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Aminated gelatin as a nasal absorption enhancer for peptide drugs: evaluation of absorption enhancing effect and nasal mucosa perturbation in rats

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Abstract

This study was carried out to evaluate the potential of aminated gelatin as a nasal absorption enhancer for peptide drugs. The absorption-enhancing effect was investigated in rats using insulin and fluorescein isothiocyanate-dextran with a molecular weight of 4.4 kDa (FD-4) as model drugs. The absorption of insulin was estimated by measuring the changes in plasma glucose levels following intranasal administration, and that of FD-4 was determined by measuring its plasma concentration after dosing. The hypoglycaemic effect after intranasal administration of insulin with aminated gelatin significantly increased compared with that after intranasal administration of insulin in phosphate buffered saline, indicating that aminated gelatin effectively enhanced the nasal absorption of insulin. In contrast, neither kind of native gelatin (isoelectric point = 5.0 and 9.0) showed any absorption-enhancing effect. The pH of the formulations and the concentration of aminated gelatin were found to affect the hypoglycaemic effect. In addition, aminated gelatin at a concentration of 0.2% significantly enhanced the absorption and the efflux of FD-4 through the rat nasal mucosa. The possible perturbation of aminated gelatin to nasal mucosa was evaluated by measuring the leaching of lactate dehydrogenase (LDH) using an in-situ perfusion rat model. Aminated gelatin presented a concentration-dependent (0.1–0.4%) but relatively small effect on the LDH leaching from the rat nasal epithelial membrane. These results suggest that positively charged aminated gelatin could be a new absorption enhancer for nasal delivery of peptide drugs.

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Introduction

Nasal drug delivery is one of the most attractive non-invasive routes for peptide and protein drugs because of the relatively high permeability of nasal epithelial membrane, avoidance of the first-pass metabolism and improvement of patient compliance. However, the bioavailability achieved following nasal administration is usually very low for hydrophilic and high molecular weight drugs, such as insulin. Different strategies have been explored to improve the absorption of these drugs through nasal mucosa, including using chemical penetration enhancers (Verhoef & Merkus 1994) and proteolytic enzyme inhibitors (Morimoto et al 1995), and designing suitable dosage formulations (Dondeti et al 1996). Of these approaches, the use of absorption enhancers has proven to be effective. Nevertheless, many studies have shown that the absorption-enhancing effects of the classical enhancers, including surfactants, bile salts and fatty acids, are often accompanied by damage

to the epithelial cell membrane when they are used at an effective concentration (Merkus et al 1993; Marttin et al 1995). Looking for new, effective and safe penetration enhancers is therefore an important approach in the development and application of these dosage forms.

It has recently been reported that chitosan, a cationic polymer, was able to improve the nasal absorption of peptide drugs while causing negligible damage to the nasal mucosal membrane at a physiological level (Illum et al 1994; Aspden et al 1996). Natsume et al (1999) investigated the effect of a series of cationic compounds as nasal absorption enhancers. They found that all of the positively charged compounds under investigation could enhance the absorption of FITC-dextran (MW 4.4 kDa) through the rat nasal mucosa. The most significant absorption-enhancing effect and the least damage to the nasal mucosa were achieved by using poly-L-arginine hydrochlorides with molecular weights of 45.5 and 92 kDa. These results implied that cationic polymers might be suitable candidates for improving the absorption of peptide and protein drugs through the nasal mucosal membrane with relatively low toxicity.

In this study, aminated gelatin, a new positively charged polymer, was evaluated as a nasal absorption enhancer in rats. The absorption-enhancing effect was investigated using insulin and FD-4 as model drugs, and the potential perturbation of aminated gelatin to the nasal mucosal membrane was estimated by measuring lactate dehydrogenase (LDH) leaching using an in-situ perfusion rat model. These results were compared with those of two kinds of native gelatin and a chitosan.

Materials and Methods

Materials

Gelatin (isoelectric point (IEP) 5.0 and 9.0; MW 100 kDa) was kindly supplied by Nitta Gelatin Co. Ltd (Osaka, Japan). Recombinant human insulin (28.6 IU mg^{-1}), fluorescein isothiocyanate-dextran (FITC-dextran) with a molecular weight of 4.4 kDa (FD-4) and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride were purchased from Sigma Chemical Co. (St Louis, MA). Chitosan 500 with a deacetylation rate of about 80%, glucose B-test kit and LDH C II test kit were obtained from Wako Pure Chemical Industries (Osaka, Japan). All other chemicals were of reagent grade and were used as received.

