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Chitosan microparticles for oral bioavailability improvement of the hydrophobic drug curcumin

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The aim of this study was to assess the feasibility of microparticles for dissolution enhancement and oral bioavailability of curcumin (Cur). Microparticles were prepared by the ionic crosslinking interaction with the use of tripolyphosphate (TPP) and chitosan (Cs). The physicochemical characteristics of microparticles were investigated. The *in vivo* performance was assessed by a pharmacokinetic study. The microparticles had an average diameter of 58.50 μm. Acceptable drug loading and encapsulation efficiency of microparticles were obtained to be 33.5% and 85.2%, respectively. Dissolution of Cur enhanced in the microparticles in comparison with pure drug. Drug release profile of Cur from microparticles fitted the first-order model. Microparticles provided improved pharmacokinetic parameters (C_{max} 270.24 ng/ml, T_{max} 1.30 h) in rats as compared with pure drug (C_{max} 87.06 ng/ml, T_{max} 0.66 h). The AUC value of microparticles was 8.4 fold that of the pure drug. The information from this study suggests that the developed microparticles successfully enhanced dissolution of the poorly water-soluble drug Cur, and eventually, improved its oral bioavailability effectively.

1. Introduction

Curcumin (Cur), a hydrophobic polyphenol extracted from the rhizomes of the herb *Curcuma longa* has a wide spectrum of biological and pharmacological activities including antioxidant, anti-inflammatory, anticarcinogenic, antimicrobial, hepatoprotective, cardiovascular, hypoglycemic, and antiarthritic effects on various experimental models (Anand et al. 2007). However, Cur is slightly absorbed in the gastrointestinal tract because it is practically insoluble in water (Cui et al. 2009). Extensive intestinal and hepatic metabolism and rapid elimination additionally restrain Cur's bioavailability (Shaikh et al. 2009). Therefore, in spite of its efficacy and safety, Cur has not yet been approved as a therapeutic agent, and the relative bioavailability of Cur has been highlighted as a major problem for this (Anand et al. 2007). Among modern drug delivery systems, matrix-type microparticles containing uniformly dispersed or dissolved drug, are a promising multi-particulate system for improvement of the oral bioavailability of water-insoluble drugs (Huang et al. 2006). Several drugs, e.g. nitrendipin and nifedipine, were reported to obtain an enhanced bioavailability and a prolonged constant drug plasma concentration after administration of this multiparticulate disperse system to rats (Yang et al. 2004; Huang et al. 2006).

Chitosan (Cs) is a natural polysaccharide with positive electricity. It has been attracting increasing interest as a biomaterial for drug delivery because of its excellent biocompatibility, biodegradability, bioactivity and nontoxicity (Zhou et al. 2006). According to previous studies, drug release from Cs microparticles could be controlled by crosslinking the matrix using a chemical crosslinking agent such as glutaraldehyde. However, chemical crosslinking agents may cause undesirable mucosal irritation (Ko et al. 2002). To overcome this disadvantage, ionic crosslinking interaction with tripolyphosphate (TPP) has been applied. TPP is nontoxic and multivalent anions. It can interact with cationic Cs by electrostatic forces (Shu and Zhu 2000). Taking into account the potential of microparticles to improve the bioavailability of lipophilic drugs, the aim of this work was to prepare microparticles with TPP and Cs and assess the feasibility of Cs microparticles to enhance the oral bioavailability of Cur.

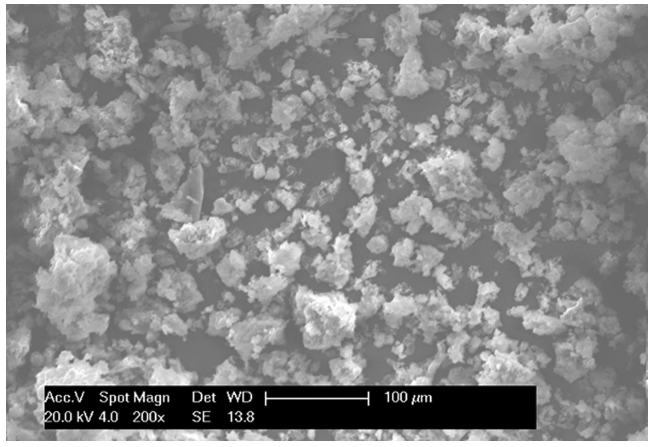

Fig. 1: Microparticles under scanning electron microscopy

2. Investigations, results and discussion

2.1. Characterization of microparticles

In this study, microparticles presented acceptable drug loading of 33.5 ± 1.15% and encapsulation efficiency of 85.2 ± 0.92%. SEM micrographs (Fig. 1) showed that the microparticles were not completely spherical in shape, and had a rough surface. Due

