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Development and evaluation of a novel phytosome-loaded chitosan microsphere system for curcumin delivery

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ABSTRACT

In this study, we developed a novel drug delivery system, curcumin-phytosome-loaded chitosan microspheres (Cur-PS-CMs) by combining polymer- and lipid-based delivery systems. Curcumin exhibits poor water-solubility and is rapidly eliminated from the body. We aimed to use our novel delivery system to improve the bioavailability and prolong the retention time of curcumin in the body. The Cur-PS-CMs were produced by encapsulating curcumin-phytosomes (Cur-PSs) in chitosan microspheres using ionotropic gelation. The final microsphere was spherical, with a mean particle size of $23.21 \pm 6.72\,\mu m$ and drug loading efficiency of $2.67 \pm 0.23\%$. Differential scanning calorimetry and Fourier transform infrared spectroscopy demonstrated that the integrity of the phytosomes was preserved within the polymeric matrix of the microspheres. The *in vitro* release rate of curcumin from the Cur-PS-CMs was slower than that from curcumin-loaded chitosan microspheres (Cur-CMs) in pH 1.0, 4.0, 6.8, and 7.4. Pharmacokinetic studies in rats dosed with Cur-PS-CMs showed a 1.67- and a 1.07-fold increase in absorption of curcumin compared with Cur-PSs and Cur-CMs, respectively. The half-life of curcumin orally administration of Cur-PS-CMs (3.16 h) was longer than those of Cur-PSs (1.73 h) and Cur-CMs (2.34 h). These results indicated that the new Cur-PS-CMs system combined the advantages of chitosan microspheres and phytosomes, which had better effects of promoting oral absorption and prolonging retention time of curcumin than single Cur-PSs or Cur-CMs. Therefore, the PS-CMs may be used as a sustained delivery system for lipophilic compounds with poor water-solubility and low oral bioavailability.

1. Introduction

Curcumin, a hydrophobic polyphenol derived from the rhizome of *Curcuma longa* L. (Zingiberaceae or dietary turmeric) has been shown to exhibit antioxidant, anti-inflammatory, antimicrobial, anti-amyloid, and antitumor activities (Maheshwari et al., 2006; Anand et al., 2008; Srivastava et al., 2011). In addition, the non-toxic nature of curcumin has been demonstrated by its long history of dietary use and clinical trials (Cheng et al., 2001; Sharma et al., 2004). To date, there are no studies that show toxicity associated with the use of curcumin, even at very high doses.

Despite its promising therapeutic efficacy and favorable safety profile, the clinical application of curcumin has been obstructed by its poor solubility in water, rapid half-life, and low bioavailability after oral administration. Its maximum solubility is 11 ng/mL in aqueous buffer (pH 5.0) (Tonnesen et al., 2002). Following oral administration (up to 8 g/day), only a trace amount of curcumin was detected in blood. In addition, the oral bioavailability was only 1% in rats (Pan et al., 1999; Yang et al., 2007). Curcumin undergoes a very high first-pass metabolism and is eliminated rapidly. Thus, its retention time in circulation is very short (Pan et al., 1999; Sharma et al., 2007; Yang et al., 2007).

To overcome these limitations, various formulations and techniques have been investigated over the past decades. Such studies included the use of solid dispersions (Paradkar et al., 2004), complex formation with cyclodextrins (Yallapu et al., 2010), copolymeric micelles (Song et al., 2011), polymeric nanoparticles (Shaikh et al., 2009; Das et al., 2010; Anitha et al., 2011; Kim et al., 2011), lipid-based nanoparticles (Sou et al., 2008; Dadhaniya et al., 2011), liposomes (Chen et al., 2009), a phospholipid complex (Maiti et al., 2007), and self-microemulsion (Cui et al., 2009; Setthacheewakul et al., 2010). However, to our knowledge, these techniques were used alone and only addressed one of the shortcomings of curcumin. For example, the curcumin-phospholipid complex increased the AUC of curcumin by 3.37 times. However, it could only lengthen the half-life of curcumin from 1.45 h to 1.96 h.

Several studies have shown that an appropriate combination of different carrier systems can achieve the advantages of each system while avoid the shortcomings. Feng et al. (2004) fabricated a novel drug delivery device called liposomes-in-microsphere (LIM), in which liposomes could protect loaded therapeutic proteins from the harsh conditions involved in the subsequent

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fabrication process, as well as the acidic microenvironment inside the PLA-PEG-PLA microspheres. Cai et al. (2012) formulated the huperzine A–phospholipid complex and loaded the complex into PLGA–PEG–PLGA gel in order to reduce the burst effect of the gel and control the huperzine A release. To encapsulate hydrophilic insulin into strong lipophilic poly (hydroxybutyrate-co-hydroxyhexanoate) (PHBHHx), insulin was first complexed with phospholipids to enhance the lipophilicity and then loaded into PHBHHx nanoparticles (Peng et al., 2012).

To extend curcumin delivery time using a phospholipid complex, phytosomes (PSs), or self-assembling bilayer vehicles of phospholipid complex, were further encapsulated within polymeric microspheres. The main purpose of the microspheres was to control the exposure of phytosomes, and consequently provide sustained release of a drug. Chitosan was chosen because of its unique biological properties, including favorable biocompatibility, biodegradability, polycationicity, and mucoadhesiveness (Dasha et al., 2011). Chitosan can enhance the absorption of drugs into gastric mucosa by mucoadhesion or by opening tight junctions between epithelial cells (Senel et al., 2000; Thanou et al., 2001). The positive charge of chitosan makes it suitable to combine with negatively charged PSs. The preparation of chitosan microspheres is entirely aqueous and should not affect phytosome stability.

