Chitosan as a Novel Nasal Delivery System for Peptide Drugs

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A nasal solution formulation of the cationic material chitosan was shown to greatly enhance the absorption of insulin across the nasal mucosa of rat and sheep. The absorption promoting effect was concentration dependent with the optimal efficacy obtained for concentrations higher than 0.2% and 0.5% in rats and sheep, respectively. The absorption promoting effect was reversible with time in a "pulse-chase" study. Histological examination of the nasal mucosa of rats exposed to a chitosan solution for 60 minutes showed little change.

KEY WORDS: chitosan; nasal delivery; insulin; sheep.

INTRODUCTION

Chitosan is a high molecular weight cationic polysaccharide derived from naturally occurring chitin in crab and shrimp shells by deacetylation. The primary unit in chitin is a 2-deoxy-2-(acetyl-amino) glucose combined by glycosidic linkages into a linear polymer. Chitosan, with a unit formula of $C_6H_{11}O_4N$, has one primary amino and two free hydroxyl groups for each C_6 building unit. By desolving the chitosan in organic or inorganic acids positively charged chitosan (salts) are obtained. Chitosan has previously been employed as an pharmaceutical excipient in oral drug formulations in order to improve the dissolution of poorly soluble drugs (1,2,3) or for the sustained release of drugs by a process of slow erosion from a hydrated compressed matrix (4,5,6). The compound is considered to be non-toxic with an oral LD50 in mice of more than 16g/kg (7). Chitosan has been shown to have mucoadhesive properties, probably mediated by ionic interaction between the positively charged amino groups in chitosan and the negatively charged sialic acid residues in mucus (8).

There is an increasing interest in the development of safe, efficient and reliable nasal delivery systems for poorly absorbable drugs such as peptides and proteins. Presently marketed formulations of for example calcitonin have bioavailabilities of 1% or less (9). Illum and coworkers have introduced the principle of employing bioadhesive microsphere systems for nasal delivery of such poorly absorbed drugs in order to improve the systemic bioavailability without the use of enhancer systems (10,11,12,13). Starch microspheres have been suggested to exert two different effects on the nasal membrane. The bioadhesive effect of the microspheres decreases the rate of clearance of the drug from the nasal cavity and thereby allow a longer contact time with the absorptive epithelium (14). Further, it has been shown in a study employing monolayers of Caco-2 cells that the microspheres promote a transient widening of the tight junctions between cells thereby allowing larger hydrophilic molecules to pass through the membrane (15).

The aim of the present work was to study whether the properties of the bioadhesive and cationic material chitosan could be exploited to obtain a novel nasal delivery system that could significantly improve the absorption of the model peptide drug insulin in animal models. The optimal concentration for absorption promoting effect was investigated in the rat and the sheep models and a "pulse-chase" study was performed to investigate the reversibility of the absorption promoting effect. The influence of chitosan on the nasal tissue was studied in a histological investigation using a rat nasal model.

MATERIALS AND METHODS

Preparation of chitosan formulations: Medium viscosity chitosan glutamate (Sea Cure+) from Protan Laboratories Inc. was dissolved in 14.65 mM phosphate buffer of pH 7.4 and mixed with a 14.65 mM phosphate buffer solution of semisynthetic sodium insulin (Novo-Nordisk, Denmark) to obtain final concentrations of chitosan of 0.1%–1.0% w/v. The insulin concentrations were 40 IU/ml and 200 IU/ml for the rat and the sheep studies, respectively. The pH was adjusted to 4.4 with hydrochloric acid. For control, a solution of insulin (200 IU/ml) in phosphate buffer adjusted to pH 4.4 with hydrochloric acid was used. For the histology studies the chitosan solutions were prepared as above in a concentration of 0.5% w/v without the addition of insulin.

Rat absorption studies: Male Wistar rats of about 250g (JABU, Sutton Bonington, UK) were fasted overnight and anaesthetised by intraperitoneal injection of sodium pentobarbitone (60 mg/kg). The rats were tracheotomised to divert the air flow from the nasal passages and aid breathing. The oesophagus was closed by ligation. 0.1 ml/kg of the formulation was then administered to the right nostril only. The left carotid artery and the right external jugular vein were cannulated for blood sampling and fluid replacement, respectively. Blood samples (200μl) were collected at −15 and −5 minutes before formulation administration and at various time intervals up to 4 hours post administration. The plasma glucose levels were determined by the glucose oxidase method using a Yellow Springs Instrument 23 AM analyser.

