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Effect of absorption enhancers on pulmonary absorption of fluorescein isothiocyanate dextrans with various molecular weights

Toshihide Ohtani, Masahiro Murakami, Akira Yamamoto, Kanji Takada and Shozo Muranishi

Department of Biopharmaceutics, Kyoto Pharmaceutical University, Kyoto 607 (Japan)

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Summary

The effects of absorption enhancers on the pulmonary absorption of fluorescein isothiocyanate-labelled dextrans (FDs) with different average molecular weights (4000–150000) were examined by means of an in situ absorption method. Absorption enhancers used in this study were linoleic acid-surfactant mixed micelles (MM), *N*-lauryl-β-D-maltopyranoside (LM), diethyl maleate (DEM) and Na_2EDTA. In the absence of absorption enhancers, the plasma concentration and percent absorption of FDs after intrapulmonary administration decreased with increase in their molecular weight. Of the absorption enhancers, MM and LM appeared to be more effective at enhancing the pulmonary absorption of FD with an approximate molecular weight of 40000 than DEM and Na_2EDTA. MM showed the strongest enhancing effect on FD with an approximate molecular weight of 40000 while maximal effect of LM was observed in pulmonary absorption of the FD with an approximate molecular weight of 70000. These results suggest that lung may afford a favorable route for systemic delivery of macromolecules and absorption enhancers such as MM and LM may increase their pulmonary absorption.

Introduction

The oral bioavailability of peptides and proteins is typically poor due to their extensive hydrolysis and poor membrane penetration characteristics (Lee and Yamamoto, 1990; Lee, 1991). Consequently, almost all peptides and proteins are administered by frequent injections, which are poorly accepted by patients and may cause an allergic reaction. Thus, alternatives, such as the nasal (Hirai et al., 1981), buccal (Ishida et al., 1981), rectal (Nishihata et al., 1983), vaginal (Okada et al., 1982), conjunctival (Yamamoto et al., 1989) and transdermal (Burnette and Marrero, 1986) routes, are being investigated for peptide and protein delivery.

Correspondence: S. Muranishi, Dept of Biopharmaceutics, Kyoto Pharmaceutical University, Misasagi, Yamashina-ku, Kyoto 607, Japan.

Of the various alternative routes, intrapulmonary administration of peptides and proteins may offer a potentially useful means of systemic delivery of these compounds, since a number of drugs which are poorly absorbed from enteral and other topical sites are well absorbed from lung due to the large surface area of the alveolar epithelium and the short distance of the air-blood pathway (O'Hagan and Illum, 1990). In addition, drugs which are administered to the lung will avoid hepatic first-pass elimination. It has been reported that absorption of heparin (Kavanagh and Jaques, 1976), salbutamol (Schenfield et al., 1976), disodium cromoglycate (Gardiner and Goodman, 1976), cyanocobalamin (Schanker and Burton, 1976), gentamicin (Trnovec et al., 1978), kanamycin (Teske and Miller, 1970) and insulin (Jones et al., 1988) which are poorly absorbed from the gastrointestinal tract, is observed after intrapulmonary administration. However, the pulmonary epithelium appears to be extremely impermeable to proteins and peptides with relatively greater molecular weights. Therefore, it is important that basic information about the absorption characteristics of macromolecules from the lung and the effectiveness of absorption enhancers for these macromolecules is established.

In this study, fluorescein isothiocyanate-labelled dextrans (FDs) with different average molecular weights (4000–150000) were chosen as model macromolecules and their pulmonary absorption was examined in rats. Further, the effects of various absorption enhancers such as diethyl maleate (DEM), ethylenediaminetetraacetic acid disodium salt (Na_2EDTA), linoleic acid (LA)-polyoxyethylated (60 mol) hydrogenated castor oil (HCO60) mixed micelles (MM) and *N*-lauryl-β-D-maltopyranoside (LM), on pulmonary absorption of FDs were investigated.

