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Research paper

Sodium hyaluronate as a mucoadhesive component in nasal formulation enhances delivery of molecules to brain tissue

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ABSTRACT

Intranasal administration of molecules has been investigated as a non-invasive way for delivery of drugs to the brain in the last decade. Circumvention of both the blood–brain barrier and the first-pass elimination by the liver and gastrointestinal tract is considered as the main advantages of this method. Because of the rapid mucociliary clearance in the nasal cavity, bioadhesive formulations are needed for effective targeting. Our goal was to develop a formulation containing sodium hyaluronate, a well-known mucoadhesive molecule, in combination with a non-ionic surfactant to enhance the delivery of hydrophilic compounds to the brain via the olfactory route. Fluorescein isothiocyanate-labeled 4 kDa dextran (FD-4), used as a test molecule, was administered nasally in different formulations to Wistar rats, and detected in brain areas by fluorescent spectrophotometry. Hyaluronan increased the viscosity of the vehicles and slowed down the in vitro release of FD-4. Significantly higher FD-4 transport could be measured in the majority of brain areas examined, including olfactory bulb, frontal and parietal cortex, hippocampus, cerebellum, midbrain and pons, when the vehicle contained hyaluronan in combination with absorption enhancer. The highest concentrations of FD-4 could be detected in the olfactory bulbs, frontal and parietal cortex 4 h after nasal administration in the mucoadhesive formulation. Intravenous administration of a hundred times higher dose of FD-4 resulted in a lower brain penetration as compared to nasal formulations. Morphological examination of the olfactory system revealed no toxicity of the vehicles. Hyaluronan, a non-toxic biomolecule used as a mucoadhesive in a nasal formulation, increased the brain penetration of a hydrophilic compound, the size of a peptide, via the nasal route.

1. Introduction

Targeting of drugs to the central nervous system (CNS) is still a difficult task to fulfill because the blood–brain barrier (BBB), segregating the brain interstitial fluid from the circulating blood, and the blood–cerebrospinal fluid barrier, separating the blood from the cerebrospinal fluid, prevents the influx of hydrophilic compounds with a molecular weight above 600 Da from the systemic circulation into the brain. Hence, these barriers actively controlling cellular and molecular trafficking prevent the utilization of many novel therapeutic agents for treating CNS disorders such as Parkinson's and Alzheimer's diseases [1,2]. In order to enhance the blood–brain transport and to deliver drugs to the brain in an effective concentration, several approaches have been attempted. These methods include the manipulation of BBB (hyperosmotic shock, vasoactive substances, inhibition of efflux transporters) and the modification of the drug molecules (lipophilic or cationic molecules, prodrugs, binding of drugs to carriers, for example, transferrin, or targeted vesicle systems) [1–8]. However, the increased transport of drugs to the central nervous system can be carried out not only by the modification of the BBB function or the drug molecule itself, but also via an alternative route by the selection of an application site circumventing the BBB.

In recent years, nasal route for delivery of drugs to the brain via the olfactory region has received a lot of attention [9–11]. Olfactory sensory neurons are the only first-order neurons whose cell bodies are located in a distal epithelium. Their dendritic processes are directly exposed to the external environment in the

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upper nasal passage, while their axons project through perforations in the cribriform plate of the ethmoid bone to synaptic glomeruli in the olfactory bulb. These unique anatomic and physiologic properties of the olfactory region provide both extracellular and intracellular pathways into the CNS that bypass the BBB [9–13]. Intranasal delivery of drugs offers several advantageous properties. This method is non-invasive, essentially painless, and can be easily and readily administered by the patient or a physician. Furthermore, it ensures rapid absorption, the avoidance of first-pass metabolism in gut and liver and does not require sterile preparation [12,14,15].

During the formulation of a dosage form intended for intranasal application, several aspects should be taken into consideration [16]. The olfactory region in man is situated in the upper part of the nasal cavity, an area that is difficult to reach with presently available nasal spray or powder devices. Furthermore, the nasally administered drugs will normally be cleared rapidly from the nasal cavity into the gastrointestinal tract by the mucociliary clearance system [9–11]. Another important factor limiting the nasal absorption of large molecular weight or polar drugs is the low membrane permeability. The epithelial cells of nasal mucosa are closely connected on the apical surface by intercellular junctions [17], which hinder the paracellular transport of polar drugs between the cells. Moreover, the nasal mucosa has a metabolic capacity as well, which can contribute to the low transport of peptides and proteins across the nasal membrane [12,14–16].

Considering the above-mentioned aspects, our aim was to formulate a carrier system, which combines bioadhesive and absorption enhancer properties to prolong retention time and to increase membrane permeability of a test molecule [18,19].

Polyethoxylated 40 hydrogenated castor oil (Cremophor RH40) is a non-ionic solubilizing and emulsifying agent. This surfactant can be used to increase bioavailability of drugs by solubilizing of poorly soluble compounds and by increasing of cell membrane fluidity. Furthermore, Cremophors have been shown to inhibit P-glycoprotein activity, therefore, they increase the bioavailability of drugs which are known substrates of this efflux transporter [20–23].

