Journal of Drug Delivery Science and Technology 37 (2017) 184–193

Contents lists available at ScienceDirect

Journal of Drug Delivery Science and Technology

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

Self-microemulsifying sustained-release pellet of Ginkgo biloba extract: Preparation, *in vitro* drug release and pharmacokinetics study in beagle dogs

Zengquan Gu [1], Xiaolei Shi [1], Emmanuel Omari-Siaw, Yuan Zhu, Huihua Li, Min Guo, Xia Yang, Jiangnan Yu**, Ximing Xu*

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

ARTICLE INFO

Article history:
Received 25 September 2016
Received in revised form
16 December 2016
Accepted 4 January 2017
Available online 5 January 2017

Chemical compounds studied in this article:
Ginkgo biloba extract (PubChem CID: 174)
quercetin (PubChem CID: 5280343)
kaempferol (PubChem CID: 5280863)
isorhamnetin (PubChem CID: 5281654)
Ethyl oleate (PubChem CID: 5363269)
Isopropanol (PubChem CID: 3776)
Oleic acid (PubChem CID: 445639)
Tween 80 (PubChem CID: 5281955)
1,2-propanediol (PubChem CID: 1030)
Isopropyl myristate (PubChem CID: 8042)
Cremophor EL (PubChem CID: 5849927)
Polyvinylpolypyrrolidone (PubChem CID: 6917)
Ethyl cellulose (PubChem CID: 24832091)

Keywords:
GBE50
SMEDDS
SMEDDS-SR pellets
Solubility
in vitro drug release
Bioavailability

ABSTRACT

Ginkgo biloba extract (GBE50), the fifth generation extract of *Ginkgo biloba*, has been widely used in clinical treatment. This study was aimed at developing self-microemulsifying drug delivery system sustained-release pellets (SMEDDS-SR pellets) to achieve sustained release and increased the oral bioavailability of GBE50. Solubility studies and Pseudo-ternary phase diagrams were investigated to optimize the suitable compositions of SMEDDS-SR pellets. The optimal formulation of GBE50-SMEDDS consisting of oil (16.7% MCT), surfactant (33.4% Cremophor EL35), cosurfactant (33.4% PEG 400) and drug (16.7% GBE50) was obtained. The GBE50-SMEDDS-SR pellets were prepared by mixing GBE50-SMEDDS with diluent agent (MCC), disintegration agent (PVPP) and coating material of ethyl cellulose (EC) using extrusion spheronization method. The Morphology study of GBE50-SMEDDS-SR pellets after dispersion in water revealed a spherical and homogeneous structure of droplets (51.6 ± 1.8) nm. The *in vitro* release data revealed the sustained-release effect of GBE50-SMEDDS-SR pellets. Pharmacokinetics study in beagle dogs after oral administration yielded relative bioavailability (*Fr*) of 160.24% and 236.18% for GBE50-SMEDDS and GBE50-SMEDDS-SR pellets, respectively. Collectively, these results indicated that the SMEDDS-SR pellets could be an effective delivery system to achieve sustained release and improved oral bioavailability of GBE50.

1. Introduction

Ginkgo biloba, one of the oldest species of trees grown in extratropical, warm and subtropical zones, has lived on the earth for two hundred million years, and predominantly (70%) cultivated in China [1,2]. Long-life and resistance to a series of diseases are its unique characteristics, and the total value of ginkgo products in the world has been more than $7 billion dollars since 2008 [3]. The fifth generation of *Ginkgo biloba* extract, GBE50, possesses numerous

* Corresponding author. Department of Pharmaceutics, School of Pharmacy, Center for Nano Drug/Gene Delivery and Tissue Engineering, Jiangsu University, Zhenjiang 212013, People's Republic of China.
** Corresponding author. Department of Pharmaceutics, School of Pharmacy, Center for Nano Drug/Gene Delivery and Tissue Engineering, Jiangsu University, Zhenjiang, 212013, People's Republic of China.
E-mail addresses: yjn@ujs.edu.cn (J. Yu), xmxu@ujs.edu.cn (X. Xu).
[1] These authors contribute equally to this work.

