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Nasal drug delivery: Design of a novel mucoadhesive and *in situ* gelling polymer

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ABSTRACT

The aim of the present study was to establish a novel polymeric excipient for liquid nasal dosage forms exhibiting viscosity increasing properties, improved mucoadhesion and stability towards oxidation in solution.

In order to achieve this goal, 2-mercaptonicotinic acid was first coupled to L-cysteine by disulfide exchange reaction and after purification directly attached to the polymeric backbone of xanthan gum by carbodiimide mediated amide bond formation. The resulting conjugate was characterized with respect to the amount of coupled ligand, the *in situ* gelling behavior, mucoadhesive properties and stability towards oxidation. Furthermore, the influence of preactivated polymers on ciliary beat frequency (CBF) of porcine nasal epithelial cells was investigated.

Results showed, that 252.52 ± 20.54 μmol of the ligand was attached per gram polymer. No free thiol groups could be detected on the polymeric backbone indicating entire preactivation. Rheological investigations of polymer mucus mixtures revealed a 1.7-fold and 2.5-fold enhanced mucoadhesion of entirely preactivated xanthan (Xan-Cys-MNA) compared to thiolated xanthan (Xan-Cys) and unmodified xanthan (Xan). Tensile force evaluation reported a 2.87 and 5.11-fold higher total work of adhesion (TWA) as well as a 1.63 and 2.41-fold higher maximum detachement force of Xan-Cys-MNA compared to Xan-Cys and Xan. In the presence of H_2O_2 as an oxidizing agent Xan-Cys-MNA showed unlike Xan-Cys no increase in viscosity, indicating high stability towards oxidation. Addition of $CaCl_2$ to Xan-Cys-MNA solutions caused a decrease in viscosity at nevertheless higher total viscosity. Results from CBF studies proved nasal safety for the novel conjugate.

According to these results, entirely preactivated thiolated xanthan gum seems to be a promising excipient for nasal dosage forms in order to improve drug bioavailability.

1. Introduction

Among non-invasive drug delivery systems the nasal route has become a notable alternative to oral drug application with comparable drug absorption variabilities (Kumar et al., 2016; Studd et al., 1999). Besides comfortable and painless way of application major advantages of intranasal drug delivery are the large absorption area and vasculature nature of the nasal mucosa as well as the avoidance of hepatic first-pass effects (Kammona and Kiparissides, 2012; Peppas and Carr, 2009). However, several mechanisms contribute to a reduced popularity of nasal dosage forms: mucociliary clearance, enzymatic degradation and low permeation often result in low bioavailability (Dimova et al., 2003; Lee et al., 2016). On the other hand, especially liquid formulations are not only associated with unpleasant stability but also with lower absorption because of their tendency to flow down the oral cavity (Andrews et al., 2009; Kublik and Vidgren, 1998). Over the last years, several strategies were approached in order to overcome these shortcomings. Besides the development of powder formulations and nanoparticles for nasal drug delivery, the use of mucoadhesion enhancing excipients plaid a major role (Andrews et al., 2009; Karakosta et al., 2015). Increasing the viscosity of nasal formulations by the addition of water-soluble thickening compounds, such as xanthan gum or galactomannan provides prolonged mucoadhesion of formulations on the nasal mucosa (Sherafudeen and Vasantha, 2015). Another promising approach is the concept of thiolated polymers providing an intimate contact between dosage form and nasal mucosa by disulfide bond

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formation (Bernkop-Schnürch et al., 2006). However, these so called thiomers are prone to intramolecular oxidation reactions in form of disulfide bond formations, which occur preferably in solutions at pH values above 5 (Bernkop-Schnürch et al., 2003; Peppas et al., 2009). Even the advancement of thiomers by introducing reactivity increasing protecting aromatic ligands, could reduce but not completely prevent these side reactions. Especially in liquid nasal dosage forms this effect leads to a limited stability and a loss of sprayability. More recently, the concept of entirely preactivated thiomers, which do not undergo intramolecular oxidation reactions while providing increased reactivity towards mucosal thiol groups, has been established (Hintzen et al., 2013). So far, however, this strategy has not been applied for liquid nasal formulations.

Therefore it was the aim of this study to establish a mucoadhesive excipient for nasal dosage forms by applying both mentioned strategies: increasing the viscosity using a gel forming agent as well as the concept of entirely preactivated thiomers. In order to achieve this goal, the polysaccharide xanthan gum was chosen as polymer backbone because of its well-studied properties as a rheology modifier and stabilizer. By attaching the ligand 2-((2-amino-2-carboxyethyl)disulfanyl)nicotinic acid (Cys-MNA) to the polymeric backbone a novel polymer was generated. Mucoadhesive properties as well as rheological behavior were investigated and the effect of preactivated thiomers on the nasal ciliary beat frequency was analyzed in the present study for the first time.

