International Journal of Biological Macromolecules 50 (2012) 523–529

Contents lists available at SciVerse ScienceDirect

International Journal of Biological Macromolecules

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

In vitro and in vivo evaluation of oridonin-loaded long circulating nanostructured lipid carriers

Lejiao Jia [a], Jingyi Shen [a], Dianrui Zhang [a,*], Cunxian Duan [a], Guangpu Liu [a], Dandan Zheng [a], Xiaona Tian [a], Yue Liu [a], Qiang Zhang [b]

[a] Department of Pharmaceutics, College of Pharmacy, Shandong University, 44 Wenhua Xilu, Jinan 250012, PR China
[b] State Key Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical Sciences, Peking University, 38 Bei Xueyuan Road, Beijing 100083, PR China

ARTICLE INFO

Article history:
Received 13 December 2011
Received in revised form 8 January 2012
Accepted 16 January 2012
Available online 24 January 2012

Keywords:
Oridonin
Nanostructured lipid carriers
Long circulating
In vitro release properties
Pharmacokinetics

ABSTRACT

The purpose of this study was to develop poly(ethylene glycol)-coated nanostructured lipid carriers (PEG-NLC) for parenteral delivery of oridonin (ORI) to prolong drug circulation time in blood. Oridonin-loaded PEG-NLC (ORI-PEG-NLC) consisting of PEG_{2000}-stearate, glycerol monostearate and medium chain triglycerides were prepared by emulsion-evaporation and low temperature-solidification technique. Oridonin-loaded NLC (ORI-NLC) were also prepared as control. ORI-PEG-NLC were observed by transmission election microscope and the morphology was in rotiform shape. The mean particle size of ORI-PEG-NLC was 329.2 nm and entrapment efficacy was 71.18%. The results of differential scanning calorimetry and X-ray diffraction revealed a low-crystalline structure of ORI and verified the incorporation of ORI into the nanoparticles. In vitro drug release of ORI-PEG-NLC exhibited biphasic drug release patterns with burst release initially and prolonged release afterwards. Pharmacokinetic analysis showed that the mean residence time of ORI-PEG-NLC was prolonged and AUC (area under tissue concentration–time curve) value was also improved compared with ORI-NLC and ORI solution. In conclusion, ORI-PEG-NLC could be a potential carrier to get prolonged retention time of oridonin in blood.

1. Introduction

Oridonin, a diterpenoid compound (molecular structure shown in Fig. 1), isolated from Chinese herb *Rabdosia rubescens*, has been demonstrated to have remarkable anti-neoplastic effects against a number of cancers such as primary liver cancer, pancreatic cancer, carcinoma of the esophagus, etc. At the present, commercial preparations include tablets, syrups and injections. However, the tablets and syrups are crude extracts with a very low content of oridonin (<1.5%), and the injection may cause serious adverse effects due to containing large quantity of organic solvents and surfactants [1–5]. Hence, its poor water-solubility and short biological half-time hamper its successful application in clinic. New techniques have been investigated to overcome these pharmaceutical problems, which involve in solid dispersions [6], nanogels [7] and nanosuspensions [8].

In the last decade, solid lipid nanoparticles (SLN) have been looked upon as promising carriers for several attractive features such as the good biocompatibility, improving the solubility of drugs, the high bioavailability and easy production in a large scale [9,10]. Nevertheless, due to the high crystallization of the solid lipids or blends of solid lipids, drugs tend to be released from the SLN, thus leading to low loading capacity and drug expulsion during storage [11]. To overcome the limitation of SLN, nanostructured lipid carriers (NLC) were developed, which consist of solid lipids and liquid lipids. In addition to having strengths of SLN, NLC possess properties of high drug loading and good stability, because the liquid lipids incorporation impacted the crystalline state of lipid matrix [12–14]. Additionally, it was reported that PEG coating or modifying the surfaces of drug carriers could improve the surface hydrophilicity. Thus, the hydrophilic layer creates a barrier preventing the adsorption of lipoproteins and opsonins to surfaces of drug carriers, so that let the PEG-coated/modified drug carriers circulate in blood for longer time [15–17].

