Insoluble Powder Formulation as an Effective Nasal Drug Delivery System

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Purpose. To evaluate the utility of insoluble powder formulation for nasal systemic drug delivery.

Methods. To compare the efficacy of liquid and powder formulations, the nasal absorption of drugs was examined in rats using hydrophilic compounds with various molecular weights (MW) such as phenol red, cyanocobalamin, and fluorescein isothiocyanate (FITC)-Dextrans, and several kinds of powder. Intranasal residence time was also compared among the different formulations.

Results. All the drugs examined were absorbed through the nasal mucosa to varying extent; their systemic bioavailability decreased with increasing MW. Insoluble calcium carbonate ($CaCO_3$) powder formulation provided increased absorption of drugs over the wide range of MW from 354 to 77,000 Da. In the case of phenol red, intranasal administration as a $CaCO_3$ powder formulation resulted in a plasma concentration profile similar to that of an intravenous bolus dose due to its very rapid and complete absorption from the nasal cavity. Furthermore, improved bioavailability of FITC-Dextran (MW 4,400; FD-4) was also achieved with other insoluble powders as well as $CaCO_3$, but not with soluble powders such as lactose, *d*-sorbitol, and *d*-mannitol. Insoluble powder formulation prolonged the residence time of FD-4 within the nasal cavity.

Conclusions. Insoluble powder formulations improve nasal bioavailability predominantly by retarding drug elimination from the absorption site and appear to be effective for nasal systemic drug delivery.

KEY WORDS: nasal delivery; powder; formulation; phenol red; cyanocobalamin; FITC-Dextran.

INTRODUCTION

The nasal route for systemic drug delivery has received great attention as an alternative to oral administration for drugs with low membrane permeability and drugs that readily undergo intestinal and hepatic first-pass metabolism, and also as an alternative to invasive parenteral administration, which has poor patient acceptance (1). However, nasal administration of peptides such as insulin and calcitonin without an absorption modifier results in unacceptably low bioavailability, possibly owing to low permeability, enzymatic degradation in the nasal epithelium, and/or mucociliary elimination from the nasal cavity. To overcome these difficulties, many attempts have been made to increase the absorption by using permeation enhancers, protease inhibitors, and mucoadhesive agents (2–4). However, these additives may cause morphologic changes and membrane perturbation, and even unexpected adverse reactions.

The small volume of the nasal cavity, one of the limiting factors in nasal drug delivery, restricts the amount of formulation that can be administered, so that only a low-dose drug or a highly soluble drug is administrable with a simple liquid formulation (5). Therefore, we have taken a great interest in powder formulation as a means of solving the above-mentioned problems because this formulation, which allows us to administer a larger dose intranasally, can result in both saturation of enzymatic degradation processes due to the high drug concentration on the nasal mucosa and retarded outflow of the drug from the nasal cavity (6). In addition, powder formulation is preferable to liquid formulation in terms of increased chemical stability and no requirement for preservatives (7,8). Despite the advantages of powder formulation, water-soluble lactose powder, which has generally been used in pulmonary dry powder inhalation (9), did not increase the nasal absorption of polar molecules (10,11), though some special powder formulations containing potent permeation enhancers or mucoadhesives have succeeded in improving bioavailability (6–8,10–12). In contrast, although several reports and patents concerning feasible water-insoluble powder formulations have appeared (10,13,14), nasal drug delivery by insoluble powder formulations has not been evaluated in detail. Recently, we observed that elcatonin, a synthetic calcitonin analog with molecular weight (MW) of 3,364, was extensively absorbed from the nasal cavity by administering as mixture with insoluble calcium carbonate ($CaCO_3$) powder (15). The supposed mechanism for an increase in nasal bioavailability was ascribed to an enhanced residence time in the absorption site and an increased topical concentration of the peptide by attaching to the powder particle.

In the present study, we investigated the nasal absorption of hydrophilic model compounds of various MW using insoluble powder formulation in comparison with liquid or soluble powder formulation to elucidate how insoluble powder results in good bioavailability in rats.

MATERIALS AND METHODS

Materials

All chemicals were commercial products and were used without further purification. Phenol red and cyanocobalamin were obtained from Wako Pure Chemical Industries (Osaka, Japan). Fluorescein isothiocyanate (FITC)-labeled Dextrans with an average MW of 4,400 (FD-4), 9,500 (FD-10), 19,500 (FD-20), 42,000 (FD-40), and 77,000 (FD-70) Da, containing FITC in the range of 0.004 to 0.015 mole per mole of glucose, were from Sigma Chemical Co. (St. Louis, MO). Precipitated calcium carbonate ($CaCO_3$), ethylcellulose, talc, barium sulfate, lactose, *d*-sorbitol, and *d*-mannitol used as drug carriers (excipients) were of JP grade. All other chemicals and solvents were of special reagent grade or HPLC grade.