2. Materials and methods

2.1. Materials

Xanthan gum (Keltrol® CG-SFT) was purchased from CP Kelco (Atlanta, Georgia, USA). All other chemicals were purchased from Sigma Aldrich (Vienna, Austria)

2.2. Methods

2.2.1. Synthesis of Xan-MNA

First, the ligand 2-((2-amino-2-carboxyethyl)disulfanyl)nicotinic acid (Cys–MNA) for conjugation with xanthan was synthesized and purified according to a method described previously (Hintzen et al., 2013). Then, the entirely preactivated xanthan conjugate (Xan-Cys-MNA) was synthesized by carbodiimide mediated amide bond formation between primary amino groups of Cys–MNA to the carboxylic acid group of xanthan according to a modified method, which was described previously by our research group (Bernkop-Schnürch and Steininger, 2000). In brief, 2 g of xanthan was dissolved in 300 mL of demineralized water and pH was adjusted to 5.5. Afterwards, carboxylic acid moieties were activated by adding *N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride (EDAC) in a final concentration of 150 mM and *N*-hydroxysuccinimide (NHS) in a final concentration of 40 mM to the reaction mixture. After stirring for 15 min the pH was raised to 7 and 8.26 mmol of prehydrated Cys-MNA was added to the reaction mixture within 60 min. The reaction mixture was stirred for 6 h at room temperature and dialyzed ten times against 5 L of demineralized water (MWCO 10,000–12,000 Da). The purified product was finally obtained by lyophilization at −30 °C and 0.01 mbar.

2.2.2. Synthesis and purification of thiolated xanthan (Xan-Cys)

Xan-Cys was synthesized according to a method described previously (Bernkop-Schnürch and Steininger, 2000): Briefly, the pH of a solution of 2 g of xanthan in 300 mL of demineralized water was adjusted to pH 5.5. EDAC was then added in a final concentration of 150 mM and the solution was stirred for 15 min. Afterwards, 8.26 mmol of prehydrated L-cysteine was added and the solution was stirred at room temperature at pH 5.5. After 6 h, the reaction mixture was filled in a dialysis tube (MWCO 10,000–12,000 Da) and dialyzed ten times against 5 L of HCl (200 μM) in the dark at 10 °C to prevent oxidation of free thiol groups. The product was lyophilized and stored at 4 °C in the dark under dry conditions until further use.

2.2.3. Characterization of Xan-MNA and Xan-Cys

The amount of free thiol groups was determined spectrophotometrically using Ellman's reagent according to a method described previously (Bernkop-Schnürch et al., 1999). Furthermore, the amount of free thiol groups after reduction with $NaBH_4$ was quantified with Ellman's reagent in order to analyze the extent of disulfide bond formation in the thiolated xanthan derivative and also to determine the amount of coupled ligand for Xan-Cys-MNA. Moreover, the amount of conjugated MNA was analyzed with reduced glutathione at 354 nm (Iqbal et al., 2012). To verify the successful purification *via* dialysis, the amount of primary amino groups was determined by 2,4,6-trinitrobenzenesulfonic acid (TNBS) detecting unbound L-cysteine and L-cystine (Bernkop-Schnürch et al., 2001).

2.2.4. Preparation of a porcine nasal epithelial cell culture

Porcine nasal epithelial cells for culture were scratched from the middle turbinate of freshly slaughtered pigs with the nasal brushing method (Toskala et al., 2005). The cells were immersed in DMEM-Ham's F12 1:1 medium and 50 μg/ml streptomycin, 50 IU/ml penicillin and 2.5 μg/ml Amphotericin B were added. After incubation for 4–6 h at 37 °C in a 5% CO_2 atmosphere the medium was centrifuged at 900 rpm for 5 min and the supernatant was thrown away. The obtained cells were suspended in 1 ml of a new DMEM-Ham's F12 1:1 medium containing 2% Ultroser G, retinoic acid, 50 μg/ml streptomycin, 50 IU/ml penicillin, 2.5 μg/ml Amphotericin B and 1% non-essential amino acids. Afterwards, 2 ml were filled into 0.2% collagen type 1 pre-coated 12-well plates and incubated at 37 °C using an atmosphere of 95% oxygen and 5% carbon dioxide. The medium was changed every 24 h.

2.2.5. Cell treatment and CBF measurement

Obtained cells were preconditioned for 60 min at 23 °C room temperature before the initial ciliary beat frequency was measured. Therefore a combination of a microscope (Motic Type AE31, Motic GmbH, Germany) and a high speed camera system (Motion Scope M1, Imaging Solutions GmbH, Germany) was used to identify cilia in motion. Afterwards, cells were incubated with test solutions of Xan, Xan-Cys and Xan-MNA in order to investigate their influence on CBF. Therefore, all excipients were dissolved in Locke-Ringer solution in a concentration of 0.5% (m/v). The ciliary beat frequency for each formulation was measured 20 min after xanthan supplementation. In order to investigate reversibility, the experiment was repeated 60 min after the cell layers were rinsed with cell culture medium.

CBF changes caused by xanthan formulations were classified like described by Merkus et al. (1998).

- no effect: less than 10% or statistically insignificant
- mild: 10–20% cilio-stimulation: inhibition and statistically significant
- moderate: 20–50% cilio-stimulation: inhibition and statistically significant
- severe: greater than 50% and statistically significant