International Journal of Pharmaceutics 497 (2016) 268–276

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Pharmaceutical potential of tacrolimus-loaded albumin nanoparticles having targetability to rheumatoid arthritis tissues

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ARTICLE INFO

Article history:
Received 5 October 2015
Received in revised form 10 November 2015
Accepted 3 December 2015
Available online 4 December 2015

Keywords:
Albumin
Nanoparticles
Tacrolimus
Rheumatoid arthritis
Collagen-induced arthritis
Targeting

ABSTRACT

Albumin is considered an attractive dug carrier for hydrophobic drugs to target inflamed joints of rheumatoid arthritis. This study focused on the pharmaceutical potential of albumin-based nanoparticles (NPs) on delivery of tacrolimus (TAC) to enhance targetability and anti-arthritic efficacy. TAC-loaded human serum albumin (HSA) nanoparticles (TAC HSA-NPs) were prepared using the nab^{TM} technology. The resulting NPs were 185.8 ± 6.8 nm in diameter and had a zeta potential value of −30.5 ± 1.1 mV, as determined by dynamic light scattering. Particles were uniformly spherical in shape as determined by transmission electron microscopy. The encapsulation efficacy of TAC was 79.3 ± 3.7% and the water solubility was over 46 times greater than that of free TAC. TAC was gradually released from NPs over 24 h, which is sufficient time for targeting and treatment of the NPs in inflamed arthritis via intravenous injection. *In vitro* study using splenocytes excised from spleens of mice following induction of arthritis using collagen clearly demonstrated the anti-proliferative activity of TAC HSA-NPs on activated T cells compared with non-activated T cells. Furthermore, TAC HSA-NPs displayed significantly more anti-arthritic activity than TAC formulations including intravenously administered TAC solution or oral TAC suspension, as reflected by the incidence of arthritis and clinical score (1.6 *vs.* 3.2 and 5.0, respectively). These improvements were due to the targetability of HSA that facilitated the accumulation of TAC HSA-NPs at inflamed arthritis sites. TAC HSA-NPs are a promising drug delivery system to enhance water solubility and increase accumulation in joints for treatment of rheumatoid arthritis.

1. Introduction

Albumin is a versatile attractive biomaterial or carrier for many therapeutics in treating a variety of diseases including cancer, arthritis and diabetes (Kratz, 2008; Kratz and Warnecke, 2012). It is used as a conjugation counterpart for many drugs like doxorubicin, methotrexate and exendin-4 (Bae et al., 2012; Choi et al., 2014; Stehle et al., 1997; Kim et al., 2010), and is extensively utilized in preparing nanoparticles (NPs) including anti-cancer agents because albumin NPs display highly selective targetability to tumor tissues due to the enhanced permeability and retention effect and gp60-mediated transcytosis (Sage et al., 1984; Elsadek and Kratz, 2012). Interestingly, albumin has special ability for arthritis targeting because it markedly accumulates in inflamed tissues in arthritis. Inflamed tissues require much more albumin as a relevant energy source than normal tissues (Wilkinson et al., 1965). Therefore, synovial cells are highly up-regulated in the up-take of albumin, and the permeability of blood-joint barrier in inflamed joint of patients with rheumatoid arthritis (RA) is remarkably enhanced (Ballantyne et al., 1971; Levick, 1981).

Tacrolimus (FK 506; TAC) is a highly lipophilic 23-member macrolide lactone antibiotic isolated from *Streptomyces tsukubaensis* that is primarily used clinically as an immunosuppressant (Patel et al., 2012; Spencer et al., 1997). TAC is 10–100 times more potent than cyclosporine A in inhibiting the production of lymphokines involved in interleukin (IL)-2 production and so has become widely used in organ transplantations (Letko et al., 1999; Tocci et al., 1989). Especially, TAC has demonstrable anti-arthritic activity in rodents through the suppressing of inflammation and expression of tumor necrosis factor-alpha (TNF-α) and IL-1β, which reduces damage to bone and cartilage (Magari et al., 2003; Sakuma et al., 2000). TAC is therapeutically effective in RA

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http://dx.doi.org/10.1016/j.ijpharm.2015.12.004
0378-5173/

patients following the failure methotrexate treatment (Furst et al., 2002).

Among many methods to prepare albumin-based NPs including desolvation, thermal gelation, emulsification, self-assembly or nano spray drying, the nanoparticle albumin-bound (nab™) technology is the most effective and cutting-edge preparation method (Fanciullino et al., 2013; Min et al., 2015; Elzoghby et al., 2012). This NP formulation uses only albumin, hydrophobic drugs, and a small quantity of organic solvents that can be completely removed by evaporation. The NPs can be easily formed using several cycles of high-pressure homogenization (Desai et al., 2006). In particular, the paclitaxel-bound albumin NP formulation has available commercially as Abraxane® since 2005, and a variety of hydrophobic drugs including rapamycin and docetaxel are in clinical phases of testing in conjunction with the nab™ technology (Elsadek and Kratz, 2012).

Here, we sought to develop an injection-based TAC-loaded albumin NP formulation for the treatment of RA. TAC is a good drug candidate for albumin NPs because it is practically insoluble and binds well to albumin molecules. Furthermore, albumin is effectively accumulated in inflamed synovial tissues in RA; NPs loaded with albumin and TAC should selectively target arthritis tissues. This study examined the physicochemical properties, *in vitro* bioactivity in mouse splenocytes, targetability and anti-arthritic efficacy of the TAC-loaded human albumin serum NPs (TAC HSA-NPs) in a collagen-induced mouse model of arthritis.