For the "pulse-chase" study, chitosan was prepared without insulin in a concentration of 0.5% w/v. Insulin was prepared as above in a concentration of 40 IU/ml. The rats were given 0.1 ml/kg of the chitosan solution in one nostril and at time points 0, 15, 30, 45 and 60 minutes after the chitosan administration 0.1ml/kg insulin solution to give 4 IU/kg was administered in the same nostril. Blood samples were collected and analysed as above.

Sheep absorption studies: The solutions were administered nasally to groups of three or four cross-bred (Suffolk and Texel) sheep (about 40 kg). The sheep were cannulated in the jugular vein for blood sampling and sedated during dosing (3 min) by use of an intravenous dose of ketamine hydrochloride at 2.25 mg/kg to prevent sneezing. The nasal

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formulations (0.01 ml/kg) were administered to the sheep as described previously (Farraj et al, 1990) at doses of 2 IU insulin/kg and concentrations of chitosan glutamate of 0.1–1.0% w/v as appropriate. Plasma samples were collected at 15 and 5 min prior to administration and at various time points up to 240 min post-administration. The plasma glucose levels were determined by the glucose oxidase method using a Yellow Springs Instrument 23 AM analyser and the plasma insulin were determined by a double-antibody radioimmunoassay as described previously (12).

Histological studies: Four male Wistar rats were prepared as above without the artery and vein cannulation. 60 minutes after administration into the right nostril of a 0.5% chitosan solution, prepared as above but without the insulin, the tissue was fixed by cardiac perfusion of Bouin Hollandes fixative solution and processed as described by Chandler et al. (16). The left nostril was used as control. Complete cross-sections of the nasal cavity were obtained. The histological effect of the formulation was determined by analysis of cross-sections randomly selected from each animal. The epithelium on each side of the septum was qualitatively compared under light microscopy.

RESULTS

The effect of chitosan on the absorption of insulin from the nasal cavity in sheep is shown in Fig. 1 and Table 1. The administration of insulin in a simple phosphate buffer solution resulted in very low plasma concentrations of insulin and hence a lowering of the plasma glucose levels was minimal (83.2% of control level). However, when the insulin was administered in the chitosan formulation the plasma insulin level showed a surprisingly sharp and rapid rise in plasma insulin levels peaking at 75 min and correspondingly, the plasma glucose level fell to 43% of the control level within 90 min.

When concentrations of 0.01%–1.0% of chitosan were administered to rats in combination with 4 IU/kg of sodium insulin a concentration related absorption of insulin was found as expressed by the corresponding decreases in plasma glucose (Fig. 2). The lowest plasma glucose levels were obtained for concentrations of chitosan of 0.2% and higher after which no significant differences in absorption promoting effect were seen. Similarly, when different concentrations of chitosan (0.1–1.0%) were administered to sheep in combination with insulin (2 IU/kg), the decreases in plasma glucose showed a similar pattern with the lowest plasma glucose values reached for concentrations of chitosan higher than 0.5% (Fig. 2).

In order to elucidate the duration of the absorption promoting effect of chitosan, a "pulse-chase" study was performed in which rats were administered insulin nasally at specific time periods after the administration of the chitosan solution. The effect of the time interval between the two administrations is shown in Fig. 3. It can be seen that after 30 minutes the absorption promoting effect of chitosan starts decreasing rapidly and 60 minutes after the administration there is very little or no effect remaining.

The effect of chitosan on the nasal membrane of rats after 60 minutes exposure was negligible as seen in Fig. 4. There was slightly increased mucus discharge and a slight reduction in epithelium thickness in the dosed side of the cavity as compared with that of the undosed side. However, no cell loss was observed and cilia were still present on the luminal cell surfaces. Nasal epithelium exposed to the chitosan formulation appeared to be more "disordered" than the control tissue with the line of cilial basal bodies at the luminal surface interrupted at intervals but nuclei appeared normal in both treated and untreated tissue.

