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Therapeutic advantage of inhaled tacrolimus-bound albumin nanoparticles in a bleomycin-induced pulmonary fibrosis mouse model

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ABSTRACT

Tacrolimus (Tac) is an immunosuppressant that inhibits translocation of nuclear factor of activated T cells and has therapeutic potential for pulmonary fibrosis. Here, we investigated the therapeutic efficacy of a sustained-release type inhaled Tac formulation for treating bleomycin-induced pulmonary fibrosis. Inhalation has many meaningful advantages over injections, such as improved patient compliance, safety, and therapeutic effect. To this end, we fabricated inhalable albumin nanoparticles with bound Tac (Tac Alb-NPs) at a daily therapeutic dose (60 μg/mouse) using a high-pressure homogenizer via nanoparticle albumin-bound technology. The Tac Alb-NPs were spherical, ~182.1 ± 28.5 nm in size, with a zeta potential of −34.5 ± 0.3 mV, and the Tac incorporation efficiency was as high as ~85.3%. The bound tacrolimus was released gradually from Tac Alb-NPs for ~24 h, which was sufficient time for pulmonary delivery. Most of all, the inhaled Tac Alb-NPs displayed remarkable anti-fibrotic efficacy in mice with bleomycin-induced pulmonary fibrosis, which was much better than the efficacy resulting from intraperitoneal administration of Tac (60 μg/mouse) based on histopathological results (hematoxylin and eosin and Masson's trichrome staining). Furthermore, the inhaled Cy5.5-labelled Tac Alb-NPs were visualized throughout the lungs of mice for ~48 h, indicating direct exposure to fibrotic tissues in lung lesions. In conclusion, Tac Alb-NPs offer great potential as an inhalation delivery formulation for treating pulmonary fibrosis. Additionally, these NPs would be particularly useful as an effective and safe prototype for delivering practically insoluble therapeutic agents into the lungs.

1. Introduction

Idiopathic pulmonary fibrosis (IPF) is a chronic lung disease associated with poor survival of 2–4 years after diagnosis [1–3]. IPF is characterized and develops as a series of processes, i.e., alveolar epithelial cell injury and hyperplasia, inflammatory cell accumulation, fibroblast hyperplasia, deposition of extracellular matrix, and scar formation. Eventually, such processes cause loss of lung elasticity and alveolar surface area, leading to severe pulmonary function damage [4]. Furthermore, the mechanism for onset and progression of IPF remains poorly understood; thus, no effective drugs for treating IPF have been available for many decades until an oral formulation of pirfenidone (Esbriet®) was approved in Europe and United States in 2011 and 2014, respectively [5]. Due to such poor treatment modalities, IPF remains a challenging disease to develop new drugs or formulations.

The calcineurin inhibitor tacrolimus (FK506; Tac) is a macrolide isolated from Streptomyces tsukubaensis (Fig. 1) and is used as an immunosuppressant that inhibits translocation of nuclear factor of activated T cells into the nucleus [6–8]. This drug inhibits T lymphocytes and thus can be used to improve respiratory dysfunction and formation of edema [9]. The therapy mechanism of Tac is associated with TGF-β. Binding TGF-β to TGF-β receptors enhances

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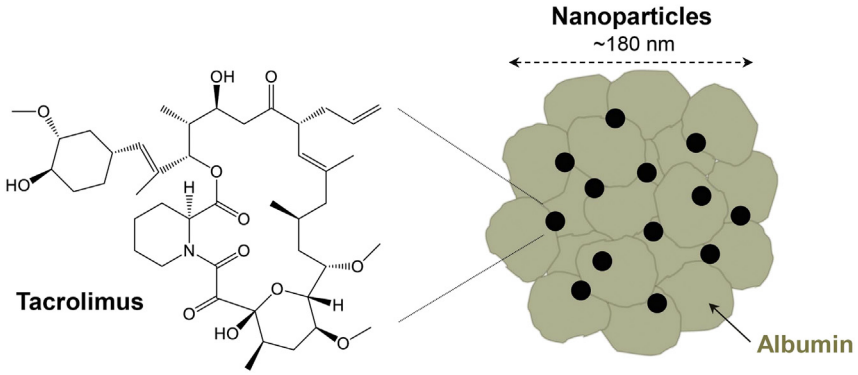

Fig. 1. Chemical structure of tacrolimus and an illustration of the tacrolimus-bound albumin nanoparticles.

lung fibrosis. MAPKs, including ERK1/2, p38, and JNK, play an important role in signal transduction of TGF-β. Tac strongly inhibits activation of p38 MAPK that increases type I collagen expression stimulated by TGF-β. So Tac markedly reduces inflammation by inhibiting TGF-β [10,11]. Importantly, oral co-administration of Tac with methyl prednisone alleviated acute IPF exacerbations in a small cohort of patients [12]. Tac has clear therapeutic effects for mitigating IPF in a bleomycin-induced lung fibrosis model and does not promote inflammation when administered by inhalation, unlike systemic administration [12–14].