2. Materials and methods

2.1. Materials

TAC with a purity of 99.6% was obtained from the Research Laboratories of ChongKunDang Pharm. Inc. (Yongin, Korea). HSA (~99% pure, 66.5 kDa) was purchased from Sigma–Aldrich (St. Louis, MO, USA). Cy5.5-NHS ester dye was purchased from GE Healthcare (Piscataway, NJ, USA). Cell Counting Kit-8 (CCK-8) was purchased from Dojindo Molecular Technologies Inc. (Gaithersburg, MD, USA). Bovine type-II collagen (CII, 2 mg/ml), complete Freund's adjuvant (CFA) and incomplete Freund's adjuvant (IFA) were purchased from Chondrex, Inc. (Redmond, USA). RPMI 1640 medium, fetal bovine serum (FBS) and penicillin/streptomycin (P/S) were obtained from Corning (Corning, NY, USA). Unless specified all other reagents and chemicals were purchased from Sigma–Aldrich.

2.2. Animals

DBA/1 mice (male, 7–8 weeks old, 20–22 g) were purchased from the Hanlim Experimental Animal Laboratory (Seoul, Korea). All experiments related to animals were conducted according to the guidelines of the National Institute of Health (NIH) on the use of laboratory animals (NIH publication 80–23, revised in 1996). Animals were acclimated for a week before use and housed with free access to feed and water. This animal study was approved by the Ethical Committee on Animal Experimentation of Sungkyunkwan University.

2.3. Preparation of TAC HSA-NPs

TAC HSA-NPs were prepared using the nab™ technology (Fu et al., 2009; Zhao et al., 2015) with slight modifications. Briefly, HSA (150 mg) was dissolved in 15 ml deionized water (DW). TAC (7.5 mg) and cholesterol (15 mg) were separately dissolved in 0.3 ml of a 9:1 solution of chloroform and ethanol. Especially, cholesterol was added to increase the hydrophobic attraction between albumins combined with TAC in the process of nanoparticle formation (Zhao et al., 2015). These two solutions were then mixed at low rotating speed to form a crude emulsion prior to high-pressure homogenization using an EmulsiFlex-B15 device (Avestin, Ottawa, Ontario, Canada) at 20,000 psi for nine cycles. The resulting colloid was rotary evaporated to remove chloroform at 40 °C for 15 min under reduced pressure. The obtained NPs were centrifuged at 6000 rpm for 10 min, the supernatant was collected and solvent was removed by lyophilization. The product was stored at −70 °C until required.

2.4. Characterization of TAC HSA-NPs

The particle size (mean particle diameter and size distribution) and zeta potential of the NPs were determined by dynamic light scattering with a Zetasizer Nano ZS90 (Malvern Instruments, Malvern, UK) using a 633 nm He-Ne laser beam with a fixed scattering angle of 90°. Measurements were performed in triplicate with a NP concentration of 1 mg/ml on HSA weight basis in DW at 25 °C. The morphology of the NPs was investigated by transmission electron microscopy (TEM) using negative staining with phosphotungstic acid (PTA, 1.0% w/v). Briefly, 20 μl of NP (1.0 mg/ml) solution was deposited onto the carbon side of 200-mesh, formvar/carbon coated copper grid (TED PELLA, Redding, CA, USA) and the excess solution was removed using filter paper after 2 min. The grid was left to dry for 30 min prior to being floated on the surface of a drop of PTA. The excess was blotted away after 30 sec using filter paper. Subsequently, the air-dried grid was examined by TEM using a model H-7600 microscope (Hitachi, Tokyo, Japan).

2.5. Encapsulation efficiency and the in vitro release profile of TAC

To determine the encapsulation efficiency (EE) and loading content (LC) of TAC in NPs, 5.0 mg of lyophilized NPs were dissolved in 2.0 ml of a 9:1 solution of acetonitrile (ACN) and DW, and thoroughly shaken for 5 min for precipitation protein. The solution was centrifuged at 13,500 rpm for 10 min. TAC in the supernatant was collected for measured using high-performance liquid chromatography (HPLC) with a 1260 infinity system (Agilent, Sunnyvale, CA, USA) using a LiChrospher 100 RP-18 reverse phase column (250 mm × 4.0 mm, 5 μm particle size; Merck, Darmstadt, Germany) at 50 °C. The mobile phase consisted of 80% ACN and 20% water, as previously described with slight modifications (Li et al., 2012). The system was run isocratically at a flow rate of 1 ml/min and TAC was detected at 215 nm. Each sample was assayed in triplicate. EE and LC were calculated as:

$$EE = \left[\frac{\text{amount of drug entrapped}}{\text{amount of drug loaded}}\right] \times 100.$$

$$LC = \left[\frac{\text{amount of drug entrapped}}{\text{total amount of NPs}}\right] \times 100.$$

In vitro drug release of TAC from NPs was investigated using the dialysis diffusion method. Sixty milligrams of NPs were suspended in 2 ml DW and dialyzed against 200 ml of phosphate buffered saline (PBS, pH 7.4) containing 0.1% (v/v) Tween 80 at 37 °C using a semipermeable membrane with a 10 kDa cut-off. At predetermined times, 2 ml of released medium was collected and the same volume replaced with fresh medium. TAC in the released fluid was measured by HPLC as described above. Each experiment was performed in triplicate. Cumulative release was expressed as percentage *vs.* initial loading amount at each time point.