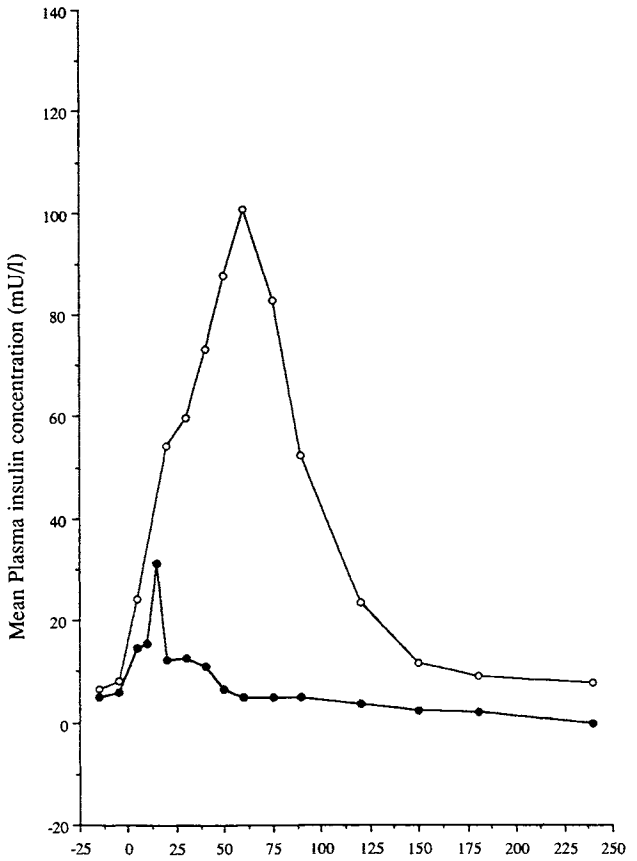

Fig. 1 Mean plasma concentration of insulin following intranasal administration to sheep of 2 IU/kg of insulin (●) as a simple solution and (○) in combination with 0.5% chitosan.

DISCUSSION

The remarkable increase in the absorption of insulin across the nasal mucosa was similar to or better than that obtained previously by our group using a powder system consisting of bioadhesive starch microspheres (13). For the chitosan system a nadir of 43% of the initial glucose level was reached slightly later, 90 minutes as compared to 75 minutes for the microsphere system. At 4 hours after administration the glucose level was still significantly lower than normal levels for the chitosan system, whereas normal levels were reached at 3 hours for the microsphere system. A similar trend was seen in the insulin levels where the chitosan system gave increased levels of insulin for 3 hours as compared to 75 minutes for the microsphere system.

The study of the effect of concentration on the promotion of insulin transport across the membrane showed that minimal concentrations are necessary in order to obtain the

Table I. Effect of Chitosan Delivery System on Absorption of Intranasally Administered Insulin in Sheep

	AUC—glucose curve ±SEM (% · min)	Mean glucose nadir ±SEM (% of basal)	AUC—insulin curve ±SEM (mIU/l)	Mean C_{max} ±SEM (mIU/l)
SHI sol (2 IU/kg)	22940 (±953)	83 (±3)	1346 (±120)	34 (±4)
SHI sol (2 IU/kg) + Chitosan (0.5%)	16548 (±1331)	43 (±10)	9809 (±2816)	191 (±16)

AUC: Area under curve; SHI: Sodium human insulin; sol: solution.
All results are calculated from individual data.

maximal effect. The difference in concentration required for the two animal species can be explained by the fact that the sheep used in these experiments were fully conscious as opposed to the rats and hence their mucociliary clearance mechanisms would be expected to be fully functional. For lower concentrations of chitosan the viscosity and the degree of mucoadhesion may have been insufficient to obtain and maintain the required deposition characteristics in the nasal cavity. In the mucoadhesion studies performed by Lehr et al. (9) on pig intestinal mucosa in vitro, the concentration of chitosan used to obtain strong mucoadhesion was 1% w/v.

