The Role of Mucoadhesion of Trimethyl Chitosan and PEGylated Trimethyl Chitosan Nanocomplexes in Insulin Uptake

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Received 16 July 2008; revised 20 December 2008; accepted 10 March 2009

Published online 30 April 2009 in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/jps.21783

ABSTRACT: The aim of this work was to investigate the role of mucoadhesion in the insulin uptake of nanocomplexes (NC) based of trimethyl chitosan (TMC) and poly(ethylene glycol) (PEG)-*graft*-TMC copolymers. Self-assembled insulin NC were prepared by polyelectrolyte complexation. The effects of PEGylation and positive charge density on mucoadhesion were assessed using a mucin assay and mucus-secreting HT29-MTX-E12 (E12) monolayers. The behaviors of corresponding insulin NC after adhesion to E12 were also established. All PEGylated TMC copolymers showed significantly higher levels of adhesion to mucus than unmodified TMC. The copolymer composed of 298 PEG chains per TMC macromolecules exhibited the highest level of mucoadhesion, being 3.4 times higher than TMC. The higher mucoadhesive properties of PEGylated TMC copolymers resulted from the synergistic effects of interpenetration of PEG chains into the mucus and electrostatic interaction between positive charged TMC and anionic glycoproteins present in the mucus layer. Compared to TMC, insulin NC based on PEGylated TMC copolymers demonstrated no evidence of insulin uptake improvement due to complete release of insulin from NC after adhering to mucus. CLSM revealed the localization of TMC and its corresponding insulin NC at cell surface membranes of E12. © 2009 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 98:4818–4830, 2009

Keywords: mucoadhesion; PEGylation; trimethyl chitosan; HT29-MTX-E12 cells; insulin nanocomplexes; uptake

INTRODUCTION

During the past decade, considerable research attempts have aimed to develop peptide and protein delivery systems for oral, nasal, buccal, pulmonary and vaginal routes based on mucoadhesive polymers such as polycarbophil,[1] chitosan,[2,3] and thiolated polymers.[4,5] Mucoadhesive polymers are thought to provide an intimate contact with mucosae and prolonged residence times of drug carriers at the absorption site, thereby reducing drug degradation between the delivery system and the absorbing membrane. However, mucoadhesive drug delivery systems have so far not reached their full potential because the mucoadhesive properties of such polymers are sufficient only in a limited pH range.[6–8] Moreover, some of them possess cytotoxic effects.[9,10] It is therefore desirable to develop mucoadhesive polymers with good biocompatibility and mucoadhesiveness in a wide pH range.

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Journal of Pharmaceutical Sciences, Vol. 98, 4818–4830 (2009)

Mucoadhesion is a complex phenomenon which may be classified into a two steps.[11] Initially, an intimate surface contact between the bioadhesive polymers and mucus tissue has to be established (contact stage). In a consecutive step, both phases may interdiffuse or interpenetrate to a certain extent and formation of secondary chemical bonds, such as electrostatic and hydrophobic interactions, hydrogen bonding and van der Waals interactions, which occur to consolidate and strengthen the adhesive joint, leading to prolonged adhesion (consolidation stage). Interpenetration of polymer chains at the interface is an important mucoadhesion mechanism which can be promoted by using a mucoadhesive promoter, a molecule that is not mucoadhesive in itself but contributes to the adhesion process, such as poly(ethylene glycol) (PEG). Sahlin and Peppas[12] found that incorporation of PEG into polymer networks (hydrogels) enhanced polymer/mucus interactions. Recently, Serra et al.[13] reported that grafting PEG onto poly(acrylic acid) gave fivefold improvement on the mucoadhesion. Besides, grafting of PEG to a cationic polymer is well known to increase biocompatibility of the polymer.[14]

Novel polymer, PEGylated trimethyl chitosan copolymer has been developed based on this concept. By grafting activated PEG onto trimethyl chitosan (TMC) *via* amino groups, water soluble and biocompatible polymer has been obtained. It was found that PEGylation with PEG 5 kDa is sufficient to increase the biocompatibility of TMC and appears to be beneficial for drug carrier containing insulin[10,15,16] and plasmid DNA.[17] Since the copolymers are positively charged and consist of PEG chain, they would increase the mucoadhesive properties *via* (i) interpenetration of PEG chains into the mucus and (ii) ionic interactions between cationic groups of modified TMC and the anionic moieties within the mucus layer. PEGylated TMC copolymer is therefore an appropriate model polymer to use in adhesion studies. Although the effect of TMC and PEGylated TMC copolymer on the uptake and transport of insulin NC has been reported,[18] little has been known about the role of mucoadhesive properties of the polymers in the insulin uptake and transport of the NC.

There are few reliable cell culture models, such as Calu-3 and HT29-MTX-E12 (E12) cell lines, that are able to provide a mucus gel lining to which adherence can be measured.[19,20] Since E12 sub-clone of HT29-MTX forms tight junctions, confluents monolayers and elaborates a 150 μm continuous mucus gel layer that corresponds to *in vivo* measurement of human small intestine,[21] it is a promising model with regard to investigation of mucus interaction with drugs, polymers or particles. Recently, Keely et al.[19] studied mucoadhesion of poly(methacrylate) and TMC polymers using E12 monolayers compared to excised *non-everted* rat intestinal sacs. The study demonstrated that E12 monolayers displayed adhesive properties similar to isolated rat intestinal sac.

Therefore, in this contribution, we focused on investigations of mucoadhesive mechanisms of TMC and PEGylated TMC and behaviors of corresponding insulin NC after adhering to mucus layer. The mucoadhesion and uptake studies of polymers and NC were assessed with the E12 monolayers under physiological condition. Localization of polymers and insulin NC on the E12 was visualized by confocal laser scanning microscopy (CLSM).

MATERIALS AND METHODS

Materials

Chitosan (400 kDa) with a degree of deacetylation of 84.7% was purchased from Fluka (Schnelldorf, Germany). A series of three TMC with quaternization degree (DQ) of 10%, 40%, and 80% were prepared by reductive methylation of the parent chitosan based on one reaction step with or without subsequent addition steps using procedures described earlier.[22] PEG-*graft*-TMC copolymers were synthesized and characterized as previously described.[10] For abbreviation, $PEG(5k)_{298}$-g-TMC400-40, $PEG(5k)_{640}$-g-TMC400-40, and $PEG(5k)_{680}$-g-TMC400-40 represented the copolymers with 298, 640, and 680 chains of 5 kDa PEG per TMC macromolecules of 40% DQ, respectively. The properties of the polymers used in the present work are summarized in Table 1.

Human recombinant insulin powder (26.2 IU/mg) was a gift from Aventis Pharma AG (Frankfurt, Germany). Mucin type III was purchased from Sigma (Deisenhofen, Germany). Tetramethyl-rhodamine isothiocyanate (TRITC) was obtained from Fluka. Oregon Green carboxylic acid succinimidyl ester (Oregon Green 448) was purchased from Molecular Probes (Eugene, OR). Tissue culture reagents were supplied by Gibco (Eggstein, Germany). Tissue culture materials