Regular Article

Nasal Drug Absorption from Powder Formulations: Effect of Fluid Volume Changes on the Mucosal Surface

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Nasal drug application has gained a great deal of attention over the last few decades because of its great potential utility for systemic drug delivery. The range of drugs investigated for possible nasal application varies from lipopholic drugs to polar and hydrophilic molecules, including peptides and proteins.1–3 It offers an attractive alternative application for drugs that have limited oral bioavailability, are degradable by gastrointestinal fluid, or undergo high hepatic first-pass or gut-wall metabolism. In addition, nasal administration results in quick onset of action than oral and transdermal applications.4,5 Because nasal administration is easier and less invasive than oral delivery, not only for the elderly but also for long-term-care patients with swallowing difficulties, it has considerable clinical potential for improving the QOL of both patients and care givers.

One of the important factors associated with nasal drug absorption is mucociliary clearance (MC).6,7 In general, when exogenous substances and infectious microorganisms are inhaled into the nasal cavity, most particles larger than 5 μm are trapped by the nasal mucosa and smaller particles (<5 μm) pass through the nasal cavity to reach the bronchus and lungs.7,8 The particles trapped by the nasal mucosa are translocated to the gastrointestinal tract via the post-nasal drip and nasopharynx, together with the surface mucus, by the coordinated movement of cilia. Infectious microorganisms swallowed into the stomach are inactivated by the severe acidic environment. This protective function of the nasal epithelium is called MC. MC can affect the drug retention time in the nasal cavity8–12 and may alter drug absorption from the nasal cavity.

Various formulations such as solutions,13,14 powders,15,16 and gels17 are used clinically for nasal administration. Among these formulations, liquid formulations are the most commonly used. However, powder formulations have many advantages such as improved drug stability18 and application in higher dosages. Additionally, powder formulations are known to have longer retention time in the nasal cavity when compared with other formulations, thus resulting in better drug absorption.19,20

When solid formulations such as tablets and capsules are administered orally, they are initially disintegrated and dissolved in the water with which the solid formulation is taken (usually 200 mL). After nasal application of powder formulations, the drug must be dissolved in the fluid on the nasal mucosa before epithelial permeation. The dissolution of a drug is important in both oral and nasal applications. In comparison with orally administered drugs, the fluid volume of a drug to be dissolved in the nasal cavity is much smaller. This is the marked difference between oral and nasal applications. Therefore, the effect of the drug dissolution on nasal absorption may be different from that on intestinal absorption. However, the relationship between the drug dissolution and nasal ab-
The aim of this study was to evaluate the relationship between the nasal drug absorption and drug dissolution of powder formulations by changing the mucosal fluid volume. To change the mucosal fluid volume, the excipients, lactose and sodium chloride (NaCl), were added to the powder formulations because they are water soluble and their molecular size is small, water can be withdrawn from epithelial cells or underneath tissues by the high osmotic pressure generated by the dissolution of excipients. The model drugs used in the study were warfarin (WF, highly permeable and highly soluble), piroxicam (PXC, highly permeable and poorly soluble), and norfloxacin (NFX, poorly permeable and poorly soluble).

MATERIALS AND METHODS

Materials

WF, PXC, NFX, lactose, NaCl, and a lactate dehydrogenase (LDH) CII-assay kit were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Fluorescein isothiocyanate-labeled dextran with an average molecular weight of 4400 (FD4) and fluorescein isothiocyanate-labeled dextran with an average molecular weight of 71600 (FD70) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, U.S.A.). Madin–Darby canine kidney (MDCK) cells obtained from Dainippon Sumitomo Pharmaceuticals Co. (Osaka, Japan). All other chemicals were of reagent grade and were commercially available.

Preparation of Powder Formulations

The bulk drug powder was mixed with lactose or NaCl at the weight ratio of 1:1. The bulk drug powder, lactose, NaCl and the mixture of the drug and the excipient were milled using a mortar and pestle. The primary particle sizes of each powder after milling were measured with a microscopy. The primary particle sizes of the drug (WF, PXC and NFX), lactose and NaCl were in a range of 5–10, 100–200 and 100–200 µm, respectively.