Eight-week-old male Wistar rats (about 200 g) were purchased from Japan SLC (Shizuoka, Japan), and were housed in a room with controlled temperature

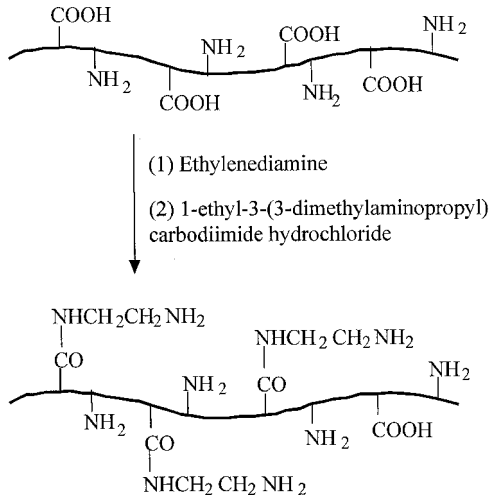

Figure 1 Synthesis of aminated gelatin (Wang et al 2000).

(24±4 °C) and humidity (55±5%). Animal experiments were carried out in accordance with *Guiding Principles for the Care and Use of Experimental Animals*, Hokkaido College of Pharmacy (1998).

Synthesis of aminated gelatin

Aminated gelatin was synthesized by the reaction of native gelatin (IEP = 9.0) with 1,2-ethylenediamine in the presence of a soluble carbodiimide as described previously (Wang et al 2000). In brief, gelatin was dissolved in phosphate buffer (pH 5.3) and ethylenediamine solution was added under stirring. After adjusting the pH to 5.0 with 5 M HCl, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride was added. The resultant solution was incubated at 37 °C for 1 h, followed by dialysis against purified water for 48 h, and then freeze-dried to obtain the aminated gelatin (Figure 1). The amino group contents of the native and aminated gelatin were determined using a 2,4,6-trinitrobenzenesulfonic acid method. Aminated gelatin presented an amino group content of 1.627±0.121 μmol mg^{-1} aminated gelatin, and the amino group content of native gelatin (IEP = 9.0) was 0.866±0.025 μmol mg^{-1} gelatin (Wang et al 2000).

Preparations

Recombinant human insulin and aminated gelatin were dissolved in phosphate-buffered saline (PBS) at different pHs (4.0, 6.0 and 7.4) in proper concentrations so that a required concentration of insulin and candidate enhancers could be obtained by mixing these solutions.

The final concentration of insulin for nasal administration was 60 IU mL^{-1}, and the concentrations of aminated gelatin were 0.1, 0.2 and 0.4% (w/v), respectively. Native gelatins were used at a concentration of 0.2% and at pH 7.4 for comparison.

Since chitosan does not dissolve at pH 7.4, a chitosan solution at pH 4.0 was prepared as a positive control. Chitosan was first dissolved in 1% acetic acid at a concentration of 0.4%, the pH was then adjusted to 4.0 with 1 M NaOH, and NaCl was added to adjust the isotonicity. This solution was mixed with insulin solution in PBS (pH 4.0) to obtain a solution with a chitosan concentration of 0.2% and insulin concentration of 60 IU mL^{-1} for nasal administration.

FD-4 was dissolved in PBS (pH 7.4) or PBS containing 0.2% (w/v) aminated gelatin to give a concentration of 200 mg mL^{-1} for nasal administration.

Intranasal administration

Male Wistar rats, weighing 210–260 g, were fasted for 24 h before the experiment, but were allowed free access to water. Rats were anaesthetized by intraperitoneal injection of sodium pentobarbital at a dose of 50 mg kg^{-1}, with an additional dose given intraperitoneally to maintain a general anaesthesia as necessary. The body temperature of the rats was maintained at about 37°C throughout the experiment by using a light. A surgical operation was performed according to the method of Hirai et al (1981a). Briefly, rats were placed in a supine position, a trachea cannulation was performed to maintain respiration, and the trachea leading to the nasal cavity was ligated to prevent the drainage of the transuding liquid oozing from the surgical cut. Another polyethylene tube, with a closed top, was inserted through the oesophagus to the posterior part of the nasal cavity to prevent the drainage of drug solution towards the nasopharynx. Insulin preparations in PBS (pH 4.0, 6.0 and 7.4) or containing candidate enhancers were administered to the nasal cavity through the nostril using a pipette, which was inserted into the nasal cavity by about 5 mm. The dose of insulin was 10 IU kg^{-1}. PBS (pH 7.4) was used as a blank control, and the insulin preparation containing chitosan was administered as a positive control. Blood samples of 0.2 mL were withdrawn from the femoral vein 10 min before administration and at predetermined time intervals after dosing for up to 5 h. After centrifugation of the blood samples at 10000 rpm for 5 min, the plasma samples were isolated and kept at –20°C until analysis. The plasma glucose concentration was determined by using a glucose B-test kit (glucose oxidase method) according to the manufacturer's instructions.

