Journal of Functional Foods 44 (2018) 137–145

Contents lists available at ScienceDirect

Journal of Functional Foods

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

Enhanced oral bioavailability, reduced irritation and increased hypolipidemic activity of self-assembled capsaicin prodrug nanoparticles

Yingshu Feng[1], Yuan Zhu[1], Jinyi Wan, Xia Yang, Caleb Kesse Firempong, Jiangnan Yu*, Ximing Xu*

Center for Nano Drug/Gene Delivery and Tissue Engineering, School of Pharmacy, Jiangsu University, Zhenjiang 212013, China

ARTICLE INFO

Keywords:
Capsaicin
Prodrug
Self-assembling
Bioavailability
Gastrointestinal irritation
Antihyperlipidemic effect

ABSTRACT

The study was aimed at formulating self-assembled capsaicin prodrug nanoparticles (Cap-SSVE NPs) with improved bioavailability, increased antihyperlipidemic activity and less gastrointestinal mucosa irritations. The *in vitro* release profile of the developed Cap-SSVE NPs was stable in HCl solution (pH 1.2) and distilled water, but very susceptible in PBS (pH 6.8 and 7.4). The relative oral bioavailability of Cap-SSVE NPs was 3.2 folds higher than free capsaicin, with an extended T_{max} (0.25–1 h). The Cap-SSVE NPs decreased certain lipid profile indices of both the liver tissue (Total lipids and TG) and the blood sample (TC, TG, LDL and TBA) with elevated levels of HDL in high fat diet induced hyperlipidemic rats. The nanoparticles also predominantly accumulated in the liver with reduced irritations in the gastrointestinal mucosa. Collectively, the formulated Cap-SSVE NPs showed an enhanced bioavailability, increased hypolipidemic effects and reduced mucosa irritations.

1. Introduction

Capsaicin (8-methyl N-vanillyl-6-nonenamide, Cap), the main pungent ingredient in hot peppers (*Capsicum annuum*), belongs to the Solanaceae family. The *Capsicum annuum* is native to South America and also widely cultivated all over the world. As a spice, capsaicin has been commonly used to treat diarrhea, emesis, chilblain and other skin disorders in China, spanning over several centuries. The capsaicin has also recently shown a variety of *in vitro* and *in vivo* pharmacological activities including antioxidant (Alvarez-Parrilla, La, Amarowicz, & Shahidi, 2011), anti-inflammatory (Chen & Kang, 2013), analgesic (Gawecka & Viken, 2012), hypolipidemic (Manjunatha & Srinivasan, 2007, Kempaiah & Srinivasan, 2006) and anti-tumor (Chen et al., 2016, Garufi, Pistritto, Cirone, & 'Orazi, 2016) properties. The promising compound from food product has therefore attracted much attention due to its great future prospects in treating diseases of mankind.

Interestingly, the full application of capsaicin has been limited by its low water solubility, which restricts its dispersion in aqueous medium for effective release. Therefore, the poor absorption and low bioavailability of capsaicin continue to present an enormous challenge to the use of this drug. The capsaicin also produces strong burning sensation and related gastrointestinal mucosa irritations when ingested (González, Antonio, Uuh-Chim, & Vázquez-Flota, 2010, Hayman & Kam, 2008). In this regard, it is imperative to find an effective oral delivery system to enhance the solubility, reduce the irritations and significantly increase the biological functions of capsaicin. Several attempts have been made to address the issue via different formulations such as liposomes, micelles and microemulsions (Zhu, Wang et al., 2015; Zhu, Zhang et al., 2015; Zhu et al., 2014). However, these nanoparticles produced low entrapment efficiency and virtually unacceptable burst release which led to their poor application.

Self-assembled drug delivery systems (SADDS) refer to self-assembled micelles, vesicles and other highly dispersed aggregates of drugs or prodrugs with a certain active surface. Compared with conventional nano-formulations, SADDS have obvious advantages such as excipients free, higher stability and lower burst release phenomenon (Zou et al., 2017). Some studies have successfully prepared prodrugs by linking undissolved drugs like PTX and Dox to hydrophobic groups with disulfide bond bridges (Wang et al., 2014). The disulfide-linked prodrugs then became prone to forming relatively stable self-assembled nanoparticles in aqueous medium due to their flexible structure and appropriate electrostatic potential.

