International Journal of Pharmaceutics 472 (2014) 40–47

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Solid self-microemulsifying dispersible tablets of celastrol: Formulation development, charaterization and bioavailability evaluation

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ARTICLE INFO

Article history:
Received 26 March 2014
Received in revised form 27 May 2014
Accepted 10 June 2014
Available online 11 June 2014

Keywords:
Celastrol
Solid self-microemulsifying system (S-SMEDDS)
Dispersible tablets
Oral bioavailability

ABSTRACT

The aims of this study were to choose a suitable adsorbent of self-microemulsion and to develop a fine solid self-microemulsifying dispersible tablets for promoting the dissolution and oral bioavailability of celastrol. Solubility test, self-emulsifying grading test, droplet size analysis and ternary phase diagrams test were performed to screen and optimize the composition of liquid celastrol self-microemulsifying drug delivery system (SMEDDS). Then microcrystalline cellulose KG 802 was added as a suitable adsorbent into the optimized liquid celastrol-SMEDDS formulation to prepare the dispersible tablets by wet granulation compression method. The optimized formulation of celastrol-SMEDDS dispersible tablets was finally determinated by the feasibility of the preparing process and redispersibility. The *in vitro* study showed that the dispersible tablets could disperse in the dispersion medium within 3 min with the average particle size of 25.32 ± 3.26 nm. *In vivo* pharmacokinetic experiments of rats, the relative bioavailability of celastrol SMEDDS and SMEDDS dispersible tablets compared to the 0.4% CMC-Na suspension was $569 \pm 7.07\%$ and $558 \pm 6.77\%$, respectively, while there were no significant difference between the SMEDDS and SMEDDS dispersible tablets. The results suggest the potential use of SMEDDS dispersible tablets for the oral delivery of poorly water-soluble terpenes drugs, such as celastrol.

1. Introduction

Celastrol (also known as tripterine), a quinone methide triterpene, is a potent anti-inflammatory (Pinna et al., 2004; Tang et al., 2008), antioxidant and neuroprotective (Cleren et al., 2005) agent extracted from the traditional Chinese medicinal herb, *Tripterygium wilfordii* Hook (also known as thunder of god vine). In recent years, it has attracted interest for its potential antitumor effects. Various cancer cell lines including breast cancer MCF-7cell (Kim et al., 2013), human prostate cancer (Yang et al., 2006), non-small-cell lung cancer A549cells (Mou et al., 2011), melanoma (Abbas et al., 2007), pancreatic cancer cells (Yadav et al., 2010), human chronic myelogenous leukemia (Lu et al., 2010), etc., are reported to be inhibited by celastrol. However, oral bioavailability of celastrol is low mainly due to its poor water solubility (13.25 ± 0.83 μg/mL at 37 °C). To improve the solubility of celastrol, some solubilization strategies have been explored, such as PAMAM dendrimer nanocarriers (Boridy et al., 2012), liposomes (Huang et al., 2012; Song et al., 2011), block copolymer micelles (Peng et al., 2012) and CPP-coated nanostructured lipid carriers (Li et al., 2012). Although many efforts have been done, no tripterine monomer preparations have overcome the research phase and been marketed.

SMEDDS, an effective pharmaceutical technology to increase the solubility and absorption of poorly water-soluble drugs, has recently received increasing attention (Balakrishnan et al., 2009; Chen et al., 2008; Cui et al., 2009; Wu et al., 2006). The SMEDDS is an isotropic mixture of oil, surfactant and cosurfactant which is capable of forming fine o/w microemulsion under gentle agitation similar to the movement of the gastrointestinal tract (Bok Ki Kang et al., 2004). This system not only improves drug solubilization, but also enhances release and absorption properties, due to the already dissolved form of the drug in the formulation and the small droplet size providing a large interfacial surface area. Therefore, SMEDDS is a potential strategy for enhance the oral bioavailability of poorly

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http://dx.doi.org/10.1016/j.ijpharm.2014.06.019
0378-5173/

water-soluble drug. However, SMEDDSs are liquid formulations, which have several disadvantages, such as few choices of dosage forms, low stability and portability during the manufacturing process and the interaction between the filling and the capsule shell.

To overcome these problems, lipid formulations could be transformed to solid dosage forms by using a suitable adsorbent. Different hydrophilic and hydrophobic solid adsorbents, such as colloidal sillica, magnesium trisilicate and dextran, etc., are reported to absorb the liquid SMEDDS and various techniques (*e.g.*, spray drying, extrusion-spheronization and granulation etc.) are adopted to prepare them into pellets, powders or other solid dosage forms, which could be further filled in capsules or compressed into tablets (Cho et al., 2013; Hu et al., 2012; Oh et al., 2011; Tang et al., 2008). The solid SMEDDS combine the advantages of liquid SMEDDS with those of solid dosage forms, such as improved oral bioavailability, storage stability and patient compliance (Lei et al., 2011; Sermkaew et al., 2013). Solid SMEDDS could spontaneously form oil-in-water microemulsions with droplet sizes of less than 200 nm upon mild agitation in aqueous media (such as gastrointestinal fluids). These fine microemulsion droplets have the advantage of presenting the drug in a dissolved form with a large interfacial surface area for drug absorption.

