European Journal of Pharmaceutical Sciences 48 (2013) 301–306

Contents lists available at SciVerse ScienceDirect

European Journal of Pharmaceutical Sciences

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

A novel permeation enhancer: *N*-succinyl chitosan on the intranasal absorption of isosorbide dinitrate in rats

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ARTICLE INFO

Article history:
Received 30 July 2012
Received in revised form 12 October 2012
Accepted 27 October 2012
Available online 16 November 2012

Keywords:
N-succinyl chitosan
Intranasal
Absorption enhancer

ABSTRACT

The purpose of this paper is to study the potential of *N*-succinyl chitosan as a novel permeation enhancer for the intranasal absorption of isosorbide dinitrate (ISDN). A series of *N*-succinyl chitosan (NSCS) with different degree of succinylation (DS) and molecular weight were synthesized. An *in situ* nasal perfusion technique in rats was utilized to investigate the effect of NSCS substitution degree, NSCS molecular weight and concentration on the intranasal absorption of ISDN. The absorption enhancing effect of NSCS was compared with that of chitosan. It was found that all the NSCS investigated improved the intranasal absorption of ISDN remarkably. Better promoting effect was observed for 0.1% NSCS 50 (63) compared with 0.5% chitosan 50. In nasal ciliotoxicity test, both NSCS and chitosan investigated showed good safety profiles. Thereafter, *in vivo* studies of the selected formulations were carried out in rats and the pharmacokinetic parameters were calculated and compared with that of intravenous injection. Both *in situ* and *in vivo* studies demonstrated that NSCS is more effective than chitosan in promoting intranasal absorption of ISDN. Taking both absorption enhancing and safety reason into account, we suggest NSCS is a promising intranasal absorption enhancer.

1. Introduction

Recently, intranasal administration attracted more and more attention as a viable option for local or systemic delivery of diverse therapeutic compounds (Malerba et al., 2011; Florence et al., 2011). Intranasal route allows a rapid onset of therapeutic effect, potential for direct-to-central nervous system delivery, avoidance of first-pass metabolism, and it is convenient for drug administration (Henkin, 2011). However, two barriers limit efficient nasal absorption of drugs. One is the low membrane permeability and the other is the rapid clearance of the administered formulation from the nasal cavity (Illum, 2003). So far, various intranasal absorption enhancers have been reported to increase nasal membrane permeability (Chandler et al., 1995; Duan and Mao, 2010). Anyhow, despite of their effectiveness, some of them were limited to use in nasal formulations due to their unacceptable membrane toxicity (Chandler et al., 1995; Duan and Mao, 2010). In view of reports in the literature, only limited permeation enhancers, such as hydropropel-β-cyclodextrin (HP-β-CD), chitosan, and poloxamer 188, alkylsaccharides, low methylated pectin, polyglycol mono- and diesters of 12-hydroxystearate (70%), polyethylene glycol (30%) are regarded as safe (Illum, 2012; Na et al., 2010). Therefore, it is absolutely essential to search for novel safe and effective permeation enhancers applicable for mucosal drug delivery.

Chitosan was evaluated for the enhancing effect on *in vivo* nasal absorption of salmon calcitonin (sCT) in rats and the results were subsequently compared with beta-cyclodextrins, one of the most commonly studied enhancers. It was shown that the inclusion of 1% CS resulted in twofold increase in the AUC(0–180) of plasma sCT relative to that of the control group. Addition of 5% DM-beta-CD led to 1.56-fold increase in absorption over the control group (Sinswat and Tengamnuay, 2003), implying that chitosan may have a better absorption enhancing effect. Chitosan is a biocompatible, biodegradable and low toxic cationic polysaccharide derived by partial deacetylation of chitin isolated from crustacean shells (Illum, 1998). Every deacetylated subunit of chitosan contains a primary amine group with a pK_a value of about 6.5 (Mao et al., 2010). Thus, chitosan is soluble in acidic media with positive charge. However, it becomes insoluble at neutral and alkaline pH values, which limits its application to specified conditions. To improve the solubility of chitosan, a quaternized derivative, *N*-trimethyl chitosan (TMC) was synthesized, which is freely soluble over a wider pH range and showed absorption-enhancing effects even in neutral and basic pH environments (Van der Merwe et al., 2004). Unfortunately, TMC was shown to be cytotoxic in L929 mouse fibroblast cells as indicated by MTT assay (Mao et al., 2005). Similarly, it was reported that

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http://dx.doi.org/10.1016/j.ejps.2012.10.030

reversibility of transepithelial resistance at 0.5% concentrations of TMC with different degree of quaternization could not be demonstrated at pH 6.2 and 7.4 in Caco-2 cells (Kotze′ et al., 1999) and the cytotoxicity of TMC is molecular weight and quaternization dependent (Jintapattanakit et al., 2008), although some other studies indicated TMC was safe (Thanou et al., 1999; du Plessis et al., 2010). Therefore, it is desirable to search for other possibilities to increase the solubility of chitosan, and in the meantime with good safety profile and permeation enhancing effect.

N-succinyl-chitosan (NSCS), which can be obtained from a simple reaction between chitosan and succinic anhydride, has been found to exhibit several biological properties, such as good solubility at various pH, nontoxicity, biocompatibility and long systemic circulation in mice, and the maximum tolerable dose for the intraperitoneal injection of Suc-Chi to mice was greater than 2 g/kg (Kato et al., 2004). For this reason, NSCS has been applied as drug delivery carriers such as microspheres and hydrogel beads (Kato et al., 2004; Ubaidulla et al., 2007; Dai et al., 2008) and as gene delivery carrier for improved chitosan solubility and gene transfection (Toh et al., 2011). Therefore, we assume NSCS might be a potential mucosal permeation enhancer with acceptable safety. This hypothesis was tested in this study.