Preparation of Formulation

To prepare nasal liquid formulation, phenol red or cyanocobalamin was dissolved in isotonic phosphate buffer solution, pH 7.4, and all the FITC-Dextrans were dissolved in

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normal saline (Otsuka Pharmaceutical Co., Tokyo, Japan) at the concentration of 20 mg/ml. Powder formulation (20% content) was made by homogeneously mixing each hydrophilic drug and $CaCO_3$ in the ratio of 1:4. In addition, FD-4 powder formulations using other excipients such as lactose and talc were prepared similarly for comparison of nasal absorption between soluble and insoluble powder forms. Each solution for intravenous administration was obtained by a method similar to that used for nasal liquid formulation, but at a concentration of 5 mg/ml. All formulations were freshly prepared and the content was checked prior to use by means of HPLC or with a fluorescence spectrophotometer as described below.

Animals

All animal experiments adhered to the Principles of Laboratory Animal Care (NIH publication no. 85-23, revised 1985), and the protocols were reviewed and approved by the Internal Animal Welfare Committee at Dainippon Pharmaceutical Co. Male Wistar rats (Japan SLC Inc., Hamamatsu, Japan) weighing 230 ± 14 g (mean ± SD) were used. They were acclimated in groups of three per cage under environmentally controlled conditions (20°C–-26°C, 45%–65% relative humidity, and a 12-h light/dark cycle) with free access to tap water and commercial diet (CE-2, CLEA Japan Inc., Tokyo, Japan) prior to use in experiments.

Surgical Operation for Nasal Administration

Nonfasted rats were anesthetized by intramuscular injection of 50 mg/kg of sodium pentobarbital (Nembutal; Abbott Laboratories, North Chicago, IL). Surgical operation for nasal dosing was performed according to the procedure by Hirai *et al.* (16) with minor modifications. Briefly, the rats were placed on their backs, and the right femoral artery was cannulated with SP-31 polyethylene tubing (Natsume, Tokyo, Japan) for serial blood sampling. Subsequently, the trachea and esophagus were exposed by making a medial line incision in the neck. A PE-205 polyethylene cannula (Intramedic, Becton Dickinson, Sparks, MD) was inserted into the trachea toward the lungs to maintain respiration, and the esophagus was occluded by tying it with a suture onto this cannula. The nasopalatine tract was sealed off with a drop of cyanoacrylate adhesive (Aron Alfa A; Sankyo, Tokyo, Japan) to avoid the drainage of drug from the nasal cavity to the oral cavity. Rats used in the intravenous administration experiment also underwent sham operation on their airways.

Nasal Absorption Studies

A 250-μl/kg aliquot of nasal liquid formulation, corresponding to a dose of 5 mg/kg, was instilled into the nasal cavity via the left nostril using a 100-μl microsyringe (Hamilton, Reno, NV) through SP-45 polyethylene tubing (Natsume) attached to the needle. About 6 mg of powder formulation in a 100-μl micropipette tip (Eppendorf, Hamburg, Germany), corresponding to a dose of 5 mg/kg, was administered into the nose via the left nostril with compressed air using a syringe (Terumo, Tokyo, Japan) joined to the micropipette tip. An intravenous bolus dose was directly given into the left femoral vein using a disposable syringe with a 26-gauge needle (Terumo). Rats were kept under anesthesia throughout the experiments with 10 mg/kg pentobarbital i.m. given periodically. The amount of drug remaining in the micropipette tip after powder dosing was measured to calculate the exact dose administered. Arterial blood (200 μl) was withdrawn at 5, 15, 30, 60, 120, 180, 240, and 360 min after dosing and was placed in heparinized micro test tubes (Eppendorf). Plasma samples were obtained by centrifugation of the blood at 15,000 rpm for 5 min and were stored at below –20°C until analysis.

Comparative Drug Duration within the Nasal Cavity in Different Formulations

To evaluate the duration of residence of drug in the nasal cavity, the remaining amount of FD-4 within the nasal cavity after administration was measured. The powder or liquid form of FD-4 was given intranasally to rats that had undergone the surgical operation described above. Immediately before the designated time (30, 60, or 120 min) postdosing, PE-260 tubing (Intramedic; Becton Dickinson) was inserted into the esophagus toward the nasopharynx, and at the appropriate times, the rats were sacrificed by administration of 100 mg/kg i.v. pentobarbital. The nasal cavity was then perfused twice with 10 ml each of 1/15 M phosphate buffer solution, pH 7.4, through PE-260 tubing to collect the remaining FD-4. In addition, FD-4 was recovered by thoroughly washing the nasal cavity exposed by incision of the nostrils. The nasal perfusate recovered was combined with the wash solution and was made up to 100 ml with the buffer solution, followed by quantitation of the remaining amount of FD-4.

