Usefulness of Liposomes as an Intranasal Dosage Formulation for Topical Drug Application

Kazunori IWANAGA,*,‡ Sumiyo MATSUMOTO,‡ Kazuhiro MORIMOTO,‡,1) Masawo KAKEMI,* Shinji YAMASHITA,‡ and Toshikiro KIMURA‡

Department of Pharmaceutics, Osaka University of Pharmaceutical Sciences,* 4–20–1 Nasahara, Takatsuki, Osaka, 569–1094, Japan, Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, Setsunan University,* 45–1 Nagatoohge-cho Hirakata, Osaka, 573–0101, Japan, and Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, Okayama University,† 1–1–1 Tsushima-naka, Okayama, 700–8530, Japan.

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The potential of liposomes as an intranasal dosage formulation for topical application was investigated in rats. When 5(6)-carboxyfluorescein (CF), a model absorbable drug, dissolved in phosphate-buffered saline (PBS) was administered intranasally, CF was rapidly absorbed into the systemic circulation and no adhesion of CF to the nasal mucosa was observed. The fraction of CF absorbed from the nasal mucosa reached about 48% 1 h after administration. On the other hand, only 3% of the dose was absorbed when CF was encapsulated in liposomes consisting of dipalmitoylphosphatidylcholine and cholesterol (DPPC-liposomes). In addition, the amount of CF adhering to the nasal mucosa after administration as DPPC-liposomes was 20–28 fold greater than that in PBS solution. In particular, positively charged liposomes markedly enhanced the adhesion of CF to the nasal mucosa. Differences in the lipid composition of liposomes did not affect the absorption of CF. However, the ability of liposomes to adhere to the nasal mucosa was consistent with the fluidity of the liposomal membrane. Furthermore, the action of liposomes on the anti-histaminic effect of diphenhydramine hydrochloride (DH) was studied in rats by measuring the amount of protein leaking into the nasal cavity under quasi-allergic conditions. The anti-histaminic effect of DH was strong but of short-duration when DH was administered as a PBS solution. However, liposomes prolonged the anti-histaminic effect of DH, suggesting that liposomes may adhere to the nasal mucosa and release DH slowly. In conclusion, liposomes suppress drug absorption into the systemic circulation and concurrently increase drug retention in the nasal cavity.

Key words nasal administration; liposome; topical application; local effect; diphenhydramine hydrochloride

The selection of an appropriate site of drug administration is one of the most important factors for optimizing its therapeutic effects. For example, as novel sites of drug administration, the nose, rectum, eye and lung have been studied for drugs which undergo extensive first-pass metabolism in the liver.2–5 Of these sites, the nasal mucosa was expected to be a good alternative site of drug administration, especially for peptide and protein drugs, since the nasal mucosa presents a low enzymatic barrier and is highly permeable.6,7

The nasal mucosa is also the site of drug administration for local therapy of pollinosis and allergic rhinitis. Because most of these drugs, e.g. anti-histaminics and anti-inflammatory agents, are highly lipophilic, they cross the nasal mucosa rapidly and enter the systemic circulation. This is followed by a rapid increase in the plasma drug concentration causing side-effects such as thirst, somnolence and exanthema. Therefore, it is important to develop intranasal dosage formulations to maintain higher drug concentrations in the nasal mucosa and concurrently suppress drug absorption into plasma.

Recently, many kinds of formulations, such as liposomes,8 microspheres9 and viscous solutions,10 have been developed as possible nasal dosage formulations. Of these formulations, liposomes are particulate carriers enabling the controlled release of drugs. We have already reported that liposomes have great potential for the oral dosage of peptides.11,12

In the present study, the effects of liposomes on the absorption and disposition of drugs in the nasal cavity were investigated in rats. Furthermore, a solution of sodium alginate was used for comparison.

* To whom correspondence should be addressed.

MATERIALS AND METHODS

Materials Dipalmitoylphosphatidylcholine (DPPC), phosphatidylcholine from egg yolk (Egg-PC), dimyristolphosphatidylcholine (DMPC), cholesterol (CHOL), dicetylphosphate (DCP), stearylamine (SA), 5(6)-carboxyfluorescein (CF) and diphenhydramine hydrochloride (DH) were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Dithiothreitol was obtained from Nacalai Tesque Co. (Kyoto, Japan). Sodium alginate (M.W. 50000 and 696000) was a gift from Kyosei Pharmaceutical Co. (Hokkaido, Japan). The other chemicals were of analytical grade.

