European Journal of Pharmaceutics and Biopharmaceutics 75 (2010) 381–387

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Research paper

Freeze-dried chitosan/pectin nasal inserts for antipsychotic drug delivery

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ARTICLE INFO

Article history:
Received 21 December 2009
Accepted in revised form 12 April 2010
Available online 29 April 2010

Keywords:
Chitosan/pectin polyelectrolyte complexes
Nasal delivery
Mucoadhesive inserts
Chlorpromazine hydrochloride

ABSTRACT

The objective of this investigation was the development of chitosan/pectin based nasal inserts to improve bioavailability of antipsychotic drugs in the treatment of psychotic symptoms. In fact, the nasal route of administration ensures systemic availability avoiding the first-pass metabolism and obtaining more efficacious treatments. Chitosan/pectin polyelectrolyte complexes were prepared at pH 5.0 with different polycation/polyanion molar ratios and lyophilized in small inserts in the presence of chlorpromazine hydrochloride. The results show that higher amount of pectin in the complexes, with respect to higher amount of chitosan, produced a more evident porous structure of the nasal inserts, improving water uptake ability and mucoadhesion capacity. Finally, the presence of increasing amounts of pectin allowed the interaction with chlorpromazine hydrochloride inducing the formation of less hydratable inserts thus limiting drug release and permeation. This investigation verifies the formation of polyelectrolyte complexes between chitosan and pectin at pH values in the vicinity of the p*Ka* interval of the two polymers and confirms the potential of these complexes, capable of achieving antipsychotic drug delivery in the nasal cavity.

1. Introduction

Novel formulations and new routes of administration for psychotropic drugs can offer advantages over older formulations in terms of efficacy, tolerability and compliance. Short-acting and long-acting preparations are useful alternatives to the traditional formulations, which can provide more acceptable forms of medication for patients affected by schizophrenia or other psychotic disorders [1]. Actually, the antipsychotic drug chlorpromazine is available on the market in injectable, oral and rectal dosage forms. The intramuscular route is used primarily when rapid action is required to control acute severe symptomatology, while oral administration of sustained release capsules is used for the long-term treatment of psychiatric illness. In addition, conventional oral tablets, suppositories and syrups are available for the administration of the antipsychotic drug chlorpromazine. Recently, nasal delivery systems have been investigated with the aim of altering the pharmacokinetics of orally and parenterally administered drugs in a fashion that can enhance their pharmacologic profiles [2]. In fact, the large surface area, porous endothelial basement membrane and high total blood flow of the nasal mucosa ensure systemic availability of compounds under circumvention of the hepatic first-pass metabolism. Nasal administration of chlorpromazine hydrochloride can be useful for either acute treatment setting or long-term treatment of psychiatric illness. In the first case, pharmaceutical formulations able to provide immediate drug permeation across nasal mucosa and a rapid onset of action can be good candidate particularly for patients who may have difficulty swallowing tablets or for patients who refuse intramuscular therapy. In the second case, pharmaceutical formulations able to provide an extended drug permeation across nasal mucosa can be useful to reduce the number of required daily doses, thus improving compliance, and to eliminate peak-to-valley fluctuations, thus reducing the risk of adverse effects.

However, the mucociliary clearance mechanism that rapidly removes applied dosage forms from the absorption site can be a problem in nasal drug delivery. Generally, conventional nasal formulations such as liquid drops or sprays are rapidly cleared from the nose, and residence times in man of 12–15 min have been described [3]. Although the residence time of a liquid vehicle can be increased by increasing its viscosity [4], viscous solutions are difficult to administer as drops or sprays. Powder formulations have been shown to have longer nasal residence times [5] than solutions but require sophisticated delivery devices for deposition and accurate dosing. The purpose of this study was the preparation and characterization of lyophilized nasal inserts [6] able to deliver a unique dose of drug in the nasal cavity and achieve a controlled release of the active principle according to hydration/diffusion mechanisms. As mucoadhesive polymers can be used to prevent the rapid clearance of the drug formulation, chitosan/pectin polyelectrolyte complexes [7] were used in this study for the preparation of nasal inserts able to increase the residence time and control

