INHALABLE DRUG DELIVERY SYSTEM BASED ON POLY(SEBACIC ACID) AND AZITHROMYCIN FOR THE TREATMENT OF BACTERIAL INFECTIONS

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Introduction

Bacteria-related respiratory infections are commonly occurring in patients suffering from e.g. chronic obstructive pulmonary disease or cystic fibrosis [1]. As a conventional treatment, i.e. oral or intravenous administration of antibiotics, which are burdened with a number of negative side effects followed by limited bioavailability of the drugs, novel methods of treatments allowing for more effective delivery of the antimicrobials to the site of action are being developed [2].

The aim of this study was to develop an inhalable drug delivery system based on poly(sebacic acid) (PSA) microparticles loaded with azithromycin (AZ). The MPs should be spherical with diameters in 1-5 μm range, as such particles are the most effective for deposition in lower respiratory tract when delivered via inhalation [3].

Materials and Methods

PSA microparticles (MPs) were fabricated using oil-in-water emulsification/solvent evaporation method. In brief, 60 mg of PSA was dissolved in 3 ml of dichloromethane (DCM) and mixed with 3 mg (5% w/w), 6 mg (10% w/w) or 12 mg (20% w/w) of AZ. As prepared oil phase was then emulsified into 20 ml of aqueous 8% w/v poly(vinyl alcohol) solution on magnetic stirrer (1500 rpm). After 5 h, the suspension of MPs was collected, centrifuged and rinsed with deionized water (3x). Purified MPs were frozen at -80°C and freeze-dried for 24 h.

The MPs were characterized in terms of their morphology (scanning electron microscopy – SEM), size, polydispersity and zeta potential (dynamic light scattering – DLS) and AZ encapsulation efficacy (high-performance liquid chromatography – HPLC). Degradation of selected MPs (i.e. unloaded MPs and those containing 10% w/w AZ) was also evaluated for up to 96 h. Finally, cytocompatibility of the MPs was determined in contact with human lung epithelial cells of malignant (A549) and non-malignant (BEAS-2B) origin via resazurin reduction assay and live/dead fluorescent microscopy staining.

Results and Discussion

SEM investigation revealed that all types of unloaded MPs and those containing 5-20% w/w AZ were spherical in shape with a smooth appearance at micrometre scale (FIG. 1). The average diameters of MPs ranged from 1.5 ± 0.1 μm (MP+10%AZ) to 2.7 ± 0.2 μm (MP+5%AZ), as evidenced by DLS. No particles smaller than 0.9 μm or larger than 4.1 μm were found, thus the MPs should be suitable for inhalation. Zeta potential of the freeze-dried MPs was negative for all samples (-15.5 to -6.3 mV). Due to hydrophobic nature of both PSA and AZ, the encapsulation efficacy was close to 100% as measured by HPLC.

The degradation behaviour of unloaded MPs and MP+10%AZ was evaluated. The pH of phosphate buffered saline (PBS) decreased significantly even within the first 2 h of degradation of both types of MPs in comparison to PBS alone (around 7.3 for unloaded MPs and 6.9 for MP+10%AZ). In the course of time, further drop in pH was observed for both samples to below 5.4 for unloaded MPs and 5.7 for MP+10%AZ after 96 h of degradation. The morphology of the MPs also changed during the degradation. It was observed that with longer incubation time, the MPs become smaller, less regular and their surface began to wrinkle. The changes were more pronounced in the case of MP+10%AZ, than in unloaded MPs.

Cytotoxicity tests were performed first for unloaded MPs at concentrations ranging from 0.1 μg/ml to 1000 μg/ml. It was found out that BEAS-2B cells were more sensitive to MPs than A549. Statistically significant differences in viability were forum at ≥500 μg/ml for A549 and ≥100 μg/ml for BEAS-2B. The second evaluation focused at comparison of cytotoxicity of unloaded MPs and AZ-loaded MPs at one concentration. None of the MPs showed cytotoxicity against A549 cells, while in the case of BEAS-2B the decrease in viability was observed in MP+20%AZ.

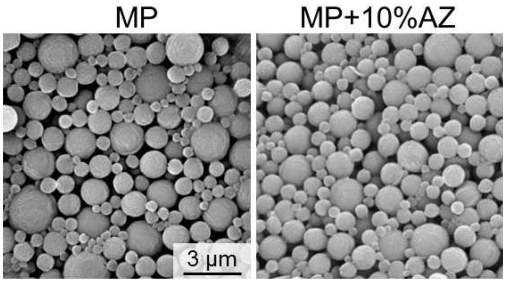

FIG. 1. SEM images of unloaded MPs (left) and MPs containing 10% AZ (right).

Conclusions

Oil-in-water emulsification/solvent evaporation method was suitable for fabrication of PSA-based microparticles loaded with azithromycin. The morphology and size of all types of MPs were suitable for administration via inhalation, as the MPs were spherical in shape, smooth and their diameters were in 1-5 μm range. The encapsulation efficacy of the drug was close to 100%. The MPs underwent rapid degradation as evidenced by changes in their morphology and decrease of buffer pH due to the release of acidic degradation products. The MPs were cytocompatible with human lung epithelial cells of malignant and non-malignant origin. Further studies on azithromycin release kinetics and antimicrobial efficacy of the MPs are necessary to fully evaluate the potential of the developed drug delivery system in the treatment of bacterial infections in lungs.

Acknowledgments

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