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Surface-active properties of the antitumour ether lipid 1-*O*-octadecyl-2-*O*-methyl-*rac*-glycero-3-phosphocholine (edelfosine)

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Abstract

The surface activity and interaction with lipid monolayers and bilayers of the antitumour ether lipid 1-*O*-octadecyl-2-*O*-methyl-*rac*-glycero-3-phosphocholine (edelfosine) have been studied. Edelfosine is a surface-active soluble amphiphile, with critical micellar concentrations at 3.5 μM and 19 μM in water. When the air–water interface is occupied by a phospholipid, edelfosine becomes inserted in the phospholipid monolayer, increasing surface pressure. This increase is dose-dependent, and reaches a plateau at ca. 2 μM edelfosine bulk concentration. The ether lipid can become inserted in phospholipid monolayers with initial surface pressures of up to 33 mN/m, which ensures its capacity to become inserted into cell membranes. Upon interaction with phospholipid vesicles, edelfosine exhibits a weak detergent activity, causing release of vesicle contents to a low extent (<5%), and a small proportion of lipid solubilization. The weak detergent properties of edelfosine can be related to its very low critical micellar concentrations. Its high affinity for lipid monolayers combined with low lytic properties support the use of edelfosine as a clinical drug. The surface-active properties of edelfosine are similar to those of other "single-chain" lipids, e.g. lysophosphatidylcholine, palmitoylcarnitine, or N-acetylsphingosine.

Keywords: Antitumour lipid; Edelfosine; Surface activity; Lipid monolayers; Critical micellar concentration

1. Introduction

The antitumour drug 1-*O*-octadecyl-2-*O*-methyl-*rac*-glycero-3-phosphocholine (ET-18-OCH_3, edelfosine) represents the prototype of a group of synthetic antitumour ether lipids that show promising and selective antitumour activity and that, unlike most conventional chemotherapeutic drugs, do not target the DNA [1–3]. Edelfosine is accumulated in lipid rafts in tumour cells [4–6], and induces apoptosis through a redistribution of lipid raft protein composition [4,7–9]. However, little is known about the physical properties of edelfosine, and of its interaction with membrane lipids. Its structure, containing defined hydrophobic and hydrophilic moieties, suggests a potent surface activity, as found with structurally related biomolecules, e.g. lysophosphatidylcholine [10], palmitoylcarnitine [11], or N-acetylsphingosine [12]. All these molecules partition preferentially into the lipid bilayers in mixed aqueous dispersions of surfactant and phospholipid vesicles and some, e.g. N-acetylsphingosine, have a mild detergent activity, i.e. they are able to form phospholipid–surfactant mixed micelles [13]. Sánchez-Piñera et al. [14] described the effects of edelfosine on the polymorphic properties of dielaidoylphosphatidylethanolamine, demonstrating clear effects of the ether–lipid on the phospholipid phase behaviour. More recently, Torrecillas et al. [15] have described the effects of edelfosine and various analogues on the thermotropic phase transitions of phospholipids.

The present study is aimed at describing the surface-active properties of edelfosine in aqueous and bilayer environments. This ether lipid is found to interact with pure phosphatidylcholine monolayers and bilayers. Some of these properties may be relevant in the antitumour activity of edelfosine.

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2. Materials and methods

Edelfosine was obtained from INKEYSA (Barcelona, Spain). 1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) was supplied by Avanti Polar Lipids (Alabaster, AL). Egg yolk phosphatidylcholine (ePC) was grade I from Lipid Products (South Nutfield, UK). 2-Anilinonaphthalene-6-sulphonic acid (ANS), lissamine™ rhodamine B 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine triethylammonium salt (rhodamine DHPE), 8-aminonaphthalene-1,3,6-trisulfonic acid (disodium salt) (ANTS) and *p*-xylene-bis-pyridinium bromide (DPX) were from Molecular Probes, Inc. (Eugene, OR). Merocyanine 540 and Triton X-100 were provided by Sigma (St. Louis, MO). All assays were carried out in 10 mM HEPES, 150 mM NaCl, pH 7.4 buffer. All lipids were used without further purification. All other reagents were of analytical grade.

For liposome preparation, the proper amount of lipid was dissolved in chloroform:methanol (2:1, v/v) solution and the solvent evaporated exhaustively. Multilamellar vesicles (MLVs) were then prepared by hydrating the lipid film with buffer by vigorous vortexing. Large unilamellar vesicles (LUVs) were prepared by the extrusion method, passing the MLV suspension 10 times through polycarbonate filters 0.1 μm in pore diameter. Average size of the vesicles was ca. 0.1 μm as measured by quasi-elastic light scattering in a Malvern Zeta-Sizer 4 spectrometer. Final lipid concentration was measured in terms of lipid phosphorus.

2.1. Lipid monolayers and fluorescence measurements

Monomolecular layers at the interface were studied at 22 °C using a μTrough-S equipment (Kibron, Helsinki, Finland) consisting on a small 2-cm rounded multi-well plate that allowed for 1 mm subphase measurements. Monolayers were formed by spreading a small amount of lipid (about 2 nmol) in chloroform:methanol (2:1, v/v) solution over assaying buffer. After allowing for solvent evaporation, edelfosine in buffer was added into the subphase through an adjusted hole. When formation of edelfosine monolayer was tested edelfosine was injected into the subphase without previous lipid spreading, following edelfosine transition to the interface in terms of changes in surface pressure. For fluorescence measurements, Rh-PE monolayers were formed at the interface at π=20 mN/m and edelfosine was then injected into the subphase. After equilibration, aliquots were extracted from the subphase and assayed for rhodamine fluorescence by using an Aminco Bowman Series 2 luminescence spectrometer. The Rh-PE emission was followed at 590 nm (excitation wavelength at 530 nm) with a cut-off filter at 560 nm. Buffer was used as a zero for fluorescence signal.