Materials and Methods

Materials

FDs were obtained from Sigma Chemical Co. (St. Louis, MO). The mean molecular weights of the FDs employed are listed in Table 1. DEM was purchased from Kanto Chemical Co. (Tokyo, Japan). Na_2EDTA was obtained from Nacalai Tesque Inc. (Kyoto, Japan). LA of high purity grade (> 99.0%) was kindly supplied by Nippon Oil & Eats Co. (Tokyo, Japan). HCO60 was a gift from Nikko Chemical Co. (Tokyo, Japan). LM was kindly supplied by Japan Fine Chemical Co. (Osaka, Japan). All other chemicals and solvents were of reagent grade quality.

TABLE 1

Average molecular weights of FITC-dextrans

Abbreviation	Molecular weight	Stokes radius (Å)
FD- 4k	4400	
FD- 10k	9000	23
FD- 20k	18900	32
FD- 40k	40500	44
FD- 70k	71600	58
FD-150k	154200	84

Preparation of test solutions

Dosing solutions containing FDs were prepared in isotonic phosphate buffer (PBS) at pH 7.4 to yield a final concentration of 50 mg/ml. In certain experiments, the dosing solutions were added with absorption enhancers such as DEM, Na_2EDTA, LA-HCO60 MM or LM to yield a final concentration of 1–50 mM. DEM, Na_2EDTA or LM was dissolved in PBS at pH 7.4. A solution of LA-HCO60 MM was prepared by dispersing LA and HCO60 (molar ratio of LA : HCO60, 30 : 4) in PBS, followed by sonication at 40 W for 5 min in an ice bath using an Ohtake model 5202 sonicator (Ohtake Seisakusho Co., Tokyo, Japan). The fatty acid was neutralized with aqueous 1 N NaOH prior to dispersion.

Animal experiments

Absorption of drugs from rat lung was investigated according to the method of Enna and Schanker (1972a,b). Male Wistar strain rats (Japan SLC, Inc., Hamamatsu, Japan) weighing 240–300 g were anesthetized with sodium pentobarbital (32 mg/kg body weight) given by intraperitoneal injection. Animals were fasted for about 16 h prior to experiments but allowed water ad libitum. 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) of length 2.5 cm, 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.

Body temperature was maintained at 37 ± 1 °C by heat from a 40 W incandescent lamp in a reflector suspended over the animal at a distance of about 25 cm during the experiment. Body temperature was monitored continuously at the rectum using a thermistor probe and thermometer.

100 μl of drug solution at 37 °C was injected into the lungs through an 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. With the syringe thus positioned, the solution was injected over a period of 1–2 s, with the rat being maintained at an angle of 80°. Immediately thereafter, the tubing was withdrawn completely, and after 45 s on administration, the animal was returned to an angle of 10°.

The animal was maintained under light anesthesia for the remainder of the experimental period.

Analytical methods

For determination of the FD concentration in plasma, 200-μl blood samples were taken from the left femoral artery periodically after dosing, centrifuged at 10000 rpm for 3 min, and the plasma (50 μl) was collected and added to an equal volume of 10 (w/v)% Triton X-100.

For estimation of the percentage of FD remaining in the lung, the animals were killed by intravenous injection with sodium pentobarbital and the lung was excised. At the end of the absorption period, the blood supply to the pulmonary artery was quickly severed by cutting around both sides of the organ, and the pulmonary artery, together with the heart, trachea

