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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

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Abstract

The effect of poly-L-arginine (poly-L-Arg) on the in vivo nasal absorption of FITC-dextrans with a mean molecular weight ranging from 4.3 to 167 kDa and recombinant human granulocyte colony-stimulating factor (rhG-CSF) in rats were studied. When FITC-dextrans were co-administered intranasally with 1.0 w/v% poly-L-Args of different molecular weight (MW, ca. 45.5 and 92 kDa, poly-L-Arg (50) and poly-L-Arg (100)), the bioavailability (F_∞) increased markedly compared with that after administration of FITC-dextran alone. However, the F_∞ decreased exponentially with the increasing molecular weight of FITC-dextrans. There was no significant difference between the enhanced nasal absorption of FITC-dextrans achieved by the co-administration of poly-L-Arg (50) and poly-L-Arg (100). Moreover, the relationship between the F_∞ and the molecular weight of FITC-dextrans indicated that the molecular weight of protein drugs, which exhibited efficient absorption with poly-L-Arg, was about 20 kDa, when the lower limit of bioavailability for developing a potent transnasal delivery system was assumed to be about 10%. Indeed, the nasal absorption of rhG-CSF, which has a molecular weight of 18.8 kDa, was also increased after co-administration of 1.0 w/v% poly-L-Arg (50) and the F_∞ was about 11%. It seems likely that poly-L-Arg can be used to provide adequate nasal absorption of various protein drugs which have a molecular weight of about 20 kDa, thereby allowing the successful development of a variety of transnasal drug delivery systems.

Keywords: Poly-L-arginine; Absorption enhancer; FITC-dextran; Nasal absorption; Intranasal drug delivery system; Recombinant human granulocyte colony-stimulating factor (rhG-CSF)

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1. Introduction

The enhancement of the transport of macromolecules through biological membranes has been investigated using polycationic materials such as poly-L-arginine (poly-L-Arg), poly-L-lysine (poly-L-Lys) and chitosan (Hammes and Singh, 1994; Illum et al., 1994; Artursson et al., 1994; Uchida et al., 1996; Kotze et al., 1997; Soane et al., 1999). These polycations have the potential to promote the transmucosal delivery of macromolecules without any toxic effects on epithelial cells (Coyle et al., 1993a,b; Illum et al., 1994; Aspen et al., 1996). We also demonstrated that poly-L-arginine can enhance the nasal absorption of a model drug, fluorescein isothiocyanate–labeled dextran (FITC-dextran, MW 4.4 kDa) without producing any membrane damage in in vivo rat and in vitro rabbit models (Natsume et al., 1999a). This advantage of being able to use polycations is ideal for drug transport through biological membranes because most classical chemical enhancers (surfactants, bile salts, fatty acids, etc.) cause irreversible damage to epithelial cells and irritate the mucosa, when used at a concentration that provides adequate absorption of biologically active peptides and proteins to allow them to exhibit effective pharmacological actions (Merkus et al., 1993; Jabbal Gill et al., 1994a,b; Kubo et al., 1994; Hosoya et al., 1994a; Marttin et al., 1995). Also, the degree of enhancement of transmucosal absorption of drugs is closely correlated with the release of membrane components (Hosoya et al., 1994a; Uchiyama et al., 1996; Yamamoto et al., 1996; Quan et al., 1998).

In our previous study, we also showed that the enhancing effect of poly-L-Arg on the nasal absorption of FD-4 in an in vivo rat model was dependent on its concentration and the molecular weight as associated with the charge density in a single poly-L-Arg molecule (Miyamoto et al., 2001). The resultant bioavailability (F) of FD-4 was more than 60%, when poly-L-Arg with a molecular weight of about 45.5 and 92.0 kDa (poly-L-Arg (50) and poly-L-Arg (100)) was administered at a concentration of more than 0.5 w/v%. Also, the pharmacological responses of α-human atrial natriuretic peptide (MW ca. 3.0 kDa) and salmon calcitonin (MW ca. 3.5 kDa) are improved dramatically following i.n. administration with 1.0 w/v% poly-L-Arg (50) in rats (Natsume et al., 1999b). The calculated pharmacological availability of these peptide drugs was equivalent to that achieved following their i.v. administration in solution form. Thus, poly-L-Arg appears to be capable of achieving sufficient nasal absorption of peptide drugs which have a molecular weight of less than 5 kDa. However, McMartin et al. (1987), have shown that the bioavailability of drugs and macromolecules following i.n. administration decreased exponentially depending on the molecular weight in in vivo human and animal studies. Hosoya et al., also found that the permeation of FITC-dextrans of different molecular weight through the excised nasal membrane of rabbits, as measured by the in vitro Ussing chamber technique, decreased exponentially with increasing molecular weight (Hosoya et al., 1993, 1994b). These results suggest that, even if the nasal absorption of protein drugs with a higher molecular weight (perhaps, more than 10 kDa) is dramatically improved by co-administration of poly-L-Arg, it may be insufficient with the increasing molecular weight of protein drugs to allow an effective pharmacological action to be obtained.

