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Enhanced systemic exposure of fexofenadine via the intranasal administration of chitosan-coated liposome

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ABSTRACT

The present study aimed to develop the intranasal delivery system of fexofenadine for the prolonged drug release via the preparation of mucoadhesive liposome. By using thin layer film hydration method, liposome of fexofenadine was prepared with DPPC/DPPG, resulting in the small lipid vesicles (359 ± 5.5 nm) with narrow size distribution (PI < 0.1). Subsequently, the surface of anionic liposome was coated by chitosan and in vitro characteristics of liposomes were evaluated along with the pharmacokinetic studies in rats. Chitosan coated liposomes were stable for 6-month storage at 4 °C without any significant size change and drug leakage. Furthermore, it exhibited strong mucoadhesive properties in mucin adsorption test, which was 3-fold higher than uncoated liposomes. Compared to the oral delivery of powder formulation, the intranasal delivery of fexofenadine significantly ($p < 0.05$) increased systemic exposure of fexofenadine in rats. Particularly, the intranasal administration of chitosan coated liposome exhibited approximately 5 fold enhancement of AUC with more sustained drug release in rats compared to the oral delivery. In conclusion, intranasal administration of chitosan coated liposome appeared to be effective to enhance the bioavailability as well as prolonged exposure of fexofenadine in rats.

1. Introduction

Allergic rhinitis is a common disease having classical symptoms such as sneezing, nasal pruritus, congestion and rhinorrhea, but it has a significant impact on the quality of life (Greiner et al., 2011). Antihistamines play an important role in the clinical treatment of allergic rhinitis. Especially, among the second-generation antihistamines, fexofenadine is a selective histamine H1 receptor antagonist and clinically effective in the treatment of seasonal allergic rhinitis (Hay and Kaliner, 2009; Simpson and Jarvis, 2000). As the first line treatment of allergic rhinitis, fexofenadine has few adverse effects compared to the first-generation antihistamines (Bernstein et al., 1997; Dhar et al., 2002; Meeves and Appajosyula, 2003; Yap and Camm, 2002). The most common adverse effect of fexofenadine is headache (Dhar et al., 2002), which is associated with greatly elevated drug exposure in blood. Fexofenadine belongs to the Biopharmaceutics Classification Systems Class III (BCS III), which has high solubility and low permeability (Tannergren et al., 2003). Due to the low permeability, its oral bioavailability was determined as low as 2–7% in preclinical species (Olsén et al., 2006; Piao et al., 2010; Qiang et al., 2009). Furthermore, since fexofenadine is the substrate of P-glycoprotein and several organic anion transporting polypeptides (OATPs), food and co-administration of other drugs significantly affect its oral bioavailability (Cvetkovic et al., 1999; Dresser et al., 2002; Lemma et al., 2006). Therefore, there is a need to improve the permeation of fexofenadine across the mucosal membrane and also develop the effective delivery systems for the alternative route of administration other than oral administration.

Intranasal administration offers a noninvasive alternative for the treatment of allergic rhinitis. Over the years, there has been a sharp increase in the reports on the nasal drug delivery for the treatment of allergic rhinitis, since intranasal delivery provides some benefits in terms of drug absorption (Bitter et al., 2011; Pires et al., 2009; Türker et al., 2004). The advantage of nasal delivery includes that (i) numerous microvilli of the epithelial surface in nasal cavity provides the large surface area available for drug absorption, (ii) subepithelial layer is highly vascularized for rapid absorption and (iii) drugs absorbed from the nose directly move into the systemic circulation to avoid the first-pass metabolism (Bitter et al., 2011; Pires et al., 2009; Türker et al., 2004). However, the rapid removal of formulation by mucociliary clearance often limits the bioavailability of drugs after intranasal delivery. Therefore, retaining the drug longer in the nasal cavity is important to maximize the utility of intranasal delivery. In addition to the prolonged drug residence time, for BCS III drugs such as fexofenadine, the enhanced permeability across the epithelial membrane is needed for achieving

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the improved drug delivery to the systemic circulation. Therefore, designing the effective intranasal delivery system which can offer the enhanced permeability as well as prolonged residence time in the nasal cavity is critical for improving the bioavailability of fexofenadine.