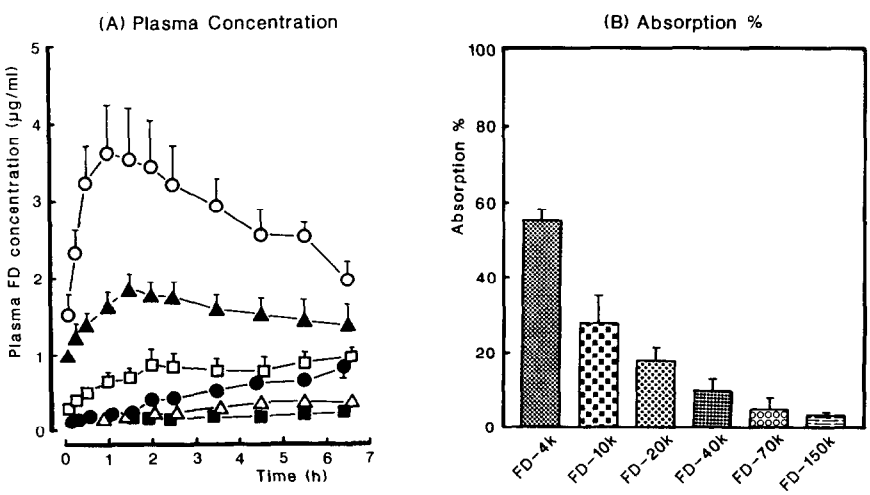

Fig. 1. Plasma concentration (A) and absorption percentage (B) of FDs after intrapulmonary administration to rats at a dose of 5 mg/250 g. (○——○) FD-4k; (▲——▲) FD-10k; (□——□) FD-20k; (●——●) FD-40k; (△——△) FD-70k; (■——■) FD-150k. Each point or value is the mean for 3–8 animals. Vertical bars indicate the S.E.; the absence of bars indicates that the S.E. is within the size of the symbol.

and attached cannula, were removed from the body. The pleural cavity was carefully opened and the trachea was gently pulled away from the surrounding tissues to a point near its entrance into the pulmonary artery. After the cannula had been removed, the heart, surrounding fatty tissue and esophagus were trimmed away, and the pulmonary artery and trachea were weighed immediately and prepared for assay of unabsorbed compound. The percent absorption of FDs from rat lung was estimated as the difference between the dosing amount of drug in the initial solution and the percentage of drug remaining in the lung at the end of experiments. The pulmonary sample was homogenized in three times its weight of PBS using a Polytron® homogenizer (Kinematica, GmbH, Switzerland) and then a Teflon homogenizer (Iuchi Co., Japan). The homogenate (0.5 ml) was thoroughly mixed with 3 ml of 12% trichloroacetic acid, followed by centrifugation at 3000 rpm for 30 min. The supernatant was then neutralized with aqueous 1 N NaOH.

The plasma and pulmonary homogenate samples were appropriately diluted with Atkins-Pantin buffer (85 mM H_3BO_3, 85 mM KCl, 15 mM Na_2CO_3; pH 8.2) (Atkins and Pantin, 1926). The concentration of FD in such samples was determined on a spectrofluorometer (Hitachi model 650-10S, Tokyo, Japan) using excitation and emission wavelengths of 495 and 512 nm, respectively. The percent recovery of the drugs is almost 100% in each experiment.

Results

Absorption of FDs after intrapulmonary administration

Fig. 1A shows the plasma concentration profiles of FDs with various molecular weights after intrapulmonary administration in rats. As is evident from Fig. 1A, the plasma concentrations of FDs gradually decreased with increasing molecular weight of the compounds. Similarly, the percent absorption of FDs from rat lung diminished with increase in the molecular weight of the FDs (Fig. 1B).

Fig. 2A illustrates the relationship between the molecular weight and bioavailability of FDs after intrapulmonary administration. A linear correlation exists between log bioavailability and log molecular weight for the FDs over the molecular weight range 4000–150000 (correlation coefficient: 0.987). Similar results were also noted between log percent absorption and log molecular weight for FDs, with a correlation coefficient of 0.996 (Fig. 2B). Further, the data on percent bioavailability of FDs correlated well with the results for the percent absorption of the compounds from the lung (data not shown).

