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International Journal of Pharmaceutics 330 (2007) 23–31
INTERNATIONAL JOURNAL OF PHARMACEUTICS
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Enhancing effect of surfactants on fexofenadine·HCl transport across the human nasal epithelial cell monolayer

Hongxia Lin [a], Matthias Gebhardt [b], Shengjie Bian [c], Kyoung Ae Kwon [d], Chang-Koo Shim [a], Suk-Jae Chung [a], Dae-Duk Kim [a,*]

[a] *College of Pharmacy and Research Institute of Pharmaceutical Sciences, Seoul National University, Seoul 151-742, South Korea*
[b] *Joint Graduate Program in Biotechnology, Dongseo University and Technical University of Berlin, Berlin 13355, Germany*
[c] *Department of Biology, Pusan National University, Pusan 609-735, South Korea*
[d] *Pharm. Develop. Res. Team, Handok Pharmaceuticals Co. Ltd., Chungbuk 369-820, South Korea*

Received 1 February 2006; received in revised form 16 August 2006; accepted 28 August 2006
Available online 1 September 2006

Abstract

The effect of various surfactants (sodium cholate, sodium taurocholate, Tween 80 and Poloxamer F68) on enhancing the transepithelial permeability of fexofenadine·HCl was evaluated in a human nasal epithelial cell monolayer model. The cytotoxicity of the surfactants on the human nasal epithelial cells was evaluated by the MTT assay. A dose-dependent reduction of cell viability was observed at higher than critical micelle concentration (CMC) of the surfactants, and the IC_{50} of non-ionic surfactants (Tween 80 and Poloxamer F68) was higher than that of ionic surfactants (sodium cholate and sodium taurocholate). The TEER values significantly decreased after 2 h incubation with the ionic surfactants, but were recovered after 24 h in the fresh culture media. Ionic surfactants significantly increased the transepithelial permeability (P_{app}) of fexofenadine·HCl compared to the non-ionic surfactants. The reduction of TEER values upon exposing the cell monolayer to the surfactants for 2 h correlated well with the P_{app} of fexofenadine·HCl, which suggests that the permeation-enhancing mechanism of the ionic surfactants is by altering the tight junction property of the paracellular pathway. F-actin staining showed that the effect of ionic surfactants on the tight junction is temporary and reversible, which is consistent with the TEER value recovery within 24 h. These results imply that ionic surfactants are potentially useful permeation enhancers for nasal delivery of hydrophilic compounds, such as fexofenadine·HCl. This study also indicated the usefulness of the human nasal epithelial cell monolayer model not only for evaluating the *in vitro* nasal drug transport but also for studying the mechanism and toxicity of enhancers.

Keywords: Human nasal epithelial cell monolayer; Fexofenadine·HCl; Surfactants; Enhancer

1. Introduction

Fexofenadine·HCl (Fig. 1), a second generation non-sedating histamine H1 receptor antagonist, is an active metabolite of terfenadine. It is known to be safer than terfenadine in treating seasonal allergic rhinitis. Currently only oral formulations at a dose of 60–120 mg/day are available for fexofenadine·HCl, despite the need for a nasal delivery system (*i.e.*, at the site of action). The large surface area of the nasal cavity and the relatively high blood flow, thereby achieving a rapid absorption and avoidance of hepatic first-pass elimination are attractive features of nasal drug administration (Turker et al., 2004; Arora et al., 2002).

Recently, *in vitro* models of human nasal epithelium offer the opportunity to study mechanisms related to drug absorption, metabolism and toxicity on cellular level. Although *in vivo* animal models have been widely used for nasal delivery studies, they show a significantly different structure in nasal cavity compared to humans, which sometimes misleads in predicting drug uptake and absorption. The nasal cell culture models also have attracted the attention of pharmaceutical researchers as promising tools for testing novel strategies to enhance drug transport and absorption (Kissel and Werner, 1998; Dimova et al., 2005). Cell culture systems show some prominent advantages including the illustration of transport pathways, minimization of drug metabolism and rapid evaluation of various penetration strategies. The primary cultured cells are more significant in predicting drug transport than cancerous cell lines, which do not have the morphology or biochemical

* Corresponding author. Tel.: +82 2 880 7870; fax: +82 2 873 9177.
E-mail address: ddkim@snu.ac.kr (D.-D. Kim).

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doi:10.1016/j.ijpharm.2006.08.043

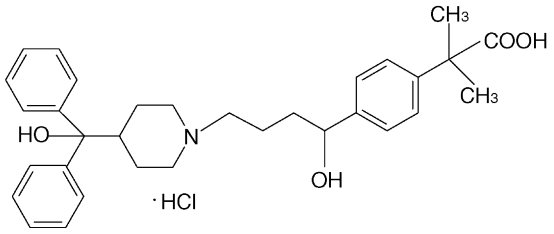

Fig. 1. Chemical structure of fexofenadine·HCl.

characteristics of the original tissue. Previously, we reported that the passaged air–liquid interface (ALI) culture system of human nasal epithelial cell monolayer developed a tight junction with the differentiation of cilia and mucin secreting cells, thus was suitable for rapid evaluation of drug transport studies (Lee et al., 2005).