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doi:10.1016/j.ejpb.2010.04.013

drug release due to the formation of a gelled system in which the drug can diffuse. Pectin is an anionic polysaccharide present in the cell wall of most plants, consisting mainly of D-galacturonic acid and its methyl ester linked via α(1–4) glycosidic bonds. Chitosan is a natural derivative of chitin consisting of glucosamine and N-acetylglucosamine. These polymers show interesting biological properties, including biocompatibility, biodegradability and mucoadhesivity. A suspension of chitosan/pectin complexes, with or without chlorpromazine hydrochloride, was lyophilized into small inserts. Morphological characteristics, water uptake, mucoadhesion, release and permeation studies were performed in order to investigate insert ability to deliver antipsychotic agents in the nasal cavity.

2. Materials and methods

2.1. Materials

Pectin from citrus peel (*M*r. 30,000–100,000; esterification degree 60%; p*K*a, 4.0), chitosan (*M*r. 150,000; deacetylation degree 97%; p*K*a, 6.3) and chlorpromazine hydrochloride used for this study were obtained commercially from Fluka (Milan, Italy). All other chemicals and solvents were of analytical grade and purchased from Carlo Erba (Milan, Italy). Water uptake, mucoadhesion, release and permeation studies were carried out in aqueous buffers with the following compositions (mM): 65.0 NaOH, 30.6 $C_6H_8O_7{\cdot}H_2O$, 68.8 HCl 37% for buffer solution pH 2.0; 4.2 $Na_2HPO_4{\cdot}10H_2O$, 100.0 KH_2PO_4, 45.5 NaCl for buffer solution pH 5.5; 8.4 $Na_2HPO_4{\cdot}10H_2O$, 7.4 KH_2PO_4, 94.0 NaCl for buffer solution pH 6.8; 6.7 $Na_2HPO_4{\cdot}10H_2O$, 1.4 KH_2PO_4, 136.9 NaCl for buffer solution pH 7.4.

2.2. Preparation of chitosan/pectin complex nasal inserts

Chitosan/pectin complexes were prepared as reported in a previous work [7]. Briefly, chitosan (0.50 mmoles of monomer) and pectin (0.50 mmol of monomer) were dissolved separately in 100 ml of acetate buffer pH 5.0 at the ionic strength of 50 mM. The chitosan solution was then added to pectin solution in various molar ratios (1:9, 3:7, 1:1, 7:3, 9:1; mol chitosan/mol pectin) and stirred at room temperature for 24 h. The precipitates were separated by ultracentrifugation at 10,000 rpm for 10 min (ALC 4239R centrifuge; Milan Italy), washed with deionised water, homogenized at 17,500 rev min^{-1} for 5 min (Ultra-Turrax, T 25 basic homogenizer; IKA, Dresden, Germany), suspended again in deionised water and finally freeze-dried (Christ Freeze Dryer ALPHA 1–2, Milan, Italy), obtaining five different chitosan/pectin complexes: $CH/PEC_{(1:9)}$, $CH/PEC_{(3:7)}$, $CH/PEC_{(1:1)}$, $CH/PEC_{(7:3)}$ and $CH/PEC_{(9:1)}$. As described in Bigucci et al., 2008, complex weight measurements, FT-IR spectra and TGA thermograms confirmed the formation of ionic bonds between chitosan and pectin. Loaded inserts (average diameter 5 mm, height 8 mm) were prepared by adding 100 μl of chlorpromazine hydrochloride (25 mg/ml, 50 mg/ml, 100 mg/ml or 200 mg/ml) aqueous solution (pH 5.5 phosphate buffer) to 10 mg of different complex/mannitol mixtures (9:1; w/w) obtaining four different complex/drug weight ratios (2:0.5, 2:1, 2:2 and 2:4). Mannitol was added, as a bulking agent in order to improve mechanical strength of lyophilized nasal inserts when handled [8]. The resultant suspensions were filled into polypropylene microcentrifuge tubes, allowed to settle to swell and remove air and finally lyophilized, obtaining cone-like shaped solid inserts. The inserts were stored in a desiccator until use. Unloaded inserts (10 mg) were prepared by the same procedure without the presence of chlorpromazine hydrochloride. Moreover, loaded inserts (10 mg) were produced only with mannitol and chlorpromazine hydrochloride as control formulations for release and permeation studies. Finally, in order to perform *in-vitro* water uptake studies, loaded and unloaded inserts (average diameter 7 mm, height 20 mm) were prepared starting from 100 mg of the different complex/mannitol mixtures (9:1; w/w).