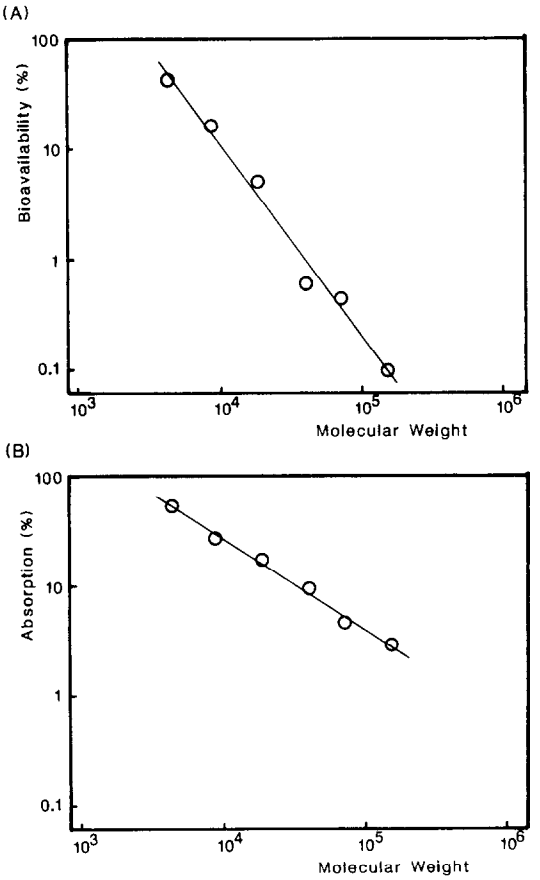

Fig. 2. Relationship between percent bioavailability (A) or percent absorption (B) and molecular weight of FDs on absorption from rat lung.

Effect of absorption enhancers on pulmonary absorption of FDs

Fig. 3 indicates the effects of various absorption enhancers on the plasma concentration of

approximate molecular weight 40000 (henceforth referred to as FD-40k) after intrapulmonary administration in rats. As shown in Fig. 3A and B, no significant increase in plasma concentration of FD-40k was observed in the presence of DEM over the concentration range 1–50 mM. Similarly, Na_2EDTA exerted no significant influence on the plasma concentration of FD-40k, although we did observe a slight enhancement effect at 50 mM Na_2EDTA. In contrast, a significant increase in plasma concentration of FD-40k occurred in the presence of MM or LM at higher concentrations, and maximal enhancement was obtained at 20 mM of each absorption enhancer (Fig. 3C,D).

Table 2 summarizes the effects of the various absorption enhancers on the pharmacokinetic parameters of FD-40k after intrapulmonary administration. Absorption of FD-40k from the lung into the bloodstream was almost negligible unless absorption enhancers were included in the dosing solution. In the presence of MM or LM at higher concentrations, the plasma concentration of FD-