In our preliminary transport study, the permeability of fexofenadine·HCl across the nasal cell monolayer was quite low, probably due to its hydrophilicity (log $P = 0.49$) (Lin et al., 2005). Although surfactants can be considered as enhancers to increase nasal absorption, a major limiting factor is their potential toxicity to the nasal mucosa. Thus, in selecting a suitable surfactant for a given formulation, it is important to investigate both the enhancing-effect of the surfactant as well as its possible adverse effects on biological barriers. Although nasal absorption enhancers should be nonirritating, nontoxic and nonallergenic, if these adverse effects are at least immediately reversible, they can be considered as candidates for nasal formulations. However, most reports on toxicity studies of nasal absorption enhancers are limited to *in vivo* animal models (Ugwoke et al., 2000). Thus, herein we report on the evaluation of several surfactants as possible enhancers for fexofenadine·HCl nasal delivery, together with a feasibility study on the toxicity as well as mechanism of these enhancers in the ALI cultured human nasal epithelial cell monolayer. Sodium cholate and sodium taurocholate were selected as model ionic surfactants while Tween 80 and Poloxamer F68 were chosen as model non-ionic surfactants.

2. Materials and methods

2.1. Materials

Sodium cholate (NaC), sodium taurocholate (NaTC), 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES) and D-glucose were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Tween 80 was obtained from Kasei Kogyo Co. (Tokyo, Japan). Poloxamer F68 was kindly supplied by BASF Co. (Ludwigshafen, Germany). Fexofenadine·HCl was a gift from Handok-Aventis Pharmaceutical Co. (Seoul, Korea). Cell culture reagents and supplies were obtained from GIBCO Invitrogen Co. (Grand Island, NY, U.S.A.). BEGM (Bronchial Epithelial Cell Growth Medium) Bulletkit was obtained from Cambrex Bio Science Inc. (Walkersville, MD, U.S.A.). Transwells® (0.4 μm, 12 mm diameter, polyester) were obtained from Costar Co. (Cambridge, MA, U.S.A.). All other materials were of analytical grade or better, and were used as purchased.

2.2. ALI culture of human nasal epithelial cell monolayer

The passaged human nasal epithelial cell monolayers were cultured by the method previously reported (Lee et al., 2005). When nasal epithelial cells of passages 2 and 3 reached approximately 70–80% confluency, the cells were detached with 0.1% trypsin–EDTA and were seeded on Transwell® insert at densities of 2×10^5 to 3×10^5 cells/cm^2. The apical side and the basolateral side were filled with 0.5 and 1.5 ml, respectively, of BEGM:DME/F12 (Dulbecco's Modified Eagle Medium: nutrient mixture F-12) (50:50) supplemented with hydrocortisone (0.5 μg/ml), insulin (5 μg/ml), transferrin (10 μg/ml), epinephrine (0.5 μg/ml), triiodothyronine (6.5 μg/ml), gentamycin (50 μg/ml), amphotericin-B (50 μg/ml), retinoic acid (0.1 ng/ml), and epidermal growth factor (0.5 ng/ml human recombinant). Culture incubator was maintained at 37 °C in an atmosphere of 5% CO_2 and 95% relative humidity. The media in both sides were changed after 24 h, and then the apical surface of the monolayer was directly exposed to air after reaching confluence on day 3, after which only the medium in the basolateral side was changed every 2 days.

2.3. Cytotoxicity of surfactants on human nasal epithelial cells

The cytotoxicity of various surfactants on the human nasal epithelial cells was evaluated by the MTT colorimetric assay (Prior et al., 2002). Briefly, 200 μl of 1×10^5 cells/ml was seeded into 96-well plates. After 48 h culture with BEGM, the cells were further incubated with different concentration of surfactants for 2 h. Then, incubation medium was withdrawn and the cells were washed twice with PBS. Aliquots (100 μl) of MTT solution (1.0 mg/ml) and 100 μl BEGM were added to each well. After 4 h of incubation, the supernatant was discarded and formazan crystals were dissolved in DMSO followed by vigorous mixing. Control wells were incubated with BEGM only without surfactant, and were treated similarly as above. The optical density was determined by microplate reader at 560 nm (Emax, Molecular Devices Co., Sunnyvale, CA, U.S.A.). The percent viability of the cells was determined from the absorbance values considering that of the control as 100%. The surfactant concentration that produced 50% viability (IC_{50}) was determined from the percent viability curves as a function of surfactant concentration.

2.4. Change of TEER values

The change of TEER value was monitored using the EVOM voltohmmeter (WPI, Sarasota, FL, U.S.A.) after exposing the nasal cell monolayer with various concentrations of surfactants for 2 h at 37 °C. After 2 h of incubation, cell monolayers were washed with PBS and cultured in fresh BEGM:DME/F12 (50:50), and the TEER was measured at 4, 6 and 24 h while

incubating in fresh media at 37 °C in order to observe the TEER recovery.

