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Thiomer-coated liposomes harbor permeation enhancing and efflux pump inhibitory properties

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ABSTRACT

An ideal oral drug carrier should facilitate drug delivery to the gastrointestinal tract and its absorption into the systemic circulation. To meet these requirements, we developed a thiomer-coated liposomal delivery system composed of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and a maleimide-functionalized lipid, to which chitosan-thioglycolic acid (CS-TGA) was covalently coupled. In addition to conventional 77 kDa CS-TGA (CS-TGA77), we tested the 150 kDa homologue (CS-TGA150) as well as an S-protected version of this polymer (CS-TGA150-MNA), in which some of the free SH-groups are conjugated with 6-mercaptonicotinamide to protect them from oxidation. Coupling of CS-TGA to the liposomal surface led to an increase in the particle size of at least 150 nm and an increase in the zeta potential from approximately −33 mV to a maximum of about +36 mV, depending on the polymer. As revealed by fluorescence dequenching the formulations have a storage stability of at least two weeks without releasing any encapsulated compounds. In simulated gastric fluid, the system was shown to be stable over 24 h, while in simulated intestinal fluid, a slow, sustained release of encapsulated compounds was observed. According to our experiments, thiomer-coated liposomes did not induce immunogenic reactions after an oral administration to mice. To evaluate the permeation enhancing and efflux pump inhibiting properties of CS-TGA coated liposomes we monitored the transport of fluoresceinisothiocyanate-dextran (FD_4) and rhodamine-123 (Rho-123), respectively, through rat small intestine. Permeation studies showed a 2.8-fold higher permeation of FD_4 in the presence of CS-TGA77 coated liposomes and an even 4-fold higher permeation in the presence of CSA-TGA150-MNA coated liposomes. The latter also performed best when we evaluated P-glycoprotein inhibiting properties by monitoring the transport of Rho-123, revealing a 4.2-fold enhancement respective to the buffer control. Taken together, thiomer-coated liposomes were shown to protect encapsulated drugs in the stomach, slowly release them in the small intestine and enhance their absorption through the intestinal tissue by opening tight junctions and inhibiting efflux pumps.

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1. Introduction

The oral delivery of drugs is generally the most convenient route, as it allows for painless and easy administration, and therefore high patient compliance. However, many drugs cannot be administered orally due to the harsh environment and/or low absorption from gastrointestinal (GI) tract. An optimal oral delivery system should therefore (1) protect compounds from degradation and (2) improve their permeation through GI-barriers; enhancing their oral bioavailability. Different nanoparticulate systems have been developed for the protection of drugs during gastrointestinal transit — among them, liposomes. Despite several successful studies [1,2], however, liposomes

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have not yet reached their full potential as oral drug carriers, though in recent years several strategies have been developed to enhance the stability of liposomes and improve their properties for oral delivery — one of which is coating them with multifunctional polymers such as chitosan, Carbopol®, Eudragit® or silica [3–6]. Following this concept, we have recently generated liposomes coated with thiolated chitosan (CS-TGA) [7].

Different thiolated polymers – designated thiomers – have been previously designed, which commonly consist of SH-group-bearing agents anchored to polymeric backbones. Thiomers have also been shown to exhibit several promising properties for drug delivery, including mucoadhesion; permeation enhancement; efflux pump inhibition; and enzyme inhibition [8–11]. Despite these effects being well-established for thiomers themselves, it remained questionable as to whether thiomer-coated liposomes will still exhibit permeation enhancing and efflux pump inhibiting properties, given that the mucus layer lining the small intestine functions as a barrier refractive to access by larger particles. To address this question, liposomes were prepared by utilizing what was expected to be a more stable composition by comparison with those used in our previous study [7]. These newly designed liposomes were evaluated in the context of their storage stability, release kinetics, permeation enhancing and efflux pump inhibitory properties, as well as regarding their immunogenic behavior. To achieve even higher permeation enhancing and efflux pump inhibitory properties, liposomes were coated with 'S-protected thiomers', as this new type of thiomers is stable towards oxidation [12].

2. Materials and methods

2.1. Materials

1,2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-N-[4-(p-maleimidomethyl) cyclohexane-carboxamide] (DPPE-MCC) were purchased from Avanti Polar Lipids (Alabaster, AL). Chitosan-thioglycolic acid of two different molecular weights (CS-TGA77; molecular weight: 77 kDa, 550 μmol SH-groups/g polymer and CS-TGA150; molecular weight: 150 kDa, 660 μmol SH-groups/g polymer) and the chitosan-thioglycolic acid 6-mercaptonicotinamide-conjugate (CS-TGA150-MNA; molecular weight: 150 kDa, 380 μmol S-protected thiol groups and 280 μmol free SH-groups/g polymer) were synthesized according to methods described previously [12,13]. Fluoresceinisothiocyanate-dextran (FD_4, 4400 Da) was supplied from TdB Consultanca AB (Uppsala, Sweden). All other chemicals were of reagent grade or of the best grade available and purchased from Sigma-Aldrich (Vienna, Austria).