It seems likely that polycations, such as poly-L-lysine, poly-L-Arg and chitosan, enhance mainly the intercellular transport of macromolecules through the epithelial membrane because of a reduction in transepithelial resistance leading to an increase in the flux of extracellular markers in a series of cell monolayers and an increase in the fluorescence intensity of FD-4 in the intercellular spaces as monitored by three-dimensional imaging using confocal laser scanning microscopic technique (McEwan et al., 1993; Uchida et al., 1996; Schipper et al., 1997). If poly-L-Arg enhances mainly the intercellular transport of drugs through the mucosal membrane in an in vivo nasal absorption study, the permeation rate of drugs in the intercellular spaces is increased, while the diffusion of macromolecules in the solvent of

the intercellular spaces will be reduced with increasing molecular volume (molecular weight) because the diffusion coefficient is inversely proportional to the molecular volume. In addition, the reflection coefficient associated with controlling the diffusion of macromolecules in the intercellular spaces will be also affected by the degree of widening of the cell-cell junctions, in particular, tight junctions (Schultz, 1980). Hence, even although poly-L-Arg will enhance mainly the intercellular transport of peptides and proteins of various molecular weights, it will be difficult to provide sufficient absorption of protein drugs with a higher molecular weight allowing the development of a successful transnasal drug delivery system. Therefore, it is important to explore the effect of poly-L-Arg on the nasal absorption of macromolecules of different molecular weight.

In the present study, we evaluated the effect of poly-L-Arg on the nasal absorption of macromolecules of different molecular weights in an in vivo rat model involving poly-L-Args of different mean molecular weights (poly-L-Arg (50) and poly-L-Arg (100)). FITC-dextrans of different mean molecular weights (MW ca. 12.0, 38.3, 50.7 and 167.0 kDa, FD-10, FD-40, FD-70 and FD-150, respectively) and recombinant human granulocyte colony-stimulating factor (rhG-CSF, MW 18.8 kDa) were used as model water-soluble macromolecule and protein drug, respectively.

2. Experimental

2.1. Materials

FITC-dextrans (MW ca. 12.0, 38.3, 50.7 and 167.0 kDa, FD-10, FD-40, FD-70 and FD-150, respectively) and poly-L-Arg hydrochloride (MW ca. 45.5 and 92.0 kDa, poly-L-Arg (50) and poly-L-Arg (100), respectively) were obtained from Sigma (St. Louis, MO). rhG-CSF (MW 18.8 kDa) was a kind gift from Kyowa Hakko Kogyo (Tokyo, Japan). The EIA kit for rhG-CSF determination was obtained from R & D System, Inc. (Quantikine human G-CSF kit, MN). All other reagents were of reagent grade.

2.2. Methods

2.2.1. I.V. bolus injection study

Drug solutions containing FITC-dextrans of different molecular weights and rhG-CSF (0.8 ml/kg) were injected in a femoral vein of anesthetized male Wistar rats (urethane, 1 g/kg, 25 w/v%, Saitama Laboratory Animals, Saitama, Japan), weighing 250–300 g, undergoing the same surgical procedure as that in the nasal absorption study ($n = 3$–4). Blood samples (0.5 ml) were collected from a jugular vein at predetermined times and plasma samples (0.1 ml) containing FITC-dextrans were obtained by centrifugation (18 000 × g, 4 °C, 5 min). Plasma samples (0.3 ml) containing rhG-CSF were obtained by centrifugation (2800 × g, 4°C, 10 min) and then were stored in a refrigerator below − 20°C until use.

FITC-dextrans of different molecular weight were dissolved in 0.9% NaCl solution to give concentrations of 0.025, 0.05, 0.10 and 0.31 w/v% (0.2, 0.4, 0.8 and 2.5 mg/kg), respectively. FITC-dextran solutions were used within 3 h of their preparation.

The rhG-CSF stock solution (1.8 mg/ml) was diluted with potassium dihydrogenphosphate buffer (1/15 mM). Sodium chloride and 1 N HCl were added to produce an isotonic state and to adjust the pH to 5.0 (Kuwabara et al., 1991; Machida et al., 1993). The concentration of rhG-CSF in the final solution was 18.8 μg/ml (15 μg/kg). Glass test-tubes, vials and pipettes were used to prevent adsorption of rhG-CSF (Johnston, 1996).

