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NMR and ESR study of amphotericin B interactions with various binary phosphatidylcholine/phosphatidylglycerol membranes

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ABSTRACT

Several biologically relevant phospholipids are considered as potential excipients for IV administration liposome's formulation of AMB (Biopharmaceututics Classification System Class IV). On the basis of in vivo bioavaibility studies, DMPC and DMPG were ranked as the first potent encapsulation enhancers for this model drug, especially if one expects to target DMPG rich systems as pulmonary surfactant. Subsequently, dispersions (multilayers) of DMPC, DMPG or in binary systems with various molar ratios were prepared with or without AMB (molar ratios AMB/lipid) and further investigated using the ^{1}H-,^{31}P-NMR methods. It was found that equimolar preparations of DMPG/DMPG exhibited both a good encapsulation of AMB, while also probably able to target pulmonary surfactant. Besides DMPG did not exhibit the same solubilization properties. Conversely, no targeting by DMPC dispersion alone was expected, even if a good solubilization was obtained.

1. Introduction

Polyene macrolide amphotericin B (AMB, Fig. 1) is a well-known antifungal agent. Due to a very low rate of resistance (especially against systemic candidiasis) AMB has been extensively clinically used since the early 60's (Tilley, 1962). Despite more than half a century of intensive study, the mechanisms of action and toxicity of AMB are still not completely understood (Barwicz et al., 1992). The selective affinity of AMB for membrane sterols have been early identified (Baginski et al., 2002; Khutorsky, 1992): with cholesterol of eukaryotic cells, leading to haemolytic activity/toxicity; with ergosterol, the fungal sterol, by forming the ionophoric structures involved in the antifungal activity. However, even the most basic components of the leading model remain unclear, including the structure/function relationships that underlie channel self-assembly (Baginski et al., 1997; Ernst et al., 1981) the importance of AMB/phospholipid interactions. The role of membrane sterols in forming ion channels and even whether the channel formation is causatively linked to antifungal activity (Andreoli, 1974; Brajtburg et al., 1990; Baginski et al., 1997, 2002; Umegawa et al., 2007). AMB is very hydrophobic (LogP = −2,3 (DrugBank, 2014)), almost insoluble in aqueous solution and thus requiring a vehicle (carrier) before any use in the treatment of systemic mycosis. Also, AMB toxicity, mainly hemolytic activity and nephrotoxicity, has been soon related with its self-aggregation properties, leading to research dispersions methods in vivo (Barwicz et al., 1992). Such properties were obtained, for instance, by using detergents (e.g. deoxycholate and buffer, to form the commercial preparation FUNGIZONE®). Organic solvents have also been tested, whereas their use was severely limited by cellular toxicity. Synthetic membrane systems (liposomes, i.e. uni or multi-lamellar phospholipid bilayers) entrapping AMB, that exhibited less toxicity and enhanced dispersion, were used in the treatment of murine histoplasmosis (Taylor et al., 1988), cryptococcosis (Graybill et al., 1982), and candidiasis (Lopez-Berestein et al., 1983). Liposomal AMB was shown to be more effective than free AMB. Furthermore, clinical studies and trials performed on cancer patients with fungal infections showed that liposomal AMB was better tolerated than Fungizone®, and could be used at higher amounts, thus giving a better effectiveness (Lopez-Berestein et al., 1987, 1985). In fact, the term liposomal vesicle is a very unprecise and coarse definition.

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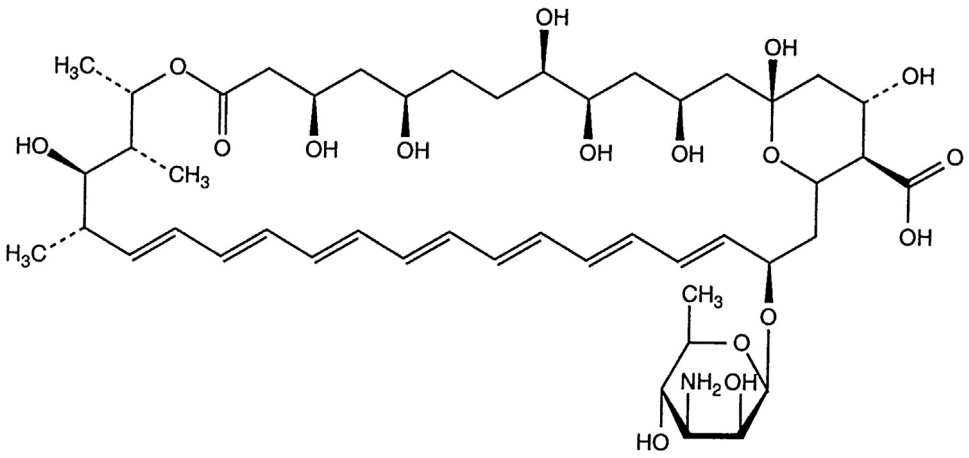

Fig. 1. Amphotericin B structure.

