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Self-assembly of green tea catechin derivatives in nanoparticles for oral lycopene delivery

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ABSTRACT

Lycopene is a natural anti-oxidant that has attracted much attention due to its varied applications such as protection against loss of bone mass, chronic diseases, skin cancer, prostate cancer, and cardiovascular disease. However, high instability and extremely low oral bioavailability limit its further clinical development. We selected a green tea catechin derivative, oligomerized (-)-epigallocatechin-3-O-gallate (OEGCG) as a carrier for oral lycopene delivery. Lycopene-loaded OEGCG nanoparticles (NPs) were prepared by a nano-precipitation method, followed by coating with chitosan to form a shell. This method not only can easily control the size of the NP to be around 200 nm to improve its bioavailability, but also can effectively protect the lycopene against degradation due to EGCG's anti-oxidant property. OEGCG was carefully characterized with nuclear magnetic resonance spectroscopy and mass spectrometry. Lycopene-loaded polylactic-*co*-glycolic acid (PLGA) NPs were prepared by the same method. Chitosan-coated OEGCG/lycopene NPs had a diameter of 152 ± 32 nm and a ζ-potential of 58.3 ± 4.2 mv as characterized with transmission electron microscopy and dynamic light scattering. The loading capacity of lycopene was 9% and encapsulation efficiency was 89%. FT-IR spectral analysis revealed electrostatic interaction between OEGCG and chitosan. Freeze drying of the NPs was also evaluated as a means to improve shelf life. Dynamic light scattering data showed that no aggregation occurred, and the size of the NP increased 1.2 times (S_f/S_i ratio) in the presence of 10% sucrose after freeze drying. The in vitro release study showed slow release of lycopene in simulated gastric fluid at acidic pH and faster release in simulated intestinal fluid. In an in vivo study in mice, lycopene pharmacokinetic parameters were improved by lycopene/OEGCG/chitosan NPs, but not improved by lycopene/PLGA/chitosan NPs. The self-assembled nanostructure of OEGCG combined with lycopene may be a promising application in oral drug delivery in various indications.

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1. Introduction

Lycopene is a natural anti-oxidant and is a major component found in human serum. It has attracted much attention due to its varied applications such as protection against loss of bone mass, chronic diseases, skin cancer, prostate cancer, lung cancer, and cardiovascular disease [1–4]. However, similar to other natural anti-oxidants, lycopene's poor water solubility, high instability, and extremely low intestinal permeability limit its further clinical development [5]. Encapsulating hydrophobic compounds in polymeric nanoparticles not only improves solubility but also prevents degradation caused by low pH condition and enzymes in the gastrointestinal tract [6–8]. Nano-carriers have significantly improved the pharmacokinetics, but the large amount of excipient used without therapeutic effect is still a major concern. For this reason, some new formulations have not received US Food and Drug Administration (FDA) approval.

To overcome the limitations of safety, toxicity, and insufficient drug deposition of conventional nano-carriers, one emerging approach is self-assembly of therapeutically effective macromolecules with drugs for the bottom-up construction of nano-formulations. Epigallocatechin-3-O-gallate (EGCG) and oligomerized (-)-EGCG (OEGCG) are found in green tea and red wine, and those from food-grade ingredients could be choices from which to generate nanoparticles (NPs) [9]. Chung's group has pioneered the design of OEGCG as a nano-carrier for loading chemotherapy drugs in the aqueous phase [10]. An in vivo study from his group suggested that the combination of OEGCG carrier and drug also dramatically increased the treatment effect compared with the drug alone.

Green tea-based NPs with small sizes (50–200 nm) and anti-oxidant, anti-inflammation, and anticancer properties have attracted

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enormous attention due to their applications in the fields of cosmetic and drug delivery [11]. Liang et al. found that hyaluronic acid-green tea catechin conjugates could self-assemble into NPs with plasmid DNA and branched polyethylenimine via electrostatic interaction; this complex more efficiently transfected genes to colon cancer cell line due to their enhanced stability [12]. Because the EGCG is hydrophilic while many anticancer drugs are hydrophobic, it is difficult to load anticancer drugs directly to EGCG. Cheng et al. have reported success with first binding EGCG with an amphiphilic block polymer and then encapsulating doxorubicin through π − π stacking interactions. The cardiotoxicity of doxorubicin was reduced and attributed to anti-oxidative effects of EGCG [13]. Using the same new concept, Yan and Chen's group recently reported using therapeutic agents to prepare a nano-carrier [14]. NPs assembled from doxorubicin and chlorine associated via electrostatic, π − π stacking, and hydrophobic interactions. This assembly successfully suppressed tumor growth in mice after injection of different formulations. However, compared to parenteral administration, the oral administration of lycopene still represents a major challenge [15–18].