In the present work, PEG-coated NLC were prepared to load oridonin, aiming to improve the solubility of oridonin and extend the circulating time in blood. The morphology of ORI-PEG-NLC was observed by transmission election microscope (TEM) and other characteristics such as the particle size, zeta potential, drug loading capability, crystalline form, etc., were also investigated. Besides, in vitro drug release and pharmacokinetics were evaluated in detail. ORI-NLC were prepared as the control group.

* Corresponding author. Tel.: +86 531 88382015; fax: +86 531 88382015.
E-mail addresses: zhang.dr2006@yahoo.com.cn, zhangdianrui2006@163.com (D. Zhang).

0141-8130/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijbiomac.2012.01.024

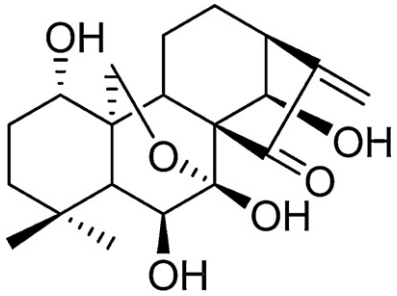

Fig. 1. Molecular structure of oridonin.

2. Materials and methods

2.1. Materials

Oridonin (ORI) (99%) was purchased from Shanxi Xuhuang Biotechnology Co. Ltd. (China). Glycerol monostearate (GMS, Beijing Chemistry Reagent Company, China) was used as solid-lipid material of NLC. Medium chain triglyceride (MCT, Tieling North Asia Officinal Oil Co. Ltd., China) was chosen as liquid-lipid material of NLC. PEG_{2000}-stearate (PEG-SA) and Pluronic F_{68} (F_{68}) were obtained from Sigma (USA). Lecithin (Injection grade) was provided by Shanghai Taiwei Pharmaceutics Co. Ltd., China. The methanol (Shanghai Siyou Co., Ltd., China) was of high performance liquid chromatography (HPLC) grade. All other reagents and solvents were of analytical reagent grade.

2.2. Preparation of ORI-PEG-NLC and ORI-NLC

ORI-PEG-NLC and ORI-NLC were prepared by the method of emulsion evaporation at a high temperature and solidification at a low temperature. Briefly, 5 wt.% oridonin (weight percentage of drug to the total amount of lipids), 600 mg of lipids (GMS:PEG-SA:MCT = 3:2:1, w/w/w) and 500 mg of lecithin were weighted and then completely co-dissolved into ethanol (5 mL) at 75 °C in a water bath. The obtained organic phase was added dropwise into 25 mL of aqueous phase containing 1% F_{68} under mechanical agitation (ETS-D4 stirrer, IKA, Germany) with 1000 rpm. After stirring for 4 h, the resultant hot nanoemulsion was quickly dispersed into 20 mL of cold distilled water (0–2 °C) under stirring at 1000 rpm for 2 h in order to acquire ORI-PEG-NLC dispersions. The ORI-NLC dispersions were prepared by the same process, except that PEG-SA was replaced by an equivalent amount of GMS.

Mannitol was chosen as a cryoprotectant in the freeze-drying procedure at a concentration of 5 wt.%. First the obtained ORI-PEG-NLC and ORI-NLC dispersions were pre-frozen using an ultra-cold freezer (MDF-382E, SANYO, Japan) at −80 °C. After 24 h, the samples were transferred to the lyophilizer (LGJ0.5, Beijing Sihuan Instrument Company, China) at temperature of −40 °C and pressure of 0.1 mbar for 48 h. The freeze-dried powders were collected for further experiments.