2.2.2. Nasal absorption study

Anesthetized Wistar rats (urethane, 1 g/kg, 25 w/v%), weighing 250–300 g, were surgically prepared as described by Hirai et al. (1981). 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 escaping into the buccal cavity. Intranasal (i.n.) doses of FITC-dextrans of different molecular weight (200 mg/ml

in 0.9% NaCl solution, 33.0 mg/kg) containing different concentrations of poly-L-Arg (50) and poly-L-Arg (100) were administered via a flexible polyethylene tube attached to a microsyringe ($n = 3$). The rhG-CSF solution (0.4 mg/ml, 50 μg/kg) containing 1.0 w/v% poly-L-Arg (50) was also administered by the same surgical procedure ($n = 3$). Blood samples (0.5 ml) were collected from a jugular vein at predetermined times. Plasma samples containing FITC-dextrans and rhG-CSF were obtained by the same procedure as described above. Plasma samples containing rhG-CSF were stored in a refrigerator below −20°C until use.

Poly-L-Arg (50) was used at a concentration of 0.5 and 1.0 w/v% for FD-10, FD-70 and FD-150. Poly-L-Arg (100) was also used at a concentration of 1.0 w/v%. In the nasal absorption study of FD-40, poly-L-Arg (50) and poly-L-Arg (100) were used at a concentration of 0.1, 0.25, 0.5, 1.0 and 2.0 w/v%.

After the end of all the animal experiments, rats were sacrificed according to the guidelines for animal use in the Life Science Research Center, Josai University.

2.3. Sample analysis

Plasma containing FITC-dextran of different molecular weights was diluted about 100 times with potassium dihydrogenphosphate-sodium borate buffer (pH 8.5). The fluorescence intensity of FD-4 was determined in a fluorescence spectrofluorometer (RF-5000, Shimadzu, Kyoto) at an excitation wavelength of 495 nm and an emission wavelength of 515 nm.

Plasma containing rhG-CSF was diluted about 50 times with isotonic phosphate buffer containing 1% bovine serum albumin (pH 7.4). The rhG-CSF was analyzed using an EIA kit (Quantikine human G-CSF kit). The amount of rhG-CSF was determined using a microplate reader (EL 312e, BIO-TEX Instruments, YT, USA) at a wavelength of 450 nm.

2.4. Data analysis

The i.v. and i.n. blood data of FITC-dextrans of different molecular weight and rhG-CSF were analyzed using a non-linear least squares regression method (Algorithm: Damping Gauss–Newton method) (Yamaoka et al., 1981). The C_{max} and T_{max} were obtained from the drug concentration-time curve, and the area under the plasma concentration versus time curve (AUC) was calculated by the trapezoidal rule. The plasma concentrations of FITC-dextrans and rhG-CSF following i.v. and i.n. administrations were extrapolated exponentially to infinity (∞).

The elimination kinetics of FITC-dextrans fitted a two-compartment model following i.v. administration at four doses (0.2, 0.4, 0.8 and 2.5 mg/kg). The area under the plasma concentration versus time ($0-\infty$) curve (AUCi.v.$_\infty$) after i.v. administration of FITC-dextrans was proportional to the dose administered and linear relationships between AUCi.v.$_\infty$ and the administered dose were obtained. At these doses, the elimination kinetics were linear and the kinetic parameters were also almost exactly identical for these doses. The α (min^{-1}), β (min^{-1}), volume of distribution (V_{dss}, ml/kg) and total clearance (ml/min per kg) for the elimination parameters obtained from the data at these doses were as follows: for FD-10; 0.291, 0.036, 16.5 and 0.436, for FD-40; 0.063, 0.006, 21.4 and 0.123, for FD-70; 0.148, 0.005, 14.6 and 0.065, for FD-150; 0.038, 0.003, 11.9 and 0.035, respectively. The AUCi.v.$_\infty$ after i.v. administration of FITC-dextrans at 33 mg/kg was calculated using the total clearance and these linearity. The calculated AUCi.v.$_\infty$s after i.v. administration (33 mg/kg) for FD-10, FD-40, FD-70 and FD-150 in rats (280 g body weight) were 21 200 (μg min/ml), 75 100 (μg min/ml), 142 000 (μg min/ml) and 264 000 (μg min/ml), respectively (Miyamoto et al., 2001).

The bioavailability (%) of the formulation based on the period 0–9 h (F_{0-9h}) and 0–infinity (F_∞) was calculated as follows:

$$F_{0-9h} = (\mathrm{AUC}_{\mathrm{i.n.\ 0-9h}} / \text{calculated AUC i.v.}_\infty \text{ for each FITC-dextran}) \times 100 \quad (1)$$

$$F_{\infty} = (\mathrm{AUC}_{\mathrm{i.n.}\,\infty} / \text{calculated AUC i.v.}_{\infty} \text{ for each FITC-dextran}) \times 100 \quad (2)$$

where $\mathrm{AUC}_{\mathrm{i.n.\,0-9h}}$ is the area under the plasma FD-4 concentration versus time (0–9 h) curve after i.n. administration of FD-4. $\mathrm{AUC}_{\mathrm{i.n.}\,\infty}$ is the area under the plasma FD-4 concentration versus time (0–infinity) curve after i.n. administration.

