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Influence of molecular weight and charge on nasal absorption of dextran and DEAE–dextran in rabbits

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Summary

Two dextran derivatives, fluorescein isothiocyanate-dextran (DT) and fluorescein isothiocyanate diethylaminoethyldextran (DE), were administered intranasally to study how the molecular weight and charge influence permeation through nasal mucosa in rabbits. These compounds were used as macromolecular models of water-soluble drugs. The concentration in plasma was determined using a high-performance liquid chromatograph equipped with a fluorescence spectrometer by means of a fluorescein isothiocyanate-dextran derivative conjugate. The concentration of DT in plasma decreased as the molecular weight of DT increased. The administration of DE (molecular weight less than 10,000) resulted in a significant increase of the concentration of DE in plasma, compared with DT. The concentration of DE in plasma increased conversely to the molecular weight of DE.

Introduction

In recent years, intranasal (i.n.) administration of peptides has received great attention. In addition to offering advantages such as rapid absorption and avoiding the first-pass effect, it provides for delivery of drugs which are degraded in the gastrointestinal (GI) tract and cannot be given orally such as peptides and proteins. It has been shown that low-molecular weight peptides (TRH, LHRH) (Anik et al., 1984), enkephalins (Su et al., 1985) as well as high-molecular weight polypeptides (insulin) (Hirai et al., 1978), GH-releasing factor (Evans et al., 1983) human interferon-β (Maitani et al., 1986) can be absorbed intranasally. But some drugs with a molecular weight and peptides were reported to have a very low bioavailability after intranasal administration.

In a previous paper, we reported that the bioavailability was only 3% for human interferon-β by nasal administration, compared with i.v. administration. Also, the mechanism of nasal absorption of human interferon-β is not fully understood. Therefore, we investigated the macromolecular permeability of the nasal mucosa of rabbits. Fluorescein isothiocyanate-dextrans (FITC-dextran, mol. wt. 4100, 9000, 17,500) were used as macromolecular models of water-soluble drugs. FITC-dextran of mol. wt. 4100, 9000 and 17,500 have been abbreviated to DT-4100, DT-9000 and DT-17500, respectively.

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To investigate the role of various functional groups of dextran, diethylaminoethyldextrans (DEAE-dextran, mol. wt. 6000, 9000, 17,200) were also used. Fluorescein isothiocyanate-DEAE-dextran of mol. wt. 6000, 9000 and 17,200 have been abbreviated as DE-6000, DE-9000 and DE-17200, respectively. These compounds were studied because they are non-toxic, non-immunotoxic and biodegradable for parenteral administration (Mckernan and Ricketts, 1960). The biological properties of dextran and DEAE-dextran were well investigated in vivo (Arturson and Wallenius, 1964; Rice et al., 1973). In addition, many molecular weights of dextran are also commercially available. The concentration of DT and DE in plasma was determined by a high-performance liquid chromatograph (equipped with a fluorescence spectrophotometer).

Materials and Methods

FITC-dextran (DT-4100, DT-9000, DT-17500) and dextran (mol. wt. 6000, 9000, 17,200) were purchased from Sigma Chemical Co. (U.S.A.) and used without further purification. Fluorescein isothiocyanate (isomer-I) and sodium glycocholate (G) were purchased from Wako Pure Chem. and Nakarai Chem., respectively.

Preparation of DEAE-dextran

DEAE-dextran was synthesized according to the method reported by Mckernan and Ricketts (1960). Dextran (mol. wt. 6000, 9000, 17,500; 6 g) was dissolved in a sodium hydroxide solution (4 g of sodium hydroxide in 17 ml of water) and then cooled to 0 °C. Next, 2-chlorotriethylamine hydrochloride solution (3.5 g in 4.5 ml of water) was added with efficient stirring. The temperature was raised to 80–85 °C for 35 min. After several precipitations in ethanol, a solution of the final precipitate was neutralized with hydrochloric acid, dialyzed by using 1-7/8 type Seamless Cellulose Tubing (Union Carbide Co.) and concentrated under reduced pressure. The basic dextran was isolated as its hydrochloride by freeze-drying. The precipitate was dissolved in distilled water, and chromatographed on a Sephadex G-75 column (3.5×40 cm) twice, after which the macromolecular fraction was dried in vacuo at room temperature. Products, DEAE-dextran (mol. wt. 6000) DEAE-dextran (mol. wt. 9000) and DEAE-dextran (mol. wt. 17,200) containing 0.55%, 0.50% and 0.51% of N, respectively, were obtained by elemental analysis. The average number of basic groups per glucose unit was calculated from the N content. DEAE-dextrans (mol. wt. 6000, 9000 and 17,200) were found to have 0.075, 0.068 and 0.069 basic groups per glucose, respectively.

