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Selection of Volume Indicator for the Study of Nasal Drug Absorption

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The nasal absorption of inulin and polyethylene glycol (PEG) 4000, which are not absorbed in the intestinal lumen and are used as volume indicators for intestinal drug absorption experiments, was investigated. No absorption of inulin was detected either by the *in situ* recirculating perfusion method in the nasal cavity or by the deposit method in the nasal cavity. On the other hand, PEG 4000 showed an absorption ratio of approximately 40 to 50% in the *in situ* recirculating perfusion method. The absorption ratio of PEG 20000 was approximately equal to that of PEG 4000 but fluorescein isothiocyanate-dextran (FITC-dextran, MW 20000) was not absorbed. Such apparent absorption of PEG in the nasal cavity might be due to the interaction between PEG and the nasal membrane, that is, the adsorption of PEG on the nasal membrane, since a higher concentration of PEG 4000 decreased the apparent absorption. The simultaneous perfusion of inulin and PEG 4000 also decreased the apparent absorption of PEG 4000, suggesting a possible interaction between inulin and PEG 4000. Consequently, it was concluded that inulin and FITC-dextran can be used as volume indicators in nasal absorption experiments, but PEG 4000 and PEG 20000 cannot.

Keywords——nasal absorption; inulin; PEG 4000; PEG 20000; FITC-dextran; volume indicator; solvent drag effect

Hirai *et al.* reported that various compounds poorly absorbed in the intestinal lumen, such as phenol red, cefazolin and insulin, were well absorbed through the nasal mucosa in dogs and rats.[1–4] Considering that the nasal mucosa characteristically has more secretory glands than the intestinal membrane, and water transport through such secretory glands may occur, it is of interest to compare the solvent drag effect on drug nasal absorption with that on intestinal absorption.[5,6]

In the present study, as the first step for investigating the solvent drag effect in nasal absorption, the nasal absorptions of inulin, polyethylene glycol (PEG) 4000, PEG 20000 and fluorescein isothiocyanate-dextran (FITC-dextran) were investigated to determine their suitability for use as volume indicators in the same manner as in the case of intestinal absorption.[5,6]

Experimental

Materials——Inulin was obtained from Wako Pure Chemical Ind., Ltd., Osaka, Japan. PEG 4000, PEG 20000 and FITC-dextran (MW 20000) were purchased from Sigma Chemical Co., Missouri, USA. Other reagents were of analytical grade or higher quality.

Animal Surgery——Male Wistar rats (body weight 250±20 g) were anesthetized by the intraperitoneal administration of 32.5 mg/kg sodium pentobarbital (Nembutal, Abbott Laboratories, Illinois, USA). Surgery was carried out according to Hirai *et al.*[2]

***In Situ* Nasal Recirculating Perfusion Method and Deposit Method**——(i) Inulin: In the *in situ* nasal recirculating perfusion method, 20 ml of 1.7 or 0.085% inulin in phosphate buffer (pH 6.5) adjusted to sodium chloride equivalent values of 0.45, 0.9 or 1.8 with NaCl and prewarmed at 37°C was perfused in the recirculating mode at a constant flow rate (2.5 ml/min) using a pump (Perista mini-pump SJ-1211H type, Atto Co., Ltd., Tokyo, Japan) for 60 min. At 60 min after the start of perfusion, the whole perfused solution was recovered and washed out into the reservoir with 2 ml of the phosphate buffer at 2.5 ml/min. The procedure in the deposit method followed Hirai *et al.*'s *in vivo* method.[2] A 20 μl aliquot of 1.7% inulin solution was administered through the nostril into the nasal cavity with a microsyringe and left for 60 min. At 60 min,

the whole drug solution was washed out with 2 ml of the phosphate buffer at 10 ml/min. Finally the samples recovered in the *in situ* method and in the deposit method were diluted to 25 ml and 5 ml with the phosphate buffer, respectively. Thereafter, the samples were centrifuged for 10 min at 3000 rpm and 50 μl of the supernatants were supplied for determination.

