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Isoniazid interaction with phosphatidylcholine-based membranes

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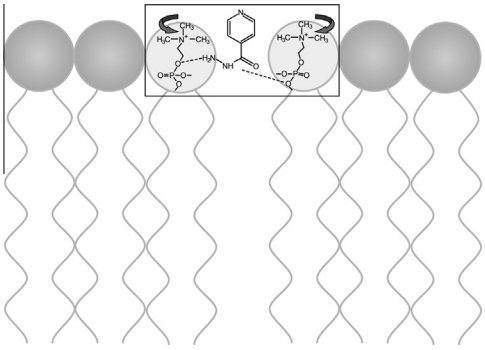
HIGHLIGHTS

- Isoniazid (INH) effects on specific regions of phosphatidylcholine (PC) membranes were studied.
- INH affinity constant to a phosphatidylcholine (PC) bilayer was detected by PWR.
- INH-induced changes in PC liposomes dynamics were determined by FTIR, NMR and DSC.
- INH interacts with the lipid head groups and enhances its motional freedom.
- At 170 μM, INH increases the mobility of first lipid acyl chain methylenes.

GRAPHICAL ABSTRACT



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ABSTRACT

Interaction between the anti-tuberculosis drug isoniazid (INH) and phosphatidylcholine membranes was investigated in terms of: (i) drug affinity to a lipid bilayer and (ii) drug-induced changes in the dynamic properties of liposomes, such as membrane hydration state, polar head and non-polar acyl chain order and lipid phase transition behavior. These parameters were studied by plasmon waveguide resonance spectroscopy (PWR), UV–visible, horizontal attenuated total reflectance–Fourier transform infrared (HATR–FTIR), nuclear magnetic resonance (NMR) and differential scanning calorimetry (DSC) techniques. PWR measurements showed an INH membrane dissociation constant value of 0.031 μM to phosphatidylcholine bilayers. INH induced higher membrane perturbation in the plane which is perpendicular to the membrane plane. The INH saturation concentration in phosphatidylcholine liposomes was 170 μM. At this concentration, HATR–FTIR and NMR findings showed that INH may interact with the lipid polar head, increasing the number of hydrogen bonds in the phosphate region and enhancing the choline motional freedom. DSC measurements showed that, at 115 μM, INH was responsible for a decrease in lipid phase transition temperature of approximately 2 °C and had no influence in the lipid enthalpy variation (ΔH). However, at 170 μM, INH induced the reduction of the ΔH by approximately 52%, suggesting that the drug may increase the distance among lipid molecules and enhance the freedom of the lipid acyl chains

Abbreviations: DMPC, dimyristoylphosphatidylcholine; DSC, differential scanning calorimetry; egg PC, egg yolk phosphatidylcholine; ΔH, enthalpy variation; FTIR, Fourier transform infrared spectroscopy; HATR–FTIR, horizontal attenuated total reflectance–Fourier transform infrared spectroscopy; INH, isoniazid; Kd, dissociation constant; MIC, minimum inhibitory concentration; MLV, multilamellar large vesicles; NMR, nuclear magnetic resonance; PWR, plasmon waveguide resonance spectroscopy; TB, tuberculosis; T_1, spin lattice relaxation time; Tm, phase transition temperature; TSP, sodium 3-(trimethylsilyl)-[2,2,3,3-2H4]-1-propionate.

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methylene groups. This paper provides information on the effects of INH on membrane dynamics which is important to understand liposome targeting of the drug and for the development of anti-TB pharmacologic systems that not only are less susceptible to resistance but also have low toxicity.

1. Introduction

Mycobacterium tuberculosis resistant to isoniazid (pyridine-4-carbohydrazide, INH, Fig. 1) is related to a rise in tuberculosis (TB) prevalence, in treatment failure and in the increase of the costs of therapy. Albeit its simple structure, the mode of action and the resistance basis of INH are complex and pleotropic. An important INH mechanism of action is related to the synthesis of mycolic acids whereas the clinical resistance is mainly associated with mutations in *katG* and *inhA* genes [1,2]. Besides problems concerning resistance, INH also has toxic side effects, such as severe hepatotoxicity, gastrointestinal disorders, optic and peripheral neuritis and even convulsions [3,4].

