasma membrane. Time evolution of GUV morphology was followed by video microscopy after incubation in glucose solutions containing ODPC at concentrations below *cmc* [19]. We first describe the responses observed by phase contrast and fluorescence microscopy from GUVs containing DOPC, binary mixtures of DOPC:Chol (8:2) and DOPC:SM (8:2) and ternary mixtures of DOPC:SM:Chol (6:1:3). All these compositions correspond to homogeneous lipid mixtures in Lo phases [22]. Then, we will consider the effect of 100 μM ODPC in a membrane containing DOPC:SM:Chol (1:1:1) that displays Lo–Ld phase separation [23]. The latter results are compared with those promoted by 100 μM perifosine.

### 3.1. Homogenous lipid mixtures in fluid phases

GUVs of DOPC and mixtures containing Chol and SM are spherical, exhibiting usually small undulations typical of fluid-like membranes [22,24,25]. The influence of ODPC was investigated at increasing concentrations. We observed that ODPC at concentrations less than 50 μM did not modify the lipid bilayers for all studied homogeneous mixtures during 1 h of continuous observation. On the other hand, we observed that a percentage of GUVs of DOPC were not altered while a significant number of them suddenly burst when they

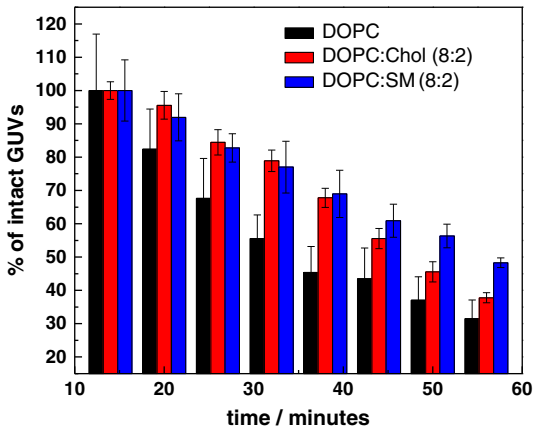


**Fig. 2.** Stability of GUVs composed of DOPC, DOPC:Chol (8:2), DOPC:SM (8:2) in contact with an outer solution containing 100 μM ODPC. The number of GUVs that remained intact was counted over time. The error bars correspond to the standard deviations of experiments done in triplicate. 100 GUVs were inspected for each membrane composition from each experiment.

were exposed to solutions containing from 50 to 170 μM ODPC. The percentage of GUVs that exploded with time increased with ODPC concentration. Furthermore, we also noted that ODPC exerted a similar effect on membranes containing 20 mol% of either SM or Chol, although to a lesser extent. Fig. 2 presents a comparison of the number of GUVs that remained unaltered with time in the presence of 100 μM ODPC, as an example, for membranes of DOPC and the binary mixtures.

As one can note, only circa 60% and 30% of GUVs composed of pure DOPC remained intact under the influence of 100 μM ODPC after 30 min and 1 h of incubation time, respectively (Fig. 2). Moreover, ODPC is less effective in disrupting DOPC bilayers containing a certain amount of SM and Chol. Note that SM is indeed more effective than Chol in protecting the membrane from the effect of ODPC for longer periods. It is worth noting that experiments of carboxyfluorescein leakage from large unilamellar vesicles, LUVs, composed of saturated lipid DPPC revealed that circa 60% of LUVs suffer rupture in the presence of 100 μM ODPC [19]. Such an effect was 4-fold less efficient in LUVs composed of DPPC:Chol (6:4) [19]. As one can see, ODPC (Fig. 1) structurally resembles DOPC and DPPC, so that it has the same polar head and a single long apolar hydrophobic chain. This similarity must facilitate its insertion into the membrane due to a favorable affinity. Once inserted, it can accumulate in the outer membrane causing an increase in the membrane tension. This would result in membrane destabilization and rupture. In fact, calorimetric data from LUVs of DPPC revealed a decrease of the liquid-to-gel transition temperature upon ODPC interaction [19], which is theoretically correlated to an increase in the membrane tension [26]. On the other hand, if ODPC is able to perform a flip–flop from the outer to the inner leaflet, it can promote a membrane solubilization via a detergent-like mechanism [27]. The presence of Chol and SM in the PC-membranes prevents somehow the GUV destabilization. Previous study focused on the interaction of up to 20 mol% of edelfosine on large multilamellar vesicles composed of DOPC:SM (1:1) by DSC demonstrated that this lipid analog may have interaction with both phospholipids, but no membrane destabilization has been reported [10].

