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Control of pulmonary absorption of water-soluble compounds by various viscous vehicles

Akira Yamamoto[a,*], Keigo Yamada[a,b], Hideaki Muramatsu[a], Asako Nishinaka[a], Shigeki Okumura[a], Naoki Okada[a], Takuya Fujita[a], Shozo Muranishi[a]

[a] *Department of Biopharmaceutics, Kyoto Pharmaceutical University, Misasagi, Yamashina-ku, Kyoto 607-8414, Japan*
[b] *Otsuka Pharmaceutical Co. Ltd., 224-18 Ebisuno Hiraishi Kawauchi-cho, Tokushima 771-0182, Japan*

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Abstract

Effects of various viscous vehicles on the pulmonary absorption of water-soluble drugs were examined by an in situ pulmonary absorption experiment. Gelatin, polyvinylacohol (PVA), hydroxypropylcellose (HPC), chondroitin sulfate A sodium salt (CS), polyacrylic acid (PAA), methylcellulose #400 (MC400) and hyaluronic acid sodium salt (HA) were used as models of viscous vehicles. 5(6)-Carboxyfluorescein (CF) and fluorescein isothiocayanate-labeled dextran with an average molecular weight of 4000 (FD4) were used as water-soluble drugs. The plasma concentration of CF was controlled and regulated in the presence of these viscous vehicles, especially gelatin (1–5%) and polyvinyl alcohol (PVA) 1%. In the pharmacokinetic analysis, the C_{max} values of CF significantly decreased, and its T_{max} values increased in the presence of these viscous vehicles compared with the control. The MRT and MAT values of CF with these vehicles were significantly higher than those without these vehicles. Therefore, these findings indicated that the viscous vehicles were effective to regulate the absorption rate of CF. On the other hand, the pulmonary absorption of FD4 was not so much affected even in the presence of gelatin and PVA, although PVA slightly decreased MRT value, and significantly decreased T_{max} value. Furthermore, we examined the release rate of CF from the cellulose tube containing various concentrations of gelatin. The release rate of CF from the cellulose tube with gelatin was inversely related to the viscosity of gelatin. In addition, the release rate of CF was inversely related to ΔMAT (ΔMAT = MAT_{gel}(MAT with gelatin)−MAT_{sol}(MAT without gelatin)) in the presence of varying concentrations of gelatin. These findings indicated that these viscous vehicles were effective to control the pulmonary absorption of CF, a water-soluble drug with low molecular weight and they might be useful to increase the local concentration of drugs in the lung.

Keywords: Pulmonary absorption; Gelatin; Viscosity; Excipient; Mucoadhesion; Controlled release

* Corresponding author. Tel.: +81 75 595 4661; fax: +81 75 595 4761.
E-mail address: yamamoto@mb.kyoto-phu.ac.jp (A. Yamamoto).

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1. Introduction

The pulmonary route seems to be the most promising alternatives for the delivery of macromolecular drugs, since a number of drugs which are poorly absorbed from enteral and other sites are well absorbed from the lung, due to the large surface area of the alveolar epithelium and the short air to blood pathway (O'Hagan and Illum, 1990). Indeed, insulin and calcitonin, macromolecular peptide drugs, administered intrapulmonarily can be absorbed, even though they are poorly absorbed from the gastrointestinal tract (Enna and Schanker, 1972a; Wigley et al., 1971). Moreover, the bioavailability of these peptides can be improved in the presence of various adjuvants such as absorption enhancers and protease inhibitors (Okumura et al., 1992; Komada et al., 1994; Kobayashi et al., 1994; Morita et al., 1994; Yamamoto et al., 1994, 1997, 2001; Todo et al., 2001). However, rapid absorption of these peptides, especially insulin may cause hypoglycemia and this low plasma glucose level may sometimes cause convulsions, a coma and other serious side effects. Consequently, the control of absorption rate of drugs from the lung is very important for effective and safe drug therapy.

On the other hand, drugs, which can be expected to cause the local pharmacological activities in the lung, should be localized for a long period in the lung tissues. These local acting drugs include antiasthmatic agents, bronchodilators and expectorants. Moreover, there is recently a great increase in patients having chronic obstructive pulmonary disease (COPD) and the development of drugs for the treatment of COPD is one of the most important areas in the field of respiratory diseases. Therefore, it is necessary for these drugs to retain a high concentration of drugs in the lung tissues. Nevertheless, few studies have been examined to control and regulate the absorption rate of such drugs after intrapulmonary administration (Morimoto et al., 2001).

