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Research paper

Surface modification of solid lipid nanoparticles for oral delivery of curcumin: Improvement of bioavailability through enhanced cellular uptake, and lymphatic uptake

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ABSTRACT

Curcumin has been reported to exhibit potent anticancer effects. However, poor solubility, bioavailability and stability of curcumin limit its in vivo efficacy for the cancer treatment. Solid lipid nanoparticles (SLN) are a promising delivery system for the enhancement of bioavailability of hydrophobic drugs. However, burst release of drug from SLN in acidic environment limits its usage as oral delivery system. Hence, we prepared N-carboxymethyl chitosan (NCC) coated curcumin-loaded SLN (NCC-SLN) to inhibit the rapid release of curcumin in acidic environment and enhance the bioavailability. The NCC-SLN exhibited suppressed burst release in simulated gastric fluid while sustained release was observed in simulated intestinal fluid. Furthermore, NCC-SLN exhibited increased cytotoxicity and cellular uptake on MCF-7 cells. The lymphatic uptake and oral bioavailability of NCC-SLN were found to be 6.3-fold and 9.5-fold higher than that of curcumin solution, respectively. These results suggest that NCC-SLN could be an efficient oral delivery system for curcumin.

1. Introduction

Curcumin is a lipophilic polyphenolic compound extracted from Curcuma Longa (turmeric) and has shown pharmacological activities including anti-amyloid, anti-oxidant, anti-malarial, anti-inflammatory, anti-HIV, and anti-cancer properties [1,2]. Recently, the anti-cancer effect of curcumin has been widely investigated and it has been suggested as a potential agent for both prevention and treatment of breast cancer. It was known that curcumin not only reduces cell proliferation and metastasis, but also accelerates apoptosis by modulating a few pro-inflammatory factors, receptors, human epidermal growth factor receptor, transcription factors and peroxisome proliferator-activated receptor [3–5,1].

Despite their potential therapeutic effects, clinical application of curcumin via the oral route was hindered due to poor aqueous solubility, rapid degradation, extensive metabolism, inadequate tissue absorption, degradation at alkaline pH, and low bioavailability [4,6,7]. Hence, a new formulation system that improves stability of curcumin, aqueous solubility and bioavailability as well as allows controlled and sustained release of curcumin is required.

SLN made from biodegradable solid lipids exists in the submicron size range (50–1000 nm) and has attracted increasing attention in recent years. The advantages of SLN are as follows: possibility of controlled drug release and drug targeting, protection of incorporated compound against chemical degradation, no biotoxicity of the carrier, and no problems with respect to large scale production [8,9]. However, unstable release profile of drug from SLN in the gastrointestinal tract has been a major problem for oral administration because conventional SLN has shown a burst release of drugs in the acidic environment of the stomach (pH 1–3) and a sustained release in the intestinal environment (pH 6.8–7.4) [10–12]. Recently, lipid-based nanoparticles such as SLN [13–15] and micelle [16] have been used to improve an oral bioavailability of curcumin. However, additional technique such as coating method would be needed to further improve oral bioavailability of curcumin because the lipid nanoparticles are known to show burst release in acidic condition like stomach [11].

Chitosan is a cationic natural polysaccharide derived from deacetylated chitin and shows high biocompatibility, biodegradability, mucoadhesion, and low toxicity [17]. It is soluble at acidic pH, and its protonated form is only active as an absorption enhancer in acidic condition. Therefore, the use of chitosan for oral formulation has been limited by its low solubility above pH 5 [18]. In order to resolve this problem, modified chitosan which is able to be soluble above pH 5 could be used as coating layer onto

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SLN. There was a publication that TMC-SLCNs exhibited significantly improved oral bioavailability of curcumin in mice [15]. On the other hand, N-carboxymethyl chitosan (NCC) dissolved at above pH 5 was synthesized [19] and introduced as a coating layer to SLN encapsulating CUR in this study while TMC is soluble at wide range of pH. As such, NCC coating can deliver curcumin to intestine so that large quantity of curcumin is able to be absorbed by lymphatic uptake and mucus on intestine [11].

The first objective of this study was to fabricate NCC-SLN for preventing burst release of curcumin in simulated gastric fluid and to evaluate their physicochemical properties. The second objective was to explore the lymphatic uptake and oral bioavailability as well as cytotoxicity and cellular uptake against MCF-7 cells.

2. Materials and methods

2.1. Materials

Stearylamine, curcumin, chitosan (MW 140,000–220,000, deacetylation 60%), and 4′,6-diamidino-2-phenylindole (DAPI) were purchased from Sigma (Steinheim, Switzerland). Glyceryl monostearate (GMS) was obtained from Gattefosse (Lyon, France). Poloxamer 188 from BASF (Ludwigshafen, Germany) and soy lecithin was purchased from Junsei (purity 99.5%) (Tokyo, Japan). All other chemicals and reagents used were of analytical grade.

