Enhancement of Oral Bioavailability of Tripterine Through Lipid Nanospheres: Preparation, Characterization, and Absorption Evaluation

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ABSTRACT: Oral delivery of anticancer drugs remains challenging because of limited water-solubility and/or poor permeability. Here, we aimed to enhance the oral bioavailability of tripterine (TRI, a plant-derived anticancer compound) using lipid nanospheres (LNs) and to determine the mechanisms of oral absorption. TRI-loaded LNs (TRI–LNs) were prepared by rapid dispersion of an ethanol mixture of TRI, lecithin, sodium oleate, and soybean oil into water. The obtained LNs were 150 nm in size with a high value of entrapment efficiency (99.95%). TRI–LNs were fairly stable and the drug release was negligible (<0.2%) in simulated physiological fluid. The pharmacokinetic results showed that LNs significantly enhanced the oral bioavailability of TRI with a relative bioavailability of 224.88% (TRI suspensions was used as a reference). The mechanistic studies demonstrated that improved intestinal permeability and post-enterocyte lymphatic transport were mainly responsible for the enhanced oral absorption. Our findings suggested that LNs may be a viable oral carrier for poorly bioavailable drugs. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci

Keywords: tripterine; lipids; nanospheres; oral drug delivery; bioavailability; permeability; absorption potential

INTRODUCTION

In recent years, numerous active chemical entities have been isolated from medicinal plants. Evidently, these new chemical entities, if developed into drugs, will provide an alternative choice to treat malignant diseases. However, majority of newly discovered compounds are poorly soluble and/or membrane impermeable, leading to failures in drug administration.[1] In an attempt to circumvent these problems, various strategies have been employed such as prodrug development with improved permeability,[2] pulmonary delivery,[3] transdermal delivery,[4] and nanocarrier delivery systems.[5] Of these, nanocarriers such as liposomes,[6] micelles,[7] microemulsions,[8] solid lipid nanocarriers (SLNs),[9] and nanostructured lipid carriers (NLCs)[10] are becoming a versatile platform for oral delivery of many drugs with solubility and permeability issues. Because of the superior permeability and lymphatic transport, lipid-based formulations play an important role in enhancing oral bioavailability of poorly absorbed drugs.[11]

Tripterine (TRI, also known as celastrol), a bioactive compound extracted from the medicinal plant *Tripterygium wilfordii* Hook. F, is traditionally used for the treatment of inflammatory and autoimmune diseases in China. In recent years, a variety of pharmacological activities have been reported for TRI, including antioxidant,[12] antirheumatoid arthritis,[13] and anticancer properties.[14] However, TRI is concerned with poor water solubility (ca. 0.3 μg/mL) and limited bioavailability, precluding its further development in medical applications. To achieve systemic administration, TRI was usually injected intraperitoneally to the animals. For instance, Huang et al.[15] prepared TRI-loaded liposomes and evaluated the pharmacokinetics and tissue distribution by intraperitoneal injection.

Oral administration is a preferred and compliant route for patients. Oral bioavailability has been improved through formulation of TRI into NLCs coated with cell-penetrating peptides.[16] However, there are limitations to these NLCs. The active pharmaceutical ingredients have to withstand a high temperature (usually above 60°C) during the preparation. Also, the preparation requires the use of organic solvents such as acetone and tetrahydrofuran, which are toxic to humans if not completely removed from the product.[17,18] Moreover, compared with the "liquid" lipids (e.g., medium chain triglycerides), the "hard" ones (e.g., stearic acid glycerides) have a weaker ability to enhance drug permeability.

To date, the choices for oral delivery of TRI are still rather limited; there is a need to develop a novel system that can deliver TRI orally with an acceptable bioavailability. In this article, we formulated a TRI-loaded carrier, namely, lipid nanospheres (LNs), through spontaneous self-assembly of the cargo and lipid materials based on the solvent-diffusion method. The engineered nanocarriers entirely comprise lipid components of soybean lecithin, sodium oleate, and a relatively small quantity of soybean oil, most of which are in the solid state under the ambient condition. The TRI-loaded LNs (TRI–LNs) were characterized by the particle size, entrapment efficiency (*EE*), drug leakage, differential scanning calorimetry (DSC), and so on. To predict the pre-enterocyte behaviors of TRI–LNs, lipolytic experiments were performed using fasted-state stimulated intestinal fluid (FaSSIF) containing lipase. The oral bioavailability was evaluated in rats and the absorption mechanisms were explored in a single-pass perfused intestine model.

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MATERIALS AND METHODS

Materials

Tripterine was purchased from Baoji Herbest Bio-Tech Company, Ltd. (Baoji, China). Soybean lecithin (S100), soybean oil, and sodium oleate were supplied by Lipoid (Ludwigshafen, Germany). Sodium taurocholate, lipase from porcine pancreas (>20,000 units/mg), glyceryl monooleate, chlorpromazine, simivastasin, cycloheximide, and filipin were obtained from Sigma–Aldrich (Shanghai, China). Sucrose was a kindly gift from Guangzhou Standard Pharma Ltd.(Guang zhou, China). Deionized water was prepared by a water purifier (Chengdu, China). HPLC-grade acetonitrile was from Merck (Darmstadt, Germany). All other chemicals were of analytical grade and used as received.