The elimination kinetics of rhG-CSF after i.v. administration was evaluated using one- and two-compartment models. The bioavailability (%) of the formulation based on the period 0–8 h (F_{0-8h}) and 0–infinity (F_{∞}) was calculated by dividing the AUC_{∞} after i.n. administration by the AUC_{∞} after i.v. administration obtained using a one-compartment models.

Statistical analyses were performed using Student's *t*-test for the AUC of FITC-dextrans and rhG-CSF of non-treatment (control) versus treatment with poly-L-Arg. The variation in the AUC obtained with poly-L-Arg (50) and poly-L-Arg (100) was compared using a two-way analysis of variance (two-way ANOVA). A *P*-value of 0.05 was considered significant.

3. Results

3.1. Effect of poly-L-Arg on the nasal absorption of FITC-dextrans of different molecular weight

Figs. 1 and 2 show the plasma concentration-time curves of FITC-dextrans of different molecular weight after i.n. co-administration of poly-L-Arg (50) and poly-L-Arg (100) at a concentration of 1.0 w/v% in rats, respectively. Table 1 lists the pharmacokinetic parameters of FITC-dextrans obtained in all experiments including data for FD-4 from our previous work (Miyamoto et al., 2001). The plasma concentrations of FD-10, FD-40 and FD-70 after i.n. co-administration of poly-L-Arg (50) and poly-L-Arg (100) increased markedly compared with those of FITC-dextran alone. The plasma concentration of FD-150 also increased following i.n. co-administration with poly-L-Args, although the plasma concentration was undetectable after administration of FD-150 alone. There was a significant difference between $\mathrm{AUC}_{\mathrm{i.n}}$s of control (FITC-dextran alone) and poly-L-Arg treatment. The $\mathrm{AUC}_{\mathrm{i.n.\,0-9h}}$ and $C_{\max}$ tended to fall with increasing molecular weight of FITC-dextrans, while the F_{∞} fell markedly with increasing molecular weight.

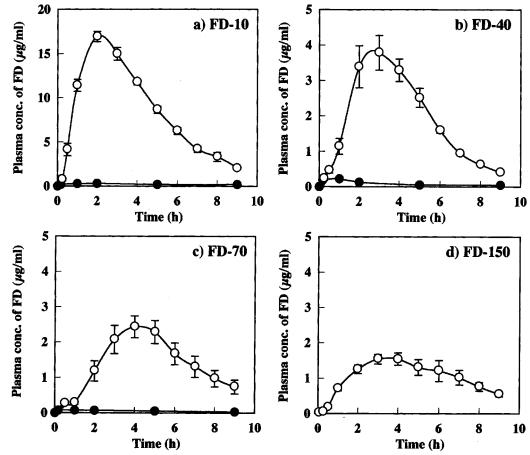

Fig. 1. Plasma concentrations of FITC-dextrans after i.n. co-administration with 1.0 w/v% poly-L-Arg (50) to rats. ●, control (FITC-dextrans alone); ○, with 1.0 w/v% poly-L-Arg (50). Each data point represents the mean ± S.E. ($n = 3–4$).

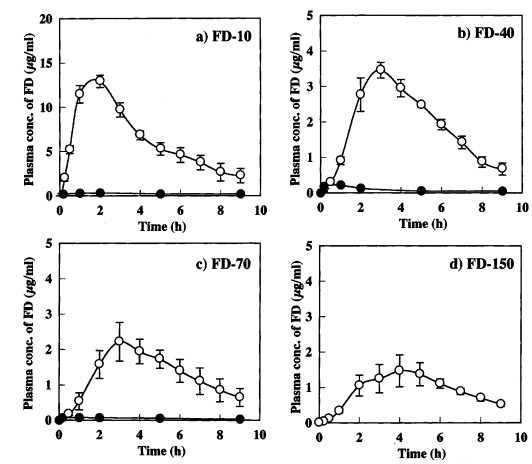

Fig. 2. Plasma concentrations of FITC-dextrans after i.n. co-administration with 1.0 w/v% poly-L-Arg (100) to rats. ●, control (FITC-dextrans alone); ○, with 1.0 w/v% poly-L-Arg (100). Each data point represents the mean ± S.E. ($n = 3–4$).

