Development of a Transnasal Delivery System for Recombinant Human Growth Hormone (rhGH): Effects of the Concentration and Molecular Weight of Poly-l-arginine on the Nasal Absorption of rhGH in Rats

Ryo Kawashima,a Masaki Uchida,a Tsutomu Yamaki,a Kazuo Ohtake,a Tomomi Hatanaka,a,b Hiroyuki Uchida,a Hideo Ueda,a Jun Kobayashi,a Yasunori Morimoto,a,c and Hideshi Natsume*a,c

Faculty of Pharmaceutical Sciences, Josai University; 1–1 Keyakidai, Sakado, Saitama 350–0295, Japan; Tokai University School of Medicine; 143 Shimokasuya, Isehara, Kanagawa 259–1193, Japan; and Research Institute of TTS Technology, Josai University; 1–1 Keyakidai, Sakado, Saitama 350–0295, Japan.

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A novel system for delivering recombinant human growth hormone (rhGH) that is noninvasive and has a simple method of administration is strongly desired to improve the compliance of children. The aim of this study was to investigate the potential for the intranasal (i.n.) co-administration of rhGH with poly-l-arginine (PLA) as a novel delivery system by evaluating the effects of the concentration and molecular weight of PLA on the nasal absorption of rhGH. The influence of the formation of insoluble aggregates and a soluble complex in the dosage formulation on nasal rhGH absorption was also evaluated by size-exclusion chromatography and ultrafiltration. PLA enhanced the nasal absorption of rhGH at each concentration and molecular weight examined. Nasal rhGH absorption increased dramatically when the PLA concentration was 1.0% (w/v) due to the improved solubility of rhGH in the formulation. A delay in rhGH absorption was observed when the molecular weight of PLA was increased. This appeared to be because the increase in molecular weight caused the formation of a soluble complex. It seems that the PLA concentration affects the absorption-enhancing effect on rhGH, while the molecular weight of PLA affects the time when the maximum plasma rhGH concentration was reached (Tmax) of rhGH after i.n. administration, mainly because of the interactions among rhGH, PLA, and additives. Therefore, the transnasal rhGH delivery system using PLA is considered to be a promising alternative to subcutaneous (s.c.) injection if these interactions are sufficiently controlled.

Key words poly-l-arginine; nasal absorption; enhancer; recombinant human growth hormone

Most peptide and protein medicinal drugs are administered clinically by injection. This invasive administration with a needle and syringe is associated with various problems, such as pain for the patients, the risk of trouble at the injection site, increased medical waste and the potential for complicated operation. Hence, an alternative route of administration is strongly desired. There have been numerous studies on the mechanism of enhanced absorption by PLA in recent years, especially in the gastrointestinal tract, and have a limited permeation across the mucous membrane. Therefore, these drugs have low bioavailability.

Recombinant human growth hormone (rhGH), a single polypeptide chain of 191 amino acids and a molecular mass of 22.1 kDa, is used to treat short stature in children caused by growth hormone deficiency, Turner’s syndrome, or chronic renal failure. At present, rhGH is administered daily by subcutaneous (s.c.) injections. Therefore, compliance with rhGH therapy is often low, because children hate the pain caused by such injections. The compliance of patients affects the therapeutic outcome, because it is necessary to regularly administer injections of rhGH for the limited period of growth.4–5) Hence, a novel rhGH delivery system that is noninvasive and has a simple method of administration is strongly desired. Such a system is expected to lead to improvements in the QOL of patients and to increase the therapeutic effects.

The use of the nasal cavity as an administration site for drugs is associated with several advantages over other sites, such as the potential concentration of the drug at the local application site, an ample blood supply to the mucosa, a large surface area for permeation, avoidance of first-pass metabolism, rapid onset of action, and painless and simple administration.4–6) However, the penetration of hydrophilic macromolecular drugs is not sufficient to provide therapeutic effects, because the penetration across the nasal mucosa also depends on the molecular weight and lipophilicity of the drugs. Therefore, it is necessary to use an absorption enhancer to develop an effective nasal rhGH delivery system.

