Available online at www.sciencedirect.com
ScienceDirect
European Journal of Pharmaceutics and Biopharmaceutics 70 (2008) 270–278
European Journal of Pharmaceutics and Biopharmaceutics
www.elsevier.com/locate/ejpb

Research paper

Permeation enhancer effect of chitosan and chitosan derivatives: Comparison of formulations as soluble polymers and nanoparticulate systems on insulin absorption in Caco-2 cells

A.M.M. Sadeghi [a,c], F.A. Dorkoosh [b], M.R. Avadi [c], M. Weinhold [d], A. Bayat [e], F. Delie [f], R. Gurny [f], B. Larijani [g], M. Rafiee-Tehrani [e], H.E. Junginger [a,h,*]

[a] *Division of Pharmaceutical Technology, Leiden University, Leiden, The Netherlands*
[b] *Department of Pharmaceutics, Isfahan University of Medical Sciences, Isfahan, Iran*
[c] *Hakim Pharmaceutical Company, Tehran, Iran*
[d] *UFT – Center for Environmental Research and Technology, Bremen, Germany*
[e] *Tehran University of Medical Sciences, Tehran, Iran*
[f] *Department of Pharmaceutics and Biopharmaceutics, University of Geneva, Geneva, Switzerland*
[g] *Endocrinology Research Center, Shariati Hospital, Tehran, Iran*
[h] *Naresuan University, Phitsanulok, Thailand*

Received 7 December 2007; accepted in revised form 5 March 2008
Available online 12 March 2008

Abstract

In this study four quaternized derivatives of chitosan: trimethyl chitosan (TMC), dimethylethyl chitosan (DMEC), diethylmethyl chitosan (DEMC) and triethyl chitosan (TEC) with degree of substitution of approximately 50 ± 5% were synthesized and their effect on the permeability of insulin across intestinal Caco-2 monolayers was studied and compared with chitosan both in free-soluble form and in nanoparticulate systems. Transepithelial electrical resistance (TEER) studies revealed that all four chitosan derivatives in free-soluble forms were able to decrease the TEER value in the following order TMC > DMEC > DEMC = TEC > chitosan, indicating their abilities to open the tight junctions. Recovery studies on the TEER showed that the effect of the polymers on Caco-2 cell monolayer is reversible and proves the viability of cells after incubation with all polymers. A similar rank order was also observed when measuring the zeta-potentials of the various polymers in solution form. Transport studies of insulin together with the soluble polymers across Caco-2 cell layers showed the following ranking: TMC > DMEC > DEMC > TEC > chitosan which is in agreement with the strength of the cationic charge of the polymer. In comparison to the free-soluble polymers, the nanoparticles prepared by ionic gelation of the chitosan and its quaternized derivatives had much lower effect on decreasing the TEER by opening of the tight junctions. This can be explained by the reduced available amount of positive charge at the surface of the nanoparticles. In accordance with these results, the insulin loaded nanoparticles showed much less permeation across the Caco-2 cell monolayer in comparison to the free-soluble polymers. Mass balance transport studies revealed that a substantial amount of the nanoparticles has been entrapped into the Caco-2 monolayer or attached to the cell surface. It can thus be stated that while free-soluble polymers can reversibly open the tight junctions and increase the permeation of insulin, the nanoparticles had basically only a low effect on the opening of the tight junction and the paracellular transport of insulin across the Caco-2 cell monolayer. These data convincingly show that nanoparticles consisting of chitosan and its quaternary ammonium derivatives loaded with insulin are less effective in facilitating paracellular transport across Caco-2 cell monolayers than the corresponding free polymers.

Keywords: Caco-2 cells; Insulin nanoparticles; Trimethyl chitosan; Dimethylethyl chitosan; Diethylmethyl chitosan; Triethyl chitosan; Uptake; Transcellular transport; Paracellular transport; Mass balance studies.

* Corresponding author. Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok 65000, Thailand. Tel.: +49 642121164; fax: +49 642121772.
E-mail address: hejunginger@yahoo.com (H.E. Junginger).

