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Liposomes containing cyclodextrins or meglumine to solubilize and improve the bioavailability of poorly soluble drugs

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ABSTRACT

Poorly soluble drug-loaded liposomes are well known for their ability to solubilize and improve the bioavailability of the carried molecules, and may provide benefits as oral drug delivery systems. In this work, we aim to evaluate the effect of the incorporation of β-cyclodextrin (βCD), methyl-βCD (MβCD), hydroxypropil-βCD (HPβCD) and meglumine (MEG) in liposomes for the oral delivery of the poorly water-soluble drugs, sulfamerazine (SMR) and indomethacin (INM). Liposomes with egg phosphatidylcholine (PC) and cholesterol (CHO), incorporating SMR or INM as plain drug or inclusion complexes, were prepared using the thin film hydration method or dehydration-rehydration method, respectively. The systems were characterized by particle size, polydispersity and zeta potential measurements, and drug-component interaction studies were performed by ^{1}H NMR. Liposome stability in presence of SMR, INM, CD and MEG was determined by the retention of vesicle encapsulated calcein after incubation in solutions of pH 7.4, at 37 °C for up to 48 h. Drug entrapment, as well as drug release, were estimated for all liposome types prepared. The ^{1}H NMR studies revealed that the drugs presented interaction with lipids of the liposomes, suggesting the location of the drugs in the lipid bilayer. The liposomes presented high stability in the presence of the drugs, βCD, HPβCD or MEG. The highest entrapment values were achieved for SMR and INM with PC:CHO 3:1 liposomes when MEG and HPβCD were used, respectively (5636.28 and 439.54 mmol/mol), meaning that 18 and 43 times higher incorporation of SMR and INM were achieved in comparison with the ligand-free formulation. The in-vitro release studies showed a strong influence of the ligands on the delivery of the drugs from the liposomes.

1. Introduction

Several approaches have been investigated to develop nanosized drug delivery systems in recent years. Lately, a great deal of interest has been focused on lipid-based carriers due to the lack of suitable large-scale production methods for polymeric nanoparticle based products and to the toxicity of polymers [1]. Liposomes are colloidal vesicles ranging from few nanometers to several micrometers in diameter with one or more lipid bilayers surrounding aqueous compartments [2]. It is well known that liposomes offer many advantages for the delivery and/or targeting of drugs [3–6], since they are biodegradable, biocompatible, non-toxic and non-immunogenic [2]. Liposomal drug formulations can be used to overcome a drug's non-ideal properties, by loading the hydrophilic drugs into the inner aqueous phase, whereas hydrophobic drugs can be inserted into the hydrophobic lipid bilayers [7,8].

Although drug-loaded liposomes have been mainly used for the parenteral administration, their potential application as oral drug delivery systems has also been studied [8–10]. These systems are well known for their ability to solubilize and deliver poorly soluble drugs with significant improvements in bioavailability, observed following liposomal encapsulation [8,11]. Besides, incorporation of poorly permeable small molecule drugs into liposomes also yielded improved oral absorption [5,12–17]. Oral liposomes may also provide protection from the hostile environment in the gastrointestinal tract [8,12] and enable sustained release of the carried drugs [18–22]. Moreover, these systems offer the advantage of reducing toxicity [21,23–25], another side effect that can be observed using similar or lower concentrations compared to the required dose for therapeutic activity [25–27]. Furthermore, they may improve biodistribution [24,28,29] and produce specific site delivery [27,28,30].

Abbreviations: βCD, β-cyclodextrin; CD, cyclodextrins; CHO, cholesterol; Δ, chemical shifts in ^{1}H NMR; Δδ, induced chances in the ^{1}H NMR chemical shifts; D/L, molar ratio of drug over the total lipid concentration; DCL, drug-in- cyclodextrin-in-liposome; DRV, dried-reconstituted vesicles; F_{AT}, calcein fluorescence value measured after the addition of Triton X-100; F_{BT}, calcein fluorescence value measured before the addition of Triton X-100; HPβCD, hydroxypropil-βCD; INM, indomethacin; MEG, meglumine; MLV, multilamellar vesicles; MβCD, methyl-βCD; PBS, phosphate buffer solution; PC, egg phosphatidylcholine; SMR, sulfamerazine; SUV, small unilamellar vesicles; TFH, thin film hydration method.

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Encapsulating a sufficient amount of the active ingredient is one of the most desirable properties for the usage of liposomes. Factors affecting the encapsulation efficiency of the drug in the liposomes are various and come from the properties of both the liposomes (as the preparation method) and encapsulated drugs (as the hydrophilic or lipophilic properties or tendency to interact with the membrane bilayer) [11,31]. Accommodation of a poorly water-soluble drug in the lipid bilayer of liposomes is often limited in terms of drug to lipid mass ratio [11]. Cyclodextrins (CD) are well known for their ability to form inclusion complexes with a variety of guest molecules providing solubility-enhancing properties for lipophilic poorly-soluble drugs [32–35]. The strategy of combining liposomes and cyclodextrin complexes of lipophilic drugs, by forming drug-in- cyclodextrin-in-liposome (DCL) formulations, provided a novel system in drug delivery for the entrapment of water-soluble cyclodextrin–drug inclusion complexes in the aqueous interior of liposomes [11,20,36]. This could potentially increase the drug to lipid mass ratio to levels above those attained by conventional drug incorporation into the lipid phase [11].