Recently, it has been demonstrated that poly cationic materials, such as poly-l-arginine (PLA), poly-l-lysine, chitosan and trimethyl chitosan, have the transmucosal absorption-enhancing effect of hydrophilic macromolecules.7–9) These materials are known to enhance the paracellular permeability via the reversible opening of tight junctions. In a previous study, we reported that PLA, a cationic polymer that consists of polymerized l-arginine, enhanced the nasal absorption of flurorescein isothiocyanate–dextran (FDs) with various molecular weights (MW 12.0–167.0 kDa) and recombinant human granulocyte colony-stimulating factor (rhG-CSF) (MW 18.8 kDa) in rats.10–12) We also reported that the absorption-enhancing effect of PLA was dependent on its concentration and molecular weight, which was associated with the charge density of the PLA molecule. Moreover, the increased absorption was reversible, without toxic effects on epithelial cells.11,13) In the studies on the mechanism of enhanced absorption by PLA in the mucosa, it has been found that PLA induces the enhanced paracellular permeability by the dispersion of tight junctions

* To whom correspondence should be addressed. e-mail: natsume@josai.ac.jp

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proteins (claudin-4, occludin, zonula occludens-1 (ZO-1) and tricellulin) into the cytoplasm through the cell–cell junction. In particular, it was known that occludin was important for macromolecular permeation among tight junction proteins. In addition, it has been also demonstrated that adherens junction protein (E-cadherin, β-catenin) is dispersed by PLA from the cell–cell junction. Hence, it is expected that the intranasal (i.n.) co-administration of rhGH with PLA can become a novel rhGH delivery system.

In the present study, using rats, we investigated the potential of performing the i.n. co-administration of rhGH with PLA, which we found to be a mucosal absorption enhancer, as a novel rhGH delivery system. The effects of the concentration and molecular weight of PLA, on the rhGH absorption across the nasal mucosa were also evaluated. rhGH and PLA respectively have negative and positive charges under physiological conditions, and a polyelectrolyte complex may be formed when both materials are mixed. Therefore, the appearance of insoluble aggregates and soluble complex in the mixture of rhGH and PLA after preparation of the dosage formulation was also evaluated by size exclusion chromatography and ultrafiltration to explain the influence of the formation of insoluble and soluble complex on the absorption enhancing effect by PLA after the i.n. administration of rhGH.

MATERIALS AND METHODS

Materials
Saizen®, obtained from Merck Serono (Tokyo, Japan), was used as recombinant human growth hormone (rhGH). Poly-L-arginine hydrochloride (MW ca. 14.8 and 147.8kDa, called PLA (15) and PLA (150), respectively), was purchased from Sigma-Aldrich Co. (MO, U.S.A.). The MW ranges of PLA (15) and PLA (150) were 5–15 and >70kDa, respectively. The Quantikine® ELISA Human Growth Hormone Immunoassay kit was purchased from R&D Systems, Inc. (MN, U.S.A.).

Animals
Male Wistar rats (eight weeks old, weight: 250–300 g) were supplied by Sankyo Labo Service Co., Ltd. (Tokyo, Japan). The animals were provided food and water ad libitum, and were kept under a 12/12 h light/dark cycle with at least 7 d of local vivarium acclimatization before experiments were carried out. All of the experiments were performed in accordance with the guidelines for animal use of the Institutional Animal Care and Use Committee at the Life Science Research Center of Josai University.

Intravenous (i.v.) Injection Study
Saizen® was dissolved according to the manufacturer’s suggestions. The rhGH solutions (0.0971, 0.194, 0.583 and 1.17 mg/mL) were prepared with physiological saline. The solution was injected into the left jugular vein of anesthetized rats (25% (w/v) urethane, 1.0 g/kg, intraperitoneally (i.p.)) which then underwent the same surgical procedure as that used in the i.n. administration study (described below) (0.0971, 0.194, 0.583 and 1.17 mg/kg all in 1.0 mL/kg).

Subcutaneous Injection Study
Saizen® was dissolved according to the manufacturer’s suggestions. An rhGH solution (0.194 mg/mL) was prepared with physiological saline. The solution was injected subcutaneously into the back of the necks of anesthetized rats as mentioned above, and then they were subjected to the same surgical procedure used in the i.n. administration study (0.194 mg/kg in 1.0 mL/kg).

Formulation for Intranasal Delivery
Saizen® was dissolved according to the manufacturer’s suggestions (rhGH: 5.83 mg/mL). The rhGH solution was mixed with or without a PLA solution (0.5, 1.0, and 2.0% (w/v), PLA (15) and PLA (150)) prepared with succinate buffer (50 mm, pH 4.4) at a ratio of 1:1, then the mixtures containing rhGH (2.91 mg/mL) and PLA (0.25, 0.5, and 1.0% (w/v)) were prepared for administration.