Surprisingly, the interaction between 100 μM ODPC and the ternary mixture of DOPC:SM:Chol (6:1:3) did not destabilize the membrane over a period of 1 h of observation. Therefore, these results point to the conclusion that the combined effect of SM and Chol may inhibit the disrupting effect of 100 μM ODPC upon fluid bilayers.

### 3.2. Lo–Ld phase coexistence

Focusing now our attention on GUVs that displayed liquid order (Lo)–liquid disorder (Ld) coexistence, we investigated membranes composed of DOPC:SM:Chol (1:1:1) [22]. Fig. 3 shows a set of GUVs in phase contrast (a) and fluorescence (b) modes to emphasize the coexistence of Ld–Lo lipid phase on the prepared membrane. The bright regions on fluorescent GUVs (Fig. 4b) correspond to Ld phases where the fluorophore Rh-DOPE is preferentially located [23].

Interestingly, by adding 100 μM ODPC to these GUVs the domains rapidly disappeared in less than 1 min of contact. This result is shown in Fig. 4, which presents a time evolution of a GUV starting from 3 min of ODPC incubation (Fig. 4a–b), with abolition of Lo–Ld phase separation indicated by the homogeneous fluorescence distribution on the membrane surface. Of course, nanodomains may still exist but they are not observable due to the optical resolution of 1 μm. However, after 14 min of ODPC–membrane interaction (Fig. 4d) the domains started to reappear, becoming evident at 18 min of ODPC contact (Fig. 4e). Extensive lipid reorganization in the membrane was observed with time until two large separated fluid domains are observed on the membrane surface after 26 to 30 min of ODPC interaction (Fig. 4g–h). More intriguing, the GUV exploded suddenly in the time sequence, as shown in the last snapshots (Fig. 4l–m).

In order to compare the ODPC action mechanism with that of perifosine on lipid rafts, we also performed GUVs experiments with 100 μM perifosine. Fig. 5 shows the results for a representative GUV. Similarly to ODPC, perifosine also disrupts Lo–Ld phase coexistence in less than 1 min. On the other hand, just a subtle tendency of lipid reorganization is identified as small bright dots on the membrane contour at 20 min of perifosine incubation (Fig. 5f), followed by membrane shrinking (Fig. 5h–i) and bursting (Fig. 5j).

Noteworthy, it has been proposed for edelfosine that its mechanism of action is mediated by the high affinity of the ALP molecule for Chol present in raft domains, leading to a redistribution of Chol from the plasma membrane to the cells after edelfosine treatment [10,11]. As a consequence, changes in the raft composition in terms of lipids and proteins must take place. We demonstrated here that the lipid raft-containing model membrane responds to ODPC action in three distinct

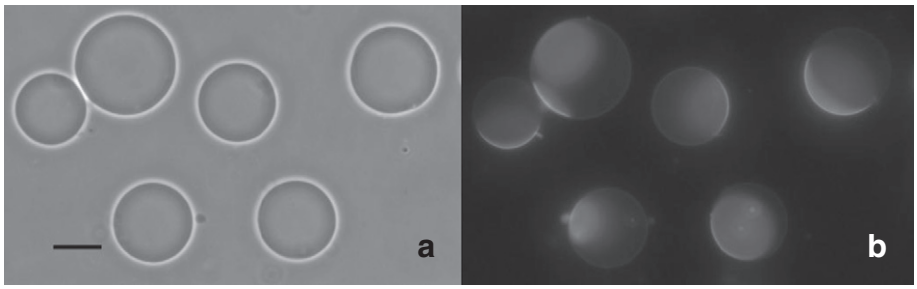


**Fig. 3.** GUVs composed of DOPC:SM:Chol (1:1:1) and labeled with 0.2 mol% of Rh-DOPE: (a) phase contrast and (b) fluorescence microscopy. The fluorescent dye, Rh-DOPE, partitions preferentially into the liquid disordered phase [23]. Objective 60×. The scale bar corresponds to 20 μm.