Sodium hyaluronate is the sodium salt of the hyaluronic acid, a naturally occurring linear polysaccharide composed of alternating disaccharide units of *N*-acetyl-D-glucosamine and D-glucuronic acid [24]. Hyaluronic acid, a non-sulfated glycosaminoglycan, can be found in the extracellular tissue matrix of vertebrates, including connective tissue, synovial fluids, vitreous humour and aqueous humour [25]. It plays a critical role as a signaling molecule in cell motility, cell differentiation, and wound healing. This natural anionic polysaccharide has an excellent mucoadhesive capacity [24,26] and many important applications in formulation of bioadhesive drug delivery systems [27]. Besides its mucoadhesive properties, it was found that this biopolymer may enhance the absorption of drugs and proteins via mucosal tissues [26,28,29]. While hyaluronan is used in diverse drug delivery systems e.g. ophthalmic, pulmonary and vaginal [24,26,30], it has not been widely exploited for nasal drug delivery to the nervous system.

In this study, the nose-to-brain transport was investigated in case of different carrier systems. Fluorescein isothiocyanate (FITC)-labeled dextran, a hydrophilic compound with an average molecular weight of 4.4 kDa (FD-4), the size of a peptide, with no transporter at the BBB, was selected as a test molecule. Vehicles containing absorption enhancer polyethoxylated 40 hydrogenated castor oil or both mucoadhesive polymer sodium hyaluronate and absorption enhancer were formulated and tested in rats.

2. Materials and methods

2.1. Materials

All reagents, including FITC-labeled dextran (Mw = 4.4 kDa) were purchased from Sigma-Aldrich Chemical Co. (MO, USA), unless otherwise indicated. Polyethoxylated 40 hydrogenated castor oil (Cremophor RH40) was obtained from BASF (Germany). Sodium hyaluronate (Mw = 1400 kDa) was obtained as a gift from Gedeon Richter Ltd. (Hungary). All other materials were of reagent grade.

2.2. Preparation of dosing solutions

Cremophor RH40 was dissolved in the physiological saline solution (PhS; 0.9% w/v sodium chloride in sterile distilled water). In case of sodium hyaluronate-containing samples the mucoadhesive polymer was added in small amounts to the solution. In order to ensure the complete solvation of polymers, samples were rehomogenized after 24 h. The FD-4 was dissolved in the prepared vehicles. The concentration of the test molecule was 1 mg/ml for intranasal and 8 mg/ml for intravenous administration. The compositions of the dosing solutions were as follows: (i) physiological saline (PhS) 100% w/w, (ii) absorption enhancer solution (AE) 10% w/w Cremophor RH40 and 90% w/w physiological saline, (iii) hyaluronate solution (HA) 1.5% w/w sodium hyaluronate and 90% w/w physiological saline, (iv) mucoadhesive solution (MA) 1.5% w/w sodium hyaluronate, 10% w/w Cremophor RH40 and up to 100% w/w physiological saline.

2.3. Rheological measurements

Rheological measurements were carried out with a Rheostress 1 Haake instrument. A cone-plate measuring device was used in which the cone angle was 1°, and the thickness of the sample was 0.048 mm in the middle of the device. The flow and viscosity curves of the samples were determined by changing the shear rate between 0.01 and 100 s^{-1} at 37 °C.

2.4. In vitro drug release studies

In case of samples with 1 mg/ml FD-4 content, in vitro drug release experiments were performed as well. The dissolution studies were carried out using ointment cells and small volume dissolution vessels in a Hanson SR8plus dissolution apparatus (Chatsworth, CA). Samples, 0.4 g each, were placed as donor phase on the Porafil membrane filter (pore diameter 0.45 μm). The effective diffusion surface area was 1.767 cm^2. Phosphate-buffered saline solution (PBS; pH = 7.4, 100 ml) was used as dissolution medium at a temperature of 37 °C and a paddle speed of 50 rpm. Samples (3 ml each) were taken and immediately replaced with fresh dissolution medium at 15, 30, 60, 120, 180, 240, 300, 360, 420 and 480 min, and further analyzed by spectrofluorometry. Six parallel measurements were performed in case of each dosing solution. Dissolution profiles were compared by using difference factor f_1 and similarity factor f_2. For two similar preparations, the value of f_1 must be between 0 and 15 and that of f_2 must be in the range of 50–100. The factors can be calculated according to the following equations: [31–33],

$$f_1 = \left\{ \left[\sum_{t=1}^{n} |R_t - T_t| \right] \Big/ \left[\sum_{t=1}^{n} R_t \right] \right\} \times 100 \quad (1)$$

$$f_2 = 50 \times \log \left\{ \left[1 + (1/n) \sum_{t=1}^{n} (R_t - T_t)^2 \right]^{-0.5} \times 100 \right\} \quad (2)$$