2.3. Transmission election microscope (TEM) examination

The morphologies of ORI-PEG-NLC and ORI-NLC were determined by TEM (H-7000, Hitachi, Japan). A drop of NLC re-dispersions was spread on a 200-mesh copper grid and negatively stained with 2% phosphotungstic acid for 30 s. The grid was dried at room temperature and then observed by TEM.

2.4. Particle size and zeta potential (ζ) analysis

The particle sizes of ORI-PEG-NLC and ORI-NLC re-dispersions were measured by Zetasizer (3000 HS, Malvern Instruments Ltd., UK) and the zeta potentials were analyzed by Zeta potential analyzer (ZEN2600, Malvern Instruments Ltd., UK). All samples were diluted with double distilled water to have a suitable concentration to test and each sample was determined in triplicate.

2.5. Entrapment efficiency and drug loading determination

The method of centrifugation ultrafiltration [14] was used for determination of entrapment efficiency and drug loading. 1 mL of ORI-PEG-NLC or ORI-NLC re-dispersions was added into an Ultra-4 ultrafiltration device (molecular weight cut-off was 10 K, Millipore, Bedford, MA, USA) and then the unit was centrifuged at 814 × *g* for 15 min. The filtrate was withdrawn as sample A. On the other hand, 1 mL of ORI-PEG-NLC or ORI-NLC re-dispersions from the same batch and 5 mL of methanol were placed in 10 mL test tube. The resultant sample was surged by ultrasound for 10 min to make drug release thoroughly from nanoparticles and then centrifuged at 13,034 × *g* for 10 min. The supernatant was withdrawn as sample B. Samples A and B were diluted with methanol to obtain suitable concentrations and determined by HPLC method. The result values were denoted by W_{free} and W_{total}, respectively. The entrapment efficiency (EE) and drug loading (DL) of nanoparticles were calculated by Eqs. (1) and (2).

$$\text{EE} \quad (\%) = \frac{W_{total} - W_{free}}{W_{total}} \times 100 \tag{1}$$

$$\text{DL} \quad (\%) = \frac{W_{total} - W_{free}}{W_{total} - W_{free} + W_{lipid}} \times 100 \tag{2}$$

where W_{total}, W_{free} and W_{lipid} were the weight of total drug in the system, analyzed weight of drug in the filtrate and weight of lipid added in the system, respectively.

To check the potential accumulation of oridonin in the ultrafiltration membrane, 1 mL of ORI solution (10 μg/mL, the solvent was the mixture of methanol/water (1/4)) was added in an Ultra-4 ultrafiltration device and the filtrate was withdrawn after centrifuging at 814 × *g* for 15 min. UV/vis spectroscopy was applied to determine the absorbance of samples before and after permeating the ultrafiltration membrane. The detention wavelength was set at 242 nm and the permeation rate (PR) was calculated by Eq. (3).

$$\text{PR} = \frac{A_{before}}{A_{after}} \tag{3}$$

where A_{before} is the absorbance of ORI solution before permeating the ultrafiltration membrane and A_{after} is the absorbance of ORI solution after permeating the ultrafiltration membrane.

2.6. Differential scanning calorimetry (DSC) analysis

Thermograms were recorded with differential scanning calorimetry (DSC) (CDR-4P, Shanghai, China) to evaluate the crystalline state of NLC. For DSC measurement, 10 mg of sample was put in an aluminium pan. A scanning rate of 10 °C/min was employed over a temperature range between 0 and 400 °C. Magnesia was used as the standard reference material to calibrate the temperature and energy scale of the DSC apparatus.

2.7. X-ray diffraction analysis

Wide-angle X-ray scattering (WAXS) investigations were performed by an X-ray diffractometer (D/max γ-B, Rigaku, Japan). A Cu Kα radiation at 40 kV and 100 mA was used. Diffractograms were performed from the initial angle $2\theta = 3^\circ$ to the final angle $2\theta = 50^\circ$ with the steps of 0.02° at a scanning speed of 4°/min (2θ).