Note

Enhancement Effect of Poly-L-ornithine on the Nasal Absorption of Water-Soluble Macromolecules in Rats

Shigehiro Omori,[a] Yusuke Kamiya,[a,b] Tsutomu Yamaki,[a] Masaki Uchida,[a] Kazuo Ohtake,[a] Mitsutoshi Kimura,[a] and Hideshi Natsume*,[a]

[a] *Faculty of Pharmacy and Pharmaceutical Sciences, Josai University; 1–1 Keyakidai, Sakado, Saitama 350–0295, Japan: and* [b] *Laboratory of Drug Metabolism and Pharmacokinetics, Showa Pharmaceutical University; 3–3165 Higashi-tamagawa Gakuen, Machida, Tokyo 194–8543, Japan.*

Received August 31, 2018; accepted October 29, 2018

The transnasal route for the delivery of water-soluble macromolecules, such as bioactive peptides and proteins, has attracted interest, although the use of permeation enhancers is required due to the poor permeabilities of these macromolecules across the nasal mucosa. With polycationic compounds, such as poly-L-arginine and chitosan, the nasal absorption of hydrophilic macromolecules is molecular weight- and concentration-dependently enhanced without causing cytotoxicity. In the present study, we evaluated the effect of various molecular weights and concentrations of poly-L-ornithine (PLO), a polycationic compound, on the nasal absorption and the damage to the nasal mucosa *in vivo*. PLO enhanced the nasal absorption of fluorescein isothiocyanate-dextran (FD-4), used as a model drug, and the bioavailability of FD-4 increased with the concentration of PLO. The enhancement effect was also dependent on the molecular weight. The administration of PLO at a concentration that sufficed for enhancing the nasal absorption had no effect on the activity of lactic dehydrogenase and the protein leakage in the nasal fluid, as indices of nasal mucosa damage. These findings suggest that a transnasal delivery system using PLO is a useful strategy for improving the nasal absorption of water-soluble macromolecules without toxicity to the nasal mucosa.

Key words poly-L-ornithine (PLO); nasal absorption; enhancement effect; molecular weight

INTRODUCTION

Water-soluble macromolecules, including peptide and protein drugs, have been developed as injection formulations that are administered invasively. However, there are some problems associated with injection formulations, such as an increased risk of inflammation and infection at the injection site as well as a decrease in medication compliance of patients due to pain from the injection.[1,2] Administration across the nasal mucosa might be able to solve these problems and has therefore recently been explored as an alternative route for delivering water-soluble macromolecules.

In addition to simple handling and painless administration, there are many advantages associated with administering molecules across the nasal mucosa, where many vessels exist, such as the efficient and rapid absorption and the avoidance of hepatic first-pass metabolism.[3–5] However, the low bioavailability of water-soluble macromolecules is mainly caused by their poor permeability across the nasal mucosa.[6] Therefore, various efforts to improve the nasal absorption of these macromolecules have been made using many kinds of permeation-enhancing candidates, including surfactants, bile salts, and chelating agents.[7–10] However, many of these candidates, which sufficiently improve the bioavailability, are also strong irritants and toxic to the mucosa, thereby hampering their application in clinical practice.

Chitosan is a cationic polysaccharide that improves the nasal permeability of insulin without exerting either mucosal membrane or cellular damage.[11,12] In addition, poly-L-arginine (PLA), a cationic polymer, has also been shown to increase the permeability of various hydrophilic macromolecules without exerting any cytotoxic effect on the nasal membrane.[13–15] All of these permeation enhancers, which both have an enhancing ability and are safe, are cationic polymers. We therefore focused on the cationic polymer poly-L-ornithine (PLO) as a promising permeation enhancer. Moreover, we previously reported that the polyethylene glycol (PEG) modification of PLO was relatively easy compared to PLA, indicating that PLO has the potential for gaining another function by the modification in accordance with various purposes.[16] Of note, it has been reported that the enhancing effects of chitosan and PLA are dependent on the molecular weight and concentration applied.[11–15,17] This suggests that the enhancement effect of PLO may also be influenced by its molecular weight and concentration, but there were only few reports about its effect of PLO.