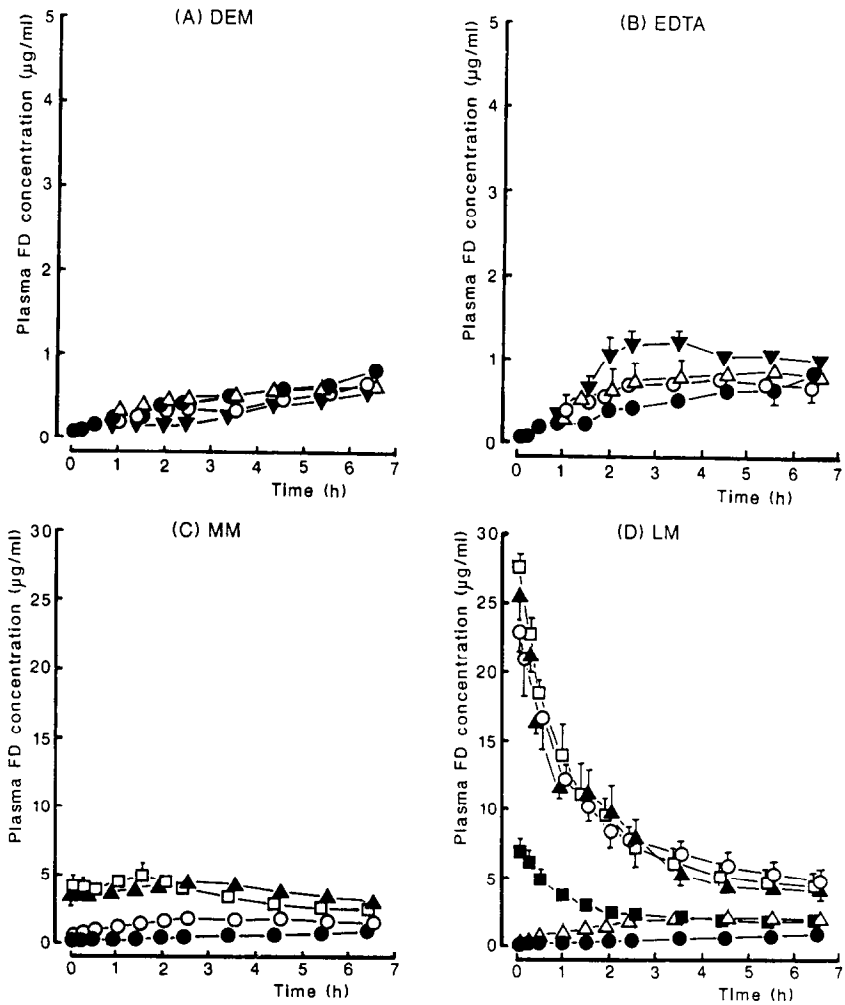

Fig. 3. Effect of various absorption enhancers on plasma concentration of FD-40k after intrapulmonary administration to rats. (●——●) No additive; (△——△) 1 mM; (■——■) 5 mM; (○——○) 10 mM; (▲——▲) 20 mM; (□——□) 30 mM. Each point is the mean for 3–8 animals. Vertical bars indicate the S.E.; the absence of bars indicates that the S.E. is within the size of the symbol.

TABLE 2

Comparison of bioavailabilities of FD-40k after intrapulmonary administration with various absorption enhancers (means ± S.E., n = 3–5)

Adjuvant	Concentration (mM)	C_{max} (μg/ml)	T_{max} (min)	$AUC_{0-6.5\ h}$ (mg min ml^{-1})	Bioavailability (%)	Absorption (%)
None	–	–	–	0.18 ± 0.02	0.58	7.14 + 2.62
DEM	1	–	–	0.19 ± 0.02	0.59	11.15 ± 2.00
	10	–	–	0.14 ± 0.04	0.44	14.56 ± 1.06
	50	–	–	0.11 ± 0.02	0.35	14.79 ± 1.30
Na_2EDTA	1	–	–	0.25 ± 0.06	0.81	12.36 ± 0.05
	10	–	–	0.24 ± 0.09	0.76	12.79 ± 1.52
	50	–	–	0.35 ± 0.03	1.11	15.76 ± 0.17
MM	10	1.95 ± 0.32	197.46 ± 38.46	0.58 ± 0.09	1.87	18.16 ± 5.28
	20	4.38 ± 0.77	123.66 ± 51.54	1.51 ± 0.16	4.84	20.62 ± 3.72
	30	5.52 ± 0.12	54.60 ± 23.40	1.37 ± 0.12	4.38	21.14 ± 3.04
LM	1	1.89 ± 0.96	311.04 ± 78.96	0.65 ± 0.17	2.06	10.44 ± 1.62
	5	9.51 ± 1.48	7.32 ± 1.20	1.30 ± 0.18	4.15	14.82 ± 7.00
	10	23.50 ± 2.73	6.72 ± 0.60	3.15 ± 0.39	10.05	26.42 ± 6.76
	20	27.77 ± 2.74	5.22 ± 0.66	3.17 ± 0.14	10.13	22.10 ± 2.48
	30	24.38 ± 3.15	7.80 ± 1.98	3.05 ± 0.41	9.73	25.38 ± 6.92

40k reached a peak within 60 or 10 min, respectively, of solution administration, whereas no peak was observed in the presence of the absorption enhancers DEM or Na_2EDTA, the maximal plasma concentration of FD-40k being achieved as indicated above in the case of 20 mM LM. Overall, MM and LM appeared to be more effective at enhancing the pulmonary absorption of FD-40k than DEM and Na_2EDTA. Similar results were also noted concerning the effect of absorption enhancers on the percent absorption of FD-40k from the lung, although some discrepancies between percent bioavailability and percent absorption were found.

