Short Communication

Influence of Formulation Viscosity on Drug Absorption Following Nasal Application in Rats

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Summary: The aim of this research is to clarify the influence of the viscosity of the nasal formulation on in vivo nasal drug absorption and its mechanism using an in vitro Caco-2 system. The drug solution was made viscous by the addition of dextran (Dex). The disappearance of FITC-labeled Dextran (FD, a marker of the dosing solution) applied with control solution followed monoexponential kinetics, while FD applied with Dex solution showed biexponential elimination. The mean residence time of FD in the nasal cavity was increased with the increase in Dex concentration. The nasal absorption of acyclovir was similar in the formulation with low viscosity, increased in the formulation with moderate viscosity and markedly decreased in the formulation with high viscosity. The result from the normal Caco-2 transport study could not explain the relation of in vivo drug absorption with viscosity, while the modified Caco-2 system provided data partly reflecting the change in in vivo absorption in rats. In conclusion, the residence of the applied solution in the nasal cavity was enhanced by the addition of Dex in a viscosity-dependent manner. Moderate viscosity of the dosing solution improved the in vivo nasal absorption of acyclovir, while higher viscosity decreased it.

Key words: nasal application; mucociliary clearance; viscosity; dextran; viscous formulation

Introduction

Nasal delivery is a promising alternative for the systemic application of drugs that are poorly absorbed via the oral route. The nasal epithelium has relatively high permeability and only two cell layers separate the nasal lumen from the dense blood vessel network in the lamina propria.1-3 Consequently, the nasal route has drawn the attention of many researchers as a systemic delivery route of peptide drugs. In addition, avoidance of the first hepatic passage makes nasal administration a promising alternative, especially for drugs exhibiting high metabolism in the intestine and/or liver.3

A drug applied in the nasal cavity is translocated to the nasopharynx and thereafter to the GI tract by the coordinated beat of the cilia of respiratory epithelial cells.4-6 This is an important nonspecific defense mechanism of the respiratory tract and is called mucociliary clearance (MC). In order to improve nasal drug absorption, a powder formulation7-10 and/or bioadhesive gel formulation11-14 are applied nasally. Since the nasal absorption of drugs is generally better than intestinal absorption, the aim of powder and gel formulations is to enhance the nasal retention of the drug by the inhibition of MC. The use of a powder or gel formulation very simply and easily enhances drug absorption; however, little research has clarified the quantitative relation of drug retention in the nasal cavity and nasal drug absorption to the viscosity of the dosing solution.

In our previous report, the theory and calculation to evaluate the nasal and intestinal absorption following nasal drug administration in the normal physiologic condition were described.15 It was also clarified that the contribution of the nasal cavity and the GI tract to the total absorption of nasally applied drugs is dependent on the physicochemical property of the drug. MC is a very important factor to determine the absorption profile of nasally applied drugs. The aim of this research is to clarify the influence of the viscosity of the formulation on MC and drug absorption following nasal application. The modified Caco-2 transport experiment was employed to clarify the mechanism of the change in the in vivo absorption of the drug.
Materials and Methods

Materials

Acyclovir and FITC-dextran (average Mw: 70 kDa; FD) were purchased from Sigma-Aldrich (St Louis, MO, USA). Dextran (Mw: 50,000–70,000; Dex), dithiothreitol (DTT) and phosphate-buffered saline (pH7.4; PBS) were purchased from Nacalai Tesque Co. (Kyoto, Japan). Caco-2 cells were obtained from Dainippon Pharmaceuticals Co. (Osaka, Japan). Reagents and the medium used for Caco-2 culture, and the preparation of the monolayer were purchased from Sigma-Aldrich (St Louis, MO, USA), Gibco Laboratories (Lenexa, KS, USA) and Beckton Dickinson Biosciences (Bedford, MA, USA). All other chemicals were of reagent grade and commercially available.

Measurement of viscosity

The viscosity of Dex solutions was measured by the rheometer (HADV-III, LVDV-III, Brookfield Engineering, Middleboro, MA, USA) at 37°C.