Preparation of TRI–LNs

Tripterine-loaded LNs were prepared by the solvent-diffusion technique. A typical formulation consisted of 50 mg TRI, 300 mg soybean lecithin, 100 mg sodium oleate, and 100 mg soybean oil. Briefly, all lipid components were dissolved in 0.5 mL ethanol and then rapidly injected into 20 mL water with a syringe. The materials were spontaneously assembled into nanospheres upon the solvent diffusion into the aqueous phase. Subsequently, the resultant nanosuspensions were evaporated under reduced pressure by a rotatory evaporator to remove the residual ethanol until the volume was condensed to 10 mL.

Characterization of TRI–LNs

The particle size of TRI–LNs was measured by dynamic light scattering using Zetasizer Nano ZS (Malvern, Worcestershire, UK) at 25°C. To determine the particle size, a 50 μL sample of TRI–LNs was diluted with deionized water to 1 mL and then subjected to laser diffraction. The data were analyzed with Mastersizer 3000 software to calculate the size of the particles.

The morphology of TRI–LNs was observed by negative transmission electron microscopy (TEM). TRI–LNs was first placed on a carbon-coated copper grid and then anchored to the supporter. After removal of excessive water using absorbent paper, a drop of 1% phosphotungstic acid was added to stain the sample for about 1 min. The pigmented particles were allowed to dry at ambient atmosphere and analyzed with TEM (JEM-1230, JEOL, Tokyo, Japan) at an acceleration voltage of 100 kV.

Entrapment efficiency of TRI in LNs was determined after separating free TRI from TRI–LNs using the centrifugal filter devices Amicon® Ultra-0.5 with a molecular weight cutoff (MWCO) of 50 kD (Millipore, Billerica, Massachusetts). The concentration of free TRI (M_{fre}) was determined by HPLC analysis. The *EE* was defined as the ratio of LN-entrapped TRI (M_{ent}) to total TRI (M_{tot}): $EE\ (\%) = (1 - M_{fre}/M_{tot}) \times 100\%$.

To assess the leakage of TRI from LNs, 1 mL of TRI–LNs was dialyzed against 2.5% Tween 80 solution (a sink condition was maintained) using the ready-to-use dialysis device Float-A-Lyzer® G2 with a MWCO of 100 kD (SpectrumLabs, Shanghai, China). The cumulative TRI amounts in dialyzate were monitored at 12 and 24 h, respectively. The percentage of drug leakage was calculated and expressed as mean ± SD ($n = 3$).

HPLC Assay

Tripterine in LNs and other samples (unless otherwise specified) was determined by the Dionex Ultimate 3000 HPLC system (Thermo Scientific, Tewksbury, Massachusetts) that was composed of a quaternary pump, a degasser, an autosampler, a column heater, and a multichannel rapid scanning UV–VIS detector. TRI was separated by a C18 column (SinoChrom ODS-BP, 5 mm, 4.6 × 200 mm^2; Elite, Dalian, China) guarded with a precolumn at 45°C. The injection volume was set to 20 μL and the detection wavelength was 425 nm. The mobile phase consisted of 89% methanol and 11% water with 0.25% phosphoric acid pumped at a flow rate of 1.0 mL/min.

Differential Scanning Calorimetry

An aliquot of the samples (TRI, lecithin, sodium oleate, physical mixture, and lyophilized TRI–LNs) (about 5 mg) was weighed into a nonhermetically sealed aluminum pan, and subjected to differential calorimetric scanning on a DSC 204 A/G phoenix instrument (Netzsch, Baveria, Germany). The samples were heated from 25°C to 250°C at a stepping rate of 10°C/min. The instrument was calibrated using indium. All the DSC measurements were carried out in the nitrogen atmosphere at a flow rate of 100 mL/min.

FTIR

FTIR spectrum was collected to further assess the possible interactions between TRI and lipid components in the LNs. In brief, the samples of TRI, lecithin, sodium oleate, physical mixture, and lyophilized TRI–LNs were ground and mixed thoroughly with KBr to obtain an infrared transparent matrix. FTIR scanning was performed on a Nicolet Avatar 360 spectrometer (Thermo Scientific), and the spectra were recorded from 4000 to 600 cm^{-1} with a resolution of 1.0 cm^{-1}.

Lipolytic Experiments

To predict the pre-enterocyte behavior of TRI–LNs, the lipolytic experiments were conducted as described with minor modifications.[19] The digestive medium mimicking the extraintestinal condition was FaSSIF,[20] which comprised 3.0 mM sodium taurocholate, 0.2 mM lecithin (based on the amount of phosphatidylcholine), 19.12 mM maleic acid, 34.8 mM glyceryl monooleate, 34.8 mM sodium hydroxide, and 68.62 mM sodium chloride (pH 6.5) supplemented with porcine pancreatic lipase (100 IU/mL). Experiments were performed at 37°C in a stirred plug-sealed flask and initiated by the addition of 1 mL TRI–LNs into 30 mL digestive medium. The particle size of LNs and pH were measured at specified time points. Release of TRI from LNs was determined by HPLC analysis. The percentage of lipolysis was estimated based on the amount of fatty acids produced, which was equivalent to the titrated amount of NaOH (in mol) for maintaining a steady pH of 6.5.

Bioavailability Studies

All animal experiments were conducted according to the Guidelines on the Care and Use of Animals for Scientific Purposes (2004). The protocols for the animal studies were also reviewed and approved by the Experimental Animal Ethical Committee of Jinan University. Male Sprague–Dawley rats (250 ± 20 g) were randomly divided into three groups ($n = 6$ per group). Rats were fasted for 12 h prior to the experiments but allowed free access to water. Two groups of rats were respectively

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