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Research paper

Preparation of estradiol chitosan nanoparticles for improving nasal absorption and brain targeting

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ABSTRACT

The estradiol(E_2)-loaded chitosan nanoparticles (CS-NPs) were prepared by ionic gelation of chitosan with tripolyphosphate anions (TPP). The CS-NPs had a mean size of (269.3 ± 31.6) nm, a zeta potential of +25.4 mV, and loading capacity of E_2 CS-NPs suspension was 1.9 mg ml^{-1}, entrapment efficiency was 64.7% on average. Subsequently, this paper investigated the levels of E_2 in blood and the cerebrospinal fluid (CSF) in rats following intranasal administration of E_2 CS-NPs. E_2-loaded CS-NPs were administered to male Wister rats either intranasally or intravenously at the dose of 0.48 mg kg^{-1}. The plasma levels achieved following intranasal administration (32.7 ± 10.1 ng ml^{-1}; t_{max} 28 ± 4.5 min) were significantly lower than those after intravenous administration (151.4 ± 28.2 ng ml^{-1}), while CSF concentrations achieved after intranasal administration (76.4 ± 14.0 ng ml^{-1}; t_{max} 28 ± 17.9 min) were significantly higher than those after intravenous administration (29.5 ± 7.4 ng ml^{-1} t_{max} 60 min). The drug targeting index (DTI) of nasal route was 3.2, percent of drug targeting (DTP%) was 68.4%. These results showed that the E_2 must be directly transported from the nasal cavity into the CSF in rats. Finally, compared with E_2 inclusion complex, CS-NPs improved significantly E_2 being transported into central nervous system (CNS).

1. Introduction

Alzheimer's disease (AD), the most common cause of dementia, affects millions of people over the age of 65 in the Western world and an increase in the occurrence of AD is expected in the future, as the proportion of older people in the population grows. AD is a chronic neurodegenerative disorder accompanied by the gradual and progressive loss of functional and psychomotor abilities [1].

There has been speculation that a person's sex could be a risk factor for AD, 17β-estradiol concentrations in women with AD have been reported to be decreased in comparison with healthy controls. 17β-Estradiol, the most potent female sex hormone, belongs to the family of steroid hormones. Apart from its influence on primary and secondary sexual characteristics, it is also involved in the regulation of brain development. Long-term oestrogen replacement has proved to be beneficial in the prevention and treatment of Alzheimer's disease [2–4].

In the recent years, the nasal route has received a great deal of attention as a convenient and reliable method for the systemic administration of drugs. Nasal delivery has been explored as an alternative administration route to target drugs directly to the brain via the olfactory neurons [5–8] providing more opportunities for estradiol to enter the central nervous system (CNS) and then treat Alzheimer's disease. Therefore, how to improve the amount of drug transported into CNS becomes the focus of attention.

Nasal mucociliary clearance is one of the most important limiting factors for nasal drug delivery. It severely limits the time allowed for drug absorption to occur and effectively rules out sustained nasal drug administration. However, bioadhesive polymers can be used to increase the nasal residence time, thus allowing longer absorption times, and to achieve a more intimate contact with the nasal mucosa, which results in a higher concentration gradient and subsequent increased absorption [9].

Another important limiting factor in the nasal application is the low permeability of the nasal mucosa for the drugs. It seems to be necessary to consider an absorption enhancement mechanism for co-administration of drugs with either mucoadhesive polymers or penetration enhancers or combination of the two [10–12].

Chitosan, a cationic polysaccharidc, is commercially available in a range of grades with different molecular weights and degrees of deacetylation. Industrially, they are produced from chitin (the schemes of chitin and chitosan are shown in Fig. 1), the world's second most abundant biopolymer, by a deacetylation process involving alkaline hydrolysis. The term chitosan refers to a family

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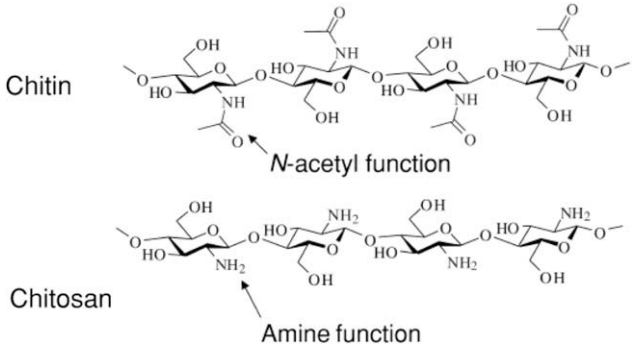

Fig. 1. Chitin and chitosan.

of polymers, individually characterized by their ratio of acetylated to deacetylated units and molecular weight, both parameters being equally responsible for the properties of the polymer.

Chitosan has been used for a range of applications as diverse as for water purification, as a food ingredient and as a pharmaceutical excipient in oral drug formulations for the improvement of the dissolution of poorly soluble drugs or to obtain controlled drug release [13,14]. It has previously been shown that chitosan has a great potential as a nasal delivery system, facilitating the passage of large hydrophilic molecules such as salmon calcitonin and insulin, through the nasal mucosa and into the systemic circulation [15]. However, the clinical use of chitosan in nasal preparations necessitates the evaluation of their effect on the nasal epithelium and the mucociliary clearance system.

On the basis of our former study [16], we prepared estradiol chitosan nanoparticles, aiming at reducing nasal mucociliary clearance and enhancing permeation of E_2 into the nasal mucosa, and then improving the E_2 bioavailability, especially for brain targeting.