Analytical Procedures

A phenol red plasma sample (100 μl) was mixed with an equal volume of methanol and was centrifuged, and 50 μl of isopropanol was then added to the 100-μl supernatant followed by mixing. The resulting specimen was again centrifuged, and the supernatant was subjected to chromatographic analysis.

A cyanocobalamin plasma sample (100 μl) was mixed with 1 ml of water, and the sample was loaded onto a conditioned and equilibrated Waters OASIS® HLB Extraction Cartridge 60 mg (Waters, Milford, MA), rinsed with 2 ml of water, and then eluted with 2 ml of methanol. The eluate was evaporated to dryness *in vacuo*, and the residue was dissolved in 100 μl of water, followed by HPLC analysis.

Quantitative determinations of plasma concentration of phenol red and cyanocobalamin were carried out on an HPLC system (LC-VP; Shimadzu, Kyoto, Japan) consisting of an LC-10AD_{VP} pump, an SIL-10AV_{VP} autoinjector, a CTO-10AC_{VP} column oven, an SPD-10AV_{VP} detector, and a YMC-Pack ODS-A column (6 mm × 150 mm; YMC, Kyoto, Japan) with a flow rate of 1.0 ml/min for phenol red and 1.2 ml/min for cyanocobalamin; an injection volume of 20 μl; a temperature of 40°C; and a detection wavelength of 427 and 361 nm for phenol red and cyanocobalamin, respectively. The mobile phases consisted of 0.2 M sodium dihydrogen phosphate solution and acetonitrile (3:1) for phenol red and 0.1 M sodium dihydrogen phosphate solution and methanol (3:1) for cyanocobalamin.

Plasma concentrations of all FITC-Dextrans were determined by fluorescence detection at 495 nm (excitation) and

516 nm (emission). The plasma was diluted with 30 volumes of 1/15 M phosphate buffer solution, pH 7.4, and the fluorescence intensity was measured with a fluorescence spectrophotometer (RF-5300PC, Shimadzu).

Concentration of FD-4 in the sample recovered from the nasal cavity was analyzed by means of the above-mentioned HPLC system, except for the detector, the column, and the mobile phase: fluorescence monitoring at excitation 495 nm and emission 516 nm (RF-10A_{XL}; Shimadzu); 7.8 mm × 300 mm Ultrahydrogel 500 GPC column (Waters); and eluting with 1/15 M phosphate buffer solution, pH 7.4. The flow rate was set at 1.0 ml/min.

Pharmacokinetics and Statistics

In experiments on the powder formulation, the measured plasma concentration was corrected for the actual dose and the body weight to obtain the value at a dose of 5 mg/kg. The corrected concentration was used as the observed concentration in pharmacokinetic analysis.

A noncompartment analysis was performed for determining the maximum concentration (C_{max}), the time at which it occurred (t_{max}), and the area under the plasma concentration-time curve from zero to infinity (AUC) from the individual data. Nasal systemic bioavailability (drug amount absorbed up to the relevant time) was calculated by integration of nasal absorption rate (input function) derived by the numerical deconvolution method in which the plasma profiles following nasal and intravenous doses were regarded as output and weight functions, respectively. The weight function was approximated by a biexponential equation with constants estimated by nonlinear least-squares regression analysis. The regression procedure was conducted by the Gauss-Newton method using weighting of $1/Cp^2$, where Cp is the observed concentration. The numerical deconvolution was performed by a computer program written in BASIC language by the authors. The other pharmacokinetic analyses were carried out using the WinNonlin Standard (ver. 1.5) software package (Pharsight, Mountain View, CA).

To compare bioavailability between different nasal formulations, we performed Student's *t* test or Welch's *t* test, or one-way analysis of variance (ANOVA) with Dunnett's multiple comparison, using the SAS statistical analysis software package (SAS Institute, Cary, NC). Differences in average values were considered to be statistically significant if $p < 0.05$.

RESULTS

In all cases of administering powder formulation, the drug amount remaining in the device was less than 5% of the nominal dose, and the actual dose was between 4.5 and 5.5 mg/kg, which deviated little from 5 mg/kg. Consequently, we judged that correction of measured data for actual dose and body weight to obtain the plasma concentration at a 5-mg/kg dose was unlikely to cause any problem.

Nasal Absorption of Drugs with Various MWs

The nasal absorption values in rats given liquid formulation and the $CaCO_3$ powder formulation of hydrophilic drugs with MW from 354 to 77,000 were compared. Figure 1 and Table I show the plasma concentration time profiles and the pharmacokinetic parameters, respectively, following intranasal administration of phenol red, cyanocobalamin, and various FITC-Dextrans. All drugs were absorbed via the nasal mucosa to varying extents; the systemic bioavailability decreased logarithmically with increasing MW (Fig. 2). When phenol red was administered in the $CaCO_3$ powder formulation, rapid and high absorption was observed: the nasal bioavailability was nearly 100% and the plasma concentration profile was comparable with that of intravenous administration. The insoluble powder formulations of all the other drugs also exhibited increased nasal bioavailability by factors of 1.5 to 3, and the C_{max} and t_{max} were or tended to be higher and earlier, respectively, than those of the liquid formulations. These results revealed that $CaCO_3$ powder formulation is effective for increasing the nasal absorption of hydrophilic drugs, regardless of MW.