In Vitro Study Using MDCK Cell Monolayers

Culture of MDCK Cells and Preparation of Cell Monolayers

For the in vitro study, MDCK cells were grown in α-minimum essential medium (MEM) containing 10% fetal bovine serum and 1% antibiotic-antimycotic mixed stock solution at 37°C in an atmosphere of 5% CO2. They were seeded onto the apical side of cell culture inserts (BD Falcon Cell Culture Insert, 6-well, Beckton & Dickinson, Franklin Lakes, NJ, U.S.A.) at a density of 4.0×10^5 cells/well. Culture medium (α-MEM) was added to apical (2.5 mL) and basolateral (3.2 mL) sides, and was replaced three times a week.

In Vivo Nasal Absorption

Animal Study

All animal studies were previously approved by the animal ethics committee at Kyoto Pharmaceutical University and were carried out in accordance with their guidelines. Male Wistar rats (Shimizu Laboratory Supplies, Kyoto, Japan), weighing 220–250 g, were used in all animal experiments. Rats were anesthetized with intraperitoneal pentobarbital sodium (52 mg/kg) and the right femoral artery was cannulated with polyethylene tubing. The powder formulation (1 mg, 100% bulk powder or 50% powder containing lactose or NaCl) was administered nasally with the hand-made powder application device. Briefly, the powder was placed into a disposable micropipette tip connected with a plastic syringe. The powder was dispersed into the nasal cavity of the rat under light ether anesthesia, by the release of air compressed in the syringe by opening a three-way stopcock between the disposable tip and the syringe. The dose of the drug was determined from the difference in the weight of the disposable micropipette tip before and after the administration. Thereafter, the rats were kept in a cage (KN-326-III, Natsume, Tokyo, Japan) throughout the experiment. Blood samples were collected 6 h after drug administration, and were centrifuged at 13000 rpm for 5 min to obtain the plasma. Plasma samples were stored frozen at −40°C until required.

Transport Study under Air-Interface Condition

Before the transport study, the culture medium was replaced with a transport medium (TM, 136.89 mM NaCl, 5.36 mM KCl, 0.34 mM NaHPO4, 0.44 mM KH2PO4, 0.41 mM MgSO4, 19.45 mM Glucose, 1.26 mM CaCl2, 0.49 mM MgCl2, 4.17 mM NaHCO3, 10 mM N-(2-hydroxyethyl)piperazine-N’-2-ethanesulfonic acid (Hepes)), preheated at 37°C, and then removed to wash the monolayer. Thereafter, TM (4.0 mL) was added only to the basal compartment (air-interface condition, AIC). The surface of basal TM was adjusted to the cell monolayer to avoid hydrostatic pressure on the monolayer. After preincubation for 10 min, the powder was sprayed on the apical side using a plastic syringe connected to a disposable micropipette tip. At predetermined time intervals, 0.2 mL of TM was collected from the basal compartment for a period of 6 h, and was replaced with the fresh medium. Because the temperature in the nasal cavity is around 25–37°C, the temperature of the monolayer was maintained at 25–37°C throughout the experiment. To prevent the surface of the monolayers from drying up, the humidity was maintained at 80–90% with an electric humidifier (Ultrasonic Humidifier, KX-80UP, CCP Co., Ltd., Tokyo, Japan). The TEER before and after the experiment was approximately 0.9 and 0.4 kΩ cm², respectively.

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Nasal Residence of the Formulation

FD70 was used as a non-absorbable marker to investigate the nasal residence of the formulation. Under light ether anesthesia, 1 mg of powder formulation containing FD70 (NFX:FD70=9:1 or NFX:FD70:lactose or NaCl=4:1:5) was sprayed into the nostril of male Wistar rats. The formulation containing 90% NFX served as a control. According to the method of Hirai et al., the esophagus and trachea were surgically operated 5 and 10 min after application to collect FD70 in the nasal cavity. Briefly, under pentobarbital anesthesia, the trachea was cannulated with polyethylene tubing and another tube was inserted from the esophagus to the posterior part of the nasal cavity. The nasopalatine was closed with a surgical adhesive.
agent (Aron Alpha®, Sankyo Co., Tokyo, Japan). The nasal cavity was washed via polyethylene tubing from the esophagus. Collection procedures of FD70 were as follows: the nasal cavity was washed with 4 mL of phosphate buffered saline (PBS), and then filled with an appropriate volume of PBS containing 10 mM dithiothreitol (DTT) for 10 min. DTT is a solubilizer of mucin. The nasal cavity was washed again with 4 mL of PBS containing DTT. All the wash solutions were collected together to adjust the volume to precisely 25 mL. FD70 in the wash solution was analyzed fluorometrically.