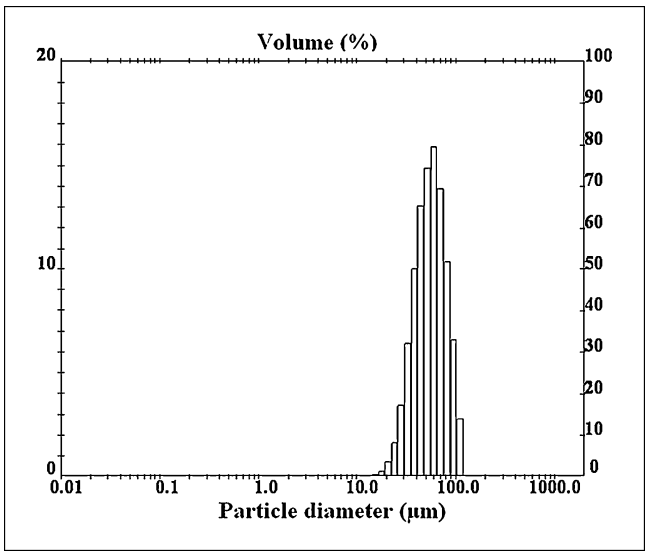

Fig. 2: Size distribution of microparticles

to the ionic interaction nature of crosslinking, the charge density of Cs and TPP under preparation conditions affected microparticle formation. pH of the TPP solution was determined to be 8.6. In this solution, TPP was dissociated into OH^- and TPP ions ($HP_3O_{10}^{4-}$ and $P_3O_{10}^{5-}$). Cs is a weak polybase, the ionization of the amine groups decreases in the solution with high pH. Therefore, TPP-Cs microparticles prepared in the original TPP solution were dominated by deprotonation and slightly ionic-crosslinking and eventually, formed rough surface (Shu and Zhu 2000; Ko et al. 2002). As shown in Fig. 2, microparticles had a mean particle size ($d_{4.3}$) of 58.50 μm with a dispersity ($d_{0.1}$–$d_{0.9}$) of 33.71–87.90 μm.

2.2. *In vitro release*

The drug release behaviors of Cur and microparticles were studied and shown in Fig. 3. It was evident that the rate of dissolution of pure drug was low: only 20% within 12 h. In the case of microparticles, the dissolution of Cur was increased. The cumulative drug release was found to be 90%. During the testing, it was noted that microparticles sank immediately, whereas pure drug kept floating on the surface of the dissolution medium for a longer period of time, indicating the enhanced hydrophilicity of the drug incorporated into microparticles. Moreover, according to a previous report, an open pore structure of Cs microparticles

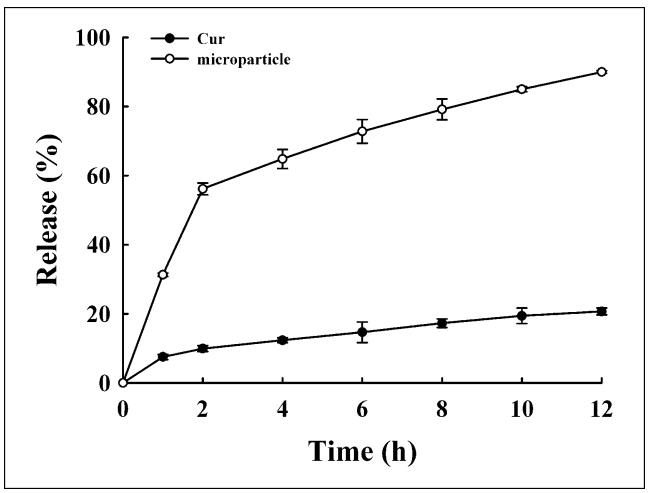

Fig. 3: *In vitro* release profiles of Cur and microparticles (n = 3)