In this study, therefore, 5(6)-carboxyfluorescein (CF) and fluorescein isothiocyanate-labeled dextran with an average molecular weight of 4 kDa (FD4) were chosen as models of water-soluble compounds and the effects of various viscous vehicles including gelatin, polyvinylacohol (PVA), hydroxypropylcellose (HPC), chondroitin sulfate A sodium salt (CS), polyacrylic acid (PAA), methylcellulose #400 (MC400) and hyaluronic acid sodium salt (HA) on the absorption of these water-soluble compounds after intrapulmonary administration were examined in rats.

2. Materials and methods

2.1. Materials

CF was kindly supplied from Eastman Kodak Co. (Rochester, NY, USA). FD4 was purchased from Sigma–Aldrich Chemical Co. (St Louis, MO, USA). Gelatin, PVA, CS, MC 400 and HA were obtained from Nacalai Tesque Inc. (Kyoto, Japan). HPC and PAA were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). All other chemicals were of analytical grade.

2.2. Preparation of drug solution

Dosing solutions containing CF and FD4 were prepared in isotonic phosphate buffer (PBS) at pH 7.4 to yield a final concentration of 200 μg/ml and 20 mg/ml, respectively. In certain experiments, the dosing solutions were added with various viscous vehicles such as gelatin, PVA, HPC, CS, PAA, MC 400 and HA at a concentration of 1–5%. For the viscosity measurement of drug solution, a Ostwald–Auerbach viscometer was used to determine the viscosity of drug solution with or without various viscous vehicles.

2.3. Absorption studies

Absorption of the drugs from rat lungs was investigated according to the methods outlined by Enna and Schanker (1972b). Male Wistar strain rats (Japan SLC, Inc., Hamamatsu, Japan), weighing 240–300 g, were anaesthetized with an intraperitoneal injection of sodium pentobarbital (32 mg/kg). All the animals were fasted for 16 h before the experiments but they were allowed free access to water. After the animal had been secured on its back on an animal board, the trachea was exposed through a longitudinal incision along the ventral aspect of the neck. The trachea was then cut transversely, halfway through, between the fourth and fifth tracheal rings caudal to the thyroid cartilage. A section of polyethylene tubing (i.d., 1.5 mm; o.d., 2.5 mm) 2.5 cm in length, which served as a tracheal cannula, was inserted through the tracheal incision caudally for

a distance of 0.6 cm so that 1.9 cm of the cannula protruded from the trachea. The incision in the skin was then closed with wound clips after drawing the skin up close to the sides of the cannula. Animal body temperatures were maintained at 37±1 °C with a 40 W incandescent heat lamp and the use of a reflector suspended over the animal at a distance of about 25 cm during the experiment.

One hundred microliters of drug solution at a temperature of 37 °C were injected into the lungs through the obtuse needle of a calibrated 100 μL syringe (Microliter® no. 710, Hamilton Co.). For the injection, the needle was inserted through the tracheal cannula to a depth of 2.5 cm below the tracheal incision. Then, at this distance of insertion, the tip of the syringe needle was located 1–2 mm above the bifurcation of the trachea. Then, the solution was injected over a period of 1–2 s, with the rat being maintained at an angle of 80 °C. Immediately thereafter, the tubing was withdrawn completely and 45 s after administration the animal was returned to an angle of 10°.

2.4. Pharmacokinetic analyses

The peak plasma concentration (C_{max}) and the peak plasma concentration time (T_{max}) were obtained from the plasma concentration–time curve from individual animals. The plasma concentration profiles of CF were analyzed based on the statistical moment theory. Moment parameters for the plasma concentration profiles (the area under the plasma concentration–time curve (AUC) and mean residence time (MRT)) were calculated by numerical integration using a linear trapezoidal formula and extraporation to infinite time based on a monoexponential equation (Yamaoka et al., 1978). Mean plasma appearance time (MAT) was calculated from the following equation

$$\mathrm{MAT} = \mathrm{MRT}_{\mathrm{i.p.}} - \mathrm{MRT}_{\mathrm{i.v.}} \quad (1)$$

where $\mathrm{MRT}_{\mathrm{i.p.}}$ and $\mathrm{MRT}_{\mathrm{i.v.}}$ are the mean residence time for intrapulmonary and intravenous administration, respectively.