Membrane Damage by Lactose and NaCl The in vivo toxicity of lactose and NaCl on the integrity of epithelial cells was evaluated. Lactose or NaCl (1 mg powder) was administered into the nostrils of male Wistar rats. Six hours after application, the nasal cavity was washed with 8 mL of PBS. As an index of membrane damage, the activity of LDH in the wash solution was determined by an LDH CII assay (Wako Pure Chemical Industries, Ltd., Osaka, Japan). The wash solution was also taken from untreated rats in the control study.

Drug Assay

HPLC Analysis of WF, PXC and NFX

Acetonitrile (1000 µL) was added to the plasma (100 µL) for deproteination. The mixture was vortexed for 10 min and centrifuged at 16000×g for 5 min. The supernatant (1000 µL) was evaporated to dryness at 60°C. The residue was reconstituted with 100 µL of the mobile phase and was analyzed using a Shimadzu HPLC system LC-20 (Shimadzu, Kyoto, Japan). The setup of the HPLC system for the analysis of each drug was as follows:

WF

Column: ODS column (Wakopak, 5 mm, 4.6×150 mm, Wako Pure Chemical Industries, Ltd.).
Mobile phase: 10 mM tetrabutyl ammonium in 10 mM phosphate buffer (pH 7.4)–methanol (50:50).
Flow rate: 0.5 mL/min.
Detection: Fluorescence detection at excitation and emission wavelengths of 310 and 390 nm, respectively.

PXC

Column: ODS column (Wakopak, 5 µm, 4.6×150 mm, Wako Pure Chemical Industries, Ltd.).
Mobile phase: 50 mM KH₂PO₄ buffer (pH 2.5)–acetonitrile=68:32.
Flow rate: 1.0 mL/min.
Detection: Photometric detection at 326 nm.

NFX

Column: ODS column (Inertsil ODS-4, 3 µm, 4.6×150 mm, GL Sciences Inc., Tokyo, Japan).
The mobile phase: 0.1 M acetic acid–acetonitrile=80:20.
Flow rate: 1.0 mL/min.
Detection: Fluorescence detection at excitation and emission wavelengths of 278 and 448 nm, respectively.

FD70

The concentration of FD70 in the nasal washing fluid was determined fluorometrically at excitation and emission wavelengths of 495 and 520 nm, respectively, using a fluorescence spectrophotometer (F-2000, Hitachi High-Technologies, Tokyo, Japan).

Date Analysis All the experiments in this study were performed in at least triplicate and the data are expressed as the mean±standard error (S.E.). The statistical comparisons were conducted using an ANOVA with subsequent Dunnett’s multiple comparison tests. The fractional absorptions of each drug

Fig. 1. The Profiles of the Plasma Concentration of the Model Drugs after Intravenous Administration

Results are expressed as the mean±S.E. of at least three experiments.
were calculated based on deconvolution from zero to the final sampling time using WinNonlin (Certara G.K., Tokyo, Japan). For the numerical deconvolution, the time course of the concentration of each drug after intravenous administration was determined. The profiles are shown in Fig. 1.

RESULTS

**In Vitro Study**

Effects of Lactose and NaCl on the Epithelial Fluid Volume

Figure 2 shows the effect of lactose and NaCl on the epithelial fluid volume of MDCK monolayers. NaCl increased the fluid volume by 74.7 µL/mg, 15 min after application, while lactose increased it by 15.8 µL/mg. The fluid volumes gradually decreased up to 60 min after application. These results clearly indicate that NaCl was more effective in increasing fluid volume than lactose. This difference is due to the smaller size and ionization of NaCl.