The effect of 20 mM MM or 10 mM LM on plasma concentrations of FDs with various molecular weights after intrapulmonary administration was also examined. As shown in Fig. 4, the plasma concentration of all FDs increased on coadministration with MM or LM. Fig. 5 depicts the relationship between the enhancement effect and the molecular weight of the FDs on pulmonary absorption. The absorption enhancement by LM or MM was evaluated from the ratio of AUC between control and enhancers. As demonstrated in Fig. 5, MM showed the strongest enhancement effect on drug with an approximate molecular weight of 40000, while the maximal effect of LM was observed in the pulmonary absorption of drug with an approximate molecular weight of 70000.

Discussion

The present study has demonstrated that a close correlation exists between the log bioavailability and log molecular weight for the pulmonary absorption of FDs. This result is fairly consistent with the previous findings of Enna and Schanker (1972a) who reported that the absorption rate constants of saccharides and urea from rat lung ranked in the same order as the diffusion coefficients of the compounds, although the two parameters were not directly proportional in some instances. Further, Folkesson et al. (1990) examined the pulmonary absorption of macromolecules such as bovine IgG (Mol.Wt = 150000), bovine serum albumin (BSA, Mol.Wt = 67000) and 1-deaminocysteine-8-D-arginine vasopressin

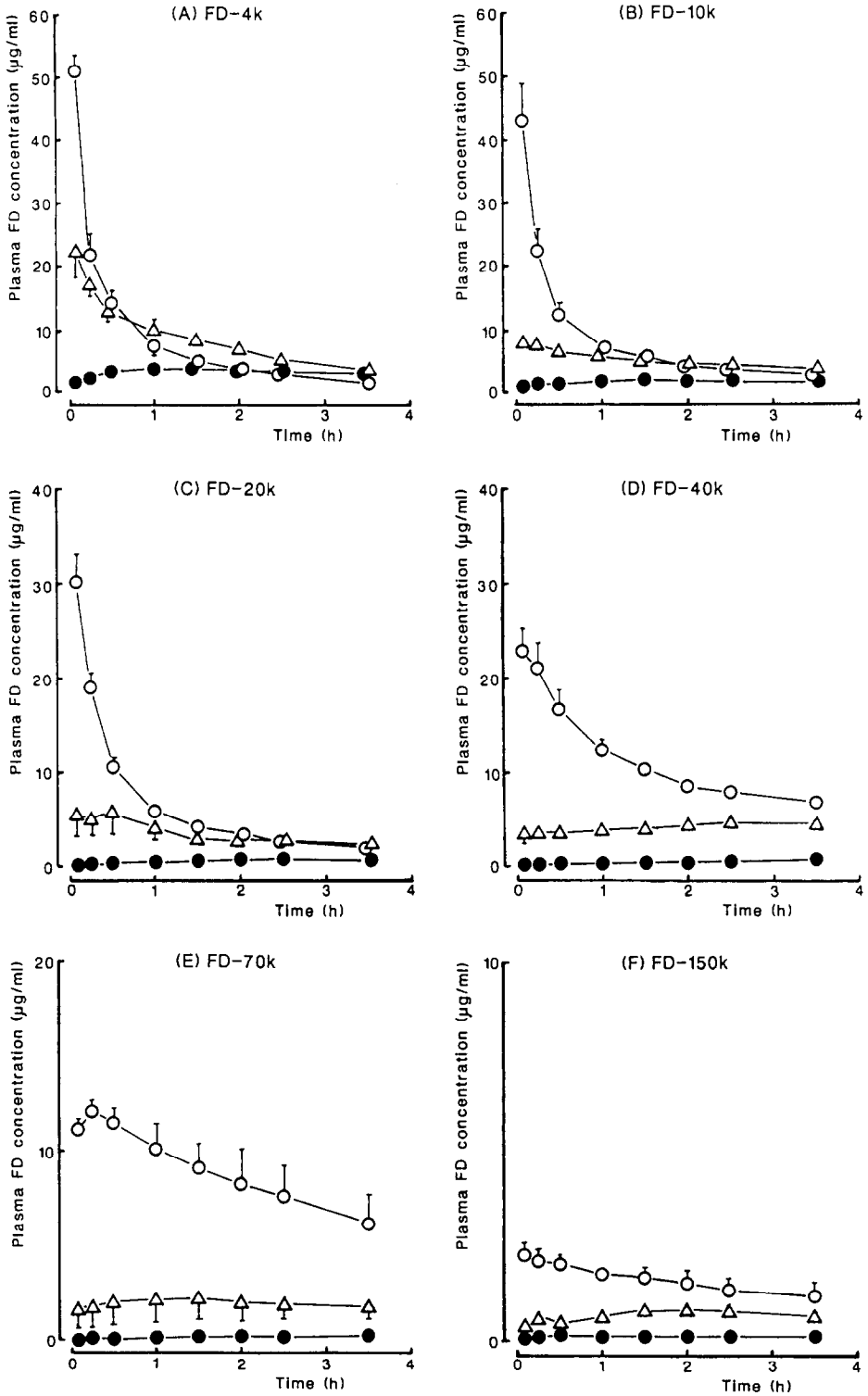