Preparation of Liposomes The composition of the liposomes used in this study is listed in Table 1. Liposomes (Lip) were prepared by the method previously reported (Iwanaga et al., 1999). Briefly, a mixture of phospholipid, cholesterol and SA or DCP dissolved in chloroform was evaporated to dryness in a rotary evaporator. The lipid film was further dried in vacuo for 8 h to remove the solvent completely. Then, the lipid film was hydrated with drug dissolved in phosphate-buffered saline (PBS, pH 7.4). After 3 cycles of freeze-thawing, the liposomal suspension was diluted with PBS to adjust the lipid concentration (50 nmol). Just before starting the experiment, the liposomal suspension was centrifuged three times at 15000 rpm for 12 min to remove any untrapped drug. No sizing of the liposomes was performed because multilamellar vesicle (MLV) is easier to prepare and has a higher drug entrapment efficiency compared with small unilamellar vesicle (SUV) or large unilamellar vesicle (LUV). The entrapment efficiency of CF and DH was

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Table 1. Lipid Composition of Liposomes

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<th>DPPC</th>
<th>DMPC</th>
<th>Egg-PC</th>
<th>Chol</th>
<th>SA</th>
<th>DCP</th>
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<tbody>
<tr>
<td>DPPC(+)-Lip</td>
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<td>DPPC(−)-Lip</td>
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<td>DPPC(N)-Lip</td>
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<td>DMPC(+)-Lip</td>
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</tr>
<tr>
<td>Egg-PC(+)-Lip</td>
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Each value is expressed as a molar ratio.

34±8%, 16±6%, respectively. Neither the charge nor lipid composition of the liposomes affected the entrapment efficiency of these drugs. The mean particle size of the liposomes used in this study was in the range of 348 nm to 411 nm, and no significant difference in particle size was observed among the different liposomes.

**In Situ Nasal Perfusion Experiment** In this study, CF was chosen as a model absorbable compound because good absorption of CF through the nasal mucosa had been confirmed in our preliminary experiments (data not shown). Under sodium pentobarbital anesthesia, the trachea and esophagus of male Wistar rats (B.W.280—320 g) were incised and cannulated by the method of Hirai.13 Then, the test solutions were instilled in all nostrils (20 μl each). The amount of phospholipid and CF administered to rats were 2 μmol and 40 nmol, respectively. Blood was taken from the femoral vein for a period of 60 min at predetermined times. Samples were centrifuged for 4 min at 10000 rpm and the CF concentration in plasma was determined. The fraction of the dose of CF absorbed over the 60 min period was calculated by applying moment analysis and determining the "absorbed fraction". One hour after administration, the nasal cavities were perfused with PBS (flow rate; 0.2 ml/min) for 10 min. The amount of CF in this perfusate was determined as the "washable fraction". Immediately afterwards, the rats were decapitated and the nasal mucosa was washed and with 20 ml PBS. The amount of CF in this solution was determined as the "adhering fraction". The CF concentration in each sample was determined by fluorospectrometry.

**Anti-histaminic Effect after Intranasal Administration of DH** The action of the intranasal dosage formulation on the anti-histaminic effect of DH was investigated in rats. This experiment was carried out using the method of Kaise with some modifications. Briefly, test solutions including 0.2% DH, an anti-histamine, were prepared by the method described above and administered to rats (80 μg/rat). Then, the nasal cavities were perfused with histamine solution in PBS (250 mg/ml) for 3 min, 30, 60, 120, 240 min after DH administration, producing quasi-allergic conditions. Immediately after perfusion of the histamine solution, 10 mm dithiothreitol, a mucolytic agent, in PBS was perfused for 15 min. The amount of protein leaking into this perfusate was determined by the modified method of Bensadoun to evaluate the pharmacological effect of DH.