Effect of Other Powder Formulations on Nasal Absorption of FD-4

To examine whether enhancement of nasal absorption could be achieved by other powder formulations or not, we evaluated the bioavailability of FD-4 in several powder formulations, including water-soluble (lactose, *d*-sorbitol, and *d*-mannitol) and -insoluble excipients (ethylcellulose, talc, and barium sulfate). The plasma concentration time profiles of FD-4 following nasal administration of different powder formulations are shown in Fig. 3, and the evaluated parameters are summarized in Table II. Intranasal administration of the insoluble powder formulation, in all cases, resulted in significantly increased plasma levels of FD-4 with earlier t_{max} as compared with the liquid formulation. Furthermore, the extents of increase of nasal absorption were comparable among the insoluble powders examined; the C_{max} values were between 1.33 and 1.73 μg/ml and the systemic bioavailability was between 37.1% and 38.8%. In contrast, the water-soluble powder formulation gave only slightly higher bioavailability, 13.7%, 19.0%, and 21.5% for lactose, *d*-mannitol, and *d*-sorbitol, respectively, than the liquid formulation (12.7%), with no statistically significant difference, although rapid absorption via the nasal mucosa was observed with these formulations. Therefore, it appears that water-insoluble powder formulations equally provide increased absorption of hydrophilic drugs from the nasal cavity.

Comparative Drug Duration within Nasal Cavity

To evaluate drug duration within the nasal cavity in rats, we compared the amount of FD-4 remaining in and disappeared from the nasal cavity following administration of three formulations, i.e., liquid formulation, lactose powder formulation, and $CaCO_3$ powder formulation. Figure 4 represents the fractional FD-4 amount absorbed from and remaining in the nasal cavity up to 30, 60, and 120 min postdosing relative to the administered dose; these values were determined by numerical deconvolution and size exclusion chromatography, respectively. In this experiment, HPLC analysis showed that fluorescence due to degraded FD-4, e.g., free FITC or smaller fragments of FD-4, was negligible in both the nasal cavity and the blood stream for all of the dosage forms, implying that FD-4 is stable on nasal epithelium. Consequently, we determined the amount of FD-4 possibly eliminated from the nasal

