European Journal of Pharmaceutics and Biopharmaceutics 70 (2008) 874–881

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

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

Research paper

Effect of chitosan structure properties and molecular weight on the intranasal absorption of tetramethylpyrazine phosphate in rats

Dan Mei [a], Shirui Mao [a,*], Wei Sun [a], Yanjun Wang [a], Thomas Kissel [b]

[a] School of Pharmacy, Shenyang Pharmaceutical University, Shenyang, China
[b] Department of Pharmaceutics and Biopharmacy, Philipps-University Marburg, Marburg, Germany

ARTICLE INFO

Article history:
Received 30 January 2008
Accepted in revised form 23 June 2008
Available online 8 July 2008

Keywords:
Intranasal
2,3,5,6-Tetramethylpyrazine phosphate
Chitosan
Trimethyl chitosan
Thiolated chitosan

ABSTRACT

The objective of this work was to assess and compare the absorption promoting effect of different molecular-weight chitosans, trimethyl chitosans and thiolated chitosans for intranasal absorption of 2,3,5,6-tetramethylpyrazine phosphate (TMPP). An in situ nasal perfusion technique in rats was utilized to test the rate and extent of TMPP absorption in situ. In vivo studies were carried out in rats and the pharmacokinetic parameters were calculated and compared with that of intravenous injection. All the chitosan derivatives investigated could enhance the intranasal absorption of TMPP significantly. However, thiolation could not improve the absorption-enhancing capacity of chitosan remarkably even when the thiolation ratio was as high as 152 μmol/g. In contrast, trimethylated chitosan exhibited stronger absorption-enhancing ability than the homopolymer chitosan. The permeation enhancing effect of chitosan increased with increasing molecular weight up to M_w 100 kDa. In vivo studies indicated that chitosan 100 kDa and TMC 50 kDa had comparable absorption-enhancing effect but chitosan 100 kDa functioned for more than 120 min versus 90 min for TMC. A good correlation was found between the in situ absorption data and plasma concentration in vivo for the polymers investigated. This study demonstrated that both chitosan structural features and chitosan molecular weight play a key role on promoting the intranasal absorption of TMPP. Taking safety reason into account, chitosan 100 kDa is the most promising as an intranasal absorption enhancer.

1. Introduction

Systemic drug delivery by the nasal route is currently receiving considerable attention because this route allows for a rapid onset of therapeutic effect, potential for direct-to-central nervous system delivery, avoidance of first-pass metabolism, and an easy way for drug administration [1]. To improve the intranasal delivery of challenging drugs, several strategies have been taken [2,3]. Among them, coadministration of chemical enhancers has been extensively exploited in recent years [4]. However, although numerous classical enhancers have been demonstrated to be able to promote the nasal absorption of biologically active peptides and proteins effectively, the successful application of these compounds is limited by their undesirable physiological effect on epithelial cells and irritation to the nasal mucosa [5,6]. Therefore, new absorption enhancers are expected to achieve safe permeation enhancement.

Chitosan, produced by partial deacetylation of chitin, has been found to be able to improve the intranasal absorption of peptides and reduce the clearance of liquid formulations from the nasal cavity through its bioadhesive characteristics, while causing negligible damage to the nasal mucosal membrane [7]. However, chitosan is only soluble in acidic milieu, in which the amino groups at the C-2 position are protonated [8]. To improve the poor water-solubility of chitosan, trimethyl chitosan was synthesized and has been proven to be a potent intranasal absorption enhancer of insulin in rats, especially at neutral pH, where chitosan salts are ineffective [9]. While the absorption-enhancing effect of chitosan was attributed to the capacity to open the tight junction and bioadhesive properties, thiolated chitosans were synthesized in order to further increase the bioadhesion of chitosan, and were found to have posed a significantly higher bioavailability of insulin after intranasal administration than that of unmodified chitosan [10]. The overall absorption-enhancing mechanism of thiolated chitosans is attributed to the formation of disulfide bonds between thiol moieties of thiomers and sulfhydryl groups of cysteine-rich subdomains of mucus glycoproteins providing prolonged residence time at the absorbing membranes [10]. However, the contribution of bioadhesion versus opening the tight junction in the absorption-enhancing effect of chitosan is unclear. So far, although numerous works have reported the promising potential of chitosan and its derivatives as safe and effective nasal absorption enhancers, the influence of chitosan