Animal study

All animal studies were previously approved by the Committee of the Animal Care of Shujitsu University and conducted under the Guidelines. Male Wistar rats (B.W. 200–260 g) were used in all animal experiments. Rats used for nasal absorption studies were fasted overnight before the experiment.

Mucociliary clearance: FD70 was used as a non-absorbable marker of the dosing solution to investigate MC. Under light ether anesthesia, 5 μL of 0.1% FD70 dissolved in Dex solution was instilled 1 cm from the nostril with a microsyringe. The animal was kept in a cage (KN-326-III, Natsume, Tokyo, Japan) throughout the experiment. The animal usually became completely conscious 5–10 min after instillation. Blood samples were collected for 240 min after drug administration. During this period, the animal was allowed free access to water. Plasma was obtained by centrifugation.

Culture of Caco-2 and preparation of Caco-2 monolayers

Caco-2 cells were grown in Dulbecco’s modified Eagle’s medium (Sigma-Aldrich, St Louis, MO, USA) supplemented with 10% fetal bovine serum 1% l-glutamine, 1% non-essential amino acid and 5% antibiotic-antimycotic solution (all from Gibco Laboratories, Lenexa, KS, USA) in a culture flask.

Preparation of Caco-2 monolayer for normal transport study: Caco-2 monolayers were prepared according to the short-term culture method(19) using a culture kit, BIOCOAT® HTS Caco-2 Assay System (Beckton Dickinson Bioscience, Bedford, MA, USA). Briefly, the cells were harvested with trypsin-EDTA and seeded on a fibrillar collagen pre-coated cell culture insert (pore size: 1.0 μm, growth area: 0.9 cm², 12 wells/plate) with seeding medium at a density of 4×10⁵ cells/well. The seeding medium was changed with differentiation medium 2 days’ post-seeding. The differentiation medium was changed everyday. The monolayer was used for the transport study 5–7 days after seeding.

Preparation of Caco-2 monolayer for modified transport study: The cells were harvested with trypsin-EDTA and seeded on polycarbonate filters (pore size: 0.3 μm, growth area: 4.2 cm², 6 wells/plate, Beckton Dickinson Bioscience, Bedford, MA, USA) with culture medium at a density of 2×10⁶ cells/well. The culture medium was changed every 2 days. The monolayer was used for in vitro modified transport study 16 days after seeding.

In vitro study on transepithelial transport

Hank’s balanced salts solution (HBSS) supplemented with 15 mM glucose was used for transport studies after adjusting pH to 7.4 with HEPES. Dex (5%, 10%, 20% and 40%) and acyclovir (1 mM) were dissolved in transport medium and used in the study described below. Before the transport study, Caco-2 monolayers were preincubated with drug-free transport medium for 10 min at 37°C. All experiments were carried out at 37°C.

Normal transport study: Apical transport medium was replaced with transport medium (0.8 mL) containing acyclovir and Dex. Drug-free transport medium (2.0 mL) was added to the basal chamber; thereafter, an aliquot of the sample was taken from the basal chamber over 120 min. The same volume of fresh transport medium was added to maintain the basal volume constant. At the end of the experiments, apical solutions
were taken to check the mass balance.

**Modified transport study:** After 10 min preincubation, the apical solution was completely removed. The volume of the basal solution was adjusted to 1.0 mL to avoid hydrostatic pressure on the monolayer. Each well of a 6-well plate was previously filled with 1.0 mL of normal transport medium. The transport study was started by applying 5 μL of the solution to the surface of the monolayer. The insert was transferred to the next well in the plate every 2 min for the initial 12 min, and 16 and 20 min thereafter. The transport medium in the well was taken for the analysis of acyclovir.

**Calculation of the permeability of acyclovir:** The permeability (apparent permeability coefficient, $P_{Caco-2}$ (cm/sec)) of acyclovir was calculated according to the following equation:

$$P_{Caco-2} = \frac{dQ/dt}{(A \cdot C_0)}$$

where $dQ/dt$ is the appearance rate of drugs in the basal chamber (μmol/sec), $C_0$ is the initial drug concentration in the apical chamber (mM), and $A$ is the surface area of the monolayer (0.9 cm²).