Abbreviations: VE, DL-α-Tocopherol; DMAP, 4-dimethylaminopyridine; Cap-SSVE, capsaicin prodrug; Cap-SSVE NPs, self-assembled capsaicin prodrug nanoparticles; TC, total cholesterol; TG, triglyc-erides; HDL, high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol; TBA, total bile acid; PDI, polydispersity index

* Corresponding authors at: Department of Pharmaceutics, School of Pharmacy, Center for Nano Drug/Gene Delivery and Tissue Engineering, Jiangsu University, Zhenjiang 212013, China.

E-mail addresses: yjn@ujs.edu.cn (J. Yu), xmxu@ujs.edu.cn (X. Xu).

[1] These authors contribute equally to this work.

https://doi.org/10.1016/j.jff.2018.03.006
Received 2 November 2017; Received in revised form 18 January 2018; Accepted 2 March 2018

In the present report, capsaicin prodrug self-assembled nanoparticles (Cap-SSVE NPs) were successfully prepared, characterized and their enhanced activities such as improved bioavailability, less gastrointestinal mucosa irritation and increased hypolipidemic activity in animal models also determined.

2. Materials and methods

2.1. Materials

Capsaicin (99.5% purity) was supplied by Chengdu Mansite Biotechnology Co., Ltd. (Sichuan, China). Dithiodiglycolic acid, DL-α-Tocopherol (VE), 98%, and methanol (chromatographic grade) were purchased from J & K Scientific Ltd. 4-Dimethylaminopyridine (DMAP) and formic acid (chromatographic grade) were purchased from Aladdin Chemistry Co., Ltd. Acetic anhydride, N,N′-Dicyclohexylcarbodiimide (DCC), ethyl alcohol and other chemicals of analytical grade were also obtained from Sinopharm Chemical Reagent Co., Ltd.

2.2. Synthesis of capsaicin prodrug

Capsaicin prodrug (Cap-SSVE) was synthesized according to previously described report (Wang et al., 2014). Dithiodiglycolic acid (1) was converted to the corresponding anhydride (2) with acetic anhydride as dehydration agent (Fig. 1A). Excessive acetic anhydride was removed with toluene under high vacuum at room temperature. The residue was made to react with VE, using DMAP as catalyst, to rapidly produce the acid (3). The product was condensed with capsaicin under DCC and DMAP to obtain the target compound, Cap-SSVE.

Mass spectral (MS) data was obtained using an analytical thermo HPLC system and an Ion Trap Mass Spectrometer (Thermo LXQ, USA) equipped with electrospray ionization (ESI) source, and analyzed using Thermo Fisher Xcalibur 2.0.7 SP1 software. The MS was recorded across the range of *m/z* 50–1000 in the positive mode. The sheath gas flow rate was 37 arb with aux gas flow rate of 9 arb, Ion Spray Voltage of 4.5 kV, capillary temperature of 305 °C, capillary voltage of 30 V and the tub lens of 120 V. Nuclear magnetic resonance (NMR) spectra were recorded on Bruker AV 400 spectrometer (Rheinstetten, Germany) with Methyl Sulfoxide-d6 as solvent and TMS as internal standard.

The product was purified using silica column chromatography to obtain a pale yellow solid with final yield of 60.7%. MS (*m/z*): 904.93 $[M+Na]^+$, 1785.66 $[2M+Na]^+$. The ^1H NMR (400 MHz, $CDCl_3$, ppm): δ 7.04 (d, J = 8.0 Hz, 1H), 6.93 (d, J = 1.9 Hz, 1H), 6.90–6.80 (m, 1H), 5.71 (s, 1H), 5.48–5.23 (m, 2H), 4.53–4.37 (m, 2H), 3.94 (s, 2H), 3.90 (s, 2H), 3.84 (s, 3H), 2.61 (t, J = 6.8 Hz, 2H), 2.32–2.20 (m, 3H), 2.14–1.96 (m, 12H), 1.90–1.75 (m, 2H), 1.72–1.58 (m, 9H), 1.48–1.38 (m, 4H), 1.31–1.25 (m, 9H), 1.20–1.11 (m, 5H), 0.98 (d, J = 6.6 Hz, 6H), 0.92–0.82 (m, 12H). ^{13}C NMR (400 MHz, $CDCl_3$): δ = 173.2, 168.2, 167.2, 182.1, 178.9, 140.5, 138.5, 137.8, 137.1, 127.5, 125.6, 123.5, 122.8, 121.1, 118.5, 111.3, 74.6, 53.2, 42.1, 40.5, 39.0, 36.9, 35.2, 33.9, 32.0, 31.1, 29.8, 28.6, 25.6, 25.2, 24.9, 22.3, 21.5, 21.0, 20.0, 13.0, 12.1, 11.1.