In order to reduce drug resistance and toxicity, studies of the activity of drug-loaded liposomes against *M. tuberculosis* have been carried out [5,6]. Liposomes are considered biocompatible carriers for drug delivery. Some of the major advantages of liposome formulations as carriers of antimicrobial drugs are that, *in vivo*, these systems protect the drug from enzymatic and immunological attacks which cause premature degradation and decrease toxicity [7]. Liposomes composed of phosphatidylcholine (Fig. 2) have been tested to improve the stability of different drug delivery systems [8,9]. Liposomal formulations composed of INH and an association with phosphatidylcholine, cholesterol and O-stearylamylopectin were tested in *M. tuberculosis* infected mice. This formulation reduced the mycobacterial load in murine lungs, liver and spleen significantly [10]. Liposomes containing egg phosphatidylcholine, cholesterol, dicetylphosphate, O-stearylamylopectin, monosialogangliosides, and distearylphosphatidyl-1-ethanolamine-polyethylene glycol 2000 were also tested to load INH and rifampicin and the *in vivo* anti-TB efficiency of this system was discussed in terms of the effect of each liposome constituent in the stability of the formulation [11].

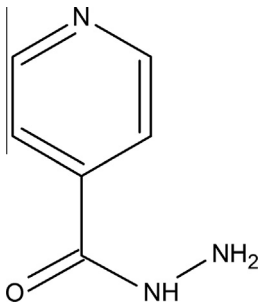

Fig. 1. Structure of isoniazid (pyridine-4-carbohydrazide).

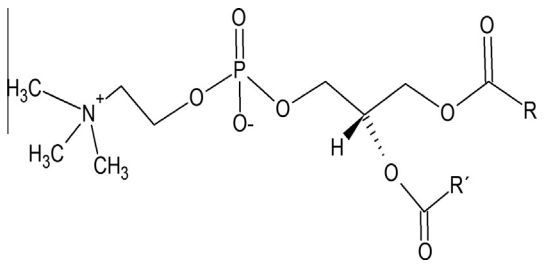

Fig. 2. Structure of phosphatidylcholine. For DMPC, R = R′ = myristic acid (C 14:0); for egg phosphatidylcholine: R and R′ = a mixture of oleic (C 18:1, 32%), linoleic (C 18:2, 18%), palmitoleic (C 16:1, 2%) and arachidonic (C 20:4, 3%) acid; for palmitoyl-oleoyl phosphatidylcholine (POPC), R = palmitic acid (C 16:0) and R′ = oleic acid (C 18:1).

In order to improve the targeting of drugs by liposomes, as well as the stability of the membrane, considerable understanding of the physicochemical characteristics of the system is required [12,13]. It includes the basic analysis of the drug effect on the membrane dynamics. Several works reported the INH preferential location in a lipid bilayer, INH-incorporated liposomes loading-capacity, encapsulation percentage or release properties as well as the membrane insertion efficiency of the drug in monolayers [2,14–16]. However, there are few works that bring information about the influence of INH on membrane dynamic parameters. Steady-state fluorescence intensity as well fluorescence energy transfer studies were performed in dimyristoylphosphatidylcholine (DMPC) and dimyristoyl-L-alpha-phosphatidylglycerol liposomes loaded with INH and no changes in bilayer dynamics induced by the drug were detected [14]. However, in dipalmitoylphosphatidylcholine monolayers, it was reported that higher concentrations of INH caused a membrane destabilization effect [16].

In this study, the interaction between INH and phosphatidylcholine-based bilayers was investigated in terms of drug affinity and effects on membrane dynamic properties, by plasmon waveguide resonance spectroscopy (PWR), UV–visible, horizontal attenuated total reflectance–Fourier transform infrared (HATR–FTIR), nuclear magnetic resonance (NMR) and differential scanning calorimetry (DSC) techniques. PWR was used to determine the dissociation constant (Kd) of INH to egg phosphatidylcholine (egg PC) bilayers. HATR–FTIR, NMR and DSC were used to investigate the effects of INH on the (i) hydration state of the phosphate and carbonyl regions; (ii) membrane polar choline group and hydrophobic acyl chain order; and (iii) lipid phase transition behavior of liposomes composed of DMPC. All responses concerning the interaction between INH and phosphatidylcholine-based membranes were compared with control studies by using the membranes in the absence of the drug.

2. Materials and methods

2.1. Chemicals

Egg yolk phosphatidylcholine (egg PC) and 1,2-dimyristoylphosphatidylcholine (DMPC) were purchased from Avanti Polar Lipids (Alabaster, AL, USA). Isoniazid (INH), salts, tricine and deuterated water/sodium 3-(trimethylsilyl)-[2,2,3,3-2H4]-1-propionate (TSP, 0.05%) were bought from Sigma–Aldrich (St. Louis, MO, USA). Lipids were used without further purification and all other chemicals were of analytical grade.

2.2. Preparation of egg PC bilayers

The self-assembled egg PC bilayers were formed by a solution of butanol/squalene (0.95/0.5 v/v) with 10 mg/mL lipid. The method which was applied to make the lipid bilayers is based on Mueller and Rudin's procedures to prepare black lipid membranes across a small hole in a Teflon block [17].