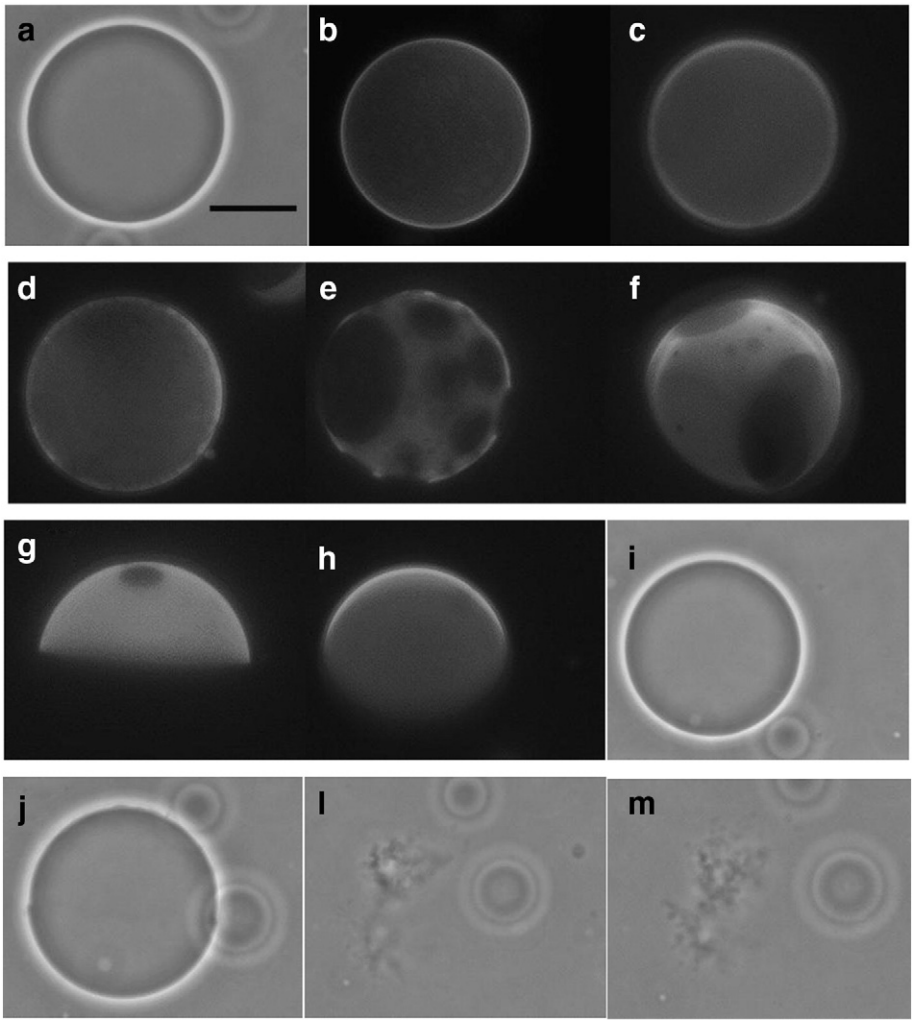


**Fig. 4.** Images obtained in phase contrast (a, i, j–m) and fluorescence (b–h) modes from a GUV composed of DOPC:SM:Chol (1:1:1) showing the effect of 100 μM ODPC upon the lipid membrane until its disruption. The fluorescent dye, Rh-DOPE, partitions preferentially into the liquid disordered phase [23]. Objective 60×. The scale bar corresponds to 20 μm. The beginning of ODPC-membrane interaction was set as time 0 min. Time sequence: (a–b) 3 min; (c) 10 min; (d) 14 min; (e) 18 min; (f) 22 min; (g) 26 min; (h–i) 30 min; (j) 35 min; (l–m) 36 min.

