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Evaluation of the effect of rifampicin on the biophysical properties of membranes: Significance for therapeutic and side effects

Marina Pinheiro *, Sílvia Pisco, Ana Sofia Silva, Cláudia Nunes, Salette Reis

REQUIMTE, Departamento de Ciências Químicas, Faculdade de Farmácia, Universidade do Porto, Portugal

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ABSTRACT

This work aims to study the biophysical interactions of rifampicin (RIF) with three-dimensional macrophage membrane models, under environments with physiological and pathological relevance in tuberculosis (TB). The interaction of RIF with liposomes formed by 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) in different pH values (i.e., 5.0, 6.2 and 7.4) was investigated by several biophysical techniques. The RIF's membrane concentration was quantified by partition coefficient (K_p) using derivative spectrophotometry. To predict the drug's location across the membrane, fluorescence quenching studies were performed using liposomes labeled with two different fluorescence probes. The effect of the drug on the biophysical parameters of the membrane was carried out by dynamic light scattering (DLS), and small-angle X-ray scattering (SAXS). The overall results confirm that the interactions of RIF with membranes are pH-dependent, being much more pronounced at the acidic pH. A correlation between the effect of RIF on the biophysical properties of the membranes and the pH was found, which may be useful in the development of novel analogs with higher efficacy and fewer side effects, and also to understand the higher selectivity of RIF to the membranes of the infected cells, as well as some of its side effects.

1. Introduction

Tuberculosis (TB) is an infectious disease and among the communicable diseases is the second leading cause of illness and death worldwide after HIV/AIDS (human immunodeficiency virus/acquired immunodeficiency syndrome) (WHO, 2011; Zumla et al., 2013). It is estimated that one-third of the world's population is infected with the etiological agent of TB (i.e., *Mycobacterium tuberculosis* (MTb) (Abubakar et al., 2013). Generally, the contamination is spread through the air when sick people with pulmonary TB expel MTb, for instance by coughing and sneezing (Dube et al., 2012). Once in the lung, one of the first interactions between MTb and the host is with the innate immune system, more specifically the resident alveolar macrophages, responsible for the phagocytosis mediated by various host receptors. Most immunocompetent individuals either eliminate MTb or contain it in a latent state (Dube et al., 2012). Following appropriate stimuli, alveolar macrophages activate, and respond effectively by transferring the phagocytized MTb to the destructive environment of lysosomes (Melo and Dvorak, 2012; Kaufmann, 2001). To persist in the host, MTb partially inhibits the activation of infected macrophages by interferon (IFN)-γ, residing in an environment that is only slightly acidic, with a pH of ~6.2 (Kaufmann, 2001). As a result, MTb persists in the lung in a latent state within the granulomas, which are structured clusters containing different types of immune cells (Pieters, 2008). Nevertheless, the inactivation of the macrophages and the arrest of phagosomal maturation are not all-or-nothing events. Some macrophages can become activated and phagosomes can proceed and develop to more mature stages of the phagolysosomes, acidifying to a pH of approximately 5.0, being a proportion of the bacteria resistant to the acidic pH inside the phagolysosomes (Vandal et al., 2009). Herein, three diferent pH-values were chosen to mimic the pH with physiological relevance (i.e., 7.4), and the acidic pH-values inside the macrophages encountered by the etiological agent of TB in a latent state (i.e., 6.2) and in the active state (i.e., 5.0).

Rifampicin (RIF) (Fig. 1) is a first-line anti-TB drug used in the treatment of TB. This anti-TB drug acts via the inhibition of deoxyribonucleic (DNA)-dependent ribonucleic (RNA) polymerase, leading to a suppression of RNA synthesis, protein synthesis and consequently bacterial cell death (Villemagne et al., 2012). RIF is usually administered by oral route, presenting high bioavailability and being well distributed through the body, diffusing freely into tissues, and bacteria cells (Campbell et al., 2001). Common side effects include fever, gastrointestinal disturbances, rashes, discoloration of the skin and body fluids and immunological reactions

* Corresponding author at: REQUIMTE, Departamento de Ciências Químicas, Faculdade de Farmácia, Universidade do Porto Rua de Jorge Viterbo Ferreira no. 228 4050-313 PORTO Portugal. Tel: +351 220428672; fax: +351 226093483.
E-mail address: marinabppinheiro@gmail.com (M. Pinheiro).