For evaluation of nasal absorption of FD-4, FD-4 preparations were administered to rats using the same method as described above, except that the rats did not undergo the fasting procedure. The dose of FD-4 was 30 mg kg^{-1}. FD-4 solution in PBS (3 mg mL^{-1}) was intravenously injected into the rats at a dose of 4 mg kg^{-1} for evaluation of bioavailability. Blood samples were taken immediately before and at different times after administration for up to 8 h. Plasma samples obtained after centrifugation were stored at 0°C until analysis. For determination of FD-4, the plasma samples were diluted with potassium dihydrogenphosphate/sodium borate buffer (pH 8.5), and the FD-4 concentration was measured using a fluorescence spectrophotometer (FP-770, JASCO, Tokyo, Japan) at an excitation wavelength of 495 nm and an emission wavelength of 515 nm (Natsume et al 1999).

In-situ perfusion

FD-4 efflux from nasal mucosa

Male Wistar rats, 210–260 g, were anaesthetized by intraperitoneal injection of sodium pentobarbital at a dose of 60 mg kg^{-1}. A surgical operation (Hirai et al 1981a) was performed in a similar way to that described in the section on intranasal administration, except that the polyethylene tubing for oesophagus cannulation was open for the purpose of perfusion. In addition, the passage of the nasopalatine was sealed with an adhesive to prevent the drainage of the drug solution from the nasal cavity to the mouth. FD-4 solution in PBS (100 mg mL^{-1}) was injected into the femoral vein at a dose of 80 mg kg^{-1}. Five minutes after the injection, 20 μL of absorption-enhancer solution was administered intranasally into each of the two nostrils using a pipette, and PBS (pH 7.4) was administered as a blank control. Fifteen minutes later, the nasal cavity was perfused with 10 mL of PBS (pH 7.4) at a flow rate of 1 mL min^{-1}. The amount of FD-4 in the lavage was determined using a fluorescence spectrophotometer at 515 nm (ex. 495 nm).

LDH leaching from nasal mucus

Male Wistar rats, 210–260 g, underwent the same surgical operation as described in the section on the FD-4 efflux test. Ten millilitres of PBS (pH 7.4) or PBS containing different concentrations of candidate enhancers was circulated through the nasal cavity using a

peristaltic pump at a flow rate of 2 mL min^{-1}. The perfusing solutions were maintained at 37°C using a water bath. After 2 h of perfusion, the LDH activity in the perfusate was determined using a LDH C II test kit according to the manufacturer's instructions.

Data analysis

The plasma glucose concentration of each rat before nasal administration was taken as the baseline level, and the changes in plasma glucose concentrations (percentage of baseline level) at different times after dosing were calculated and plotted against time. The AUC (area under the plasma glucose level versus time curve) was obtained according to the trapezoidal rule, and the total decrease in plasma glucose level ($D\%$) was calculated using the following equation (Hirai et al 1981b):

$$D(\%) = \frac{AUC_c - AUC_s}{AUC_c} \times 100\% \quad (1)$$

where AUC_c represents the AUC after intranasal administration of pH 7.4 PBS (blank control) and AUC_s represents the AUC of insulin formulations at different pHs or containing candidate absorption enhancers.

The plasma FD-4 concentration was plotted against time, and the AUC was calculated according to the trapezoidal rule.

Data were expressed as mean ± s.d. Statistical analysis was performed using the analysis of variance method. Differences between group means were judged to be significant at $P < 0.05$.

