Biomedicine & Pharmacotherapy 78 (2016) 226–233

Available online at
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com/en

Original article

Treatment of bleomycin-induced pulmonary fibrosis by inhaled tacrolimus-loaded chitosan-coated poly(lactic-*co*-glycolic acid) nanoparticles

Changkyu Lee[1,a], Jisoo Seo[1,a], Ha Shin Hwang[a], Le Quang Thao[a], Seunghyun Lee[a], Eun Seong Lee[b], Eun Hee Lee[c], Han-Gon Choi[d], Yu Seok Youn[a,*]

[a] School of Pharmacy, Sungkyunkwan University, 300 Cheoncheon-dong, Jangan-gu, Suwon 16419, Republic of Korea
[b] Division of Biotechnology, The Catholic University of Korea, 43-1 Yeokgok 2-dong, Wonmi-gu, Bucheon-si, Gyeonggi-do 14662, Republic of Korea
[c] College of Pharmacy, Korea University, 2511 Sejong-ro, Sejong 30019, Republic of Korea
[d] College of Pharmacy, Hanyang University, 55, Hanyangdaehak-ro, Sangnok-gu, Ansan 15588, Republic of Korea

ARTICLE INFO

Article history:
Received 5 October 2015
Accepted 20 January 2016

Keywords:
Tacrolimus
PLGA nanoparticle
Chitosan
Pulmonary fibrosis
Inhalation

ABSTRACT

Pulmonary fibrosis is a chronic lung disease characterized by inflammation and collagen deposition, with an estimated mortality rate exceeding 70%. Here, we evaluated the therapeutic effectiveness of inhaled tacrolimus-loaded chitosan-coated poly(lactic-*co*-glycolic acid) nanoparticles (chitosan TAC PLGA-NPs) in a bleomycin-induced pulmonary fibrosis mouse model. Chitosan TAC PLGA-NPs were fabricated using an o/w emulsification diffusion method, and uncoated TAC PLGA-NPs and chitosan TAC PLGA-NPs were spherical with approximate diameters of 320 and 441 nm, respectively. The zeta potential of chitosan TAC PLGA-NPs (+13.6 mV) was increased significantly by chitosan-coating versus uncoated TAC PLGA-NPs (−28.3 mV). The incorporation efficiency of tacrolimus was 37.7%, and the tacrolimus was gradually released until about 5 day. Direct inhalation of chitosan TAC PLGA-NPs (TAC 180 μg/mouse) twice a week produced marked anti-fibrotic efficacy in mice with bleomycin-induced pulmonary fibrosis, which was much better than the efficacy resulting from daily oral administration (TAC 300 μg/mouse) on the basis of hematoxylin/eosin and Masson's trichrome staining assessments. Imaging of lung deposition showed that chitosan TAC PLGA-NPs were located well in the lungs and gradually faded over 96 h. The pulmonary delivery of tacrolimus could be therapeutically efficacious for treating pulmonary fibrosis. TAC-loaded PLGA nanoparticles should be considered to be an efficient sustained-release type inhalation system that reduces administration frequency and relevant side effects.

1. Introduction

Pulmonary fibrosis is a chronic lung disease characterized by loss of lung epithelial cells and accumulation of fibroblasts that promotes collagen synthesis. Pulmonary fibrosis markedly impairs respiration and gas exchange. The respiratory condition of patients often becomes impaired suddenly, and the mortality rate of pulmonary fibrosis exceeds 70% [1,2].

Tacrolimus (FK506; TAC) has great potential in treating pulmonary fibrosis because this drug has a strong immunosuppressive effect. TAC specifically suppresses T-lymphocyte signal transduction and interleukin-2 transcription by inhibiting calcineurin. Although the treatment mechanism is similar to that of cyclosporine, the initial effective serum level of TAC is 100 times less than that of cyclosporine. This suggests that the required therapeutic dose and relevant side effects caused by TAC can be significantly reduced versus cyclosporine [3].

Although oral formulations of TAC are preferred by patients, pulmonary formulations have many advantages in treating pulmonary fibrosis. Especially, the oral bioavailability of TAC is as low as 21% and clinical problems include large inter-subject variability and narrow therapeutic window via the oral route [4]. The physiological environment of respiratory tract is favorable to inhalation delivery of TAC due to the thin epithelial barrier (0.1–0.5 μm in alveoli), slow mucociliary clearance, and insignificant enzymatic activity [5]. Moreover, the direct delivery of TAC via the pulmonary route increases the local concentration of TAC around fibrosis lesions and reduces distribution of TAC to other organs [4,6]. Therefore, inhalation of TAC could reduce relevant systemic

* Corresponding author. Fax: +82 31 290 7724.
E-mail address: ysyoun@skku.edu (Y.S. Youn).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.biopha.2016.01.027
0753-3322/

side effects that otherwise occur upon oral or parenteral administration [6]. In this respect, the pulmonary delivery of TAC can be considered to be an effective way of treating lung fibrosis.

The use of TAC has been limited by its low water solubility and narrow therapeutic index [7,8]. To surmount this problem, nanotechnology have been developed by using poly(lactic-*co*-glycolic acid) (PLGA) copolymers due to its biocompatibility, biodegradability, and controlled release kinetics [9]. Owing to such good physicochemical properties, PLGA nanoparticles (PLGA-NPs) have been extensively used as a delivery system for a variety of drugs for many therapeutic indications [10].