Furthermore, we introduced a new parameter to estimate the release rate of CF from gelatin matrix as follows.

$$\Delta\mathrm{MAT} = \mathrm{MAT}_{\mathrm{gel}} - \mathrm{MAT}_{\mathrm{sol}} \quad (2)$$

where $\mathrm{MAT}_{\mathrm{gel}}$ and $\mathrm{MAT}_{\mathrm{sol}}$ represent the mean plasma appearance time at the administration of gelatin viscous solution and normal solution, respectively.

2.5. Dissolution test

The release of CF dissolved into the various concentrations of gelatin in the seamless cellulose tube was determined in 100 mL of PBS solution. One hundred microliters of aliquot of each solution was removed periodically for analysis, and the same volume of PBS solution was immediately replaced to maintain a constant volume. These samples were analyzed on a spectrofluorometer (HITACHI F-2000, Tokyo, Japan), using the excitation and emission wavelengths of 490 and 520 nm, respectively. The apparent permeability coefficient (P_{app}) in the presence of various concentrations of gelatin was calculated from the following equation.

$$P_{app} = \frac{dX}{dt}\frac{1}{C_0 A} \quad (4)$$

where P_{app} is apparent permeability coefficient of CF across the cellulose tube, X is the amount of drug released from the cellulose tubing to the PBS medium, C_0 is the initial concentration of drug, A is the surface area of cellulose tube.

The real permeability coefficients across the cellulose tube (P_g) in the presence of various concentrations of gelatin were calculated from the relationship

$$\frac{1}{P_g} = \frac{1}{P_{g+m}(P_{app})} + \frac{1}{P_m} \quad (5)$$

where P_m is the permeability coefficient of CF across the cellulose membrane without gelatin (control experiment).

2.6. Analytical methods

For determination of the CF and FD4 concentrations in plasma, 200 μl blood samples were taken from the femoral artery periodically after dosing, centrifuged at 10,000 rpm for 3 min, and the plasma (50 μl) was collected and added to an equal volume of 10% (w/v) Triton X-100. The plasma samples were appropriately diluted with Atkins-Pantins buffer (85 mM H_3BO_3, 85 mM KCl, 15 mM Na_2CO_3; pH 8.2) (Atkins and Pantins, 1926). The concentrations of drugs in such

samples were determined on a spectrofluorometer (Hitachi F-2000, Tokyo, Japan) using excitation and emission wavelengths of 490 and 520 nm for CF and 495 and 512 nm for FD4, respectively.

2.7. Statistical significance

Results are expressed as the mean ± S.E. and statistical significance was performed by the Student's *t*-test with the minimum levels of significance with $P < 0.05$ or Dunnett's test for multiple comparison.

3. Results

Fig. 1 shows the concentration–time profiles of CF after intratracheal administration with various viscous vehicles in rats. As shown in this figure, C_{max} value of CF decreased and the plasma concentration of CF was prolonged in the presence of various viscous vehicles as compared with the control without any viscous vehicle.

Fig. 2 shows the concentration–time profiles of CF after intratracheal administration with varying concentrations of gelatin in rats. As shown in Fig. 2, the plasma concentration of CF was prolonged and the absorption rate of CF decreased with increasing the concentrations of gelatin.

Table 1 summarizes the pharmacokinetic parameters of CF, which were calculated from the results of Figs. 1 and 2. As shown in Table 1, each C_{max} value significantly decreased and T_{max} values increased in the presence of various viscous vehicles compared with the control. MRT and MAT values significantly increased with these vehicles, and 5% gelatin and 1% PVA markedly increased the MRT and MAT values. Therefore, these findings suggested that these viscous vehicles were effective to regulate the absorption rate of CF. Table 1 also indicates the viscosity of drug solutions in the presence or absence of various viscous vehicles. Overall, the viscosity of drug solutions with