2.2. Synthesis and characterization of NCC

NCC was synthesized according to the previously established method [11,15]. Briefly, chitosan was dissolved in 0.7% (v/v) acetic acid solution (pH 3) overnight and the solution was filtered to remove undissolved chitosan. And then, glyoxylic acid monohydrate was added to the solution and stirred for 2 h. After formation of the imine compound, the pH was adjusted to 4.5 by addition of 1 N NaOH under stirring for 1 h. Then, 5% (w/v) of sodium borohydride was added to reduce the formed imine under magnetic stirring for 1 h and the product was isolated by precipitation through addition of excess of ethanol. The product was washed on a glass filter under vacuum. The product was purified by dissolving in water (pH 9) and subsequent acidification to pH 5 using 1 N HCl. Final product was collected by centrifugation at 5000 rpm. The product was converted into its sodium salt by dissolving the product in water and adjusting the pH to 10.5 using 1 N NaOH. The final NCC product was lyophilized.

2.3. Preparation of curcumin-loaded SLNs

Blank SLN and curcumin-loaded SLN (C-SLN) were prepared using modified hot homogenization and sonication method (Table 1). Curcumin (20 mg), GMS (200 mg), and soya lecithin (100 mg) were dissolved in 10 mL of chloroform. The organic solvent mixture was completely evaporated at 60 °C using rotary evaporator. Nitrogen was further blown on the lipid layer for 10 min to remove traces of chloroform completely. And then, drug-embedded lipid layer onto flask was melted by heating at 10 °C above the melting point of lipid. 1.5% (w/v) of poloxamer 188 solution (300 mg in 40 mL of distilled water) as aqueous phase was added to melted lipid and homogenized (Ultra Turrax T25, IKA, Ohio, USA) at 12,000 rpm for 3 min, followed by sonication for 10 min. The obtained solution was cooled down at 4 °C to obtain C-SLN. For blank SLN, all procedures were same without adding curcumin. For positively charged SLN (CC-SLN), stearylamine (20 mg) was added to the lipid phase. The formulations prepared were lyophilized using freeze-dryer (FD-1000, EYELA, JAPAN) for 48 h and stored at 4 °C for further studies.

2.4. Surface modification

NCC-SLN were surface-modified with NCC by surface charge interaction. Positively charged CC-SLN (200 mg) were dispersed in 1% (w/v) NCC solution (10 mL) and stirred for overnight. Surface modified nanoparticles were collected after centrifugation at 14,137.5*g* for 30 min and lyophilized using freeze-dryer (FD-1000, EYELA, JAPAN) for 48 h and stored at 4 °C for further studies. The ^{1}H-NMR spectrum of the synthesized NCC was obtained using Avance III (Bruker Analytik, Rheinstetten, Germany). Freshly synthesized NCC (1 mg) was dissolved in D_2O (700 μL) for analysis. FT-IR spectra of NCC was collected using Nicolet 380 FT-IR spectrometer (Thermo Scientific, USA) in reflectance mode, from 4000 cm^{-1} to 650 cm^{-1}.

2.5. Analysis of drug loading and encapsulation efficiency (EE) of SLNs

EE of C-SLN, CC-SLN and NCC-SLN was measured by ultracentrifugation with Amicon® filter tubes (Millipore, Carrigtwohill, Ireland). C-SLN, CC-SLN and NCC-SLN (0.5 mL) were transferred to a 0.5 mL of centrifugal filter tube and centrifuged at 14,137.5*g* for 30 min at 4 °C. The amount of unencapsulated curcumin in the supernatant was determined by HPLC. An Agilent 1100 liquid chromatography system with an autosampler and UV detector were used. The column used was a C18 column (4.0 × 250 mm, 5 μm particle size, Supelco™; MetaChem, USA). The flow rate of the mobile phase was 1 mL/min and the detection wavelength was set to 425 nm. The mobile phase was a mixture of 0.1% (w/v) formic acid and acetonitrile (50:50, v/v). EE was calculated as follow:

$$\text{EE}\ (\%) = (\text{total amount of curcumin} - \text{amount of curcumin in the supernatant}) / \text{total mass of lipids} \times 100$$

2.6. Measurements of particle size and polydispersity

The particle size and zeta potential analysis of blank SLN, C-SLN, CC-SLN, and NCC-SLN were measured by laser scattering analyzer (ELS-8000, Otasuka Electronics, Osaka, Japan). The lyophilized SLN (5 mg) was dispersed in distilled water (pH 7.0), added to the sample dispersion unit and sonicated in order to minimize the inter-particle interactions. The obscuration range was maintained between 20 and 50%. The instrument was set to measure the sample 50 times and the average volume mean diameter was obtained. As for zeta potential, the Helmholtz-Smoluchowski equa-

Table 1
Compositions of different formulations (unit: mg in 40 mL of suspension).

Formulation	Curcumin	GMS	Stearylamine	Lecithin	Poloxamer 188	NCC
Blank SLN	–	200	–	100	300	–
C-SLN	20	200	–	100	300	–
CC-SLN	20	200	40	100	300	–
NCC-SLN	20	200	40	100	300	200