http://dx.doi.org/10.1016/j.jddst.2017.01.002
1773-2247/

pharmacological activities such as anti-hyperlipidemia [4,5], anti-arteriosclerotic [6,7], anti-oxidation, and anti-angiogenic effects [8–10], so it has been widely used for the treatment and prevention of cardiovascular diseases and cognitive disorders [11,12]. In addition, GBE50 has also been found to alleviate myocardial ischemia in coronary artery ligation via reducing the scope of myocardial ischemia [13,14]. The main active pharmaceutical component in GBE50 was the total flavonol glycosides (TFG) including quercetin, kaempferol, and isorhamnetin [15,16]. However, poor water solubility of GBE50 (<0.20 mg/mL) led to low bioavailability. The pharmacokinetic parameters (($t_{1/2}$, MRT, C_{max}, T_{max}) of GBE50 were (2.39 ± 1.41) h, (2.85 ± 0.34) h, (107.09 ± 16.70) ng/mL, and (1.33 ± 0.52) h) indicating that GBE50 would be sustained *in vivo* for a shorter time and be easily eliminated. All said outcomes could lead to a low absorption after oral administration of GBE50 and thus limit its clinical application [17]. Therefore, it is necessary to develop a novel formulation for GBE50 to improve its water solubility and oral bioavailability.

So far, a number of solubilizing formulations have been developed for hydrophobic drugs, such as solid dispersion, inclusion complexes, nanoparticles, etc [18]. In previous studies, several formulations were designed to increase the bioavailability of GBE50, such as SMEDDS [19] and proliposome [20]. SMEDDS is thermodynamically stable, isotropic mixtures composing of oil, surfactant, cosurfactant and drug [21,22] which can be successfully used to increase the solubility, chemical stability and bioavailability of hydrophobic drugs [23,24]. In addition, SMEDDS can rapidly deliver large amounts of dissolved hydrophobic drugs through the gastrointestinal tract [25,26]. However, the cumulative release of GBE50-SMEDDS (85.0%) achieved complete release after 30 min that indicates a greater drug release rate without sustained release effect as the drug sustained *in vivo* for a shorter time [19]. Besides, the half-life of SMEDDS is not significantly longer than free drug. Therefore, it is reasonable to infer that SMEDDS might result in a faster absorption as well as elimination *in vivo*, so that its oral bioavailability could be further enhanced. Some studies regarding SR pellet containing SMEDDS have been reported that SMEDDS-SR pellets were prepared via self-microemulsifying as well as extrusion-spheronization method and thus remarkably improve their oral bioavailability [27,28]. It has been found that converting liquid SMEDDS into solid forms, that is GBE50-SMEDDS-SR pellets, would combine the advantages of better stability, improved oral bioavailability, ease of handling, decreased the gastrointestinal tract irritation and increase the absorption of drugs *in vivo* [29–32]. Therefore, the investigation of GBE50-SMEDDS-SR pellets could be helpful for improving oral bioavailability and attaining sustained-release effect.

In this paper, GBE50-SMEDDS was prepared by self-microemulsifying technology, which was then used to prepare GBE50-SMEDDS-SR pellets via extrusion spheronization technique [33,34]. The stability and the release properties of GBE50-SMEDDS-SR pellets *in vitro* were investigated alongside physical characterization including the determination of droplets size and its distribution, transmission electron microscopy and drug content. More importantly, *in vivo* pharmacokinetic study of GBE50-SMEDDS-SR pellets in beagle dogs was evaluated.

