Biomaterials 33 (2012) 9117–9126

Contents lists available at SciVerse ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Influence of surface charge and inner composition of nanoparticles on intracellular delivery of proteins in airway epithelial cells

Christophe Dombu, Rodolphe Carpentier, Didier Betbeder*

EA4483, IMPRT, IFR 114, Faculté de médecine pôle recherche, Université de Lille Nord de France, 1 place Verdun, 59000 Lille, France

ARTICLE INFO

Article history:
Received 29 June 2012
Accepted 28 August 2012
Available online 14 September 2012

Keywords:
Porous nanoparticle
Endocytosis
Protein delivery
Airway epithelium
Cell trafficking

ABSTRACT

The delivery of protein in the airway using nanoparticles (NP) is an emerging strategy that shows encouraging results *in vivo* for several applications. However, the mechanisms by which NP deliver proteins to the inside of cells remain poorly understood. In this study, we investigated the intracellular delivery of ovalbumin (OVA) in human airway cells by two porous cationic polysaccharides nanoparticles. These NP have the same surface charge density but differ in that their inner core contains either cationic or anionic charges (respectively: NP^+ and $DGNP^+$). Confocal microscopy showed a rapid uptake of both NP by human airway cells, followed by a significant accumulation in clathrin vesicles and early endosomes. Both NP were found to associate OVA in a quantitative manner, and this association was stable even in presence of serum proteins. We observed that the two NP greatly increased OVA uptake by human airway cells, meanwhile FRET studies using FITC-labelled NP and TRITC-labelled OVA showed a gradual release of OVA from NP within cells, and this was much faster with $DGNP^+$ than NP^+. These results were confirmed using OVA–DQ to follow OVA degradation fragments within cells. Both NP increased intracellular proteolysis of OVA, however $DGNP^+$ facilitated OVA escape from endosomes. Studies with trypsin and pepsin at different pH strongly suggested that both NP can protect (in the extracellular medium) or promote (in acidic endosomes) protein proteolysis, depending on the environment. Interestingly, the mechanisms involved could be explained as a function of protein global charge at different pH. All these results confirm the importance of not only the surface charge but also the inner composition of NP in determining their efficacy as tools for the delivery of proteins to different cellular compartments.

1. Introduction

The strategy of employing nanoparticles (NP) to administer drugs via the respiratory tract is receiving significant attention as an alternative to the more common oral or intravenous methods. Indeed, delivery via the airway is an easily accessible and non-invasive route of drug administration which allows molecules to avoid the first-pass effect in the liver and/or gut. Moreover, the respiratory tract constitutes a very large surface for drug absorption, with a vast underlying vascularization, a relatively thin epithelial barrier and a low proteolysis activity compared to other administration routes [1]. All these characteristics make the airway potentially suitable for both local and systemic delivery. However, the airway's mucosa also constitutes a tight, selective barrier which complicates the delivery of macromolecules. Nanoparticles have a proven ability to overcome this barrier by passing through the mucus and strongly interacting with airway epithelial cells. The association of drug molecules with NP is therefore a promising strategy for efficient drug delivery via this route. Nanoparticles as protein carriers are expected to provide many advantages such as improved protein stability, protection from proteases, cell targeting, deeper airway deposition, and higher uptake and bioavailability [2,3]. However, the mechanisms involved in NP interactions with airway epithelial cells, their endocytosis and their ability to deliver proteins within cells still remain unknown. We recently developed porous nanoparticles with different inner core compositions, and found that they were able to be loaded with large amounts of proteins, and are thus interested in evaluating their behavior after mucosal administration [4].

The aim of this study was to characterize the mechanisms of endocytosis and protein delivery of two cationic nanoparticles that differ in their inner core composition and hence evaluate their potential as drug delivery systems.

* Corresponding author. Tel.: +33 320 62 68 83; fax: +33 320 62 69 93.
E-mail addresses: didier.betbeder@univ-lille2.fr, didier.betbeder@neuf.fr (D. Betbeder).

0142-9612/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biomaterials.2012.08.064