2.5. Transport and cellular uptake studies of fexofenadine·HCl

The transport experiments were carried out after 8–10 days of ALI culture when TEER value was higher than 500 Ω cm^2. The transport studies were performed by initially incubating the monolayers in transport medium (HBSS supplemented with 15 mM glucose and 15 mM HEPES, pH 7.4) for 20 min at 37 °C. Each transport experiment was performed by adding 0.4 ml of transport medium containing fexofenadine·HCl (100 μg/ml) with various surfactants in the apical side, and 1.0 ml of blank transport medium in basolateral side. At predetermined time intervals, samples of 1.0 ml were withdrawn from the basolateral side and replaced with an equal volume of fresh transport medium. The integrity of the cell monolayers was checked at the beginning and end of each transport experiment by measuring the TEER using an EVOM voltohmmeter, which was expressed as the percentage of the initial value. Samples were analyzed by HPLC and the cumulative amount of fexofenadine·HCl permeated was plotted as a function of time. The apparent permeability coefficients were calculated from the linear slope of the plot using the equation shown below:

$$P_{app} = \frac{dQ}{dt}\frac{1}{AC_0}$$

where P_{app} is the apparent permeability coefficient (cm/s), dQ/dt the steady state flux, A the surface area of membrane (cm^2) and C_0 is the initial concentration of fexofenadine·HCl in the apical side.

The amount of cellular uptake of fexofenadine·HCl after 2 h of the transport studies was measured following the method in the literature with slight modification (Sudoh et al., 1998). Briefly, the monolayers were rinsed three times with ice-cold PBS and were cut off from the Transwells®. The cells were trypsinized with 0.5% trypsin–EDTA for 30 min. Then, the cell suspension was added with 2.0 ml acetonitrile. After centrifuging at 4000 rpm for 20 min, the supernatant was evaporated under N_2. The sample residues were reconstituted with 500 μl water and analyzed by HPLC.

2.6. HPLC analysis of fexofenadine·HCl

The samples of fexofenadine·HCl were directly analyzed using an isocratic HPLC system equipped with a pump (Waters 515), an automatic injector (Waters 717plus) and a fluorescence detector (Series 200, Perkin-Elmer). A reversed phase C-18 column (Lichrospher®100, RP-18, 125 mm × 4 mm, 5 μm, Merck Darmstadt, Germany) was used as a stationary phase. The mobile phase was a mixture of phosphate buffer and acetonitrile (62:38, w/w) at a flow rate of 1.0 ml. Excitation and emission wavelength were set at 230 and 290 nm, respectively. The retention time of fexofenadine·HCl was about 7.9 min. The detection limit was 10 ng/ml.

2.7. F-actin staining of cytoskeleton before and after transport studies

The filamentous actin (F-actin) staining was performed with FITC-labeled phalloidin (Anderberg et al., 1993) on the nasal epithelial cell monolayer before and after 2 h transport studies. In order to observe the recovery of tight junction, the F-actin staining was also performed after culturing the monolayer in the fresh media for 24 h. Briefly, the human nasal cell monolayers were rinsed three times with PBS, and were fixed for 10 min with 3.7% formaldehyde in PBS on ice. After rinsing three times with PBS, they were treated with 0.1% Triton X-100 (Gibco BRL) on ice for 5 min. Then, they were rinsed with PBS twice and air-dried. The cells were then stained with FITC-labeled phalloidin (2 U/400 μl PBS) for 30 min in the dark. The monolayers were rinsed again with PBS three times and then mounted on glass slides with gelvatol and covered with a cover slip. The slides were examined under Leica TCS NT confocal systems (Leica Microsystems Heidelberg GmbH, Heidelberg, Germany) at 200× magnification.

2.8. Statistics

Each study was performed at least in triplicates using different nasal epithelial cell monolayers and the data were expressed as the mean ± standard deviation (S.D.). A two-tailed Student's t-test was performed at $p < 0.05$.

3. Results and discussion

3.1. Cytotoxicity of surfactants on human nasal epithelial cells

The cytotoxicity of four different model surfactants on the human nasal epithelial cells was investigated by MTT assay, an indicator of cellular mitochondrial dehydrogenase activity. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) is a tetrazolium salt that is oxidized by mitochondrial dehydrogenase in living cells to give a dark blue formazan product. Damaged or dead cells show reduced or no dehydrogenase activity (Mossman, 1983). Thus, the optical density of the cell lysate after MTT treatment is linearly correlated with the dehydrogenase activity, and also reflects the cell viability. Fig. 2 shows the changes of the cell viability with the incubation of various concentrations of surfactants for 2 h. A dose-dependent reduction of dehydrogenase activity was observed with micellar system of ionic surfactant, *i.e.* at higher than critical micelle concentration (CMC) of the surfactants. The concentration that inhibited 50% of the enzyme activity (IC_{50}) was 3.49 ± 1.45 and 12.02 ± 3.30 mM for NaC and NaTC, respectively (Table 1), which were consistent with the value of 6 and 10 mM reported on Caco-2 cells (Meaney and O'Driscoll, 2000). This was also in agreement with a report that NaC was more toxic than the conjugated NaTC to the epithelial cell membrane of Caco-2 cells (Martin et al., 1992). It was reported that higher toxicity of NaC than NaTC is due to the difference in polarity between

