Pharmaceutical nanotechnology

Bovine serum albumin nanoparticles for delivery of tacrolimus to reduce its kidney uptake and functional nephrotoxicity

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ABSTRACT

The purpose of the present study was to develop a new nanoparticulate formulation for delivery of tacrolimus to reduce its kidney distribution and functional nephrotoxicity. Tacrolimus (TAC)-loaded bovine serum albumin (BSA) nanoparticles (TAC-BSA-NPs) were prepared by emulsification-dispersion technique. The obtained TAC-BSA-NPs, with 189.50 ± 7.15 nm of diameter and -20.86 ± 0.45 mV of Zeta potential determined by DLS, were spherical in shape observed by TEM. The drug loading content and encapsulation efficiency were $(1.7 \pm 0.13)\%$ and $(85 \pm 3.0)\%$, respectively. The in vitro release of TAC-BSA-NPs exhibited biphasic drug release pattern with an initial burst release and subsequently sustained release. Pharmacokinetic analysis displayed that TAC-BSA-NPs could enhance the drug blood level and prolong the circulation time in comparison to Prograf®. Meanwhile, compared with Prograf®, TAC-BSA-NPs could deliver less TAC to kidney and simultaneously reduce the functional nephrotoxicity of TAC to kidney. In conclusion, BSA nanoparticles might be a more safe carrier for delivery of hydrophobic drug TAC.

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1. Introduction

Tacrolimus (TAC) (Fig. 1), a macrolide immunosuppressant, has been widely used to prevent acute rejection in organ transplants (Wallemacq and Reding, 1993). Similar to cyclosporine A, a cyclic undecapeptide immunosuppressant, the action mechanism of TAC has been demonstrated to bind to the TAC binding protein (immunophilin FKBP-12) inside the activated T-cells (Cardenas et al., 1995), which inhibits the activity of calcineurin for dephosphorylating the nuclear factor of activated T cells (NFAT), thereby reduces the generation of IL-2 and inhibits activation and proliferation of T cells, leading to suppressed immune response (Ruff and Leach, 1995; Shaw et al., 1995). TAC exhibits much greater immunosuppressive activity than cyclosporine A (Geissler and Schlitt, 2009) and provides a better side effect profile and increases long-term survival in patients (Jurewicz, 2003; Wiesner, 1998). However, its therapeutic efficacy is limited due to its poor water solubility. In addition, TAC is known to exhibit some adverse events, such as nephrotoxicity, neurotoxicity, hypertension and diabetogenic effects (Bottiger et al., 1999). Its commercial formulation Prograf® contains high amount of polyethylene hydrogenated castor oil (HCO-60) which has been found to associate with severe side effect of anaphylaxis and still showed nephrotoxicity to some extent (Nicolai and Bunyavanich, 2012). Therefore, many efforts have been devoted to improve the solubility of TAC and reduce the side effects of TAC, including encapsulation of TAC in liposomes (Ishii et al., 2013), polymeric micelles (Wang et al., 2011), cyclodextrin inclusion (Brewster and Loftsson, 2007), nanoparticles (Xu et al., 2014), nanocapsules (Nassar et al., 2009) and self-microemulsifying drug delivery system (von Suesskind-Schwendi et al., 2013). Among these drug delivery vehicles, nanoparticles have gained much attention in recent years.

Nanoparticles are made of a variety of polymers, such as polysaccharides (Fernandez-Urrusuno et al., 1999; Liu et al., 2008), proteins (Elzoghby, 2013; Harsha, 2013) and synthetic polymers (Breunig et al., 2008; Fattal et al., 1998). Among all the available materials, the versatile albumin is an ideal material to fabricate nanoparticles for drug delivery due to its nontoxic, nonimmunogenic, biocompatible and biodegradable properties (Kratz, 2008). Additionally, albumin nanoparticles exhibited high binding capacity of various drugs (Jithan et al., 2011; Kratz, 2014) and were well tolerated without any serious side effects (Ibrahim et al., 2002). Moreover, their polymeric nature controls the release of drug in a sustained and controlled manner for a longer time (Roney et al., 2005). Accordingly, albumin nanoparticles have received

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considerable attention (Elzoghby et al., 2012). Encouragingly, paclitaxel-loaded nanoparticles human serum albumin (Abraxane®) was approved by FDA for clinic use in 2005. It improved water solubility of paclitaxel with enhanced efficacy and tolerability compared with Cremophor based paclitaxel formulation (Cortes and Saura, 2010). To the best of our knowledge, albumin nanoparticles containing TAC has not been reported. Hence, this study was aimed to formulate TAC-loaded bovine serum albumin nanoparticles and evaluate their physicochemical properties, *in vitro* release, *in vivo* pharmacokinetics and biodistribution, and find out their effectiveness in reduction of functional nephrotoxicity.

2. Materials and methods

2.1. Materials

Tacrolimus (TAC) was supplied by Guangzhou Yibang Company (Guangdong, China). Bovine serum albumin (BSA, purity 99%) was purchased from Beijing Xinjingke Company (Beijing, China). Cholesterol was obtained from Tianjin Bodi Company (Tianjin, China). All other reagents were of analytical grade.

The kits used to assay blood urea nitrogen, serum creatinine and creatinine clearance were purchased from Nanjing Kaiji Biological Company (Jiangsu, China).