2.3. Scanning electron microscopy (SEM) and porosity measurements

The morphology of nasal inserts was performed by SEM analysis. Inserts (10 mg) were cut with a razor blade to expose the inner structure, fixed on supports and coated with gold–palladium under an argon atmosphere using a gold sputter module in a high-vacuum evaporator. Samples were then observed with LEO 420 (LEO Electron Microscopy Ltd., England) using secondary electron imaging at 15 kV in order to examine the surface morphology and structure of the inserts.

Variation in porosity due to chitosan/pectin complex composition was determined using lyophilized samples, by means of a mercury porosimeter (Autopore IV 9500, Micromeritics Instrument).

2.4. Water uptake ability

Accurately weighed unloaded inserts (100 mg) were placed on filter papers (d = 40 mm) soaked in different media (pH 2.0, pH 5.5 and pH 7.4 phosphate buffers) and positioned on top of a sponge (5 cm × 5 cm × 2 cm) previously soaked in the hydration medium and placed in a Petri dish filled with the same buffer to a height of 0.5 cm. Water uptake was determined, as weight increase of the insert after 6 h, according to the following equation:

$$\%\ \text{Water Uptake}\ (\%\text{WU}) = (W_{Hip} - W_{Hp} - W_{Di}) \times 100/W_{Di}$$

where W_{Hip} is the weight of hydrated insert and wet filter paper, W_{Hp} is the weight of wet filter paper and W_{Di} is the initial weight of the dry insert. The influence of chlorpromazine hydrochloride on the water uptake behaviour of loaded inserts ($CH/PEC_{(1:9)}$ with different complex/drug weight ratios: 2:0.5, 2:1, 2:2 and 2:4) was also studied at pH 5.5, particular to human nasal secretions [9,10].

2.5. Insert mucoadhesion properties

The *in-vitro* mucoadhesion was measured in terms of the force needed to pull out a freshly excised sheep nasal mucosa (surface area 1 mm^2) from a tablet (100 mg) with an adapted tensiometer (Krüss 132869; Hamburg, Germany) as reported in a previous work [7]. For this study, tablets (weight of 100 mg) were prepared by direct compression of freeze-dried chitosan/pectin complex (compaction force: 18 kN) with a single-punch press (type Korsch, Korsch Maschinenfabrik No. 1.0038.86, Berlin, Germany). The nasal mucosa was fixed to a support with cyanoacrylate adhesive and then suspended from the tensiometer spring. The mucosa was lowered until it just contacted the surface of the tablet, previously immersed in phosphate buffers at pH 5.5 for 15 min. A 100-dyne force, measured by the torsion balance of the instrument as a negative force, was applied to the tablet for 30 s. Then, the nasal mucosa was raised until it was separated from the tablet. This point represents the adhesive bond strength between these elements and is expressed as a positive force in dyne.

2.6. In-vitro release studies

Loaded inserts (10 mg) were placed on the sintered-glass filter plate (pore size 90–150 μm) of a Borosil® glass filter crucible (inner diameter = 2.0 cm, capacity 15 ml), and the whole system was closed with Parafilm® film to avoid evaporation of release medium. The crucible was placed vertically into a release medium container