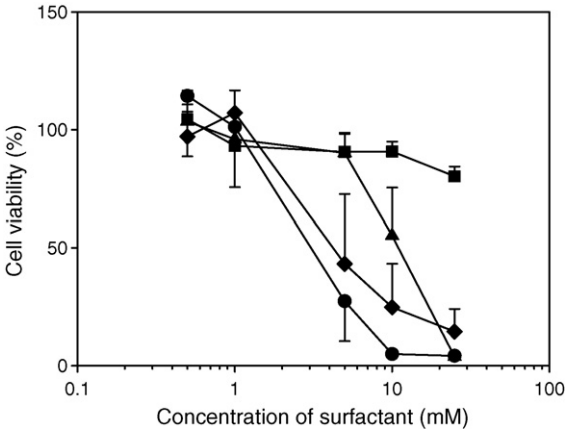

Fig. 2. Viability of human nasal epithelial cells after 2 h exposure to various concentrations of surfactants. (●) Sodium cholate; (▲) sodium taurocholate; (♦) Tween 80; (■) Poloxamer F68.

the unconjugated NaC and the conjugated NaTC (Murakami et al., 1984).

Generally, the toxicity of non-ionic surfactants is reported to be lower than that of ionic surfactants, thus they are widely used in foods, cosmetics and pharmaceutical preparations. However, the IC_{50} of Tween 80 (5.95 ± 0.54 mM) was similar to that of the ionic surfactants in this study, while Poloxamer F68 was non-toxic at higher concentration than its CMC. Since CMC of Tween 80 (0.01 mM) is 10-fold lower than that of Poloxamer F68 (0.1 mM) (Table 1), more micelles of Tween 80 are to be formed at the same concentration, and showed more cytotoxic effect than Poloxamer F68.

Human intestinal epithelial cell *(i.e.*, Caco-2 cells) monolayer has been commonly used in many cytotoxicity studies including the MTT assay of ionic/non-ionic surfactants (Ekelund et al., 2005; Legen et al., 2005; Meaney and O'Driscoll, 2000; Martin et al., 1992; Shappell, 2003). However, since the nasal epithelium plays an important defense system against mucosal infections (Agu et al., 2002a,b), and since they are anatomically and physiologically different from Caco-2 cells, it is particularly important that toxicity of enhancers in nasal epithelial cells were evaluated when studying nasal drug formulations.

3.2. Effect of surfactants on TEER value

The change of TEER was monitored upon exposure to the human nasal cell monolayer as a quantitative indicator of tight junction integrity, which is the major barrier of the paracellular permeability. Fig. 3A illustrates the changes in TEER value induced by 2 h incubation of NaC at the concentration above its CMC (5–15 mM). Because NaC was the most cytotoxic surfactant tested in the current study (Table 1), it was necessary to closely examine its dose dependence. The results indicate that the effect of NaC on TEER value was dependent on both concentration and time. TEER reduction increased remarkably when the surfactant concentration increased from 5 to 15 mM. At the concentration of 15 mM NaC, the TEER was approximately 30% of its original value after 2 h incubation. However, as shown in Fig. 3B, the TEER pattern with 2 h incubation of non-ionic surfactants was significantly different from that of the ionic surfactants. For example, higher than 80% of the initial TEER value was maintained upon exposure to 10 mM Tween 80 for 2 h. It is interesting to note that the TEER value increased up to 129% of its initial value with 10 mM Poloxamer F68. The decease of TEER could be an indirect indication of cytotoxicity and the order of the TEER reduction was as follows: NaC > NaTC > Tween 80 > Poloxamer F68.

The cell viability was almost less than 10% when the liquid covered culture in 96-well plates was used for the MTT assay with 10 and 15 mM NaC (Fig. 2). However, it should be noted that results in Fig. 3 were from ALI cultured monolayers using

