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Research paper

Mechanistic basis for unexpected bioavailability enhancement of polyelectrolyte complexes incorporating BCS class III drugs and carrageenans

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Dedicated to Hans Peter Merkle on the occasion of his 70th birthday

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ABSTRACT

The objective of this study was to investigate the potential of λ-carrageenan to work as an absorption modifying excipient in combination with formulations of BCS class 3 substances. Trospium chloride was used as a model BCS class 3 substance. Polyelectrolyte complexes of trospium and λ-carrageenan were produced by layer-by-layer complexation. A λ-carrageenan-containing formulation was administered either in capsules size 9 to rats by gavage or directly into ligated intestinal loops of rats. Exceptionally strong variations were observed in the plasma concentrations of the rats that received λ-carrageenan compared to the control group, but enhanced plasma concentrations were observed only in some of the rats. In vitro permeability studies were performed across Caco2-monolayers and across excised segments of rat jejunum in a modified Ussing chamber to learn more about the mechanism of absorption enhancement. The complex did not show any effect in Caco2-cells, but led to a major enhancement of permeability across excised segments in modified Ussing chambers. Carrageenan did not lead to alterations of tight junctions. The bioavailability enhancing effect thus was most likely due to an interaction of the polyelectrolyte-drug complex with the mucus, which provided an intimate contact between the drug and the absorbing surface. A similar effect was also achievable with other types of carrageenan and was also transferable to other compounds.

In conclusion, λ-carrageenan-drug complexes show interesting excipient-drug-epithelium interactions – however, for full utilization of the permeation enhancing potential, an intimate and reproducible contact between absorbing epithelia and the complex is needed.

1. Introduction

The oral bioavailability of a drug substance mainly depends on its solubility and its permeability. Even though considerable effort has been made to establish new ways to improve low drug permeability, many promising compounds are not further developed as therapeutics because of their low permeability. Specifically, substances of high molecular weight, hydrophilic, or charged compounds often show low permeability.

A number of excipients affect drug absorption at the level of the intestinal membrane. Some compounds, such as sodium dodecyl sulfate (SDS), may alter the epithelial permeability [1]. Other compounds, such as tocopheryl polyethylene glycol succinate (TPGS), have been shown to change the absorption of drugs by inhibiting P-glycoprotein (PGP) [2]. Most of the excipients in the scientific focus take effect on tight junctions (TJs) between the cells in the gastrointestinal tract [3]. Chitosans, for example, open tight junctions by binding to the cell-surface and interact with the cytoskeletal F-actin and the zonula occludens [4]. Sodium decanoate, another promising substance already in clinical use, takes effect on tight junctions through the activation of phospholipase C [5,6]. Recently, highly specific zonula occludens toxin analogs were developed that reversibly disassemble intestinal tight junctions as well [7,8]. It should be considered that the opening of tight junctions is not specific to absorption enhancement of a single compound and that other xenobiotics may also be systemically absorbed upon widening of the TJs. Moreover, it is not completely clear whether some of

Abbreviations: SDS, sodium dodecyl sulfate; TPGS, tocopheryl polyethylene glycol succinate; PGP, P-Glycoprotein; TJs, tight junctions; API, active pharmaceutical ingredient; PECs, polyelectrolyte complexes; KBR, Krebs-Ringer-Bicarbonate-buffer. pH 7.4; R, transsegmental electrical resistance; PD, potential difference; TEER, transepithelial electrical resistance.

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these permeation modifiers might permanently harm the membrane [9].

Another approach to enhance the permeability is the use of mucoadhesive substances that increase drug absorption by attaching to the mucosal membrane, prolongation of transit time, and creation of a steeper concentration gradient of the active pharmaceutical ingredient (API) over the membrane. The use of mucoadhesive substances has been studied for the nasal [10], buccal [11], vaginal [12], and gastrointestinal [13] pathways. Several polymer classes have been examined. Mainly positively charged polymers such as chitosan [14] and negatively charged polymers such as poly(acrylic acid) [15] have received significant attention. Furthermore, dendrimers, amphoteric polymers, and synthetic glycopolymers were investigated. Different approaches have been made to study the mucoadhesive strength of polymers as well as the mechanism of mucus–polymer-interaction [16].

One of the classes of anionic polymers that have been studied is carrageenans. The structure of λ-carrageenan is shown in fig. 1. Carrageenans are sulfated polysaccharides. These are extracted from red seaweeds and are widely used as thickening or stabilizing agents in food products. From the different classes of carrageenans, the most important are I-, K-, and λ-carrageenan. The main chemical difference between the single classes is number and position of sulfate ester groups. While I- and K- carrageenans form gels in the presence of certain ions, λ-carrageenan does not [17]. The use of carrageenans as mucoadhesive excipients for drug delivery has also been studied utilizing the vaginal [18], buccal [19], or ophthalmic [20] routes. Song and Eddington showed that carrageenan alone does not enhance the permeation of mannitol, but it led to an enhancement in permeation in the co-presence of the permeation enhancer AT1002 [21]. To the best of our knowledge, the effect of this polymer for improvement of gastrointestinal permeation has not been investigated yet.