The aim of our study was to prepare a combined drug delivery system to promote the absorption and slow down the elimination of curcumin after oral administration. The phytosomes and chitosan microspheres were integrated to fabricate a new vehicle in which both components could promote the absorption of curcumin. In addition, the matrix of the microspheres could delay the release of curcumin. The new curcumin-phytosome-loaded chitosan microspheres (Cur-PS-CMs) were prepared by encapsulating curcumin-phytosomes (Cur-PSs) in chitosan microspheres. We then characterized the Cur-PSs and Cur-PS-CMs. We determined the *in vitro* drug release behavior of the microspheres at different pH levels to demonstrate the mechanism of action of the combined drug delivery system. In addition, the pharmacokinetics was evaluated in rats after oral administration of natural Cur, Cur-PSs, curcumin-loaded chitosan microspheres (Cur-CMs), and Cur-PS-CMs.

2. Materials and methods

2.1. Materials

Soybean phospholipids (94% purity) were purchased from Shanghai Taiwei Co., Ltd (Shanghai, China). Chitosan (viscosity of 300 cps and deacetylation degree of 93%) was purchased from Golden-shell Biochemical Co., Ltd. (Qindao, China). Curcumin (98% purity) was purchased from Xi'an Rongsheng Biotechnology Co., Ltd. (Xi'an, China). Emodin (98% purity) was purchased from Chroma-standard Medical technology Co., Ltd. (Tianjing, China). Other solvents and chemicals were of analytical or chromatographic grade.

2.2. Preparation of Cur-PSs

The Cur-PSs were prepared as previously reported (Maiti et al., 2007), with slight modifications. Briefly, 20 mL of absolute alcohol was mixed with 100 mg of curcumin and 214 mg of soybean phospholipids in a 100 mL round bottom flask. The mixture was stirred at 50 °C for 2 h. Afterwards, the solution was added to 40 mL of 2% (w/v) acetic acid. The resulting mixture was continuously stirred at 50 °C until the odor of alcohol was no longer apparent. All procedures were protected from light.

2.3. Preparation of Cur-PS-CMs

The Cur-PS suspension was diluted with 2% (w/v) acetic acid until the final curcumin concentration was 0.1 mg/mL. Chitosan (400 mg) was dissolved in 200 mL of the diluted suspension. The resulting solution was then fed through a 2 mm diameter nozzle *via* peristaltic pump (BT-100, Shanghai Huxi Analysis Instrument Factory Co., Ltd., China) at a flow rate of 2.0 mL/min and dropped into 400 mL of strongly agitated sodium tripolyphosphate (TPP) solution (0.3%, w/v). The resulting chitosan microspheres were left in the dark overnight. The TPP solution was then decanted. The microspheres were washed several times with deionized water, laid out on aluminum trays, and oven dried at 40 °C until their weight remained constant. Cur-CMs and blank chitosan microspheres (CMs) were prepared using the procedures just described, except a curcumin suspension or 2% (w/v) acetic acid with no solute was used instead of the Cur-PS suspension.

2.4. Characterization of Cur-PSs and Cur-PS-CMs

2.4.1. Complex formation efficiency and drug loading

The percentage of curcumin complexed with phospholipids was determined as follows: Cur-PS suspension was diluted 1-fold with 0.5% (w/v) Tween-80 and then centrifuged at 30,000 rpm for 2 h at 4 °C. The supernatant was isolated and the amount of free curcumin was determined by UV/Vis spectroscopy at 420 nm. To determine the total amount of curcumin, 0.1 mL of the Cur-PS suspension was diluted in proper methanol, adjusting the volume to 50 mL. The complex formation efficiency was calculated according to the following formula:

$$\text{Complex formation efficiency (\%)} = \left[\frac{\text{Total amount of curcumin} - \text{amount of free curcumin}}{\text{Total amount of curcumin}}\right] \times 100$$

To determine the loading capacity of the microspheres, 10 mg of microspheres were added to 5 mL of 0.1 mol/L HCl and sonicated for 20 min in a water bath. One milliliter of the suspension was immediately withdrawn and diluted to 10 mL with methanol. After filtration through 0.45 μm Millipore filters, the samples were analyzed by UV/Vis spectroscopy at 420 nm.

2.4.2. Particle size and morphology

The average diameter and zeta potential of the Cur-PSs were both measured using a Zetasizer ZEN 3600 (Malvern Instruments Ltd., UK) at a fixed scattering angle of 90° at 25 °C. The average diameter of the microspheres was measured by laser diffraction using a Malvern Mastersizer 2000 particle sizer (Malvern Instruments Ltd., UK) at a fixed scattering angle of 90° at 25 °C.

The morphology of the Cur-PSs was analyzed by atomic force microscopy (AFM; SPM, CE Ltd., USA) and transmission electron microscopy (TEM; H-7500, Hitachi Scientific Instruments Ltd., Japan). The suspension was diluted 10-fold with deionized water. For AFM, one drop of suspension was placed on freshly cleaved mica. The sample was air-dried at room temperature and mounted on the microscope scanner. The shape was observed and imaged in tapping mode. For TEM, one drop of suspension was placed on a 400 mesh copper grid coated with carbon. After 20 min, the grid was tapped with filter paper to remove surface water and stained using a solution of phosphotungstic acid (2%, w/v) for 20 min. Then the stained sample was dried in air and the morphology was observed.

The morphology of Cur-PS-CMs was evaluated by scanning electron microscopy (SEM). The microspheres were suspended in absolute alcohol and sonicated for 5 s to break up the aggregates