(ii) PEG 4000, PEG 20000 and FITC-dextran: Isotonic phosphate buffer solutions (25 ml) of 0.25% and 2% PEG 4000, 0.25% PEG 20000 and 0.1% FITC-dextran (MW 20000) were perfused into the nasal cavity with recirculation at 2.5 ml/min and 37° in the same way as for inulin. Finally, the samples recovered with the phosphate buffer at 60 min after the start of the perfusion were diluted to 25 ml and centrifuged for 10 min at 3000 rpm. The supernatants, 150 μl for PEG and 50 μl for FITC-dextran, were subjected to assay.

(iii) Coadministration of PEG 4000 and Inulin: The mixture of 0.25% PEG 4000 and 0.085% inulin (final concentration) was perfused into the nasal cavity with reciculation as mentioned above. The recovered samples were also treated as before.

***In Situ* Recirculating Perfusion Method for Measuring Intestinal Absorption of PEG 4000**——Thirty ml of 0.25% isotonic phosphate buffer solution (pH 6.5) of PEG 4000 was perfused (with reciculation) into the jejunal loop (length 30 cm, starting at the proximal end of the jejunum, *i.e.* at the duodenum-jejunum flexure) for 60 min at 2.5 ml/min. The whole perfused solution was recovered and washed out with 10 ml of the phosphate buffer at 2.5 ml/min into the reservoir. The recovered sample was diluted to 50 ml with the same buffer and centrifuged for 10 min at 3000 rpm. A 150 μl aliquot of the supernatant was used for assay.

Analytical Method——(i) Inulin: The inulin concentration was determined by the reported method.[5]

(ii) PEG 4000 and PEG 20000: The turbidity method used by Ochsenfahrt and Winne[7] was adopted for the determination of PEG 4000 and PEG 20000. To 150 μl of the samples prepared as stated above was added 3 ml of 20% trichloroacetic acid (TCA). Exactly 5 min after mixing the above solutions for 10 s, the turbidity was measured at 650 nm against TCA solution as the blank. The linear region of the standard curve (0.0625—0.25% PEG solution) was used.

(iii) FITC-dextran: After further dilution of the samples (50 μl) to 10 ml with the phosphate buffer, the fluorescence intensity was measured at an excitation wavelength of 480 nm and at an emission wavelength of 516 nm. No hindrance from the perfused solution without drugs was detected and the linear region of the curve over 0.03—0.1% FITC-dextran was used.

Results and Discussion

Unabsorbed Ratio of Inulin through the Nasal Mucosa

Hirai *et al.* stated that the nasal absorption of phenol red in the *in situ* recirculating perfusion method was apparently higher than that found by the deposit method.[2] However, in the present study, all the unabsorbed ratios of 1.7% inulin at various NaCl equivalent values were approximately 100% independently of the experimental method (Table I). Furthermore, no absorption of 0.085 w/v% inulin, *i.e.*, the concentration practically used as a volume indicator in intestinal absorption, could be detected. From these results, it is concluded that inulin at any concentration is not absorbed through the nasal mucosa and can be used as a volume indicator in the nasal cavity.

Table I. Unabsorbed Amounts (Percent) of 1.7 w/v% Inulin from Hypertonic, Isotonic and Hypotonic Solutions through the Nasal Mucosa Using the *In Situ* Recirculating Perfusion Method (*In Situ* Method) and the Deposit Method

	In situ method[b]	Deposit method[c]
Hypertonic[a]	96.5±0.7	91.9±7.5
Isotonic[a]	97.4±2.5	91.5±8.7
Hypotonic[a]	90.8±3.5	91.9±5.5

Values represent means ± S.D. of three rats, except for the value in the *in situ* method with isotonic solution, which is the mean ± S.D. of four rats.

a) Osmotic pressures were adjusted with sodium chloride to the following sodium chloride equivalent values; hypertonic 1.8: isotonic 0.9: hypotonic 0.45.

b) The dose was obtained as (initial concentration of inulin) × (perfused volume, *i.e.*, 20 ml).

c) The dose was obtained as the amount of inulin washed out with the phosphate buffer immediately after administration into the nasal cavity.

