Chitosan and Randomly Methylated β-cyclodextrin Combined to Enhance the Absorption and Elevate the Bioavailability of Estradiol Intranasally: in situ and in vivo Studies

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Abstract: Chitosan and randomly methylated β-cyclodextrin were the most to be studied absorption enhancers for nasal administration recently. The enhancing absorption mechanisms of each other were different. Authors chosen estradiol as the model drug used in situ and in vivo methods to study whether or not they could combine to enhance the nasal absorption. In situ study data indicated that the optimal concentration and pH for chitosan to enhance the nasal absorption of estradiol in rats were 0.5% and 5, and in this condition the remaining percentage of estradiol was significant difference with RAMEB formulation after perfusion 30 min (P<0.05). The absolute bioavailabilities after intranasal administration were estradiol ethanol (70% w/w) solution: 22.24±7.88%; RAMEB: 58.78±11.19%; chitosan + RAMEB: 78.51± 23.13%. They were different significantly from each other (P<0.05). Based on the in situ and in vivo studies results, it's clear that chitosan and randomly methylated β-cyclodextrin could combine to enhance the absorption and elevate the bioavailability of estradiol after nasal administration. [The Journal of American Science. 2006;2(1):61-65]

Keywords: chitosan; randomly methylated β-cyclodextrin; estradiol; absorption enhancers; in situ; in vivo

1. Introduction

Chitosan is a polymer obtained from deacetylation of chitin, a naturally occurring structural polymer abundant in crab and shrimp shells. It is a cationic polysaccharide with linear chain con- sitting of b-(1,4)-linked 2-acetamino-2-deoxy-β-Dglucopyranose (GlcNAc) and 2-amino-2-deoxy-β- D-glucopyranose (GlcN) [1]. The greater the extent of deacetylation, the smaller is the proportion of GlcNAc in the polymer chain.

Recently, chitosan has been shown to enhance nasal and intestinal absorption of hydrophilic drugs like peptide hormones in both the in vitro and in vivo models [2-4]. According to the study using an in vitro Caco-2 cell model, its absorption enhancing mechanisms were reported to be a combination of mucoadhesion and an effect on the opening of the tight junctions [2]. Schipper et al. [5], using the same in vitro model, reported that the structural properties of chitosans such as degree of acetylation and molecular weight are very important for its drug absorption enhancement . They found that a low degree of acetylation (i.e. high percent deacetylation with greater charge density) and/or a high molecular weight appear to be necessary for chitosans to increase the epithelial permeability. Toxicity of chitosan also depends on its high charge density but appears to be less affected by the molecular weight.

With respect to mucosal drug delivery, chitosans show strong mucoadhesive properties [6]. In addition, interaction of the positively charged amino group at the C-2 position of chitosan with the negatively charged sites on the cell surfaces and tight junctions allows paracellular transport of large hydrophilic compounds by opening the tight junctions of mucosal membrane barriers [2, 5, 7]. The interaction with the opening mechanism of the tight junctions has been demonstrated by a decrease in ZO-1 proteins and the change in the cytoskeletal protein F-actin from a filamentous to a globular structure [8, 9]. These characteristics reveal the potential of chitosan and chitosan salts as penetration enhancers for mucosal paracellular pathways. Chitosans have been found to enhance the nasal absorption of degracalcitonin and insulin in rats and sheep [3], morphine-6-glucuronide and goserelin in sheep [10] and D-Arg -kyotorphin in rats [11], and the intestinal absorption of buserelin in rats [4].

Most of studies utilized chitosan alone as absorption enhancer. Currently, it is not known if the combination of chitosan and other absorption enhancers, as well as some other factors could exhibits a synergistic effect in the nasal absorption of drugs. Cyclodextrins (CDs) could also extract the phospholipids and proteins from membrane [12], so there was opportunity that chitosan and cyclodextrins combined to enhance drug nasal absorption.

The purpose of this paper was to evaluate the effects

of chitosan concentrations and pH in chitosan solutions on the estradiol permeation across the rat nasal mucosa in situ and the plasma estradiol concentrations after nasal administration of estradiol to normal rats. Moreover, tried to identify whether chitosan and randomly methylated β-cyclodextrin had a synergistic effect in enhancing nasal absorption of estradiol or not.