Results

Absorption-enhancing effect of aminated gelatin

The potential of aminated gelatin as a nasal absorption enhancer for peptide drugs was evaluated in rats using insulin and FD-4 as model drugs. The absorption of insulin was detected by measuring its hypoglycaemic effect, i.e. the changes in plasma glucose concentration. Figures 2–4 show the time-course of plasma glucose levels after intranasal administration of insulin (10 IU kg^{-1}) in PBS (pH 4.0, 6.0 and 7.4) or containing candidate absorption enhancers (native gelatins, aminated gelatin and chitosan). Table 1 lists the relevant pharmacokinetic parameters. As shown in Figure 2 and Table 1, a significant decrease ($P < 0.05$) in plasma glucose levels was achieved when insulin was administered with 0.2% aminated gelatin, and the effect was

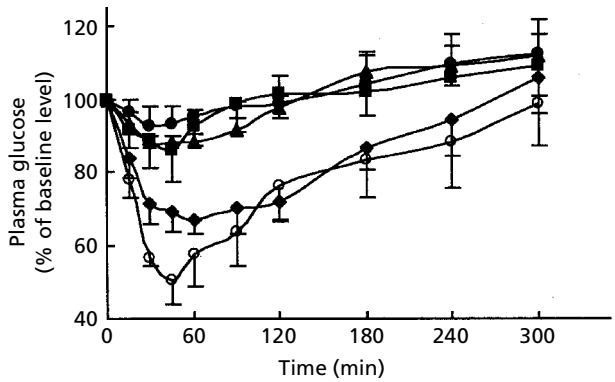

Figure 2 Effect of absorption enhancers (0.2% w/v) on the changes in plasma glucose levels after intranasal administration of insulin (10 IU kg^{-1}) in rats. The pH of all preparations was 7.4 except for that of chitosan (pH 4.0). (●) PBS; (○) chitosan; (▲) gelatin (IEP = 5.0); (■) gelatin (IEP = 9.0); (◆) aminated gelatin. Each point represents mean ± s.d. (n = 3–5) (Wang et al 2000).

comparable to that of chitosan at the same concentration. For both kinds of native gelatin (IEP = 5.0 and 9.0), however, the plasma glucose levels did not decrease significantly after administration, and they were nearly the same as in the case of PBS (pH 7.4). These results indicate that aminated gelatin effectively enhances the absorption of insulin through the rat nasal mucosa, whereas native gelatins present no penetration-enhancing effect.

A trend towards concentration dependency was observed for the nasal insulin absorption-enhancing effect of aminated gelatin (Figure 3 and Table 1). With the increase of the aminated gelatin concentration from 0.1 to 0.4%, the total decrease in plasma glucose levels ($D\%$) increased from 19.9 to 29.9%.

Figure 4 shows the influences of the pH of the formulations on the hypoglycaemic effect after intranasal administration in rats. A greater decrease in plasma glucose levels was obtained at pH 7.4 and pH 4.0 than at pH 6.0, in either the presence or absence of aminated gelatin. Nevertheless, aminated gelatin was able to increase the nasal absorption of insulin at all the pH values investigated, although an apparently smaller effect was observed at pH 6.0.

To further demonstrate the nasal absorption-enhancing effect of aminated gelatin, we investigated its effect on the absorption of FD-4 from rat nasal cavity. As shown in Figure 5, the plasma FD-4 concentration following intranasal administration with 0.2% (w/v) aminated gelatin increased significantly in comparison with that after administration of FD-4 in PBS (pH 7.4). The $AUC_{0-8\,h}$ in the case of aminated gelatin

Table 1 Pharmacokinetic parameters following intranasal administration of insulin (10 IU kg^{-1}) in PBS or containing candidate absorption enhancers in rats.

Formulations	T_{min} (min)	C_{min} (% of baseline)	D (%)
pH 7.4			
PBS	30	92.2±5.4	6.2±0.8
0.2% gelatin (IEP = 5.0)	45	88.2±1.8	5.7±0.5
0.2% gelatin (IEP = 9.0)	45	86.0±8.9	6.4±1.2
0.1% aminated gelatin	60	72.2±4.3	19.9±6.2[a]
0.2% aminated gelatin	60	66.9±3.6	22.2±3.0[a]
0.4% aminated gelatin	60	62.3±2.4	29.9±5.8[a]
pH 6.0			
PBS	30	95.6±4.5	1.5±0.1
0.2% aminated gelatin	60	74.1±5.2	14.9±3.8[b]
pH 4.0			
PBS	45	84.2±3.1	7.2±0.9
0.2% aminated gelatin	45	69.7±4.7	21.2±6.7[c]
0.2% chitosan	45	50.7±7.1	27.0±5.7[d]

