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Pharmacokinetics, tissue distribution and anti-tumor effect of low density lipoprotein peptide conjugated submicron emulsions

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ABSTRACT

Docetaxel (Doc) is a potent chemotherapy for cancer but its application is limited by poor water solubility and high risk of side effects. To improve these issues, low density lipoprotein receptor (LDLR) targeted peptide-RLT (CEKLKEAFRLTRKRGLKLA) modified Docetaxel-loaded submicron emulsions (RLT-DocSEs) had been developed. Docetaxel-loaded SEs (DocSEs) and cationic DocSEs (DocCSEs) were also prepared for comparison. To evaluate the tumor-targeting ability and anti-tumor efficacy, DocSEs, DocCSEs, and RLT-DocSEs were administrated intravenously to rats respectively. The pharmacokinetic parameters of three formulations were significantly different. *In vivo* distribution study was conducted in mice and the results indicated that RLT-DocSEs possessed increased tumor targeting ability than DocSEs and DocCSEs. RLT-DocSEs also resulted in a higher tumor inhibition rate and a better anti-tumor efficacy in mice. All the results suggested that RLT-DocSEs could be a potential formulation for the injection of Doc with enhanced tumor targeting and anti-tumor efficacy.

1. Introduction

Docetaxel (Doc) is the gold standard chemotherapy for prostate cancer (PC) and the first-line treatment for advanced castration-resistant PC [1]. However, Doc is a lipophilic compound with poor water solubility, which results in the poor bioavailability. Furthermore, because of the unspecific distribution in the body and high content of surfacants, Doc injection causes a series of side effects, such as allergic reactions, extreme weakness, severe vomiting or diarrhea, fever. To improve Doc application in clinic, new formulations, such as liposomes, polymeric nanoparticles, micelles, and submicron emulsions (SEs), have been developed [2–7]. SEs has been an advanced formulation for injection due to its highly biocompatibility and low risk of side effects [8–11]. However, SEs needs improvement for its poor tumor-targeting.

RLT, a polypeptide (CEKLKEAFRLTRKRGLKLA), shows high affinity to the low density lipoprotein receptor (LDLR), a membrane glycoprotein that is overexpressed on prostate cancer cells [12,13], glioblastoma multiforme [14,15], etc. Recently, we developed Docetaxel-loaded submicron emulsions (DocSEs), cationic DocSEs (DocCSEs) and LDLR targeted peptide-RLT conjugated DocSEs (RLT-DocSEs). We found that RLT-DocSEs showed more intracellular delivery and slower intracellular elimination of Doc than that of DocSEs and DocCSEs. In addition, RLT-DocSEs resulted in the highest tumor inhibition in vitro (data unpublished).

In this work, the tumor-targeting ability and anti-tumor efficacy of the DocSEs, DocCSEs and RLT-DocSEs were explored. Pharmacokinetics, *in vivo* distribution and anti-tumor efficacy of these formulations were also investigated.

2. Materials and methods

2.1. Materials

2.1.1. Chemicals

Docetaxel (purity > 99.38%) was provided by Beijing Yi-He Biotech Co, Ltd (Beijing, China). DSPE-PEG (2000) maleimide was purchased from Avanti Polar Lipids Inc (Alabama, USA). Poloxamer188 were supplied by BASF (Ludwigshafen, Germany). Oleic Acid, Soybean oil and Lipoid E80 was obtained from Lipoid GmbH (Ludwigshafen, Germany). RLT was synthesized by AC Scientific

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(Xi'an, China). Octadecyl amine was purchased from Sigma (St. Louis, USA). Sodium heparin injection was obtained from Wanbang Biopharmaceuticals (Xuzhou, China). Methanol, acetonitrile and *tert*-Butyl methyl ether were obtained from Shield Specialty Chemical Ltd. Co. (Tianjin, China). All chemicals were in analytical or chromatographic grade.

2.1.2. Animals and cell culture

Sprague–Dawley rats (200 ± 20 g) and male SPF mice (20 ± 2 g) were purchased from the Laboratory Animal Center of Zhengzhou University (Zhengzhou, China). All animal experiments were evaluated and approved by the animal and ethics review committee of Faculty of Zhengzhou University.

Sarcoma 180 (S-180) cells was kindly provided by the Department of Pharmacology, Henan Institute of Medical Science (China). S-180 cells were maintained by intraperitoneal transplantation of 2×10^6 cells suspended in Hanks' balanced solution (0.1 mL) per mouse. Cells were harvested from the peritoneal cavity of a tumor-bearing mouse 9–11 days after inoculation [10]. In anti-tumor experiments, S-180 cells (1×10^6 cells) were suspended in Hanks' balanced solution (0.1 mL) and inoculated subcutaneously to each mouse in its axillary region. The experiment was begun when the tumor size reached an average diameter of 6–8 mm, at 7–10 days after inoculation.