In this study, we assessed the effect of the molecular weight and concentration of PLO on the nasal absorption of fluorescein isothiocyanate (FITC)-dextran (molecular weight (MW), *ca.* 4 kDa; FD-4) used as a model drug of water-soluble macromolecules in rats. The irritation and toxicity of PLO for the nasal epithelium were also evaluated.

MATERIALS AND METHODS

Reagents Poly-L-ornithine hydrobromide (MW, *ca.* 5.8, 20 and 78 kDa for PLO (5), PLO (20) and PLO (80), respectively) was purchased from Alamanda Polymers, Inc. (Huntsville, AL, U.S.A.). Fluorescein isothiocyanate-dextran (FD-4, MW 4.0 kDa) and poly-L-arginine hydrochloride (PLA, MW 44.3 kDa for PLA (45)) were obtained from Sigma-Aldrich Co. (St. Louis, MO, U.S.A.). Sodium deoxycholate (DC) was sup-

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

plied by Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All other reagents used were of reagent grade.

Animals Male 8-week-old Wistar rats (Sankyo Labo Service Co., Ltd., Tokyo, Japan), weighing 240–280 g, were used in all animal experiments. They were allowed food and water *ad libitum*, and were housed in group cages ($n = 3$–4) in a room under controlled temperature and a 12-h light-dark cycle. All of the experiments were approved by the Institutional Animal Care and Use Committee at the Life Science Research Center of Josai University and were performed in accordance with the guidelines stipulated by the same committee.

Pharmacokinetic Study

Intravenous (i.v.) Injection Study

The rats were anesthetized with intraperitoneal injections of urethane (25% (w/v), 1.0 g/kg) and treated with the same surgical procedure used in the nasal absorption study (described below). An FD-4 solution (3.3 mg/kg, 3.3 mg/mL in saline) was injected into the left jugular vein.

Nasal Absorption Study

The rats anesthetized as mentioned above were then surgically treated using the method of Hirai *et al.*[18] In brief, a polyethylene tube was inserted into the trachea to secure the airway. The nasal cavity was occluded by inserting another tube from the esophagus to the throat and sealing the nasopalatine duct with a medical adhesive (Aron Alpha A®; Daiichi Sankyo Co., Ltd., Tokyo, Japan) to prevent the escape of any test solution. Intranasal (i.n.) doses of FD-4 (33 mg/kg, 165 mg/mL in saline) containing different concentrations (0.025, 0.05, 0.1, 0.5 and 1.0% (w/v)) of PLO (5), PLO (20) and PLO (80) were administered at a distance of 8 mm from the entrance of the left nostril *via* a polyethylene tube attached to a microsyringe. For comparison, FD-4 solutions with/without 0.5% (w/v) PLA (45) were also administered at the same dose as that in the groups co-administered with PLO. At the appropriate time after FD-4 administration, blood samples were collected from the right jugular vein using a heparinized syringe and then centrifuged at 15000 rpm for 5 min at 4°C to obtain the plasma.

Determination of the Plasma FD-4 Concentration

The fluorescence intensity of FD-4 in the plasma samples was determined fluorometrically (excitation 495 nm and emission 515 nm) using a spectrofluorophotometer (RF-5300PC; Shimadzu, Kyoto, Japan).

Data Analyses

A noncompartmental analysis was performed for the plasma FD-4 profiles to calculate the pharmacokinetic parameters, including the maximum plasma concentration (C_{max}), the time of maximum plasma concentration (T_{max}) and the mean residence time (MRT). The area under the plasma concentration–time curve (*AUC*) from time zero to the last measured concentration was determined by the trapezoidal rule, and the absolute bioavailability (F) following i.n. administration was calculated. The plasma FD-4 profiles after i.v. administration were fitted to a two-compartment model using a non-linear least squares regression program (algorithm: Damping Gauss–Newton method) to obtain the elimination kinetic parameters. The absorption FD-4 profile after i.n. administration was calculated based on a deconvolution method applied to the i.n. and i.v. data and the elimination parameters. The maximum absorption rate (MAR) was obtained from the slope of this profile.