C_{min} and T_{min} represent the minimum blood glucose level and the time to reach C_{min} after nasal administration, respectively. The D (%) value, which represents the total decrease in plasma glucose concentration, was calculated from equation (1) and expressed as mean±s.d. (n = 3–6). [a]$P < 0.05$ versus nasal administration of insulin in pH 7.4 PBS. [b]$P < 0.05$ versus nasal administration of insulin in pH 6.0 PBS. [c]$P < 0.05$ versus nasal administration of insulin in pH 4.0 PBS. [d]$P > 0.1$ versus 0.2% aminated gelatin at pH 4.0. No significant difference was observed between the two kinds of native gelatin and PBS groups.

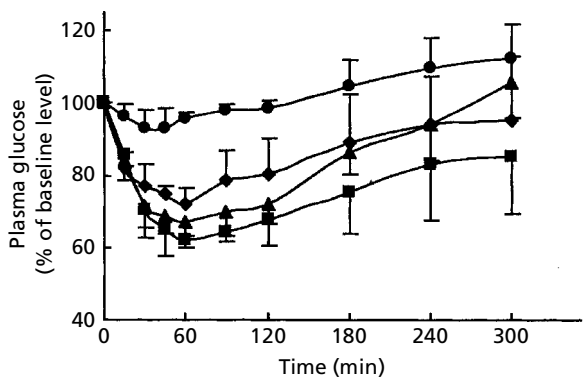

Figure 3 Effect of the concentration of aminated gelatin on the changes in plasma glucose levels after intranasal administration of insulin (10 IU kg^{-1}) at pH 7.4 in rats. (●) PBS; (◆) 0.1% aminated gelatin; (▲) 0.2% aminated gelatin; (■) 0.4% aminated gelatin. Each point represents mean±s.d. (n = 3–5).

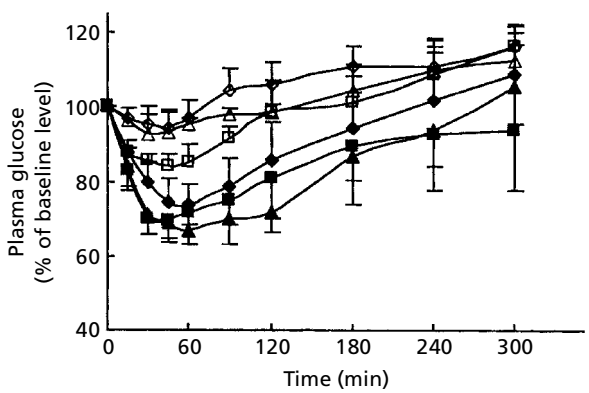

Figure 4 Effect of the pH of the formulations on the changes in plasma glucose levels after intranasal administration of insulin (10 IU kg^{-1}) in rats. (□) pH 4.0 PBS; (◇) pH 6.0 PBS; (△) pH 7.4 PBS; (■) 0.2% aminated gelatin at pH 4.0; (◆) 0.2% aminated gelatin at pH 6.0; (▲) 0.2% aminated gelatin at pH 7.4. Each point represents mean±s.d. (n = 3–6) (Wang et al 2000).

(1230.8 $\mu g\ mL^{-1}$ per min) increased 2.5-fold compared with that of the PBS group (490.6 $\mu g\ mL^{-1}$ per min), and a bioavailability of 23.8% was achieved by using aminated gelatin (0.2%) as an absorption enhancer. This result further confirmed that aminated gelatin could increase the absorption of soluble macromolecular compounds through the rat nasal mucosa.

FD-4 efflux from nasal mucosa

In order to elucidate the mechanism of the absorption-enhancing effect of aminated gelatin, we investigated the effect of intranasally administered aminated gelatin on the efflux of a paracellular transport marker compound FD-4, which was intravenously injected before