Table: Pharmacokinetic parameters of Cur after oral administration of pure drug and microparticles (n = 6)

Pharmacokinetic parameters	Cur	Microparticles
T_{max} (h)	0.66	1.30
C_{max} (ng/ml)	87.06	270.24
K_e (/h)	1.431	0.307
$T_{1/2}$ (h)	0.493	2.257
AUC (ngh/ml)	156.363	1311.785

Data are means, $^*P < 0.05$ compared with pure drug

was observed when the microparticles were prepared in TPP solution of pH 8.6 (Ko et al. 2002), which was also responsible for the improved drug dissolution. The drug release profile of Cur fitted Higuchi equation, indicating the diffusion mechanism. For microparticles, the drug release mechanism could be explained by the first-order model. First-order law is usually applied to drugs released from porous matrices where the release rate of the drug is proportional to the amount of drug remaining in the dosage form (Tanaka et al. 2005). The correlation coefficients of the equations were 0.9962 and 0.9831, respectively.

2.3. *In vivo pharmacokinetic study*

A sensitive reversed-phase HPLC method was used for the detection of Cur in plasma after oral administration. The lowest detectable limit of Cur was 8.0 ng/ml. There were no endogenous components that interfered with the detection of Cur. The extraction recovery at low, medium and high concentrations were between 80.4% and 88.0% (n = 3). Good linearity of the standard curves having Cur concentrations was obtained ranging from 25.0 to 500.0 ng/ml, and correlation coefficients over this concentration range were 0.9994 (n = 3). The assay was accurate and reproducible with coefficients of variation ranging from 1.3% to 3.4%.

Cur loaded microparticles were designed to improve the oral bioavailability of the drug. The profiles of the plasma concentrations of Cur versus time after oral administration of the pure drug and the microparticles are depicted in Fig. 4. The pharmacokinetic data between Cur and microparticles were compared for statistical significance by Student's "t" test. $P < 0.05$ was considered as significant. The plasma concentration of the

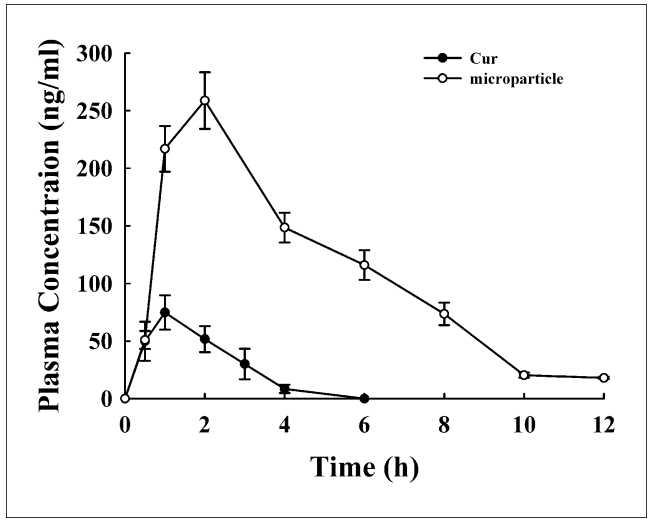

Fig. 4: Plasma concentration profile of Cur in rats following administration of pure drug and microparticles (50 mg/kg). Data are means ± S.D., n = 6

microparticles was significantly higher than that of Cur at each time point. The parameters of Cur in the microparticles were compared with those of the parent drug and are summarized in Table.

Microparticles obtained prolonged T_{max} value compared with that of Cur, indicating an extended therapeutic period of microparticles. Cur suspension upon oral administration resulted in sharp C_{max} within 0.66 h and the plasma concentration of the drug decreased rapidly, indicating rapid metabolism of Cur. Whereas, relatively slow increase and sustained plasma concentration of Cur for a longer time was observed after administration of the microparticles. Meanwhile, microparticles retarded the elimination of Cur and prolong the drug transit time *in vivo* with lower K_e and longer $T_{1/2}$. The AUC value of Cur after oral administration of microparticles was 8.4 times that of the pure drug meant an improved relative bioavailability.

