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Liposomes coated with thiolated chitosan as drug carriers of curcumin

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ABSTRACT

Liposome is one of a promising delivery system to improve water solubility, stability, and bioavailability of curcumin. But its instability is not favorable for long-circulating treatment, controlled release or conservation. To overcome the disadvantages, thiol derivatised chitosan (CSSH) were synthesized and utilized to coat liposomes. The CSSH coated curcumin liposomes (Cur-Lip-CSSH) had an encapsulation efficiency (EE) of 93.95%, a drug loading (DL) of 7.95%, an average particle size of 406.0 nm, and a positive zeta-potential of 36.6 mV, which were all higher than that of Cur-Lip. Cur-Lip-CSSH showed slower in vitro release than Cur-Lip at pH 5.5 and pH 7.4, and the higher retention of curcumin would be remained for the following uptake of cells. The stability of the both liposomes at 4 °C was almost the same, but Cur-Lip-CSSH displayed a higher stability at room temperature and higher temperature by DSC characterization. Curcumin can inhibit the growth of cancer cells under certain conditions. MCF-7 cell line was used to study its inhibition and proliferation after treating with curcumin and Cur-Lip-CSSH. Treatment of MCF-7 with curcumin and Cur-Lip-CSSH showed dose and time dependent cytotoxicity, with growth suppression at 200 μM, 72 h, obviously. These results indicate that the proper coating of liposomes is able to improve the stability of liposomes and the Lip-CSSH can function as potential drug delivery system.

1. Introduction

1,7-Bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-2,5-dione, commonly known as curcumin is a yellow pigment found in the rhizomes of *Curcuma longa* (turmeric). Besides its wide application as food spices, especially in South Asia since ancient time, recently focus on this molecule has been tremendously rising for its pharmaceutical applications (including for anti-cancer, anti-amyloid, anti-inflammatory and anti-oxidant activities) [1], as well as wound healing characteristics. Although, curcumin has numerous advantages, the therapeutic application of curcumin is limited because of its low bioavailability, due to its poor aqueous solubility, low stability against alkaline pH conditions, extensive first-pass metabolism and rapid systemic elimination [2]. Therefore, a carefully designed carrier could significantly facilitate curcumin delivery and broaden the range of its possible pharmaceutical applications.

Liposomes with phospholipid bilayer structure, are biocompatible, completely biodegradable, nontoxic, and non-immunogenic, which make them an ideal carrier system with applications in different fields including food, cosmetics, pharmaceutics, and tissue engineering [3].Some researchers have reported that liposomes could improve the bioactivity and water solubility of curcumin, and increase drug uptake into cells [4,5]. However, one of the main disadvantages of "conventional" liposomes as drug carriers is their fast elimination from blood circulation [6] and reticulo-endothelial system macrophages [7]. In addition, liposomes are physical and chemical instable, suffering such problems as aggregation, fusion, degradation, hydrolysis and oxidation of phospholipids [8]. Zhang et al. reported that pH and temperature impact the extent of hydrolysis of phospholipids and thus the shelf-life of liposomes [9]. Membrane fluidity increases with increasing pH and temperature [10]. Similarly, with increasing temperature, hydrocarbon chain packing of the bilayer becomes randomized, thereby reducing membrane rigidity because of pore like defect and bilayer disc formation [11].

Polymer coating is a promising way to modify the surface characteristics of liposomes in order to improve their applicability. Some researchers have reported chitosan-coated can improve their properties and applicability [12–14]. Y. Wang et al. anchored the cholesterol succinyl chitosan on the surface of liposomes to improve their physical stability and sustain the release of epirubicin in vitro [15]. But such ionization linkages are still not stable enough in blood circulation or complicated physiological environment, due to the weak combination of anti-charges.

The present study will be focused primarily on the preparation of thiol derivatised chitosan (CSSH) and the coating on liposomes, wherein the free –SH groups forms –S—S– linkages to stabilize the liposomes coatings. The particles size, chemical structures, release of curcumin and stability of the liposomes will be studied and discussed in details.

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2. Materials and methods

2.1. Materials

Chitosan (CS, medium molecular mass, degree of deacetylation = 75–85%, viscosity = 200–800 cps), L-cysteine hydrochloride monohydrate (Cys), L-α-Phosphatidylcholine (PC) (from soybean, reagent grade) and Cholesterol (reagent grade, ≥92.5%) were purchased from Sigma-Aldrich. N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDAC), N-Hydroxysuccinimide (NHS), 5,5′-Dithiobis-(2-nitrobenzoic acid) (DTNB) (99%) were of analytical grade and purchased from Qi Yun Biotechnology Co., Ltd. (China). Curcumin (98% pure) were purchased from Guangzhou Shuoheng Biotechnology Co., Ltd. (Guangzhou, China).

All other reagents were commercially available, of analytical grade, and used without further purification.

2.2. Synthesis and characterization of thiol derivatised chitosan (CSSH)

Chitosan was functionalized via the reaction of its carboxylic acid with L-cysteine hydrochloride monohydrate, which was performed as described in literature [16–19]with slight modification. 1 g chitosan was dissolved in 100 mL 0.05% (*v*/v) acetic acid to obtain a 1% (*w*/*v*) polymer solution, the EDAC and NHS were added in at final concentrations of 50 mM, and stirred for 15 min at room temperature. Then, Cys was added and the pH was readjusted to 5.0–6.0 with 1 M NaOH. After incubated for 5 h at room temperature under stirring, the resulting conjugate was dialysed (MWCO 8–14 kDa) twice against 0.2 mM HCl, twice against 0.2 mM HCl containing 1% NaCl, twice against 0.2 mM HCl at 4 °C in the dark, and finally lyophilized. Samples were stored at 4 °C until further use.

