Colloids and Surfaces B: Biointerfaces 133 (2015) 108–119

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

The mechanism of self-assembled mixed micelles in improving curcumin oral absorption: *In vitro* and *in vivo*

Jinling Wang[a], Wenzhuan Ma[a,b], Pengfei Tu[a,*]

[a] *Modern Research Center for Traditional Chinese Medicine, Beijing University of Chinese Medicine, Beijing 100029, PR China*
[b] *School of Chinese Materia Medica, Beijing University of Chinese Medicine, Beijing 100102, PR China*

ARTICLE INFO

Article history:
Received 24 December 2014
Received in revised form 22 May 2015
Accepted 26 May 2015
Available online 8 June 2015

Keywords:
Curcumin
Polymeric micelles
Intestinal absorption
Endocytosis
Transcytosis
Oral bioavailability

ABSTRACT

Curcumin-loaded self-assembled polymeric micelles (Cur-PMs) were designed to increase oral bioavailability of curcumin and investigate the oral absorption mechanism *in vitro* and *in vivo*. The Cur-PMs were spherical nano-size particles 17.82 ± 0.33 nm in size, with a drug loading of 3.52 ± 0.18%, and encapsulation efficiency as high as 93.08 ± 2.23%. The intestinal absorption of Cur-PMs in the duodenum, jejunum, and ileum was 3.09-, 6.48-, and 1.78-fold greater than that of curcumin solution (Cur-Sol) at 0.5 h. The cellular uptake of Cur-PMs in Caco-2 cells was significantly enhanced in comparison with Cur-Sol by caveolae-mediated and clathrin-mediated endocytosis. Moreover, the apparent permeability coefficient (Papp) of Cur-PMs was 3.50-fold higher than that of Cur-Sol in Caco-2 transport studies. The transport mechanism of Cur-PMs into the system circulation was not paracellular transport through opening the tight junctions, but was by energy-dependent, macropinocytic transcytosis and lymphatic transport pathways. Furthermore, the $AUC_{(0-t)}$ value of Cur-PMs was improved 2.87-fold compared with that of Cur-Sol after oral administration in rats. Therefore, self-assembled polymeric micelles could be a promising vehicle to efficiently improve the oral absorption of curcumin.

1. Introduction

Curcumin (Cur), a natural polyphenolic compound derived from *Curcuma longa* L (jianghuang), has multiple biological activities such as anti-inflammatory [1], antineoplastic [2], antioxidant [3], and chemopreventive activities [4,5]. On the basis of these activities, more attention has been focused on curcumin due to its low cost and pharmacologic safety. Unfortunately, despite having promising pharmacological properties, curcumin oral therapy has been greatly restricted in clinical applications due to poor hydrophilic activity, low stability, rapid metabolic elimination, and low intestinal permeability [6].

Although in the past decades, several strategies have been used to improve the oral absorption of curcumin, such as microspheres[7], phospholipid complexes [8], solid lipid nanoparticles [9], nanoemulsions [10], and solid dispersions [11,12], there are still limitations and challenges in drug transport across epithelium and systemic absorption. Therefore, it is still necessary to find new approaches to improve oral delivery of curcumin to achieve its desirable pharmacological effects.

Currently, more attention has been focused on self-assembled polymeric micelles to improve the oral absorption of hydrophobic drugs. Polymeric micelles could self-assemble in aqueous medium and encapsulate drugs into their hydrophobic core. Polymeric micelles are widely used as drug carriers because of their advantages in changing the pharmacokinetic properties and improving the therapeutic efficacy of hydrophobic drugs by encapsulating hydrophobic drugs in hydrophobic core [13,14]. The hydrophilic shell could effectively protect drugs from hydrolysis and enzymatic degradation. Micelles have some advantages for oral drug delivery: (1) delivery of hydrophobic drugs, (2) improvement of drug stability in the gastrointestinal tract, (3) facilitation of transport across the intestinal barrier, and (4) promotion of endocytosis and transcytosis [15,16]. Therefore, polymeric micelles are a promising vehicle to improve oral absorption of hydrophobic drugs, mainly due to their unique structure and inherent properties, such as nanometer size, biocompatibility, sustained release of incorporated drugs, and improved solubility and intestinal permeability [17–19].

