European Journal of Pharmaceutics and Biopharmaceutics xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

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

Research paper

N-alkylation of highly quaternized chitosan derivatives affects the paracellular permeation enhancement in bronchial epithelia *in vitro*

Berglind Eva Benediktsdóttir [a], Thórarinn Gudjónsson [b], Ólafur Baldursson [c], Már Másson [a,*]

[a] *Faculty of Pharmaceutical Sciences, School of Health Sciences, University of Iceland, Reykjavik, Iceland*
[b] *Biomedical Center, School of Health Sciences, University of Iceland, Reykjavík, Iceland*
[c] *Department of Pulmonary Medicine, Landspitali – The National University Hospital of Iceland, Reykjavík, Iceland*

ARTICLE INFO

Article history:
Received 9 November 2012
Accepted in revised form 5 April 2013
Available online xxxx

Keywords:
Airway drug delivery
Chitosan
N-quaternary derivatives
TMC
Permeation enhancement
Tight junctions

ABSTRACT

This study describes the structure–activity relationship for carefully characterized *N*-alkyl-*N*-quaternary chitosan derivatives as permeation enhancers for drugs that are mainly absorbed through the paracellular pathway, such as macromolecular drugs and hydrophilic drugs, in a well defined bronchial epithelial cell line. The *O*-methyl free derivatives used in the study were fully trimethylated (100%) *N*,*N*,*N*-trimethyl chitosan (TMC) and *N*-propyl-(QuatPropyl), *N*-butyl-(QuatButyl) and *N*-hexyl (QuatHexyl)-*N*,*N*-dimethyl chitosan, with 85–91% degree of quaternization. The fully trimethylated TMC, from 0.25 mg/ml, decreased transepithelial electrical resistance (TER) in a reversible manner and enhanced the permeation of the macromolecule FITC–dextran 4 kDa (FD4) 2–5 fold. TMC did not cause any alterations in the tight junction (TJ) protein claudin-4 or in F-actin architecture. QuatHexyl was the most effective polymer to produce enhanced permeation and decreased TER from 0.016 mg/ml. Nevertheless, this enhanced permeation was accompanied by reduced viability and dissociation of F-actin and claudin-4 proteins. The structure–activity relationship suggests that more lipophilic derivatives show more permeation enhancement, TJ disassembly, and less viability in the order of hexyl ≈ butyl > propyl > methyl and demonstrates that the permeation effect is not only mediated by permanent positive charge but also by the extent of *N*-alkylation. These results are relevant to elucidate the structural factors contributing to the permeation enhancement of chitosan derivatives and for potential use in pulmonary applications.

1. Introduction

Biotechnological medicines, such as vaccines and monoclonal antibodies, are an expanding field within the pharmaceutical industry, with currently around 1200 medicines in development in Europe [1]. Due to their macromolecular nature and lack of stability, these medicines are usually given in parenteral dosage forms, such as injections, that can result in reduced patient compliance. This necessitates the development of novel delivery systems that can deliver therapeutic agents in a more accessible dosage form. Inhalation of macromolecular drug candidates for systemic delivery results in higher systemic bioavailability than any other noninvasive route of delivery [2,3] and can therefore be considered an interesting alternative to the conventional oral route. The increased systemic bioavailability can be attributed to large surface area, small aqueous volume at the epithelial surface and relatively low enzymatic activity [2], attractive for compounds that have physicochemical properties unfavorable to the conventional oral route.

