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Ciprofloxacin-loaded lipid-core nanocapsules as mucus penetrating drug delivery system intended for the treatment of bacterial infections in cystic fibrosis

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ABSTRACT

Treatment of bacterial airway infections is essential for cystic fibrosis therapy. However, effectiveness of antibacterial treatment is limited as bacteria inside the mucus are protected from antibiotics and immune response. To overcome this biological barrier, ciprofloxacin was loaded into lipid-core nanocapsules (LNC) for high mucus permeability, sustained release and antibacterial activity. Ciprofloxacin-loaded LNC with a mean size of 180 nm showed a by 50% increased drug permeation through mucus. In bacterial growth assays, the drug in the LNC had similar minimum inhibitory concentrations as the free drug in *P. aeruginosa* and *S. aureus*. Interestingly, formation of biofilm-like aggregates, which were observed for *S. aureus* treated with free ciprofloxacin, was avoided by exposure to LNC.

With the combined advantages over the non-encapsulated drug, ciprofloxacin-loaded LNC represent a promising drug delivery system with the prospect of an improved antibiotic therapy in cystic fibrosis.

1. Introduction

Cystic fibrosis (CF) is one of the most common autosomal recessive genetic disorders among Caucasians with an incidence of 1 case per 2500 births (Müller et al., 2015; Cohen-Cymberknoh et al., 2011). Due to mutations in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene, chloride ion transport through the CFTR ion channel is defective, leading to dehydrated, thickened secretions in several organs of the human body. CF patients are mainly affected by pulmonary symptoms, as a consequence of the production of highly viscous mucus in the lungs. With the mucociliary clearance being impaired, mucus is easily colonized by pathogenic bacteria, among which *Pseudomonas aeruginosa* and *Staphylococcus aureus* are the most prevalent (Ahlgren et al., 2015).

P. aeruginosa is a Gram-negative bacterium, capable of forming biofilms. Inside those microcolonies, the pathogen is protected against immune response and antibiotics. Bacteria inside the biofilm are difficult to target by antibiotics and far less susceptible in comparison to planktonic pathogens (Høiby, 2002; Forier et al., 2014). Up to 60–75% of adult CF patients are chronically infected with *P. aeruginosa*, which is strongly associated with inflammatory

Abbreviations: CF, cystic fibrosis; LNC, lipid-core nanocapsules; LNC-blank, blank lipid-core nanocapsules; LNC-CIP, ciprofloxacin-loaded lipid-core nanocapsules; PCL, poly(ε-caprolactone).

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responses, decreased lung function and lower survival rates (Müller et al., 2015; Ahlgren et al., 2015). *S. aureus*, a Gram-positive bacterium, is known to be one of the first pathogens colonizing CF lungs and the most prevalent bacterium in younger patients, associated with a decline in lung function and inflammatory responses. In adults, *S. aureus* has a high prevalence as well, however it is considered less detrimental in comparison to *P. aeruginosa* (Ahlgren et al., 2015; Marks, 1990). Overall, bacteria are not only causing recurrent airway infections and pneumonia, exaggerated inflammatory responses are also leading to bronchiectasis and progressive obstructive airways disease. The resulting pulmonary insufficiency is the cause of most CF-associated cases of death (Cohen-Cymberknoh et al., 2011; Sens and Stern, 2012). Therefore, new treatments are urgently needed and a variety of different approaches to tackle especially infections with *P. aeruginosa* are currently being developed (Wagner et al., 2016).

Given the negative influence of bacterial infections on the outcome of CF patients, their treatment and prophylaxis are main pillars of CF therapy. Ciprofloxacin is a broad spectrum fluoroquinolone antibiotic and effective against *P. aeruginosa* and *S. aureus*. Intravenous and oral application of ciprofloxacin are already established in CF therapy, however systemic administration involves as well systemic side effects. Ciprofloxacin is mainly limited to adult patients, due to safety concerns regarding a long-term use in children (Principi and Esposito, 2015). In contrast to oral or intravenous application, administration via inhalation delivers the antibiotic directly to the site of infection. This leads to a higher local concentration and lower systemic side effects. Ciprofloxacin applied as dry powder inhaler is currently investigated in clinical studies and its efficacy in chronic *P. aeruginosa* infections has already been shown (Antoniu, 2012). By using inhaled ciprofloxacin, dose and systemic side effects can be reduced and pediatric therapy might benefit from this antibiotic.

However, even if the antibiotic is locally distributed in the lungs, it still has to overcome the mucus as biological barrier to reach its site of action. In infected lungs of CF patients bacteria are located inside the mucus, which significantly impedes the successful treatment of pulmonary infections by inhaled drug (Günday Türeli et al., 2014).