In the present study, curcumin-loaded polymeric nanomicelles (Cur-PMs) were fabricated with TPGS2 K, HS15, and Pluronic F127 to enhance the oral absorption of curcumin and elucidate the oral absorption mechanism of Cur-PMs. Therefore, the physicochemical properties and intestinal absorption of Cur-PNs were investigated. Furthermore, Caco-2 cells were used to investigate cellular

* Corresponding author. Tel.: +86 10 82802750; fax: +86 10 82802750.
E-mail address: pengfeitu@163.com (P. Tu).

http://dx.doi.org/10.1016/j.colsurfb.2015.05.056
0927-7765/

uptake and transport mechanisms of Cur-PMs in comparison with curcumin solution (Cur-Sol). In particular, oral pharmacokinetic behavior and lymphatic pathways were studied in rats to evaluate the effects of Cur-PMs on oral absorption and lymphatic transport.

2. Materials and methods

2.1. Materials

Curcumin (Cur) was obtained from Sigma-Aldrich (St. Louis, MO, USA). HS15 (Octadecanoic acid, 12-hydroxy-polymer with alpha-hydro-omega-hydroxypoly (oxy-1,2-ethanediyl) and Pluronic F127 were gifted from Beijing Fengli Jingqiu Commerce and Trade Co., Ltd (China, Beijing). Di-tocopherol polyethylene glycol 2000 succinate (TPGS2 K) was synthesized by our group [20,21]. Penicillin–streptomycin solution and Hank's buffered salt solution (HBSS) were purchased from Hyclone (Logan, USA). 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) and trypsin were also purchased from Sigma–Aldrich (St. Louis, MO, USA). All solvents used in the investigation were of analytical reagent grade.

2.2. Preparation and characterization of Cur-PMs

Cur-PMs were prepared *via* a solvent evaporation method. Briefly, 20 mg of curcumin, 200 mg of TPGS2 K, 200 mg of HS15, and 40 mg of Pluronic F127 were co-dissolved in 5 mL dehydrated alcohol under mild stirring and evaporated to form a thin film by rotary vacuum evaporation. Subsequently, the co-evaporation was hydrated in 4 mL PBS (pH7.4), stirring 3 h at 37 °C, to form curcumin polymeric micelles (Cur-PMs) spontaneously. The curcumin micellar solutions were centrifugated (12,000 rpm, 10 min, and 4 °C) and filtered through a 0.22 μm filter (Milipore Co., USA) to remove unencapsulated curcumin. Blank polymeric micelles were prepared in the same way, but without curcumin.

Size distribution and zeta potential of blank and curcumin polymeric micelles were evaluated by dynamic light scattering (DLS) by Zetasizer (Nano ZS 90, Malvern Co., UK). Measurements were repeated in triplicate.

The morphology of blank and Cur-loaded micelles was evaluated on transmission electron microscopy (TEM, Tecnai 20 200 kV, FEI). Briefly, micelles were diluted and deposited on a copper grid. Then, the grid was washed with double-distilled water twice and negatively stained with 1% uranyl acetate before observation by TEM.

The physical state of Cur in Cur-loaded micelles was characterized by DSC analysis on a TA-60 WS Thermal Analyzer (Shimadzu, Japan) at a temperature range of 30 −200 °C, with a rate of 10 °C/min.

Encapsulation efficiency (EE) and drug-loading (DL) of Cur micelles were measured by high performance liquid chromatography (HPLC, Agilent 1260). The HPLC conditions were as follows: Eclipse XDB-C18 (4.6 × 250 mm, 5 μm, Agilent); mobile phase: acetonitrile-0.3% acetic acid water (70:30, (v/v)); detection wavelength 227 nm and flow rate 1 mL/min. Briefly, Cur-PMs were diluted with methanol and ultrasonic energy was used to destroy micellar integrity. Moreover, a certain volume of Cur-PMs was freeze-dried and weighed. The EE and DL was calculated using the following equation:

$$\text{EE}\% = \frac{\text{amount of the drug encapsulated}}{\text{amount of drug added}} \times 100\%$$

$$\text{DL}\% = \frac{\text{amount of drug encapsulated}}{\text{weight of micelles}} \times 100\%$$