Liposome is a highly biocompatible and biodegradable drug carrier (Lian and Ho, 2001; Sharma and Sharma, 1997). As a highly permeable vesicle, it can entrap both hydrophobic and hydrophilic compounds, protect entrapped compounds from chemical or enzymatic degradation and enhance the drug permeation across the membrane (Lian and Ho, 2001; Sharma and Sharma, 1997). Furthermore, the liposome coated by mucoadhesive polymers appeared to be effective to prolong the drug residence time in mucosa. Among the mucoadhesive polymers, chitosan is a natural cationic polymer having favorable biocompatibility and mucoadhesiveness (Kas, 1997; Liu and Park, 2009). Therefore, in this study, chitosan-coated liposome was prepared and evaluated as an effective intranasal delivery system to improve the bioavailability of fexofenadine.

2. Materials and methods

2.1. Materials

Fexofenadine, DPPC (1,2-ditetradecanoyl-sn-glycero-3-phosphocholine), cholesterol, chitosan, DPPG (1,2-dipalmitoyl-sn-glycero-3-phosphoglycerol sodium salt), piroxicam and mucin were obtained from Sigma–Aldrich (St. Louis, MO, USA). VIVASPIN 500 was purchased from Sartorius Stedim (Aubagne, France). Triethylamine was obtained from Junsei Chemical Co. (Tokyo, Japan). Acetonitrile, methanol, ethanol and acetic acid were obtained from Merck Co. (Darmstadt, Germany). All other chemicals were of analytical grade and all solvents were of HPLC grade.

2.2. Preparation of liposomes

Liposomes were prepared by modified thin film hydration method. Briefly, the mixture of DPPC, DPPG, cholesterol and fexofenadine in the molar ratio of 8:1:2.25:0.45 was dissolved in a small amount of chloroform and placed in a rotary evaporator at 45 °C until a thin film was obtained. And then, it was allowed to stand in a vacuum chamber for 4 h. Thin films were hydrated with 1 mL of HBS buffer (pH = 7.4) and agitated for 2 h at 45 °C. The obtained liposome was then passed through an extruder (LipoFast-Pneumatic, Avestin, Canada) using 0.4 μm polycarbonate membrane filters. After the centrifugation at 25,000 × *g* for 1 h using VIVASPIN 500 at 4 °C, the collected liposomes were resuspended by using 0.9% NaCl. Last two steps were repeated twice for the effective washing of free drug.

For the preparation of chitosan-coated liposome, liposome suspended in HBS buffer was added dropwise into the chitosan solution (0.1% (w/v) in 0.1 M acetic acid, pH adjusted to 5.5) and stirred for 1 h. The suspension was left overnight at 4 °C. Chitosan-coated liposome was harvested from the reaction mixture by centrifugation at 15,000 × *g* for 30 min and resuspended in HBS buffer. The last two steps were repeated twice for the effective washing of free drug.

2.3. Characterization of liposomes

The average particle size and the surface charge of liposomes were determined by ZETA Sizer 3000 HSa (Malvern Instrument Ltd., UK). The average particle size was calculated from 5 measurements and displayed with a polydispersity index. The sample was diluted with the filtered deionized water prior to the determination of surface property.

The encapsulation efficiency (EE %) of drug in uncoated liposome or chitosan-coated liposome was determined by HPLC assay. Briefly, after the centrifugation of the liposomal suspension at 25,000 × *g* for 45 min at 4 °C, the free drug concentration in supernatant portion was analyzed by HPLC. The encapsulation efficiency (EE %) was then calculated as follows.

$$\text{EE } \% = \frac{A_{\text{int}} - A_{\text{sup}}}{A_{\text{int}}} \times 100$$

where A_{int} is the initial amount of fexofenadine and A_{sup} is the amount of fexofenadine in the supernatant portion.