2. Materials and methods

2.1. *Materials*

Estradiol (17β-estradiol) was purchased from Xianju pharmaceutical factory, China. Randomly methylated β-cyclodextrin (RAMEB) was purchased from Wacker-Chemie, Germany. The 98% deacetylated Chitosan was obtained from Xindie Chitosan Company, China. All other reagents were of analytical grade or highest grade commercially available. All the chemicals were used without further purification.

Male Sprague–Dawley rats weighing 250–300 g were procured from the local animal house. Animals were further acclimatized to the environment of the experimental room for 2 days before starting the experiments.

2.2. *Methods*

2.2.1 *Preparation of RAMEB - estradiol complexes chitosan solution*

Chitosan was first dissolved in 1% v/v acetic acid normal saline to make stock solutions of 2.0% w/v. Following overnight swelling, the chitosan solutions of different concentrations (0.3%, 0.5% and 0.8%) were obtained by diluting the stock solution with normal saline, the pH was adjusted to 5.0 by drop wise addition of either 1 mol/L hydrochloric acid or 1 mol/L sodium hydroxide. The different pH of 0.5% solution was adjusted to 3.0, 4.0, 5.0 or 6.0 by drop wise addition of either 1 mol/L hydrochloric acid or 1 mol/L sodium hydroxide, too. Isotonicity was further achieved by gradual addition of sodium chloride and was checked by osmometer (Osmomat 030-D, Gonotec, Germany).

Estradiol was dissolved in 95% (w/w) ethanol with RAMEB (molar ratio 1:2) to form inclusion complexes [13]. Ethanol was evaporated under a mild nitrogen stream (50℃) and added resolved in the chitosan solutions to obtain the final estradiol formulations for nasal administration. The estradiol formulations contained the following: 2 mg/ml eatradiol and 2%(w/v) RAMEB for nasal delivery. For intravenous perfusion, the inclusion complexes were dissolved in normal saline .The estradiol and RAMEB concentrations were 0.01 mg/ml and 0.01% (w/v), respectively.

2.2.2 *In- situ drug absorption study*

The absorption studies were carried out according to the in-situ nasal perfusion technique [14]. Rats were anesthetized by intraperitoneal injections of urethane (2 g/kg body weight). An incision was made in the neck of the rats laid on their backs and placed under a heating lamp to maintain body temperature. The trachea was cannulated with a polyethylene tube to aid breathing. Another tube was inserted through the esophagus into the posterior part of the nasal cavity. The nasopalatine duct was closed with an adhesive agent (cyanoacrylate glue) to prevent the drainage of the solution from the nasal cavity into the mouth. The tube inserted into the esophagus was connected to a reservoir of 5 ml drug solution under magnetic stirring and immersed in a water-bath at 37°C. The solution was circulated, by means of a peristaltic pump (DDB－600 electric peristaltic pump, Shanghai Zhixin instrument limited company) from the reservoir through the nasal cavity and out of the nostrils back into the reservoir. Flow rate was set at 2.5 ml/min. Aliquots (100 µl) were sampled after 30 min and stored at -20°C until the assay. Three rats were used for each condition tested. The estradiol concentration was determined by HPLC (HITACHI) using a C_{18} column and UV detector at 205 nm. These studies were performed in triplicate for each of the samples, but the average values were considered for data analysis. The S.D. was less than 5%.

2.2.3 In vivo drug absorption study

In vivo studies were performed as earlier reported [15]. Briefly, The rats were fasted overnight and anaesthetized by intraperitoneal injection of urethane (2 g/kg). The rats were tracheotomised to divert the airflow from the nasal passages and aid breathing. The oesophagus was closed by ligation onto the tracheal cannula. The right external jugular vein was cannulated for blood sampling and fluid (physiological saline) replacement. The estradiol preparation (2 mg/ml) were delivered through the right nostril using a PVC tube connected to a microliter syringe to give an estradiol dose of 160 µg/Kg. The preparation administered nasally was about 20–24µl, depending on the weight of the rat. Blood samples (0.3 ml) were taken at various time intervals up to 3 h after administration, After each blood withdrawal, the same volume of sterile normal saline was put back into the circulation to maintain total blood volume. Plasma samples were separated by centrifugation at 8000 rpm for 10 min and kept frozen at －20◦C for subsequent analysis of estradiol. Each group contains six rats. The estradiol in plasma was completely extracted with diethyl ether. Ether containing estradiol was evaporated to dryness in a clean beaker and the residue was dissolved in methanol. The estradiol content was then estimated by HPLC, using a C_{18} column and fluorescence detector at

excitation 267 nm and emission 302 nm.