Chitosan, the *N*-acetylated derivative of chitin, is also biodegradable, biocompatible, little toxic, and cationic [11]. Surface modification of PLGA-NPs with chitosan has several significant pharmaceutical advantages. First, chitosan coating decreases the burst effect of drugs. Second, positively-charged chitosan effectively adheres to the negative-charged cellular membrane and enhances the retention and permeation of PLGA-NPs in the relevant tissues [12,13]. In our previous study, chitosan-modified PLGA-NPs were retained in a longer period than uncoated PLGA-NPs at the bronchial mucus tissue and increased the sustained-release of exendin-4 when administered via the pulmonary route [14].

In this study, our purpose was to evaluate the therapeutic effectiveness of inhalable TAC-loaded PLGA-NPs (TAC PLGA-NPs) in a bleomycin-induced pulmonary fibrosis mouse model. These NPs were fabricated using a conventional oil/water emulsification method, and further modified with mucoadhesive chitosan in an attempt to increase their retention time and the uptake of TAC in the lungs. Most of all, these PLGA NPs were expected to gradually release TAC in a sustained pattern, which would reduce the frequency of TAC inhalations. The physicochemical properties and lung deposition of inhaled chitosan-coated tacrolimus-loaded PLGA-NPs (chitosan TAC PLGA-NPs) were investigated, and the anti-fibrotic efficacy was compared with an oral formulation of TAC.

2. Materials and methods

2.1. Materials

TAC was obtained from the Research Laboratories of Chong Kun Dang Pharm. (Yongin, Korea). PLGA (RG502, M_w: 7000–17,000; lactic acid:glycolic acid, 50:50) was purchased from Boehringer-Ingelheim (Ingelheim, Germany). Low molecular weight chitosan (20,000 cps) was purchased from Sigma–Aldrich (St. Louis, MO, USA). The Cy5.5 NHS ester dye was purchased from GE Healthcare (Piscataway, NJ, USA). All other reagents were obtained from Sigma–Aldrich unless otherwise specified.

2.2. Animals

Male C57BL/7 mice (7 weeks old) were purchased from Hanlim Experimental Animal Laboratory (Seoul, South Korea). Animals were cared for in accordance with the guidelines issued by the National Institutes of Health (NIH) regarding the care and use of laboratory animals (NIH publication 80-23, revised in 1996). Animals were housed in groups of 6–8 under a 12-h light/dark cycle (lights on 6 am), allowed food and water ad libitum, and acclimatized for 1 week. This study was approved by the Ethical Committee on Animal Experimentation at Sungkyunkwan University.

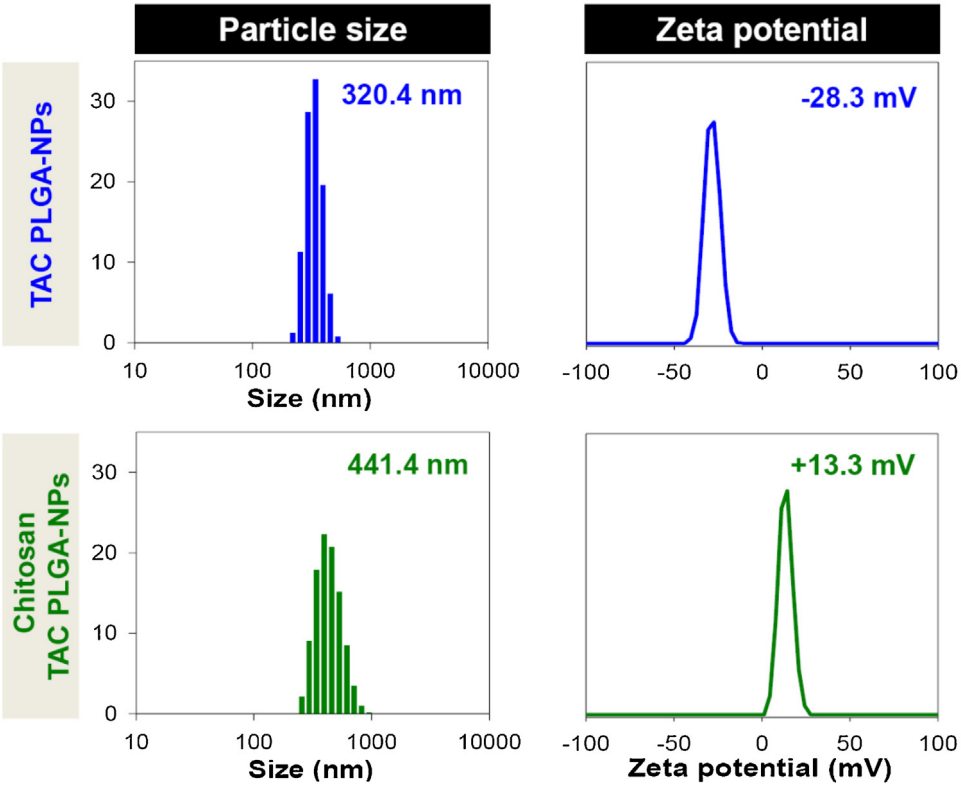

Fig. 1. Histograms of particle size (left) and zeta potential (right) of tacrolimus-loaded PLGA nanoparticle (TAC PLGA-NPs) and chitosan-coated tacrolimus-loaded PLGA NPs (chitosan TAC PLGA-NPs).