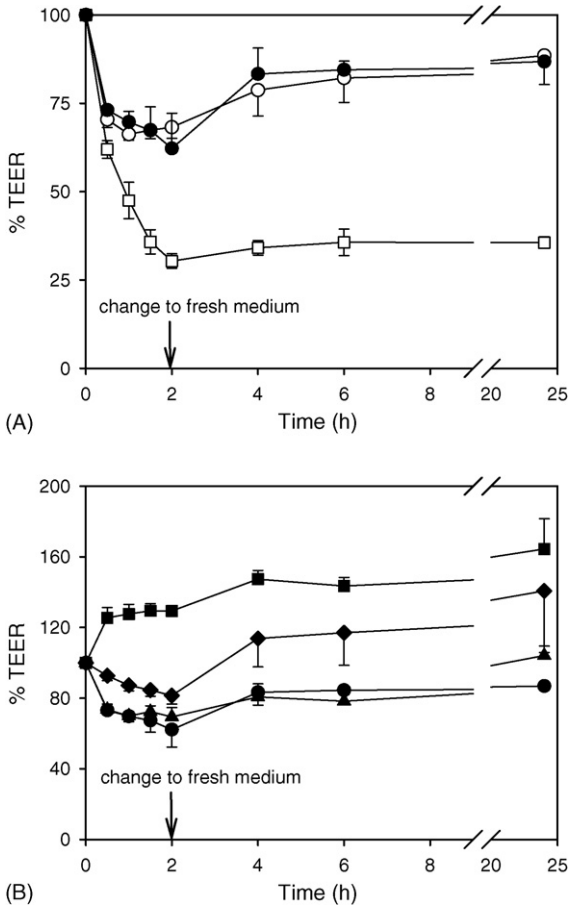

Fig. 3. Change of TEER values during the 2 h transport study with various surfactants and its recovery in fresh media after 24 h. (A) Sodium cholate: (◯) 5 mM; (●) 10 mM; (□) 15 mM. (B) (●) 10 mM sodium cholate; (▲) 10 mM sodium taurocholate; (♦) 10 mM Tween 80; (■) 10 mM poloxamer F68.

Table 1
IC_{50} of various surfactants on human nasal epithelial cells measured by MTT assay

Surfactants	CMC (mM)	IC_{50} (mM)
Sodium cholate	4[a]	3.49 ± 1.45
Sodium taurocholate	8[a]	12.02 ± 3.30
Tween 80	0.01[b]	5.95 ± 0.54
Poloxamer F68	0.1[b]	>25

[a] Data from Small (1971).
[b] From the data of the product supplier.

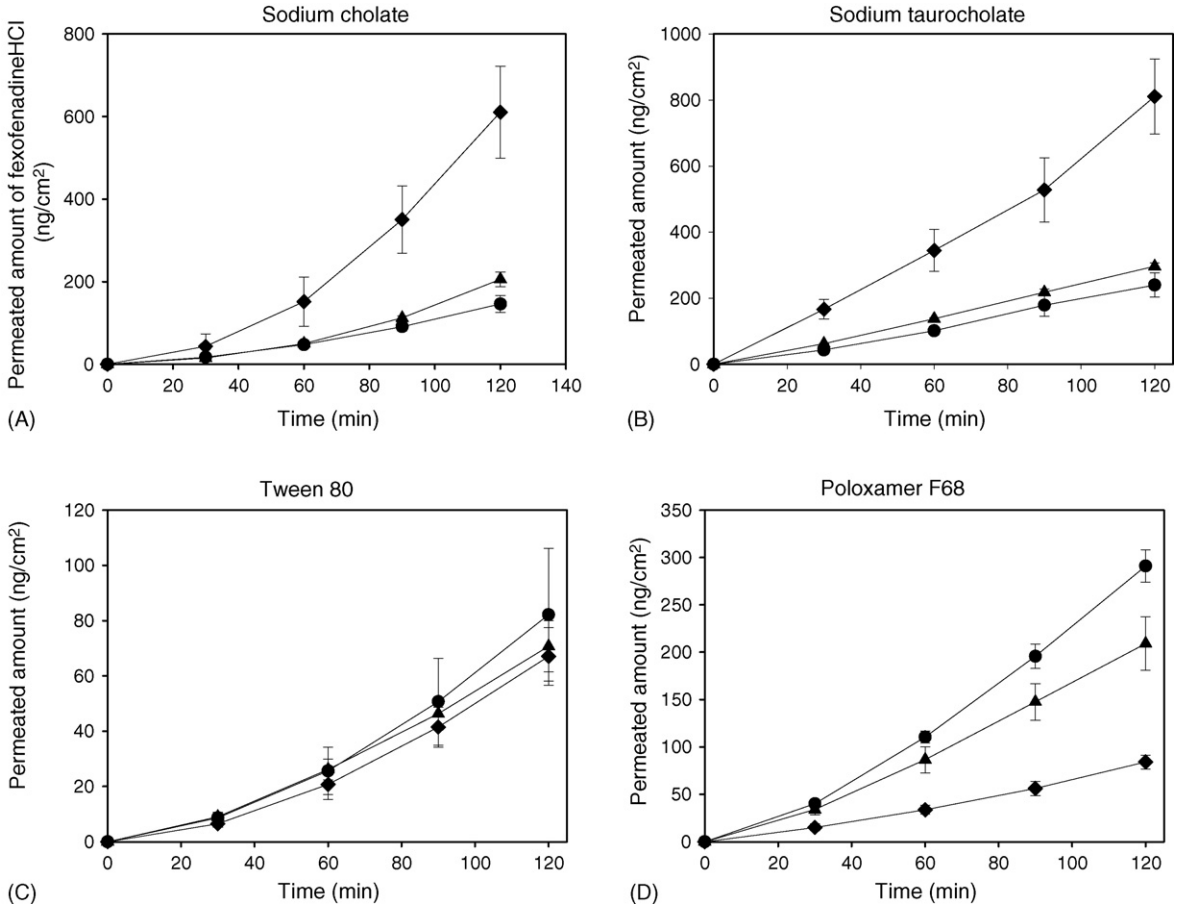

Fig. 4. Effect of various surfactants on the transport profiles of fexofenadine·HCl across the human nasal epithelial cell monolayer. (A) Sodium cholate: (●) 5 mM; (▲) 10 mM; (♦) 15 mM. (B) Sodium taurocholate: (●) 5 mM; (▲) 10 mM; (♦) 15 mM. (C) Tween 80: (●) 2.0 mM; (▲) 5.0 mM; (♦) 10.0 mM. (D) Poloxamer F68: (●) 0.5 mM; (▲) 2.0 mM; (♦) 5.0 mM.