The release profiles of Cur from Cur-PMs were conducted by a modified dialysis method. The Cur-PMs were sealed in dialysis bags (MWCO = 14,000) and incubated in simulated intestinal fluid (SIF) or simulated gastric fluid (SGF) medium containing 0.5% (w/v) Tween 80 at 37 °C with gentle shaking (100 rpm) in the dark[22]. A volume of 1000 mL SGF contained 3.2 g pepsin and 2.0 g sodium chloride with a hydrochloric acid adjusted pH of 1.2. SIF contained 10 g trypsin and 6.8 g monopotassium phosphate with a sodium hydroxide adjusted pH of 6.8 per 1000 mL volume. At desired time intervals, 1 mL of medium was withdrawn and analyzed by HPLC as described above. Experiments were carried out in triplicate.

2.3. Caco-2 cell culture

Human colon cancer Caco-2 cells were cultured in a culture flask containing Dulbecco-modified Eagle's minimal essential medium (DMEM) supplemented with 10% (v/v) fetal bovine serum, 1% (v/v) nonessential amino acids, 100 U/mL penicillin, and 100 μg/mL streptomycin, with a constant incubated temperature of 37 °C in 5% CO_2.

2.4. Cytotoxicity study and LDH release assay

A cell counting kit-8 (CCK-8) assay was applied to evaluate cytotoxicity of Cur-PMs on Caco-2 cells. Caco-2 cells were seeded at a density of 1×10^5 cells/mL in 100 μL DMEM culture medium in a 96-well plate at 37 °C and 5% CO_2. After incubation, Caco-2 cells were exposed to a series of Cur-Sol, Cur-PMs, and the physical mixture of curcumin and corresponding carriers with different Cur concentrations for 2, 24, 48, and 96 h. Meanwhile, serum-free medium (SFM) incubation without drug was utilized as a control. Then, CCK-8 solutions were added and incubated for another 4 h. Ultimately, the fluorescence intensity was assayed at 450 nm in a microplate reader (Bio-Rad 500, USA). Relative cell viability (R %) was calculated as follows:

$$\text{R}\% = \frac{\text{absorbance}_{\text{test}}}{\text{absorbance}_{\text{control}}} \times 100\%$$

A lactate dehydrogenase (LDH) assay kit (Beyotime, Haimen, Jiangsu, China) was used to determine whether Cur-PMs destroy cell membranes. Briefly, Caco-2 cells seeded in a 96-well plate were incubated with Cur-Sol and Cur-PMs for 2 h. Sole SFM solution was used as a control. After 1 h of incubation, 20 μL LDH release medium was added, followed by 1 h of co-incubation. At a determined time interval, 120 μL supernatant was transferred to a new plate. A 60 μL of LDH test medium was added and incubated for 30 min with shaking. Finally, the absorption was measured at 490 nm in a microplate reader (Bio-Rad 500, USA). The relative percentage (R %) was calculated as above.

2.5. Cellular uptake

The cellular uptake measurement was investigated by a flow cytometry system (FACS) and confocal laser scanning microscopy (CLSM). Caco-2 cells were seeded at a density of 1×10^5 cells per well in 24-well plates, and cultured for 15 days. The culture medium was exchanged every 2 days for the first week and everyday thereafter. Before the experiments, cells were washed with HBSS twice and pre-incubated for 30 min at 37 °C. Cur-Sol and Cur-PMs were incubated with different curcumin concentrations of 10, 20, and 30 μg/mL on Caco-2 cellular monolayers for 1 h to test the effects of concentration on cellular uptake. In addition, to identify the time effect on uptake, Cur-Sol and Cur-PMs with equivalent curcumin concentrations (30 μg/mL) were incubated for 30, 60, and 90 min, respectively. At determined intervals, the medium was removed and the cellular monolayer was washed with 4 °C PBS three times.