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The effect of furazolidone on the physico-chemical properties of dimyristoylphosphatidylcholine bilayers: Relevance to anti-leishmanial therapy

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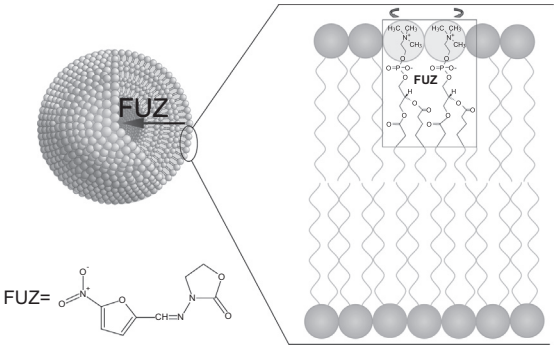
HIGHLIGHTS

- Furazolidone changes the dynamic of specific dimyristoylphosphatidylcholine regions.
- Furazolidone disorders lipid polar/interfacial groups, as shown by FTIR and NMR.
- Furazolidone disorders the first methylene groups of lipid acyl chains.
- Our findings may explain liposome-loaded furazolidone activity against *Leishmania*.
- Findings may improve strategies to use liposomal furazolidone against *Leishmania*.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, the influence of furazolidone, an anti-leishmanial drug, on dimyristoylphosphatidylcholine (DMPC) liposome hydration degree, mobility and thermodynamics was investigated by Fourier transform infrared spectroscopy (FTIR), nuclear magnetic resonance (NMR) and differential scanning calorimetry (DSC). FTIR results showed that furazolidone was responsible for an increase in the hydrogen bound number and mobility of the lipid phosphate group. Furazolidone also affected the lipid choline group by increasing its motional freedom, as shown by FTIR and ^{1}H NMR spin–lattice relaxation time measurements. At the DMPC interfacial region, FTIR results showed a drug-induced reduction of the carbonyl hydration and order degrees. Very weak interaction among furazolidone and the hydrophobic lipid chains was also observed. However, no furazolidone-induced changes on thermodynamical parameters, such as phase transition temperature (Tm) and enthalpy variation (ΔH), were detected by the DSC technique. Thus, furazolidone seems to interact preferentially with lipid polar and interfacial regions, enhancing the freedom for gauche-trans isomerization of the first methylene groups of DMPC acyl chains. Responses described in this paper may explain the improved activity of furazolidone-encapsulated liposomes by comparison with the effect of the free drug, described in literature. The findings can also improve further

Abbreviations: DMPC, dimyristoylphosphatidylcholine; DSC, differential scanning calorimetry; ΔH, enthalpy variation; FTIR, Fourier transform infrared spectroscopy; FUZ, furazolidone; HATR-FTIR, horizontal attenuated total reflectance-Fourier transform infrared spectroscopy; HIV, human immunodeficiency virus; MLVs, multilamellar large vesicles; NMR, nuclear magnetic resonance spectroscopy; T_1, spin–lattice relaxation time; Tm, phase transition temperature; TSP, sodium 3-(trimethylsilyl)-[2,2,3,3-2H$_4$]-1-propionate.

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strategies for the potential therapeutic application of liposomal furazolidone as a drug delivery system and minimize the risk of drug resistance and collateral effects related to high toxicity.

1. Introduction

Leishmaniasis is a neglected parasitic disease that afflicts 12 million people worldwide and threatens 350 million in endemic countries in America, Africa, the Middle East and Asia. Widespread misuse of drugs and the human immunodeficiency virus (HIV)/leishmaniasis coinfection are related to resistance of the parasite *Leishmania* to conventional drug therapy [1,2]. New pharmacological strategies need to be developed not only to reduce the cases of resistance and toxicity, but also to enhance the efficacy of the anti-leishmanial treatment. In this context, studies of drugs that are being clinically used against other diseases have been conducted. Furazolidone (FUZ, Fig. 1), a nitrofuran derivative with antibacterial and antiprotozoal properties, had its anti-leishmanial activity reported in the 80's. FUZ also seems to have limited toxicity by comparison with other anti-leishmanial drugs [2–4].

Incorporation of anti-leishmanial drugs into lipid bilayers, such as liposomes, has improved their activity against the *Leishmania* parasite [5]. Phosphatidylcholine-based liposomes containing FUZ have demonstrated to be effective inhibitors of the *L.* (*L.*) *chagasi* parasite, which is responsible for visceral leishmaniasis [2,6–9]. Phosphatidylcholines are frequently used as lipid matrix of liposomes for drug delivery [10].