It is clearly demonstrated in the "pulse-chase" study in rats that the absorption promoting effect of chitosan on the nasal membrane is transient. Already between 30 and 45 minutes after administration of the chitosan solution the absorption enhancing effect was decreasing and after 60 minutes the nasal membrane apparently regained normal permeability properties. Since the studies were performed in a rat model with impaired mucociliary clearance, it is not likely that the chitosan was removed within this time from the site of absorption. Earlier studies on the effect of classical enhancer systems such as surfactants and bile salts on the rat

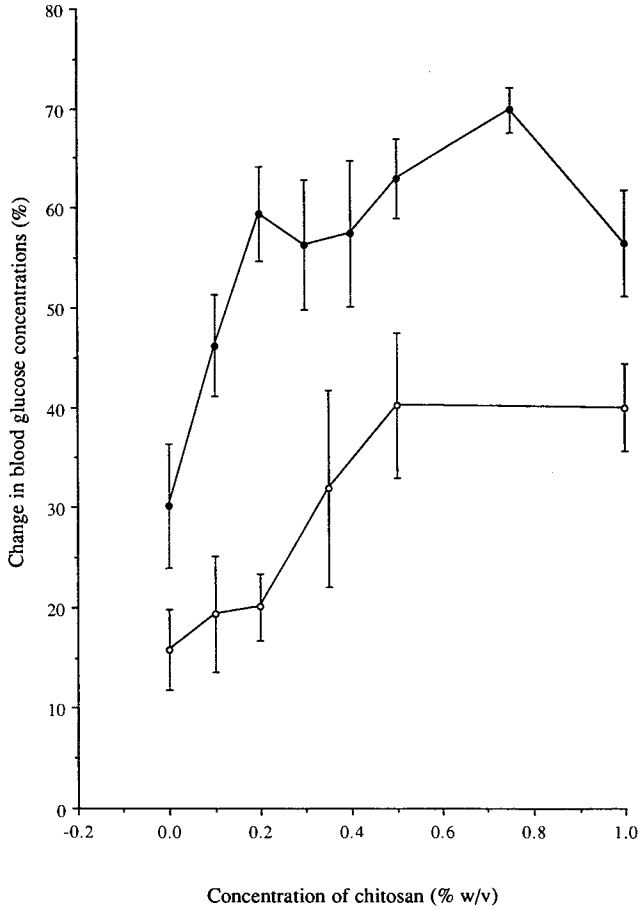

Fig. 2 The influence of chitosan concentration on the mean maximum percent decrease in plasma glucose in (●) rats and (○) sheep following intranasal administration of 4 IU/kg and 2 IU/kg of insulin, respectively. (±SEM)