Cytotoxic Study To evaluate the irritation and toxicity caused by the application of PLO to the nasal mucosa, PLO (20) (0.1% (w/v), 0.1 mL/kg) was applied to the nasal cavity of a surgically treated rat in the same manner as that described in the nasal absorption study (described above). The nasal cavity was washed with phosphate-buffered saline (PBS, 10 mL) 2 h after PLO application. The wash solution was collected and then centrifuged at $200 \times g$ for 7 min at 4°C to obtain the supernatant. The activity of lactic dehydrogenase (LDH) and the protein leakage were measured in the supernatant using a CytoTox 96® Non-Radioactive Cytotoxicity Assay (Promega Co., WI, U.S.A.) and a Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific Inc., MA, U.S.A.), respectively, as indices of nasal mucosa damage.[9,13,19] For comparison purposes, DC (1.0% (w/v) in saline), which is toxic to the nasal mucosa, was applied to the nasal cavity.[7,8,13,19] In addition, saline alone and PLA (45) (0.1% (w/v) in saline), which have no cytotoxicity, were also applied to the nasal cavity, and the results were compared to that of PLO (20).[13,14]

Statistical Analyses The data were expressed as the mean ± standard error (S.E.). The statistical differences between each group were analyzed by one-way ANOVA with the *post-hoc* Student's *t*-test and Dunnett's multiple comparisons test. The variation in the *AUC* achieved for PLO of different molecular weights was compared using a two-way ANOVA. The level of significance was considered to be $p < 0.05$.

RESULTS

Effect of Various Molecular Weights and Concentrations of PLO on Nasal Absorption of FD-4 Figure 1 shows the plasma concentration profiles of FD-4 after i.n. administration of PLO at various molecular weights and concentrations.

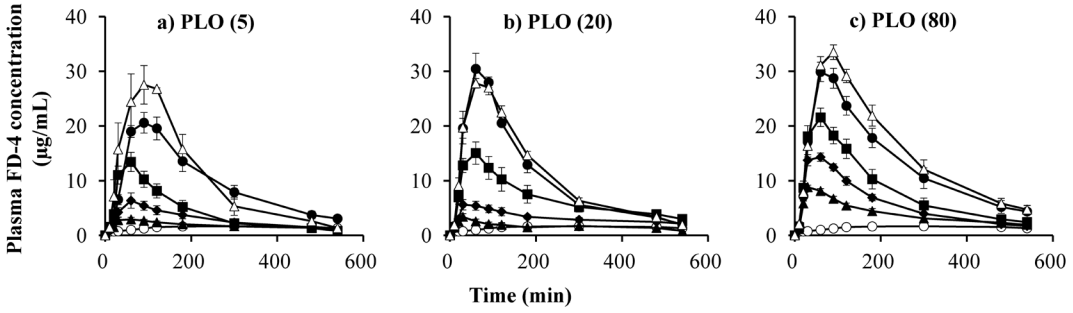

Fig. 1. Plasma FD-4 Profiles after i.n. Co-administration with Poly-L-ornithine of Different Molecular Weights and Concentrations in Rats

PLO molecular weight: a) PLO (5), b) PLO (20), c) PLO (80). ○: control (FD-4 alone), ▲: 0.025% (w/v) PLO, ◆: 0.05% (w/v) PLO, ■: 0.1% (w/v) PLO, ●: 0.5% (w/v) PLO, △: 1.0% (w/v) PLO. The results are expressed as the mean ± S.E. ($n = 3$–6).