The amount of thiol groups remaining on CS was determined using ^{1}H NMR and Ellman's method [20,21]. ^{1}H NMR (500 MHz) spectra were recorded on an AVANCE III 500 NMR spectrometer (Bruker, Germany).

Ellman's method: 1 mg CSSH was dissolved in 250 μL deionized water, and then was added in 250 μL of 0.5 mol/L pH = 8.0 phosphate buffer and 500 μL DTNB. After incubation in the dark for 2 h at room temperature, absorbance at 450 nm was measured with the UV–Vis absorption spectra were recorded by a UV-2550 spectrophotometer (Shimadzu, Japan). The amount of free thiol groups was calculated using a standard curve obtained by the sulfhydryl group determination of a series of solutions containing Cys.

2.3. Curcumin-loaded liposome (Cur-Lip) preparation

Cur-Lip was prepared according to the thin-film hydration method [22]. A weight ratio at 5:1 of PC and cholesterol was dissolved in the ethanol. PC and curcumin were mixed in a ratio of 10:1 (*w*/w). The solvent was evaporated on a rotary evaporator at 45 °C resulting in the formation of a lipid film over the inner surface of a round-bottom flask. The film was hydrated with the phosphate buffer solution (PBS) and stirred at 500 rpm for 60 min at room temperature, and then the solution was homogenized using a probe sonicator treatment for 10 min (300 W, 3 s on, 3 s off). The final concentrations of PC, cholesterol and curcumin were kept at 10 mg/mL, 2 mg/mL and 1 mg/mL, respectively.

2.4. CSSH coating of Cur-Lip (Cur-Lip-CSSH)

The CSSH aqueous solution (1%, *w*/*v*) was added dropwise into Cur-Lip suspension at equal volume with magnetic stirring of 500 rpm for 60 min. The final concentration of CSSH was 0.5% (*w*/*v*). Excess polyelectrolytes and curcumin were then removed by ultracentrifugation at 20,000*g* for 30 min followed by a single washing with DW [22]. The resulting CSSH-coated liposomes were resuspended in PBS.

2.5. Curcumin content analysis

Curcumin content in the liposomes was measured by an ultraviolet spectrophotometry detector (Shimadzu, Japan). The solution was diluted 40 times before analyzing using UV system at the wave length of 425 nm. All samples were analyzed in triplicate. The drug loading (DL) was calculated by the following equation:

$$\mathrm{DL}(\%) = \frac{\mathrm{M_{EC}}}{\mathrm{M}} \times 100\%$$

where M_{EC} is the mass of the curcumin encapsulated in the liposomes, and M was the gross mass of the liposomes.

To evaluate the drug availability of the liposomes, the entrapment efficiency (EE) of the curcumin in liposomes was determined by analyzing the encased amount of the curcumin. The liposomes suspension was centrifuged to remove the curcumin attached possibly on the surface of liposomes, and then re-dispersed with the PBS. This procedure was repeated several times to make sure that the curcumin in the supernatant was hardly detected. Finally, the sedimentary curcumin liposome was dissolved in ethanol. The content of encapsulated curcumin was determined by UV. The EE of liposomes was calculated via the following equation:

$$\mathrm{EE}(\%) = \frac{\mathrm{M_{EC}}}{\mathrm{M_{Cur}}} \times 100\%$$

where M_{Cur} was the gross mass of the curcumin for encapsulation in Cur-Lip or Cur-Lip-CSSH.

2.6. Differential scanning calorimetry (DSC)

DSC was performed on a differential scanning calorimeter (Q20, TA instruments, USA) for liposomes without curcumin (Lip), Cur-Lip, Lip-CSSH and Cur-Lip-CSSH, respectively. 50 μL samples was placed in the aluminum pan with the liposome concentration of 10 mg/mL. Then, the aluminum pan was sealed by a tablet press machine. The heating scan was carried out from 10 °C to 150 °C at a scanning rate of 5 °C/min under dry nitrogen. A baseline was recorded with blank hermetic aluminum pan.

2.7. Particle size distribution and zeta potential measurements

The particle size distribution and zeta potential of Lip, Cur-Lip, Lip-CSSH and Cur-Lip-CSSH were determined using dynamic light scattering (DLS) technique employing a Zetasizer Nano ZS (Malvern Instruments Ltd., UK). Samples dispersion was added to polystyrene latex cells, and the mean particle size and zeta potential were measured at 25 °C with a detector angle of 90°. The average values from at least three measurements were reported, with the polydispersity index (PDI).

2.8. Transmission electron microscopy (TEM)

Transmission electron microscopy was employed to observe the structure of nanoliposomes and CSSH-coated liposome with a staining method. Briefly, the samples were diluted 20-folds with distilled water to reduce the concentration of the particles. Samples were dropped into a carbon-coated copper grid and dried for 1 min. Excess sample was removed using filter paper. Then, the samples were instantly stained with 2% (*w*/*v*, %) phosphotungstic acid, allowed to stand for 1 min at room temperature, and drained, then the sample was examined using a (PHILIPS TECNAI 10) Transmission Electron Microscope.