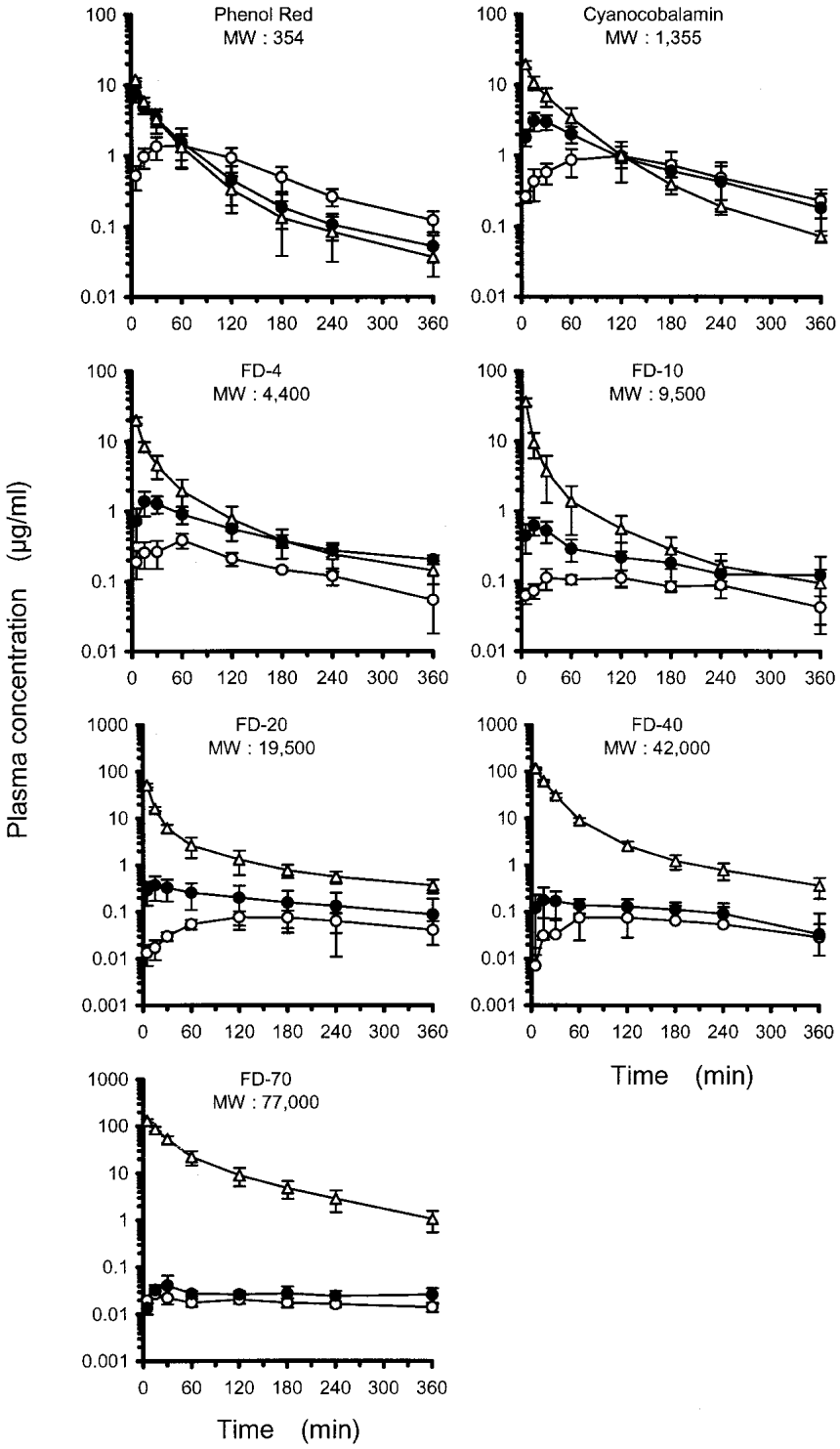

Fig. 1. Plasma concentration-time curves of phenol red, cyanocobalamin, and various FITC-Dextrans after intravenous and intranasal administration at a dose of 5 mg/kg in rats. Each point represents the mean ± SD of results from five rats. △, intravenous; ○, intranasal (liquid); ●, intranasal ($CaCO_3$ powder).

cavity due to mucociliary clearance by subtracting the sum of remaining and absorbed amounts from the given dose. As shown in Fig. 4, FD-4 administered in the liquid formulation readily disappeared from the nasal cavity, with 17% and 50% of the dose having been eliminated at 30 and 120 min, respectively. The lactose powder formulation also had a propensity to be eliminated similarly to the liquid formulation up to 60 min, though the absorption (percentage of dose) was slightly higher. In contrast, the $CaCO_3$ powder formulation decreased the elimination of FD-4 from the nasal cavity and increased

Table I. Pharmacokinetic Parameters of Hydrophilic Drugs with Various Molecular Weights in the Liquid and Insoluble Powder ($CaCO_3$) Formulations Administered Intranasally at a Dose of 5 mg/kg in Rats

Drug	Molecular weight	Form	C_{max} (μg/ml)	t_{max} (min)	AUC (μg min/ml)	Bioavailability (BA)[a] (%)
Phenol red	354	Liquid	1.44 ± 0.49	72.0 ± 26.8	240 ± 73	69.6 ± 21.9
		Powder	7.52 ± 1.79[b]	5.0 ± 0.0[b]	321 ± 83	96.3 ± 25.7
Cyanocobalamin	1,355	Liquid	1.01 ± 0.54	96.0 ± 32.9	255 ± 123	35.5 ± 17.8
		Powder	3.23 ± 0.75[b]	21.0 ± 8.2[b]	376 ± 91	55.3 ± 14.5
FD-4	4,400	Liquid	0.40 ± 0.09	54.0 ± 13.4	75 ± 20	12.7 ± 2.4
		Powder	1.49 ± 0.42[b]	21.0 ± 8.2[b]	235 ± 22[b]	37.1 ± 3.4[b]
FD-10	9,500	Liquid	0.135 ± 0.009	54.0 ± 39.1	48.5 ± 14.9	5.4 ± 0.3
		Powder	0.638 ± 0.182[b]	13.0 ± 4.5	86.6 ± 34.6	13.8 ± 4.9[c]
FD-20	19,500	Liquid	0.090 ± 0.019	120 ± 42	40.1 ± 23.0	2.08 ± 0.64
		Powder	0.431 ± 0.164[b]	30 ± 18[b]	96.3 ± 92.4	6.24 ± 4.54
FD-40	42,000	Liquid	0.105 ± 0.074	120 ± 74	22.1 ± 22.5	0.69 ± 0.84
		Powder	0.236 ± 0.103[c]	45 ± 46	53.7 ± 19.1[c]	1.25 ± 0.34
FD-70	77,000	Liquid	0.027 ± 0.004	15 ± 0	6.4 ± 0.4	0.13 ± 0.01
		Powder	0.050 ± 0.020	189 ± 169	13.9 ± 0.9[b]	0.21 ± 0.02[b]

Note. Each value represents the mean ± SD of results from five rats.
[a] BA denotes the systemic bioavailability calculated by deconvolution up to 360 min after dosing.
[b, c] Significantly different from the liquid formulation ([b]$p < 0.01$, [c]$p < 0.05$; Student's *t* test or Welch's *t* test).

the absorption; the elimination (percentage of dose) was less than 0.5% at 30 min and was only 8% at 120 min. These results indicate that insoluble powder formulation prolongs the residence time available for drug absorption on the nasal epithelium.