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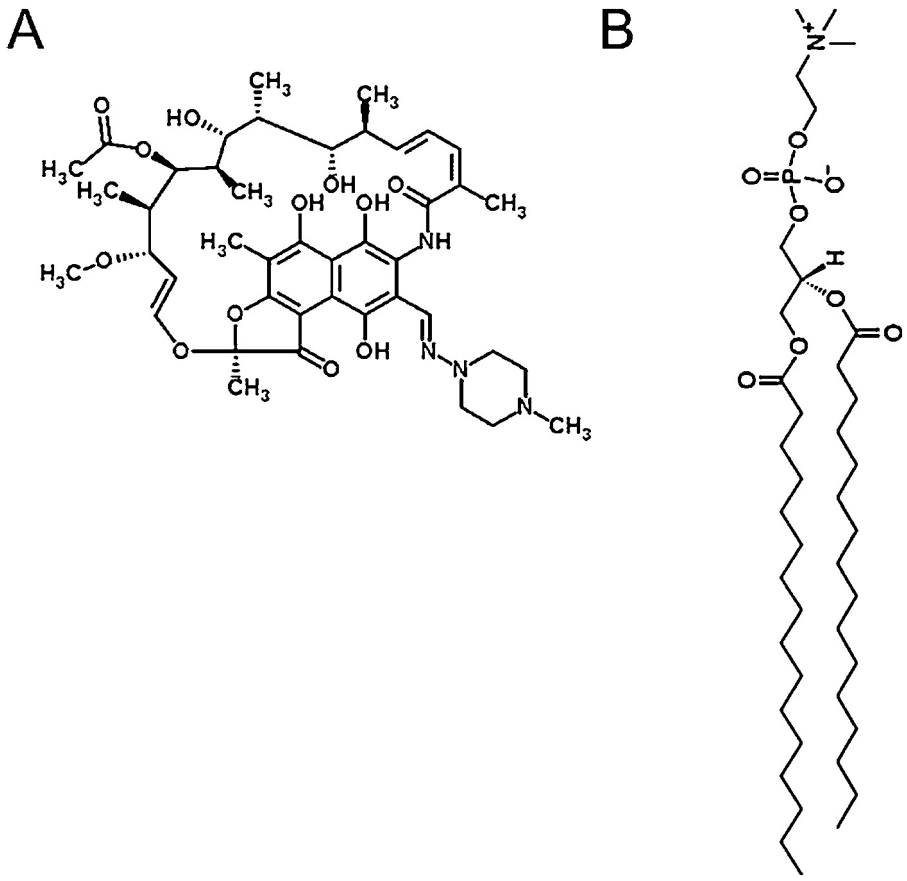

Fig. 1. Chemical structure of Rifampicin (RIF) and 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC).

(Harn and Hui, 2011). Hepatotoxicity although rare, is the most serious adverse effect related with RIF (Harn and Hui, 2011).

In this study, liposomes were used as membrane models of macrophage membranes. Liposomes possess an ordered molecular arrangement and can account for the electrostatic forces, making them excellent models to predict the interaction of drugs with the biological membranes (Mouritsen, 2011). The liposomes were formed by a zwitterionic phospholipid, namely 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) (Fig. 1), since phosphatidylcholines make up about one-third of the total of phospholipids present in the body (Mohapatra and Mishra, 2010), being also in high amounts in the macrophage membranes (Nishiyama-Naruke and Curi, 2000).

Previous works reported the pronounced interactions of RIF with membrane models at the physiological pH, being the drug deeply inserted within the lipid bilayer (Rodrigues et al., 2003; Rodrigues et al., 2001). To the best of our knowledge, this is the first report of anti-TB drug-membrane interaction studies, using several values of pH with relevance in the TB disease. In fact, it is well-known that the MTb resides inside the macrophages, surrounded by an acidic-pH environment and therefore the systematic interaction with biological membranes surrounded by different pHs occurs after the intake of RIF (Melo and Dvorak, 2012). Moreover, the most common side-effects seem to be related with the drug's interaction with the biological membranes since this drug at the therapeutic concentrations has a low affinity binding to the eukaryotic RNA polymerases, and hence does not interfere with the RNA synthesis in human cells (Aristoff et al., 2010).

The aim of this work is to study the interactions of RIF, with 3D membrane models at different pH values (i.e., 5.0, 6.2 and 7.4), aiming to assess the membrane partition of the drug, understand how the drug penetrates into the membrane, what are the membrane biophysical consequences of the drug, and its preferential location within the lipid bilayer. The RIF liposome/water partition coefficient (K_p) was determined by UV/Vis derivative spectrophotometry, and the RIF location within the lipid bilayer was studied by fluorescence quenching studies. For the RIF location studies, two probes that report different locations in the lipid bilayer were used. The influence of the RIF on the biophysical parameters of the membrane, such as cooperativity and the main phase transition temperature (T_m) was assessed by dynamic light scattering (DLS) and small-angle X-ray scattering (SAXS).

2. Material and methods

2.1. Reagents

RIF was obtained from Sigma–Aldrich Co. (St. Louis, MO, USA). 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) was purchased from Avanti Polar Lipids (Alabaster, AL, USA). The probes 1,6-diphenyl-1,3,5-hexatriene (DPH) and 1-(4-trimethylammonium)-6-phenyl-1,3,5-hexatriene (TMA-DPH) were obtained from Molecular Probes (Invitrogen, Paisley, UK). All other chemicals were purchased from Merck. Drug solutions were prepared with phosphate buffer at pH values of 5.0, 6.2, or 7.4. The phosphate buffer was prepared with double-deionized water (conductivity less than 0.1 $\mu S\,cm^{-1}$) from a Millipore system, and the ionic strength ($I = 0.1$ M) was adjusted with NaCl.