The shape of liposome was examined by field emission transmission electron microscope (FE-TEM). A 2 μL of each liposomal suspension was applied to carbon-coated copper grid. The excess was drawn off with filter paper. After drying overnight at room temperature, the shape was examined using transmission electron microscopy (Tecnai G2 F30, Philips, USA).

2.4. In vitro stability studies

Stability of liposome during the storage was evaluated for 6 months. Samples were prepared in the form of freeze-dried powder including 10% of lactose as a cryoprotective agent and vials containing samples were filled with nitrogen gas. Samples were stored at 4 °C and 25 °C for 6 months and collected at day 0, day 1, day 3, day 7, day 15, 1 month, 3 months and 6 months. Encapsulation efficiency and vesicle size of each sample were determined as described above.

2.5. Mucin adsorption studies

Adsorption of mucin (extracted from porcine stomach, type III) on the surface of liposome was measured to evaluate the mucoadhesive properties of prepared liposomes (Hägerström and Edsman, 2003). Briefly, 1 mL of mucin solution (1 mg/mL) was stirred with 1 mL of chitosan-coated liposomes (1 mg/mL) for 1 h at 37 °C. Uncoated liposome also underwent the same procedure. Then, the suspension was centrifuged at 25,000 × *g* for 30 min at 4 °C. The amount of free mucin was determined by using Bradford colorimetric method (Bradford, 1976) in order to assess the amount of mucin adsorbed on the liposome from the difference between total and free mucin. Briefly, mucin standard solutions (62.5, 125, 250, 500, and 1000 μg/mL) were prepared for mucin calibration curve. Bradford reagent (Sigma–Aldrich, St. Louis, MO, USA) was added to the samples and incubated at 37 °C for 10 min. Then, solutions were transferred into a 96-well plate and the absorbance of solutions was detected at 595 nm.

The adsorption % was estimated by using the mathematical equation described below.

$$\text{Adsorption } \% = \frac{\text{total amount of mucin used} - \text{free mucin}}{\text{total amount of mucin used}} \times 100$$

2.6. Pharmacokinetic studies

Male Sprague-Dawley rats (250–260 g) were purchased from Samtako Bio Co. (Osan, Korea). All animal studies were performed in accordance with the "Guiding Principles in the Use of Animals in Toxicology" adopted by the Society of Toxicology (USA). Rats had free access to normal standard chow diet (Jae Il Chow, Korea) and tap water. Animals were fasted for 24 h prior to the experiments. At the day of experiment, rats were divided into five groups ($n = 6$ per each group). Groups 1–3: intranasal administration of each drug solution (untreated drug (non-liposome), uncoated liposome or chitosan coated liposome) at the dose equivalent to 2 mg/kg of fexofenadine, Group 4: oral administration of

Table 1
Characteristics of liposomes (mean ± SD, $n = 5$).

	Size (nm)	Zeta potential (mV)	PI	EE (%)
Uncoated liposome	359 ± 5.5	−110 ± 5.4	<0.1	65.9 ± 1.4
Chitosan coated liposome	716 ± 14.2	11.8 ± 1.5	0.1	66.1 ± 1.0

fexofenadine (10 mg/kg), Group 5: intravenous administration of fexofenadine (5 mg/kg). Fexofenadine was dissolved in ethanol and water (1:9) for oral and nasal administration and in saline (0.9%) for intravenous administration. Liposome suspended in water was used for the intranasal administration. Dosing solutions for the intranasal administration were prepared at the drug concentration of 20 mg/mL and 25 μL of dosing solution was administered onto the nostrils of rats. For the oral administration, dosing solution of non-liposomal formulation was prepared with 2.5 mg/mL and the dosing volume was 0.9 mL. Blood samples were collected from the femoral artery at 0.05, 0.16, 0.25, 0.5, 1, 2, 4, 8, 12, and 24 h following an intravenous administration. Blood samples were also collected from the femoral artery at 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 8, 12, and 24 h following an oral or intranasal administration. Blood samples were centrifuged and the obtained plasma was stored at −70 °C until analyzed.

