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Chitosan microspheres as an alveolar macrophage delivery system of ofloxacin via pulmonary inhalation

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ABSTRACT

Because *Mycobacterium tuberculosis*, which causes tuberculosis, survives mainly in the alveolar macrophages, the remedial efficiency of anti-tuberculosis drugs such as ofloxacin may be improved by their direct delivery to the lungs via pulmonary inhalation. For this purpose, ofloxacin-loaded, glutaraldehyde-crosslinked chitosan microspheres (OCMs) were prepared using a water-in-oil emulsification method. The particle size of the OCMs was around 1–6 μm, and the content of ofloxacin was 27% (w/w). A twin-stage impinger (TSI) study revealed that the device-removal efficiency of the drug from the capsule and the arrival rate of the drug to stage II of the apparatus were substantially improved for OCMs compared to ofloxacin itself (i.e., 81 vs. 98% and 13 vs. 45%, respectively). Also, the *in vitro* uptake of ofloxacin from the OCMs to alveolar macrophages (NR8383) was substantially accelerated: the cellular ofloxacin concentrations at 4 and 24 h after the application were >3.5-fold greater than those for free ofloxacin. The above results indicate that pulmonary inhalation of OCMs might improve the delivery efficiency of ofloxacin to the alveolar macrophages, thereby shortening the length of time that is required to cure tuberculosis with the drug—usually at least 6 months when administered orally.

1. Introduction

Tuberculosis (TB) is a serious infectious disease worldwide that is caused by *Mycobacterium tuberculosis*. The important thing is that *M. tuberculosis* can survive in alveolar macrophages for extended periods of time by preventing the fusion of phagosomes and lysosomes—a bactericidal mechanism of macrophages (Bermudez, 1994). *M. tuberculosis* causes granuloma and pneumorrhagia in the lung, and necrosis of other organs by the infection through lymph or plasma. To treat TB, several anti-TB drugs such as ethambutol, isoniazid and rifampinusually are administered orally.

Although these drugs demonstrate an adequate antibiotic effect against *M. tuberculosis*, they cannot sufficiently reach the lung and alveolar macrophages via oral administration (Vyas et al., 2004). For that reason, the oral administration of the drugs should be continued for at least 6 months to 2 years to cure TB (Vilarica et al., 2010). In addition to inconvenience and irritability, the administration of drugs for such long periods of time can provoke adverse effects. Also, problems with tolerance or resistance by *M. tuberculosis* to the administered drugs may be induced. Indeed, multidrug resistant tuberculosis (MDR-TB) strains of bacteria that are resistant to at least two anti-TB drugs have caused serious problems in efforts to control TB (Tomioka, 2000).

Therefore, it seems desirable to shorten the period of drug administration to cure TB by elevating the delivery efficiency of relevant drugs by any means. Coincidentally, inhalation of drugs via the respiratory route has been a frequent approach for delivery. Therefore, pulmonary inhalation is expected to shorten the drug treatment period for a regimen that can cure TB, if an efficient delivery of the drugs to the alveolar macrophages can be achieved (Vyas et al., 2004). The pulmonary delivery of drugs would be achievable by the inhalation of appropriately sized microspheres that contain relevant drugs, because the microspheres are likely to be actively phagocytosed by the alveolar macrophages, enhancing the influx of the drugs into the macrophages (Champion et al., 2007).

In the present study, the development of an alveolar macrophage delivery system for ofloxacin was the aim. In order to develop the system, ofloxacin-loaded, glutaraldehyde-crosslinked chitosan microspheres (OCMs) were prepared using a water-in-oil emulsification method. There have been various attempts to encapsulate anti-tuberculosis drugs to micro-sized formulation for pulmonary delivery, and poly-lactic-co-glycolic acid (PLGA) represents the polymers that have been used to encapsulate the drugs (Muttil et al., 2009). For example, rifampicin-loaded PLGA microspheres enhanced the delivery of rifampicin to alveolar macrophages compared to free rifampicin (Hirota et al., 2010).

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PLGA is a biocompatible polymer that is useful in sustaining the release of entrapped drugs *in vivo* (Gupta et al., 2010). However, the problem with this polymer is that because it is hydrophobic, it is difficult to load a sufficient amount of hydrophilic drugs, such as fluoroquinolones, into PLGA particles. Furthermore, the degradation rate of the polymer—in other words, the drug release rate—is difficult to control, which can frequently provoke drug release problems for PLGA particles (Meenach et al., 2012).

However, chitosan, another biocompatible polymer, is hydrophilic and soluble in acidic solvents, and thus it is easy to encapsulate hydrophilic drugs. In addition, due to its mucoadhesive properties, chitosan formulations are likely to adhere to mucous membranes (El-Shabouri, 2002). In fact, the clearance of inhaled chitosan-coated PLGA nanospheres in the lung tissue was retarded because of the enhanced mucoadhesion of the nanospheres by the chitosan coating (Yamamoto et al., 2005). Also, chitosan particles interact with the mannose receptors of macrophages, which results in the phagocytosis of the particles in macrophages followed by the degradation of lysozymes and N-acetyl-β-D-glucosaminidase in phagosomes (Bianco et al., 2000; Shibata et al., 1997). Because of these strong points, chitosan has attracted the attention of many researchers as a polymer that can encapsulate hydrophilic anti-tuberculosis drugs in the development of alveolar drug delivery systems.