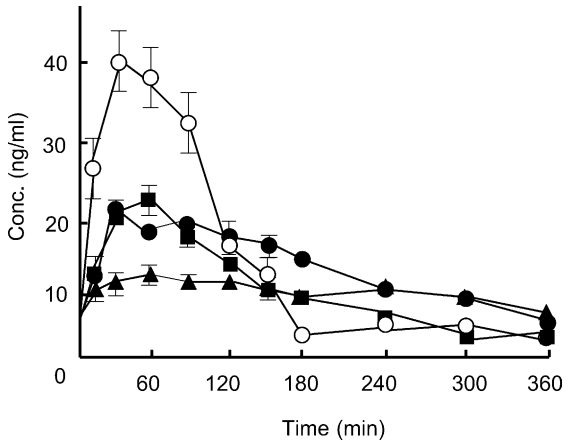

Fig. 2. Plasma concentration–time profiles after intratracheal administration of 5(6)-carboxyfluorescein (CF) with varying concentrations of gelatin in rats. Results are expressed as the mean ± S.E. of three to four rats. Keys: (○) control, (■) 1% gelatin, (●) 2% gelatin, (▲) 5% gelatin.

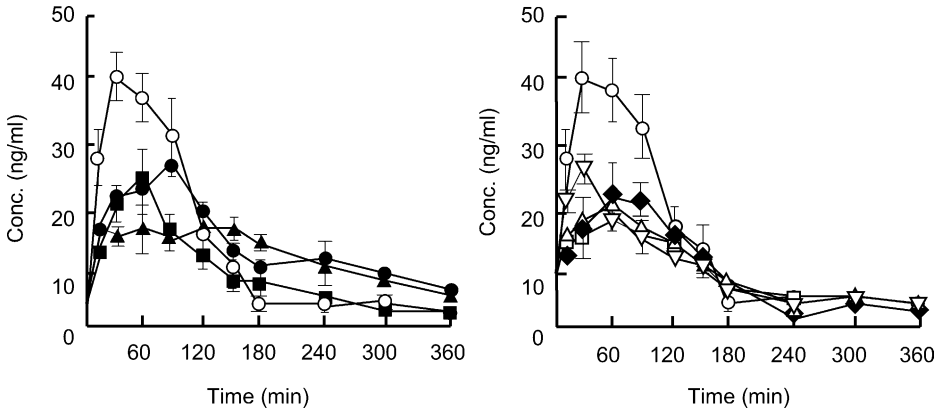

Fig. 1. Plasma concentration–time profiles after intratracheal administration of 5(6)-carboxyfluorescein (CF) with various viscous vehicles (1%) in rats. Results are expressed as the mean ± S.E. of three to four rats. Keys: (○) control, (■) 1% gelatin, (▲) 1% PVA, (●) 1% HPC, (♦) 1% CS, (▽) 1% PAA, (△) 1% MC400, (□) 1% HA.

Table 1
Pharmacokinetic parameters of CF after intratracheal administration in the presence of various viscous vehicles

	Relative viscosity	C_{max} (ng/ml)	T_{max} (min)	MRT (min)	MAT (min)	$AUC^{0-\infty}$ (ng min/ml)
Control	1.00	39.4 ± 8.8	40.0 ± 17.3	103.0 ± 18.0	63.0	6084.0 ± 1100.0
Gelatin 1%	1.43	26.3 ± 2.5*	45.0 ± 8.7	125.0 ± 21.3	85.0	3731.0 ± 400.0**
Gelatin 2%	2.15	22.7 ± 2.9*	50.0 ± 34.6	176.0 ± 28.4*	136.0*	4962.0 ± 200.0
Gelatin 5%	4.11	14.8 ± 1.8**	62.5 ± 45.0	267.0 ± 31.8***	227.0***	4438.0 ± 400.0*
PVA 1%	1.39	21.8 ± 1.2**	62.5 ± 22.5	178.0 ± 9.0***	138.0***	5003.0 ± 200.0
HPC 1%	8.43	25.6 ± 3.5**	82.5 ± 37.7	207.0 ± 46.9*	167.0*	5870.0 ± 1300.0
PAA 1%	1.02	26.1 ± 2.5*	25.0 ± 5.0	155.0 ± 9.0	115.0	3631.0 ± 900.0*
MC400 1%	2.78	22.3 ± 1.2**	45.0 ± 17.3	141.0 ± 14.3*	101.0*	3502.0 ± 20.0**
HA 1%	36.75	21.4 ± 2.3**	40.2 ± 12.2	147.0 ± 9.6**	107.0**	3254.0 ± 700.0**
CS 1%	1.44	24.5 ± 2.4*	62.5 ± 18.9	112.0 ± 7.1	72.0	3415.0 ± 300.0*

Results are expressed as the mean ± S.E. of three to four rats.
* $P < 0.05$, compared with the control.
** $P < 0.01$, compared with the control.
*** $P < 0.001$, compared with the control.

the viscous vehicles was higher than that without any viscous vehicle, especially 1% HA has the greatest effect for increasing the viscosity of drug solution.

