Pulmonary delivery of tobramycin-loaded nanoparticles for the treatment of Pseudomonas aeruginosa infection in cystic fibrosis patients

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Abstract

Pseudomonas aeruginosa is the main pathogen that affects the respiratory tract of cystic fibrosis (CF) patients. As an ally to fight against this infection, nanotechnology has emerged over the last decades as a promising alternative to overcome the resistance in respiratory tract of cystic fibrosis (CF) patients. As a way to fight against this infection, nanotechnology has emerged over the last decades as a promising alternative to overcome the resistance in cystic fibrosis. The aim of this work was to elaborate and characterize lipid nanoparticles for pulmonary delivery of tobramycin. Tobramycin-loaded nanostructured lipid carriers (Tb-NLCs) showed to be active against P. aeruginosa displaying a MIC (minimum inhibitory concentration) of 0.5 µg/mL. Moreover, an in vivo study on an intratracheal administration in mice, nanoparticles presented a wide distribution in lungs. Therefore, Tb-NLCs could represent an alternative drug delivery system for pulmonary infection treatment.

Introduction

Cystic fibrosis (CF) is a genetic disorder that affects nearly 70,000 patients worldwide. Pseudomonas aeruginosa is the most frequent pathogen identified in CF patients. Over the last decades, antibiotic-resistant strains have increased due to the misuse and overuse of anti-infectious drugs. In this regard, nanotechnology has emerged as a new alternative to drug encapsulation in order to overcome the limitations of conventional drugs. Nanoparticles (NPs) are currently being extensively investigated for antibiotic delivery therapy. Pulmonary drug delivery has gained much attention as a non-invasive route for the delivery of high amounts of therapeutically active agents directly to the desired site of action minimizing systemic exposure and adverse effects [1].

Methods

Two NPs loaded with tobramycin were elaborated by a hot melt homogenization technique using Precirol® ATO5 (Tb-NLC P) or mixture of Precirol® and Compritol® ATO 888 (Tb-NLC PC) and Miglyol as core materials. Infra-red labeled NLCs (IR-NLC) were prepared for the biodistribution assays. In both cases, the nanoparticles were washed by centrifugal filtration units (Amicon®) followed by freeze-drying using trehalose (15%, w/v) as cryoprotectant (3). Size and zeta potential were determined using Zetasizer ZS. Encapsulation efficiency (EE) and release profile were assessed by UV-VIS spectrophotometer (2) and NPs morphology was examined by transmission electron microscopy (TEM).

The minimum inhibitory concentration (MIC) of nanoparticles and free tobramycin were tested against P. aeruginosa strains isolated from sputum of cystic fibrosis patients (mucoid and non-mucoid) by broth microdilution method in 96-well microwells. P. aeruginosa ATCC was used as control strain.

For an in vivo biodistribution study in BALB/c OlaHsd mice, 1 mg of IR-NLCs was administered intratracheally to each mouse by a MicroSprayer® nebulizer (Penn Century® Liquid). The mice were placed in an intubation platform and the trachea and epiglottis of the animals were visualized by using a small animal laryngoscope. At pre-established time points, mice were sacrificed and lungs and other organs were removed and analyzed by LI-COR Pear® impulse small animal imaging system.

Results

Tb-NLCs displayed a mean diameter size around 250 nm and a zeta potential of -23 mV. Likewise, IR-labeled NLCs displayed a similar size and charge, around 289 nm and -26 mV. Tobramycin sustained release lasting 92 hours (Figure 1C).

Both types of Tb-NLCs showed to be active against clinically isolated P. aeruginosa displaying a MIC of 0.5 µg/mL in most of the planktonic bacteria tested. In the same experimental conditions, free tobramycin displayed the same or higher MIC indicating that the encapsulation of the drug did not affect the antimicrobial activity (Figure 2B).

Figure 1. Characterization of NLCs

Figure 2. A) Images of Pseudomonas aeruginosa clinical isolates. B) MIC values of Tb-NLCs and free drug against strains of clinically isolated P. aeruginosa samples. M. mucoid clinical strain and NM. non-mucoid clinical strain.

Conclusions

Tb-NLCs demonstrated efficacy against P. aeruginosa in vitro and large pulmonary distribution and retention in the in vivo studies. Tb-NLCs (both Tb-NLC P and Tb-NLC PC) can provide the advantage of a sustained drug release in the target site, resulting in reduced-dose schedule and improved patient compliance. Other clear advantages of these nanoparticles are the use of biocompatible and biodegradable lipids and the avoidance of organic solvents during their preparation leading to economic efficiency and an environmental friendly process. Therefore, Tb-NLCs could represent an alternative drug delivery system for pulmonary infection treatment. Yet, the results presented in this study are sufficient to predict the effectiveness of the lipid-based nanosystem in CF patients although the features of the developed formulation so far examined could be considered promising in a perspective of an efficacious CF inhalable therapy.

Bibliography


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