2.7. HPLC assay

Plasma concentration of fexofenadine was determined by the HPLC method described as follows. In brief, 10 μL of piroxicam (10 μg/mL) as an internal standard was added to 90 μL of each plasma sample and then the mixture was deproteinized by adding 200 μL of acetonitrile. After centrifugation of the samples at 13,000 × *g* for 10 min, the supernatant was evaporated and the residue was reconstituted with 120 μL of the mobile phase, and then 50 μL of aliquots were injected directly into the HPLC system (Perkin Elmer Series 200; Waltham, MA, USA). The octadecylsilane column (Gemini C18, 4.6 mm × 150 mm, 5 μm; Phenomenex, Torrance, CA, USA) was eluted with the mobile phase consisting of 0.1 M triethylamine: acetonitrile: methanol (61:19.5:19.5, v/v/v%, pH 3.0 adjusted with phosphoric acid) at a flow rate of 1.0 mL/min. The UV detector set at 195 nm. The calibration curve from the standard samples was linear over the concentration range of 0.01–0.5 μg/mL. The detection limit of fexofenadine was 0.01 μg/mL. For in vitro samples, the mobile phase consisted of 0.1 M triethylamine:acetonitrile:methanol (50:25:25, v/v/v%, pH 3.3 adjusted with phosphoric acid).

2.8. Pharmacokinetic data analysis

Noncompartmental pharmacokinetic analysis was performed using the WinNonlin® version 5.2 (Pharsight Corporation, Mountain View, CA, USA). The elimination rate constant (K_{el}) was estimated from the slope of the terminal phase of the log plasma concentration-time points fitted by the method of least-squares and the terminal half-life ($T_{1/2}$) was calculated by $0.693/K_{el}$. The peak concentration (C_{max}) and the time to reach peak concentration (T_{max}) of drug in plasma were obtained by visual inspection of the data from the concentration–time curve. The area under the plasma concentration-time curve (AUC_{0-t}) from time zero to the time of last measured concentration (C_{last}) was calculated by the linear trapezoidal rule. The AUC from time zero to infinite ($AUC_{0-\infty}$) was obtained by the addition of AUC_{0-t} and the extrapolated area determined by C_{last}/K_{el}. The absolute bioavailability (A.B.) of drug was calculated by $AUC_{p.o.}/AUC_{i.v.} \times Dose_{i.v.}/Dose_{p.o.} \times 100$.

2.9. Statistical analysis

All mean values were presented with their standard deviation (mean ± SD). Statistical analysis was conducted using a one-way ANOVA followed by a posteriori testing with Dunnett correction. A P value less than 0.05 was considered statistically significant.

3. Results and discussion

3.1. Preparation and characterization of liposomes

The physicochemical properties of the drug, especially solubility, permeability and partition coefficient, can be important determinants for the extent of its liposomal incorporation. Considering the high solubility of fexofenadine in chloroform, anionic liposome was prepared by dissolving fexofenadine in chloroform with DPPC, DPPG and cholesterol. Subsequently, the obtained anionic liposome was coated with chitosan to provide mucoadhesiveness to the liposomal surface.

The encapsulation efficiency, size and charge of the obtained liposomes were summarized in Table 1. Before coating with chitosan, negatively charged liposomes were obtained as relatively

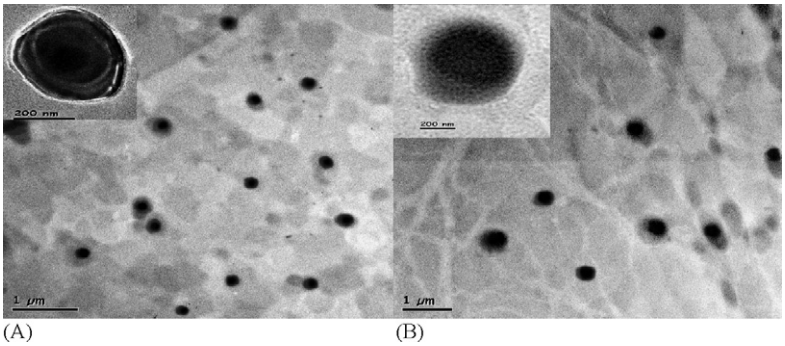

Fig. 1. FT-TEM images of uncoated liposomes (A) and chitosan coated liposomes (B).

