Colloids and Surfaces B: Biointerfaces 171 (2018) 377–382

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Lipids mediating the interaction of metronidazole with cell membrane models at the air-water interface

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ARTICLE INFO

Keywords:
Metronidazole
DPPE
DPPC
Air-water interface

ABSTRACT

In this paper, metronidazole, a known compound with microbicide effect, was incorporated in Langmuir monolayers of selected phospholipids in order to investigate the interactions of this compound with cell membrane models by using tensiometric, spectroscopic and morphological techniques. Surface pressure-area isotherms showed that selected amounts of metronidazole condense DPPC monolayers, but expand DPPE monolayers. Vibrational spectroscopy pointed that metronidazole adsorbs on the polar heads of the phospholipids, affecting the gauche conformations of the aliphatic chains, and different patterns were observed for DPPC and DPPE monolayers containing metronidazole in terms of Brewster angle microscopy images. As a result, distinctive interactions with DPPC and DPPE could be pointed regarding the incorporation of metronidazole with each lipid, leading to particular molecular arrangements at the air-water interface.

1. Introduction

Metronidazole (Fig. 1) belongs to the class of nitroimidazoles and is an antibacterial and antiprotozoal drug [1,2]. It can be used alone or with other antibiotics to treat pelvic inflammatory disease, endocarditis, bacterial vaginosis, dracunculiasis, giardiasis, trichomoniasis, and amebiasis [3], being effective against gram-positive and gram-negative bacteria [4]. This drug was firstly used commercially in 1960 in France, but its antibacterial activity was discovered by accident when, in 1962, it was successfully employed for the treatment of a patient with vaginal trichonomia and bacterial gingivitis [5]. Their in-depth use and research came about when Tally and collaborators demonstrated that this drug was suitable in the treatment of systematic anaerobic infections, including those caused by *Bacteroides fragilis* [6]. Its proposed mechanism of action is related to the inhibition of nucleic acid synthesis by the breakdown of DNA from microbial cells [7]. This function only occurs when metronidazole is reduced, which usually happens only in anaerobic cells [8], having relatively little effect on human cells or aerobic bacteria. The mechanism of action is then related to its redox potential and involves four steps [9–11]: 1) entry into the bacterial cell, 2) reduction of the nitro group, 3) cytotoxic effect of the reduced product, and 4) release of inactive products.

Although this fact is well known and discussed in the literature, the process associated with its uptake into bacterial cells has not yet been fully elucidated [12–14]. Absorption of metronidazole in the human body has been considered as a passive process, although some studies suggest that its absorption is also linked to the need for a facilitator (such as transmembrane proteins and protein channels) [15,16].

Current studies [17,18] bring a possible mechanism of drug activation. The activation of the nitro group occurs through the formation of a nitro radical, followed by the formation of hydroxylamine, which must interact in the intracellular environment of bacterial membranes and lead to the DNA rupture [19,20]. In view of such uncertainties, and knowing that the mechanism of action of metronidazole is focused on its interaction with respect to microbial DNA, it is fundamental to study the interaction of metronidazole with the plasma membrane at the molecular level since the action of this compound involves the passage of the drug through the membrane to reach the cell nucleus. In addition, metronidazole interactions on lipid surfaces are interesting from the point of view of the possible application of the drug in liposomes for controlled delivery systems [21].

Therefore, considering that compounds of this nature attack cell membranes of parasites, it is interesting to use simplified models of biomembranes to study their effects at the molecular level, as already reported in literature for other bioactive compounds in Langmuir lipid films formed at the air-water interface [22–24]. This paper then investigates the interactions between metronidazole and cell membrane models composed of phospholipids to evaluate possible drug-lipid interactions due to the differences in the structures of the lipids.

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https://doi.org/10.1016/j.colsurfb.2018.07.057
Received 7 June 2018; Received in revised form 16 July 2018; Accepted 24 July 2018
Available online 25 July 2018
0927-7765/

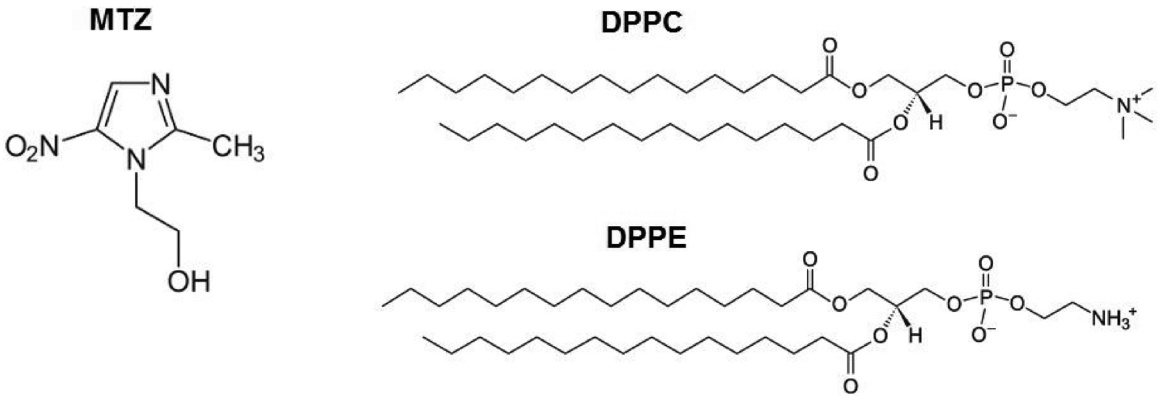

Fig. 1. Structures of DPPC, DPPE and MNZ.

