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Daunorubicin and doxorubicin molecular interplay with 2D membrane models

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ABSTRACT

The anthracyclines daunorubicin and doxorubicin are widely used antineoplastic agents due to their therapeutic activity against a broad variety of human cancers. Although, the classical model to explain anthracyclines' cytotoxicity has been based in the direct interference with nucleic acid function, evidence suggests that the plasma membrane is also involved in the drug's mechanism of action.

In this work, the interaction of these drugs with two-dimensional membrane models were studied in order to gain further insights at the molecular level regarding anthracyclines membrane interactions. For that purpose, Langmuir monolayers composed of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), sphingomyelin (SM) and cholesterol (Chol) were used, since these are the most common lipids found in biological membranes. Several biophysical techniques were employed: surface pressure (π) – area (A) isotherms measurements were used to investigate the adsorption and penetration of drugs, polarization-modulation infrared reflection-absorption spectroscopy (PM-IRRAS) to acquire structural information and Brewster angle microscopy (BAM) to record images of the monolayers on the micrometer scale.

The interactions of anthracyclines were assessed by alterations in the monolayers' shape, characteristic parameters (C_s^{-1} values and area per lipid molecule at 30 $mN{\cdot}m^{-1}$ and under maximum packing conditions) and morphology of each 2D model studied. The presence of the drugs in the interface led to the production of less ordered monolayers, as evidenced by the decrease in the compressibility modulus. In addition, the drugs' effect on the membrane organization is related with their chemical structure and depends on the membranesí phase. For lower surface pressures, both electrostatic and hydrophobic interactions led to significant modifications in the monolayer order. With further compression, the impact of such interactions is reduced, resulting in the squeezing-out of some drug molecules from the interface. Furthermore, BAM images showed a clear anticancer drug interplay with the lipid monolayer by changes in the domains shape and appearance of bright dots, which are located in the frontier between the condensed and expanded lipid phases.

1. Introduction

Anthracyclines are considered one of the most effective group of anticancer drugs ever developed [1], wherein daunorubicin (DAN) and doxorubicin (DOX) where the first isolated antibiotics from cultures of *Streptomyces peucetius* [1]. DAN and DOX (Fig. 1) share a quinone containing a rigid planar aromatic ring structure (dihydroxyanthraquinone) bound to an amino sugar moiety and the only difference between these two molecules lies in the end of the side chain: DOX ends with a hydroxyl group, whereas DAN ends with a methyl group. This small structural difference has relevant consequences on their spectrum of activity and pharmacokinetics [1]. Thus, while DAN shows activity in acute lymphoblastic or myeloblastic leukemia's [2], DOX is used in the treatment of breast cancer, childhood solid tumors, soft tissue sarcomas and aggressive lymphomas [1].

Although, the mechanism of anthracyclines' cytotoxicity may involve multiple pathways, the DNA has held the center stage as primary target. In fact, anthracyclines have been thought to act either by directly binding to nucleic acids or by interfering with the enzymes that are involved in their biosynthesis [3]. Nevertheless,

Abbreviations: BAM, Brewster angle microscopy; Chol, Cholesterol; DPPC, dipalmitoylphosphatidylcholine; IR, infra-red; LC, liquid-condensed; LE, liquid-expanded; LO, liquid-ordered; SM, sphingomyelin; PM-IRRAS, Polarization modulation infrared reflection-absorption spectroscopy.

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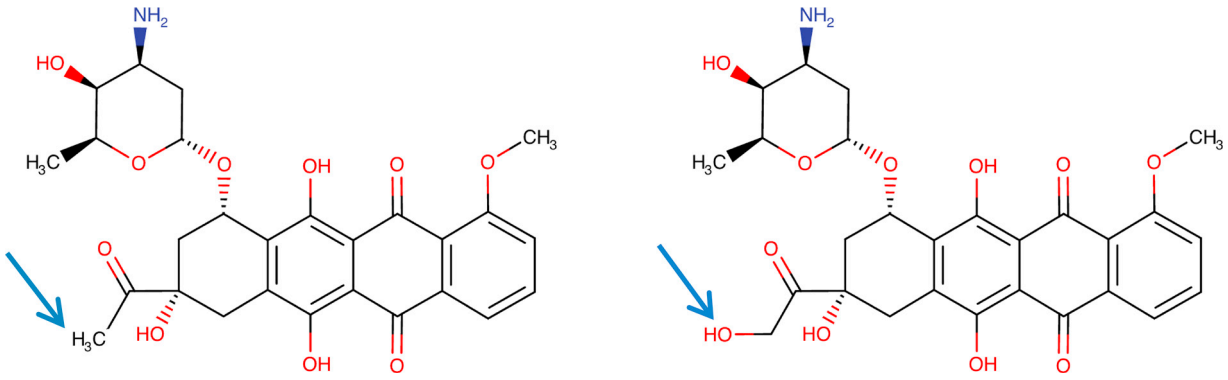

Fig. 1. Structure of daunorubicin (left) and doxorubicin (right): same dihydroxyanthraquinone and sugar moieties, but doxorubicin ends with a hydroxyl group instead of the methyl group that is present in daunorubicin.

growing evidence has contributed to the idea that the cell membrane can itself be a target for these drugs and can also be involved in their mode of action [4–6]. For example, in 1982 Tritton et al. have demonstrated that anthracyclines, without entering the cell, could exert their cytotoxic effect only through their interplay with the membrane [4].