2. Materials and methods

2.1. Materials

GBE50 was purchased from Shanghai XingLing Sci. &Tech. Pharmaceuticals Co., Ltd (Shanghai, China). Quercetin, Kaempferol and Isorhamnetin were purchased from the National Institutes for Food and Drug Control (Beijing, China). Medium chain (consisting of caprylic acid and capric acid) triglyceride (MCT), containing a 98% mixture of C8:0 and C10:0 fatty acids (66% and 32%, respectively) and soybean oil were purchased from Tieling Beiya Medicinal Oil Co., Ltd. (Tieling, China). Ethyl oleate was provided by Shanghai Feixiang Chemical Co., Ltd. (Shanghai, China). Isopropanol, Oleic acid, PEG 400, Tween 80, 1,2-propanediol and isopropyl myristate (IPM) were provided by Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Cremophor EL was purchased from BASF (Ludwigshafen, Germany). Microcrystalline cellulose PH101 (MCC), Polyvinylpolypyrrolidone K30 (PVPP K30) and Ethyl cellulose (EC, Ethocel Standard 10 FP Premium) were purchased from Foremost Farms. Pure chromatographic methanol was obtained from Hanbon Science and Technology (Jiangsu, China). Chromatographic grade acetonitrile was purchased from Honeywell Burdick & Jackson (Muskegon, USA). Distilled water was produced by a Millipore water purification system (Millipone Corporation, USA). All organic reagents in this study were of high performance liquid chromatography (HPLC) grade and all other chemicals were of analytical grade which obtained commercially.

2.2. Establishment of HPLC analysis method in vitro

The quantitative analysis of GBE50 was performed by HPLC. The HPLC analysis was carried out using a Shimadzu Scientific Instruments which consisted of an LC-20AT pump and an SPD-20A UV–Vis detector (Shimadzu, Japan). The chromatographic conditions were as follows: a Hypersil C18 column (4.6 mm × 250 mm, 5 μm) set at 30 °C; mobile phase, 55% methanol and 45% acetonitrile containing 0.5% phosphoric acid; flow rate, 1.0 ml/min; and a detection wavelength, 368 nm. Three standards of quercetin (1 g), kaempferol (0.9 g), and isorhamnetin (0.25 g) were mixed together in a glass tube and then dissolved in methanol (5 ml) to obtain the mixed standards solution. A 0.6 g of pure GBE50 and 0.5 g of blank excipients (the mixture of SMEDDS and SR-pellets excipients) were dissolved in 1 ml of methanol-25%HCL (4:1) solution, respectively, and heated at 80 °C with the aid of water bath for 1 h. Afterwards, 3 ml of methanol were added and vortex-mixed for 5 min. All samples were centrifuged at 3500 r/min for 5 min. Finally, 20 μL of the supernatant was injected into the HPLC for analysis. The blank excipients had no interference in the determination of quercetin, kaempferol, and isorhamnetin in all the chromatogram. The linear regression equation of quercetin was Y = 157211 C + 178192, r = 0.9998, ranging from 5 to 100 μg/mL. The linear regression equation of kaempferol was Y = 158969 C + 45126, r = 1, ranging from 4.5 to 90 μg/mL. The linear regression equation of isorhamnetin was Y = 115707 C−27080, r = 0.9998, ranging from 1.25 to 25 μg/mL. The results of precision, recovery and repeatability of standard drug were validated differences which were within acceptable range. These results confirmed that this method could be used to determine the GBE50 contents successfully *in vitro*.

2.3. Solubility studies

The solubility of GBE50 in different carriers was determined. Briefly, an excess amount of GBE50 (approximately 1 g) was added into 1 g of each selected oils, surfactants, cosurfactants and water phases. These mixtures were vortexed for 5 min, and then oscillated continuously in a Water-bathing Constant Temperature Vibrator (TH2-82A, Jintan Zhongda Instrument Corporation, Jiangsu, China) at 37 °C for 72 h. The different resulting equilibriums were centrifuged at 3000 r/min for 10 min to observe the presence of phase separation or otherwise. The insoluble GBE50 was removed in cases of phase separation. Afterwards, 20 μL of supernatant was analyzed by HPLC method as mentioned in section 2.2.