steps: i) the ODPC molecule initially provokes a disturbance in the lipid domains by favoring lipid mixing in a fast way (less than 1 min), as shown in GUVs composed of DOPC:SM:Chol (1:1:1) (Fig. 4). Probably, its insertion in the membrane alters the phase boundaries of the lipid mixture of DOPC, SM and Chol, by lowering the miscibility transition temperature $T_{mix}$ [22]; ii) the lipids subsequently segregate to liquid

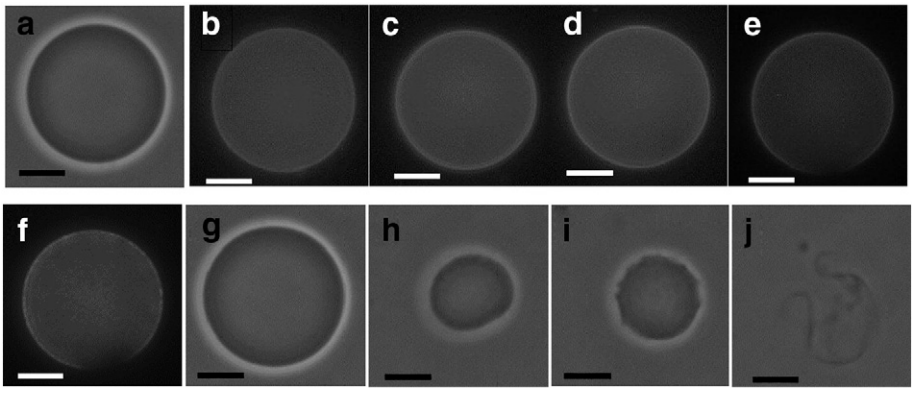


**Fig. 5.** Images obtained in phase contrast (a, g–j) and fluorescence (b–f) modes from a GUV composed of DOPC:SM:Chol (1:1:1) showing the effect of 100 μM perifosine upon the lipid membrane until its disruption. The fluorescent dye, Rh-DOPE, partitions preferentially into the liquid disordered phase [23]. Objective 60×. The scale bar corresponds to 10 μm. The beginning of perifosine–membrane interaction was set as time 0 min. Time sequence: (a–b) 3 min; (c) 6 min; (d) 10 min; (e) 17 min; (f–g) 20 min; (h) 22 min; (i) 25 min; (j) 26 min.

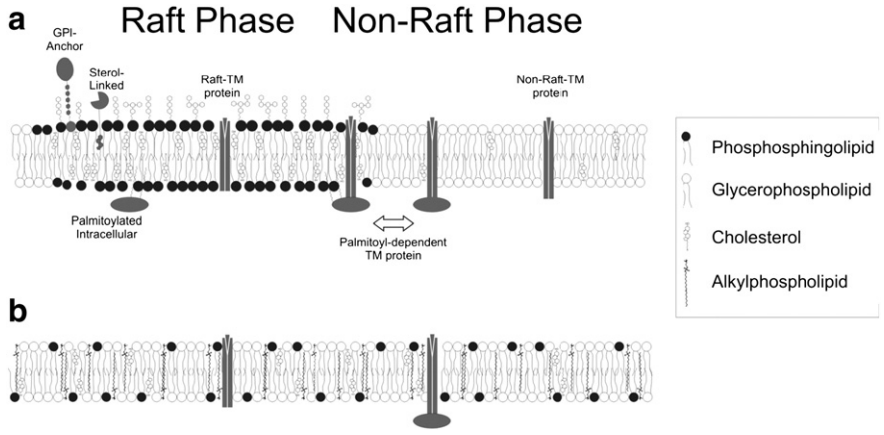


**Fig. 6.** Proposed mechanism of alkylphospholipids (here represented by ODPC) that induces raft disorganization. (a) Assembly of lipid rafts containing domains of varying orders and compositions (TM-protein: transmembrane protein). (b) Treatment with ODPC disrupts the assembly of lipid-rafts, resulting in a displacement of raft-associated proteins. ODPC-induced modifications in the plasma membrane, by which lateral organization and fluidity are compromised, may lead to cell death.

domains within 14–18 min (Fig. 4d–e), reflecting that the homogenous phase was not thermodynamically stable. Thus, the pseudo-ternary homogeneous phase should be very close to the boundary of one-liquid phase to coexisting liquid phases transition; iii) the lipids continue to evolve into two segregated fluid domains (Fig. 4f–h); this redistribution of lipids in the membrane leads to rupture of the GUVs (last panels on Fig. 4l–m).

