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Probing the interactions of mitoxantrone with biomimetic membranes with electrochemical and spectroscopic techniques

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ABSTRACT

Mitoxantrone – an anticancer drug – was used to probe the interactions of this class of cytostatic molecules with biomimetic monolayers. The drug effect was monitored with electrochemical (cyclic voltammetry and electrochemical impedance spectroscopy), as well as spectroscopic techniques (surface enhanced Raman scattering), during its passive partitioning/penetration through the mixed Langmuir and Langmuir–Blodgett monolayers after their transfer on gold electrodes. This approach allowed us to discriminate between the drug interactions with hydrophilic head-group region and hydrophobic alkyl chains moiety of such monolayers.

1. Introduction

In search of the new anticancer drugs, much attention is paid to the drug specificity and efficacy against their intracellular target, e.g. DNA molecules, for the case of the double helix intercalating agents such as anthracyclines.

There are several structurally related anthracyclines in common chemotherapeutic use, with mitoxantrone (MTX), an anthracenedione in frequent use as substitute for other anthracyclines, such as daunorubicin, doxorubicin or idarubicin, particularly in pediatric acute lymphoblastic leukemia and lymphoblastic lymphoma [1]. The structural variety existing among the anthracyclines has been shown to be related to their therapeutic efficacy and toxicity – primarily – cardiotoxicity [2,3]. Mitoxantrone is an amino-anthraquinone anticancer drug, designed and synthesized to improve the clinical response of other anthracyclines in conjugated chemotherapies of several leukemias, ovarian and breast cancer [4–6]. The biological activity of mitoxantrone is related to its interaction with DNA and the inhibition of topoisomerase II [2,7], giving rise to double-stranded breaks or intercalation into DNA, preferentially at the CpG base sequence, with side chains of MTX in the minor groove of a double helix, thus inhibiting DNA replication and transcription to mRNA [8–10].

Yet before the drug molecule reaches its target inside the cancer cell, it has to pass through the cellular membrane. Unfortunately, little is known about the mechanisms involved in such processes, being generally divided into passive and active transport pathways. The manner in which mitoxantrone can penetrate plasma membrane structures is, of course, central to its chemotherapeutic action because the plasma membrane acts as a barrier to the permeation of polar molecules and this effect is mainly due to the hydrophobicity of membrane interior. The partitioning of MTX molecules into the lipid bilayer must be thus the basis for its passive transport across the biological membrane. Many questions still remain as to how MTX and other structurally related anthracyclines are transported into the cells across the plasma membrane and subsequently into the cell nucleus. In a series of our previous papers we investigated passive partitioning of another anthracycline drug – doxorubicin (DOX) – through the biomimetic membranes [11–13]. Using a variety of techniques ranging from Langmuir, Langmuir–Blodgett, electrochemistry, spectroscopy, surface plasmon resonance (SPR) and time resolved fluorescence lifetime and anisotropy imaging microsopies (FLIM/FLAIM) we have found that DOX can be used as probe molecule, recognizing various organization sites of biomimetic monolayers and bilayers. Moreover, the mode of DOX interaction with these systems strongly depends on the method of the formation of such layer and whether its hydrophobic or hydrophilic regions are exposed to an aqueous, DOX-containing, solution. What differs MTX from DOX molecules is that it belongs to a chemically different class of anthracenedions and apart from containing the same

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hydroquinone/benzoquinone redox centers in their condensed ring system, its chemical structure is different than that of DOX. MTX possesses two alkyl side-chains, each containing secondary amines and terminated with hydroxyl functionalities, rendering this molecule slightly more hydrophobic than DOX (solubility in water up to ca. 10 mM, DOX solubility is up to ca. 50 mM, both compounds in a form of hydrochlorides). MTX molecule also lacks daunosamine sugar, as compared to DOX (Fig. 1).

In this paper we present our electrochemical and spectroscopic results on the interaction of MTX with several biomimetic films, obtained by Langmuir and Langmuir–Blodgett (L–B) techniques. The focus of this paper is on the electrochemical and spectroscopic characterization of interactions of mitoxantrone with monolayers used as models of biomembranes.

Mitoxantrone is a redox-active molecule with its metabolism involving enzymatic reduction of the quinone moiety by one or two electrons, leading to the formation of semiquinone or hydroquinone, respectively. The one-electron process carried out by reductase and followed by oxidation by molecular oxygen is known as redox cycling and is thought to be responsible for the possible formation of reactive oxygen species (ROS), damaging to biological systems. The two-electron reduction product – hydroquinone, is secreted by the organism along a detoxification pathway [14,15]. This redox behavior, under controlled conditions of $2e^-/2H^+$ transformations for each of the redox centers of MTX molecules (quinone and hydroquinone) [16–20] is advantageous in monitoring the interactions of MTX with biomimetic layers deposited on the electrode surface in a similar way as was done for the case of DOX in our previous works [11–13].