Transwell® for evaluating the effect of surfactants on TEER. Thus, while the MTT assay only deals with cells that are proliferating without forming a tight junction, cells were allowed to differentiate on the Transwell® inserts allowing more cells to be viable even at 10 mM, showing detectable TEER values as shown in Fig. 3.

Surfactants as permeation enhancers may increase the permeability by altering and/or damaging the tight junction of nasal epithelium. Consequently, the reversibility of TEER value is an important issue when choosing penetration enhancers. The surfactants were removed after 2 h incubation and the TEER value recovery of monolayers was investigated up to 24 h in the fresh culture media. As shown in Fig. 3, recovery of TEER values was observed after removing the surfactants, except when 15 mM NaC was used. The TEER value was restored up to 72% and 104% of their original values for NaC and NaTC, respectively, at 10 mM concentrations, while TEER value increased up to 140% and 164% of their initial values for Tween 80 and Poloxamer F68, respectively, at the same concentrations. The recovery of TEER value implies that the epithelial cell monolayer was in its growth phase and that alteration and/or damage caused by the surfactants are mild and reversible within 24 h. These results were consistent with other reports on Caco-2 cell monolayers (Anderberg et al., 1992; Lindhardt and Bechgarrd, 2003), which indicated that non-ionic surfactants are generally less cytotoxic and less irritant than ionic ones.

3.3. Effect of surfactants on transepithelial permeability of fexofenadine·HCl

Fig. 4 shows the effect of various surfactants on the transport profiles of fexofenadine·HCl across the human nasal epithelial cell monolayer. The P_{app} values of fexofenadine·HCl increased with the increase of ionic surfactant concentration in a dose-dependent manner, and were significantly higher than the control (without surfactant) when the concentrations of ionic surfactant were higher than their CMC (Table 2). However, it was interesting to observe that the P_{app} significantly decreased with the addition of Tween 80. This result is consistent with a previous report of Tween 80 in the Caco-2 cells monolayer (Takahashi et al., 2002). It is widely accepted among pharmaceutical scientists in the membrane transport field that hydrophilic drugs permeate through the paracellular route (Davis and Illum, 2003). Since Tween 80 did not decrease the TEER value of human nasal epithelial cell monolayer (Fig. 3), it does not seem to be a suitable enhancer for the paracellular permeability of the hydrophilic fexofenadine·HCl. Poloxamer F68 significantly enhanced P_{app} at a low concentration (0.5 mM), but decreased up to 50% of

Table 2
Effect of various concentrations of surfactants on permeability coefficient (P_{app}) and cellullar uptake of fexofenadine·HCl (100 μg/ml) across the human nasal epithelial cell monolayer

Surfactant	Concentration of surfactant (mM)	P_{app} ($\times 10^{-7}$ cm/s)	Cellular uptake after 2 h (ng/cm^2)
Control		2.37 ± 0.76 (n = 26)	61.15 ± 7.75 (n = 9)
NaC	5	2.39 ± 0.37	162.23 ± 16.77**
	10	3.52 ± 0.33**	155.39 ± 36.25**
	15	10.56 ± 1.49**	190.64 ± 22.17**
NaTC	5	3.34 ± 0.34**	135.72 ± 37.20**
	10	4.35 ± 0.19*	177.66 ± 35.60**
	15	11.75 ± 2.00*	160.90 ± 43.71**
Tween 80	2.0	1.36 ± 0.41*	146.42 ± 14.94**
	5.0	1.14 ± 0.17**	148.94 ± 7.66**
	10.0	1.19 ± 0.17*	127.77 ± 11.18**
Poloxamer F68	0.5	4.64 ± 0.31*	61.26 ± 1.86
	2.0	3.26 ± 0.41	49.18 ± 3.86*
	5.0	1.28 ± 0.09*	22.82 ± 5.43**

Each value is the mean ± S.D. (n > 3). $^{*}p < 0.05$, $^{**}p < 0.01$, compared to control.

the control at 5.0 mM, which is probably due to the increased viscosity of the media. Further studies are still needed, however, to understand the exact effects of Tween 80 and Poloxamer F68 on the nasal epithelial cell monolayer.