**RESULTS**

**Effects of Liposomal Surface Charge** Figure 1 shows the changes in plasma concentration of CF in rats after intranasal administration of CF dissolved in PBS solution, or entrapped in various surface-charged DPPC-Lip. When CF was administered as a PBS solution, CF was rapidly absorbed from the nasal mucosa and the amount absorbed over 60 min was about 48% of the dose. Administration of CF, as any of the DPPC-liposomal formulations, significantly reduced the plasma concentration of CF. The fraction of the dose of CF absorbed was 3.3%, 3.3% and 2.7% for DPPC(+)-Lip, DPPC(−)-Lip and DPPC(N)-Lip, respectively. The effect of the surface charge of liposomes on their adhesion to the nasal mucosa is shown in Fig. 2. When PBS solution was administered, most of the CF remaining in the nasal cavity was recovered in the washable fraction and the amount recovered in the adhering fraction was less than 4.0%. On the other hand, DPPC-Lip, especially DPPC(+)-Lip, significantly increased the amount of CF in the adhering fraction, compared with PBS solution.

**Effect of Lipid Composition of Liposomes** The effect of the lipid composition of liposomes on both the absorption and adhesion to the nasal mucosa is shown in Fig. 3. The fraction of CF absorbed after administration of DPPC(+)-Lip, DPPC(−)-Lip and Egg-PC(+)-Lip was 3.3%, 3.6% and 3.7%, respectively. However, the amount of CF recovered in the adhering fraction was affected by the lipid composition. Egg-PC(+)-Lip significantly increased the amount of CF in adhering fraction compared with DMPC(+)-Lip and DPPC(+)-Lip.
Comparison of Dosage Formulations  The effect of sodium alginate solutions (ALG-5; M.W.: 50000, ALG-69.6; M.W.: 696000) on both the amount of CF absorbed and adhering to the nasal mucosa is shown in Fig. 4. When CF was administered as ALG-5 solution, the plasma concentration of CF increased rapidly. The fraction of the dose of CF absorbed reached 52.3%, which is in the same range as that after administration of PBS solution. On the other hand, the absorption of CF from ALG-69.6 solution was much slower and the fraction of the dose absorbed was significantly lower than that from PBS solution. ALG-69.6 significantly increased the amount of CF recovered in the adhering fraction compared with PBS solution and ALG-5.

Plasma Concentration of DH after Intranasal Administration  The time-course of the plasma concentration of DH after intranasal administration as various dosage formulations is shown in Fig. 5. When DH was administered as PBS solution, it was rapidly absorbed and reached a $C_{\text{max}}$ 10 min after administration. The absorption profile of DH from ALG-5 solution was comparable with that from PBS solution. Both ALG-69.6 solution and Egg-PC(+)-Lip markedly suppressed the absorption of DH from the nasal mucosa.

Local Effects of DH  Figure 6 shows the effects of the dosage formulation on the anti-histaminic effect after intranasal administration of DH. When DH was administered in PBS solution, the amount of protein leaking into the nasal cavities was reduced to 63% of the control value 15 min after administration. However, the anti-histaminic effect had disappeared 60 min after administration. Intranasal administration of DH encapsulated in Egg-PC(+)-Lip gradually reduced the amount of protein leaked and the anti-histaminic effect was significantly prolonged. When DH was administered as ALG-5 and ALG-69.6 solutions, the amount of protein leaked was only slightly reduced.

DISCUSSION

Recently, the nasal mucosa has been considered as a site for the administration of peptide and protein drugs because of its high permeability and low enzymatic activity. Indeed, nasal dosage formulations of luteinizing hormone releasing hormone (LHRH) and a vasopressin derivative are already on the market.\textsuperscript{16,17} The nasal mucosa is also the site of administration of drugs used to alleviate local symptoms such as rhinitis and allergic coryza. In the case of drugs for such local therapy, the high permeability of the nasal mucosa might be a disadvantage, since the rapid absorption of drugs into the systemic circulation sometimes causes severe side-effects and reduces the local therapeutic effects. Therefore, it is important to develop formulations which avoid rapid absorption and increase the retention of a drug in the vicinity of the nasal mucosa.
DPPC-Lip markedly suppressed the absorption of CF from the nasal mucosa, regardless of the surface charge of the liposomes (Fig. 1). Since the phospholipase activity of the nasal mucosa is relatively low, these liposomes remain stable in the nasal cavities and release CF gradually. DPPC-Lip enhanced the adhesion of CF to the nasal mucosa (Fig. 2). In particular, DPPC(+)-Lip had the highest ability to enhance adhesion. Generally, the adhesion ability of formulations is thought to be affected by their charge, because the surface of the biomembrane is covered with a mucin layer which is negatively charged. In this study, the adhesion ability of positively charged liposomes was significantly greater than that of negatively charged or uncharged liposomes (Fig. 2). This may be caused by an electrostatic interaction between the surface of the mucosa and liposomes. A similar result concerning the effect of charge on the adhesion has also been reported by Vyas et al.\(^\text{18}\).