Intranasal Administration Study
The rats anesthetized as mentioned above were treated surgically using the method reported by Hirai et al. Briefly, a cannula was inserted into the trachea to maintain respiration, and the esophagus was occluded by another cannula inserted in the direction of the throat. The nasopalatine duct was sealed with medical adhesive (medical Aron Alpha A® superglue, Sankyo Pharmaceuticals Co., Ltd., Osaka, Japan) to prevent any test solution from escaping into the buccal cavity. The mixture was applied at a distance of 8 mm from the entrance of the left nostril via a pipette and pipette tip (0.583 mg/kg in 0.2 mL/kg).

Collection of Plasma
Blood samples were collected from the right jugular vein using a heparinized syringe at predetermined times after the administration of rhGH to rats. The samples were centrifuged at 1000×g for 15 min at 4°C to obtain plasma within 30 min of collection. The plasma was stored at −20°C until analysis.

Determination of the Plasma rhGH Concentration
The plasma rhGH concentrations were determined according to the instruction manual provided with the Quantikine® ELISA Human Growth Hormone Immunoassay kit. Briefly, a mouse monoclonal antibody against hGH was coated onto a 96-well microplate. The plasma was diluted appropriately with the diluent included in the kit, then the sample was pipetted into a well in the microplate. After incubation, a horseradish peroxidase-linked polyclonal antibody specific for hGH was added to the well as a secondary antibody. After incubation, a substrate solution containing hydrogen peroxide and tetramethylbenzidine was used to develop the color. The amount of rhGH was determined using a microplate reader (Multiskan Ascent, Thermo Fisher Scientific Inc., GA, U.S.A.) at wave-lengths of 450 and 540 nm. The sensitivity of the assay was 7.2 pg/mL with a coefficient of variation of <10%.

Evaluation of the Formation of Insoluble Aggregates and Soluble Complex after Preparation
The appearance of insoluble aggregates in the mixture of rhGH and PLA was evaluated by size exclusion chromatography. The supernatants were obtained by centrifuging the different dosage solutions at 1000×g for 15 min after their preparation, followed by dilution with mobile-phase solution. Thereafter, these samples were injected into a HPLC system to determine the rhGH and PLA concentrations in the supernatants. The HPLC system included a pump (LC-10AT), a UV detector (SPD-10A), a column oven (CTO-10A), a system controller (SCL-10A) (Shimadzu Corp., Kyoto, Japan), and a size exclusion column (Asahipak GS-320 HQ, 7.5 mm i.d. × 300 mm, Showa Denko K.K., Tokyo, Japan). The mobile phase was 0.5 M acetate buffer (pH 4.0) and methanol (20:1), and the flow rate was 0.5 or 1.0 mL/min. The UV detector was operated at 230 nm, and the column temperature was maintained at 40°C.

The formation of soluble complex in the mixture of rhGH and PLA (150) was confirmed by ultrafiltration. The dosage solution containing 1.0% (w/v) PLA (150) and 2.91 mg/
mL rhGH for i.n. administration was centrifuged at 1000×g for 15 min. The supernatant was ultrafiltered using Amicon Ultra-0.5 100K (molecular weight cut-off: 100 kDa, Millipore, Billerica, MA, U.S.A.) by centrifuging at 14000×g for 30 min. The ultrafiltrate was injected into the HPLC system mentioned above. In order to determine the retention time and intensity (peak height or area) of rhGH, rhGH only solution (2.91 mg/mL) was prepared. Phosphate buffered saline (PBS) was used as a solvent to dissolve it completely. The prepared solution was analyzed with the same procedure as mentioned above. The PLA (150) only solution (1.0% (w/v)) was also analyzed as mentioned above.

Data Analysis The plasma data for rhGH after i.v. administration were analyzed by a non-linear least squares regression program (algorithm: damping Gauss–Newton method). The plasma rhGH concentrations following i.v., s.c., and i.n. administration were extrapolated exponentially to infinity (∞). The maximum plasma rhGH concentration (C_{max}) and the T_{max} were obtained from the plasma rhGH concentration–time curve, and the area under the plasma concentration–time (0–∞) curve (AUC_{0–∞}). The mean residence time (MRT) was calculated by a moment analysis. The elimination kinetics of rhGH fitted a two-compartment model following i.v. administration. The AUC_{0–∞} was proportional to the dose administered and linear relationships between the AUC_{0–∞} after i.v. administration. The AUC_{0–∞} was proportional to the dose administered and linear relationships between the AUC_{0–∞} and the administered dose were observed. At these doses, the elimination kinetics were linear and the kinetic parameters were also almost identical for the three doses. The k_{12} (h^{-1}), k_{21} (h^{-1}), k_{10} (h^{-1}), V_{dss} (mL) and total clearance (CL_{tot}, mL/h) for the elimination parameters obtained from the i.v. data were 0.78±0.32, 1.13±0.09, 5.47±0.56, 13.37±1.43, and 44.71±3.51, respectively. The bioavailability (%) of rhGH following s.c. and i.n. administration were extrapolated exponentially to infinity (∞).