Fig. 4. Effect of 20 mM MM (△) or 10 mM LM (○) on plasma concentration of FDs after intrapulmonary administration to rats. Each point is the mean for 3–8 animals. Vertical bars indicate the S.E.; the absence of bars indicates that the S.E. is within the size of the symbol.

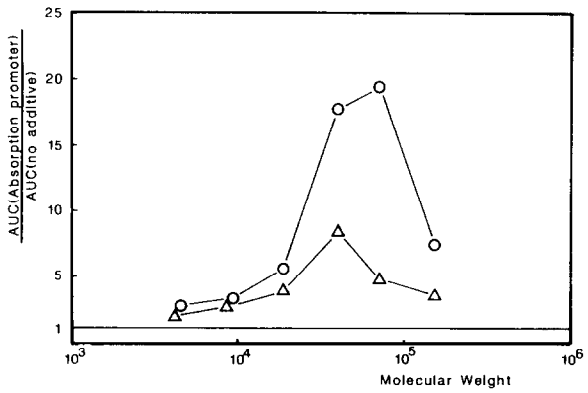

Fig. 5. Relationship between enhancement effect of MM or LM and molecular weight of FDs on absorption from rat lung. (△——△) 20 mM MM; (○——○) 10 mM LM.

(dDAVP, Mol.Wt = 1067) and found that an inverse relationship appears to exist between the molecular weight of instilled macromolecules and the transferred amount. These results suggest that the absorption of macromolecules such as FDs from rat lung depends on their average molecular weights. Similar findings have also been reported on the nasal absorption of drugs with various molecular weights by Fisher et al. (1987), Maitani et al. (1989), McMartin et al. (1987) and Donovan et al. (1990).

We observed some discrepancies between percent bioavailability and percent absorption from the lung in the presence of absorption enhancers (Table 2), although a close correlation exists between the parameters in the absence of enhancers as described in Results. The reason for these discrepancies is as yet unknown. It may be considered that the probable cause is that the percent bioavailability was calculated from the plasma data, while the percent absorption was based on the data on percentage remaining in lung tissue, which include tissue accumulation and binding of the drugs.