The lipid composition of liposomes did not affect the absorption of CF, but had a significant effect on the adhesion of liposomes to the nasal mucosa (Fig. 3). The adhesion of liposomes increased in the order of DPPC(+)-Lip(52.7±2.2%)>DMPC(+)-Lip(55.9±2.0%)>Egg-PC(+)-Lip(64.7±3.1%). It is well known that the fluidity of the liposomal membrane increases with a reduction in the phase transition temperature (Tc) of phospholipid liposomes. The Tc of the phospholipids used in this study was in the order of DPPC (42°C)>DMPC(24°C)Egg-PC (−7→15°C). Therefore, it is speculated that the adhesive force may be related to the membrane rigidity of the liposomes. Further studies are now underway to clarify this point.

As far as an effective dosage formulation for topical drug application is concerned, not only the mucoadhesion but also the ability to provide a controlled release of drug is an important feature. It is known that sodium alginate solutions (ALGs) are transformed to the gel state and exhibit mucoadhesion under acidic conditions.\(^\text{20}\) However, ALGs do not possess adhesive ability but do allow the controlled release of drugs due to their high-viscosity under neutral and basic conditions. Therefore, we chose ALGs as dosage formulations in order to confirm the importance of mucoadhesion for the use of dosage formulations for topical drug application. The effects of ALGs on the absorption and adhesion of CF to the nasal mucosa were investigated and compared with those of liposomes. ALG-69.6 (M.W.: 696000) solution, but not ALG-5 (M.W.: 50000) solution, significantly reduced CF absorption compared with PBS solution (Fig. 4). The viscosity of ALGs increased on increasing the molecular weight of sodium alginate.\(^\text{20}\) The increase in viscosity produced by ALG-69.6 caused a reduction in the diffusion of CF molecules, resulting in the suppression of CF absorption. On the other hand, neither ALG-5 nor ALG-69.6 exhibited any strong effect on the adhesion of CF. These results indicate that Egg-PC(+)-Lip is more suitable as a nasal dosage formulation than sodium alginate solution for drugs having a local effect. Both ALG-69.6 solution and Egg-PC(+)-Lip significantly suppressed the absorption of diphenhydramine hydrochloride (DH) from the nasal mucosa compared with PBS and ALG-5 solution (Fig. 5). This result was consistent with the observation obtained in our studies with CF.

To confirm the effect of liposomes as nasal dosage formulations, we observed the pharmacological effect of DH in vivo. Egg-PC(+)-Lip significantly prolonged the anti-histaminic effect of DH estimated by the amount of leaked protein (Fig. 6). This result indicates that Egg-PC(+)-Lip adhered to the nasal mucosa and released DH gradually, although DH was rapidly absorbed and disappeared from the nasal mucosa when administered as PBS solution. However, the duration of the anti-histaminic effect after administration of Egg-PC(+)-Lip was shorter than that expected from the results obtained in the CF experiment. The release rate of DH from Egg-PC(+)-Lip was considered to be faster than that of CF (data not shown). It may be possible to prepare liposomes with a more suitable release profile for DH by changing the physicochemical properties of the liposomes. Furthermore, we are now investigating the effect of mucociliary clearance\(^\text{21}\) on the adhesion of liposomes to the nasal mucosa using a rat model without surgery to obtain nasal perfusion.

In conclusion, liposomes suppress drug absorption from the nasal mucosa and concurrently improve the retention of drugs in the vicinity of the nasal mucosa. These findings confirm the great potential of liposomes as intranasal dosage formulations for topical drug application.

REFERENCES AND NOTES

1) Present address: Department of Pharmaceutics, Hokkaido College of Pharmacy, 7-1 Katsurazaka-cho, Otaru 047-0264, Japan.