The plasma rhGH concentrations following i.v., s.c., and i.n. administration were analyzed by a non-linear least squares regression. The statistical analysis was performed using Student's t-test. A p-value of <0.05 was considered to be statistically significant.

RESULTS

Effect of the PLA Concentration on the rhGH Absorption across the Nasal Mucosa Figure 2 shows the effects of the PLA concentration on the plasma rhGH concentration after i.n. administration in rats. Table 1 summarizes the pharmacokinetic parameters of rhGH after administration under the various conditions in rats. Following s.c. injection, which is the usual route used for rhGH, T_{max} was 1.4h and F_{0–∞} was 29.6%. After i.n. administration, the plasma rhGH concentrations were increased by adding PLA to the dosage solution, although the increase in the plasma level was only slight in the control group (rhGH only). In all of the PLA groups, AUC_{0–∞} increased significantly compared with that of the control group (p<0.05). In the PLA (15) and PLA (150) groups, although the rhGH absorption across the nasal mucosa was almost the same between 0.25 and 0.5% (w/v) (F_{0–∞}: 6.7 and 6.3% in PLA (15), 9.6% and 6.0% in PLA (150), respectively), it increased dramatically (F_{0–∞}: 11.8 and 14.7%, respectively) when the PLA concentration was increased to 1.0% (w/v).

Effects of the Molecular Weight of PLA on the rhGH Absorption across the Nasal Mucosa Figure 3 shows the effects of the molecular weight of PLA on the plasma rhGH concentration after i.n. administration in rats. Although the nasal rhGH absorption in the groups with 0.25 and 1.0% (w/v) PLA showed that there was a tendency toward increased absorption with increasing PLA molecular weight, there was no significant difference between PLA (15) and PLA (150) with 0.25, 0.5, and 1.0% (w/v). A delay in the rhGH absorption was observed as the molecular weight of PLA increased for each PLA concentration, which was associated with increases in T_{max} and MRT. In particular, T_{max} in the 1.0% (w/v) PLA (150) group was 9.6h, and a remarkable delay in the rhGH absorption was observed in comparison with that in the groups treated with other concentrations and molecular weights.

Effects of the Formation of Insoluble Aggregates and Soluble Complex on the rhGH Absorption across the Nasal Mucosa The formation of insoluble aggregates was evaluated by determining the amount of soluble PLA and rhGH in the formulations used for i.n. administration after their preparation, and the impact of these aggregates on the rhGH absorption across the nasal mucosa was investigated. Figures 4 and 5, respectively, show the amounts of PLA and rhGH in the supernatants after mixing rhGH with the PLA solutions. In the PLA (15) and PLA (150) groups, the amount of PLA in the supernatant was almost the same as that in the formulation prepared for each PLA concentration. On the other hand, the amount of rhGH in the supernatant increased with an increase in the PLA concentration. The rhGH concentration in the supernatant of the formulation prepared without PLA was about 35% of that in the prepared formulation. However, the amount of rhGH in the supernatant was the same as that in the for-
Table 1. The Pharmacokinetic Parameters of rhGH after i.n. Administration under Various Conditions in Rats