2. Materials and methods

2.1. Materials

Estradiol (17β-estradiol) was purchased from Xianju Pharmaceutical Factory, China. Randomly methylated β-cyclodextrin (RAMEB) was obtained from Wacker-Chemie, Germany. Chitosan (deacetylation degree 95.0%, molecular weight 50,000) was provided by Zhejiang Yuhuan marine biochemistry Ltd., China. Sodium tripolyphosphate (TPP) was given from Shenyang Dongxing Reagent Factory, China. All other reagents were of analytical grade or the highest grade commercially available.

Microdialysis probes were U-shaped and made of hollow cellulose fiber (DM-22, 200 μm inner diameter and 220 μm outer diameter, EICOM CORP, Japan). The membrane was 4 mm in length with a molecular weight cut-off of 5000 Da. Artificial CSF composed of 128 mM NaCl, 2.6 mM KCl, 1.26 mM $CaCl_2$ and 2 mM $MgCl_2$ was prepared using deionized distilled water. The solution was filtered through a 0.22 μm nylon filter before use.

Male Wister rats weighing 250–300 g were provided by the animal house of Shenyang Pharmaceutical University, China. These animals were allowed to acclimatize in environmentally controlled quarters (24 ± 1 °C and 12:12 h light–dark cycle) for at least 5 days before being used for experiments.

2.2. Preparation of E_2 chitosan nanoparticles (CS-NPs)

E_2-loaded chitosan nanoparticles were prepared by ionic interaction. A 0.2% w/v solution of chitosan was prepared in 1% v/v acetic acid solution. TPP (0.1% w/v) was dissolved in purified water, while E_2 inclusion complex [16] was dissolved in CS acetic acid solution to ensure the concentration of E_2 in the final CS-NPs suspension reaching 2 mg ml^{-1}.

The chitosan solution (5.0 ml) was stirred at 1500 rpm with a DF-101S magnetic stirrer (Gongyi Yuhua Instrument Co., Ltd., China) at room temperature (25 °C). The TPP solution (2.0 ml) was gently added to the system through a No. 4 syringe needle at the speed of 2 ml h^{-1}, and nanoparticles were formed, stirring for 30 min. Subsequently, a certain amount of 1 N HCl or NaOH solution was added to adjust the pH of the suspension to pH 5.

2.3. Physicochemical characterization of E_2 CS-NPs

The particle size and zeta potential were measured using a NICOMPTM 380 Zeta Potential/Particle Sizer (Particle Sizing Systems, Santa Barbara, USA). The mean particle size and distribution were measured based on photon correlation spectroscopy (PCS, dynamic light scattering, DLS) technique, which is a powerful and versatile tool for estimating the particle size distribution of fine-particle materials ranging from a few nanometers to several micrometers [17]. The zeta potential is a very useful way of evaluating the stability of any colloidal system, and it was determined based on an electrophoretic light scattering (ELS) technique.

2.4. High-performance liquid chromatographic analysis in vitro [18]

The HPLC equipment consisted of a HITACHI L-7110 Intelligent HPLC pump, and a HITACHI L-7200 Intelligent HPLC Autosampler, a HITACHI L-7420 Intelligent HPLC Detector, and a ANASTAR Chromatography Data System. Separation was achieved under room temperature on a Kromasil C_{18} column (200 mm × 4.6 mm, particle size 5 μm, Zirchrom company). The mobile phase consisted of acetonitrile–water (50:50, v/v%), filtered and degassed under reduced pressure, prior to use. The flow rate was 1.0 ml min^{-1} and peak detection was performed at 205 nm. The injection volume was 20 μl.

2.5. Evaluation of E_2 loading capacity and entrapment efficiency

For determining E_2 loading capacity, the Stand solution (about 10 μg ml^{-1}) was prepared by dissolving E_2 in methanol, and the Test solution was obtained as follows: 0.5 ml CS-NPs suspension of the three batches was shifted into a 50 ml volumetric flask, and then diluted with 1% (v/v) acetic acid solution, being ultrasound damaged for 30 min, finally, filtrated through 0.22 μm microporous membrane.

The entrapment efficiency of the process was determined upon separation of nanoparticles from the aqueous suspension containing non-entrapped E_2 by ultra centrifugation at 40,000 r min^{-1}, 10 °C for 30 min. The amount of free E_2 in the supernatant was measured by HPLC. E_2 entrapment efficiency (EE%) and loading efficiency (LC) in the CS-NPs were calculated according to the equations below:

$$EE\% = \frac{\text{Total amount of } E_2 \text{ loading} - \text{free } E_2 \text{ in the supernatant}}{\text{Total amount of } E_2 \text{ loading}} \times 100 \quad (1)$$

$$LC(\text{mg ml}^{-1}) = \frac{\text{Total } E_2 \text{ amount} - \text{free } E_2 \text{ amount}}{\text{Suspension volume}} \quad (2)$$

2.6. Animal experiments

2.6.1. Nasal cavity isolation and jugular vein cannulation

The rats were anesthetized with an intraperitoneal injection of urethane (1.2 g kg^{-1}). During the experiment, body temperature was maintained at 37 °C under an infrared lamp. The nasal cavity was isolated from the respiratory and gastrointestinal tracts using a procedure described by Hirai et al. [19] and Huang et al. [20].