The obtained pharmacokinetic parameters are summarized in Table 1. PLOs with different molecular weights improved the nasal absorption of FD-4 at all concentrations and significantly increased the C_{max} and the AUC compared to the control solution (FD-4 alone), except for 0.025% (w/v) PLO (5) and PLO (20). Furthermore, PLO increased the plasma FD-4 levels and tended to prolong the T_{max} in a concentration-dependent manner, indicating that the nasal absorption-enhancing effect was increased and prolonged with increasing PLO concentration. However, there were no significant differences between 0.5 and 1.0% (w/v) PLO at any molecular weights, suggesting that FD-4 absorption across the nasal mucosa became saturated near 0.5% (w/v), regardless of the molecular weight of PLO.

Significant differences were noted among the AUC_{0-540} values achieved for PLOs with various molecular weights (two-way ANOVA, $p<0.001$), and the F_{0-540} of PLO (80) was higher than those of PLO (5) and PLO (20) at the same concentration. Therefore, the nasal FD-4 absorption was considered to be dependent on the molecular weight of PLO, with PLO (80) providing more effective enhancement than PLO (5) or PLO (20). The $AUCs_{0-540}$ values in the PLO (20) and PLO (80) groups were higher than that in the PLA (45) group at the same concentration. Notably, the $AUCs$ obtained from applying 0.1% (w/v) PLO (20) and PLO (80) were similar to that of 0.5% (w/v) PLA (45), indicating that the enhancement effect of PLO on the nasal permeability of FD-4 was nearly 5 times that of PLA.

Figure 2 shows the typical absorption profiles of FD-4 after nasal administration, calculated based on a deconvolution method. The absorption profile after the i.n. administration of

Table 1. Pharmacokinetic Parameters of FD-4 after i.n. Co-administration with Poly-L-ornithine of Different Molecular Weights and Concentrations in Rats

Route	Dose (mg/kg)	Enhancer	C_{max} (μg/mL)	T_{max} (min)	AUC_{0-540} (μg·min/mL)	MRT_{0-540} (min)	F_{0-540} [a] (%)	MAR (μg/min)
i.v.	3.3	—	—	—	1142 ± 107	100 ± 9	—	—
i.n.	33	None (control)	1.9 ± 0.3	252 ± 48	796 ± 92	288 ± 3	6.5	2.0
		PLA (45)						
		0.5%	18.1 ± 0.8**	60 ± 15	3427 ± 319**	166 ± 12	27.9	28.3
		PLO (5)						
		0.025%	3.1 ± 0.4	60 ± 13	965 ± 94	227 ± 13	7.8	5.9
		0.05%	6.6 ± 1.4*	75 ± 7	1558 ± 321*	196 ± 13	12.7	9.6
		0.1%	13.9 ± 1.8***	50 ± 6	2285 ± 422*	149 ± 11	18.6	25.7
		0.5%	22.2 ± 1.6***	90 ± 9	5123 ± 514***	201 ± 12	41.6	27.2
		1.0%	29.4 ± 2.6***	100 ± 20	5614 ± 774***	157 ± 12	45.6	37.6
		PLO (20)						
		0.025%	3.5 ± 0.6	35 ± 5	913 ± 226	220 ± 15	7.4	6.8
		0.05%	6.3 ± 0.7*	45 ± 10	1726 ± 185*	228 ± 18	14.0	12.8
		0.1%	15.3 ± 2.0**	48 ± 7	3567 ± 492***	201 ± 17	29.0	31.1
		0.5%	30.8 ± 2.7***	70 ± 8	5507 ± 426***	160 ± 13	44.8	46.7
		1.0%	29.8 ± 0.7***	65 ± 9	5758 ± 167***	165 ± 7	46.8	47.0
		PLO (80)						
		0.025%	8.9 ± 0.3**	40 ± 6	2114 ± 198*	206 ± 11	17.2	20.0
		0.05%	15.2 ± 0.6***	55 ± 9	3091 ± 170**	180 ± 4	25.1	31.6
		0.1%	21.7 ± 1.7***	54 ± 6	4472 ± 623***	176 ± 10	36.3	43.7
		0.5%	30.0 ± 1.8***	65 ± 5	7057 ± 708***	193 ± 11	57.4	45.8
		1.0%	34.5 ± 0.9***	80 ± 6	8110 ± 596***	195 ± 9	65.9	45.8

The results are expressed as mean or mean ± standard error ($n=3$–6). *a)* F_{0-540} (%) = $[AUC_{i.n.}/(AUC_{i.v.}\times 10)]\times 100$. *$p<0.05$, **$p<0.01$ and ***$p<0.001$ compared with i.n. control. There was a significant difference among the AUC_{0-540} values for poly-L-ornithine of different molecular weights (two-way ANOVA, $p<0.001$).