Table 1
Pharmacokinetic parameters of FITC-dextrans of different molecular weight after i.n. co-administration with poly-L-Arg to rats

FD	Enhancer	C_{max} (μg/ml)	T_{max} (min)	$AUC_{i.n.\ 0-9h}$[a] (μg min/ml)	$AUC_{i.n.\ \infty}$[a] (μg min/ml)	F_{0-9h} (%)	F_{∞} (%)
FD-4[b]	Control	0.8	120	292.6 ± 48.5	402.3 ± 122.2	6.5	8.9
	0.5% poly-L-Arg (50)	16.6	60	2785 ± 164.4[c]	2831.0 ± 163.4[c]	61.9	62.9
	1.0% poly-L-Arg (50)	17.4	60	3380.1 ± 366.5[c]	3470.5 ± 380.8[c]	73.5	75.4
	1.0% poly-L-Arg (100)	16.1	60	3970.9 ± 120.8[c]	4274.3 ± 145.6[c]	86.3	92.9
FD-10	Control	0.3	120	194.4 ± 16.6	556.8 ± 19.7	0.92	2.6
	0.5% poly-L-Arg (50)	12.5	120	2925.9 ± 377.0[c]	3290.6 ± 475.9[d]	13.8	15.5
	1.0% poly-L-Arg (50)	16.9	120	4645.6 ± 199.9[c]	5024.7 ± 255.6[c]	21.9	23.7
	1.0% poly-L-Arg (100)	12.9	120	3469.0 ± 234.0[c]	4138.0 ± 667.0[d]	16.4	19.5
FD-40	control	0.2	60	50.7 ± 9.7	124.6 ± 45.5	0.07	0.17
	0.1% poly-L-Arg (50)	1.5	120	405.5 ± 58.1[c]	546.0 ± 143.0[d]	0.54	0.73
	0.25% poly-L-Arg (50)	3.4	120	737.8 ± 89.0[c]	792.8 ± 99.0[c]	0.98	1.06
	0.5% poly-L-Arg (50)	3.6	180	1040.0 ± 147.5[d]	1270.2 ± 204.9[d]	1.38	1.69
	1.0% poly-L-Arg (50)	3.8	180	1045.8 ± 73.8[c]	1099.5 ± 79.0[c]	1.39	1.46
	2.0% poly-L-Arg (50)	4.1	180	1130.8 ± 98.5[c]	1228.7 ± 92.6[c]	1.51	1.64
	0.1% poly-L-Arg (100)	1.5	120	407.0 ± 28.3[c]	483.8 ± 40.1[d]	0.54	0.64
	0.25% poly-L-Arg (100)	2.6	180	644.3 ± 41.2[c]	746.7 ± 11.5[c]	0.86	0.99
	0.5% poly-L-Arg (100)	3.2	180	1047.4 ± 125.0[c]	1179.0 ± 213.0[c]	1.39	1.57
	1.0% poly-L-Arg (100)	3.5	180	1078.6 ± 66.9[c]	1124.0 ± 129.0[c]	1.44	1.50
	2.0% poly-L-Arg (100)	3.7	180	1227.9 ± 139.0[c]	1309.0 ± 216.0[d]	1.64	1.74
FD-70	Control	0.08	60	23.0 ± 10.1	37.8 ± 19.5	0.02	0.03
	0.5% poly-L-Arg (50)	2.4	300	838.1 ± 100.3[d]	972.1 ± 89.5[c]	0.59	0.68
	1.0% poly-L-Arg (50)	2.4	240	760.2 ± 127.9[d]	926.7 ± 185.0[d]	0.54	0.65
	1.0% poly-L-Arg (100)	2.2	180	697.4 ± 58.7[c]	864.2 ± 89.9[c]	0.49	0.61
FD-150	Control[e]	n.d.[f]	n.d.[f]	–	–	–	–
	0.5% poly-L-Arg (50)	1.3	180	491.0 ± 87.1	671.3 ± 101.8	0.19	0.25
	1.0% poly-L-Arg (50)	1.5	240	570.7 ± 56.7	722.6 ± 35.4	0.22	0.27
	1.0% poly-L-Arg (100)	1.5	240	511.0 ± 67.0	654.7 ± 50.5	0.19	0.25

There was no significant difference between the $AUC_{i.n.}$s of FD-40 obtained for poly-L-Arg (50) and poly-L-Arg (100) (two-way ANOVA, $P > 0.05$).

[a] Mean ± S.E., $n = 3$–4.

[b] Data from Miyamoto et al. (2001).

[c] $P < 0.001$;

[d] $P < 0.01$ compared with control (Student's t-test).

[e] Plasma concentration of FD-150 was undetectable.

[f] Not detected.

Fig. 3 shows the relationships between the F_{∞} and the molecular weight of FITC-dextrans after i.n. co-administration of poly-L-Args of different molecular weight at a concentration of 0.5 and 1.0 w/v%, respectively. In all cases, the F_{∞}s achieved after i.n. co-administration of poly-L-Arg increased compared with the values for FITC-dextran alone, while the F_{∞}s decreased exponentially with increasing molecular weight of FITC-dexrans. There was no significant difference between the F_{∞}s obtained for 0.5 and 1.0 w/v% poly-L-Arg (50), and 1.0 w/v% poly-L-Arg (100), respectively (one-way ANOVA, $P > 0.05$). Hence, the resultant relationships were very similar to those shown in Fig. 3.

