International Journal of Biological Macromolecules 43 (2008) 320–324

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

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

Short communication

The use of solid lipid nanoparticles to target a lipophilic molecule to the liver after intravenous administration to mice

Wen Lu, Lang Chong He*, Chang He Wang, Yan Hua Li, San Qi Zhang

School of Medicine, Xi'an Jiaotong University, No. 76 Western Yanta Road, Xi'an 710061, PR China

ARTICLE INFO

Article history:
Received 27 March 2008
Received in revised form 12 June 2008
Accepted 13 June 2008
Available online 21 June 2008

Keywords:
Solid lipid nanoparticles
Liver targeting
Taspine

ABSTRACT

Taspine solid lipid nanoparticles (Ta-SLN) and taspine solid lipid nanoparticles modified by galactoside (Ta-G_2SLN) were prepared by the film evaporation-extrusion method. The nanoparticles were spherical or near-spherical particles with smooth surface, small size and high encapsulation efficiency. Ta-G_2SLN and Ta-SLN showed significant inhibition on 7721 cell growth. Intravenous injection of either Ta-SLN or Ta-G_2SLN resulted in a higher plasma and liver concentration and a longer retention time in mice compared with the administration of Ta. These results suggested that SLN tended to be preferentially delivered to the liver and Ta-G_2SLN may further enhance liver targeting.

1. Introduction

Solid lipid nanoparticles (SLN) present a more attractive new drug delivery system for lipophilic drugs than other colloidal delivery systems. SLN have a good physicochemical stability profile [1]; result in a high oral bioavailability of poorly soluble drugs [2] and can be used as the carrier for tissue targeting [3]. In addition, SLN are composed of a solid lipid matrix which can provide an effective means for control release of drugs [4].

As the exponential growth of tumor cells relies on the nutrition and oxygen, leading to an abnormally rapid growth of blood vessels in affected tissues, many studies have thus been conducted with a view to suppressing such neovascularization thereby inhibiting or delaying cancer growth [5]. Taspine was a bioactive aporphine alkaloid. From our prophase study, taspine showed retention characteristics in vascular endothelial cell (ECV304) membrane chromatography, which suggested that taspine could act on ECV304 cell membrane and its receptors [6]. Both in vivo and in vitro results showed that taspine inhibited tumor angiogenesis and controlled tumor growth [7,8]. Therefore, taspine may possess potent anti-angiogenesis and anti-tumor activities. In order to target taspine to a specific tissue (such as liver) after intravenous injection, taspine was incorporated into nanoparticles. In a previous study, we compared the in vivo disposition of liposomal taspine, with that of taspine solutions (Ta) after i.v. administration to mice. The results indicated that liposomes prolonged taspine retention within the systemic circulation, increased its distribution to the liver but reduced its distribution to the heart [9].

In the present study, galactoside was synthesized and included in taspine nanoparticles with an attempt to enhance the liver targeting of the nanoparticles. Taspine solid lipid nanoparticles (Ta-SLN) and taspine solid lipid nanoparticles with galactoside (Ta-G_2SLN) were prepared separately using the film evaporation-extrusion method. The pharmacokinetics and liver target efficiency after i.v. administrations of Ta-SLN and Ta-G_2SLN to ICR mice were finally compared.

2. Materials and methods

2.1. Materials

Taspine, a white amorphous powder with a melting point of 370 °C, was extracted from *Racix Rhicoma Leonticis* and then chemically identified using UV, IR, NMR and MS [10]. Its purity was measured using high-performance liquid chromatography and was found to be above 97%.

D-Galactose was purchased from Guoyao Chemical Reagent Co. (Xi'an, China). Compritol 888 ATO was a gift from Gattefossé (France). Polyethylene-2-stearyl ether, MTT, DMEM and trypsin were from Sigma (St. Lousi, MO, USA). HPLC grade solvents were from Fisher (Hampton, NH, USA), and all other analytical grade chemicals were obtained from Xi'an Chemical Regent Co.

Male ICR mice, weighing 18–22 g, were supplied from the Experimental Animal Centre of Xi'an Jiaotong University (Xi'an, China). All experimental protocols involving animals were reviewed and

* Corresponding author. Tel.: +86 29 82655441; fax: +86 29 82655451.
E-mail address: helc@mail.xjtu.edu.cn (L.C. He).

0141-8130/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijbiomac.2008.06.006

approved by the Institutional Animal Experimentation Committee of Xi'an Jiaotong University.

2.2. Synthesis of galactoside

The polyoxyethylene-stearyl ether galactoside (G_2SE) was synthesized according to the method studied previously in our laboratory [11].

2.3. Preparation of Ta-SLN and Ta-G_2SLN

Taspine (10 mg) and lipid (100 mg) were dissolved in chloroform (10 mL) in a around bottom flask. The organic solvent was completely evaporated at 60 °C under vacuum on a Rotary evaporator (RE-52, Shanghai, China). A lipid film was obtained on the flask wall. An aqueous phase was prepared by dissolving poloxamer 188 (100 mg) in 20 mL distilled water and heated to 80 °C. The lipid film was hydrated with 20 mL aqueous phase and rotated for 30 min at 80 °C. The obtained hot pre-emulsion was homogenized at 80 °C for 30 min and extruded for 4 extrusion cycles (the Extruder, Lipex, Northern Lipids Inc.) through a polycarbonate membrane with 0.4, 0.2 and 0.1 μm pores (Nuclepore, Whatman) to produce solid lipid nanoparticles dispersion solution. Taspine loaded SLN obtained using Compritol 888 ATO (100 mg), Compritol 888 ATO (95 mg) and G_2SE (5 mg) were abbreviated as Ta-SLN, Ta-G_2SLN, respectively.