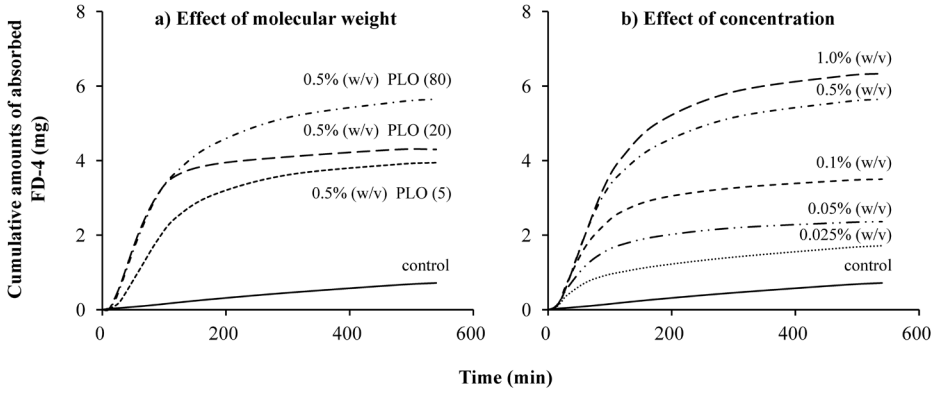

Fig. 2. Simulated Absorption Profiles of FD-4 after i.n. Co-administration with Poly-L-ornithine

The absorption profiles after i.n. administration of FD-4 were calculated using a deconvolution method with the i.n. and i.v. data and the elimination parameters. The results are expressed as the mean value ($n=3$–6).

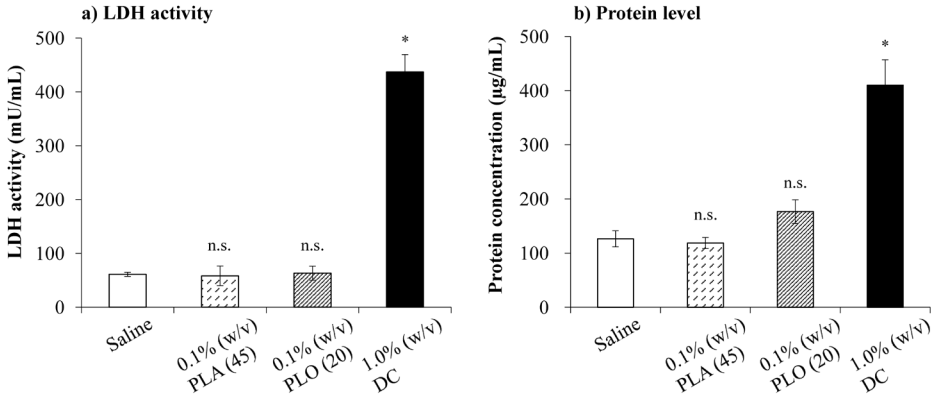

Fig. 3. a) The LDH Activities and b) the Protein Levels in the Nasal Cavity Lavage Fluid 2 h after the i.n. Administration of Various Enhancers in Rats

The results are expressed as the mean ± S.E. ($n = 3$). *$p < 0.05$ compared with saline. n.s.: not significant.