**In Vitro Transepithelial Transport Study**

Figure 3 shows the transepithelial permeation of PXC, WF, and NFX after application of the powder formulations onto the surface of the MDCK monolayer. The transport of the model drugs was increased by the addition of lactose and NaCl to the formulations. The enhancement by NaCl was larger than that by lactose; however, the differences were not significant.

**Effects of Lactose and NaCl on the in Vivo Absorption of Model Drugs after Nasal Application of Powder Formulations**

Figure 4 shows the effects of lactose and NaCl on the in vivo nasal absorption of the model drugs. The plasma concentration of WF (Fig. 4(i)) was slightly decreased by lactose and NaCl. Lactose induced no effect on the absorption of NFX, while a small increase by NaCl was observed, as shown in Fig. 4(iii). On the contrary, both NaCl and lactose increased the absorption of PXC Fig. 4(ii). The large initial increase in the fraction absorbed was observed after application with NaCl. The effect of lactose was initially small; however, the fraction absorbed reached the same level as that of the NaCl group 4h after application.

**Clearance of Formulations from the Nasal Cavity**

Figure 5 shows the clearance of FD70 from the nasal cavity after nasal administration of the formulation labelled with FD70.

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Fig. 2. Effects of Lactose and NaCl on the Fluid Volume on the Surface of MDCK Monolayers

Keys: □: NaCl powder, △: lactose powder. Results are expressed as the mean±S.E. of at least three experiments.

Fig. 3. Transepithelial Transport of the Model Drugs (Warfarin, WF; Piroxicam, PXC; Norfloxacin, NFX) across MDCK Monolayers after Spaying Powder Formulations onto the Monolayer Surface

Keys: ●: bulk powder, △: lactose powder, □: NaCl powder. Results are expressed as the mean±S.E. of at least three experiments.
The residual amount of FD70 in the nasal cavity 10 min after administering the control formulation (NFX:FD70=9:1) was 53.7%, while that after administration of the lactose formulation was 20.1%. Clearance of the NaCl formulation was very rapid. The residual amount of FD70 5 min after administering the NaCl formulation was only 5.1%. These results indicate that clearance of the formulation is enhanced under a fluid-rich environment and a change in clearance is likely dependent on the volume of the withdrawn fluid.

**Cytotoxic Effects of Lactose and NaCl on the Nasal Tissues**

Figure 6 shows the effects of lactose and NaCl administration on the LDH activity in the nasal lavage fluid, which is an index of damage to nasal epithelial cells. No significant difference in the LDH activity was observed in lactose or NaCl group in comparison with the control group.

**DISCUSSION**

Among the various nasal formulations, powder formulations have many advantages. Solid drugs are more chemically stable in comparison with solutions. High doses can be applied via nasal administration using powder formulations because the
Epithelial fluid to increase the osmotic pressure, leading to the solid formulations. Lactose and NaCl are dissolved in the nasal cavity, lactose and NaCl were used as excipients. To change the volume of the dissolution fluid in epithelium). To change the volume of the dissolution fluid in the nasal cavity, lactose and NaCl were used as excipients. Lactose and NaCl are dissolved in the epithelial fluid to increase the osmotic pressure, leading to the withdrawal of fluid from cells and/or tissues.

Generally, nasal drug absorption is faster than gastrointestinal absorption. For example, the blood concentration of propranolol following nasal application is similar to that after intravenous application. Propranolol is a highly permeable drug. A small volume of the drug solution applied nasally can reach the surface of nasal epithelium, but without being dissolved, whereas powder formulations, when administered nasally, directly reach the surface of the nasal epithelium and are dissolved in the small fluid volume. Therefore, the drug concentration near the absorptive mucosa is very high, and can be assumed to be saturated. On the contrary, after oral administration of some solid formulations, the drug is dissolved in water with which the formulation is taken (usually 200 mL). After dissolution in the stomach, the dissolved drug moves from the stomach along the intestinal tract, being diluted by gastric and/or intestinally secreted fluid. The effective drug concentration around the mucosal epithelium after nasal application is much higher than that after oral application, thus resulting in a faster and more effective nasal absorption. Thus, nasal residence of a drug is one of the factors that determines drug absorption after nasal administration.