Based on our experimental results, almost 50% of FD-4k and 10% of FD-40k were absorbed from the lung in 6.5 h. This suggests that the absorption of drugs from the lung was much more rapid than that from the small intestine, since the intestinal absorption of drugs of Mol.Wt > 10000 is considerably limited. However, our results indicate that macromolecules of molecular weights in excess of 70000 are poorly absorbed from lung. Consequently, the inclusion of absorption enhancers which could temporarily diminish the barrier resistance of structural elements of lung toward impermeable drugs, represents a useful strategy to improve the pulmonary absorption of macromolecules of high molecular weights. Recently, Niven and Byron (1990) reported that surfactants such as oleic acid, oleyl alcohol and Span 85 can produce an increase in the transfer rate of disodium fluorescein, a model compound, from the airways of the isolated rat lung. However, very few studies have been reported concerning the effect of various absorption enhancers on the pulmonary absorption of drugs. In this study, MM, LM, DEM and Na_2EDTA were chosen as model absorption enhancers, since they are known to improve the intestinal absorption of drugs. MM, which was discovered as an absorption enhancer in our laboratory, is known to improve the absorption of a number of poorly absorbable drugs such as heparin, streptomycin, gentamicin, bleomycin, interferon and carboxyfluorescein from the gastrointestinal tract while we observed slight mucosal damage in intestinal tissue (Muranishi, 1990). We have also reported that among the lipoidal substances, unsaturated fatty acids are the most effective and that the colorectum is the most sensitive portion of the gastrointestinal tract (Muranishi, 1990). As clearly demonstrated in Fig. 3 and Table 2, pulmonary absorption of FD-40k was improved by the addition of MM, indicating that MM enhanced the absorption of drugs in both the gastrointestinal tract and lung. Moreover, the maximal enhancement effect on the pulmonary absorption of FD-40k was attained in the presence of 20 mM MM. This result is in good agreement with previous data showing that the optimal concentration of MM for improving the absorption of drugs from the gastrointestinal tract was about 20 mM (Murakami et al., 1988).

LM, an alkylsaccharide, has recently been found to lower surface tension and to have absorption-enhancing activity in the gastrointestinal tract. In our previous studies, rectal absorption of CF, FDs and insulin was improved by coadminis-

tration of LM and the maximal effect was noted at 10 mM LM (Murakami et al., 1992). In addition, histological examination of the rectal tissue revealed a slight change in mucosal structural integrity at doses of LM that resulted in increased drug absorption. As demonstrated in Fig. 3 and Table 2, LM afforded a significant increase in the pulmonary absorption of FD-40k, indicating that LM is an effective absorption enhancer in both intestinal and pulmonary routes.

The mechanism by which pulmonary absorption was improved in the presence of MM or LM is still unclear. Recently, Murakami et al. (1988) demonstrated that the enhancement of permeation of carboxyfluorescein from the colon induced by unsaturated fatty acids is related to the intact SH group of membrane-associated protein. Therefore, it may be plausible to consider this SH group as contributing to the enhanced absorption of FDs from the lung as well as the intestine.

We observed no apparent increase in the absorption of FDs in the presence of DEM or Na_2EDTA, although in some cases both enhancers are effective at improving the absorption of poorly absorbable drugs from the gastrointestinal tract. DEM, which is known to increase the permeability of drugs by interacting with glutathione in membranes, is a transcellular enhancer (Nishihata et al., 1984) whereas intercellular permeability of drugs was increased in the presence of EDTA by chelation of Ca^{2+} and Mg^{2+} in the regions of the tight junction (Schanker and Johnson, 1961; Cassidy and Tidball, 1967; Kunze et al., 1972). The discrepancy in the effectiveness of the enhancers between lung and intestine may be partly explained by morphological differences, varying sensitivity of enhancers and application of enhancers for different drugs.

As shown in Fig. 5, maximal enhancement effects for MM and LM were noted in the pulmonary absorption of drugs with approximate molecular weights of 40000 and 70000, respectively. This finding may be attributed to the following reasons. In the case of FDs with lower molecular weights, we determined a low value for the ratio of AUC between control and enhancers, since the FDs were absorbed to a considerable extent from the lung without any enhancer and the AUC value for the control experiments was rather high. On the other hand, it was considered that the molecular sizes of the FDs with higher molecular weights were so large that the absorption of such FDs from the lung would not be markedly improved even in the presence of absorption enhancers.

In summary, it is suggested that macromolecules are relatively well absorbed from the lung, which may afford a favorable route for systemic delivery to peptides and proteins. MM and LM are suitable absorption enhancers for improving the pulmonary absorption of macromolecules.

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