Fig. 3 shows the concentration–time profiles of FD4 after intratracheal administration with gelatin or PVA, and Table 2 shows the pharmacokinetic parameters of FD4 calculated from the results of Fig. 3. As shown in this figure, 1% gelatin did not affect the T_{max} and MRT value of FD4, although it decreased the C_{max} and AUC values. These finding indicated that gelatin may not reduce the rate of FD4 absorption from the lung, but it may regulate the extent of pulmonary FD4 absorption. In addition, PVA slightly decreased MRT value of FD4 and significantly decreased its T_{max} value. However, its sustained effect was marginal and insignificant.

Furthermore, we examined the effect of different concentrations of gelatin on the release of CF from the cellulose tube. Their release profiles are presented in Fig. 4. More than 75% of CF was released within 240 min without any gelatin in the cellulose tube. On the other hand, the release of CF from the cellulose tube containing several concentrations of gelatin significantly decreased as compared with the control, i.e.,

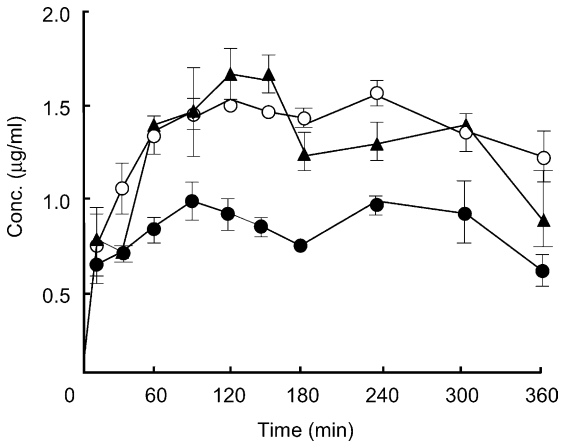

Fig. 3. Plasma concentration–time profiles after intratracheal administration of fluorescein isothiocyanate-labeled dextran with an average molecular weight of 4000 (FD4) with two viscous vehicles (1%) in rats. Results are expressed as the mean ± S.E. of four rats. Keys: (◯) control, (●) 1% gelatin, (▲) 1% PVA.

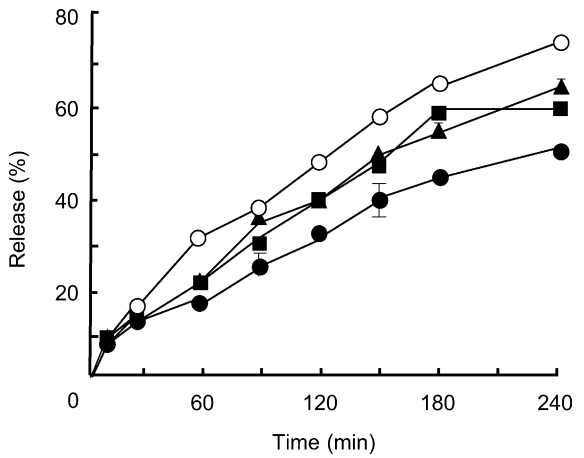

Fig. 4. Effect of gelatin concentrations on in vitro release of CF from gelatin matrices. Results are expressed as the mean ± S.E. of three to four experiments. Keys: (◯) control, (▲) 1% gelatin, (■) 3% gelatin, (●) 5% gelatin.

Table 2
Pharmacokinetic parameters of FD4 after intratracheal administration in the presence of various viscous vehicles

	C_{max} (μg/ml)	T_{max} (min)	MRT (min)	$AUC^{0-\infty}$ (μg min/ml)
Control	1.6 ± 0.1	220 ± 24.5	283 ± 71.6	673.7 ± 243.9
Gelatin 1%	1.2 ± 0.1*	156 ± 41.6	303 ± 67.0	437.8 ± 9.5
PVA 1%	1.8 ± 0.1	120 ± 12.2*	246 ± 33.7	558.4 ± 104.3

Results are expressed as the mean ± S.E. of four rats.
* $P < 0.05$, compared with the control.