Statistical analyses were accomplished using SPSS statistical package. Student's t-test was used to determine the statistically significant differences between the results. Results with P values < 0.05 were considered statistically significant.

3. Results and discussion

3.1 *In situ drug absorption*

3. 1.1 *Effect of chitosan concentration*

The remaining estradiol concentration of perfusions was detected after 30min perfusion experiment, the remaining percentages (mean ± SD) of RAMEB, 0.3%chitosan +RAMEB, 0.5% chitosan + RAMEB and 0.8% chitosan +RAMEB formulations were 63.94±9.24, 68.32±4.50, 40.24±14.61 and 45.34±8.38% respectively(Table.1). The RAMEB formulation and the 0.3% chitosan +RAMEB formulation were no significant difference on the remaining percentage of estradiol. The estradiol absorption was enhanced with increase of the chitosan concentration, the remaining percentage of RAMEB and 0.3% chitosan +RAMEB were significant difference with 0.5% chitosan + RAMEB and 0.8% chitosan +RAMEB, but there was no significant difference between 0.5% chitosan + RAMEB and 0.8%chitosan +RAMEB formulations, indicating that their enhancing activity was saturable. This was in agreement with previous reports, which suggested that the mechanism of absorption enhancement of chitosan might be different from typical membrane-disruption enhancers like sodium taurodihydrofusidate, synthetic surfactants, and bile salts [2,3]. Thus, based on the data obtained in this part, the concentration of 0.5% appeared to be optimal for chitosan to enhance the nasal absorption of estradiol in rats.

3. 1.2 *Effect of pH*

In order to study whether or not pH would influence the nasal absorption of estradiol, the 0.5% of chitosan containing RAMEB solutions of different pH were administered to rats, and the remaining percentages of estradiol were assayed after 30min perfusion. The remaining percentages (mean±SD) of pH 3.0, 4.0, 5.0 and 6.0 formulations were 52.87±0.70, 52.08±1.37, 53.72±2.49 and 62.27±1.57% respectively(Table.2). The enhancement of estradiol was declined with the increase of pH. There were no significant difference between pH 3.0, 4.0 and 5.0 formulations, but pH 6.0 formulation was significant different with the others. Considering the normal environment of the nose, pH 5.0 formulation seemed to be optimal for chitosan combined with RAMEB to enhance the nasal absorption of estradiol in rats.

Table 1. Effect of the chitosan concentration on estradiol nasal absorption clearance (n=3)

Formulations	Remaining percentages of estradiol after 30 min perfusion (%)			
	Individual values			Mean±SD
RAMEB	74.55	59.59	57.69	63.94±9.24
0.3% chitosan +RAMEB	63.55	68.93	72.48	68.32±4.50*
0.5% chitosan + RAMEB	39.41	26.06	55.26	40.24±14.61
0.8% chitosan +RAMEB	49.21	35.72	51.08	45.34±8.38

* Significant difference (P < 0.05)

Table 2. Effect of the pH on estradiol nasal absorption clearance (n=3)

pH	Remaining percentages of estradiol after 30 min perfusion (%)			
	Individual values			Mean±SD
3.0	52.06	53.27	53.27	52.87±0.70
4.0	53.16	52.53	50.54	52.08±1.37
5.0	55.29	55.02	50.85	53.72±2.49
6.0	60.97	61.82	64.01	62.27±1.57*

* Significant difference (P < 0.05)

3.2 *In vivo drug absorption*

To determine whether or not chitosan and RAMEB had a synergistic effect in enhancing the absorption and elevate the bioavailability of estradiol intranasally, these formulations were administered intranasally and intravenously in rats. The C_{max} (mean ± SD) of estradiol ethanol (70% w/w) solution, RAMEB and chitosan + RAMEB in plasma after intranasal administration were 70.56 ± 29.14, 20.51±6.02 and 62.41 ± 28.80 ng/ml, respectively; The t_{max} of the three formulations were 5, 15 and 30 min, respectively (Figure. 1). Intravenous administration of estradiol showed comparable plasma

concentration–time profiles compared to the nasal route of administration for these formulations. For all formulations the absolute bioavailabilities after intranasal delivery (estradiol ethanol (70% w/w) solution: 22.24 ± 7.88%, RAMEB: 58.78 ± 11.19%, chitosan + RAMEB: 78.51 ± 23.13%) differed significantly from each others (Table 3). The in vivo absorption data indicated the potential of chitosan combined RAMEB as effective nasal absorption enhancers of estradiol. There enhancing effects were better than single RAMEB under their corresponding optimum concentration and pH.