DISCUSSION

In the present study, we demonstrate increased nasal bioavailability of hydrophilic drugs in insoluble powder formulation using model compounds with various MW and several kinds of excipients. Many investigators have examined MW dependency in nasal drug absorption (17–24) because MW may be a determinant of the nasal epithelial permeation of large solutes of >1000 Da. We chose FITC-Dextrans as marker compounds in our study of the nasal absorption of large hydrophilic molecules because of their ease of detection and their proven utility in *in vivo* and *in vitro* studies on absorption/permeation across various biomembranes (11,21–29).

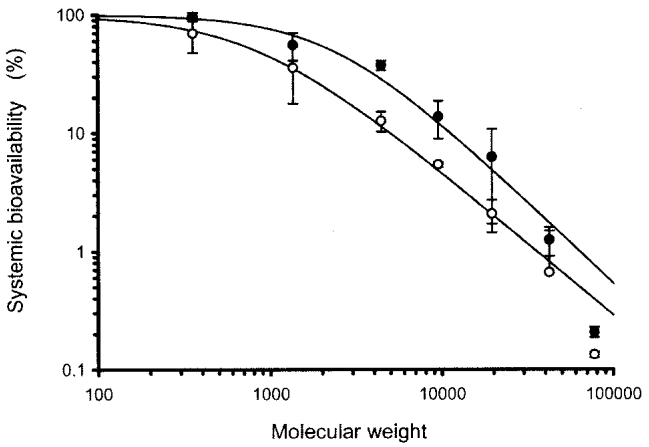

Fig. 2. Systemic bioavailability of hydrophilic drugs following intranasal administration of liquid (○) and $CaCO_3$ powder (●) formulations as a function of molecular weight in rats. Each point represents the mean ± SD of results from five rats. The solid lines are the computer-generated curves based on the kinetic model and the parameters shown in Fig. 5 and Table III, respectively.

In the range of low- to middle-MW compounds, nasal bioavailability of about 70% was obtained with liquid formulation of phenol red, which is poorly absorbed from the gastrointestinal (GI) tract. This finding for nasal phenol red is in agreement with the result reported previously (16), and again indicates that the nose should be an effective site for systemic delivery of GI nonabsorbable drugs. On the other hand, cyanocobalamin, vitamin B_{12}, is well absorbed by a specific mechanism from the ileum following its complex formation with a 59-kDa gastric glycoprotein, so-called intrinsic factor (30). We also demonstrated the good nasal absorbability of cyanocobalamin in rat here because the occlusion of the esophagus prevented the passage of drug into the GI tract. Indeed, a nasal cyanocobalamin gel formulation is now on the market (Nascobal™) for patients with vitamin B_{12} deficiency due to ileectomy or the lack of intrinsic factor.

Over a wide range of >1000 Da, MW dependency of nasal systemic bioavailability was observed in both liquid and powder formulations (Fig. 2). The bioavailability decreased logarithmically with respect to increasing MW, and this is in accordance with *in vivo* findings reported previously (17–19,22). Although ciliary beating on the mucosal epithelium plays a very significant role in removal of entrapped dust in the upper airway, this physiological defense mechanism is another factor influencing nasal absorption. Therefore, the overall absorption process can apparently be regarded as a balance between entry into the systemic circulation and removal from the absorption site, and a model for kinetic analysis of nasal absorption based on this idea has been proposed (17), as shown in Fig. 5. On the assumption first of concentration-independent permeation and homogeneity of the mucosal surface, and second, of MW-dependent permeation represented by a power function and MW-independent elimination, the systemic bioavailability (BA) can be defined as follows (refer to Fig. 5):