Cur is a poor water-soluble drug with high lipophilicity (Maiti et al. 2007). The dissolution of the drug is the rate-limiting step for its absorption *in vivo*. However, enhancing the drug dissolution is not enough to improve the oral bioavailability of Cur. Cur easily tends to be inactivated due to extensive elimination in the body and rapid degradation at basic pH, e.g. intestinal juice (Anand et al. 2007; Tiyaboonchai et al. 2007; Shaikh et al. 2009). Previous studies have reported an enhanced bioavailability and prolonged constant drug plasma concentrations obtained after oral administration of microparticles (Yang et al. 2004; Huang et al. 2006), which was also the case in this study. The longer T_{max}, higher C_{max}, lower K_e and larger AUC obtained by microparticles upon oral administration suggested that the proposed microparticles could sustain the absorption of Cur, prolong the drug transit time *in vivo*, prevent metabolism and degradation of the prototype molecules, and eventually, enhance the bioavailability of the drug, according, improve therapeutic efficacy, decrease doses and side effects of the drug after oral administration.

To improve oral bioavailability of the poorly water-soluble drug Cur, Cs-TPP microparticles were successfully developed by the ionic crosslinking technique. The characteristics were investigated by particle size distribution, morphological examination, and the drug loading and encapsulation efficiency determination. The *in vitro* release of Cur was enhanced from microparticles. The proposed microparticles significantly prolonged the absorption of Cur and enhanced its bioavailability compared with the parent drug. Our studies proved that the developed microparticle is a promising and convenient method for improving oral absorption of poorly soluble drugs like Cur.

3. Experimental

3.1. Materials

Cur was purchased from Tianjin GuangFu Fine Chemical Research Institute, China. Cs (D041013210, Zhejiang Golden-shell Biochemical Co., Ltd., China) was used as carrier. TPP (Tianjin Yuanli Chenical Co., Ltd., China) was used as ionic crosslinking agent. Methanol and acetonitrile of HPLC grade were purchased from SK chemicals agent Co., Ltd. (Ulsan, Korea). All other chemicals were of analytical grade. Double distilled water was used throughout the work.

3.2. Preparation of microparticles

Microparticles were prepared by the ionic gelation technique. Cs (0.1 g) was dissolved in 10 ml acetic acid aqueous solution (1%, v/v) at 25 °C. Due to its water-insolubility, Cur (0.1 g) was dissolved in diethylene glycol monoethyl ether (10 ml) firstly and then added into Cs solution under mechanical stirring until a transparent yellow solution was obtained. Microparticles were formed spontaneously upon the addition of 40 ml TPP aqueous solution (0.3%, w/v) to the mixed solution under magnetic stirring for 30 min. Then, the microparticles were separated by centrifugation for 10 min at 10000 rpm, washed with water repeatedly, freeze-dried for 12 h, and then stored at 4 °C before further used.

3.3. Characterization of microparticles

3.3.1. Particle size analysis

The particle size distribution was determined by laser diffractometry with a Mastersizer 2000 Coulter (Malvern Instruments Ltd, UK) at 25 °C. The particle size analysis data were evaluated using the number distribution. The microparticles were dispersed in water and diluted properly for size determination. The mean diameter over the volume distribution $d_{4.3}$ was used as a particle size distribution parameter. $d_{0.1}$ and $d_{0.9}$ were also determined and correspond to the diameters at 10% and 90% cumulative volumes, respectively.

3.3.2. Scanning electron microscopy (SEM)

The morphological examination of the microparticles was performed using SEM (JEOL JSM5200, Japan). The lyophilized microparticles were evenly distributed onto a conductive tab on a double side tape and then sputter coated with gold in a cathodic evaporator.