It is also interesting to observe that the reduction of TEER values upon exposing the cell monolayer for 2 h to the surfactants showed a linear relationship with the transepithelial permeability (P_{app}) of fexofenadine·HCl (Fig. 5). This result suggests that the change of the TEER value can be an important indicator to predict the permeation-enhancing effect of surfactants. The decrease of TEER value is known to specifically indicate the potency of increasing the paracellular permeability (Liu et al., 1999). Thus, in the case of non-ionic surfactants, their insignificant effect on the tight junction seems to result in no change in the TEER value, thus not being able to enhance the P_{app} values of fexofenadine·HCl. On the other hand, ionic surfactants seem to affect the tight junction and thus more effectively enhance the permeability of hydrophilic compounds, such as fexofenadine·HCl. Results shown in Fig. 5 supports the hypothesis of this study that fexofenadine·HCl, like previously reported hydrophilic drugs, is transported through the paracellular route (Davis and Illum, 2003). However, as shown in Fig. 3, the effect of NaC and NaTC at lower than 10 mM concentration on the tight junction was temporary and recovered within 24 h.

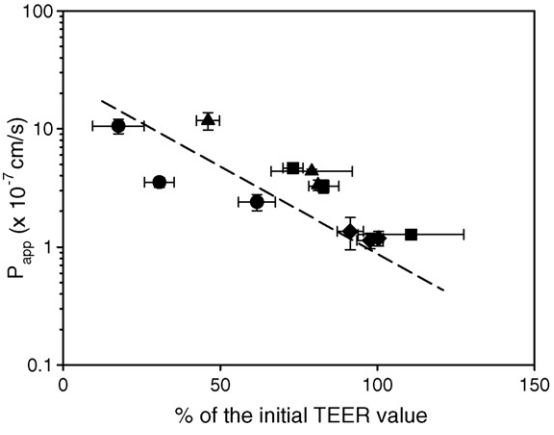

Fig. 5. Relationship between the maximum TEER value reduction and permeability coefficient (P_{app}) of fexofenadine·HCl across the human nasal epithelial cell monolayer with various surfactants. (●) Sodium cholate; (▲) sodium taurocholate; (♦) Tween 80; (■) Poloxamer F68.

3.4. Effect of surfactants on cellular uptake of fexofenadine·HCl

The amount of cellular uptake of fexofenadine·HCl after 2 h of the transport studies with various surfactants are shown in Table 2. NaC, NaTC, and Tween 80 significantly enhanced cellular uptake at all concentration ranges tested, which suggests the interaction of surfactants with the membrane lipid bilayer (*i.e.*, transcellular pathway). Surfactants are known to affect the lipid bilayer of cell membrane by perturbation as well as by a variety of intracellular mechanisms (Liu et al., 1999). Tween 80 also seems to interact with the cellular membrane, thereby enhancing the cellular uptake and increasing the cytotoxicity, but does not affect the paracellular pathway including tight junction. However, the addition of Poloxamer F68 decreased cellular uptake of fexofenadine·HCl, which was consistent with the decrease of P_{app}. Again, it could be due to the increased viscosity of the media, which decreased the thermodynamic activity of the permeation. Further studies are under way to determine more convincing mechanisms of the surfactants on the cellular uptake of fexofenadine·HCl.

3.5. Effect of surfactants on the F-actin staining

The reduction of TEER values in the presence of ionic surfactants (*i.e.*, NaC and NaTC) implied the damage on the tight junction, thereby an increase in paracellular permeability. Tight junctions are characterized as the fusion of contiguous cell membranes located in the apical side. There are many molecular components of the tight junction, such as occludin,

acludins, JAM (junctional adhesion molecule), zonula occludens, and actin. Among these components, the perijunctional F-actin is known to play a major role in controlling the paracellular permeability. Interfering with the adhesion junction interaction would allow the cells to separate and thus pull apart the tight junction, and to open the barrier (Lapierre, 2000). Therefore, F-actin staining was performed to visualize the effect of NaC on the tight junction of the nasal epithelial cell monolayer.

The silhouettes of stained F-actin contours showed well-formed tight junctions in the human nasal epithelial cell monolayer before being exposed to NaC (Fig. 6A). When the monolayer was exposed to 5 mM of NaC for 2 h, negligible deformation of tight junction was observed (Fig. 6B), while extensive disruption of monolayer occurred with the treatment of 15mM NaC for 2 h (Fig. 6C) compared with the control (Fig. 6A). However, after culturing the monolayer in the fresh media for 24 h, the contour of F-actin was recovered to the control level in both 5 and 15 mM treated groups (Fig. 6D and E), which is consistent with the recovery of the TEER values in Fig. 3. Previous reports on Caco-2 cell monolayer also observed reversible change in tight junction structure by F-actin staining and in TEER values after exposing up to 20 mM NaTC (Meaney and O'Driscoll, 1999; Werner et al., 1996).