2.2. Preparation of liposomes

Liposomes were prepared by thin lipid film rehydration method. Briefly, DPPC and the maleimide-functionalized lipid DPPE-MCC were dissolved in methanol and mixed in a molar ratio of 3:0.3. The organic solvent was evaporated under a nitrogen stream, and the resulting lipid film was dried overnight in a vacuum chamber. A 10 mM phosphate buffer containing 150 mM NaCl, pH 7.4 (PBS) was added to the dry lipid film, which was then rehydrated for 1 h at a temperature of 50 °C with repeated vortexing. The so-formed multilamellar vesicles (final lipid concentration: 30 mg/mL) were sized by freeze and thaw and size extrusion through 200 nm polycarbonate membranes (Whatman Inc., Clifton, NJ) with a mini-extruder (Avanti Polar Lipids, Alabaster, AL).

To evaluate particle stability and release behavior, the fluorophore/quencher couple 8-aminonaphthalene-1,3,6-trisulfonic acid (ANTS)/p-xylene-bis-pyridinium bromide (DPX) was encapsulated within the liposome during the hydration step. Briefly, 5.4 mg/mL ANTS and 19 mg/mL DPX were solved in PBS and added to the lipid film. All further steps were carried out as described above. After size extrusion, free ANTS/DPX was separated from ANTS/DPX-loaded liposomes by Sephadex G75 column chromatography (Amersham Biosciences, Uppsala, Sweden).

2.3. Coupling of the polymer

Three different polymers (CS-TGA77, CS-TGA150 and CS-TGA150-MNA) were coupled to the liposomal surface by covalent bond formation between maleimide groups of the liposome and free SH-groups of the polymer, as described previously [7] (see Fig. 1). Briefly, the polymer was dissolved in deionized water in a concentration of 2 mg/mL, of which an appropriate amount was added to the pre formed liposomes and incubated overnight under agitation. The molar ratio of SH-groups to maleimide groups was approximately 4:1, whereby a final lipid concentration of 1.7 mg/mL and a polymer concentration of 1.7 mg/mL were maintained throughout the study. Uncoated liposomes were diluted with deionized water to the same lipid concentration.

2.4. Particle characterization

2.4.1. Particle size

Size measurements were performed at room temperature by dynamic light scattering (DLS) using a Zetasizer 3000HS (Malvern Instruments, Herrenburg, Germany). Coated- and uncoated liposomes were measured after being diluted to a final lipid concentration of about 0.03 mg/mL with ultra-pure water (USF ELGA, High Wycombe Bucks, UK). Particle size was analyzed by calculating the auto correlation function of the detected intensity. The polydispersity index of the liposomal suspension is given by the width of the size distribution.

2.4.2. Zeta potential

Zeta potentials of coated- and uncoated liposomes were determined with a Zetasizer nano ZS (Malvern Instruments, Herrenberg, Germany) after dilution to a lipid concentration of 0.3 mg/mL with a buffer containing 10 mM Tris and 2 mM CsCl, pH 7.0. All measurements were carried out at room temperature.

2.5. Release study

Leakage and stability studies were carried out using uncoated- and CS-TGA77-coated liposomes. All stability and release measurements were carried out by measuring the fluorescence of the suspension (excitation wavelength: 360 nm; emission wavelength: 530 nm) after diluting them with PBS buffer to a final lipid concentration of approximately 50 μg/mL using a SPEX FLUOROMAX-3 fluorescence spectrometer (Jobin Yvon Horiba, Longjumeau Cedex, France). Leakage of ANTS/DPX leads to an enhanced fluorescence signal due to the greater distance between fluorophore and quencher. To determine the fluorescence intensity corresponding to 100% release of ANTS/DPX, 10 μL of 10% Triton X-100™ were added to the cuvette (2 mL) before measuring.

2.5.1. Long term storage stability

For long term storage stability, ANTS/DPX-loaded liposomes were stored at 4 °C in darkness and aliquots were measured at different time points over a time interval of 2 weeks.

2.5.2. Stability and release behavior in different simulated body fluids

To measure the release kinetics in different body fluids, freshly prepared coated- and uncoated liposomes were exposed to either simulated gastric fluid (SGF; 1 L contains 2 g sodium chloride, 3.2 g pepsin, 7 mL hydrochloric acid; pH 1.2) or simulated intestinal fluid (SIF; 1 L contains 6.8 g monobasic potassium phosphate, 10 g pancreatin, 77 mL 0.2 N sodium hydroxide; pH 6.8), which were prepared

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