Fig. 4 shows the relationships between the F_{∞} of FD-40 and the concentration of poly-L-Args of different molecular weight. The F_{∞} of FD-40 increased with increasing concentration of poly-L-Arg and reached a plateau at an applied concentration of more than 0.5 w/v% in both cases. There was no significant difference between the $AUC_{i.n}$s, used to calculate the F_{∞} of FD-40, obtained for poly-L-Arg (50) and poly-L-Arg (100) (two-way ANOVA, $P > 0.05$).

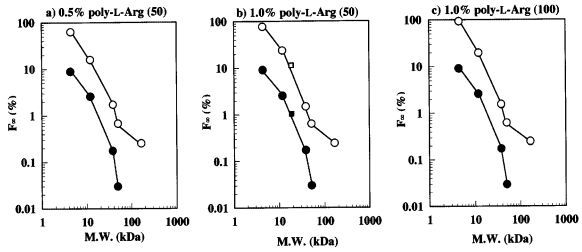

Fig. 3. Relationships between the F_{∞} and the molecular weight of FITC-dextrans. ●, control (FITC-dextrans alone); ○, with poly-L-Arg; ■, F_{∞} of rhG-CSF without 1.0 w/v% poly-L-Arg (50); □, F_{∞} of rhG-CSF with 1.0 w/v% poly-L-Arg (50). There was no significant difference between the F_{∞}s obtained for 0.5 and 1.0. w/v% poly-L-Arg (50), and 1.0 w/v% poly-L-Arg (100), respectively (one-way ANOVA, $P > 0.05$). Detailed kinetic data for rhG-CSF are given in Table 2. Each data column represents the mean value ($n = 3$–4).

3.2. Effect of poly-L-Arg on nasal absorption of rhG-CSF

Fig. 5 shows the logarithmic plasma concentration-time curves of rhG-CSF following i.v. and i.n. administrations of 15 and 50 μg/kg in rats, respectively. Table 2 lists the pharmacokinetic parameters of rhG-CSF obtained. The plasma concentration of rhG-CSF after i.v. administration fell exponentially and fitted both one- and two-compartment models. The elimination parameters obtained from both models were almost identical. The plasma concentration of rhG-CSF after i.n. co-administration with 1.0 w/v% poly-L-Arg (50) increased markedly compared with that after i.n. administration of rhG-CSF alone. The AUC_{∞} obtained after i.n. co-administration with 1.0 w/v% poly-L-Arg (50) was also significantly increased. The bioavailabilities, F_{∞}s, calculated based on the one-compartment model, after i.n. administration of rhG-CSF, with and without 1.0 w/v% poly-L-Arg (50) were 11.3 and 1.03%, respectively. These values were very similar to those predicted from the relationship between the F_{∞} and the molecular weight of FITC-dextrans as shown in Fig. 3b.

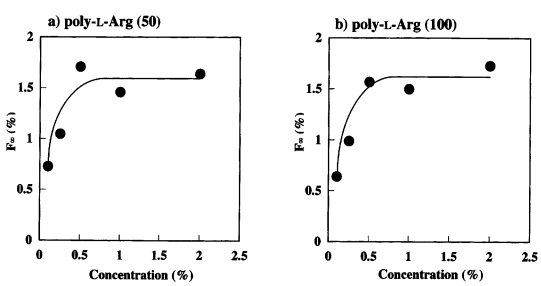

Fig. 4. Relationships between the F_{∞} of FD-40 and the concentration of poly-L-Args of different molecular weight. Each data column represents the mean value ($n = 3$–4).

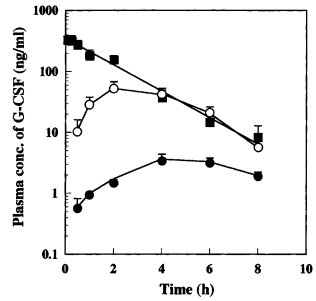

Fig. 5. Plasma rhG-CSF concentration after i.v. and i.n. administration to rats. ■, i.v. administration of rhG-CSF; ●, i.n. administration of rhG-CSF alone (control); ○, i.n. co-administration of rhG-CSF with 1.0 w/v% poly-L-Arg (50). Each data point represents the mean ± S.E. ($n = 3$).

4. Discussion

In the present study, we explored the effect of poly-L-Arg on the nasal absorption of macromolecules of different molecular weight in an in vivo rat model. When FITC-dextrans of different molecular weight were co-administered intranasally with 1.0 w/v% poly-L-Arg (50) and poly-L-Arg (100), the plasma concentration of all FITC-dextrans significantly increased compared with that after i.n. administration of FITC-dextrans alone (control), suggesting that poly-L-Arg is able to enhance the nasal absorption of FITC-dextrans which have a mean molecular weight of less than 167.0 kDa. The $AUC_{i.n}$s and the resulting bioavailability, F_{∞}, of FITC-dextrans were also increased dramatically by co-administrating of poly-L-Arg compared with control, but the F_{∞} decreased exponentially depending on the molecular weight of FITC-dextrans. These results show that the effect of poly-L-Arg in increasing the