Table 3. Mean time (t_{max}) to maximal plasma concentration (C_{max}, weight corrected), area under the curve (AUC) from 0 to 3 h and bioavailability of intranasal estradiol formulations (ethanol solution, RAMEB and chitosan + RAMEB) after administration (results expressed as mean ± S.D.) (n = 6)

Formulation	t_{max} (min)	C_{max} (ng /ml)	AUC_{0-3h}	Bioavailability (%)
Intravenous		200.58±96.01	3394.85±676.05	
Ethanol solution	5	70.56 ± 29.14	754.99±267.66	22.24±7.88*
RAMEB	15	20.51±6.02	1995.33±58.78	58.78±11.19*
Chitosan + RAMEB	30	62.41 ± 28.80	3134.52±1425.74	78.51±23.13*

* Significant difference ($P < 0.05$).

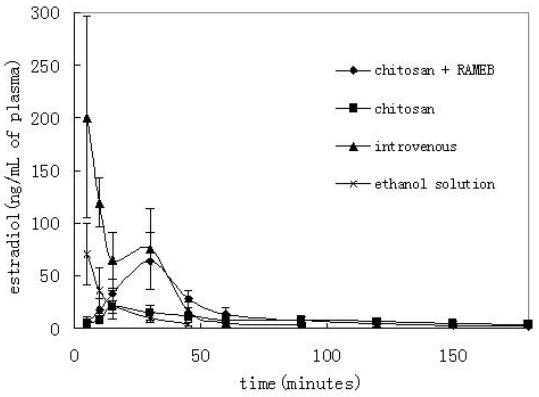

Figure 1. In vivo absorption of estradiol from intravenous and intranasal estradiol formulations [estradiol ethanol (70% w: w) solution, RAMEB and chitosan + RAMEB formulations in rats estradiol (ng/mL of plasma)]

The influence of chitosan on the effect of estradiol both in-situ and in vivo in our studies indicated that chitosan concentration and pH were the impacting factors influencing the enhancement of drugs to pass through the membrane, probably due to the mucoadhesive properties and high viscosity produced by the chitosan solutions, which make the drugs stay in the nasal cavity for a long time and be cleared slowly by mucocilia from nasal mucosa. But in this rat model, the mucociliary clearance mechanism is impaired hence the mucoadhesiveness has less importance in this studies. On the other hand, chitosan may open the tight junctions between cells due to the interaction of the positively charged amino group of it with the negatively charged sialic acid residues in mucus, leading to the transport increase of drugs across the epithelium, as it was mentioned in the introduction. Studies [2] demonstrated that an increase in chitosan concentrations resulted in an increase in the permeability coefficient of 14C-mannitol with a plateau level between 0.25 and 0.5%, using a human intestinal cell line (Caco-2) as the model epithelial cell layer. The combined effect mechanism of the absorption enhancement was when chitosan interacts with the epithelial membrane, the tight junctions are opened, and then RAMEB could penetrate into the opened gaps between cells and extract the phospholipids in biomembrane. Thus, the tight junction proteins such as occludin [16], claudin-1 and -2 [17] are naked and may collapse after the removal of surrounding phospholipids, resulting in these fusion points untied. So the opening of the tight junctions may be strengthened by co-administration of chitosan and RAMEB.

4. Conclusions

In-situ study data indicated the optimal concentration and pH for chitosan to enhance the nasal absorption of estradiol in rats were 0.5% and 5. After intranasal delivery the absolute bioavailabilitiy of chitosan + RAMEB formulation was higher than RAMEB formulation's, moreover they were different significantly (P<0.05). Based on the results above, we could conclude that chitosan and randomly methylated β-cyclodextrin could combine to enhance the nasal absorption of estradiol. This paper just studied the lipophilic and micromolecular drugs, such as estradiol; their effects on the hydrophilic and macromolecular drugs were waiting for the further studies afterward.

Preferences

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