**Drug Assay**

**FITC-dextran:** The volume of the nasal washing was precisely adjusted to 5 mL. After filtrating the samples through a membrane filter (pore size: 0.45 μm, Millex®, Millipore Co., MA), the fluorescent intensity of FD70 was determined (excitation: 495 nm and emission: 520 nm) using a fluorescence spectrometer (F-2500, Hitachi Co., Tokyo, Japan).

**Acyclovir:** Methanol (1200 μL) was added to the plasma (100 μL) for deprotenization and the mixture was centrifuged. The supernatant (1000 μL) was taken for analysis with an LC/MS system (API1100, Agilent Technologies, Boeblingen, Germany) equipped with a reversed phase column (Inertsil® ODS-3, 2.1 × 150 mm, GL Sciences Inc., Tokyo, Japan). The mobile phase consisted of 10 mM ammonium acetate–methanol (95:5) at a flow rate of 0.25 mL/min. The injection volume was 20 μL. The detection limit of acyclovir was 5 ng/mL under this analytical condition. With regard to samples from *in vitro* transport study, 20 μL was directly injected to LC/MS without any treatment.

**Results and Discussion**

**Viscosity of Dex solutions**

The Table lists the viscosity of Dex solutions. The viscosity of the dosing solution used in this study ranged between 0.1 and 147 mPa·sec and increased exponentially with the increase in Dex concentration. 40% Dex solution showed the highest viscosity (147 mPa·sec) and was one order of magnitude larger than that of 20% Dex solution (15.9 mPa·sec). The viscosity of 0.1% FD70 solution (data not shown) was the same as PBS (control in Table, 1.02 mPa·sec), indicating that the addition of 0.1% FD70 does not affect the viscosity of the solution. In addition to viscosity, the osmolarity of the solution changed by the addition of Dex. The direct measurement and calculation based on van’t Hoff equation clarified the small difference in osmolarity between Dex solutions. The effect of the osmolarity of the solution on *in vivo* absorption and *in vitro* permeation is assumed to be negligible in this study.

**Effect of viscosity of the application solution on mucociliary clearance of FD70**

Figure 1 indicates the disappearance profiles of FD70 from the rat nasal cavity after nasal administration. The profiles of the control showed mono-exponential elimination. On the other hand, the elimination of FD applied with 5% Dex, 10% Dex, 20% Dex and 40% Dex solutions showed biexponential elimination. The initial decline was similar to the control. Mean resistance times (MRT) of FD70 in the nasal cavity are shown in the Table. MRT of FD70 in the nasal cavity was approximately 20 min in the control. With the increase in Dex concentration, MRT of FD70 was increased. The slow elimination in the second phase largely contributed to the increase in MRT.

The disappearance of FD70 is based on the movement of the mucus to the nasopharynx. Mucus is driven by the ciliary beat. Due to the characteristics of the ciliary beat, the movement velocity of the mucus is dependent on the depth from the surface. The shallower the depth, the faster the mucus moves. FD70 diffused into...
the mucus after application onto the mucosal surface; therefore, the disappearance of FD70 is dependent on how fast the solution spreads over the surface, diffusion into the mucus and mucus clearance to the nasopharynx. The mechanism is very complex. The reason for the qualitative difference in disappearance profiles of FD70 by Dex addition is not clear at present. To clarify the details of the biexponential elimination of FD70, an in vitro system to investigate MC should be developed.