Inhalation has been reviewed as an effective way to deliver drugs to pulmonary lesions in patients with lung-related diseases [15,16]. First, the respiratory tract has favorable physiological environment to pulmonary delivery of Tac because of the thin epithelial barrier, slow mucociliary clearance, and trivial enzymatic activity [17]. Moreover, inhalation enables high local drug availability around disease sites at high concentrations due to direct delivery via the pulmonary route and avoids undesirable high systemic levels of drugs to other organs. Therefore inhalation delivery of Tac can reduce adverse side-effects that may occur in intraperitoneal injection [14]. In addition, inhalation is a non-invasive convenient administration method that allows self-medication by patients versus injection [18,19]. Also inhalation can avoid risk of syringe needle-related infection by injection [20]. Due to these advantages, a number of inhalation treatments have been developed and have shown successful therapeutic results in patients with lung diseases, such as, bronchitis, asthma, and chronic obstructive pulmonary disease [15,21].

Here, we investigated the therapeutic efficacy of an inhaled Tac formulation to treat pulmonary fibrosis. Albumin nanoparticles are considered to be an effective way to dissolve and deliver Tac because it is practically insoluble in water. Accordingly, we examined the physicochemical properties, release profile, aerosolization, and lung deposition characteristics of Tac-loaded albumin nanoparticles (Tac Alb-NPs). The therapeutic efficacy of Tac Alb-NPs was evaluated in a bleomycin-induced pulmonary fibrosis mouse model.

2. Materials and methods

2.1. Materials

Tac was kindly supplied by the Research Laboratories of ChongKunDang Pharm. (Yongin, Korea). Bleomycin sulfate and bovine serum albumin (BSA) were purchased from Sigma–Aldrich (St. Louis, MO, USA). All other reagents were obtained from Sigma–Aldrich.

2.2. Animals

C57BL/6 mice (males, 7 weeks old) were purchased from Hanlim Experimental Animal Laboratory (Seoul, Korea). The animals were cared for in accordance with the guidelines issued by the National Institute of Health regarding the care and use of laboratory animals (NIH publication 80-23, revised in 1996). The animals were housed in groups of 6–8 under a 12-h light/dark cycle (lights on 6 am), provided food and water *ad libitum*, and acclimatized for a week. This study was approved by the Ethical Committee on Animal Experimentation at Sungkyunkwan University.

2.3. Preparation of Tac Alb-NPs

The Tac Alb-NPs were prepared using a slight modification of nanoparticle albumin-bound technology [22,23]. Briefly, Tac (2.5 mg) and cholesterol (5 mg) were dissolved in 0.1 ml of a 9:1 solution of chloroform and ethanol, and BSA (50 mg) was dissolved in 5 ml of deionized water (DW). These two solutions were mixed and passed through a high-pressure homogenizer (EmulsiFlex-B15, Avestin Inc., Ottawa, ON, Canada) nine times at 20,000 psi. The resulting dispersion was rotary evaporated to remove chloroform and ethanol at 40 °C for 15 min under reduced pressure. The nanoparticles generated were centrifuged at 6000 rpm, and the supernatant was lyophilized for ~2 days and stored at −20 °C until required.

2.4. Characterization of the Tac Alb-NPs

The mean particle size and zeta potential of the Tac Alb-NPs were measured by dynamic light scattering (DLS) with a 90° scattering angle for optimum detection. The zeta potential of the nanoparticles and mean nanoparticle size were measured at a concentration of 1 mg/ml in DW. Tac Alb-NPs morphology was observed by transmission electron microscope (TEM) with a model H-7600 microscope (Hitachi, Tokyo, Japan).

2.5. Incorporation efficiency and release of Tac

The incorporation efficiency of Tac in the Tac Alb-NPs was measured by reverse-phase high-performance liquid chromatography (RP-HPLC). A portion (4 mg) of Tac Alb-NPs was added to acetonitrile (ACN; 4.5 ml) and DW (0.5 ml) and shaken for 15 min to extract the Tac. The sample was centrifuged at 13,500 rpm for 5 min. A 25 μl aliquot of the supernatant was injected onto a LiChrospher 100 RP-18 column (250 × 4.0 mm, 5 μm, Merck, Darmstadt, Germany) at 60 °C. The mobile phase was ACN and DW (70:30) at a flow rate of 1.0 ml/min. Eluates were monitored at