The biomimetic layers were formed from long chain aliphatic amphiphiles, under conditions of Langmuir monolayer, where the hydrophilic headgroups were exposed to an aqueous, MTX-containing solution and, in the case of Langmuir–Blodgett transfer onto the metallic surface, the hydrophobic moiety of well-organized monolayers was facing the aqueous solution with mitoxantrone.

Such layered structures are used as the simplest models of biomembranes and we have chosen to use them because of their relative ease of formation and the experimental control that can be exerted over their properties and architecture [21–25]. The choice of such structures was based upon the hydrophilic/hydrophobic regions present in the lipid matrix of the cellular membrane that forms the first barrier against the penetration of MTX into the cell. Our data point to the interaction of MTX with these biomimetic interfaces being significantly dependent on the identity of the interfacial amphiphile. The electrochemical and spectroscopic responses of MTX in these environments provide insight into the factors that are most likely to mediate the transport of anthracyclines across plasma membrane structures.

2. Experimental

All reagents were of the highest quality available commercially and were used without further purification. Octadecanethiol ($C_{18}SH$, 98%), dihexadecyl phosphate (DHP, 97%), octadecylamine ($C_{18}NH_2$), hydroxylamine hydrochloride (99.9%) and lithium perchlorate (99.9%) were purchased from Aldrich. Mitoxantrone hydrochloride was obtained from Selleck Chemicals (USA), chloroform (99.8% purity) was obtained from Chempur (Poland). Silver nitrate and sodium hydroxide of analytical reagent grade were supplied by POCH (Poland). Aqueous solutions were prepared with milli-Q water (resistivity 18.2 MΩ cm) obtained from the Millipore system.

2.1. Langmuir mixed monolayers

Mixed monolayers of octadecanethiol:octadecylamine ($C_{18}SH$:$C_{18}NH_2$) and octadecanethiol:dihexadecyl phosphate ($C_{18}SH$:DHP) were prepared by mixing stock solutions of each compound in chloroform to obtain a desired molar ratio of each constituent in the spreading solution. The octadecanethiol molecules in these monolayers served as anchors of a mixed monolayer after its transfer onto the gold substrates [26,27]. The lowest possible amount of octadecanethiol molecules that was capable of strong anchoring of such monolayers after their Langmuir–Blodgett transfer onto the electrode surface and yet unaffecting monolayer properties as judged from the isotherm shape, were evaluated in our previous paper [11]. Here, we use the same molar ratio of 1:2 ($C_{18}SH$:$C_{18}NH_2$) and 1:1 ($C_{18}SH$:DHP), as in the cited paper.

Langmuir monolayers, following the widely used procedure, were spread from 15 μl of appropriate chloroform solution (2 mg/ml) applied carefully via a microsyringe on the aqueous subphase in a Langmuir trough (NIMA Technol., Model 611, Coventry, England) equipped with a Brewster Angle Microscope (KSV NIMA MicroBAM, fixed angle, 12 μm resolution, Finland). After evaporation of the volatile solvent (ca. 10 min., as judged by the gradual return of the biased surface pressure to its zero value, pre-set for the pristine subphase), the monolayer was continuously compressed at a rate of 30 cm^2/min, to obtain pressure-area isotherms in an isotherm cycling mode set for 2 cycles of compression–decompression. This allowed us to obtain at least 2 isotherms, and to evaluate the quality of such monolayer and reproducibility of its

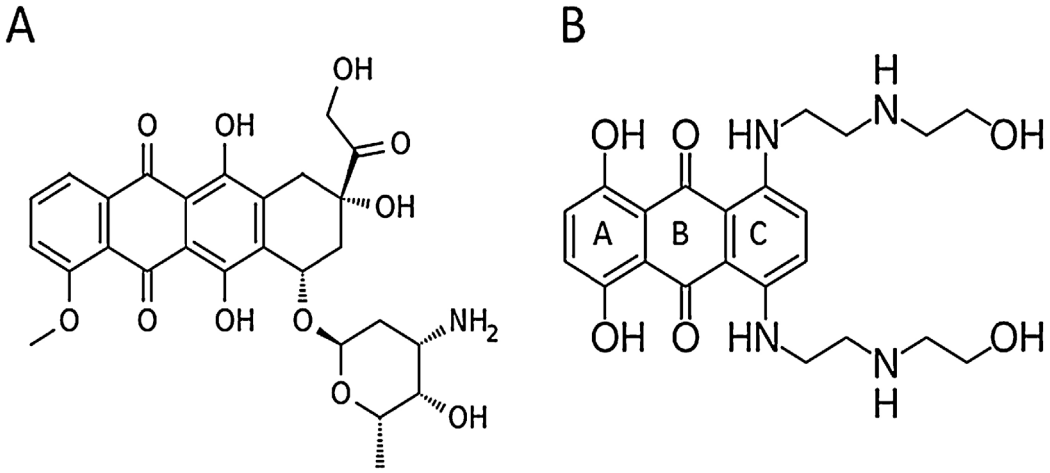

Fig. 1. Structure of doxorubicin (A) and mitoxantrone (B).