Preparation of FITC-labeled DEAE-dextran (DE)

Fluorescein isothiocyanate-labelled DEAE-dextran was synthesized according to the method reported by Belder and Granath (1973). DE (1.0 g) was dissoved in dimethyl sulfoxide (10 ml) containing a few drops of pyridine. Fluorescein isothiocyanate (0.1 g) was added, followed by dibutyltin dilaurate (20 mg), and the mixture was heated for 2 h at 95 °C. After several precipitations in ethanol to remove most of the free dye, the precipitate was dissolved in distilled water, and chromatographed on a Sephadex G-75 column (3.5×40 cm) twice, then the macromolecular fraction (column number > 16–19, $v_0 = 12$) was dried in vacuo at 80 °C.

Nasal administration to rabbits

Male Japanese white rabbits (Saitama, Experimental Animal Supply Co.; body wt. 2.6–3.2 kg) were administered in conscious condition intranasally with DT or DE (6.55 mg) and G (3 mg). The powder sample was crammed in a polyethylene tube (1.57 mm i.d., 2.08 mm o.d.) inserted in the nasal cavity at a position about 1.7 cm from the nostril, and then sprayed with a sprayer (rubber bulb with reservoir). Their venous blood (2.5 ml) was collected from the vena auricularis into heparinized blood sampling tubes before administration and at 0.25, 0.5, 0.75 and 1 h after administration. Plasma was separated by centrifugation at 3000 rpm for 15 min.

Determination of the concentration of DT and DE in the plasma by HPLC

Deproteination was carried out according to the Somogyi method. Two ml of both $Ba(OH)_2$

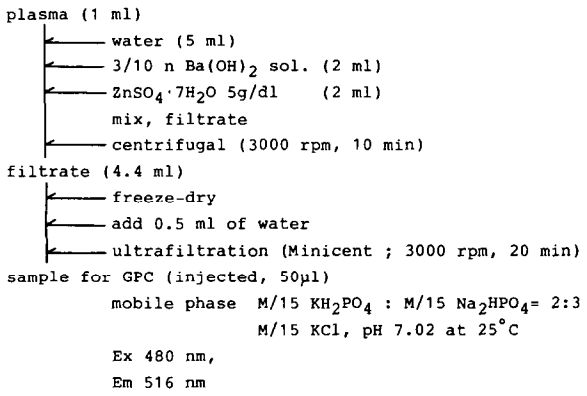

Fig. 1. Sample preparation for HPLC by the Somogyi method.

and $ZnSO_4$, were added to the collected plasma in 5 ml of water, mixed vigorously, and then filtered rapidly. The supernatant fraction was concentrated by freeze-drying and 0.5 ml of water was added, as shown in Fig. 1. After ultrafiltration using Minicent, a 50 μl portion of the supernatant was subjected to HPLC (in a Hitachi HLC 655 equipped with a gel permeation column (TSK-G 3000 PW XL column, Toyo Soda, 7.8 mm i.d. × 300 mm with a precolumn, 6.0 mm i.d. × 40 mm)), and the column was eluted with 1/15 M phosphate buffer ($KH_2PO_4 : Na_2HPO_4 = 2:3$, 1/15 M KCl, pH 7.02 at 25°C) at a flow rate of 0.4 ml/min. The quantity of DT or DE was calculated from the peak height on the chromatogram relative to a standard DT or DE solution. The fluorescence intensity of DT or DE was measured with a fluorescence spectrophotometer (Shimadzu model RFA-530) at 516 nm using an excitation wavelength of 480 nm. The intensity of the fluorescence measured in the range of the calculation curve showed linearity.