Furthermore, the use of polyelectrolyte complexes (PECs) containing mucoadhesive substances for oral drug delivery was investigated [22]. PECs consist of polymers with ionizable groups complexed with oppositely charged polymers or drug substances, for example dextran sulfate, alginate, carrageenan, or chitosan. The complexation is based on hydrogen bonding, ionic or hydrophobic interactions [23]. PECs containing mucoadhesive or permeation enhancing components can provide protection against gastric degradation of a compound, prolonged residence time at the site of absorption, absorption enhancement by opening of tight junctions, or direct uptake of a drug substance due to an intimate contact with the mucosal membrane [24]. The usage of PECs containing chitosan for the administration of insulin via the oral pathway was studied previously [25].

Trospium chloride was used as a model substance. The BCS class 3 drug shows high solubility, but low and variable permeation. Trospium has been used for the treatment of overactive urinary bladder for many years [26–28]. Trospium is a positively charged quaternary ammonium compound and therefore displays good candidate properties for complexation with a negatively charged polymer. The structure of trospium chloride is shown in fig. 1.

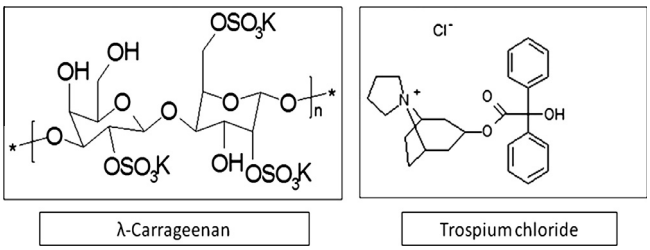

Fig. 1. Structures of λ-carrageenan (potassium salt) and trospium chloride.

The aim of this study was to test the effectiveness of PECs containing λ-carrageenan on the permeability of trospium chloride and other BCS III substances. In vivo studies were carried out by administration of powder complex filled hard capsules to rats by gavage and direct administration of powder complex into ligated loops of rat small intestine. Since the statistical analysis of the results showed unusually high standard deviations, in vitro permeation experiments were performed across excised segments of rat jejunum in modified Ussing chambers and across Caco2-monolayers to elucidate the cause of the variable bioavailability enhancement.

2. Material and methods

2.1. Material

Rats were purchased from Charles River (Sulzfeld, Germany). Caco2-cells were from the European Collection of Cell Cultures. DMEM, HBSS, Penicillin/Streptomycin, Ketamin, and Xylazin were purchased from the Pharmacy of the Medical Center of the Johannes Gutenberg-University (Mainz, Germany). Trospium chloride was a kind gift of K.H.S. Pharma Holding GmbH (Ingelheim, Germany). Polycarbonate transwells were purchased from Corning (Amsterdam, The Netherlands). Butylscopolamine hydrobromide, atenolol, and all other chemicals were obtained from Sigma–Aldrich (Schnelldorf, Germany).

2.2. Complexation

Complexation was performed by Capsulution (Berlin, Germany). Complexes were produced that consisted of alternating layers containing positively charged trospium and layers containing negatively charged polymer. Different negatively charged polymers, such as chondroitin sulfate or carboxymethylcellulose, were tested, but only the addition of λ-carrageenan to a solution of trospium chloride resulted in a considerable precipitation. The optimal mass balance between trospium and polymer was evaluated. For that purpose, the precipitate was weighed, and the concentration of trospium in the solution was determined by HPLC. Finally, a solution of 25 mg/ml λ-carrageenan was provided, and a solution of 100 mg/ml trospium slowly added. The mass-ratio trospium: λ-carrageenan was 1.85:1. The precipitate was freeze-dried. The exact procedure of a layer-by-layer complexation and the structure of the originating particles are described in the patent [29].

2.3. Complex Characterization

To measure the amount of trospium in the freeze-dried particles, the complex was dissolved in HBSS. The HPLC method used is described below. Measurement was repeated with three different samples of precipitates. Dissolution profiles of the complexes were examined in a USP type II apparatus at a paddle speed of 50 rpm and 37 °C in 500 ml of different dissolution media at pH 1.0, 4.6 and 6.8. Hard-gelatine capsules size 000 containing 100 mg of compound were used mainly for reasons of sufficient analytical sensitivity. Another size of capsules (size 9) was used for the in vivo study due to the limitations of the animal model with respect to size of the administered capsule. Samples from the dissolution media were taken after 5, 10, 20, 30, 40, 55, 70, 90, 120, and 180 min. Dissolution experiments were repeated 5 times at each pH value. Concentration in the media was determined using UV/Vis spectrophotometry at 258 nm.