The results of F-actin staining study indicate that micellar systems of NaC at high than CMC caused significant changes in tight junction, which also resulted in the decrease of TEER

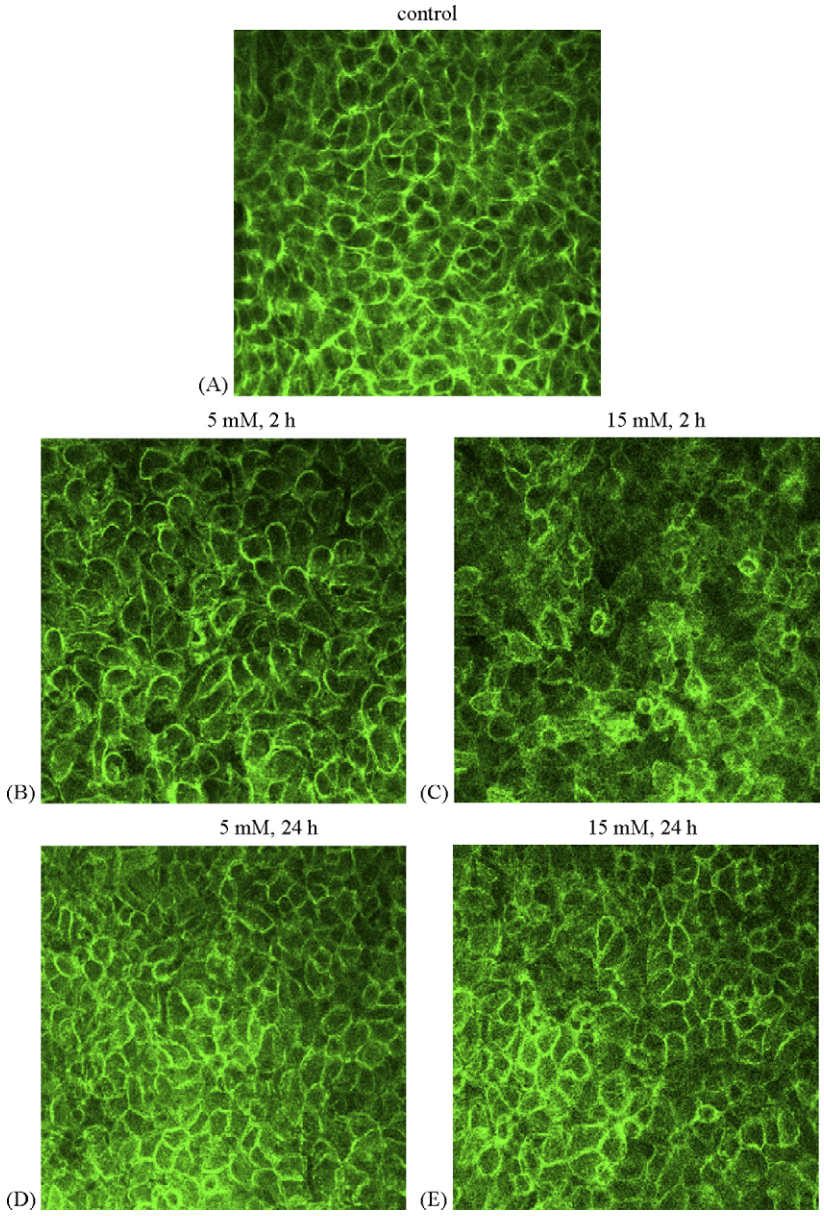

Fig. 6. F-actin staining of human nasal epithelial cell monolayer before and after treatment with sodium cholate. (A) Control, (B and C) after 2 h of treatment, (D and E) recovery after 24 h.

value. Thus, the increased P_{app} value of fexofenadine·HCl with the addition of 15 mM NaC seems to be a consequence of the damage on the tight junction, which is known as the major barrier of the paracellular pathway. Although both ionic and non-ionic surfactants significantly enhanced cellular uptake of fexofenadine·HCl (Table 2), the effect of the transcellular permeability on the overall transepithelial permeability (P_{app}) seems to be insignificant, since Tween 80 enhanced cellular uptake yet did not decrease TEER value and failed to increase the P_{app} (Table 2). The effect of surfactants on the cellular membrane rather seems to be related with their cytotoxicity. Therefore, ionic surfactants (*i.e.*, NaC and NaTC) seem to be more effective in enhancing the permeability of hydrophilic compounds, such as fexofenadine·HCl, across the nasal epithelial cell monolayer, since they work mainly on the paracellular pathway, which is known as their major permeation route. Moreover, the damaging effect of ionic surfactants was temporary and recovered to the normal state within 24 h.

4. Conclusions

The ionic surfactants (*i.e.*, NaC, NaTC) as absorption enhancers resulted in a significant increase in transepithelial permeability of fexofenadine·HCl, while non-ionic surfactants (*i.e.*, Tween 80 and Poloxamer F68) were ineffective. Change of TEER value and F-actin staining studies demonstrate that the absorption-enhancing mechanism of bile salts is the combination of membrane perturbation and opening of the tight junction between the epithelial cells. However, disrupted nasal epithelial cell monolayer was recovered within 24 h, implying that the damaging effect of ionic surfactants is temporary and reversible. Thus, ionic surfactants seem to be useful nasal absorption enhancers for hydrophilic compounds, such as fexofenadine·HCl, which mainly permeates through the paracellular pathway. Moreover, these results also demonstrate that the human nasal epithelial cell monolayer model can be a useful tool not only for the *in vitro* nasal drug permeation studies but also for evaluating *in vitro* toxicity and mechanism of enhancers.

Acknowledgement

This work was supported by research grant (No. 06072-155) from the Korean Food and Drug Administration in 2005.

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