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Colloids and Surfaces B: Biointerfaces 62 (2008) 258–264
COLLOIDS AND SURFACES B
www.elsevier.com/locate/colsurfb

Evaluation of antitubercular drug insertion into preformed dipalmitoylphosphatidylcholine monolayers

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Received 23 May 2007; received in revised form 22 October 2007; accepted 23 October 2007
Available online 4 November 2007

Abstract

Insertion profiles of antitubercular drugs isoniazid (INH), rifampicin (RFM) and ethambutol (ETH) into dipalmitoylphosphatidylcholine (DPPC) membrane models were evaluated by Langmuir monolayer technique. Maximum drug insertion into DPPC monolayer was observed with rifampicin with a surface pressure increase ($\Delta\pi_{max}$) in the range of 21–33 mN/m depending upon rifampicin concentration. Isoniazid had minimal insertion resulting in a lower $\Delta\pi_{max}$ of about 2–3 mN/m, suggestive of minimal interactions between INH and DPPC. Ethambutol surface pressure increment on insertion resulted in an intermediate rise in the $\Delta\pi_{max}$ (6–10 mN/m). Antitubercular drug combination in the ratio of 2 mM:0.7 mM:4.5 mM for INH:RFM:ETH, attained $\Delta\pi_{max}$ between 25 and 33 mN/m. Insertion profiles similar to rifampicin were exhibited by the antitubercular drug mixture suggestive of predominant rifampicin insertion into the DPPC monolayer. The extent of drug insertion into the DPPC monolayer is suggestive of the drug penetration potential into biological membranes *in vivo*. Higher RFM $\Delta\pi_{max}$ is suggestive of excellent cell membrane penetration, which explains broad reach of the drug to all the organs including the cerebrospinal fluid while lower $\Delta\pi_{max}$ of INH suggests poor membrane penetration restricting the entry of the drug in different biological membranes. DPPC membrane destabilization was observed at higher antitubercular drug concentrations indicated by the negative slopes of the surface pressure–time curves. This may correlate with the dose related toxic effects observed in tuberculosis affected patients. Drug insertion studies offer a potential tool in understanding the pharmacotoxicological behavior of the various pharmacological agents.

Keywords: Langmuir monolayers; Antitubercular drugs; Biomembranes; Drug insertion; Dipalmitoylphosphatidylcholine

1. Introduction

Tuberculosis chemotherapy is clinically associated with poor drug bioavailability and dose related adverse effects. Antibiotic-cell membrane interactions play a crucial role in understanding bioavailability of drugs, their entry into the cellular compartments and drug induced toxicity [1]. Pharmacological action of drugs to a large extent depends upon complex drug-phospholipid interactions [2]. Phospholipids are an integral part of the biological membranes. Biophysical interactions exist between different drug molecules and phospholipids. Drug insertion into preformed monolayers may have implications in drug penetration and entry into cells via biological membranes [3–5]. Langmuir monolayers offer an useful technique to understand the mechanism of action between the pharmacological agents and the biological membranes. Drugs are known to interact with the lipid monolayers by modifying the packing arrangement of the membrane phospholipids hence changing their lateral organization [6–8]. Isoniazid, rifampicin and ethambutol are the front line antitubercular drugs used currently in the tuberculosis chemotherapy regimens. Interactions of these antitubercular drugs with cell membranes may be evaluated using Langmuir monolayers. Such a study could aid in understanding the poor bioavailability and toxicity associated with administration of antitubercular drugs.

Ability of drugs to insert into phospholipid monolayers gives us an insight into molecular mechanisms responsible for interactions between drugs and host cell membranes. Isoniazid has intermediate intracellular penetration while rifampicin and ethambutol are avidly concentrated in the intracellular compartments [9]. Rifampicin is a hydrophobic molecule (aqueous solubility of rifampicin is 1 g/764 ml of water) [10] while isoniazid and ethambutol are hydrophilic (Fig. 1) (aqueous solubility of isoniazid and ethambutol is 1/8 g of water and 1/4 g of

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doi:10.1016/j.colsurfb.2007.10.010

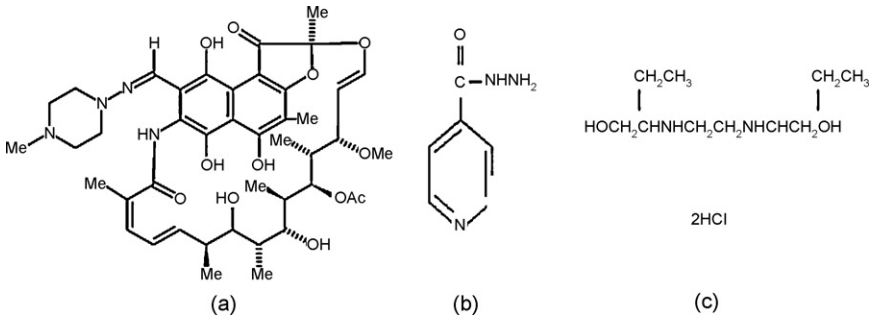

Fig. 1. Molecular structure of antitubercular drugs. (a) Rifampicin (b) isoniazid (c) ethambutol.