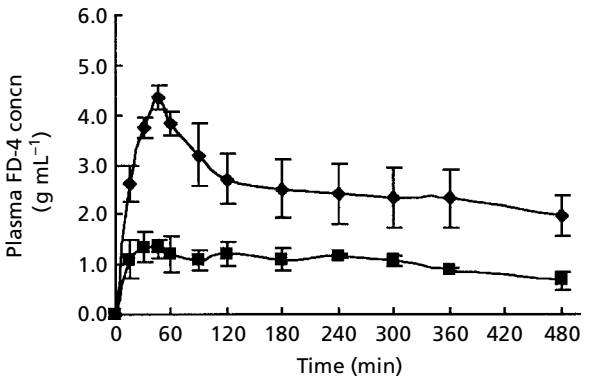

Figure 5 Effect of aminated gelatin on the plasma FD-4 concentration after intranasal administration of FD-4 (30 mg kg^{-1}) in rats. (■) pH 7.4 PBS; (◆) 0.2% aminated gelatin at pH 7.4. Each point represents mean±s.d. (n = 4).

the administration of the absorption enhancers. As shown in Table 2, the amount of FD-4 efflux in the presence of enhancers was significantly higher than that treated with PBS for all the enhancer solutions with the exception of the native gelatin (IEP = 9.0), and the efflux was proportional to their absorption-enhancing effect. A trend towards dose dependency was observed for the efflux of FD-4 induced by aminated gelatin. The amounts of FD-4 efflux induced by 0.1 and 0.4% of aminated gelatin were 2.61±0.44 and 4.05±0.14 μg respectively, whereas it was 4.30±0.85 μg in the case of 0.2% chitosan.

LDH leaching from nasal mucosa

The potential perturbation of aminated gelatin to nasal mucosa was evaluated in rats by measuring LDH leaching using an in-situ perfusion model, and the result was compared with those of native gelatin and chitosan. Table 2 shows the LDH activities in the perfusate after 2 h of perfusion in rats. PBS caused a slight leaching of LDH from the rat nasal epithelial cells, with an LDH activity of 48 IU L^{-1}, while native gelatin (IEP = 9.0) showed relatively less LDH leaching (30.7 IU L^{-1}) than in the case of PBS. For aminated gelatin, with the increase in the concentration of aminated gelatin from 0.1 to 0.4%, the amount of LDH leaching increased from 76.8 to 133.2 IU L^{-1}. At a concentration of 0.2% aminated gelatin, the LDH activity in the perfusate was 93.2 IU L^{-1}, whereas chitosan gave a value of 146.1 IU L^{-1} at the same concentration.

Discussion

In this study, aminated gelatin, a new positively charged polymer, significantly enhanced the nasal absorption of insulin in rats. The insulin absorption-enhancing effect of aminated gelatin after intranasal administration showed a trend towards concentration dependency, and was comparable to that of chitosan at the same concentration. In addition, aminated gelatin significantly enhanced the nasal absorption as well as the efflux of FD-4, a paracellular transport marker compound. The effect of aminated gelatin on the FD-4 efflux was proportional to the insulin absorption-enhancing effect. From these results it was suggested that aminated gelatin probably took effect through the electrostatic interaction with the negatively charged nasal mucosa, thus increasing the paracellular permeability of nasal epithelial membrane, as in the case of other polycations. It has been reported that the absorption-enhancing effect of chitosan on epithelial cell membrane is mediated through its positive charge (Schipper et al 1997). The electrostatic interactions between the positively charged polymers and the negatively charged epithelial cell membrane result in an alteration of cytoskeletal F-actin and redistribution of tight-junction associated proteins, therefore enhancing the transport through a paracellular pathway (Schipper et al 1997). Aminated gelatin might share the same mechanism although we have not proved this at present. McEwan et al (1993) reported that several

Table 2 Efflux of intravenously administered FD-4 and LDH leaching from nasal mucosa after intranasal administration of absorption enhancer solutions in rats.

Enhancer solutions	PBS	0.2% chitosan	0.2% gelatin (IEP = 9.0)	Aminated gelatin		
				0.1%	0.2%	0.4%
FD-4 efflux (μg)	1.15±0.38	4.30±0.85	1.97±0.36	2.61±0.44	3.71±0.65	4.05±0.14
LDH activity (IU L^{-1})	48.0±22.5	146.1±55.0	30.7±3.2	76.8±8.6	93.2±10.8	133.2±9.8

The pH of all the enhancer solutions was 7.4 except for that of chitosan solution (pH 4.0). Data are expressed as mean±s.d. (n = 3–4).

positively charged poly(amino acids), such as poly-L-lysine, poly-D-lysine, poly-L-arginine and poly-L-ornithine, share a similar effect on the transepithelial permeability of a few endothelial and epithelial monolayers. At the same time, McEwan et al (1993) suggested that the changes in permeability induced by these polycations were more dependent on the amount of positive charge (charge density) than on the type of the cationic moieties. This may partly explain why native gelatin (IEP = 9.0) did not show any absorption-enhancing effect although it also carried a positive charge under the experimental conditions. Aminated gelatin presented a much higher positive charge density (higher amino group content) than the native gelatin (IEP = 9.0), therefore it possibly presented a higher penetration-enhancing ability to nasal epithelial membrane.