The cell membrane is not just a lipid bilayer in which proteins are embedded. Such cell boundary is involved in several biological activities that lead to cell survival, differentiation, cell death, among others [7]. Therefore, alterations of its general lipid organization and structure, like the ones induced by chemotherapeutic compounds, can initiate several processes implicated in the anticancer cytotoxic effects (such as apoptosis). For this reason, the goal of this study was to perform a systematic comparison between the degree of penetration of DAN and DOX, and their ability to induce changes in the biophysical properties of lipid membranes.

Besides, the interaction of DAN and DOX with membranes is inevitable, since they must pass across membranes in order to exert their cytotoxic effects through interplay with DNA or other intracellular components (taking in consideration that the DNA is the primary target of anthracyclines). Several studies have demonstrated that anthracycline' molecules interact specifically with negatively charged membrane lipids, which have been implicated in the cardiotoxicity caused by these drugs [8,9]. Finally, evidence also shown that this type of chemotherapeutic agents has the ability to alter the membrane structure, by reducing the formation of the non-lamellar hexagonal (H_{II}) phase propensity and just by interacting with membrane phospholipids [5]. Because the H_{II} phase is involved in the location and activity of several proteins (such as, the G-protein and the kinase C) important for cell signaling, various cellular events implicated in the signal transduction are indirectly affected by these drugs [5].

In this context, the study of anticancer drug-membrane interactions is fundamental to better understand the drugs' bioavailability and the drug-induced toxicity process. Therefore, two-dimensional (Langmuir monolayers) model systems composed of different ratio of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), sphingomyelin (SM) and cholesterol (Chol) were chosen to mimic the plasma membranes and explain the drug's impact on the structure-packing behaviour and molecular organization of the model membranes. The lipids choice is related to the fact that such lipids are the major components of the outer layer of human membranes [10]. In fact, phosphatidylcholines are the most abundant phospholipids in natural cell membranes. SM is the major sphingolipid found in membranes, being involved in many cellular functions [11]. Chol is also a major component of the membrane and one of the most important regulators of lipid organization [12]. Chol has also the ability to modulate membrane fluidity and permeability, which is important for the preservation of membrane' organization, contributing to the cell membrane's physiology and function [13]. In addition, Chol and SM form liquid-ordered domains in a more fluid matrix, crucial for membrane organization and where several proteins and other biomolecules are located [14]. The use of monolayers at the air/liquid interface allows to assess the adsorption and penetration of DAN and DOX at the membrane surface without the interference of trans-bilayer events [15]. The combination of Langmuir monolayers with other methods provides information regarding the monolayer morphology, domains structure, packing properties, phase coexistence [16], structural organization [17,18] and about the effects of the anticancer drugs on such properties, important to better understand the drug's cytotoxic mechanisms. In this regard, different biophysical techniques were performed using Langmuir monolayers: pressure-area isotherm measurements, polarization modulation infrared reflection-absorption spectroscopy (PM-IRRAS) and Brewster angle microscopy (BAM).

2. Experimental section

2.1. Materials and reagents

Daunorubicin (DAN) was obtained from Biovision (Milpitas, California) and doxorubicin (DOX) from LC Laboratories (Woburn, Massachusetts, USA). The lipids 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), sphingomyelin (SM) and cholesterol (Chol) were supplied by Avanti Polar Lipids, Inc. (Alabama, USA). All compounds were used without further purification. Drug solutions were prepared with HEPES buffer (10 mM, pH 7.4). The buffer was prepared with double deionized water (conductivity inferior to 0.1 μScm^{-1}) and adjusted ionic strength with NaCl (I = 0.1 M).

2.2. Langmuir isotherms

The surface pressure area isotherms (π/A) experiments were carried out in a KSV NIMA Langmuir balance. The system was equipped with two barriers, used in compression isotherms assays, and a Wilhelmy microbalance with filter paper plate (accuracy superior to 0.1 $mN{\cdot}m^{-1}$), used as a surface pressure sensor. Monolayers of DPPC, DPPC:SM [8:2] and DPPC:SM:Chol [7:1.5:1.5] were prepared by spreading dropwise the lipid/chloroform solutions (1 mM) using a Hamilton syringe on the Hepes subphase (pH 7.4), with or without anticancer drug (DAN or DOX, 10 μM). The drug concentration choice was based on Matyszewska et al. work, which demonstrated that concentrations of DAN above 10^{-5} M did not affect the DMPC:Chol monolayer to a greater extent due to a limiting concentration of drug in the subphase [19]. For all Langmuir