2. Materials and methods

Dipalmitoylphosphatidylcholine (DPPC) and dipalmitoylphosphatidylethanolamine (DPPE) were purchased from Sigma-Aldrich and dissolved in chloroform (Synth), resulting in a final solution of 0.54 mg/mL and 0.62 mg/mL, respectively. The lipid structures can be seen in Fig. 1. Metronidazole (MNZ) was also obtained commercially from Sigma-Aldrich and dissolved in water purified by the Milli-Q® system, resulting in a concentration of 0.66 mg/mL.

The drug was incorporated in the monolayer by injecting its solution in the subsurface below the pre-formed lipid monolayer, resulting in a final concentration in the aqueous subphase of 0.1 μg/mL (5.8 μM), minimum concentration of microbicide activity reported to *C. difficile* [25]. In this paper, the killing of *C. difficile* by metronidazole was studied with the batch culture method. Total viable counts and spore counts were performed reaching the minimum concentration of metronidazole to have effect on the growth of *C. difficile*. The aqueous subphase was composed of 0.1 mol/L NaCl salt solution (Synth). Injection of the drug solution occurred few millimeters below the air-water interface to avoid long diffusive processes.

Two Langmuir troughs from KSV-Nima Instruments were used to obtain the Langmuir films and subsequent characterizations. The first was a mini-trough model (total capacity of 200 mL) and another KN 1002 (total capacity of 30 mL). For the formation of the monolayers, the trough was filled with water purified by the Milli-Q® system up to the formation of a meniscus to allow the lateral compression of the Langmuir monolayer through mobile barriers.

The phospholipid solution in chloroform was spread over the aqueous subphase in order to promote the complete spreading throughout the aqueous surface. A period of 10 min was previously waited for solvent evaporation and lateral lipid diffusion along the interface. The monolayer was then compressed at a speed of 10 mm/min, obtaining surface pressure-area pressure isotherms, with the surface tension being measured by the Wilhelmy's method. For this purpose, a plate made of filter paper of dimensions 1 × 2 cm was placed in order to intercept perpendicularly the air-water interface. This plate was hung by a sensor coupled to an electronic interface that calculated the surface pressure through the force sensed by the paper, discounting its weight.

Initially, the isotherms for the lipids were obtained without the presence of the drug, and later in its presence. To this end, the drug was inserted underneath the aqueous subphase through a syringe. After 30 min waited for homogenization, the surface pressure-area isotherms for the mixed drug-lipid monolayer were then obtained.

To obtain PM-IRRAS spectra or micrographs by Brewster angle microscopy, a sufficient amount of phospholipid was initially spread to reach the membrane pressure of 30 mN/m (achieved with subsequent monolayer compression). Subsequently, the metronidazole solution in the aqueous subphase was added. Surface images were obtained using the Brewster angle microscopy technique (KSV Instruments), and PM-RRAS spectra through the KSV PMI 550 (KSV-Nima Instruments) spectrophotometer at an angle of incidence of 75°.

All the experiments were carried out at a temperature of 25 ± 1 °C, and repeated at least three times for ensure reproducibility. Representative curves or images are shown.

3. Results and discussion

First of all, it is important to report that the air-water interface of the solution containing MNZ, in the same conditions employed in this work for the Langmuir monolayer studies, but without any lipid at the surface, was investigated in terms of surface activity as control information. No relevant increase of surface pressure was observed even compressing the interface. Also, the PM-IRRAS spectra did not detect any relevant band. Furthermore, BAM images did not show any difference in the pattern of reflectivity in relation to the interface with pure water. This indicates a low surface activity of MNZ by itself (without the presence of a lipid monolayer).

Fig. 2 shows surface pressure-area isotherms for DPPC on 0.1 mol/L NaCl aqueous subphase. The curve for the pure DPPC film is typical, and its profile does not diverge so much from those obtained for pure water [26], initiating its gas phase in molecular areas as high as 95 $Å^2$, where it reaches the liquid-expanded (LE) phase, lasting up to 80 $Å^2$, where the transition from liquid-expanded to liquid-condensed (LC) phase occurs around a surface pressure of 8–12 mN/m. The LE phase is reached at around 62 $Å^2$, denoted by an abrupt increase of surface pressure upon compression up to collapse – phenomenon attributed to the passage of molecules from two-dimensional arrangements to multilayer aggregates [27,28].

With the addition of MNZ, it is observed a shift of the curve to smaller areas, which should be attributed to the decrease of the lateral repulsions between the DPPC molecules, condensing the monolayer. It is likely therefore that the molecular interactions between MNZ and DPPC are due especially to the polar head of the lipid since a possible penetration of the drug would lead to an expansion of the monolayer. Literature shows that concentrations 10 times higher cause a discrete expansion of the monolayer [29], being therefore improbable a possible desorption of DPPC from interface caused by MNZ. Our results show therefore that smaller amounts of MNZ are not able yet to expand the monolayer. Also, it is worth mentioning the high hydrophilicity (log-$P_{octanol/water}$ 0.09) of the drug [29], which implies in its low ability to penetrate into the aliphatic tails of the phospholipid. Therefore, considering that the changes in the isotherms were only obtained when a high ionic strength of the aqueous subphase (0.1 mol/L NaCl) was used, the surface activity of MNZ was induced by the "salting out" effect. This means that the excess of ions in the aqueous subphase induced the water molecules to solvate preferentially these ions, promoting the adsorption of MNZ at the interface.

Fig. 2B shows the surface elasticity of the monolayer as a function of