2.2. CMC determination

Critical micellar concentration was measured at 22 °C by mixing increasing concentrations of edelfosine in water (0 to 34 μM) with the hydrophobic dye Merocyanine 540 (final concentration 4 μM) [16]. Absorption spectra from the samples was then measured in an Uvikon 922 spectrophotometer and cmc was determined by an increase of about 25 nm in the absorption maximum wavelength (λ_{max}) of the dye upon merocyanine entrapping into edelfosine micelles. Alternatively, edelfosine was mixed with the fluorescent probe ANS (final concentration 2 μM) [16 bis], and ANS fluorescence emission was measured in a SLM-AMINCO 8100 spectrofluorometer (λ_{ex}=390 nm, λ_{em}=550 nm). In the ANS method the cmc is determined from an abrupt increase in fluorescence emission intensity.

2.3. Vesicle content efflux measurement

Large unilamellar vesicles were prepared in the presence of ANTS and DPX [17]. The lipid suspension was passed through a Sephadex G-25 column to discard non-encapsulated dye. Final lipid concentration was always 0.3 mM. Vesicle content efflux was measured at 22 °C in an Aminco Bowman Series 2 luminescence spectrometer by following ANTS externalization after edelfosine addition. ANTS emission was recovered at 520 nm (excitation wavelength at 355 nm) with a cut-off filter at 450 nm. Initial vesicle suspension was used as the 0% fluorescence signal while 100% fluorescence was obtained after breakdown of the vesicles by addition of 1 mM Triton X-100.

2.4. Solubilization assays

Liposome suspensions were prepared in sucrose buffer (10 mM HEPES, 255 mM sucrose, pH 7.4) and diluted 10 times in assay buffer to diminish non-encapsulated sucrose concentration for a proper sedimentation. Sucrose-containing vesicles were ultracentrifuged for 1 h at 100,000×*g* and 4 °C, and the supernatant discarded. The pellet was resuspended in assay buffer and mixed with the same volumes of the appropriate edelfosine solutions in the same sucrose-free buffer. Final lipid vesicle concentration was always 1 mM. The mixtures were left to equilibrate for 1 h at 22 °C, and solubilization was assayed from changes in turbidity. Turbidity was measured as absorbance at 500 nm (A_{500}) in an Uvikon 922 spectrophotometer. Turbidity values were normalized by setting 100% as the turbidity of the LUV suspension in the absence of edelfosine, while 0% turbidity corresponded to pure buffer [18]. After turbidity measurements, the solutions were again ultracentrifuged for 1 h at 125,000×*g* and 4 °C, and vesicle solubilization followed in terms of lipid phosphorus in the supernatant.

3. Results

The surface-active properties of edelfosine were tested in a Langmuir balance. The ether lipid suspended in buffer was injected into the subphase, and the change in surface pressure (π) was recorded. Edelfosine caused a dose-dependent increase in surface pressure. Representative curves of π vs. time are shown in Fig. 1A. After a certain time, that decreased with increasing edelfosine concentrations, π remained constant. This constant value was operationally defined as an equilibrium value in this context. Equilibrium values of edelfosine-induced

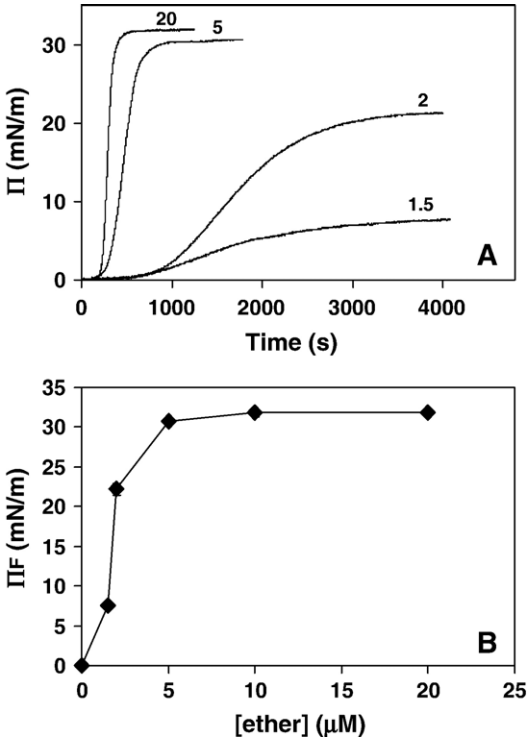

Fig. 1. Edelfosine-induced changes in surface pressure at an air–water interface. (A) Time course. Representative π vs. time curves, the figures correspond to bulk edelfosine concentrations (μM). (B) Equilibrium values obtained from experiments as those shown in panel A. Average values±S.E. (n=3). Subphase composition was 10 mM HEPES, 150 mM NaCl, pH 7.4. Edelfosine was injected into the subphase.