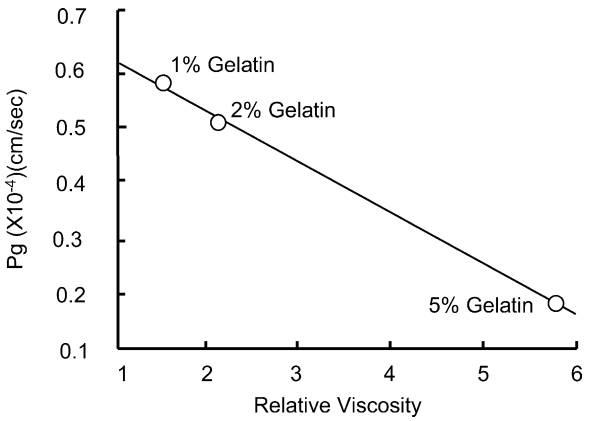

Fig. 5. Correlation between in vitro permeability coefficients of CF and relative viscosity in the presence of varying concentration of gelatin. The correlation of the coefficient was 0.999.

without any gelatin. When the amount of gelatin in the cellulose tube increased, the release of CF decreased.

Fig. 5 shows the correlation between in vitro permeability coefficients (P_g) of CF from the cellulose tube and relative viscosity in the presence of varying concentrations of gelatin. There exists a linear correlation between these parameters with a correlation coefficient of 0.999, and the permeability of CF across the cellulose membrane with gelatin was inversely related to the viscosity of gelatin.

Fig. 6 illustrates the correlation between in vitro permeability coefficients (P_g) of CF and ΔMAT in the presence of varying concentrations of gelatin. There exists a linear correlation between these parameters with a correlation coefficient of 0.986, and the permeability of CF across the cellulose membrane with gelatin was inversely related to ΔMAT.

4. Discussion

In general, pulmonary absorption of drugs was influenced by various physicochemical and biological factors. These factors include molecular size of drugs (Enna and Schanker, 1972a), lipophilicity of drugs (Enna and Schanker, 1972b), pH in drug solution (Arakawa and Kitazawa, 1987), various additives

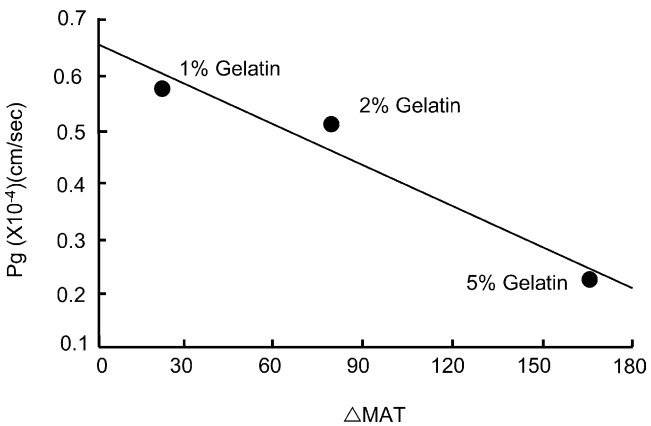

Fig. 6. Correlation between in vitro permeability coefficients of CF and ΔMAT in the presence of varying concentrations of gelatin. The correlation of the coefficient was 0.986.

(Ohtani et al., 1991; Morita et al., 1993), species and gender differences (Folksson et al., 1990), etc. On the other hand, viscosity of a drug solution is one of the most important physicochemical and pharmaceutical factors determining absorption rate of a drug. In ophthalmic therapy, polymers increasing vehicle viscosity have often been added to drug solutions to improve ocular bioavailability by prolonging contact of a drug with the eye tissues (Davies et al., 1991; Saettone et al., 1994; Sasaki et al., 1999, 2000; Nishida et al., 2000). However, few studies have been examined the effect of viscosity on the pulmonary absorption of drugs (Morimoto et al., 2001). In this study, therefore, we examined the effects of various viscous vehicles on the pulmonary absorption of drugs in rats.