Apart from the electronic charge, the high molecular weight of aminated gelatin might also exert an important role on its absorption-enhancing effect. Schipper et al (1996) reported that molecular weight was a very important factor in determining the absorption-enhancing effect of chitosans. For chitosans with a higher degree of acetylation, only those with a higher molecular weight could increase the epithelial membrane permeability. Natsume et al (1999) also showed that L-arginine only slightly increased the absorption of FD-4 through the rat nasal mucosa. In contrast, the bioavailabilities of FD-4 following nasal administration were 24.7, 62.9 and 78.7% when poly-L-arginines with molecular weights of 8.9, 45.5 and 92.0 kDa, respectively, were used as absorption enhancers. In this study, therefore, the high molecular weight of aminated gelatin might also have contributed to its absorption-enhancing effect. The mechanism of the effect of molecular weight on the nasal insulin absorption-enhancing effect of polymer penetration enhancers is not yet clear. However, as can be imagined, higher molecular weight enhancers themselves should present a low level of penetration through the membrane, thus demonstrating a stronger interaction with the mucosa and a stronger absorption-enhancing effect.

The pH of a formulation may affect the solubility, partition behaviour and/or stability of drugs, especially peptide and protein drugs. It can also affect the tissue structure and properties in the administration sites, thus affecting the absorption of drugs. We therefore investigated the absorption-enhancing effect of aminated gelatin at different pH values. It was found that aminated gelatin significantly increased the nasal absorption of insulin at all the pH values investigated, although a relatively smaller effect was observed at pH 6.0 than at pH 4.0 and pH 7.4. However, the apparently smaller effect at pH 6.0 might not result from the lower absorption-enhancing ability of aminated gelatin under this condition. The real reason may be the formation of high molecular weight aggregates at pH 6.0, which are more difficult to penetrate through the epithelial membrane than the monomeric form. Hirai et al (1978) reported that the hypoglycaemic effect of insulin following nasal administration increased with a decrease in the pH of the formulations. The greatest effect was achieved at pH 3.1, and almost no effect was observed at pH 6.1. Above pH 6.1, the hypoglycaemic effect increased with the increase in the formulation pH. Fernandez-Urrusuno et al (1999) also showed that a relatively more pronounced hyperglycaemic effect was obtained at pH 4.3 than at pH 6.4 after intranasal administration of a suspension of insulin-incorporated chitosan nanoparticles. Our results agree well with these reports. The smallest effect, at about pH 6.0, was ascribed to the low solubility of insulin and its tendency to form high molecular weight aggregates.

The irritation or toxicity of absorption enhancers remains a major concern for their clinical application. Different methods and indexes have been employed to evaluate these undesirable effects. In this study, the potential irritation of aminated gelatin to nasal mucosa was evaluated by measuring the leaching of LDH using an in-situ perfusion rat model. Since LDH is a cytosolic enzyme, its leaching into the perfusate is regarded as a result of irritation or damage to the epithelial cells. Aminated gelatin presented a concentration-dependent (0.1–0.4%) effect on LDH leaching (76.8–133.2 IU L^{-1}). However, the amount of LDH leaching induced by aminated gelatin was less than that caused by chitosan at the same concentration (Table 2), therefore aminated gelatin might not induce serious damage to the nasal epithelial cells since chitosan is generally considered to be a relatively safe absorption enhancer. The lower toxicity of high molecular weight penetration enhancers is partly ascribed to their lower level of penetration into the epithelial cells.

Conclusion

Aminated gelatin significantly enhanced the nasal absorption of both insulin and FD-4, while causing a relatively small LDH leaching from the nasal mucosa after intranasal administration in rats. The high positive charge density, high molecular weight and other physicochemical properties of aminated gelatin might contribute to these effects. These results indicate that positively charged aminated gelatin could be a useful

candidate absorption enhancer for the nasal delivery of peptide drugs.

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