Chemical Physics Letters 559 (2013) 99–106

Contents lists available at SciVerse ScienceDirect

Chemical Physics Letters

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

Langmuir films containing ibuprofen and phospholipids

Vananélia P.N. Geraldo [a], Felippe J. Pavinatto [a], Thatyane M. Nobre [a], Luciano Caseli [b], Osvaldo N. Oliveira Jr. [a,*]

[a] Universidade de São Paulo, Instituto de Física de São Carlos, CP 369, São Carlos, 13560-970 SP, Brazil
[b] Universidade Federal de São Paulo, Instituto de Ciências Ambientais, Químicas e Farmacêuticas, Diadema, SP, Brazil

ARTICLE INFO

Article history:
Received 5 November 2012
In final form 28 December 2012
Available online 8 January 2013

ABSTRACT

This study shows the incorporation of ibuprofen, an anti-inflammatory drug, in Langmuir monolayers as cell membrane models. Significant effects were observed for dipalmitoyl phosphatidyl choline (DPPC) monolayers with relevant changes in the elasticity of the monolayer. Dipalmitoyl phosphatidyl glycerol (DPPG) monolayers were affected by small concentrations of ibuprofen, from 1 to 5 mol%. For both types of monolayer, ibuprofen could penetrate into the hydrophobic part of the monolayer, which was confirmed with polarization-modulated infrared reflection–absorption spectroscopy (PM-IRRAS). Brewster angle microscopy (BAM) images showed that ibuprofen prevents the formation of large domains of DPPC. The pharmacological action should occur primarily with penetration of ibuprofen via electrically neutral phospholipid headgroups of the membrane.

1. Introduction

The cell membrane is a complex system essential for cellular functions because it mediates interactions between the cell and its external environment. The differentiation and function, for example, depend on the composition of lipids in the membrane, which is the reason why delivery of drugs varies for different cell types [1–3]. A drug administered orally or injected intravenously seldom arrives in a specific target in the body in the appropriate concentrations to cause the expected therapeutic effect. This is easily explained by the obstacles of various kinds (anatomical, chemical and biological) that must be overcome before the drug reaches the target organ or tissue [4,5]. Ibuprofen is a non-steroidal anti-inflammatory drug, with low solubility in water (maximum solubility of 0.011 g/L or 53 μM at 25 °C) [6], which displays prolonged side effects. The inconvenience in the use of ibuprofen is mostly associated with gastrointestinal complications, since 15 to 30% of patients using this drug for a long time have gastrointestinal ulcers and bleeding, in addition to renal dysfunction [7].

Interactions of drugs with cell components involve changes in the organization of the biological membrane. Therefore, it is essential to characterize the interaction between drugs and membranes, particularly obtaining molecular-level information. Currently, this is normally done with membrane model systems, such as Langmuir monolayers [8–10] and liposomes [11]. The incorporation of substances in a Langmuir film has varying effects depending on the substance location, its electrical charge, and the method for incorporation. Though lipid monolayers are much less complex than the real biological membranes, they have been useful in modeling the interactions [12,13].

This paper deals with the effects from ibuprofen interacting with Langmuir monolayers with a zwitterionic phospholipid (DPPC) and an anionic (DPPG) phospholipid. The monolayers were characterized using surface pressure, surface potential, Brewster angle microscopy (BAM), and polarization-modulated infrared reflection absorption spectroscopy (PM-IRRAS) measurements. The main objective was to identify how ibuprofen affects the molecular packing, in addition to studying the dependence on the ibuprofen concentration and type of lipid, so that biological implications can be drawn as to the mode of action for the drug and its possible incorporation in drug delivery systems.

2. Materials and Methods

Dipalmitoyl phosphatidyl glycerol (DPPG) sodium salt and dipalmitoyl phosphatidyl choline (DPPC) were purchased from Avanti Polar Lipids, and analytical grade chloroform and methanol were purchased from Merck. Ibuprofen [α-methyl-4-(2-methylpropyl) benzeneacetic acid] of the highest purity available was acquired from Sigma. Figure 1 shows the chemical structure of DPPC, DPPG, and ibuprofen.

Two methodologies were employed to study interaction between phospholipids and ibuprofen. The first was co-spreading both components from the same solution, which is suitable to verify whether the drug can interact with the phospholipid and be incorporated in liposomes as a carrier for ibuprofen delivery [14]. DPPC and ibuprofen were dissolved in pure chloroform, and a

* Corresponding author.
E-mail address: chu@ifsc.usp.br (O.N. Oliveira Jr.).