**Effect of viscosity of application solution on nasal absorption of acyclovir**

Figure 2 shows the profiles of the plasma concentration of acyclovir as a function of time following nasal application. In previous research, 15) 5 model drugs were used to develop a kinetic theory to evaluate nasal and intestinal absorption. In this research, acyclovir was selected as the model drug, since it has a moderate \( P_{\text{Caco-2}} \) among the 5 drugs. When acyclovir was nasally applied with 5% and 10% Dex solutions (abbreviated as ACV-5% and ACV-10%, respectively), the profiles were similar to the control. These findings were due to the small increase in the viscosity of 5% and 10% Dex solutions; however, when acyclovir was applied nasally with 20% or 40% Dex solution (abbreviated as ACV-20% and ACV-40%, respectively), the profiles showed a difference from the control. Absorption is enhanced at ACV-20% and inhibited at ACV-40%. The Table lists AUC as an index of nasal drug absorption.
According to AUC, absorption was similar at ACV-5\%, slightly increased at ACV-10\%, further increased at ACV-20\% and markedly decreased at ACV-40\% in comparison with the control. The increase in absorption of ACV-20\% was likely due to the longer residence of acyclovir in the nasal cavity. Since MRT of 40\% Dex solution is larger than 20\% Dex solution, the decrease in the absorption of ACV-40\% is due to the predominance of some adverse factors over longer MRT of 40\% Dex solution.

The surface of nasal epithelial cells is covered with mucus. The penetration rate of Dex solution into mucus following nasal instillation relates to how close the drug can approach the cell surface. One of the factors determining the penetration rate into mucus is the viscosity of the solution. With the increase in the viscosity of applied Dex solution, the penetration rate of the solution into mucus is considered to be decreased, resulting in a delay of the drug’s approach to the cell surface. The degree of the increase in the viscosity of the 40\% Dex solution is remarkable in comparison with other Dex solutions. This may be one reason why the in vivo absorption of ACV from 40\% Dex solution is decreased.

According to Zaki et al., the increased viscosity of the dosing solution by hydroxylpropyl methylcellulose adversely affects the nasal absorption of metoclopramide, which originally showed good absorption from the nasal cavity (more than 90\% by 60 min). Although the difference in the experimental design of the animal study such as dosing volume and anesthesia of the rat must be considered carefully, their findings on metoclopramide are in agreement with acyclovir. The effect of viscosity on the nasal absorption of the highly permeable drug is likely negative.

**Effect of viscosity of application solution on transepithelial transport of acyclovir**

Figure 3 shows the permeation profiles of acyclovir derived from normal and modified Caco-2 systems. In the normal system (left panel in Fig. 3), the permeation of acyclovir was markedly decreased by Dex. The difference in permeation is very small in Dex solution with a concentration of more than 10\%. P_{Caco-2} of 10\%, 20\% and 40\% Dex solutions was markedly small compared with the control and 5\% Dex solution, as shown in the Table. These findings suggest that a factor other than diffusion in the solution and permeation across Caco-2 is a determinant of in vivo nasal absorption. One of the factors governing drug transepithelial transport is the area through which the drug can permeate. In the normal system, the diffusion area of the drug on Caco-2 is constant (0.9 cm²). The viscosity of the solution can influence the surface area where the drug solution can spread in the rat nasal cavity in vivo. Based on these considerations, the Caco-2 system was modified. The apical solution of the transwell insert was completely removed. Five microliters of solution, the same volume as the in vivo animal study, was applied directly to the surface of the Caco-2 monolayer. Permeation profiles up to 20 min derived from the modified Caco-2 system are also indicated in Fig. 3. Differences in the permeations of acyclovir from control, 5\%, 10\% and 20\% Dex solutions were very small. In contrast, the permeation of acyclovir from 40\% Dex solution was significantly decreased and a lag time, which was approximately 2 min, was observed in the permeation profile. This finding is consistent with the decrease in the nasal absorption of ACV-40\%. Consequently, the adverse effect of 40\% Dex solution on the in vivo nasal absorption of acyclovir is partly due to the decrease in the area where the dosing solution can spread and its rate.

In conclusion, the residence of the applied solution in the rat nasal cavity was enhanced by the addition of Dex. The increase in the residence time is dependent on the viscosity of the solution. It was also clarified that moderate viscosity of the dosing solution improved in vivo nasal absorption of acyclovir, while higher
viscosity of the application solution decreased it. The results from the modified Caco-2 transport study suggest that this may be due to differences in the absorption surface area.

References