The in vitro study using MDCK cell monolayers showed that lactose and NaCl increased the fluid volume on the epithelial surface. Because the molecular weight of lactose is 342.3 Da, the isotonic solution of lactose is calculated as 96 mg/mL. Therefore, 0.5 mg of lactose can be used to prepare 5.2 µL of isotonic water. However, the concentration of NaCl in isotonic saline is 0.9% (w/v); thus, 0.5 mg of NaCl can be used to prepare 56 µL of isotonic water. Therefore, NaCl can theoretically withdraw 10 times as much water as lactose. As shown in Fig. 2, the volume increase by lactose and NaCl 15 min after application was 74.7 and 15.8 µL/mg, respectively. Consequently, the actual volume increase by 0.5 mg of lactose and NaCl was 37.4 and 7.9 µL, respectively.

The in vitro transepithelial transport of model drugs was enhanced by lactose and NaCl. Lactose effectively increases the transport of the drug. However, the difference between lactose and NaCl is not clear. Inokuchi et al. reported that NaCl and mannitol enhanced drug permeation across Caco-2 cell monolayers. The change is likely due to the enhanced dissolution of the fluid-rich epithelium by lactose and NaCl. Another mechanism is possibly due to the effective surface area because a larger volume of the solution can spread over a wider epithelial area.

According to the in vivo animal study, lactose and NaCl enhanced the absorption of PXC, slightly decreased that of WF, and slightly increased that of NFX. These data indicate that changes in the fluid volume were different, depending on the solubility and permeability of the drug. With regard to WF, the absorption can never be enhanced because the absorption from the control powder formulation (bulk WF powder) is 100%. The absorption of PXC was found to be clearly enhanced. Because PXC is a highly permeable but poorly soluble drug, the rate-limiting process for its absorption from the solid formulation is typically the dissolution. The mucosal fluid volume increase by lactose and NaCl enhanced the dissolved amount of PXC, which in turn increased its absorption. NFX is a poorly soluble and poorly permeable drug. The results of NFX from the in vivo animal study hardly agree with those from the in vitro transport study. Other in vivo factors,
such as nasal residence, to decrease the absorption should be considered.

Nasal drug absorption from the nasal cavity generally occurs within 30 min after nasal administration due to MC. To understand the mechanism of the unchanged absorption of NFX by lactose and NaCl, the nasal residence of the formulation was evaluated using FD70 as a marker of the formulation. The clearance of the formulation was expectedly enhanced by lactose and NaCl. The enhancement of clearance by NaCl was larger than that by lactose. Rich fluid on the nasal mucosa allows an improved flow of the dissolved drug out of the nasal cavity. Reduction in the viscosity of the nasal mucus by withdrawn fluid can enhance the MC. In other words, the poorly permeable drug, NFX, is cleared out into the gastrointestinal tract before a sufficient amount is absorbed by the nasal cavity. Lactose and NaCl both show a positive effect on the drug dissolution and the effective mucosal surface area, and a negative effect on the nasal residence of the drug. The overall effect on the drug absorption is determined by a balance of these effects. If one wishes to enhance absorption with lactose and NaCl, the drug should be not only poorly soluble but also highly permeable.

The acute membrane damage by lactose and NaCl was evaluated with LDH activity.2030 The safety of lactose has already been established because lactose is commonly used as a diluent for oral solid formulations. However, since the local concentration was likely very high, the acute membrane damage by lactose was checked to make sure that the enhanced absorption of PXC was not due to membrane damage. LDH levels after nasal application of lactose and NaCl were similar to that of the control group. This indicates no acute membrane damage by lactose or NaCl. For the clinical use, additional studies are required to elucidate the chronic and detailed toxicity.

In conclusion, the addition of NaCl and lactose to the powder formulation increased the mucosal fluid volume. This volume increase can enhance the absorption of poorly soluble drugs through an increase in the drug dissolution and the effective mucosal surface area, and a negative effect on the nasal residence of the drug. The overall effect on the drug absorption is determined by a balance of these effects. If one wishes to enhance absorption with lactose and NaCl, the drug should be not only poorly soluble but also highly permeable.

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Conflict of Interest The authors declare no conflict of interest.

REFERENCES