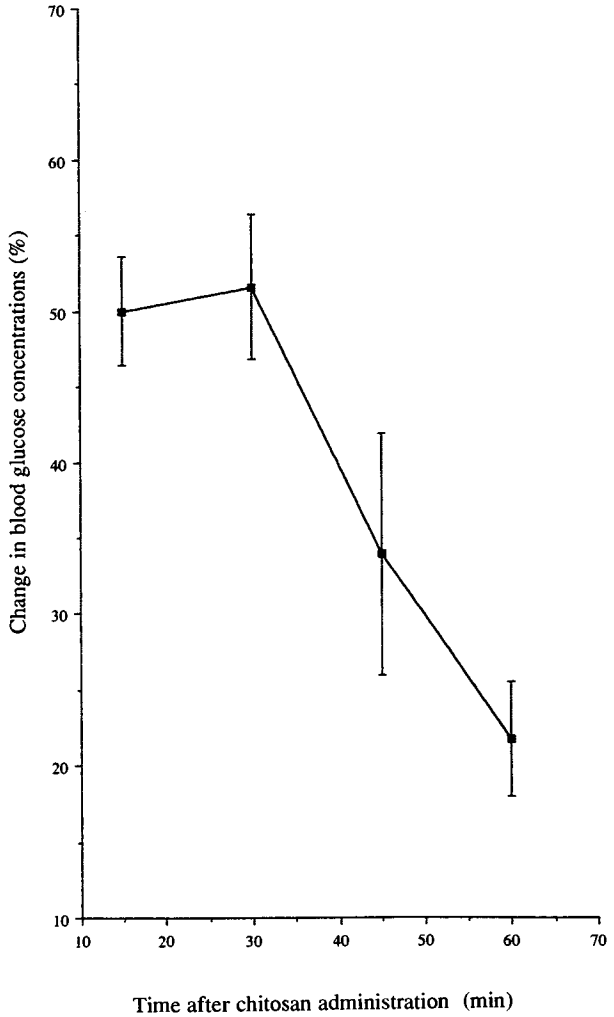

Fig. 3 A "pulse-chase" study of the effect of a time interval between the intranasal administration of a 0.5% chitosan solution to the nasal cavity of a rat and the intranasal administration of 4 IU/kg insulin to the same nostril of the rat. The data are expressed as change in percent blood glucose concentration ± SEM.

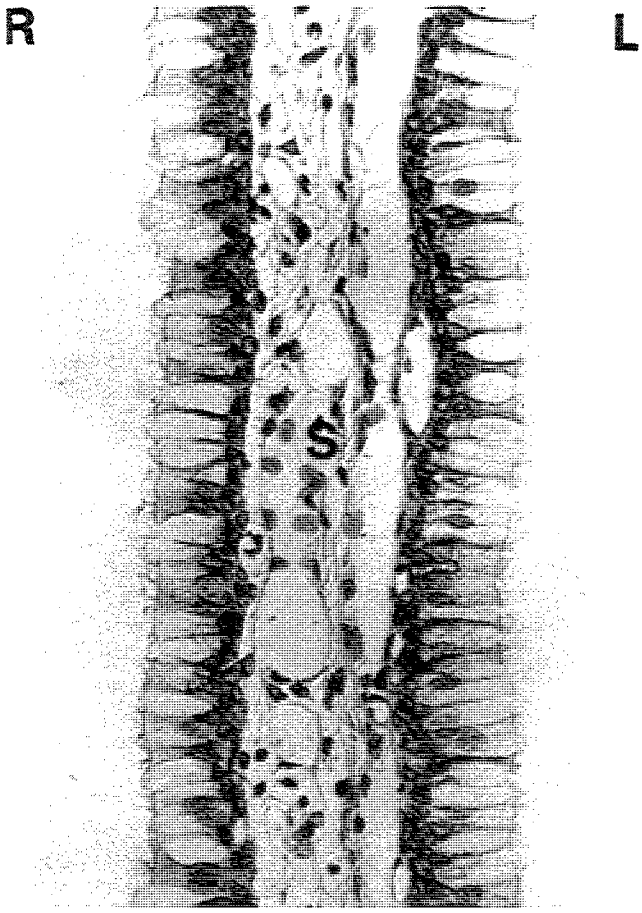

Fig. 4 Photomicrograph of section through the nasal cavity of a rat dosed with 0.5% w/v chitosan (pH = 4) and left in contact for 60 minutes. S: Septum, R: Right nasal cavity (test side), L: Left nasal cavity (control) (× 1070)

nasal mucosa show that recovery from damage to the membrane takes up to 24 hours (17). Hence, these results suggest that a different mechanism of membrane damage is responsible for the apparent increase in permeability. This was confirmed in the histological study on the effect of chitosan solution on the integrity of the nasal mucosa in the rat model (16). Exposure of the rat nasal mucosa to a chitosan solution concentration of 0.5% over a period of 60 minutes failed to cause any significant change in the morphology of the mucosa as compared to the control.

We are presently exploring in detail the mechanisms by which chitosan is increasing the absorption of polar drugs across the nasal mucosa. While mucoadhesion could be a contributing factor we believe that other effects may also come into play. It is possible that the cationic nature of chitosan could have a transient effect on the gating function of tight junctions. The cationic peptide protamine enhances the lymph flow and the transcapillary lymph protein clearance after infusion into the intestinal lymph system of the rat (18). The effect is suggested to be due to neutralisation of the fixed anionic sites on the capillary wall. The effect of chitosan on tight junction integrity remains to be investigated.

In conclusion, the nasal chitosan delivery system enhanced nasal uptake of a poorly absorbed polypeptide without the use of potentially damaging penetration enhancing agents.

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