The present study indicated that the pulmonary absorption of CF was regulated in the presence of 5% gelatin, 1% PVA, 1% HPC, 1% MC400 and 1% HA. Of these viscous vehicles, gelatin is a low-toxic, biodegradable and highly water-soluble compound. Moreover, gelatin solution has the advantage that it is relatively rapidly degraded in the body fluid and is easily prepared. It is generally known that an aqueous solution of gelatin has viscous and mucoadhesive characteristics. Nishihata et al reported that a gel containing 8% gelatin co-administered with insulin and 5-methoxysalicylate increased the intestinal absorption of insulin by restricting the movement of insulin and the adjuvant down the intestine (Nishihata et al., 1981). Therefore, the control of absorption rate of CF with gelatin from the lung in the present study may be due to the viscous gelatin formulation. Furthermore, our previous findings indicated that the antitumor effect of mitomycin C (MMC) was enhanced by using the gelatin viscous solution, which is due to the decrease in the clearance rate of MMC from the peritoneal cavity to the systemic circulation (Fujita et al., 1997). Therefore, gelatin solution can be useful to enhance the antitumor effects of anti-cancer drugs in rats.

On the other hand, PVA produced by hydrolysis of polyvinylpyrrolidone has several residues of acetic acid. PVA solution has characteristics of viscous additive owing to its complexity in molecule residue and has been used to enhance the precorneal retention of solutions and maintain an adequate concentration of the drug in the precorneal area. Davies et al demonstrated that the bioavailability of pilocarpine from PVA solution was significantly greater than that from PBS (Davies et al., 1991). Therefore, PVA is effective to enhance the ocular absorption of drugs by increasing the retention time of drug solution.

Sodium hyaluonate, which is a straight chain and unbranched polymer with repeating disaccharide units of glucuronic acid and *N*-acetylglucosamine, is a natural polymer and a major component of interstitial tissue (Hadler et al., 1982). An aqueous solution of sodium hyaluronate has viscous and mucoadhesive characteristics (Saettone et al., 1989). Morimoto et al reported that a viscous sodium hyaluronate solution enhanced the nasal absorption of vasopressin and its analogs (Morimoto et al., 1991). They recently demonstrated that the hyaluronate solutions (0.1–0.2%, w/v) significantly enhanced the pharmacological availability (PAB) of insulin from the lung compared to the aqueous solution of insulin at pH 7.0 (Morimoto et al., 2001). On the other hand, our present finding indicated that the pulmonary absorption of CF was controlled and reduced by the addition of HA, although we used higher concentration of HA(1%) as compared with the report of Morimoto et al., (2001). Therefore, the effect of HA on the pulmonary absorption of drugs may depend on the concentration of HA. Moreover, we observed a decrease in the extent and rate of absorption of CF in the presence of CS, PAA and MC400, but the reason was not clearly observed. Probably, the viscosity of these vehicles was not optimal for controlling the absorption of CF from the lung, since the viscosity value in the presence of PAA and CS was not so much different from that without any vehicle.

Unlike CF, the pulmonary absorption of FD4 was not clearly regulated by the addition of gelatin and PVA, as compared with the case of CF. The reason for the negative result of these viscous vehicles for controlling the pulmonary absorption of FD4 is not fully understood. However, the molecular weight of FD4 is considerably greater than that of CF and the absorption rate of FD4 even in the absence of viscous vehicles is relatively lower than that of CF. Therefore, it may be reasonable that the pulmonary absorption of drugs with high molecular weight and low absorption rate from the lung may not be clearly controlled by the addition of various viscous vehicles.

The present study demonstrated that the release of CF from the cellulose tube decreased as the concentration of gelatin increased. In addition, the viscosity in the solution increased with increasing the concentration

of gelatin. Cheong et al. (1992) demonstrated that the diffusion rate of propranolol from the hydroxypropyl methycellulose (HPMC) matrix was delayed with increasing HPMC concentration. From these findings, we suggested that the higher concentration of gelatin might increase the viscosity of drug solution, thereby reducing the release rate of CF from the cellulose tube. We also observed a linear correlation between the release of CF and ΔMAT in this study. This finding indicates that gelatin may reduce the diffusion rate of CF in dosing solution and this low diffusion rate of CF may be related to the increase in ΔMAT value. However, it was found that there seems to be almost no correlation between the viscosity and MRT and MAT values in the presence of other viscous vehicles. Consequently, viscosity is not a major factor controlling the pulmonary absorption of drugs in case of other viscous vehicles, although the mechanism is not fully understood.

In conclusion, we demonstrated that the pulmonary absorption of CF with low molecular weight and a highly water-soluble characteristic could be regulated in rats by using the various viscous vehicles. This method using various viscous vehicles may be useful to retain the local acting drugs to the lung tissues and to control the absorption rate of drugs from the lung to the systemic circulation.

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