Knowledge concerning the effect of a drug on the physicochemical properties of membranes, such as liposomes, contributes significantly to improve parameters of pharmacological systems to be developed, such as drug targeting and membrane stability [10,11]. Besides, understanding the changes induced by a drug on membrane dynamics may provide a broader view of its biological activity and mechanism of action, which may be useful to the development of new strategies for disease therapy [12].

To the best of our knowledge, no studies have been conducted to describe the effects of FUZ on the dynamics of phospholipids-based liposomes. Thus, this study reports the influence of FUZ on the hydration degree, mobility and thermodynamics of dimyristoylphosphatidylcholine (DMPC) multilamellar large vesicles (MLVs), investigated by Fourier transform infrared spectroscopy (FTIR), nuclear magnetic resonance (NMR) and differential scanning calorimetry (DSC). The use of DMPC reduces the complexity which can be caused by the presence of lipid associations with different head groups or unsaturation degree. For example, unsaturated lipids have transition temperature below 0 °C, which makes the interpretation of DSC results more difficult. MLVs have been employed to determine details of bilayer structure. For example, the regular array of MLV bilayers are suited for X-ray studies and their large size (around 2000 nm) enables to obtain details concerning its structural and motional features by NMR experiments [13,14]. Results described in this paper are relevant to provide knowledge which can minimize resistance risks and collateral effects of anti-leishmanial systems, being useful to the development of new strategies against leishmaniasis.

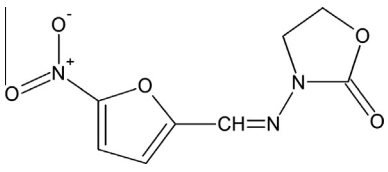

Fig. 1. Structure of furazolidone.

2. Materials and methods

2.1. Chemicals

DMPC was purchased from Avanti Polar Lipids (Alabaster, Alabama, USA). Furazolidone, salts, tricine and deuterated water (D_2O)/sodium 3-(trimethylsilyl)-[2,2,3,3-2H4]-1-propionate (TSP, 0.05%) were bought from Sigma–Aldrich (St. Louis, USA). DMPC was used without further purification and all the other chemicals were of analytical grade.

2.2. Preparation of liposomes

MLVs containing DMPC 221 μM were produced by lipid co-solubilization in chloroform followed by solvent evaporation under vacuum. The dried films were then resuspended at room temperature from the walls of a round bottom flask by vigorous vortexing in tricine10 mM/$MgCl_2$ 2.5 mM buffer, pH 7.4 and submitted to three freeze–thaw cycles [13]. In order to prepare liposomes containing FUZ, increasing initial drug concentrations, from 0 to 330 μM (which corresponds to 0–59.8 mol% of FUZ in the FUZ/DMPC mixture), were added during the solvent co-solubilization process [2].

2.3. Determination of the saturation concentration of FUZ in DMPC liposomes

To determine the highest FUZ concentration which can be loaded into the liposomes by the chosen preparation method, DMPC-based vesicles – either pure or containing different molar ratios of FUZ:DMPC, related to a drug concentration range from 0 to 330 μM – were washed with tricine/$MgCl_2$ buffer (pH 7.4). A washing procedure was performed to remove free FUZ from the liposomal suspensions, based on the sample centrifugation and replacement of the reminiscent buffer solution for a new buffer aliquot. After the second centrifugation cycle, the liposomal pellets were treated with Triton X-100 (0.6%, v/v) to dissolve the bilayers. The efflux of FUZ from the liposomes was quantified by a UV-2550 Shimadzu spectrophotometer (Kyoto, Honshu, JP), at 367 nm [15,16]. A control assay without Triton X-100 was also performed to detect any influence of free lipids or micelles on the solution. For each FUZ:DMPC molar ratio, at least five independent samples were prepared and analyzed. Results are presented as mean ± S.D.

2.4. FTIR experiments

FTIR essays were performed in the horizontal attenuated total reflectance mode (HATR-FTIR), by a Shimadzu IR Prestige-21 spectrometer (Kyoto, JP), with resolution of 2 cm^{-1} at 20 °C. DMPC (221 μM) liposomes, pure or loaded with concentrations of FUZ (from 0 to 330 μM, represented as initial FUZ:DMPC molar ratio), were deposited on ZnSe crystal support and immersed in the tricine/$MgCl_2$ buffer (pH 7.4). HATR-FTIR interferograms were averaged for 50 scans and collected in the frequency range from 400 to 4000 cm^{-1}. The spectra were analyzed by the Shimatzu IR solution software (version 1.5). Frequency variations were analyzed from the FUZ-induced shifts of vibrational bands related to specific DMPC regions: the phosphate antisymmetric stretching vibration (ν_{as} PO_2^-) in the range of frequency from 1260 to 1220 cm^{-1}; the