0939-6411/$ - see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejpb.2008.03.004

1. Introduction

The low oral bioavailability of the peptide and protein drugs is mainly due to their poor absorption through the gastrointestinal epithelium as well as the rapid hydrolytic and enzymatic degradation in contact with the gastrointestinal fluids [1,2]. In order to overcome the above obstacles, different delivery platforms have been developed for hydrophilic drugs. An effective strategy to improve peptide permeation is the incorporation of suitable permeation enhancers in the delivery system. Modern permeation enhancers are multifunctional polymers with mucoadhesive properties, the ability to locally inhibit intestinal enzymes, reversibly open the tight junctions, show no toxicity and they are ideally not absorbed. Hence they do not show perturbances of the membranes of the epithelial cells and are therefore specific for inducing only the paracellular hydrophilic drug transport. The intestinal epithelium regulates the passage of natural compounds and acts as a barrier for paracellular passive transport of large hydrophilic molecules. This absorption barrier is composed of a single layer of columnar epithelial cells joined at the apical surface by a tight junctional complex. The junctional complex forms a continuous seal, which segregates the apical from and the basolateral compartment and conveys size and charge selectivity due to the presence of negative charge in its structure [3,4]. Nanoparticulate drug delivery systems have attracted an immense attention as novel carriers for the delivery of lipophilic and hydrophilic substances as well as vaccines [5].There is a strong belief that nanoparticles of appropriate size can pass the mucosal membranes intactly and deliver their drug load into the systemic circulation. In the case of hydrophilic drugs, nanoparticles should be able to protect such drugs from degradation in the intestinal fluids and improve their penetration and permeation across the intestinal mucosal epithelium [6–8]. Suitable nanoparticles have mucoadhesive properties which are due to their particle size and the particle's superficial charge [9]. It has been shown that nanoparticles may be internalized into the intestinal epithelial cells [10].

Chitosan, a natural polyaminosaccharide, obtained by *N*-deacetylation of chitin is a non-toxic, biocompatible and biodegradable polymer that has good mucoadhesive properties in acidic environments [11–13]. Studies have shown that chitosan can promote the nasal absorption of insulin in rats and sheep and further enhance the paracellular transport of peptides in vitro and in vivo by opening the tight junctions [14–17]. However, chitosan is a polycation with an apparent pK_a of 5.5 and it loses its charge and precipitates in neutral and basic environments as prevailing in the intestine. Studies have shown that only protonated soluble chitosan in its uncoiled configuration can trigger the opening of the tight junctions and facilitate the transport of hydrophilic compounds [18,19]. Hence, chitosan can be used as an enhancer only in the proximal part of the intestine where the pH is close to its pK_a value. Subsequently, quaternized derivatives of chitosan, synthesized by introducing various alkyl groups to the NH_2-group of the chitosan molecule structure, were studied extensively. These derivatives were characterized to be drastically more soluble in neutral and alkaline environments of the intestine and hence more useful for drug delivery and absorption across the intestinal epithelium of the jejunum and ileum than the mother compound chitosan [19,20]. The permeation enhancing properties of these chitosan derivatives have been attributed to their ion pair interactions with the tight junctions and cellular membrane components to increase the paracellular permeation of hydrophilic compounds [20]. Trimethyl chitosan (TMC), Dimethylethyl chitosan (DMEC), Diethylmethyl chitosan (DEMC) and Triethyl chitosan (TEC) were synthesized by partial quaternization of chitosan as described by Sieval et al., Bayat et al. and Avadi et al., respectively [21–24]. The polymer charge density, determined by the substitution degree is a key factor in obtaining both the mucoadhesion and penetration enhancement towards the intestinal epithelium [25,26]. Chitosan derivatives were synthesized from low molecular weight chitosan and all had the same degree of quaternization of approximately $50 \pm 5\%$ which has been shown for TMC to have the highest penetration rate across the intestinal epithelium in vitro [25,27]. The molecular weight of the synthesized derivatives did not change significantly.

Accordingly, the aims of this study were to develop and compare the nanoparticulate systems based on chitosan, TMC, and the newly described DMEC, DEMC and TEC loaded with insulin generated by the polyelectrolyte complexation (PEC) method described previously [28] with a weight ratio of polymer to insulin of 50% (w/w) which has been described by the same authors as optimal and to measure their intestinal transmucosal insulin transport across Caco-2 cell layers. The flux data were compared afterwards with the insulin fluxes across Caco-2 cell layers obtained after application of the various aqueous insulin/polymer solutions. The obtained results convincingly show that nanoparticles consisting of chitosan and its quaternary ammonium derivatives loaded with insulin are much less effective in facilitating paracellular transport across Caco-2 cell monolayers than the corresponding free polymers.

2. Materials and methods

2.1. Materials

ChitoClear® chitosan (viscosity 1% w/v solution, 22 mPa s, 98% deacetylated) was purchased from Primex, Iceland. Human insulin was a generous gift from Exir Pharmaceutical Company (Lorestan, Iran). The TMC, DMEC, DEMC and TEC were synthesized in our laboratory as described previously [21–24].

2.2. Determination of the molecular weight of chitosan and its derivatives

The biopolymer analysis was performed with a triple detection size-exclusion-chromatography (SEC^3) on a