A valid model must be used to study the permeation of these potential drugs through the lungs. The VA10 human bronchial epithelial cell line was recently established as an *in vitro* drug permeation model [4], having permeability properties similar to the widely used bronchial epithelial cell lines Calu-3 and 16HBE14o-. The VA10 cell line is derived from a normal human bronchus, expresses tight junctions (TJs) and has ciliated, 2–3 cell layer morphology in air–liquid interface (ALI) culture [4,29], thereby showing *in vivo*-like phenotype and providing a valid alternative to Calu-3 and 16HBE14o-. The passage of hydrophilic and macromolecular drugs between epithelial cells is limited by the TJ

Abbreviations: A, surface area (cm^2); ALI, air–liquid interface; CSLM, confocal scanning laser microscopy; DA, degree of acetylation; DQ, degree of quaternization; DS, degree of substitution; ER, permeation enhancement ratio; FBS, fetal bovine serum; FCS, fetal calf serum; FD4, fluorescein isothiocyanate labeled dextran 4 kDa; FITC, fluorescein isothiocyanate; HBSS, Hanks balanced salt solution; MTT, (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; P_{app}, apparent permeability; QuatPropyl, *N*-propyl-*N*,*N*-dimethyl chitosan; QuatButyl, *N*-butyl-*N*,*N*-dimethyl chitosan; QuatHexyl, *N*-hexyl-*N*,*N*-dimethyl chitosan; TER, transepithelial electrical resistance; TJ, tight junctions; TMC, *N*,*N*,*N*-trimethyl chitosan.

* Corresponding author. Faculty of Pharmaceutical Sciences, School of Health Sciences, University of Iceland, Hofsvallagata 53, IS-107 Reykjavik, Iceland. Tel.: +354 525 4463; fax: +354 525 4071.
E-mail address: mmasson@hi.is (M. Másson).

0939-6411/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ejpb.2013.04.002

proteins that serve as a fence mechanism, controlling which molecules pass [5]. Absorption enhancers have been studied to circumvent this gating mechanism to enable the efficient paracellular permeation of these drugs.

Chitosan, a linear polysaccharide derived from chitin by *N*-deacetylation, has been evaluated as a potential permeation enhancer, mainly to increase absorption in the intestinal mucosa *in vitro* and *in vivo* [6–8] with disruption of TJ or related proteins as the proposed mechanism [9,10]. The feasibility of chitosan permeation enhancement studies on the intestinal mucosa can be attributed to its mucoadhesiveness [11], biodegradability [12], and safety in non-parental dosage formulations [13]. This polymer is only soluble under acidic pH, forming a polycation, at similar pH as is present in some parts of the intestine. However, studies using chitosan in airway epithelia [14,15] are scarce due to limited solubility of chitosan above its pK_a of 6.6 [16].

Introducing a permanent positive charge, such as with *N*,*N*,*N*-trimethyl chitosan (TMC), is one way of increasing physiological aqueous solubility of the polymer, thereby enabling effective permeation enhancing studies on other mucosal surfaces such as the bronchial epithelia. TMC has gained increased attention as a polymer with enhanced permeation potential for intestinal, pulmonary and ocular drug delivery [17]. Previous synthetic strategies have produced TMC with a different degree of trimethylation [18–20], often accompanied with *O*-methylation [21,22], thereby reducing the water solubility [21,22] and diminishing the permeation enhancing effect of TMC [23,24]. In addition to TMC, *N*-alkyl quaternary chitosan derivatives have been synthesized [25]. However, their structures were not fully characterized, making their structure–activity relationship difficult to elucidate. The fundamental requirement in the development of pharmaceuticals and their excipients is the use of well characterized compounds. We have synthesized *O*-methyl free *N*-alkyl-*N*,*N*-dimethyl chitosan derivatives with well defined structures [26] (Fig. 1). Increased length of the *N*-alkyl chain in these derivatives introduces more amphiphilicity to the polymer that could augment even further the permeation enhancement of macromolecules, possibly by stronger interactions with the TJs. The aim of this study was therefore to determine whether the increased amphiphilicity, caused by longer alkyl chains in *N*-quaternary chitosan derivatives, would benefit the permeation enhancement profile in the VA10 cell line.