Mucus is a gel consisting of water, mucins (glycoproteins), non-mucin proteins, salts, lipids and cellular debris (Cone, 2009; Kirch et al., 2012; Fröhlich and Roblegg, 2014). A mesh structure is formed by mucin fibers with spacings ranging from about 100–1000 nm (Cone, 2009). In the healthy state, bronchial mucus is an effective barrier to protect the bronchiolar epithelium from infectious microorganisms. However, in cystic fibrosis bacterial colonization is even favored due to a reduced mucociliary clearance. Inside the highly viscous and immobile mucus pathogens are protected from antibiotics and immune response. Besides a potential deactivation of antibiotics inside the mucus, effective antimicrobial therapy suffers from limited diffusion of drugs through mucus (Levy, 1986; Bhat et al., 1996).

For an effective inhalative antibacterial therapy in cystic fibrosis, advanced drug delivery systems protecting the drug and facilitating the transport to the site of action are required. A promising approach is to use nanoparticles as carriers to protect the drug from deactivation and increase mobility in mucus. The ability to permeate mucus has already been demonstrated for nanoparticles depending on their surface properties, charge and size (Günday Türeli et al., 2014; Nafee et al., 2014; Lai et al., 2009; Sanders et al., 2000). In addition to protect the drug and to facilitate mucus permeation, nanoparticles can sustain the drug release, enabling a reduced dosing frequency. The combined advantages of a local therapy by drug-loaded nanoparticles allow a potential decrease of the applied overall dose, resulting in a reduction of systemic toxicity.

An advanced carrier system with advantageous properties for drug delivery are lipid-core nanocapsules (LNC), which are composed of a lipid core, covered by a polymeric wall (Mora-Huertas et al., 2010; Pohlmann et al., 2013). For the polymeric shell poly(ε-caprolactone) (PCL) can be used, a biodegradable and biocompatible polymer controlling drug release. LNC can efficiently be loaded with poorly soluble drugs, such as ciprofloxacin free base, since its lipid core structure is formed by an organogel composed by sorbitan monostearate and a liquid lipid (Poletto et al., 2015; Bianchin et al., 2015). In addition, active ingredients encapsulated in LNC are protected from chemical and light induced degradation (Pohlmann et al., 2013). Furthermore, lipid-core nanocapsules exhibit the previously discussed advantages of nano-sized drug delivery systems, like decreased side effects, high pharmacological responses and the capability of crossing biological barriers (Pohlmann et al., 2013). However, the ability to permeate pulmonary mucus has not yet been shown.

In this work, we developed lipid-core nanocapsules to be loaded with ciprofloxacin. The modified LNC were evaluated toward a controlled release, the capability to permeate mucus and a high antibacterial activity against *P. aeruginosa* and *S. aureus*.

2. Materials and Methods

2.1. Materials

Ciprofloxacin, poly(ε-caprolactone) (MW = 80,000 g mol^{-1}) and sorbitan monostearate were obtained from Sigma-Aldrich (São Paulo, Brazil). Oleic acid was purchased from Dinâmica (Diadema, Brazil) and polysorbate 80 from Vetec (Rio de Janeiro, Brazil). Respiratory horse mucus was provided from horse clinic Altforweiler (Überherrn, Germany). All solvents used for HPLC analysis were of chromatographic grade. All other chemicals and solvents were of analytical or pharmaceutical grade.

2.2. Methods

2.2.1. Preparation of nanoparticles

Ciprofloxacin-loaded lipid-core nanocapsules (LNC-CIP) were prepared by interfacial deposition of polymer (Mora-Huertas et al., 2010). The organic phase consisted of poly(ε-caprolactone) and sorbitan monostearate in acetone and was supplemented by ciprofloxacin previously solubilized in oleic acid. Under magnetic stirring and light protection, the organic phase was injected into an aqueous polysorbate 80 solution. After maintaining stirring for 10 min, the acetone was removed and the nanosuspension concentrated by evaporation under reduced pressure (Rotavapor, Büchi, Flawil, Switzerland) to approximately 9 mL. Final volume was adjusted in a volumetric flask to 10 mL. Blank nanocapsules (LNC-blank) were prepared similarly, but without addition of ciprofloxacin to oleic acid. The exact composition is listed in Table 1.

Table 1
Compositions of LNC-CIP and LNC-blank yielding a final volume of 10 mL nanosuspension.

	LNC-CIP	LNC-blank
Poly(ε-caprolactone)	100 mg	100 mg
Sorbitan monostearate	38.5 mg	38.5 mg
Ciprofloxacin	8 mg	–
Oleic acid	0.165 mL	0.165 mL
Acetone	27 mL	27 mL
Polysorbate 80	77 mg	77 mg
Water	54 mL	54 mL