The type of liposomal vehicle (Sculier et al., 1988) (chemical composition, charge, structure, and mode of preparation) can strongly modify the physicochemical, biological, and pharmacological properties of liposomes and thus of the carried drug. These aspects had been evoqued under the scope of solubilization, transfer of AMB from vesicle to cell membrane ability, and size of liposome-AMB preparations. For instance, AMBISONE® that was designed to produce injectable emulsions consisted of large unilamellar vesicles (LUV) composed of mainly PC, and also PG, cholesterol and tocopherols. With regards to AMB alone, those preparations appeared less nephrotoxic and often of better therapeutic index (de Marie, 1996).

Among the different phospholipids, two species had been early identified: i) 1,2-Dimyristoyl-sn-glycero-3-phosphorylcholine (natural or synthetic DMPC), as an abundant matrix in natural membranes, taken as reference in the sterol-AMB intramembranar studies (Bolard, 1986). For instance, specific interactions and AMB-sequestered membrane domains had been clearly identified (Dufourc et al., 1984a,b) ii) 1,2-Dimyristoyl-sn-glycero-3-phosphorylglycerol (natural or synthetic DMPG) due to their specific physicochemical properties (DMPG when dispersed in water/NaCl exhibits a complex phase behaviour caused by its almost unlimited swelling properties in excess water (Loew et al., 2011). PG (phosphatidyglycerol), as surfactant agent, is greatly involved in the properties of alveolo-capillar barrier as surfactant reagent. Hence, pulmonary surfactant is a complex mixture of lipids (90%) and proteins (10%), participating in reducing surface tension at the air-liquid interface and in protecting lungs against pathogens as an innate immune system (Rooney et al., 1994; Veldhuizen et al., 1998; Kingma and Whitsett, 2006). PC and PG components play great physiological roles as a barrier for physiological exchanges, and also as target for external deleterious agents (physico-chemical, viruses, bacteria . . .) (Debouzy et al., 2010).

Another clinical interest is related with the frequency of respiratory fungal infections in immunodeficient organisms: using nebulized liposomal amphotericin B would appear as a suitable galenic form of administration (Gavaldà et al., 2005; Peghin et al., 2016).

Besides, DMPG/DMPC systems are classically used in peptides mixed membranes (bicelles, multilayers, ternary mixtures) interactions studies. From these basis, the present work used mixed phospholipid dispersions of DMPC and DMPG, in various proportions and temperature conditions, using ^{31}P-,^{1}H NMR and ESR observation techniques. Among these preparations, the commercially available (ABELCET®) composed of DMPC/DMPG 7/3 M/M with amphotericin B (Hiemenz and Walsh, 1996): this system had been investigated in the past by classical methods (UV–vis and dichroic spectrum analysis and electron microscopy) investigated, showing curious membrane structures (disks or ribbons) (Larabi et al., 2004).

For each reference mixture, DMPC/DMPG/AMB ternary preparation were realized using total phospholipid/AMB molar ratio of 2/1 under the same experimental conditions.

2. Materials and methods

2.1. Materials

2.1.1. Lipids and chemicals

Sodium salts and phospholipids (1,2-Dimyristoyl-sn-glycero-3-phosphorylcholine: DMPC, 1,2-Dimyristoyl-sn-glycero-3-phosphorylglycerol: DMPG were purchased from Sigma-Aldrich (Saint Quentin-Fallavier, France) and were used as received. Deuterated solvents and deuterium-depleted water were from Eurisotop (91191, Saint-Aubin, France). Amphotericin B (AMB) was from Bristol-Myers Squibb (Montreal-Canada).

2.1.2. Model membranes

Small unilamellar vesicles (SUV) were formed by bath sonication starting from chloroformic solutions of phospholipids after rotavapor evaporation and resuspension in D_20, for a final concentration of 10 mM. Size and homogeneity of SUV were controlled by light scattering and γ-choline and terminal methyl line width measurements on ^{1}H NMR spectra (Neumann et al., 1985) (see Fig. 7).

Multibilayers (MLV): DMPC liposomes for ^{31}P NMR experiments were prepared by successive freezing and thawing cycles until an homogenous milky sample was obtained (Loew et al., 2011). The suspensions were degassed under nitrogen gas then introduced into NMR tubes and sealed. The final lipid concentration was 50 mM. The various M/M proportions of DMPC/DMPG ranged from pure DMPC to pure DMPG. In AMB containing MLV, molar ratio AMB/lipid was $^{1}/_{2}$. In the followings, for convenience, molar ratios are identified as DMPC/DMPG/AMB molar ratios, and noted by a single number. For instance, DMPC/DMPG/AMB of 7/3/5 molar ratios is noted 735.

2.2. Methods

2.2.1. NMR experiments

^{1}H NMR experiments were recorded at 22 °C on a Bruker AVANCE III 400 NMR spectrometer using a presaturation of the