Isosorbide dinitrate (ISDN) is commonly used for therapy of stable angina pectoris and traditionally administrated via oral or sublingual routes. However, loss of consciousness appears in patients when angina pectoris breaks out, and thus it is difficult for patients to take medicine by themselves. Additionally, ISDN administrated orally has low bioavailability due to its high first-pass metabolism in the gastrointestinal tract and liver (Zhao et al., 2007). Moreover, the critical point of antianginal therapy depends, to a certain extent, on the ability of the drug to produce an immediate effect. Thus, intranasal (i.n.) delivery may be an appropriate administration route for ISDN.

Therefore, in the present work, first of all, NSCS with different degree of substitution (DS) and molecular weight were synthesized. Thereafter, taking ISDN as a model drug, the feasibility of NSCS as an intranasal absorption enhancer was studied and influence of succinylation degree of NSCS, NSCS molecular weight and concentration on the absorption permeation effect was investigated using *in situ* test. Safety properties of different NSCSs were further studied and compared with chitosan of the same molecular weight by using *in situ* toad palate model. Moreover, based on the *in situ* results, the selected intranasal formulations were evaluated *in vivo* by measuring drug blood concentration after intranasal administration in rats. To the best of our knowledge, this is the first time that NSCS was used as a permeation enhancer for mucosal drug delivery.

2. Materials and methods

2.1. Materials

ISDN was purchased from Shandong Keyuan Inc. Paracetamol (APAP, 99.6% purity, internal standard) was obtained from Shandong Xinhua Pharmaceutical Company, Ltd. (Jinan, China). The standard of isosorbide minitrate (ISMN) was purchased from National Institute for the Control of Pharmaceutical and Biological Products. Chitosan 400 kDa (CS 400) with a degree of deacetylation of 85% was purchased from Weifang Kehai Chitin Co., Ltd. Chitosan 20 kDa (CS 20), chitosan 50 kDa (CS 50) and chitosan 100 kDa (CS 100) were prepared by oxidative degradation of CS 400 with $NaNO_2$ at room temperature (Mao et al., 2004). Succinic anhydride and dimethyl sulfoxide were all purchased from Tianjin Bodi Chemical Co., Ltd. (Tianjin, China). Methanol and acetonitrile of liquid chromatographic grade were purchased from Shangdong Yuwang Tech Reagent Company (Shandong, China). All other chemicals were of analytical grade.

2.2. Preparation and characterization of NSCS

NSCS was synthesized according to the method reported previously with minor modification (Sun and Wang, 2006). Briefly, 1.0 g of chitosan powder (CS 20, CS 50 or CS 100) was added into 50 ml of dimethyl sulfoxide and dissolved, then 1.0 g of succinic anhydride was slowly added at room temperature with stirring, thereafter the mixture was placed in a 60 °C oil bath and heated for a certain period of time. The mixture was filtered to remove the solvent and then the precipitate was dispersed in ethanol for 1 h at room temperature. The pH of the dispersion was adjusted to 10. Subsequently, the product was filtered, dissolved in distilled water and purified by acetone for three times. Finally, the precipitate was washed with ethanol (70% wt.%) and acetone, and then dried. The structure of NSCS is shown in Fig. 1.

NSCS with different degree of succinylation (DS) were obtained by controlling the reaction time for 2, 4 and 6 h at 60 °C, respectively, and the DS of the samples was determined by 2, 4, 6-trinitrobenzenesulfonic acid (TNBS) method (Izco et al., 2000) and the result is listed in Table 1. The obtained polymers were denoted as NSCS X(Y) in this paper, where X represents the initial molecular weight of chitosan (kDa), Y represents the degree of substitution of succinyl groups (%) at the *N*-positions along the chitosan chains. The synthesized polymers were freely soluble at neutral and weak basic condition until pH 10.

2.3. Preparation of intranasal formulations

NSCS polymers of different Mw and DS were dissolved in physiological saline solution to obtain desired concentrations of 0.1%, 0.5% and 1.0% for *in situ* test. ISDN was dissolved in the above-mentioned solution (0.5 mg mL^{-1}) and pH of the solution was adjusted to 6 unless specially indicated. As for formulations used in *in vivo* studies, the preparation of NSCS solutions (0.1%) was the same as that for *in situ*, while the concentration of ISDN was 5 mg mL^{-1}. Intravenous injection solution was prepared by dissolving ISDN (0.25 mg mL^{-1}) in sterile saline solution and adjusting pH to 6.

2.4. 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). Male Wistar rats (7 weeks old, 200 ± 20 g) were supplied by the Lab Animal Center of Shenyang Pharmaceutical University. 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.

For the nasal absorption studies, male Wistar rats weighing 180–220 g were used and allowed free access to food and water.

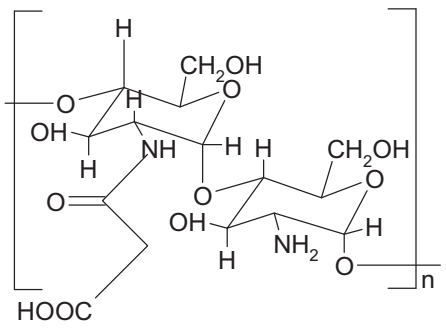

Fig. 1. The schematic structure of *N*-succinyl chitosan.