Membrane domain-disrupting effects have also been recently reported for cholesterol derivatives [28] and vitamin E [29]. In the latter case, Muddana et al. [29] showed by coarse-grained molecular dynamic simulation that vitamin E acts as a linactant, partitioning preferentially to the domain boundaries and lowering the tendency for domain formation. New experiments using a fluorescent probe linked to ODPC are currently in progress in our lab to confirm the preferential partition of the ALP in the rafts boundaries. On the other hand, Carrer et al. [28] measured the lipid diffusion coefficient of some Chol-derivatives by fluorescence correlation spectroscopy and demonstrated that these Chol analogs contribute to an increase in the diffusion coefficients, favoring the lipid mixing into the membrane. Furthermore, monolayer experiments performed with SM:Chol raft-like domains demonstrated that edelfosine has a fluidizing effect on the mixed system [30]. Based on this finding, we may think that ODPC has a similar effect on lipid bilayers, in such a way that it may promote changes in the fluidity of the membrane, as also previously revealed by DSC for ODPC interaction with DPPC membranes [19], favoring initially the lipid mixing.

Despite the fact that perifosine also disrupts the lipid domains, such as ODPC, it must promote changes in the membrane properties to a larger extent than does ODPC. Perifosine must diminish $T_{mix}$ more than ODPC, by increasing the membrane fluidity. As a consequence, the new formed homogenous fluid phase must be placed far from the pseudo-ternary phase transition boundary [22]. Nevertheless, the perifosine-containing membrane also becomes unstable over time until its total destruction. So far, there is no report in the literature about the action mechanism of ALPs upon lipid membranes that resulted in lipid de-mixing followed by membrane disruption as revealed in the present work.

It must be emphasized, however, that the initial stage of lipid raft-disruption by both ODPC and perifosine, and maybe other ALPs, by promoting lipid mixing, may be correlated with their toxicity upon neoplastic cells. Accordingly, changes in the lipid bilayer biophysical properties have been demonstrated to trigger signaling pathways [31]. As a consequence, selective (dis)association of essential proteins within lipid raft scaffolds must take place. AKT/PI3K cell signaling disruption by ALPs has been observed probably due to a primary action of the APLs on the organization of lipids in rafts and raft-associated proteins, as an example NTAL/LAB2 with function seems to be consequently altered [20]. Previous studies indicated that ODPC interferes with the interaction of lipid rafts and adaptor proteins in cells, which leads to an inefficient scaffold for signal transduction proteins. In particular, raft-transmembrane proteins which present palmitoylation sites (Fig. 6) decreased in abundance after ODPC treatment of leukemic cells [20]. Palmitoylation regulates raft affinity for the majority of integral raft proteins [32].

## Acknowledgments

The authors thank Prof. Dr. Kleber Thiago de Oliveira (Chemistry Department Federal University of São Carlos — UFSCar) for the synthesis of ODPC. We also thank the following Brazilian governmental agencies for financial support: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento do Pessoal de Ensino Superior (CAPES), Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP), and Fundação de Apoio ao Ensino, Pesquisa e Assistência do Hospital das Clínicas da FMRP-USP (FAEPA). A.B. Gomide is a recipient of a post-doctoral fellowship from the Nanobiotec-CAPES project.