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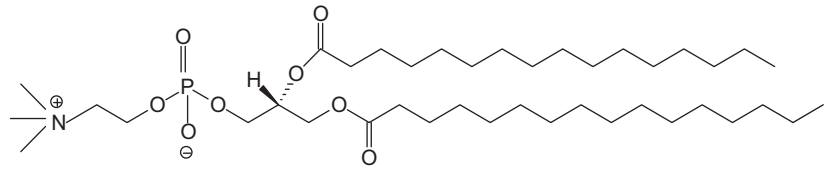
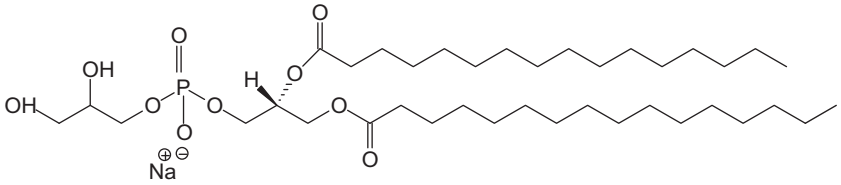
http://dx.doi.org/10.1016/j.cplett.2012.12.064



DPPC



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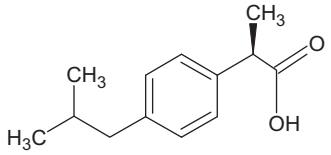

Ibuprofen

Figure 1. Chemical structures of dipalmitoyl phosphatidyl choline (DPPC), dipalmitoyl phosphatidyl glycerol (DPPG), and Ibuprofen.

chloroform:methanol (9:1 v:v) mixture was employed to dissolve solutions containing DPPG. Initially, 40μL of solutions containing pure phospholipids or phospholipids/ibuprofen mixtures in a range from 0.1 to 5.0% in mol of ibuprofen were spread on the surface to obtain a Langmuir monolayer. Milli-Q water at pH of 5.6 and temperature of 21 ± 1 °C was used as subphase. After spreading, the solvent was allowed to evaporate for 10 min. In the second methodology, the ability of ibuprofen at incorporating into the phospholipid monolayers was evaluated with the drug in the subphase at the following concentrations: 12.5, 25.0 or 50.0 μM. The surface activity of the drug was also assessed without spreading the phospholipids by compressing the barrier to observe any increase in surface pressure. All experiments were repeated several times to ensure reproducibility of the isotherms.

Surface pressure and surface potential measurements were carried out with a Langmuir minitrough from KSV Instruments which total area is 23,625.00 mm^2 in a class 10,000 clean room. The surface pressure π was determined using the Wilhelmy plate method and the surface potential ΔV was measured using the vibrating plate method (frequency 300 Hz) using a KSV Kelvin probe with both reference and vibrating plate electrodes made of platinum, and the probe located at approximately 1–2 mm above the water surface. Film compression using two symmetrically movable barriers was carried out at a constant barrier speed of 10 mm min^{-1}. The system is computer-controlled, which allows the simultaneous recording of surface-pressure (π-A) and surface-potential (ΔV-A) isotherms.

The film morphology was studied with a Brewster angle microscope (BAM), Model BAM2Plus System from Nanofilm Technologies (NFT - Germany), mounted on the trough apparatus. The BAM principle is based on the fact that a *p*-polarized light beam impinging on the water surface at the Brewster angle is not reflected. Therefore, no light reaches a camera placed in the direction of the reflected beam. The Brewster angle is determined by the refractive index of the two media that form the interface, for example, water and air, for a clean water surface [15]. If a Langmuir film is formed, this new interface changes with the refractive index being slightly modified, producing reflection of the light toward the camera. An image of the interfacial film structure is formed by contrast between regions without film (dark regions – without reflection) and spots where the water surface is covered with film molecules (bright regions – reflection).

Polarization-modulated infrared reflection absorption spectroscopy (PM-IRRAS) was performed using a KSV PMI550 instrument (KSV, Finland) at a resolution of 8 cm^{-1} with Langmuir monolayers obtained by spreading mixtures of phospholipids and ibuprofen 5% in mol on the aqueous subphase. The experimental setup used was similar to that described by Pavinatto et al. [16]. The Langmuir trough was mounted for the light beam reach the monolayer at a fixed incidence angle of 80°. As the incoming light was continuously modulated between s- and p-polarization at a high frequency, the spectra could be measured for the two polarizations simultaneously. The two channels processing the detected signal give the differential reflectivity spectrum $\Delta R=(Rp - Rs)/(Rp + Rs)$, where Rp and Rs are respectively the polarized reflectivities for parallel and perpendicular directions to the plane of incidence. The absorption of the parallel polarized light beam comes mainly from vertically oriented dipoles, while those oriented horizontally give rise to absorption of the perpendicularly polarized beam. The difference of the two spectra thus provides information on oriented vibrational dipoles, which is generally surface specific as the molecules in the subphase have random orientation. Since the spectra were measured simultaneously and the IR spectrum was divided by the corresponding spectrum of the subphase, the effect of water vapor was reduced. In the angle used in this work, positive bands indicate a transition moment preferentially in the surface plane, whereas negative bands indicate preferential orientation perpendicular to the surface.

3. Results and Discussion

The main objective in studies of pharmaceutical drugs interacting with model membranes is to obtain molecular-level