Unabsorbed Ratio of PEG 4000 and PEG 20000 through the Nasal Mucosa

The unabsorbed ratios of 0.25 w/v% PEG 4000 and PEG 20000 in the *in situ* recirculating perfusion were 53—58% in all cases independently of NaCl equivalent values, indicating that approximately 40% was apparently absorbed through the nasal mucosa (Table II).

Table II. Unabsorbed Amounts (Percent) of 0.25 w/v% PEG 4000 and PEG 20000 from Hypertonic, Isotonic and Hypotonic Solutions through the Nasal Mucosa Using the *in Situ* Recirculating Method

	PEG 4000	PEG 20000
Hypertonic[a)]	57.0±3.2	53.6±1.7
Isotonic[a)]	52.5±3.0	54.8±3.5
Hypotonic[a)]	58.4±5.3	58.4±1.6

Values represent means ± S.D. of three rats, except for the value of PEG 20000 in the hypertonic solution, which is the mean ± S.D. of four rats.
a) Osmotic pressures were adjusted with sodium chloride to the following sodium chloride equivalent values; hypertonic 1.8: isotonic 0.9: hypotonic 0.45.

Unabsorbed Ratio of FITC-dextran through the Nasal Mucosa

The nasal absorption of FITC-dextran, a large molecule (MW 20000) with a sugar chain like that of inulin, was studied by the *in situ* recirculating perfusion method. The unabsorbed ratios (mean ±S.D.) were 93.7±4.0% (n=4) in the hypertonic perfused solution, 97.5±0.6% (n=4) in the isotonic solution, and 96.5±0.6% (n=3) in the hypotonic solution, indicating that essentially no nasal absorption occurred, as in the case of inulin. The difference in the nasal absorptions of inulin and FITC-dextran and of PEG might be due to the presence or absence of sugar chains, but the details remain to be studied.

Effect of PEG Concentration on the PEG Nasal Absorption

An 8-fold increase in the initial concentration of PEG 4000 in the isotonic perfused solution (2 w/v% PEG 4000) increased the unabsorbed ratio to 85.2±3.7% (n=3) from 52.5±3.0% for 0.25 w/v% PEG 4000 (Table II). Since Bryan *et al.* reported that essentially no damage to the intestinal membrane could be found until the PEG 2000 concentration reached 10 w/v%[8)] it is unlikely that PEG damaged the nasal membrane at the concentrations used here. The fact that a higher concentration of PEG decreased the apparent PEG absorption in the nasal mucosa suggests that such apparent absorption may be mainly due to the adsorption of PEG on the nasal mucosa.

Effect of the Coadministration of Inulin and PEG 4000 on Their Nasal Absorptions

The coadministration of 0.085 w/v% inulin and 0.25 w/v% PEG 4000 did not affect the inulin absorption, where the unabsorbed ratio was 96.0±2.1% (n=3), but significantly decreased the PEG 4000 absorption, where the unabsorbed ratio was 77.0±3.6% (n=3). This result suggests that some interaction between inulin and PEG 4000 decreased the characteristic interaction between PEG 4000 and the nasal membrane. The finding that the absorption of inulin was not changed supports the conclusion that damage to the nasal membrane by PEG 4000 can be ruled out. The possible interaction between PEG 4000 and inulin (with the sugar chain) also suggests that the characteristic interaction between PEG 4000 and nasal mucosa might be closely related to the fact that the nasal mucosa is rich in glycoprotein, and secretory fluids containing sugar always cover the nasal mucosa. However, further investigations are necessary to clarify the interaction in detail.

Conclusion

Since the unabsorbed ratio of PEG 4000 in the jejunum (30 cm length) was 94.6±4.4% (n=3), intestinal absorption of PEG 4000 can be ruled out. Accordingly, the occurrence of nasal absorption of PEG 4000 suggests some characteristic interaction between PEG and nasal mucosa. In conclusion, it was shown that inulin or FITC-dextran can be used as volume indicators in nasal absorption, as in intestinal absorption, to study aspects of the absorption mechanism, such as the solvent drag effect, but PEG is not suitable for use in this way.

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