## References

[1] W.J. van Blitterswijk, M. Verheij, Anticancer alkylphospholipids: mechanisms of action, cellular sensitivity and resistance, and clinical prospects, Curr. Pharm. Des. 14 (2008) 2061–2074.
[2] J.M. Jiménez-López, P. Ríos-Marco, C. Marco, J.L. Segovia, M.P. Carrasco, Alterations in the homeostasis of phospholipids and cholesterol by antitumor alkylphospholipids, Lipids Health Dis. 9 (33) (2010) 1–10.
[3] M.R. Berger, P.G. Munder, D. Schmahl, O. Westphal, Influence of the alkyllysophospholipid ET-18-OCH3 on methylnitrosourea-induced rat mammary carcinomas, Oncology 41 (1984) 109–113.
[4] L. Lucas, R. Hernandez-Alcoceba, V. Penalva, J.C. Lacal, Modulation of phospholipase D by hexadecylphosphorylcholine: a putative novel mechanism for its antitumoral activity, Oncogene 20 (2001) 1110–1117.
[5] P. Hilgard, T. Klenner, J. Stekar, C. Unger, Alkylphosphocholines: a new class of membrane-active anticancer agents, Cancer Chemother. Pharmacol. 32 (1993) 90–95.
[6] W.J. van Blitterswijk, J.B. Klarenbeek, A.H. van der Luit, M.C. Alderliesten, M. van Lummel, M. Verheij, Fas/CD95 down-regulation in lymphoma cells through acquired alkyllysophospholipid resistance: partial role of associated sphingomyelin deficiency, Biochem. J. Eng. 425 (2010) 225–234.
[7] S.R. Vink, J.H. Schellens, W.J. van Blitterswijk, M. Verheij, Tumor and normal tissue pharmacokinetics of perifosine, an oral anti-cancer alkylphospholipid, Invest. New Drugs 23 (2005) 279–286.
[8] C. Gajate, F. Mollinedo, The antitumor ether lipid ET-18-OCH(3) induces apoptosis through translocation and capping of Fas/CD95 into membrane rafts in human leukemic cells, Blood 98 (2001) 3860–3863.
[9] H.R. Fei, G. Chen, J.M. Wang, F.Z. Wang, Perifosine induces cell cycle arrest and apoptosis in human hepatocellular carcinoma cell lines by blockade of Akt phosphorylation, Cytotechnology 62 (2010) 449–460.