2.2. Preparation and characterization of DocSEs, DocCSEs, and RLT-DocSEs

SEs was prepared by high pressure homogenization method [16]. To prepare DocSEs, Doc, Lipoid E80, Oleic acid, and Vitamin E were dissolved in ethanol. For DocCSEs and RLT-DocSEs, instead of Vitamin E, Octadecyl amine were firstly added to ethanol. Subsequently, soybean oil was added to obtain the oil phase. As for the water phase, Poloxamer188 and glycerol were dissolved in purified water. To prepare RLT-DocSEs, additional DSPE-PEG (2000) maleimide was added into the water phase. Subsequently, the water phase and the oil phase were mixed at 60 °C and emulsified by a homogenizer (FJ-200; Shanghai Specimen and Model Factory, Shanghai, China) at 18000 rpm for 5 min. The pH was adjusted to 7.2-7.4. Fine emulsions were filtered through a membrane with pore size 0.22 μm. For the preparation of RLT-DocSEs, DocCSEs that contained DSPE-PEG (2000) maleimide (0.01%) was reacted with RLT for 1 h under stirring. RLT conjugation efficiency was determined by HPLC (Agilent 1100, flow rate: 1.0 mL/min, mobile phase: acetonitrile/water 20.8%/79.2% with 0.05% trifluoroacetic acid (TFA), column: Diamonsil, 4.6 mm × 150 mm, 5 μm, detection wavelength: 215 nm and temperature: 25 °C).

The size and zeta potential of DocSEs, DocCSEs, and RLT-DocSEs were determined by zeta-sizer Nano ZS90 (Malvern Instrument, UK). TEM was used to observe the morphology of DocSEs, DocCSEs, and RLT-DocSEs (JEM2000-FX, JEOL, Japan). Temperature, light, and freezing were examined for their impact on stability. Doc loading efficiency was determined by HPLC (Agilent 1100, flow rate: 1.0 mL/min, mobile phase: acetonitrile/water 50/50, column: DiamonsilTM C18, 4.6 mm × 150 mm, 5 μm, detection wavelength: 231 nm and temperature: 25 °C).

2.3. Pharmacokinetics study in rats

The pharmacokinetic (PK) study was conducted on Sprague-Dawley Rats. The rats weighting 200 ± 20 g were divided into three groups and were given DocSEs, DocCSEs, and RLT-DocSEs at 75 mg/m^2 via tail vein, respectively. Blood samples (0.5 mL) were collected into heparinized Eppendorf tubes at 0.083 h, 0.25 h, 0.5 h, 1 h, 2 h, 4 h, 6 h and 8 h after administration by retro-orbital puncture, respectively. Plasma was obtained by centrifuging at 5000 rpm for 15 min. Subsequently, the plasma (0.2 mL) was mixed with 2 mL *tert*-butyl methyl ether, shaken by a vortex mixer for 3 min, and centrifuged at 3500 rpm for 15 min. The supernatant was transferred into glass tubes and dried at 40 °C. 50 μL methanol was added and the samples were shaken by a vortex mixer for 2 min. Concentration of Doc in the samples was determined by HPLC [17].

2.4. In vivo *distribution in mice*

Swiss mice were chosen to study the tissue distribution of DocSEs, DocCSEs, and RLT-DocSEs [18]. Briefly, Swiss mice were inoculated subcutaneously with S-180 tumor cells at the right axillary region. The sarcoma S-180-implanted mice were divided into three groups and DocSEs, DocCSEs, and RLT-DocSEs were given at 75 mg/m^2 via the tail vein injection, respectively. 0.5 mL blood samples were collected at 0.5 h, 1 h and 3 h, respectively. Subsequently, the mice were sacrificed and their organs (heart, liver, spleen, lung, kidney, brain, and tumor) were collected. The tissue samples were cleaned and rinsed with cold saline and dried with filter paper. Organs were weighed precisely. Acetonitrile solution (acetonitrile: water, 1:1, v/v) was added to the tissues and the tissue samples were homogenized by a glass homogenizer with a Teflon pestle. The homogenate (0.2 mL) was disposed like the plasma sample preparation described in 2.3 and quantified by HPLC.

2.5. In vivo *anti-tumor efficacy*

Tumor size and tumor growth inhibition rate (TGI) were used to evaluate anti-tumor efficacy. The sarcoma S-180-implanted mice were randomly assigned into 5 groups. Saline, Doc, DocSEs, DocCSEs, and RLT-DocSEs were injected intravenously (via the tail vein, dosage: 75 mg/m^2), respectively. The treatment was given every other day and four times in total. The tumor volume was measured before administration by a Vernier's caliper, and calculated by formula ($V = a \times b^2/2$), where a is the length, b is the width. The mice were executed 2 days after the last administration, and the tumors were collected and weighed to calculate TGI. TGI was expressed as $TGI\% = (1 - m_t/m) \times 100\%$, where m_t and m denoted the average tumor weight of the treated groups and the saline group, respectively. The pathological slices of tumor were dyed with HE and examined by a light microscope.

2.6. Statistical analysis

Pharmacokinetics in rats was calculated by Kinetica 4.4. Curve of biodistribution in mice was fitted by statistical matrix method. Data were presented as mean ± SD. A p value less than 0.05 (i.e., $p < 0.05$) was considered to be statistical significant.

3. Result

3.1. Characterization of DocSEs, DocCSEs, and RLT-DocSEs

The TEM images of RLT-DocSEs were shown in Fig. S1. Table 1 listed the particle size, morphology, zeta potential, and Doc loading capacity (LC) and loading efficiency (LE) of DocSEs, DocCSEs, and RLT-DocSEs. DocSEs, DocCSEs, and RLT-DocSEs possessed nano-scale diameters, small PDI, high zeta potential, and sufficient Doc encapsulation. DocSEs, DocCSEs, and RLT-DocSEs only had slight pH decrease up to 10 days at 4 °C (data not shown). Long-term stability test showed that DocSEs, DocCSEs, and RLT-DocSEs could be stored stably at 4 °C up to two months (data not shown).