2.3. Preparation of Cap-SSVE NPs

The Cap-SSVE NPs were prepared based on previous studies (Wang et al., 2014). Briefly, the Cap-SSVE was dissolved in ethanol (0.5 mL) and then added dropwise to water, under mechanical stirring (~600–800 rpm) at room temperature, to produce a total volume of 10 mL. Under these conditions, the self-assembled Cap-SSVE NPs were spontaneously obtained (Fig. 1B).

2.4. Characterization of Cap-SSVE NPs

2.4.1. Transmission electron microscopy

The morphology of Cap-SSVE NPs (2 mg/mL) was examined using transmission electron microscopy (TEM, Tecnai 12, FEI, Amsterdam, Holland). A drop of diluted Cap-SSVE NPs (20 μL, 200 μg/mL) was placed on a copper grid and stained with phosphotungstic acid (2%). After drying at room temperature, the prepared thin film was observed under TEM.

2.4.2. Particle size analysis and zeta potential

The particle size distribution of Cap-SSVE NPs (2 mg/mL) was determined using dynamic light scattering (DLS) technique, with a Brookhaven BI-90 Plus instrument (Brookhaven Instruments Corp., Holtsville, NY, USA), at 25 °C. The data were analyzed using the software provided by Brookhaven. The zeta potential of Cap-SSVE NPs was also determined using a ZetaPlus zeta potential analyzer (Brookhaven).

2.4.3. In vitro chromatographic conditions

The HPLC system consisted of an LC-20AD pump, DGU-20A degasser, SIL-20AC Autosampler, CTO-20AC Column oven and SPD-M20A detector, with the detector linked to LabSolution Data Station Software (Shimadzu Corporation, Tokyo, Japan). Chromatographic separation was performed using Shimadzu Shim-pack CLC-C8 (M) column (4.6 × 150 mm, 5 μm particle size, Japan) with 0. 075% formic acid (A) and methanol (B) as the mobile phase for gradient elution at a flow rate of 1.0 mL/min at 30 °C: 70% B for 2 min and 100% B for 2.01–15 min. The detection wavelength was 280 nm with injection volume of 20 μL.

2.4.4. Self-assembling efficiency and stability of Cap-SSVE NPs

Self-assembling efficiency was determined according to previously reported method (Zhu, Wang et al., 2015). Briefly, different concentrations (2, 4 and 8 mg/mL) of Cap-SSVE NPs, each with a volume of 2 mL, were diluted to 10 mL with double-distilled water and filtered through a 0.8 μm cellulose nitrate membrane to remove the unassembled Cap-SSVE and free capsaicin. An aliquot of the filtrate (0.1 mL) was diluted with chromatographic methanol to 1 mL. The product was then vortexed for 3 min and centrifuged at 10,000*g* for 10 min. The supernatant was injected into HPLC to measure the content of the Cap-SSVE. The self-assembling efficiency (SE%) was determined using the Eq. (1) below:

$$SE\% = C/C_T * 100\% \quad (1)$$

where C and C_T represent the calculated concentration of Cap-SSVE and theoretical concentration of Cap-SSVE NPs, respectively.

The storage stability test of Cap-SSVE NPs at different concentrations (2, 4 and 8 mg/mL) was performed at 4, 25 and 40 °C respectively, with a storage time of 15 days. The appearance of the nano-particles was observed. The particle size and self-assembling efficiency were also monitored to evaluate the stability of Cap-SSVE NPs. Three parallel experiments were carried out in these determinations.

2.5. In vitro release of Cap-SSVE NPs

The *in vitro* release profile of Cap-SSVE NPs was expressed using the concentration of Cap-SSVE and capsaicin in four different media: double-distilled water, HCl solution (pH 1.2) and PBS (pH 6.8 and 7.4). The test was conducted in a water bath with oscillator at constant temperature (37 ± 1 °C). The Cap-SSVE NPs (1 mL, 2 mg/mL) were transferred into dialysis bags (8000–14,000 Da). The dialysis bags were then immersed in 100 mL of the different dissolution media. Each sample (1 mL) of the different media was taken at appropriate time intervals (5, 10, 20, 30 min, and 1, 2, 4 h) and methanol added to it. The concentrations of Cap-SSVE and capsaicin were measured using the same HPLC method stated in Section 2.4.3. The cumulative release (%) was calculated as the weight ratio of released Cap-SSVE or capsaicin to respective total Cap-SSVE or capsaicin in theory. Data were presented as mean of the triplicate samples.