FD-4 alone increased linearly, indicating that it was absorbed according to zero-order kinetics. In contrast, the amount of absorbed FD-4 began to increase, and the MAR was observed immediately after a lag time when PLOs with different molecular weights were co-administered at the same concentration (Fig. 2a). The level of MAR and the cumulative amounts of absorbed FD-4 were dependent on the molecular weights of PLO. The absorption profiles of FD-4 after treatment with PLO (80) at different concentrations were also increased in a concentration-dependent manner (Fig. 2b).

Cytotoxicity of PLO on the Nasal Mucosa Figure 3 shows the activity of LDH and the protein leakage in the nasal lavage fluid after the application of various absorption enhancers to rat nasal cavity. The activity of LDH and the protein leakage in the DC exposure group were significantly higher than those in the saline exposure group. In contrast, the activity of released LDH and the protein leakage in the polycationic compounds (PLA and PLO) exposure group were similar to those in the saline exposure group, indicating no cytotoxic effects on the nasal mucosa by the application of 0.1% (w/v) PLA (45) or 0.1% (w/v) PLO (20). Scanning electron microphotograph of the rat nasal mucosa 2 h after nasal application of 0.1% (w/v) PLO (20) was almost the same as that of the saline (data not shown), indicating no cytotoxic effect on the nasal mucosa by the application of 0.1% (w/v) PLO (20) histologically, as well as PLA (45).[13,14]

DISCUSSION

The absorption route across the nasal mucosa has generated interest as a potential delivery route of water-soluble macromolecules that are otherwise difficult to absorb without an injection.[4,20] However, the use of absorption enhancers is essential for delivering these macromolecules due to their poor permeabilities across the nasal mucosa.[5,6] Cationic polymers, such as chitosan and PLA, enhance the *in vivo* nasal absorption of various water-soluble macromolecules without cytotoxicity to the nasal mucosa.[11–15,17] It has also been reported that these absorption-enhancing effects are dependent on the molecular weight and concentration. Thus, the molecular weight and concentration of polycationic compounds may be involved in their enhancing effect on the absorption of polar macromolecules.

Recent investigations have shown that PLO enhanced the uptake of FITC-insulin in cultured alveolar type II epithelial cells without cytotoxicity.[21] In addition, the hypoglycemic action of insulin was increased by pulmonary co-administration with PLO in an *in vivo* rat model. We also reported that PLO improved the *in vivo* nasal absorption of FD-4, and the improvement was dependent on its concentration.[22] In addition, we showed that PLO enhanced the paracellular permeability of polar macromolecules by changing the localization of tight junction proteins, including occludin and claudin-4 in Caco-2 cells. However, the relationship between the enhancement effect of PLO on the nasal absorption and the molecular weights and concentrations of PLO applied remains unclear. We therefore examined the enhancement effect of the molecular weights and concentrations of PLO on the *in vivo* nasal absorption of FD-4 used as a model drug of water-soluble macromolecules.

As shown in Figs. 1 and 2, PLO dramatically increased the plasma FD-4 levels in a concentration-dependent manner, with the exception of 0.025% (w/v) PLO (5) and PLO (20). These results suggest that the concentration of PLO that was applied may be an important factor in determining the degree of enhancement absorption. However, there were only slight differences between the pharmacokinetic parameters of the groups administered 0.5 and 1.0% (w/v) PLO, and thus the enhancing effect of PLO on nasal FD-4 absorption peaked near 0.5% (w/v). In addition, increasing the PLO concentration tended to prolong the T_{max} of FD-4, suggesting that the persistence of enhancement by PLO may be attributed to the concentration of PLO applied. Previous reports have shown that the T_{max} of FD-4 was prolonged by increasing the PLA concentration, and the enhancement effect of PLA on the nasal permeability of FD-4 was attenuated due to degradation by several proteolysis enzymes in the nasal cavity when PLA was nasally administered to rats.[14,23] Thus, PLO also seems to be decomposed by the enzymes in the nasal cavity, resulting in a prolongation of the T_{max} of FD-4, similar to PLA. Significant differences in the *AUCs* were noted among the groups treated with PLOs at different molecular weights (two-way ANOVA, $p < 0.001$). PLO with a higher molecular weight effectively enhanced the nasal absorption of FD-4, indicating that PLO at each applied concentration enhanced the nasal absorption in a molecular weight-dependent manner. These findings suggest that the absorption enhancement by PLO can be controlled by regulating its molecular weight and concentration. In com-