3.4. Drug loading (DL%) and encapsulation efficiency (EE%)

To determine the drug content, the weighed amount of freeze-dried microparticles were dissolved in acetic acid at 25 °C, followed by centrifugation. The supernatants were appropriately diluted with mobile phase and analyzed by HPLC (LabAlliance 201, China). All the manipulation was performed in triplicate. A reversed-phase C_{18} column (Thermo, 250 × 4.6 mm, 5 μm particle size) linked with a uniphase C_{18} guard column was used. The mobile phase consisted of methanol and 4% (v/v) acetic acid solution (85:15, v/v). The flow rate of the mobile phase was 1 ml/min and the injection volume was 20 μl. The ultraviolet detector was set at a wavelength of 418 nm and the temperature of the column was maintained at 30 °C. The linearity was performed with a six-point calibration curve. The method was found to be linear over the examined concentration range of 0.1–10.0 μg/ml. The drug loading and encapsulation efficiency were calculated using the following equation. Data represent the arithmetic mean ± standard deviation (S.D.) (n = 3).

DL (%) = (weight of Cur in microparticles/weight of microparticles) × 100%.

EE (%) = (weight of Cur in microparticles/weight of drug added) × 100%.

3.5. In vitro release study

The *in vitro* release characteristics of Cur were determined according to USP dissolution II paddle method at a rotation speed of 100 rpm in 900 ml 0.1 M HCl (Komal et al. 2011) containing 0.05% (w/v) polysorbate 80 at 37 ± 0.5 °C using a dissolution apparatus (RCZ-8A, Precise Apparatus of Tianjin University Co., Ltd, China). Samples of Cur and microparticles equivalent to 8 mg of Cur were placed in each vessel. At specified time intervals, a 1 ml sample was withdrawn and replaced by the fresh media. The samples were then filtered through a 0.45 μm syringe filter and the content was determined with the abovementioned HPLC method. The study was performed in triplicate. Data represent mean ± S.D. (n = 3). The mechanisms of drug release from all formulations during dissolution investigations were determined with various release kinetic models including zero-order, first-order and Higuchi model employing the following set of equations (Das et al. 2010):

Zero-order mode

$$Q = k_0 t \quad (1)$$

First-order model

$$Q = 1 - \exp(-k_1 t) \quad (2)$$

Higuchi model

$$Q = k_H t^{1/2} \quad (3)$$

where, Q refers to the fraction of drug released at a particular time, t. The terms k_0, k_1 and k_H correspond to the release kinetic constants obtained from the linear curves of zero-order, first-order and Higuchi model, respectively.

3.6. Bioavailability and pharmacokinetic study after oral administration

Twelve male Wistar rats (obtained from Tianjin Radiation Medicine Institute, Chinese Academy of Medical Sciences, 250–300 g) were used for the oral administration study. All animal experiments complied with the requirements of the National Act of the People's Republic of China on the use of experimental animals. The study was approved by the Ethics Committee on Animal Research of Chinese Academy of Medical Sciences. The rats were divided in two groups and fasted overnight. The drug and microparticles

were suspended in 1 ml of sodium carboxymethyl cellulose solution (1%, w/v) and immediately administered at an equivalent dose of 50 mg Cur/kg per animal through oral gavage.

At predetermined time intervals, blood samples (0.5 ml) were collected *via* the inner canthus into heparinized tubes and separated immediately by centrifuging at 3500 rpm for 10 min. After each sampling, 500 μl of methanol was added to 100 μl of plasma, and the solution was shaken together for 2 min. The organic layer was separated by centrifugation at 10000 rpm for 15 min. The organic solvent was evaporated under nitrogen gas, and the residue was reconstituted in 100 μl of mobile phase and 20 μl were injected onto the HPLC column.

The HPLC method was performed as previously reported (Maiti et al. 2007) on the same LabAlliance 201 system and the column described above. Methanol, 2% acetic acid and acetonitrile at a ratio of 5:30:65 (v/v) were used as mobile phase with a flow rate of 1 ml/min at a wavelength of 425 nm. Calibration curves were prepared by linear regression analysis of five blank plasma samples (0.1 ml) added with varying concentrations of Cur covering the range of 25.0–500.0 ng/ml.

Samples were analyzed as described above. The pharmacokinetic parameters, e.g. the time to reach maximum plasma concentration (T_{max}), peak plasma concentration (C_{max}), elimination rate constant (K_e), half life ($T_{1/2}$), and the area under the curve from 0 to t (AUC) were computed using the Practical Pharmacokinetic Program Version 97 (the Chinese Society of Mathematical Pharmacology, China). The relative bioavailability of microparticles compared with Cur was calculated by comparing those two AUCs.

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