* Corresponding author. School of Pharmacy, Shenyang Pharmaceutical University, 103, Wenhua Road, 32#, Shenyang 110016, China. Tel./fax: +86 24 23986358.
E-mail address: shiruimao156@hotmail.com (S. Mao).

0939-6411/$ - see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejpb.2008.06.031

structure properties on the intranasal absorption of drugs has not been elucidated systemically to the best of our knowledge.

2,3,5,6-Tetramethylpyrazine (TMPP) is a biologically active ingredient originally isolated from *Ligusticum wallichii* France in 1957 and currently used in China for the treatment of cardiovascular disease [11]. It was found to have significant therapeutic activity, including improving brain microcirculation, inhibiting thrombus formation, decreasing platelet aggregation, and improving blood viscosity [12]. It was reported recently that TMPP has appreciable blood–brain barrier (BBB) penetrability [13]. However, peroral absorption of TMPP is variable and incomplete, with a bioavailability of 10–30% and a short biological half-life of 0.5–2 h [14]. Accordingly, intranasal administration might represent a promising route to improve its bioavailability.

Therefore, taking TMPP as a model drug, influence of chitosan structure properties on the intranasal absorption of drugs was investigated using an in situ perfusion method and the absorption mechanism was discussed. Based on the in situ results, the selected chitosan formulations were further evaluated in vivo by measuring the blood concentration level after intranasal administration in rats.

2. Materials and methods

2.1. Materials

TMPP was a gift from Beijing Shuanghe Inc. Chitosan 400 kDa with a nominal degree of deacetylation of 85% was purchased from Weifang Kehai Chitin Co., Ltd. Carbamazepine (99.6% purity, internal standard) and acetic acid were obtained from Shandong Xinhua Pharmaceutical Company, Ltd. (Jinan, China). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDAC) and *N*-hydroxysuccinimide (NHS) were purchased from Shanghai Medpep Co., Ltd. Methanol of liquid chromatographic grade was purchased from Tianjin Concord Tech Reagent Company (Tianjin, China). All other chemicals were of analytical grade.

2.2. Chitosan derivatives preparation and characterization

Chitosans of different molecular weights were prepared by depolymerization as described previously [15], and the obtained polymers were nominated based on their molecular weight. For example, chitosan with molecular weight of 400 kDa is abbreviated as CS 400, the same for the other polymers. Trimethyl chitosan (TMC) derivatives were prepared according to a two-step method with a quaternization degree of 40% [16] and their molecular weights were characterized using asymmetrical flow field-flow-fractionation method as described previously [17]. Thiolated chitosans were synthesized according to a previously reported method with little modification [18]. Briefly, cysteine was covalently attached to chitosan via the formation of an amide bond mediated by EDAC and NHS to obtain chitosan–cysteine conjugates. First, 1 g of chitosan was hydrated in 8 ml of 1 M HCl and then dissolved by the addition of demineralized water to obtain a 1% (w/v) polymer solution. The pH was adjusted to 4.0 by the addition of 1 M NaOH. Afterwards, 3 g cysteine, 2 g NHS and 2 g EDC in 10 ml of demineralized water was added under stirring. The pH was readjusted to 4.0. The reaction mixture was incubated for 6 h at room temperature under permanent stirring. The resulting polymer conjugate was precipitated by adjusting the system pH to 7.5 and washed several times with water, freeze-dried and stored at 4 °C until further use. Iodine titration was used to determine the thiol group content [18]. In vitro mucoadhesion of thiolated chitosans was studied [18] and compared with that of the unmodified chitosan. Firstly, 30 mg of lyophilized polymer–cysteine conjugates and controls were compressed into 5.0-mm diameter flat-faced discs. The compaction pressure was kept constant during the preparation of all discs. Discs described above were thereby attached to freshly excised intestinal porcine mucosa, which had been spanned on a stainless steel cylinder. Thereafter, the cylinder was placed in the dissolution apparatus according to the CP containing 100 mM PBS, pH 6, at 37 ± 0.5 °C. The fully immersed cylinder was agitated with 250 rpm. The detachment, disintegration and/or erosion of test discs were observed within a time period of 12 h. Properties of various chitosans employed in this study are listed in Table 1.