Results

Dextrans were administered intranasally. The amount of DT or DE in plasma was compared using HPLC. The retention time of DT and DE in the gel permeation chromatogram depended on molecular weight, as shown in Fig. 2. The concentrations of all DT solutions are 1.750 mg in 100 ml. The concentrations of DE-6000, -9000 and

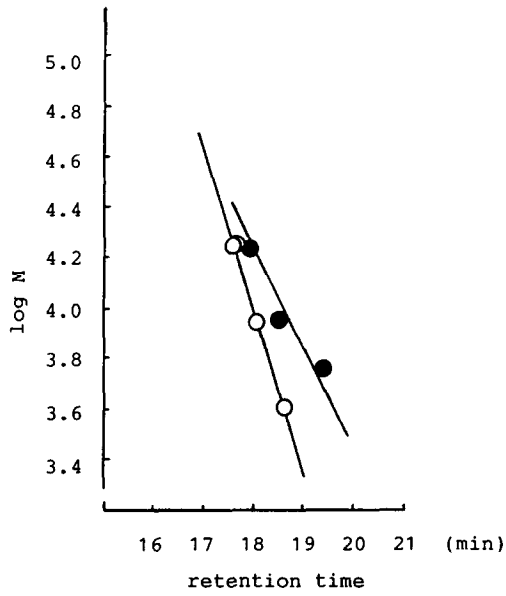

Fig. 2. Molecular weight of DT and DE versus retention time in GPC. ○, DT (-4100, -9000, -17500) ●, DE (-6000, -9000, -17200). Injection 50 μl. The concentrations of all DT solutions are 1.750 mg in 100 ml. The concentrations of DE-6000, -9000 and -17200 are 1.735 mg, 1.805 mg and 0.925 mg in 100 ml, respectively.

-17200 are 1.735, 1.805 and 0.925 mg in 100 ml, respectively.

The typical DT gel permeation chromatogram is represented in Fig. 3 (DT 6.55 mg, G 3 mg). As shown in Fig. 3, the amount of DT could be easily determined by the peak height relative to a standard DT solution with a known DT concentra-

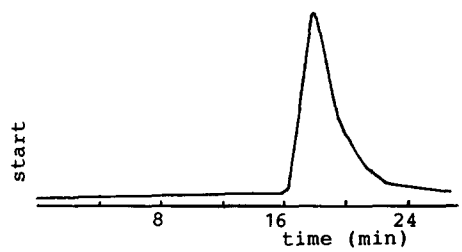

Fig. 3. Gel permeation chromatogram of DT-4100 at 30 min after nasal administration of DT-4100 (6.55 mg) and sodium glycocholate (3 mg) in rabbits. Hitachi HLC 655 equipped with a gel permeation column (TSK-G 3000 PW XL column, Toyo Soda, 7.8 mm i.d. × 300 mm with a precolumn, 6.0 mm i.d. × 40 mm), flow rate 0.4 ml/min, range 1, fluorescence spectrophotometer (Shimadzu model RFA-530, Ex 480 nm, Em 516 nm).

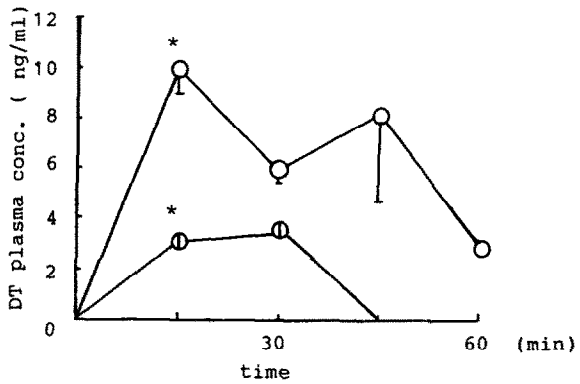

Fig. 4. Effects of sodium glycocholate (1–3 mg) on DT-4100 (6.55 mg) after nasal administration in rabbits. ⦶, sodium glycocholate, 1 mg; ○, sodium glycocholate, 3 mg. The values are expressed as mean ±S.E. of 3 rabbits. * Significantly different ($P < 0.05$).

tion. Dextrans were not absorbed without absorption promoters. But when sodium glycocholate (G) as absorption promoter was added, DT or DE was detected after nasal administration in the rabbit plasma.