$$BA\ (\%) = 100/(1 + E/A \times MW^{B})$$

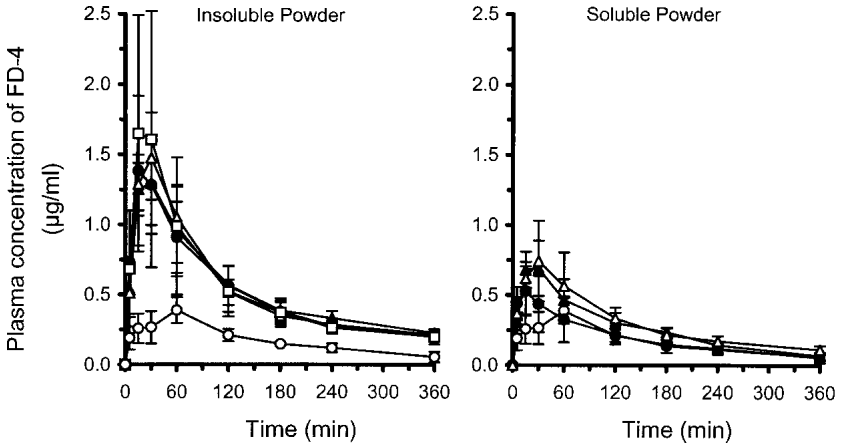

Fig. 3. Plasma concentration-time curves of FD-4 after intranasal administration of the insoluble (left) and soluble (right) powder formulations at a dose of 5 mg/kg in rats. Each point represents the mean ± SD of results from five rats. ○, liquid formulation (control); *left panel*: ●, $CaCO_3$; △, ethylcellulose; ▲, talc; □, barium sulfate; *right panel*: ●, lactose; △, *d*-sorbitol; ▲, *d*-mannitol.

where E is the mucociliary clearance, and A and B are parameters relating to transmucosal permeability. Values for E/A and B estimated by nonlinear least-squares regression analysis are summarized in Table III, and give the lines shown in Fig. 2. Values for B, which involves MW dependency, were 1.22 and 1.38 in the liquid and $CaCO_3$ powder formulations, respectively, and agreed well with the reported value of 1.35 (17), so that the insoluble powder is unlikely to affect the MW dependency in nasal permeation. In contrast, it is suggested that the $CaCO_3$ powder can suppress the mucociliary elimination because the value of E/A for the liquid formulation, 29.1×10^{-5}, was 12 times larger than that for the powder formulation, 2.4×10^{-5}. Accordingly, the increased absorption afforded by insoluble powder appears to be due to prolongation of the residence time available for absorption in the nasal cavity. This idea is consistent with the extreme insolubility of $CaCO_3$, so that it cannot facilitate permeation of the drug across the nasal epithelium, and the fact that increased FD-4 absorption was found not only with $CaCO_3$, but also with the other insoluble compounds (viz., equally with all insoluble excipients), though not soluble excipients (Fig. 3). Soluble powder formulation failed to give sufficient bioavailability, presumably owing to its being washed out by mucus in a relatively short time following rapid dissolution. However, further studies on transmucosal permeation are still needed to elucidate how insoluble powder increases nasal absorption.

Recently, we reported that $CaCO_3$ powder did not facilitate the *in vitro* permeation of elcatonin or inulin across rabbit nasal mucosa by showing no difference in the permeability between liquid and powder formulations (15). These findings suggest that the participation of specific transport mechanism for the uptake of powder particles by M-cells of the nose-associated lymphoid tissue in the enhanced nasal absorption is negligible. Also, it appears that $CaCO_3$ powder causes little membrane perturbation, implying that it does not affect the integrity of nasal epithelial tissue. Additionally, an idea that the improved nasal bioavailability may be due to an increase in chemical/biological stability of drug by insoluble powders within nasal cavity should be withdrawn in the present study because all the model drugs used were stable in both liquid

Table II. Bioavailability of FD-4 in Water-Soluble and -Insoluble Powder Formulations Administered Intranasally at a Dose of 5 mg/kg in Rats

Dosage form	C_{max} (μg/ml)	t_{max} (min)	AUC (μg min/ml)	BA[a] (%)
Liquid	0.40 ± 0.09	54.0 ± 13.4	75 ± 20	12.7 ± 2.4
Water-soluble powder				
Lactose	0.54 ± 0.18	13.0 ± 4.5[b]	81 ± 27	13.7 ± 4.7
d-Sorbitol	0.76 ± 0.26	24.0 ± 8.2[b]	139 ± 33	21.5 ± 5.1
d-Mannitol	0.72 ± 0.17	21.0 ± 8.2[b]	108 ± 17	19.0 ± 3.3
Water-insoluble powder				
$CaCO_3$	1.49 ± 0.41[b]	21.0 ± 8.2[b]	235 ± 22[b]	37.1 ± 3.4[b]
Ethylcellulose	1.54 ± 0.26[b]	27.0 ± 6.7[b]	288 ± 63[b]	38.0 ± 3.8[b]
Talc	1.33 ± 0.22[b]	27.0 ± 6.7[b]	256 ± 45[b]	38.5 ± 6.8[b]
Barium sulfate	1.73 ± 0.87[b]	24.0 ± 8.2[b]	248 ± 42[b]	38.8 ± 10.4[b]

Note. Each value represents the mean ± SD of results from five rats.

[a] BA denotes the systemic bioavailability calculated by deconvolution up to 360 min after dosing.