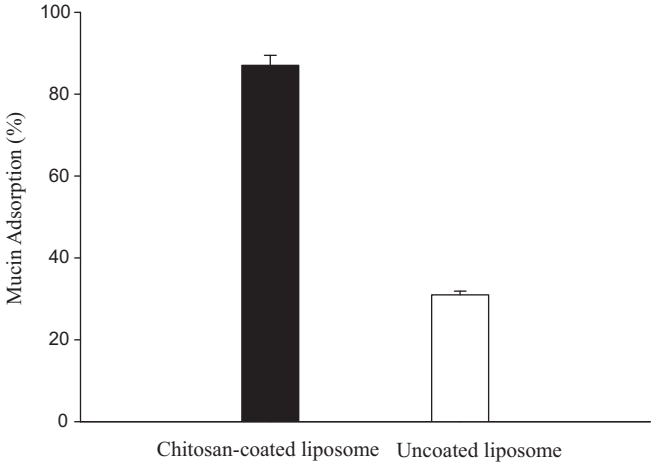

Fig. 2. Mucoadhesiveness of liposomes (mean ± SD, $n = 3$).

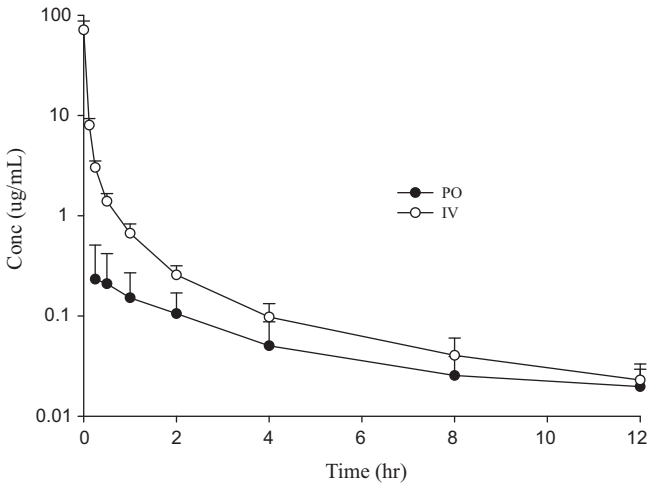

Fig. 3. Plasma concentration–time profiles of fexofenadine after an intravenous (5 mg/kg) and oral administration (10 mg/kg) of untreated drug (nonliposome) to rats (mean ± SD, $n = 6$).

small size vesicles (359 ± 5.5 nm) with narrow size distribution (PI < 0.1), while the average size of coated liposomes was about 716 nm. The spherical shape of the obtained liposomes was confirmed with FT-TEM, which also indicated narrow size distribution (Fig. 1). Zeta potential indicated that the ionization of the phosphate groups of DPPC and DPPG created negatively charged liposome which could undergo the electrostatic interaction with positively charged chitosan for the mucoadhesive coating layer on the liposomal surface.

3.2. Stability of liposomes

For the stability test during storage, vials containing samples were filled with nitrogen gas to reduce the oxidation stress. Compared to mannitol, sucrose or D-glucose, 10% of lactose was effective to maintain the stability of liposome during the freeze-drying process (data not shown). Therefore, liposomes were freeze-dried with 10% lactose as a cryoprotective agent to inhibit liposomal fusion or degradation during freezing procedures and then the stability of freeze-dried liposomal powder was determined by examining changes in vesicle size and drug leakage over time. As summarized in Table 2, under all the tested conditions, drug leakage was lower than 10% and the change in the size was minimal. Particularly, at 4 °C, liposomes were stable without any significant change in drug leakage and size for 6 months, implying the potential long-term stability of liposome.