Table 2
Pharmacokinetic parameters of G-CSF after i.v. and i.n. administration to rats

	$t_{1/2}$ (h)	$t_{1/2\beta}$ (h)	V_d (ml)	V_{dss} (ml)	CL_{tot} (ml/h)	C_{max} (ng/ml)	T_{max} (h)	AUC_{0-9h} (ng/ml h)	AUC_∞ (ng/ml h)	F_{0-9h}[a] (%)	F_∞[b] (%)
i.v. administration dose: 15 μg/kg											
One-compartment model	1.36	–	11.8	–	5.88	–	–	665.2 ± 30.8	678 ± 35.9	–	–
Two-compartment model	–	1.53	–	14.6	6.65	–	–	669.9 ± 32.5	685.0 ± 37.1	–	–
i.n. administration dose: 15 μg/kg											
Control	–	–	–	–	–	3.38	4	19.1 ± 5.2	23.3 ± 7.1	0.8	1.03
1% poly-L-Arg	–	–	–	–	–	53.9	3	245.0 ± 17.9[c]	256.2 ± 11.6[c]	10.8	11.3

The AUC represents the mean ± S.E. ($n = 3$). F was calculated using the AUC_∞ after i.v. administration obtained from the one-compartment model.

[a] F_{0-9h} (%) = (AUC_{0-9h} after i.n. administration/AUC_∞ after i.v. administration) × 15/50 × 100.

[b] F_∞ (%) = (AUC_∞ after i.n. administration/AUC_∞ after i.v. administration) × 15/50 × 100.

[c] $P < 0.001$ compared with control (Student's t-test).

efficacy (i.e. bioavailability) of nasal absorption of macromolecules, allowing them to exhibit an effective pharmacological action, is linked to the molecular weight of the macromolecules, although poly-L-Arg enhanced the nasal absorption of macromolecules of various molecular weights. Transnasal delivery systems for buserelin acetate and desmopressin acetate have been marketed and can be used to reduce the endocrinopathic effect, such as endometriosis and diabetes insipidus. The bioavailability of desmopressin acetate after i.n. administration was about 10%. If the lower limit of the bioavailability required for a potent transnasal delivery system of macromolecules is assumed to be about 10%, the molecular weight of protein drug candidates suitable for development as delivery systems containing poly-L-Arg may be about 20 kDa as predicted from the relationships between the F_∞ and the molecular weight of FITC-dextrans as shown in Fig. 3. Indeed, the F_∞ of rhG-CSF, which has a molecular weight of 18.8 kDa, obtained after i.n. co-administration with 1.0 w/v% poly-L-Arg (50) was about 11%. In addition, since the rate of enzymatic degradation of rhG-CSF in the nasal cavity is slow (Machida et al., 1994), the F_∞ of rhG-CSF obtained was very similar to that predicted from the relationship as shown in Fig. 3b.

The F_∞ of FD-40 after i.n. co-administration with poly-L-Arg (50) and poly-L-Arg (100) increased depending on the poly-L-Arg concentration applied and reached a plateau at a concentration of 0.5 w/v% (Fig. 4). A similar relationship was found for FD-4 in our previous work (Miyamoto et al., 2001), suggesting that the enhancing effect of poly-L-Arg reaches a maximum at a concentration of about 0.5 w/v%. Also, the F_∞s of FD-10, FD-70 and FD-150 obtained for poly-L-Arg (50) and poly-L-Arg (100) were also almost identical (Table 1). Therefore, similar relationships between the F_∞ and the molecular weight of FITC-dextrans were observed when poly-L-Arg (50) and poly-L-Arg (100) were co-administered at concentrations higher than 0.5 w/v%, as shown in Fig. 3. However, the F_∞ of FD-150 after i.n. co-administration with poly-L-Arg (50) and poly-L-Arg (100) was unexpectedly high regardless of the fact that the plasma concentration of FD-150 was undetectable following administration of FD-150 alone. One possible reason is that poly-L-Arg may increase not only the nasal absorption of FD-150 by simple diffusion but also that by endocytosis. Further investigations will be required to clarify this.