[b] Significantly different from the liquid formulation ($p < 0.01$; one-way ANOVA with Dunnet's multiple comparison).

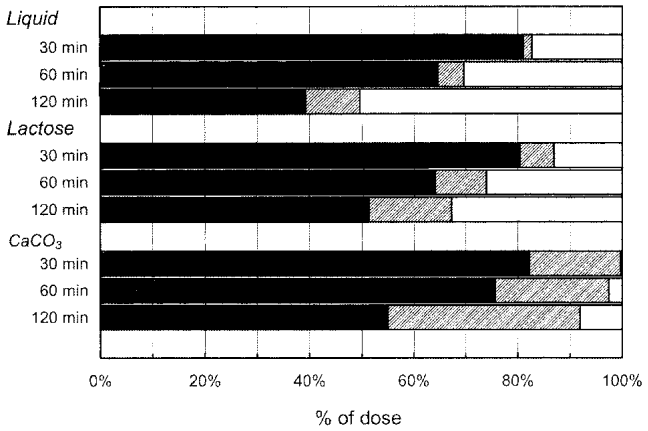

Fig. 4. Disappearance of FD-4 from rat nasal cavity as a function of time following intranasal administration of various formulations. Closed, hatched, and open columns represent the means of the fractional amounts of FD-4 remaining, absorbed, and eliminated, respectively, relative to the given dose from three rats.

and powder administrations. We confirmed that the insoluble powder formulation results in significant retardation of drug outflow from the absorption site as compared with the liquid and soluble lactose powder formulations by evaluating the absorption and recovery of FD-4 from rat nasal cavity (Fig. 4).

For intranasally administered FD-4, interestingly, the nasal bioavailability of 37.1% to 38.8% achieved with the present insoluble powder formulation is similar to the best values reported by other workers: 35.5% with the best powder formulation comprising microcrystalline cellulose and hydroxypropyl cellulose (12) and 32.7% by the mucoadhesive Carbopol 934P microspheres, vs. 9.1% with lactose microspheres (11). Therefore, our insoluble powder formulation, which can be prepared easily at low cost, should be highly effective for nasal systemic delivery of active polypeptides such as insulin and calcitonin, as well as elcatonin, as reported previously (15).

Powder formulation should be preferable to liquid formulation for rapid transmucosal diffusion because of the existence of a high concentration on the nasal mucosal surface due to direct contact. In addition, an insoluble powder formulation should provide fast onset of pharmacological action, owing to earlier t_{max} and higher C_{max} compared with a liquid formulation. The very rapid and complete absorption of phenol red in the insoluble powder formulation suggests that this formulation could be useful in the treatment of pain, migraine, seizure, nausea, and vomiting, etc. Insoluble powder formulation may also improve patient compliance if the taste

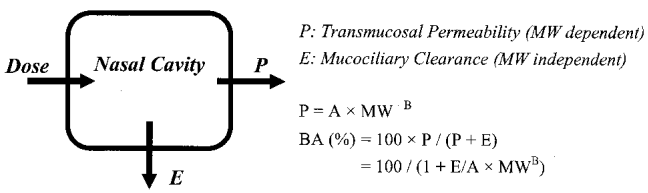

Fig. 5. A model for kinetic analysis of nasal absorption of hydrophilic drugs with various molecular weights. BA denotes the systemic bioavailability via the nasal route. This model is based on the report by McMartin et al. (17).

Table III. Kinetic Parameters for Intranasal Disposition of Hydrophilic Drugs Administered in Liquid and $CaCO_3$ Powder Formulations in Rats

Dosage form	$E/A \times 10^5$	B
Liquid	29.1 ± 12.8	1.22 ± 0.06
$CaCO_3$ powder	2.4 ± 1.8	1.38 ± 0.16

Note. Values represent the mean ± SD of the dimensionless parameters estimated by the nonlinear least-squares regression analysis according to the following equation: $BA\ (\%) = 100/(1 + E/A \times MW^B)$, and give the lines shown in Fig. 2.

of a drug administered is unacceptable. In contrast, no safety assessment of powder formulation was conducted in the present study. Nasal mucosal irritation or cytotoxicity caused by pharmaceutical excipients would be a serious determinant in the development of nasally administrable products. In the future, a comprehensive investigation including the safety of excipients should be essential to optimize nasal drug delivery with insoluble powder formulation.

In conclusion, we have demonstrated that an insoluble $CaCO_3$ powder formulation increased the nasal bioavailability of hydrophilic compounds having MWs covering the wide range of 354 to 77,000 Da. Similar increases in bioavailability were obtained with insoluble powder forms other than $CaCO_3$ powder form, presumably through retardation of the drug outflow from the nasal cavity. These results suggest that insoluble powder formulations are likely to be effective for nasal systemic drug delivery.

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