N-acetyl glutamine, also known as meglumine (MEG), is a polyhydroxy organic amine that has been demonstrated to raise solubility [37,38], drug release rate [38–41] and stabilization [41] of weakly acidic molecules. In a previous work developed in our laboratory, MEG showed a significant solubilization enhancement of sulfamerazine (SMR), which is a very slightly water-soluble (0.22 mg/ml) [38] sulfonamide, compared with the free drug and the SMR-CD complexes, and proved to be responsible for a solubility improvement via multiple factors rather than just providing a favorable pH. Moreover, it was demonstrated that the complexation of this active ingredient with βCD, MβCD, HPβCD and MEG resulted in a decrease in the release rate of the drug through cellulose acetate membrane, thereby enabling sustained drug delivery systems [38].

Following on from these studies, we now aimed to examine various liposome-based formulation approaches, from biodegradable components, and to evaluate the effect of the incorporation of βCD, MβCD, HPβCD and MEG on the oral delivery of poorly water-soluble drugs, using SMR and indomethacin (INM) as model drugs. Experimental studies applied to these molecules can also provide information for other poorly water-soluble drugs with similar physicochemical properties. The characterization of the systems was carried out by particle size and zeta potential determinations, and drug-component interaction studies were performed by ^{1}H NMR. In addition, the integrity of the liposomes was measured in order to evaluate the vesicle stability after the incorporation of the drugs and ligands. Moreover, the effect of encapsulation on the solubility and release rate of the drugs, which affects the bioavailability, was also tested.

2. Materials and methods

2.1. Materials

Phosphatidyl-choline (PC), cholesterol (CHO), calcein, βCD, MβCD KLEPTOSE® CRYSMEB (DS = 0.5), HPβCD (DS = 0.45–0.95) and Meglumine were purchased from Sigma Aldrich® Hellas, Greece. Sulfamerazine and indomethacin were obtained from Parafarm®, Argentina. All the other materials and solvents were of analytical grade or better. Purified water was obtained from Millipore Milli-Q Water Purification System.

2.2. Preparation of liposomes

2.2.1. Thin film hydration method (TFH)

Multilamellar vesicles (MLV) were prepared by the TFH method. For this, PC (20 mg/ml) and CHO (10 mg/ml) were dissolved in chloroform/methanol (2:1 v/v) and mixed using different PC:CHO ratios (3:1, 2:1, 1:1, 1:0) that were subsequently evaporated under vacuum (in a round bottomed flask connected to a rotor evaporator) until a thin lipid film was formed and the traces of the solvent were removed under a stream of nitrogen. The lipid film was hydrated with the appropriate volume of pH 7.4 Phosphate Buffer solution (PBS). Small unilamellar vesicles (SUV) were prepared by probe sonication. The large liposome suspension initially produced was sonicated for at least two 15-min cycles using a vibra cell sonicator (Sonics and Materials, UK), equipped with a tapered microtip. In all cases, the initially turbid liposomal suspension was well clarified after sonication. The Ti-fragments that leaked from the probe during sonication, as well as any Multilamellar vesicles or liposomal aggregates present in the samples, were removed by centrifugation at 14.000 *g* for 10 min at 201C (Spectrafuge 16 M, Labnet, Germany). For the preparation of drug-loaded liposomes, the same procedure as for empty liposomes was applied, and SMR or INM were incorporated in the chloroform/methanol (2:1 v/v) dispersion in a 1 mg/ml concentration. For measurement of drug entrapment in liposomes, liposomal drug was separated from the non-solubilized drug by filtration thought 1 μm pore size membrane.

2.2.2. Dehydration- rehydration method

Dried-reconstituted vesicles (DRV) were prepared by the procedure of Kirby and Gregoriadis [42], due to the demonstrated high entrapment efficiency of drugs in the liposomes. The 3:1 lipid ratio was selected since it showed high encapsulation of the drugs when the THF method was applied (Section 2.2.1). SMR or INM were incorporated in the vesicles in absence and presence of βCD, MβCD, HPβCD and in the presence of MEG for SMR, in a 1:1 stoichiometric relation, chosen from previous studies. The INM-loaded liposome formulation containing MEG was dismissed because a negative effect on INM stability, caused by the ligand, was observed in previous studies.

2.3. Particle size, polidispersity and zeta potential

The droplet size, polidispersity and zeta potential of the liposomes were determined at 25 °C using a Malvern Zetasizer Nano Series. The intensity autocorrelation function was measured at a 165-degree angle using a viscosity of 0.8878 Pa · s and a refractive index of 1.3328 for the bulk medium. The samples were appropriately diluted with water before the analysis.

2.4. Nuclear magnetic resonance (NMR) studies

^{1}H NMR studies were performed at 298 K in a Bruker® Advance II High Resolution Spectrometer equipped with a Broad Band Inverse probe (BBI) and a Variable Temperature Unit (VTU) using 5-mm sample tubes. Spectra were obtained by diluting a 0.1 ml volume of the empty, SMR-loaded and INM-loaded PC:CHO 3:1 THF liposomes to 1 ml D_2O. In order to acquire the spectra of the pure lipids, plain liposomes containing only PC or CHO were prepared and diluted to a 1 mM concentration with D_2O. All the studies were carried out at 400.16 MHz and the data were processed with the Bruker® TOPSPIN 2.0 software. The residual solvent signal (4.80 ppm) was used as the internal reference. Induced changes in the ^{1}H NMR chemical shifts (Δδ) for the drugs and the liposomes components originated due to their interaction were calculated according to the following equations:

$$\Delta\delta = \delta_{\text{loaded liposomes}} - \delta_{\text{unloaded liposomes}}$$

and

$$\Delta\delta = \delta_{\text{drug in liposomes}} - \delta_{\text{drug}}$$

2.5. Measurement of liposome integrity

The integrity of liposomes was evaluated by measuring the retention (%) of calcein in the vesicles. For this, the lipid film was hydrated with