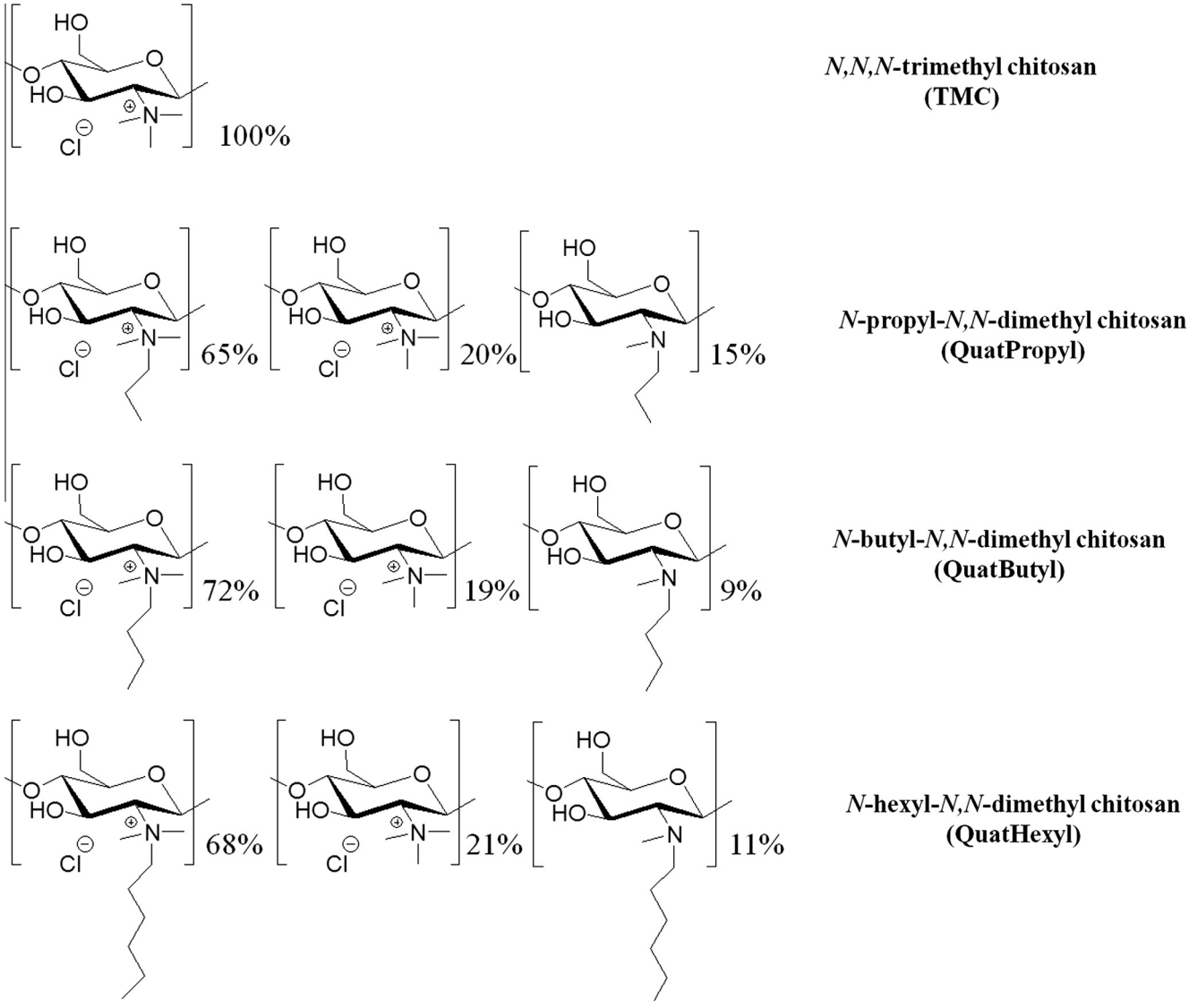

Fig. 1. Structure of the *N*-quaternary chitosan derivatives used in the current study.

Please cite this article in press as: B.E. Benediktsdóttir et al., *N*-alkylation of highly quaternized chitosan derivatives affects the paracellular permeation enhancement in bronchial epithelia *in vitro*, Eur. J. Pharm. Biopharm. (2013), http://dx.doi.org/10.1016/j.ejpb.2013.04.002