water, respectively) [10]. Differences in the chemistry of the drugs could be responsible for differential drug-membrane interactions. Evaluation of drug insertion profiles of the different antitubercular drugs at clinically relevant concentrations may explain the differential concentrating ability of antitubercular drugs in host cells. Insertion of antitubercular drugs at clinically administered concentrations into DPPC monolayers, modeled as model biomembranes, has been evaluated in the current study. Effect of antitubercular drugs were studied individually as well as in combinations as given to the tuberculosis affected patient.

2. Experimental setup

2.1. Materials

Synthetic 1,2 dipalmitoylphosphatidylcholine (>99% purity) was purchased from Sigma Chemical Co. (St. Louis, USA) and used without further purification. Indian Pharmacopoeia grade antitubercular drugs isoniazid (INH), rifampicin (RFM), and ethambutol (ETH) were obtained as kind gifts from LUPIN Laboratories, India. Rifampicin was obtained as ≥97% pure powder, isoniazid as ≥99% pure powder and ethambutol ≥97% pure powder. HPLC grade chloroform used for preparation of stock solutions was purchased from E. Merck Ltd. Mumbai. Extra pure analytical grade methanol and acetone used for cleaning the Langmuir-Blodgett trough and sodium chloride (99.9% pure), analytical grade calcium chloride used for preparing the subphase were obtained from Sisco Research Laboratories, Mumbai, India. Water used in all experiments was doubly distilled, deionized using the MilliQ UV plus system (Millipore Corp., USA) and had a resistivity of 18.2 mΩ cm.

2.2. Methods

2.2.1. Monolayer studies

Surface activity studies were performed using an automated Langmuir mini trough (KSV instrument Ltd. Finland) equipped with a Wilhelmy balance (323 mm × 75 mm). The trough is mounted on a thermo regulated base plate and the temperature is maintained at $37 \pm 0.5\,^{o}C$ by an external circulating water bath. The surface pressure (π) was measured continuously with a gold Wilhelmy plate connected to a microelectronic feedback system for surface pressure measurement. The Wilhelmy plate was roughened by a sand paper each time before the start of the experiments so as to achieve complete wetting of the plate. The trough was triply cleaned with deionized water, methanol and acetone followed again by deionized water.

The surface of the subphase was cleaned by gently aspirating the minute particulate matter. Cleanliness was confirmed by compressing the bare monolayer (in absence of lipids) and achieving a zero surface pressure reading. The subphase consisted of 0.9% sodium chloride and 2 mM calcium chloride. The pH was maintained at 7.4 throughout the experiments. The DPPC stock solution was prepared in HPLC grade chloroform. DPPC monolayers were obtained by spreading DPPC from a chloroform: methanol (2:1) solution at a surface concentration corresponding to 110 $Å^2$ area for each DPPC molecule. The monolayer was compressed after 30 min to allow solvent evaporation.

DPPC is a cell membrane lipid and used as a model biomembrane [11]. Further DPPC is one of the major lipids of the lung surfactant system [12]. At 37 °C, phase transition of DPPC takes place at ∼38 mN/m and hence at this temperature DPPC exists in a liquid expanded state, similar to that of unsaturated phospholipids. Since the aim of the paper was to study the differential interactions of the antitubercular drugs with the DPPC monolayer, the monolayer was maintained at a low initial surface pressure of 10 mN/m, in the liquid expanded phase at a constant area. Further, inhalation therapy using DPPC for pulmonary drug delivery of antitubercular drugs is also currently being investigated. Upon inhalation, the antitubercular drugs have to insert through the surfactant monolayer before reaching alveolar macrophages [13]. DPPC was thus chosen as it mimicked the liquid expanded phase of the unsaturated phospholipids which are commonly used as model membranes and forms a constituent of lung surfactant and liposomal drug carriers. Lateral pressure in biological membranes is around 20–30 mN/m [14]. Drug insertion is observed to be inversely proportional to the membrane surface pressures [15–17]. Drug interactions with lipid monolayers are strongly dependent upon molecular packing of lipids and are predominantly expressed in the liquid expanded phase of monolayers. In the liquid expanded phase phospholipid acyl chains have considerable degree of freedom and lower monolayer lipid density which facilitates interactions and insertion of the drugs [18]. Appropriate amounts of methanolic stock solution of each of the three drugs either individually or as combination were injected into the subphase outside the barriers to evaluate their insertion into a preformed DPPC monolayer.