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Research paper

The influence of gellan gum on the transfer of fluorescein dextran across rat nasal epithelium in vivo

Björn Jansson, Helene Hägerström[1], Nelly Fransén, Katarina Edsman*, Erik Björk

Department of Pharmacy, Uppsala University, Uppsala, Sweden

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Abstract

The nasal uptake of a 3000 Da fluorescein dextran (FD3) was investigated in rats, using fluorescence microscopy. The uptake from a formulation containing deacetylated gellan gum, an in situ gelling agent, was compared to that from a mannitol solution. Additionally, the rheological behavior of the gellan gum in water and saline was studied. It was shown that the gellan gum solution was easily administered owing to its low viscosity, and upon contact with the mucosa, a gel was formed. The epithelial uptake and transfer of FD3 appeared to be increased and prolonged using the gellan gum formulation. This increase was not accompanied by qualitative changes of the epithelial FD3 distribution or any visible harmful effects.

Keywords: Intranasal administration; In situ gel; Fluorescein dextran; Deacetylated gellan gum; Nasal mucosa; Olfactory mucosa; Fluorescence microscopy

1. Introduction

Nasal administration is considered to be a convenient route of administration when oral administration of a drug gives an undesirably slow effect, or when a drug is highly metabolized or incompletely absorbed in the gastrointestinal tract. Nasal sprays may be preferred to injections because of higher patient compliance and may have applications in, e.g. pain management [1] and vaccinations [2]. Moreover, several animal studies show that a large number of substances are transferred directly to the central nervous system (CNS) after nasal administration, bypassing the blood–brain barrier (BBB) [3–5]. Some studies indicate that this transfer along the olfactory nerves also occurs in man [6,7]. A field where the olfactory pathway may become important is the delivery of peptides (molecular weights around 1000 Da) intended for neuroprotection, as discussed by Gozes [8]. The olfactory pathway, and other aspects of nasal drug delivery, has been discussed by Illum [9].

In the present study, we investigate the nasal uptake of a 3000 Da fluorescein dextran (FD3) from a gellan gum formulation using a previously reported method [10]. Salmon calcitonin is an example of a substance in the same molecular weight range, currently marketed for intranasal administration.

Gellan gum is a linear, anionic polysaccharide that is secreted by the microbe *Sphingomonas paucimobilis* (formerly known as *Pseudomonas elodea*). Marketed as Gelrite® or Kelcogel®, deacetylated gellan gum is approved in the USA and EU as a gelling, stabilizing and suspending agent in food products [11]. Because of its ability to form strong clear gels at physiological ion concentration, deacetylated gellan gum has been widely investigated for use as an in situ gelling agent in ocular formulations. It has been reported to provide a significantly prolonged corneal contact time in comparison with conventional solutions [12–14] and is currently marketed in the controlled-release timolol formulation Blocadren® Depot (Timoptic-XE®). It has also been suggested that gellan gum is a promising polymer for use in nasal formulations [15]. To our knowledge, however, it has only been included in one

* Corresponding author. Department of Pharmacy, Uppsala University, Box 580, SE-751 23 Uppsala, Sweden. Tel.: 46 18 471 4268; fax: 46 18 471 4223.
E-mail address: katarina.edsman@farmaci.uu.se (K. Edsman).
[1] Present address: Cederroth International AB, Upplands, Väsby, Sweden.

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study [16] on this subject where it was shown to moderately enhance the local and serum antibody response in mice after nasal administration of viral antigens. Other in situ gelling systems, such as temperature and pH responsive gels, have, on the other hand, appeared more frequently in nasal drug delivery studies and have been shown to increase the residence time and improve drug absorption, see, e.g. Zhou and Donovan [17], Aikawa et al. [18] and Park et al. [19].

Enhanced bioavailability has been reported after administration of viscous polymer solutions in some studies (e.g. [20,21]), but it has not been clearly established whether this is caused by a prolonged residence time in the nasal cavity or an effect on the mucosa. Pennington et al. [22] reported clearance half-times of 1 and 2.2 h for 0.6% and 1.25% HPMC, respectively, although the difference was not statistically significant. The dependence of bioavailability and residence time on viscosity may be caused by the larger droplet size arising from the higher viscosity of the formulation rather than the higher viscosity itself [23]. The nasal spray pumps used today give deposition primarily in the anterior nasal cavity, and it was reported that this pattern was further amplified with increasing viscosity [23,24]. The advantage of using an in situ gelling formulation is that, owing to its low viscosity, it can be readily administered. After gelling is induced by some physiological stimulus at the absorption site, the formulation attains semisolid properties.