Thus, in the present study, ofloxacin-loaded, glutaraldehyde-crosslinked chitosan microspheres (OCMs) were prepared, and their potential to deliver ofloxacin directly to alveolar macrophages via the respiratory route was examined. Ofloxacin, afluoroquinolone antibiotic, was selected because it demonstrates superior antibiotic activity against various *M. tuberculosis* strains, including MDR-TB (Berning et al., 1995; Yew et al., 2000). Indeed, ofloxacin has been used to treat tuberculosis patients who have a resistance to other anti-tuberculosis drugs (Ziganshina and Squire, 2008). Also, the drug is effective against other respiratory pathogens, such as *Haemophilus influenzae* and *Streptococcus pneumoniae* (Plouffe et al., 1996), and has shown an effective response to acute exacerbations of chronic bronchitis (T'Jonck and Willems, 1993).

2. Materials and methods

2.1. Materials

Ofloxacin, medium molecular weight chitosan (deacetylation ratio of 75–85%), glutaraldehyde, antipyrine, phosphate buffered saline (PBS) and Percoll solution were purchased from Sigma–Aldrich (St. Louis, MO, USA) and Span 80, acetic acid, hydrochloric acid, sodium hydroxide and dichloromethane were obtained from Daejung Chemical (Gyonggi-do, Korea). Paraffin liquid and diethyl ether were purchased from Samchun Chemical (Gyonggi-do, Korea). Fetal bovine serum (FBS), penicillin (100 units/mL) and streptomycin (100 μg/mL) were from Welgene Inc., Korea.

2.2. Preparation of ofloxacin-loaded, glutaraldehyde-crosslinked chitosan microspheres (OCMs)

OCMs were prepared according to a modified water-in-oil emulsification method (Menon et al., 2010), as shown in Fig. 1. First, 240 mg of chitosan was dissolved in 24 mL of 1% (w/v) acetic acid (pH 2.72), and 240 mg of ofloxacin was dissolved in the chitosan solution with shaking and sonication. The aqueous phasewas centrifuged at 1000 rpm for 10 min to remove undissolved chitosan debris. The supernatant phase was emulsified as follows in a mixed oil phase, which was composed of 20 mL of dichloromethane and

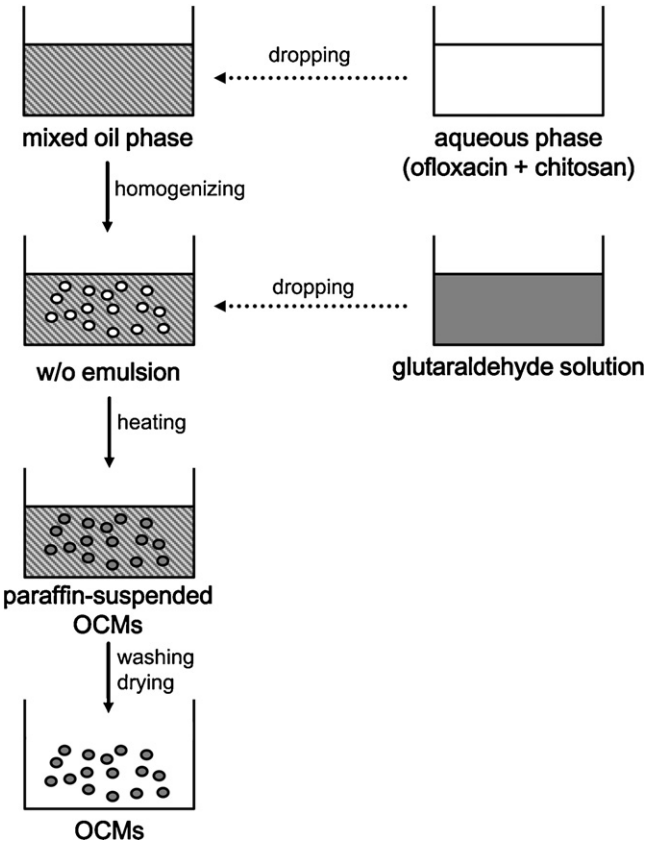

Fig. 1. Preparation process forofloxacin-loaded, glutaraldehyde-crosslinked chitosan microspheres (OCMs).

20 mL of liquid paraffin, containing Span 80 (1%, v/v, in the mixture, as an emulsifier) and lecithin (0.1%, v/v, in the mixture, as a deaggregation agent) (Menon et al., 2010): 20 mL of the aqueous phase (i.e., ofloxacin–chitosan solution) was added dropwise to 40 mL of the mixed-oil phase at the rate of 2 mL/min under continuous homogenization (IKA-Ultra-Turrax T25 basic, IKA-Labortechnik, Germany) and at a speed of 13,500 rpm. The water-in-oil emulsion thus formed was further homogenized for 10 min under the dropwise addition of 2 mL of glutaraldehyde solution (5%, v/v). The emulsion was then homogenized for another 10 min, transferred to 20 mL of pre-heated liquid paraffin, and heated at 170 °C with stirring for 1 h. The heating was to remove dichloromethane and aqueous solvent from the emulsion by evaporation.

The remaining oil phase was cooled to room temperature, and centrifuged at 2000 rpm for 10 min. That speed (2000 rpm) was found to be appropriate to separate ofloxacin debris and nano-sized particles from the paraffin-suspended OCMs. The resultant microsphere pellet was dispersed in fresh liquid paraffin and centrifuged again at 2000 rpm for 10 min to remove non-encapsulated ofloxacin debris. The obtained microsphere pellets were washed with diethyl ether three times to remove residual paraffin, and dried overnight in an oven at 50 °C.

Most of the added ofloxacin (≫85%) was recovered from the final product, suggesting insignificant degradation of the drug during the preparation of OCMs, if any. Besides, chitosan seemed to be stable during the preparation of OCMs because it was reported to be degraded at temperatures over 250 °C (Sakurai et al., 2000).

2.3. Particle size analysis

The OCMs were suspended in DDW and sonicated for 30 s to uniformly disperse the particles, and their particle size was measured