However, the dissolution rate of those solid SMEDDS preparations is inclined to delayed even incomplete for the strong adhesion interaction of lipid SMEDDS with adsorbents (such as silicate), which also lead to poor disintegration of those tablets. Compared to conventional tablets, dispersible tablets offer an advantage in administration of drug, with a fast disintegration (disintegration time $<$3 min) (Charoo et al., 2012). Herein, we developed a solid self-microemulsifying dispersible tablets of celastrol, which can disintegrate rapidly and spontaneously emulsify in the mixed aqueous gastrointestinal environment. Since celastrol is encapsulated in the lipid SMEDDS, an isotropic oil mixture of poor fluidity, it is necessary to select a suitable adsorbent and disintegrating agent to develop the dispersible tablets.

In this study, solubility test, self-emulsifying grading test, droplet size analysis and ternary phase diagrams test were performed to screen and optimize the composition of liquid celastrol-SMEDDS. Then the suitable adsorbent was added into the optimized liquid celastrol-SMEDDS formulation to prepare the dispersible tablets by wet granulation compression method. The optimal formulation of celastrol-SMEDDS dispersible tablets were finally determinated by the feasibility of the preparing process and redispersibility. Finally, an oral bioavailability study in rats was carried out to evaluate the absorption of celastrol-SMEDDS dispersible tablets compared to the celastrol-SMEDDS and 0.4% CMC-Na suspension of celastrol.

2. Materials and methods

2.1. Materials

Celastrol (99.2% purity) was purchased from Zelang Pharmaceutical Technology Co., Ltd. (Nanjing, China). Corn oil, soybean oil, castor oil, olive oil, ethyl oleate, GTCC and Masine-1 were purchased from Jingchun Corporation (Shanghai, China). Octyl polyethylene glycol phenyl ether (OP-10) was purchased from Linghai Chemical Reagent Co., Ltd. (Shanghai, China). Isopropyl palmitate (IPP) was purchased from Wumei Chemical (Zhejiang, China). Isopropyl myristate (IPM), Transcutol P, Masine-1, Labrasol® and Labrafil® M1944 were purchased from Gattefosse (Brittany, France). Cremophor EL, Cremophor RH40 and Solutol HS15 were purchased from BASF (Ludwigshafen, Germany). Microcrystalline cellulose MCC KG 802 was purchased from Asahi Kasei (Tokyo, Japan) and microcrystalline cellulose 101 was purchased from ISP (New Jersey, America), All other reagents were of analytical grade.

2.2. Solubility studies

To select a suitable oil, surfactant, and co-surfactant for SMEDDS formulation, the solubility of celastrol in various vehicles was measured. 1 g of selected vehicles was added to each cap vial containing an excess of celastrol. After sealing, the mixture was treated with ultrasonic and heated at 60 °C in a water bath for 1 h to facilitate solubilization. Then these mixtures were shaken at 37 °C for 72 h. After reaching equilibrium, each vial was centrifuged (Sigma 3K30, Harz, Germany) at 3000 $\times$ *g* for 15 min. The supernatant was filtered through a 0.45 μm syringe filter membrane and the filtrate was diluted with methanol for quantification by HPLC method on a LC-10A (Shimadzu, Japan) HPLC system with a ODS-3C_{18} column (4.6 mm $\times$ 250 mm, i.d. 5 μm), a column heater set at 35 °C and an ultraviolet detector set at wave-length of 425 nm. The flow rate of mobile phase (methanol/methyl cyanides/1% formic acid, 85/10/5) was 1.0 mL/min. Twenty microliters of sample were injected for each analysis. After validation of specificity, linearity, recovery, precision and accuracy, the method has been successfully applied in solubility studies. Solubility studies were carried out in triplicate.

2.3. Self-emulsifying grading test

The optional oils (Corn oil, Soybean oil, Ethyl oleate, GTCC, IPM, Castor oil, Masine-1, Olive oil, IPP) surfactants (Labrafil M1944, Labrasol, OP-10) and co-surfactants (Transcutol P) according to the result of solubility were homogenized at 3:4:3 (w/w/w) in tubes by vortexing for 5 min. Then put 1 mL of the mixture into 100 mL water (37 °C) with magnetic stirring to observe the emulsion forming process and final appearance. The result was divided into five grades by a visual grading system (Shui-Mei Khoo et al., 1998).

(A) denoting rapid emulsification (within 1 min) and forming clear or slightly bluish microemulsion;
(B) denoting rapid emulsification (within 1 min) and forming slightly less clear and bluish white emulsion;
(C) denoting emulsification slower within 2 min and forming a bright white milk-like liquid;
(D) denoting emulsification slower (longer than 2 min) with dark gray and slightly oily appearance;
(E) denoting emulsification difficulties or the existence of a large number of oil droplets.

2.4. Construction of ternary phase diagram

According to the result of Section 2.2 and 2.3, OP-10 and Transcutol P were selected as surfactant and co-surfactant respectively in our study. Different oil phases (ethyl oleate, GTCC, IPM, masine-1, olive oil and IPP), surfactant (OP-10) and co-surfactant (Transcutol P) were fixed as the three vertices of ternary phase diagram. Oil-surfactant-cosurfactant mixtures with different percents were prepared. Then each mixture was dispersed into water following the methods in Section 2.3 to get a visual grading and the grade A or B point was put into the ternary phase diagram.

2.5. Formulation optimization of SMEDDS

Single-factor method was adopted to optimize the selected SMEDDS system. The ratio of oil phase, surfactant and co-surfactant in liquid SMEDDS was determined by droplet size