3.3. Mucin adsorption studies

Mucin is the major component of mucus, which is primarily responsible for the viscoelastic properties of the mucus (Rubin, 2010). Positively charged chitosan has electrostatic interaction with negatively charged mucin secreted from epithelial cells. Therefore, the mucoadhesive property of obtained liposomes was determined by the extent of mucin adsorption on the surface of liposomes. As illustrated in Fig. 2, the amount of mucin adsorbed on the surface of chitosan coated liposomes was 3-fold higher than that of uncoated liposomes, suggesting that chitosan coated liposomes might retain for a longer time in the nasal cavity compared to uncoated liposomes and subsequently increase the drug transport across the nasal epithelial membrane in vivo. It was also confirmed by the pharmacokinetic study described below.

3.4. Pharmacokinetic studies

The plasma concentration time profiles of fexofenadine after an oral, intravenous or intranasal administration are illustrated in Figs. 3 and 4. The pharmacokinetic parameters are also summarized in Table 3.

As shown in Fig. 3, the oral bioavailability of fexofenadine was low as 6.2% in rats, which is comparable to the previous reports (Piao et al., 2010). Compared to the oral administration, the intranasal administration significantly increased the bioavailability of fexofenadine from all the tested formulations (Fig. 4). Even for the powder formulation (nonliposome), intranasal delivery enhanced the systemic exposure of fexofenadine by 4-folds compared to the oral administration. Bioavailability of fexofenadine was even further increased up to 34.7% via the intranasal administration of chitosan coated liposomes while uncoated liposome did not show any additional enhancement. This might be due to the decreased mucociliary clearance and the enhanced drug retention in nasal cavity via the mucoadhesiveness of chitosan coated liposomes (Ugwoke et al., 2005). In addition, chitosan could

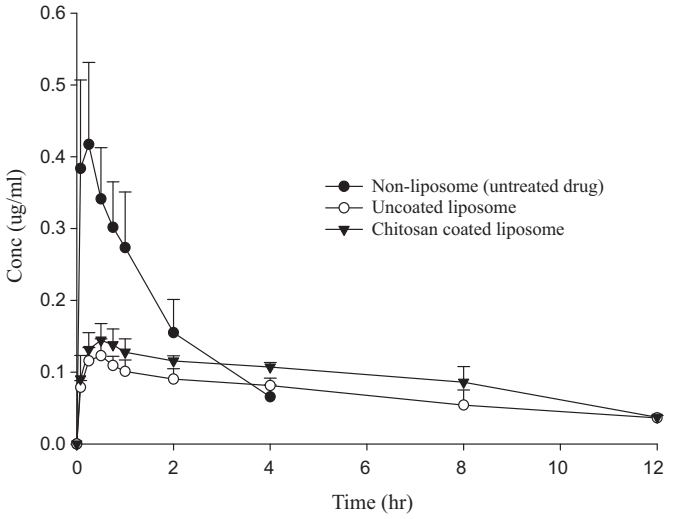

Fig. 4. Plasma concentration–time profiles of fexofenadine after an intranasal administration of fexofenadine (2 mg/kg) in different formulations to rats (mean ± SD, $n = 6$).

Table 2
Stability of liposomes (mean ± SD, $n = 3$).