2.3. Preparation of intranasal formulations

All the chitosans applied were first dissolved in 0.5% (v/v) acetic acid saline solution to obtain desired concentrations. TMPP was dissolved into the above-mentioned solution (4 mg/ml) and pH of the solution was adjusted as required. Isotonicity was adjusted by sodium chloride. For in vivo studies, the formulations were prepared according to the following method: TMC 50, CS 50, CS 100 and H-CS(100)-Cys were dissolved in 0.5% (v/v) acetic acid saline solution to reach a concentration of 0.5% (w/v). Thereafter, TMPP was added to the above solutions (20 mg/ml) at pH 6. Intravenous injection solution was prepared by dissolving TMPP (4 mg/ml) in sterile saline solution and adjusting solution pH to 6.

2.4. Analytical method of TMPP

The content of TMPP was analyzed by a HPLC method. HPLC apparatus (Schimadzu, LC10-AS liquid chromatograph) connected to an ultraviolet variable wavelength detector (Model SPD-10A) with a C-18 reversed phase column (Bondapack, 5 μm, 4.6 mm × 200 mm, Shimadzu, Japan) and isocratic pump (Model LC10-AS, Shimadzu, Japan) was used. The mobile phase consisted of 55% of methanol and 45% of water. The flow rate was 1.0 ml/min. The UV detector wavelength was 295 nm and the oven temperature was 30 °C. The injection volume was 20 μl. The limit of detection and quantitation of TMPP was 0.08 ng and 0.2 ng, respectively. A linear relationship between As/Ai and concentration was found in the concentration range of 0.2–10 μg/ml (As/Ai = 0.3212 C + 0.0009, r = 0.9999, n = 6), where As is the peak area of TMPP and Ai is the peak area of the internal standard. The method and extraction recoveries of TMPP from plasma at three different concentrations were between 99.7–101.8% and 81.7–87.6%, respectively. The inter-day RSDs were less than 2.56% (n = 6), and the intra-day RSDs were less than 5.41% (n = 6).

2.5. Nasal perfusion studies in rats

The animal experiment was carried out in accordance with the Principles of Laboratory Animal Care (NIH Publication No. 86-23, revised 1985). Male Wistar rats (7 weeks old, 200 ± 20 g) were supplied by the Lab Animal Center of Shenyang Pharmaceutical University (Grade II, Certificate No. SYXK 2006-0064). The experimental protocol was approved by the University Ethics Committee for the use of experimental animals and conformed to the Guide for Care and Use of Laboratory Animals. Rats were maintained at 22 ± 2 °C and 55 ± 5% relative humidity under a 12-h light–dark cycle for 4–6 days before experiments, with food and water available ad libitum.

Rats were surgically treated according to a previously described method [19]. A 5-ml solution was recirculated at 37 °C through the rat nasal cavity at a rate of 2.0 ml/min for 120 min (n = 5). Aliquots (50 μl) were sampled periodically and analyzed by HPLC assay. And an equal volume of isotonic saline of pH 6 was added in the meantime. The absorption-rate constants were calculated according to the absorption amount in the first 40 min using the following equation: $Q = Q_0 - k_a t$, where Q_0 and Q represent the initial and