The in situ gelling properties of deacetylated gellan gum are attributed to its responsiveness to cations. In an ion-free aqueous medium, the polymer chains form double helices, resulting in a fluid that has a viscosity close to that of water. In the presence of gel-promoting cations (Na^+, K^+, Ca^{2+}), a portion of the helices associates and the cation-mediated aggregates cross-link the gel network [25]. A rapid gelling can be expected upon contact with the mucosa since, even at low polymer concentrations, small quantities of ions suffice for the formation of a strong gel [26].

The purpose of the present study was to investigate whether the use of a gellan gum formulation would change the extent or the time profile of the uptake and transfer of FD3 across rat nasal epithelia in vivo in comparison to a plain isotonic water solution. In addition, the distribution of fluorescence and histological changes in the mucosa were to be evaluated.

2. Materials and methods

2.1. Materials

The deacetylated gellan gum (Kelcogel F®) was kindly given by the Kelco division of the Monsanto Company (USA). Stock solutions of gellan gum (0.5% w/w) were prepared by dispersing the polymer powder in ultra-pure water and then stirring the dispersion in a sealed vial for 20 min at 100 °C by using a water bath. After cooling to room temperature, 4% (w/w) of D-mannitol (Sigma Chemical Co., USA) was added to achieve isotonicity. The resultant stock solution was used within 2 days of preparation. For the rheological analysis a gellan preparation was made with both 4% mannitol and 0.9% NaCl, using the same heating and stirring procedure as for the stock solution. On the same day as the animal experiment, 0.5% (w/v) aldehyde fixable fluorescein dextran (FD3, Molecular Probes Inc., USA) was dissolved in isotonic mannitol or gellan stock solution and protected from light.

The rats were anesthetized using a 1:1:2 mixture of Hypnorm® (fentanyl citrate 0.315 mg/ml, fluanisone 10 mg/ml; Janssen Animal Health, Belgium), midazolam 5 mg/ml and water. Bouin's fluid (saturated picric acid, 40% formaldehyde and concentrated acetic acid; 15:5:1) was used for the perfusion and the immersion fixation. The stock solution of propidium iodide (1 mg/ml in water, Molecular Probes Inc., USA) was diluted to 5 μg/ml in phosphate buffered saline prior to use.

2.2. Rheological measurements

The rheological measurements on the gellan stock solution and the gellan preparation made in 0.9% NaCl were carried out at 37 °C using a Bohlin VOR rheometer (Bohlin Reologi, Lund, Sweden). The measuring systems used were a double gap cylinder (DG 24/27) and a concentric cylinder (C14). Silicone oil was added to the surface of the sample to prevent evaporation during measurements. The gellan stock solution was directly equilibrated at 37 °C, whereas the hot, freshly prepared gellan sample in 0.9% NaCl was poured into the measuring system at 90 °C and subjected to controlled cooling (0.5 °C/min) from 90 to 37 °C [26]. Having equilibrated at 37 °C, a strain sweep measurement was performed at a constant frequency of 1 Hz to determine the maximum strain amplitude. Then a frequency sweep (0.01–5 Hz) was performed at a selected strain amplitude chosen to be within the linear region of the sample. The rheological behavior of the samples was evaluated in terms of the elastic (storage) modulus (G') and the viscous (loss) modulus (G'') obtained in the frequency sweep. Furthermore, the viscosity of the gellan stock solution was determined using the rheometer in the rotational viscometry mode.

2.3. Animal experiments

A more detailed description of the procedures is provided in our previous study [10]. The rats (male Sprague–Dawley, B&K Universal, Sweden) were housed with a normal 12 h light–dark cycle for 1 week prior to the experiment and weighed 241–308 g at the time of the experiment. They were fed a standard pellet diet and tap water ad libitum and were anesthetized with intraperitoneal hypnorm-midazolam (0.27 ml/100 g body weight).