2.4. Drug content and encapsulation efficiency

The amount of taspine incorporated in Ta-SLN or Ta-G_2SLN was determined by HPLC. Thus, 0.5 mL of Ta-SLN or Ta-G_2SLN dispersion solution was diluted by a mixture of chloroform/methanol (2:1 v/v) to a total volume of 10 mL, sonicated and centrifuged at 12,000 × *g*. A 10 μL aliquot of the resultant solution was injected into a HPLC system. The HPLC system was described in our previous study [9]. Drug content was the amount of taspine in SLN expressed as the percentage of total lipid in the preparation.

The encapsulation efficiency was measured after Ta was separated from the SLN samples by dialysis [12]. 2 mL of Ta-SLN or Ta-G_2SLN dispersion solution was placed into a dialysis bag (MWCO 8–12,000, Shanghai, China), which was then transferred into a conical flask containing 250 mL of phosphate buffer solution (pH 5.0) as the receiving medium. The receiving medium was stirred for 2 h with a magnetic stirrer. It was then discarded and replaced with an equal volume of fresh phosphate buffer solution. Two receiving media were combined and taspine concentration was measured by HPLC. Encapsulation efficiency was calculated from the relationship below:

$$\text{EE}\ (\%) = \frac{Q_t - Q_m}{Q_t} \times 100$$

where EE is encapsulation efficiency, Q_t is the theoretical amount of taspine that was added whilst Q_m is the taspine content in the receiving media.

2.5. Transmission electron microscopy (TEM)

The morphology of Ta-SLN and Ta-G_2SLN was examined using a transmission electron microscope (H-600, Japan). After being diluted 50 times with distilled water, a drop of the resultant dispersion solution was placed onto a carbon-coated copper grid to form a thin liquid film. The film on the grid was then negatively stained by 2% (w/v) phosphotungstic acid for 5 min. The copper grid was dried for 15 min. The stained film was then viewed and photographed.

2.6. Measurement of particle size

Ta-SLN and Ta-G_2SLN were suspended in 50 mL of distilled water and stirred manually for 2 min. The mean particle size of the nanoparticles was determined using a Nicomp 380 Submicron Particle Sizer (Particle Sizing System, Santa Barbara, CA, USA). Data were analyzed automatically by Nicomp Distribution Analysis from the Nicomp CW380 software.

2.7. Inhibition of cell growth

7721 cell (Shanghai Institute of Cell Biology in the Chinese Academy of Sciences, Shanghai, China) sensitivity to control, Ta-G_2SLN, Ta-SLN and Ta were estimated by means of the MTT assay as described [13]. Briefly, exponentially growing 7721 cells were harvested and plated in 96-well plates at a concentration of 2×10^4 cells/well. After 24 h incubation at 37 °C, the cells were exposed to control, Ta-G_2SLN, Ta-SLN and Ta at various taspine concentrations (0.05–3.2 μg mL^{-1}) for 48 h. Then, 20 μL of MTT (5 mg mL^{-1}) was added to each well and the plates incubated at 37 °C for 4 h. After the supernatant was discarded, 150 μL of DMSO was added to each well, and optical density was assessed at 490 nm using a 96-well microplate reader (Bio-Rad instruments, USA). The percent growth inhibitory rate of treated cells was calculated by $(A_{\text{tested}} - A_{\text{control}})/A_{\text{control}} \times 100\%$, where A is the mean value of the data from five replicate tests. The IC_{50} values were determined by plotting the percentage viability versus concentration on a logarithmic graph.

2.8. The in vivo distribution

Pharmacokinetics studies were performed as described previously [14]. Mice were randomly divided into two groups (50 per group). Group 1 was treated with Ta-SLN whilst group 2 was treated with Ta-G_2SLN. Each preparation, which contained 0.5 mg mL^{-1} taspine, was injected through the tail vein at the taspine dose of 12 mg kg^{-1} mouse.

Blood samples were collected by terminal retro-orbital bleeding at various time points (0.05, 0.08, 0.25, 0.5, 1, 2, 4, 8, 12 and 24 h) into microtubes containing heparin as an anticoagulant. The heart, liver, spleen, lung, kidney and brain of each mouse were immediately excised following blood collection and then homogenized separately in 1.0 mL normal saline (0.9% NaCl). Taspine was extracted from the plasma samples or the homogenates using the liquid phase extraction method described previously [9] before it was quantified using the HPLC method.

3. Results and discussion

3.1. Characterization of Ta-SLN and Ta-G_2SLN

As can be seen in Table 1, Ta-G_2SLN appeared to have a slightly larger mean diameter than Ta-SLN although drug content and encapsulation efficiency followed a reverse trend. Thus, incorporation of galactoside into the nanoparticles might slightly increase the particle size but reduce drug entrapment within the nanoparticles.

Table 1
Physicochemical characteristics of Ta-SLN and Ta-G_2SLN (mean ± S.D., $n = 3$)

Preparation	Particle size (nm)	Drug content (%, w/w)	EE (%)
Ta-SLN	173.0 ± 62.8	10.23 ± 0.51	90.67 ± 3.32
Ta-G_2SLN	192.3 ± 91.4	9.66 ± 0.37	81.03 ± 2.57