<table>
<thead>
<tr>
<th>Route</th>
<th>Dose (mg/kg)</th>
<th>Enhancer</th>
<th>$C_{\text{max}}$ (ng/mL)</th>
<th>$T_{\text{max}}$ (h)</th>
<th>$AUC_{0-\infty}$ (ng·h/mL)</th>
<th>MRT (h)</th>
<th>$F_{0-\infty}$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>i.v.</td>
<td>0.194</td>
<td>None</td>
<td>—</td>
<td>—</td>
<td>1238.32±232.27</td>
<td>0.26±0.03</td>
<td>100.00</td>
</tr>
<tr>
<td>s.c.</td>
<td>0.194</td>
<td>None</td>
<td>85.24±10.77</td>
<td>1.25±0.24</td>
<td>365.93±33.35</td>
<td>3.68±0.53</td>
<td>29.55</td>
</tr>
<tr>
<td>i.n.</td>
<td>0.583</td>
<td>None (Control)</td>
<td>13.41±3.23</td>
<td>1.38±0.38</td>
<td>91.78±29.91</td>
<td>5.29±0.91</td>
<td>2.47</td>
</tr>
<tr>
<td></td>
<td>0.25% PLA (15)</td>
<td>49.00±3.77**</td>
<td>3.00±0.58</td>
<td>249.12±28.47*</td>
<td>5.08±0.20</td>
<td>6.69</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.5% PLA (15)</td>
<td>53.74±2.15**</td>
<td>4.00±0.00</td>
<td>234.23±45.28*</td>
<td>4.22±0.52</td>
<td>6.29</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.0% PLA (15)</td>
<td>76.48±15.17**</td>
<td>6.00±0.00</td>
<td>437.19±116.24*</td>
<td>5.44±0.41</td>
<td>11.75</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.25% PLA (150)</td>
<td>35.26±5.14*</td>
<td>6.00±0.73</td>
<td>356.81±69.22*</td>
<td>10.42±2.79</td>
<td>9.59</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.5% PLA (150)</td>
<td>29.26±5.19</td>
<td>6.00±0.73</td>
<td>223.31±25.46*</td>
<td>7.03±0.43</td>
<td>6.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.0% PLA (150)</td>
<td>63.80±6.77**</td>
<td>9.60±0.40</td>
<td>545.82±54.07**</td>
<td>9.56±0.32**</td>
<td>14.67</td>
<td></td>
</tr>
</tbody>
</table>

Each data point represents as the mean±S.E. ($n=3–6$). $F_{0-\infty}$ (%)=$\left\{\frac{AUC_{0-\infty,\text{test}}}{Dose_{\text{test}}}\right\} / \left\{\frac{AUC_{0-\infty,\text{i.v.}}}{Dose_{\text{i.v.}}}\right\} \times 100$. *$p<0.05$, **$p<0.01$ compared with the control (Student’s $t$-test).
mulation prepared when 1.0 % (w/v) PLA was used for each molecular weight (about 100%).

The formation of soluble complex in the dosage solution containing PLA (150) and rhGH was confirmed by ultrafiltration. Figure 6 shows the size exclusion chromatograms of ultrafiltrate of supernatant after centrifuging the dosage solution with 1.0 % (w/v) PLA (150) for i.n. administration. In rhGH only solution (2.91 mg/mL), the single peak of rhGH was obtained about 15 min after injecting the ultrafiltrate. In the dosage solution containing 1.0 % (w/v) PLA (150) and 2.91 mg/mL rhGH, the intensity (peak height or area) in rhGH peak around 15 min was lower than that in 2.91 mg/mL rhGH only solution, and the peak area of rhGH in the dosage solution was about 26% of that in rhGH only solution. These results indicate the possibility of formation of soluble complex of rhGH in the dosage solution containing PLA (150) and rhGH for i.n. administration.

DISCUSSION

We investigated the effects of the concentration and molecular weight of PLA on the rhGH absorption across the nasal mucosa to develop a novel delivery system for rhGH using PLA as a transmucosal absorption enhancer. Furthermore, the influence of the formation of insoluble aggregates and soluble complex on the enhancing effect of PLA after the i.n. administration of rhGH was also evaluated. Although the rhGH absorption across the nasal mucosa was almost the same between the 0.25 and 0.5 % (w/v) of PLA (15) and PLA (150), it increased dramatically when the PLA concentration was 1.0 % (w/v). This is likely because the rhGH did not dissolve completely in the dosage solution when the PLA concentration was 0.25 or 0.5 % (w/v), but it almost completely dissolved in the 1.0 % (w/v) PLA. On the other hand, PLA almost completely dissolved at all of the PLA concentrations examined. Therefore, these results suggest that the increase in the nasal absorption of rhGH in 1.0 % (w/v) PLA was caused by the improved solubility of rhGH in the dosage solution, that
is, the solution was at least partly in suspension in the 0.25 and 0.5% (w/v) PLA, but was in solution in the 1.0% (w/v) PLA. In 1.0% (w/v) PLA, the $F_{0-\infty}$ (PLA (15) and PLA (150) ; 11.8 and 14.7%, respectively) was similar to that in rhG-CSF (11.3%) after i.n. administration with 1.0% (w/v) PLA (MW ca. 45.5 kDa) and to that predicted from the relationship between the molecular weight of FDS and the $F_{0-\infty}$ in our previous study. These results suggest that PLA also has the nasal absorption enhancing effect in rhGH. Also, the $F_{0-\infty}$ was about 1.2% when the rhGH solution, dissolved completely in PBS without PLA, was administered intranasally (data not shown). Therefore, it seems that nasal rhGH absorption is increased by increasing the solubility and the paracellular permeability of rhGH by PLA as the same mechanism identified previously.