parison with PLA and PLO, the *AUCs* of PLO (20) and PLO (80) were higher than that of PLA (45) at the same concentration. Of note, *AUCs* in the 0.1% (w/v) PLO (20) and PLO (80) groups were comparable to that in 0.5% (w/v) PLA (45) group. These results indicate that the nasal absorption enhancing-effect of PLO was approximately 5 times higher than that of PLA. This could be due to the difference of the side-chain in PLA and PLO. Further study is needed to explore this difference of absorption enhancement in polycationic amino acids.

Research into the irritation and toxicity of penetration enhancers on the mucosa is important for clinical practice. We therefore evaluated the activity of LDH and the protein leakage in the nasal fluid after nasal PLO application, as these parameters are correlated with cell damage.[13,19] As shown in Fig. 3, PLO (20) induced no membrane damage when applied at 0.1% (w/v), a concentration that exhibited sufficient enhancement of nasal penetration. In our previous study, PLA with different molecular weights also showed no effects on the LDH activity and the protein leakage from extirpated rabbit nasal mucosa, whereas the amount of phospholipids leakage after nasal application of PLA with a higher molecular weight was slightly higher than that of saline.[13] However, the effect of PLA with different molecular weights on the rat nasal mucosa was not observed in the histological study.[14] Thus, further study is also needed to evaluate the effect of PLO with different molecular weights on the nasal mucosa. Although further investigations into the degradability and cytotoxicity of PLO to the nasal epithelium will be required, our findings suggest that a transnasal delivery system using PLO is a useful strategy for enhancing the *in vivo* nasal absorption of water-soluble macromolecules without inducing toxicity to the nasal mucosa.

Acknowledgments We thank Yuki Kawamura, Iori Yamazaki, and Shigeru Hashimoto for their technical assistance in the pharmacokinetic study.

Conflict of Interest The authors declare no conflict of interest.

