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Prostaglandin E_1 encapsulated into lipid nanoparticles improves its anti-inflammatory effect with low side-effect

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ABSTRACT

Prostaglandin E_1 (PGE1) shows various pharmacological activities including anti-inflammation. However, the rapid metabolization and inactivation of the intravenously administered PGE1 during the first passage through the lungs result in significant non-compliance in clinical trials which greatly limits its application. The aim of this work was to prepare the lipid nanoparticles loading PGE1 to improve its anti-inflammatory effect with low side-effect. The experimental results showed that PGE1 loaded lipid nanoparticles (PLNs) could be successfully prepared by high pressure homogenization with particle size 68.1 ± 4.7 nm, zeta potential -3.32 ± 0.37 mV and entrapment efficiency $92.1 \pm 1.3\%$. PLNs exhibited a sustained release with low burst drug release. PLNs could improve the inhibition effects of PGE1 on lipopolysaccharides (LPS)-induced TNF-α expression on macrophage RAW264.7 cells, and improve the inhibition of lymphocyte to endothelial cell adhesion and ICAM-1 adhesion molecule expression on HUVEC and MDA-MB-468 cell membrane. No allergenicity, vascular and muscle irritation were induced in animals by PLNs even at double of the highest drug concentration of clinical infusion. As a result, PLNs could be a more potential delivery system for PGE1 in the treatment of inflammation-related diseases.

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1. Introduction

Prostaglandin E_1 (PGE1), also called Alprostadil, shows various pharmacological activities such as vasodilation, inhibition of leukocyte adhesion and platelet aggregation, amelioration of the rheological property of blood, and anti-inflammation (Kerins et al., 1991). Therefore, it has been widely used in the treatment of peripheral vascular disease, ulcers, hepatopathy, pulmonary hypertension, ischaemic heart disease and so on (Mizushima et al., 1983; Murota et al., 2008; Shen et al., 2005). However, the rapid metabolization and inactivation of the intravenously administered PGE1 during the first passage through the lungs lead to the requirement of long-term infusion to reach the valid treatment concentration which causes severe clinical side effects such as hypotension, peripheral oedema, severe allergic reactions, and bleeding, bruising, pain, swelling or redness at the injection site (Golub et al., 1975; Schramek and Waldhauser, 1989).

So far, great efforts have been made to develop reliable delivery system for PGE1 such as lipid microspheres (Mizushima and Hoshi, 1993), cyclodextrin complexes (Gu et al., 2005), liposomes (Kawakami et al., 2001) and polymeric conjugates (Takeda et al., 2009) in order to improve its stability or modulate its pharmacokinetics, and finally enhance therapeutic effects and improve clinical compliance. Complexation of PGE1 by α-CD (PGE1-CD) which is clinically used worldwide could improve dissolution characteristics and chemical stability of PGE1 (Davis and Brewster, 2004). The lipid emulsions of PGE1 (Lipo-PGE1) clinically used in Japan, South Korea, and China are known to accumulate in the sites of inflammation or vascular lesions, and thus show a significantly enhanced clinical efficacy for peripheral vascular disease (Mizushima et al., 1983, 1990; Mizushima and Hoshi, 1993). However, PGE1 encapsulated in emulsion containing soybean oil as the emulsifier showed some drawbacks including chemical instability and rapid leakage of drug from emulsions in blood (Igarashi and Mizushima, 1996) and the local side effects were still found in patients receiving PGE1-CD (Toyota et al., 1993) or Lipo-PGE1 (Shen et al., 2005). So it is still a challenge to develop new delivery system of PGE1 with chemical and biological stability, enhanced pharmacological activity with low side effect.

Recently, lipid nanoparticles have attracted great attentions because this drug delivery system could effectively protect drug from inactivation, prolong drug retention time in blood thus increase its efficacy (Müller, 2007; Joshi and Müller, 2009; Puglia et al., 2008). In addition, lipid nanoparticles also show some advantages including good safety of the component materials and easy preparation over other nano-based drug delivery systems. We are interested in designing and developing a new delivery system for PGE1. In this work, the PGE1 loaded lipid nanoparticles (PLNs) were prepared, and the physiochemical characteristics, the in vitro

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anti-inflammatory effects of PLNs, and the in vivo allergenicity, vascular and muscle irritation of PLNs were evaluated.

2. Materials and methods

2.1. Materials

Prostaglandin E_1, Freund's adjuvant complete, Freund's adjuvant incomplete, hematoxylin, eosin and *Escherichia coli* lipopolysaccharide (LPS) were obtained from Sigma (St. Louis, MO, USA). Soybean lecithin was obtained from Shanghai Taiwei Pharmaceutical Co. Ltd. (Shanghai, China). Poloxmer 188 was purchased from BASF (Ludwigshafen, Germany). Lipo-PGE1 (Kaishi) was purchased from Tide Pharmaceutical Co. Ltd. (Beijing, China). Trypsin-EDTA and phosphate buffered saline (PBS, pH 7.4) were obtained from Gibco-BRL (Burlington, ON, Canada). The Dulbecco's modified Eagle medium (DMEM), RPMI 1640 medium, M199 medium, antibiotics and fetal bovine serum (FBS) were purchased from Invitrogen GmbH (Karlsruhe, Germany). Bradford protein assay kit was purchased from Beyotime Institute of Biotechnology (Jiangsu, China). PE conjugated mouse IgG1 (K Isotype control), PE conjugated anti-human CD54 (ICAM-1), and PE conjugated anti-human CD106 (VCAM-1) were purchased from eBioscience (San Diego, CA, USA). Mouse TNF-α and IL-1β ELISA kits were purchased from Bender MedSystems (Burlingame, CA, USA). Lymphocytes separation medium was obtained from Huajing Biological Hi-tech Co. Ltd. (Shanghai, China). Bovine serum albumin (BSA) was purchased from Junchuang Bioscience Co. Ltd. (Shanghai, China). Urethane and Evans Blue dye were obtained from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). All other chemicals and solvents were of analytical grade and used without further purification.