The effects of G on DT-4100 after nasal administration of DT are shown in Fig. 4. The 3 mg of G were more effective in facilitating the nasal absorption of DT than 1 mg. The time-courses of the concentration of DT or DE in plasma are

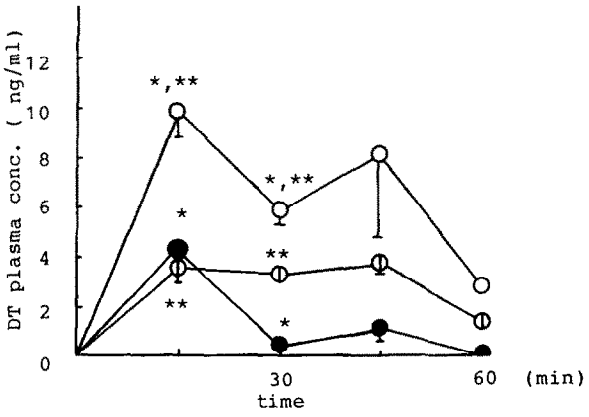

Fig. 5. Concentrations of DT in plasma after nasal administration of DT-4100, DT-9000, and DT-17500 (6.55 mg) with sodium glycocholate (3 mg) in rabbits. ○, DT-4100; ●, DT-9000; ⦶, DT-17500. The values are expressed as means ±S.E. from 3 rabbits. Significant differences from DT-4100 are represented as follows: *, **, $P < 0.05$.

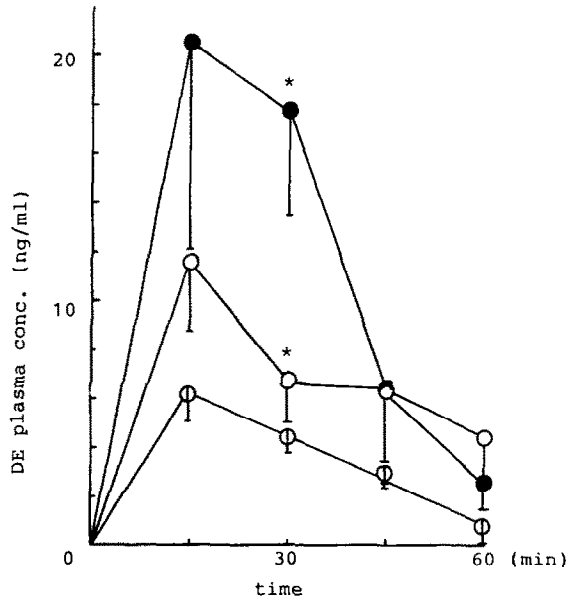

Fig. 6. Concentrations of DE in plasma after nasal administration of DE-6000, DE-9000 and DE-17200 (6.55 mg) with sodium glycocholate (3 mg) in rabbits. ○, DE-6000 ($n = 6$); ●, DE-9000 ($n = 5$); ⦶, DE-17200 ($n = 3$). The values are expressed as means ±S.E. from rabbits. * Significantly different is represented ($P < 0.05$).

represented in Figs. 5 and 6, respectively. DT was influenced significantly by the molecular weight, as shown in Fig. 5. The concentrations of DT in the plasma showed a maximum at about 15 min after the nasal administration.

A statistically significant difference ($P < 0.05$) was found between DT-4100 and DT-9000, and between DT-4100 and DT-17500 after the nasal administration. One was also found between DE-6000 and DE-9000. But DT-9000 and DT-17500 levels showed almost the same tendency. Comparing the two substances (DT-9000 and DE-9000) with the same mol. wt., DE, which carries a positively charged substituent, revealed significantly high plasma levels at 15–30 min after nasal administration.

Discussion

Dextran derivatives with sodium glycocholate were administered nasally in rabbits to investigate

the relationship between their molecular weights and their concentration in plasma. The administration of dextran derivatives with the same average molecular weight and a different charge might be anticipated to result in different concentrations in plasma due to the interaction with the nasal mucosa.

DT is a neutral polysaccharide, with the mother structure for DE. The concentration of DT in plasma decreased as the molecular weight of DT increased. This result seems quite reasonable because the pore induced by the absorption promoter may have a definite size in the membrane of nasal mucosa (Hayashi et al., 1985).

DE is a polycation. The difference in electric charge between DE (polycation) and DT must be reflected in their behaviors after administration, since the positive charges of DE can be available for complex formation with the negatively charged nasal mucosa (due to, for example, sialic acid residues) (Larsen, 1977). The concentration of DE was observed to be quite different from that of DT. The concentration of DE in plasma increased conversely with the molecular weight of DE (less than 10,000). Consequently, it cannot yet be elucidated to what extent the difference in charge explains the difference of the concentration in plasma.