REFERENCES

1) Drudge-Coates L. Improving management of patients with advanced cancer. *Patient Prefer. Adherence*, **4**, 415–424 (2010).
2) Peyrot M, Rubin RR, Kruger DF, Travis LB. Correlates of insulin injection omission. *Diabetes Care*, **33**, 240–245 (2010).
3) Mygind N, Dahl R. Anatomy, physiology and function of the nasal cavities in health and disease. *Adv. Drug Deliv. Rev.*, **29**, 13–38 (1998).
4) Harris AS. Review: Clinical opportunities provided by the nasal administration of peptides. *J. Drug Target.*, **1**, 101–116 (1993).
5) McMartin C, Hutchinson LEF, Hyde R, Peters GE. Analysis of structural requirements for the absorption of drugs and macromolecules from the nasal cavity. *J. Pharm. Sci.*, **76**, 535–540 (1987).
6) Donovan MD, Flynn GL, Amidon GL. The molecular weight dependence of nasal absorption: the effect of absorption enhancers. *Pharm. Res.*, **7**, 808–815 (1990).
7) Merkus FWHM, Schipper NGM, Hermens WAJJ, Romeijn SG, Verhoef JC. Absorption enhancers in nasal drug delivery: efficacy and safety. *J. Control. Release*, **24**, 201–208 (1993).
8) Kubo H, Hosoya KI, Natsume H, Sugibayashi K, Morimoto Y. *In vitro* permeation of several model drugs across rabbit nasal mucosa. *Int. J. Pharm.*, **103**, 27–36 (1994).
9) Marttin E, Verhoef JC, Romeijn SG, Merkus FWHM. Effects of absorption enhancers on rat nasal epithelium *in vivo*: release of marker compounds in the nasal cavity. *Pharm. Res.*, **12**, 1151–1157 (1995).
10) Marttin E, Verhoef JC, Cullander C, Romeijn SG, Nagelkerke JF, Merkus FWHM. Confocal laser scanning microscopic visualization of the transport of dextrans after nasal administration to rats: effects of absorption enhancers. *Pharm. Res.*, **14**, 631–637 (1997).
11) Illum L, Farraj NF, Davis SS. Chitosan as a novel nasal delivery system for peptide drugs. *Pharm. Res.*, **11**, 1186–1189 (1994).
12) Aspden TJ, Illum L, Skaugrud Ø. Skaugrud. Chitosan as a nasal delivery system: evaluation of insulin absorption enhancement and effect on nasal membrane integrity using rat models. *Eur. J. Pharm. Sci.*, **4**, 23–31 (1996).
13) Natsume H, Iwata S, Ohtake K, Miyamoto M, Yamaguchi M, Hosoya KI, Kobayashi D, Sugibayashi K, Morimoto Y. Screening of cationic compounds as an absorption enhancer for nasal drug delivery. *Int. J. Pharm.*, **185**, 1–12 (1999).
14) Miyamoto M, Natsume H, Iwata S, Ohtake K, Yamaguchi M, Kobayashi D, Sugibayashi K, Yamashina M, Morimoto Y. Improved nasal absorption of drugs using poly-L-arginine: effects of concentration and molecular weight of poly-L-arginine on the nasal absorption of fluorescein isothiocyanate-dextran in rats. *Eur. J. Pharm. Biopharm.*, **52**, 21–30 (2001).
15) Miyamoto M, Natsume H, Satoh I, Ohtake K, Yamaguchi M, Kobayashi D, Sugibayashi K, Morimoto Y. Effect of poly-L-arginine on the nasal absorption of FITC-dextran of different molecular weights and recombinant human granulocyte colony-stimulating factor (rhG-CSF) in rats. *Int. J. Pharm.*, **226**, 127–138 (2001).
16) Kamiya Y, Yamaki T, Uchida M, Hatanaka T, Kimura M, Ogihara M, Morimoto Y, Natsume H. Preparation and evaluation of PEGylated poly-L-ornithine complex as a novel absorption enhancer. *Biol. Pharm. Bull.*, **40**, 205–211 (2017).
17) Schipper NGM, Vårum KM, Artursson P. Chitosans as absorption enhancers for poorly absorbable drugs. 1: Influence of molecular weight and degree of acetylation on drug transport across human intestinal epithelial (Caco-2) cells. *Pharm. Res.*, **13**, 1686–1692 (1996).
18) Hirai S, Yashiki T, Matsuzawa T, Mima H. Absorption of drugs from the nasal mucosa of rats. *Int. J. Pharm.*, **7**, 317–325 (1981).
19) Uchiyama T, Yamamoto A, Hatano H, Fujita T, Muranishi S. Effectiveness and toxicity screening of various absorption enhancers in the large intestine: intestinal absorption of phenol red and protein and phospholipid release from the intestinal membrane. *Biol. Pharm. Bull.*, **19**, 1618–1621 (1996).
20) Illum L. Nasal drug delivery—recent developments and future prospects. *J. Control. Release*, **161**, 254–263 (2012).
21) Oda K, Yumoto R, Nagai J, Katayama H, Takano M. Enhancement effect of poly(amino acid)s on insulin uptake in alveolar epithelial cells. *Drug Metab. Pharmacokinet.*, **27**, 570–578 (2012).
22) Kamiya Y, Yamaki T, Omori S, Uchida M, Ohtake K, Kimura M, Yamazaki H, Natsume H. Improved intranasal retentivity and transnasal absorption enhancement by PEGylated poly-L-ornithine. *Pharmaceuticals*, **11**, 9 (2018).
23) Ohtake K, Natsume H, Ueda H, Morimoto Y. Analysis of transient and reversible effects of poly-L-arginine on the *in vivo* nasal absorption of FITC-dextran in rats. *J. Control. Release*, **82**, 263–275 (2002).