SD rats weighing 250 ± 30 g were supplied by the Experimental Animal Center of Peking University Health Science Center. All care and handling of animals were performed with approval of Institutional Authority for Laboratory Animal Care of Peking University Health Science Center.

2.2. Preparation of TAC-loaded BSA nanoparticles

TAC-loaded BSA nanoparticles (denoted as TAC-BSA-NPs) were prepared by the emulsification-dispersion technique as previously reported with minor modification (Zhang et al., 2011). Briefly, TAC (6 mg) and cholesterol (12 mg) were dissolved in a mixture of chloroform (0.55 mL) and ethanol (0.05 mL), and then added to BSA

Fig. 1. Chemical structure of tacrolimus (TAC).

aqueous solution (1%, w/v, 30 mL). The resultant mixture was under shearing for 3 min to form a crude emulsion, and then passed through a high-pressure homogenizer (NCJJ-0.007/200, Langfang, China) for 8 cycles at 90–100 MPa, followed by evaporation under vacuum at 30 °C for about 30 min to obtain the organic solvent-free dispersion of TAC-BSA-NPs.

2.3. Characterization of TAC-loaded BSA nanoparticles

The mean diameter and size distribution, and Zeta potential of TAC-BSA-NPs were determined by dynamic light scattering (DLS) using a Malvern Zeta/Sizer (Nano ZS, Malvern, UK) with a scattering angle of 90° at 25 °C.

Morphological examination of TAC-BSA-NPs was conducted using transmission electron microscope (TEM, JEM-1230, JEOL, Japan) following negative staining with a drop of 1 wt% phosphotungstic acid solution.

To determine the encapsulation efficiency (EE) and loading content (LC) of the prepared nanoparticles, 9 mL methanol was added to 1 mL TAC-BSA-NPs suspension to precipitate protein, followed by centrifugation at 3000 rpm for 5 min. An HPLC system (HP1100, Agilent, USA) with a UV detector set at 220 nm was used to determine the concentration of TAC in the supernatant (Gao et al., 2012). The separation of TAC in 20 μL of samples was performed on a reversed phase column (ODS C18, 5 μm, 4.6 mm × 250 mm; Dikma, Beijing, China) at 50 °C, eluting with a mixture of acetonitrile and water at a ratio of 3:1 (v/v) at a flow rate of 1.0 mL/min. The EE and LC of TAC-BSA-NPs were calculated as follows:

$$EE(\%) = \frac{\text{amount of TAC loaded in nanoparticles}}{\text{original feeding amount of TAC}} \times 100$$

$$LC(\%) = \frac{\text{amount of TAC loaded in nanoparticles}}{\text{total amount of nanoparticles}} \times 100.$$

2.4. In vitro *drug release profile*

The *in vitro* release behavior of TAC from TAC-BSA-NPs was monitored by using a dialysis-bag diffusion method as described previously with little modification (Li et al., 2010). Briefly, 2 mL of TAC-BSA-NPs dispersion was introduced into a dialysis bag (molecular weight cutoff of 8–14 kD). Following sealed, the dialysis bag was completely submerged into 20 mL of PBS (pH 7.4) containing 0.1% (v/v) Tween 80 at 37 °C with a shaking rate of 100 rpm. Periodically, 1 mL of the release medium was taken out at various time intervals and the same volume of fresh medium was added. The concentration of released TAC in release medium was measured by HPLC method as described above. The release experiments were repeated three times and average data were reported. The release of TAC from free TAC solution in ethanol was also tested as control.

2.5. In vivo *pharmacokinetics study*

2.5.1. In vivo *experiments*

12 healthy male SD rats weighing 250 ± 30 g, fasted for 12 h prior to the experiments but allowed free access to water, were randomly divided into two groups with 6 rats in each group to be intravenously given a single injection of TAC-BSA-NPs and commercial formulation Prograf® (served as control) through the tail vein at an equivalent TAC dose of 10 mg/kg, respectively. 1 mL of blood sample was drawn from the rat's orbit at the designated time intervals, and then 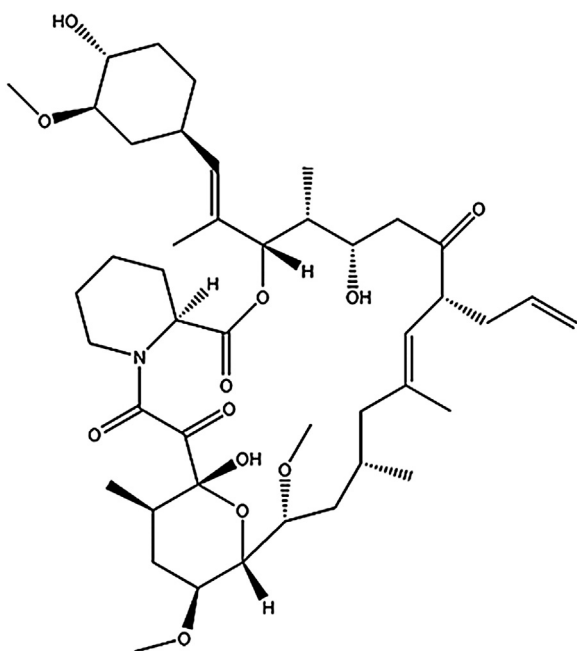stored at −20 °C until analysis.