[10] A. Ausili, A. Torrecillas, F.J. Aranda, F. Mollinedo, C. Gajate, S. Corbalan-Garcia, A. de Godos, J.C. Gómez-Fernández, Edelfosine is incorporated into rafts and alters their organization, J. Phys. Chem. B 112 (2008) 11643–11654.
[11] V. Zaremberg, C. Gajate, L.M. Cacharro, F. Mollinedo, C.R. McMaster, Cytotoxicity of an anti-cancer lysophospholipid through selective modification of lipid raft composition, J. Biol. Chem. 280 (2005) 38047–38058.
[12] A.H. van der Luit, S.R. Vink, J.B. Klarenbeek, D. Perrissoud, E. Solary, M. Verheij, W.J. van Blitterswijk, A new class of anticancer alkylphospholipids uses lipid rafts as membrane gateways to induce apoptosis in lymphoma cells, Mol. Cancer Ther. 6 (2007) 2337–2345.
[13] W.J. van Blitterswijk, M. Verheij, Anticancer mechanisms and clinical application of alkylphospholipids, Biochim. Biophys. Acta 1831 (2013) 663–674.
[14] D. Lingwood, K. Simons, Lipid rafts as a membrane-organizing principle, Science 327 (2010) 46–50.
[15] K. Gaus, E. Chklovskaia, B.F. de St. Groth, W. Jessup, T. Harder, Condensation of the plasma membrane at the site of T lymphocyte activation, J. Cell Biol. 171 (2005) 121–131.
[16] H.J. Kaiser, A. Orlowski, T. Rog, T.K.M. Nyholm, W. Chai, T. Feizi, D. Lingwood, I. Vattulainen, K. Simons, Lateral sorting in model membranes by cholesterol-mediated hydrophobic matching, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 16628–16633.
[17] E. Sezgin, H.J. Kaiser, T. Baumgart, P. Schwille, K. Simons, I. Levental, Elucidating membrane structure and protein behavior using giant plasma membrane vesicles, Nat. Protoc. 7 (2012) 1042–1051.
[18] M. Agresta, P. D'Arrigo, E. Fasoli, D. Losi, G. Pedrocchi-Fantoni, S. Riva, S. Servi, D. Tessaro, Synthesis and antiproliferative activity of alkylphosphocholines, Chem. Phys. Lipids 126 (2003) 201–210.
[19] G.A. dos Santos, C.H. Thomé, G.A. Ferreira, J.S. Yoneda, T.M. Nobre, K.R.P. Daghastanli, P.S. Scheucher, H.L. Gimenes-Teixeira, M.G. Constantino, K.T. de Oliveira, V.M. Faça, R.P. Falcão, L.J. Greene, E.M. Rego, P. Ciancaglini, Interaction of 10-(octyloxy) decyl-2(trimethylammonium) ethyl phosphate with mimetic membranes and cytotoxic effect on leukemic cells, Biochim. Biophys. Acta 1798 (2010) 1714–1723.
[20] C.H. Thome, G.A. dos Santos, G.A. Ferreira, P.S. Scheucher, C. Izumi, A.M. Leopoldino, A.M. Simao, P. Ciancaglini, K.T. de Oliveira, A. Chin, S.M. Hanash, R.P. Falcão, E.M. Rego, L.J. Greene, V.M. Faça, Linker for activation of T-cell family member (LAT2) a lipid raft adaptor protein for AKT signaling, is an early mediator of alkylphospholipid anti-leukemic activity, Mol. Cell. Proteomics 11 (2012) 1898–1912.
[21] M.I. Angelova, D.S. Dimitrov, Liposomes electroformation, Faraday Discuss. 81 (1986) 303–311.
[22] S.L. Veatch, S.L. Keller, Miscibility phase diagrams of giant vesicles containing sphingomyelin, Phys. Rev. Lett. 94 (2005) 148101.
[23] S. Semrau, T. Idema, T. Schmidt, C. Storm, Membrane-mediated interactions measured using membrane domains, Biophys. J. 96 (2009) 4906–4915.
[24] C. Dietrich, L.A. Bagatolli, Z.N. Volovyk, N.L. Thompson, M. Levi, K. Jacobson, E. Gratton, Lipid rafts reconstituted in model membranes, Biophys. J. 80 (2001) 1417–1428.
[25] C.K. Haluska, M.S. Baptista, A.U. Fernandes, A.P. Schroder, C.M. Marques, R. Itri, Photo-activated phase separation in giant vesicles made from different lipid mixtures, Biochim. Biophys. Acta Biomembr. 1818 (2012) 666–672.
[26] M.J. Uline, M. Schick, I. Szleifer, Phase behavior of lipid bilayers under tension, Biophys. J. 102 (2012) 517–522.
[27] T.P. Sudbrack, N.L. Archilha, R. Itri, K.A. Riske, Observing the solubilization of lipid bilayers by detergents with optical microscopy of GUVs, J. Phys. Chem. B 115 (2011) 269–277.
[28] D.C. Carrer, A.W. Schmidt, Hans-Joachim Knölker, P. Schwille, Membrane domain-disrupting effects of 4-substitued cholesterol derivatives, Langmuir 24 (2008) 8807–8812.
[29] H.S. Muddana, H.H. Chiang, P.J. Butler, Tuning membrane phase separation using nonlipid amphiphiles, Biophys. J. 102 (2012) 489–497.
[30] K. H¸ac-Wydro, P. Dynarowicz-Ł¸atka, P. Wydro, K. B¸ak, Edelfosine disturbs the sphingomyelin–cholesterol model membrane system in a cholesterol-dependent way — the Langmuir monolayer study, Colloids Surf. B Biointerfaces 88 (2011) 635–640.
[31] A.E. Cremesti, F.M. Goni, R. Kolesnick, Role of sphingomyelinase and ceramide in modulating rafts: do biophysical properties determine biologic outcome? FEBS Lett. 531 (2002) 47–53.
[32] I. Levental, D. Lingwood, M. Grzybek, U. Coskun, K. Simons, Palmitoylation regulates raft affinity for the majority of integral raft proteins, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 22050–22054.