Time	4 °C				25 °C			
	Uncoated liposome		Chitosan-coated liposome		Uncoated liposome		Chitosan-coated liposome	
	Size (nm)	EE (%)	Size (nm)	EE (%)	Size (nm)	EE (%)	Size (nm)	EE (%)
Day 0	357 ± 1.6	65.8 ± 0.1	717 ± 2.8	65.4 ± 1.3	358 ± 3.5	65.8 ± 0.9	721 ± 3.9	65.4 ± 0.9
Day 1	360 ± 2.4	65.7 ± 1.0	720 ± 5.1	64.8 ± 1.4	360 ± 3.8	64.2 ± 0.9	719 ± 5.8	65.3 ± 1.1
Day 3	359 ± 2.4	65.2 ± 1.3	717 ± 4.0	65.1 ± 1.0	357 ± 1.3	64.7 ± 0.5	721 ± 6.5	64.8 ± 1.0
Day 7	356 ± 2.2	65.2 ± 1.1	723 ± 6.7	65.3 ± 1.0	360 ± 2.7	65.5 ± 1.0	719 ± 2.5	64.1 ± 0.8
Day 15	359 ± 2.9	64.7 ± 0.6	721 ± 7.1	64.4 ± 2.1	361 ± 1.9	64.1 ± 1.2	725 ± 4.7	63.9 ± 1.0
1 month	360 ± 5.1	65.1 ± 0.9	726 ± 7.9	64.6 ± 0.8	362 ± 2.4	64.4 ± 0.5	731 ± 4.0	63.9 ± 1.0
3 months	359 ± 3.4	64.9 ± 0.6	722 ± 3.1	65.0 ± 0.7	362 ± 4.4	62.9 ± 1.8	742 ± 12.4	61.1 ± 1.4
6 months	361 ± 4.4	64.1 ± 0.9	721 ± 4.8	64.2 ± 0.1	375 ± 3.0	58.2 ± 2.6	759 ± 16.2	57.6 ± 1.4

Table 3
Pharmacokinetic parameters of fexofenadine in rats (mean ± SD, $n = 6$).

Route	Formulation	Dose (mg/kg)	C_{max} (ng/mL)	T_{max} (h)	$T_{1/2}$ (h)	AUC ((ng h)/mL)	BA (%)
IV	Non-liposome	5			11 ± 6.3	7970 ± 900	
PO	Non-liposome	10	340 ± 290	0.9 ± 0.8	5.3 ± 1.7	920 ± 280	6.2 ± 2.0
Intranasal	Non-liposome	2	420 ± 120	0.2 ± 0.1	1.5 ± 0.1	750 ± 190	25.0 ± 8.0
	Uncoated liposome	2	120 ± 20*	0.5*	6.6 ± 1.3*	710 ± 230	24.5 ± 7.5
	Chitosan-coated liposome	2	220 ± 30*	0.5*	6.8 ± 1.5*	1040 ± 120*	34.7 ± 6.3*

* $p < 0.05$ compared to the intranasal delivery of nonliposomal fexofenadine.

have a contribution to the improved mucosal drug transport by opening the tight junction between epithelial cells (Qian et al., 2006).

On the other hand, the maximum plasma concentration (C_{max}) of fexofenadine was significantly lower in liposomal formulations than the observed value from nonliposomal formulation, which might be due to the slow release of drug across the lipid bilayers. Considering that most common adverse effect such as headache is associated with greatly elevated drug exposure in blood (Dhar et al., 2002), avoid of sharp increase in the plasma concentration may provide a benefit to reduce the adverse effect of fexofenadine. In addition, liposomal formulation achieved longer $T_{1/2}$ with more sustained drug exposure. In the case of nonliposomal formulation, after the sharp increase in drug exposure at T_{max}, plasma drug concentration rapidly decreased and was not detectible after 4 h post-dose. In contrast, chitosan-coated liposome exhibited sustained drug exposure up to 12 h, which might have positive impact on the duration of action.

Taken all together, compared to the conventional powder formulation (nonliposome), avoid of sharp increase in the plasma concentration (lower C_{max}) and more prolonged drug exposure via the intranasal delivery of chitosan coated liposome may provide some therapeutic benefit with reduced dosing frequency and adverse effect of fexofenadine.

4. Conclusions

The intranasal delivery of chitosan-coated liposomes appeared to be effective for improving the bioavailability as well as prolonged exposure of fexofenadine in rats.

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