2.2. Cell culture

Human lymphocytes (HL) from healthy donors were separated by centrifugation with lymphocyte separation medium (Oostingh et al., 2006). The HUVEC (human umbilical vein endothelial cells) and MDA-MB-468 (human breast cancer cells) were purchased from the ATCC (American Type Culture Collection, Manassas, VA, USA). RAW264.7 cells (mouse macrophages) were obtained from CBCAS (Cell Bank of the Chinese Academic of Sciences, Shanghai, China). HUVEC were grown in M199 containing 20% fetal bovine serum (FBS), 100 Unit/ml penicillin G sodium and 100 μg/ml streptomycin sulfate. MDA-MB-468 cells were cultured in RPMI 1640 medium supplemented with 10% FBS and 1% antibiotics (100 Unit/ml penicillin G sodium and 100 μg/ml streptomycin sulfate). RAW264.7 cells were cultured in DMEM supplemented with 10% FBS and 1% antibiotics (100 Unit/ml penicillin G sodium and 100 μg/ml streptomycin sulfate). Cells were maintained at 37 °C in a humidified and 5% CO_2 incubator.

2.3. Animals

Healthy female guinea pigs (180–220 g) were purchased from Shanghai Institute of Biological Products (Shanghai, China). Male Sprague–Dawley rats (180–200 g) were purchased from Sino-British Sippr/BK Lab Animal Ltd. (Shanghai, China). Male and female New Zealand white rabbits (1.8–2.2 kg) were obtained from Shanghai Shengwang Experimental Animal Ranch (Shanghai, China). All animal procedures were performed according to the protocol approved by the Institutional Animal Care and Use Committee of the Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

2.4. Preparation and characteristics of PLNs

The PLNs were prepared by high pressure homogenization as described elsewhere with minor modification (Puglia et al., 2008). Briefly, soybean lecithin and alprostadil (w/w, 300:1) were dissolved in ethanol at a concentration of 0.2 mg/ml. The solution was mixed with 0.5% Poloxamer 188 solution containing 20% sucrose to obtain a final drug concentration of 5 μg/ml, and the mixture was ultrasonicated for 2 min. The obtained premix was then passed through a high pressure homogenizer (EmulsiFlex-C3, Avestin, Canada) at 20,000 psi for three cycles. The PLN suspension was lyophilized and stored at 4 °C.

The mean particle size and zeta potential of PLNs after dispersed in water were determined by dynamic light scattering method using Nicomp 380/ZLS analyzer (Particle Sizing Systems, Inc., Santa Barbara CA, USA). The morphological examination of PLNs was performed using a transmission electron microscope (TEM, CM12, Philips, Netherlands) after negative staining with sodium phosphotungstate solution (0.2%, w/v). The amount of alprostadil in PLNs was determined by ultrafiltration/centrifugation. Briefly, 500 μl of PLNs dispersed in water was added to Ultrafree-MC (50KD, Millipore) and centrifuged for 10 min at 5000 rpm. The amount of alprostadil in the filtrate which was not entrapped in the nanoparticles, W_{EX}, was thus determined. Total alprostadil concentration W_{TO}, was measured by HPLC as described below after dissolution of the colloidal dispersions by methanol. The encapsulation efficiency (EE) of PLNs was calculated as:

$$EE\,(\%) = \frac{W_{TO} - W_{EX}}{W_{TO}} \times 100\% \quad (1)$$

For measurement of alprostadil, a sensitive HPLC method with post-column derivatization was used. Aliquots of 20 μl were analyzed on HPLC system (Agilent 1100) with the sequence of separation, post-column reaction and detection. Separation was carried out by a column of SB-C_{18} (250 × 4.6 mm i.d., pore size 5 μm) at a flow rate of 1.0 ml/min with the mobile phase: 6.7 mM potassium dihydrogen phosphate solution (pH 6.3)–CH_3CN (3:1, v/v). The T_{98} post-column reaction system (Beijing, China) was placed between the detector and the column consisting of poly(tetrafluoroethylene) pipe (0.5 mm × 10 m) and 1 mol/l KOH as reaction solvent with reaction temperature 60 °C. The reaction product was detected at 278 nm.

2.5. In vitro release of PLNs

In vitro release of PLNs was performed using pure water as release medium. The PLNs dispersed in water (5 μg/ml) was diluted with water (1:5, v/v) and shaken horizontally (100 rpm) at 37 °C in dark place. At predetermined time intervals, 1 ml of solution was taken out and the amount of released drug was measured by HPLC after separated from nanoparticles by ultrafiltration/centrifugation. The same volume of fresh water was added to maintain the total volume after solution removal. As a control, Lipo-PGE1 was used, and in vitro release experiment was performed as PLNs.

2.6. In vitro anti-inflammation experiment

2.6.1. ELISA for detecting tumor necrosis factor alpha (TNF-α) and interleukin-1beta (IL-1β)

The concentration of TNF-α and IL-1β in the culture supernatants of RAW264.7 cells after LPS stimulation was analyzed by the commercially available ELISA kits according to the manufacturer's protocol. Briefly, RAW264.7 cells were seeded in a 24-well plate with 0.5 ml growth medium and allowed to attach for 24 h. Then, cells were incubated with free PGE1 (first dissolved in ethanol

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