In our previous study, we showed that the enhancing effect of poly-L-Args of different molecular weights on the nasal absorption of FD-4 exhibited a molecular weight-dependence, in that a significant difference between $AUC_{i.n}$s obtained for poly-L-Args was observed, although the difference in the degree of enhancement of FD-4 absorption between poly-L-Args, with a mean molecular weight of about 8.9 kDa, and poly-L-Arg (50) was marked compared with that between poly-L-Arg (50) and poly-L-Arg (100) (Miyamoto et al., 2001). It was concluded that the molecular weight-dependence of poly-L-Arg for the enhancement of FD-4 absorption was associated with the charge density of individual poly-L-Arg molecule, thereby affecting both the enhancing efficiency and the time-frame of the enhancing effect. However, no significant difference between the enhancing effects of poly-L-Arg (50) and poly-L-Arg (100) on FD-40 absorption was observed in this study (Fig. 4 and Table 1). If poly-L-Arg induces mainly a transient widening of cell-cell junctions, in particular tight junctions as observed for chitosan (Illum et al., 1994), the intercellular transport of macromolecules through the mucosal membrane will be increased. Even although the intercellular transport was increased by poly-L-Arg, drug diffusion in the solvent of the intercellular spaces will be reduced depending on the molecular volume (molecular weight). In addition, macromolecule diffusion in the intercellular spaces will be also affected by the reflection coefficient (Schultz, 1980). This reflective effect is found to increase with increasing molecular volume, thereby leading a larger reduction in intercellular transport of macromolecules of higher molecular weight. Hence, the similar enhancing effect of poly-L-Arg (50) and poly-L-Arg (100) on FD-40 absorption may be due to an inability to produce a sufficient reduction in the reflective effect, which probably relates to the degree of widening of the tight junctions to exhibit a molecular weight-de

pendent enhancing effect, although such a reflective effect is negligible for the enhanced absorption of macromolecules of lower molecular weight, such as FD-4. In addition, the difference of only a factor of two in the mean molecular weight of poly-L-Arg (50) and poly-L-Arg (100) may also affect this.

rhG-CSF can be used to enhance the differentiation and proliferation of neutrophils, which is necessary to treat a variety of diseases, such as acute leukemia, hypoplastic anemia, and leukopenia. rhG-CSF is also generally given intravenously and subcutaneously once a day, but the treatment of such diseases could also involve repeated doses of rhG-CSF for a long period. Therefore, attempts to administer rhG-CSF via routes other than the parenteral one have been made (Takada et al., 1994; Niven et al., 1993, 1994; Machida et al., 1994; Nomura et al., 1996). As shown in Fig. 5 and Table 2, when rhG-CSF was co-administered intranasally with 1.0 w/v% poly-L-Arg (50), its plasma concentrations were increased markedly compared with those after i.n. administration of rhG-CSF alone (control) and these plasma levels were maintained for a longer period. Consequently, the C_{max} and the F_{∞} were about 16 and 11 times higher than those of the control, respectively. The C_{max} of rhG-CSF after i.n. co-administration of poly-L-Arg (50) was almost the same as that after s.c. administration of rhG-CSF alone as reported in a previous study (Kuwabara et al., 1991), although the bioavailabilities were different. Since the half life ($t_{1/2}$) and the total clearance per body weight of rhG-CSF obtained after i.v. administration in rats were nearly the same as those in humans (Suzuki et al., 1991), the pharmacokinetic parameters in rats obtained in this study can be used to evaluate rhG-CSF disposition in humans, although the analytical method used on the plasma samples was different. When rhG-CSF was administered subcutaneously at a dose of 1.0 μg/kg in humans, the resulting C_{max} was about 1.5 ng/ml (Suzuki et al., 1991). If rhG-CSF, at a dose of 1.0 μg/kg, is co-administered intranasally with poly-L-Arg in rats, the C_{max} is found to be about 1.1 ng/ml, as expected from the C_{max} at a dose of 50 μg/kg obtained in this study. This level was almost the same as that following s.c. administration in humans. Hence, the pharmacological effects of rhG-CSF after i.n. administration are equivalent to those after s.c. administration, when rhG-CSF at a dose of 1.0 μg/kg was co-administered intranasally with poly-L-Arg in humans. Thus, poly-L-Arg allows adequate nasal absorption of rhG-CSF to be obtained. Also, it seems likely that poly-L-Arg can be used to provide adequate nasal absorption of protein drugs and cytokines such as human menopausal gonadotropin (MW ca. 30 kDa), somatropin (MW ca. 22 kDa), interleukins and interferons (MW of these cytokines, 15–30 kDa), thereby allowing the development of a variety of transnasal drug delivery systems.

In conclusion, poly-L-Arg (50) and poly-L-Arg (100) enhanced the nasal absorption of FITC-dextrans with mean molecular weights ranging from 4.3 to 167 kDa in an in vivo rat model. The improved efficacy (i.e. bioavailability) of nasal absorption of FITC-dextrans was also marked, but the F_{∞} fell exponentially with the increase in the molecular weight of the FITC-dextrans. In addition, an F_{∞} of more than 10%, which may be required to develop a potent nasal delivery system for peptide and protein drugs, is expected to be possible for peptide and protein drugs with a molecular weight of less than 20 kDa as predicted from the relationship between the F_{∞} and molecular weight of FITC-dextrans. An effective improvement in the nasal absorption of rhG-CSF, which has a molecular weight of 18.8 kDa, was achieved by poly-L-Arg, indicating that poly-L-Arg could be used to provide adequate nasal absorption of protein drugs and cytokines which have a molecular weight of about 20 kDa.

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