2. Materials and methods

2.1. Materials

Epigallocatechin gallate (EGCG) (>95%) and lycopene (>95%) were purchased from Vesino Industrial Co., Ltd., Tianjin, China. Poly(D,L-lactide-*co*-glycolide), PLGA, average 5000–15,000 g/mol; lactide:glycolide 50:50; chitosan oligosaccharide lactate (average 5000 g/mol); polyethylene glycol sorbitan monooleate (BioXtra); acetic acid (>99%); DMSO (99.9%); acetaldehyde (>99.5%); acetone (>99.5%); hexane (>95%); 2,6-*di-tert*-butyl-4-methylphenol (BHT, 99%); and HCl (37%) were purchased from Sigma-Aldrich, St. Louis, MO, USA and used without further purification unless otherwise noted. Simulated intestinal fluid and simulated gastric fluid were purchased from Ricca Chemical, Arlington, TX, USA. Deionized water (Millipore Milli-Q grade) with resistivity of 18.0 MΩ was used in all experiments. Immune-deficient male mice aged 5–6 weeks and weighing 18–20 g were purchased from Harlan Laboratories (Indianapolis, IN, USA). All animal studies were conducted at the animal facility of the Veteran Affairs Medical Center, Albany, NY, USA in accordance with the institutional guidelines for humane animal treatment and according to the current NIH guidelines. The animal protocol was approved by the Veteran Affairs Medical Center Institutional Animal Care and Use Committee. Mice were maintained under specific pathogen-free conditions and housed under controlled conditions of temperature (20–24 °C), humidity (60–70%), and 12 h light/dark cycle with ad libitum access to water and food. Mice were allowed to acclimatize for 5 d prior to the start of the study.

2.2. Synthesis of oligomerized EGCG

EGCG (0.3 g, 0.65 mmol) was first dissolved in 3 mL of DMSO and 10 mL of water. Then 0.84 mL of acetic acid and 0.14 mL of 1 mol/L HCl were added to decrease the pH of the solution from 7 to 2. Acetaldehyde (2.4 mL, 40 mmol) was added dropwise under vigorous stirring. The mixture was degassed under vacuum for 10 min and then filled with nitrogen. The reaction mixture was stirred for 48 h at 30 °C under nitrogen. Afterwards the solution was dialyzed to remove free EGCG. The oligomerized EGCG (OEGCG) was collected and lyophilized [9,19,20].

2.3. Preparation of lycopene/OEGCG nanoparticles via nano-precipitation

In a typical experiment, OEGCG (20 mg) and lycopene (10 mg) were first dissolved in 5 mL of acetone/ethanol yielding a 4 mg/mL OEGCG solution. Note that we slightly increased the temperature of the polymer solution to 50 °C to dissolve lycopene. Subsequently, the lycopene solution was added dropwise into 30 mL of water containing polyethylene glycol sorbitan monooleate (20 mg) and sonicated for 10 min. The entire solution was then sonicated for ~30 s using a probe sonicator. The residual acetone and ethanol were removed under vacuum rotary evaporation at 40 °C. The NP suspension was washed once with water using ultrafiltration centrifugation (9800 ×*g*, 4 °C, 60 min) and resuspended.

2.4. Preparation of lycopene/PLGA nanoparticles via nano-precipitation

PLGA (20 mg) and lycopene (4 mg) were dissolved in 5 mL of acetone, yielding a 4 mg/mL polymer solution. Note that we slightly increased the temperature of the polymer solution to 50 °C to dissolve lycopene. Subsequently, the remaining steps were done as described above.

2.5. Preparation of chitosan-coated lycopene/OEGCG nanoparticles via two components with opposite charges

One milligram of chitosan oligosaccharide lactate was dissolved in 1 mL water. Then this chitosan solution was added into the lycopene/OEGCG NPs solution under sonication and incubated for 30 min at room temperature. The solution was then washed once with water to remove free lycopene using centrifugation (14,800 ×*g*, 4 °C, 60 min) and resuspended.

2.6. Characterizations

Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Bruker Avance 800 MHz spectrometer at Rensselaer Polytechnic Institute (Troy, NY, USA). The spectra were recorded in DMSO-*d6* with water as the internal standard in ^{1}H NMR spectra. Transmission electron microscopy (TEM) studies were carried out using a JEOL 2010 transmission electron microscope with an accelerating voltage of 80 kV. TEM samples were prepared by casting the suspension of assemblies on a carbon-coated copper grid (300 mesh). Fourier transform infrared spectroscopy (FT-IR) investigations were carried out using a Thermo Nicolet Avatar 330 FT-IR spectrometer. The hydrodynamic diameters of lycopene NPs and ζ-potential were measured with dynamic light scattering (DLS) using a Zetasizer Nano ZS dedicated ζ-potential analyser (Malvern Instruments, Worcestershire, UK), and each batch was analysed in triplicate.

2.7. Freeze drying

Freeze drying was employed as a means to impart stability or improve shelf life of the developed formulations. In brief, 2 mL of the NP suspension was placed in 20 mL glass vials. Sucrose 10% (w/v) was added as a cryo-protectant to preserve the NP properties during the freezing step. Then the solution was frozen at −80 °C for 12 h, and afterward it was sublimated for 24 h under pressure of 0.110 mPa at room temperature. Finally, the NPs were collected and preserved in a freezer for later evaluation and analysis [20,21].

2.8. Determination of the lycopene encapsulation efficiency and loading capacity

The encapsulation efficiency of lycopene NPs was determined by analysing the lycopene loading in the NPs compared to the lycopene fed initially [22]. After lyophilization, the weighed NP powder was dispersed in 15 mL of hexane-water (9:1, *v*/*v*), followed by sonication for 10 min until no colour was observed in the water phase. Subsequently, the resulting solution was centrifuged at 9600 ×*g* for 10 min. The amount of lycopene in the hexane was determined at 475 nm using a NanoDrop 2000 UV–Vis spectrophotometer (Thermo Scientific, Waltham, MA, USA) and a calibration curve (Fig. S1). Lycopene

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