With regard to the effects of the molecular weight of PLA, a delay in the rhGH absorption was observed with an increase in the molecular weight. In particular, $T_{\text{max}}$ in 1.0% (w/v) PLA (150) was 9.6h, and the rhGH absorption was remarkably delayed compared with that in the solutions with other concentrations and molecular weights of PLA. In our previous study using FD-20 (MW 21.0 kDa) as a model drug and PLA (100) as an enhancer, $T_{\text{max}}$ was about 2h (data not shown), so $T_{\text{max}}$ for rhGH was longer than that for FD-20. The PLA and rhGH almost completely dissolved in the dosage solution at 1.0% (w/v) PLA (150). However, it was found that soluble complex were formed in the dosage solution by the results in ultrafiltration. It was expected that free rhGH was absorbed through the paracellular route, that was opened by PLA, between the nasal epithelial cells, but soluble complex of rhGH were difficult to be absorbed as well as insoluble aggregates. Therefore, it seems that the delay in nasal rhGH absorption in the 1.0% (w/v) PLA (150) group was due to the increase in appearance molecular weight caused by the formation of soluble complex with rhGH, that is, when free rhGH was absorbed, it was produced from soluble complex by that amount. In Fig. 6, PLA (150) peak was seen around 10 min after injecting ultrafiltrate of PLA only solution, although it was usually not obtained because of using the ultrafilter with molecular weight cut-off of 100 kDa. This is likely because PLA has a wide range of molecular weight (PLA (150): >70 kDa). Hence, it seemed that the multiform soluble complex with rhGH were formed. It was difficult to determine the multiform soluble complex and separate them. Therefore, it was impossible to analyze using the bonding constant about soluble complex in this study. The delay in rhGH absorption may also be related to the stability of PLA in the nasal cavity. It was previously demonstrated that the enhancing effect of PLA was reduced by a decrease in the molecular weight due to enzymatic degradation in the nasal cavity.

We initially expected that the polyelectrolyte complex consisting of rhGH and PLA was formed because rhGH and PLA have negative and positive charges, respectively. Actually, when mixing of rhGH and PLA solutions prepared at neutral pH, it occurred the turbidity. The turbidity seems to influence the enhancing effect of PLA and permeability of rhGH, so we attempt to reduce the turbidity of dosage solution by changing pH. The formulation used in this study was found. However, rhGH was dissolved by adding PLA in this formulation, and the PLA was almost completely dissolved in each of the concentrations examined. Therefore, the reduced solubility of rhGH in succinate buffer or the formation of insoluble aggregates consisting of rhGH and succinic acid after the preparation and administration of the dosage solution may have affected the nasal rhGH absorption. Moreover, the formation of soluble complex by rhGH also seems to affect the absorption. The mechanism of formation of insoluble and soluble complex has not been found. It might be related to the change in three-dimensional structure of rhGH by isoelectric point and low pH. Insoluble aggregate was formed when rhGH was mixed with succinate buffer at pH 4.4, but it was not formed in other solution at pH 4.4 (data not shown). Therefore, it seems that the formation is related to other reasons such as sort of solvent, ionic strength.

In the present study, PLA enhanced the nasal rhGH absorption, and the effects of the concentration and molecular weight of PLA were different from the results in our previous study using FDs largely because of the interaction between the rhGH and PLA with various concentrations and molecular weights, as well as other components present in the formulation after preparation and administration. However, it seems that the PLA concentration affects the absorption-enhancing effect of PLA on rhGH, and the molecular weight affects $T_{\text{max}}$ after i.n. administration. The interaction between rhGH and PLA may prove to be useful for developing a drug delivery system, such as a controlled-release formulation. In addition, the nasal rhGH absorption for each of the rhGH delivery systems using PLA was higher than that in the control group. It has been reported that PLA does not induce significant damage to the nasal mucosa. Therefore, a transnasal rhGH delivery system using PLA as an enhancer is a promising alternative to s.c. injection provided that the interactions among rhGH, PLA and additives are sufficiently controlled.

Conflict of Interest The authors declare no conflict of interest.

REFERENCES