Dextran derivatives and polypeptides such as insulin and interferon were absorbed by adding an absorption promoter rapidly, peak levels being achieved within 15–30 min after nasal administration. The addition of sodium glycocholate was found to produce the greatest effect at a fixed dextran dose. DT and DE might move with the transfer of water (McMartin et al., 1987). As this intercellular space between ciliated–goblet cells, goblet–goblet cells, or ciliated–ciliated cells was loosened by adding G, DT with mol. wt. 4100 could easily go through the nasal mucosa (Gordon et al., 1985; Inagaki et al., 1985). But DT with mol. wt. more than 9000 could not pass easily. Meanwhile, DE-6000 and DE-9000 have almost the same basic group per glucose unit. As the molecular weight of DE increases, the possibility of which DE comes near the cell surface may increase by interacting with the charge of the cell surface. Therefore, in DE having a positive charge, molecules with mol. wt. even more than 6000 could permeate into the nasal mucosa.

References

Arturson, G. and Wallenius, G., The intravascular persistence of dextran of different molecular sizes in normal humans. *Scand. J. Clin. Lab. Invest.*, 1 (1964) 76–80.

Anik, S.T., McRae, G., Nerenberg, C., Worden, A., Foreman, J., Jiin Yu, H., Kushinsky, S., Jones, R. and Vickery, B., Nasal absorption of nafarelin acetate, the decapeptide (D-Nal(2)^6)LHRH, in rhesus monkeys I. *J. Pharm. Sci.*, 73 (1984) 684–685.

Belder, A.N. and Granath, K., Preparation and properties of fluorescein-labelled dextrans. *Carbohydr. Res.*, 30 (1973) 375–378.

Evans, W.S., Borges, J.L.C., Kaiser, D.L., Vance, M.L., Sellers, R.P., Macleod, R.M., Vale, W., Rivier, J. and Thorner, M.O., Intranasal administration of human pancreatic tumor GH-releasing factor-40 stimulates GH release in normal men. *J. Clin. Endocrinol. Metab.*, 57 (1983) 1081–1083.

Gordon, G.S., Moses, A.C., Silver, R.D., Flier, J.S. and Carey, M.C., Nasal absorption of insulin: enhancement by hydrophobic bile salts. *Proc. Natl. Acad. Sci. U.S.A.*, 82 (1985) 7419–7423.

Hayashi, M., Hirasawa, T., Muraoka, T., Shiga, M. and Awazu, S., Comparison of water influx and sieving coefficient in rat jejunal, rectal and nasal absorptions of antipyrine. *Chem. Pharm. Bull.*, 33 (1985) 2149–2152.

Hirai, S., Ikenaga, T. and Matsuzawa, T., Nasal absorption of insulin in dogs. *Diabetes*, 27 (1978) 296–299.

Inagaki, M., Sakakura, Y., Itoh, H., Ukai, K. and Miyoshi, Y., Marcomolecular permeability of the tight junction of the human nasal mucosa. *Rhinology*, 23 (1985) 213–221.

Larsen, B., Redistribution of polycations bound to lymphocytes. *Mol. Cell. Biochem.*, 15 (1977) 117–123.

Maitani, Y., Igawa, T., Machida, Y., and Nagai, T., Intranasal administration of β-interferon in rabbits. *Drug Design and Delivery*, 1 (1986) 65–70.

Mckernan, W.M. and Ricketts, C.R., A basic derivative of dextran and its interaction with serum albumin. *Biochem. J.*, 76 (1960) 117–120.

McMartin, C., Hutchinson, L.E.F., Hyde, R. and Peters, G.E., Analysis of structual requirements for the absorption of drugs and macromolecules from the nasal cavity. *J. Pharm. Sci.*, 76 (1987) 535–540.

Rice, J.M., Davidson, J.K., Madison, R.M., Kingsbury, E.W. and Turner, W., Oncogenic water-soluble polycations. I. Induction of sarcomas in mice by diethylaminoethyl-dextran. *J. Natl. Cancer Inst.*, 50 (1973) 387–401.

Su, K.S.E., Campanale, K.M., Mendelsohn, L.G., Kerchner, G.A. and Gries, C.L., Nasal delivery of polypeptides I: Nasal absorption of enkephalins in rats. *J. Pharm. Sci.*, 74 (1985) 394–398.