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Characterization and *in vitro* evaluation of freeze-dried microparticles composed of granisetron–cyclodextrin complex and carboxymethylcellulose for intranasal delivery

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ABSTRACT

The aim of this study was to prepare microparticles (MPs) of granisetron (GRN) in combination with hydroxypropyl-β-cyclodextrin (HP-β-CD) and sodium carboxymethylcellulose (CMC-Na) by the simple freeze-drying method for intranasal delivery. The composition of MPs was determined from the phase-solubility study of GRN in various CDs. Fourier transform infrared spectroscopy (FT-IR), powder X-ray diffraction (PXRD) analysis and differential scanning calorimetry (DSC) studies were performed to evaluate possible interactions between GRN and excipients. The results indicated the formation of inclusion complex between GRN and CD, and the conversion of drug into amorphous state. The *in vitro* release of GRN from MPs was determined in phosphate buffered saline (pH 6.4) at 37 °C. Cytotoxicity of the MPs and *in vitro* permeation study were conducted by using primary human nasal epithelial (HNE) cells and their monolayer system cultured by air–liquid interface (ALI) method, respectively. The MPs showed significantly higher GRN release profile compared to pure GRN. Moreover, the prepared MPs showed significantly lower cytotoxicity and higher permeation profile than that of GRN powder ($p < 0.05$). These results suggested that the MPs composed of GRN, HP-β-CD and CMC-Na represent a simple and new GRN intranasal delivery system as an alternative to the oral and intravenous administration of GRN.

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1. Introduction

Granisetron (GRN), 1-methyl-*N*-[(3-*endo*)-9-methyl-9-azabicyclo(3,3,1)non-3-yl]-1*H*-indazole-3-carboxamide, is a kind of selective 5-HT_3 receptor antagonists. It has been used as antiemetics to treat nausea and vomiting following chemotherapy. It has improved side effect and has lower risk of drug interactions than other 5-HT_3 receptor antagonists. Thus, GRN is known to be an effective and well-tolerated agent in the management of chemotherapy-induced, radiotherapy-induced and post-operative nausea and vomit in adults and children (Aapro, 2004). Although many investigations were reported on the pharmacological activity and mechanism of action of GRN (Gregory and Ettinger, 1998; Perez, 1995), only few studies were reported regarding its delivery systems (Chaturvedula et al., 2005; Zheng et al., 2010). GRN HCl-loaded formulations for oral and intravenous administration are currently available in the market. However, since cancer patients under chemo- or radiotherapy already have distressed stomach as well as nausea and vomitting, orally administered drug tends to be discharged by vomiting. Intravenous route is also not desirable due to the invasiveness and not suitable for self-medication. Thus, an alternative approach is required to make the treatment more convenient and effective in cancer patients.

Nasal drug delivery system has acquired a great deal of attention as a convenient and reliable method for the systemic administration of drugs in the recent years (Huh et al., 2010; Piao et al., 2010). The advantages of nasal route have been suggested as follows: fast onset of therapeutic action by rapid absorption, higher bioavailability allowing lower doses, avoidance of liver or gastrointestinal metabolism, avoidance of the gastrointestinal irritation, and enhanced patient compliance by self-medication (Costantino et al., 2007). Since the dose of GRN is low (1 or 2 mg) and rapid onset of action is required, intranasal formulation of GRN would be an advantageous alternative to oral and intravenous administration. However, mucociliary clearance is known to be an important limiting factor for nasal drug delivery, which severely limits the residence time for drug to be absorbed. Therefore, mucoadhesive preparations have been developed using bioadhesive polymers, including sodium carboxymethylcellulose (CMC-Na), to increase the contact time between the dosage form and mucosal layers of nasal cavities, thereby enhancing drug absorption.

Cyclodextrin (CD) is natural cyclic oligosaccharides that are formed through enzymatic degradation of starch. Three most common CDs, α-CD, β-CD and γ-CD, are composed of six, seven and

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eight α(1,4)-linked α-D-glucopyranose units, respectively, with a hydrophilic outer surface and a somewhat lipophilic central cavity (Loftsson and Duchêne, 2007). CDs have been reported for their enhanced oral, skin and nasal bioavailability of drugs (Prabagar et al., 2007; Loftsson et al., 2007; Brewster and Loftsson, 2007). HP-β-CD was used for enhancing drug solubility by forming drug–CD complex and drug absorption across the nasal mucosa. In addition, it is known that drug–CD complex can control drug release and it may influence on the drug permeation across mucosal membrane (Carrier et al., 2007).

In this study, HP-β-CD was selected to prepare a complex with GRN, based on the phase-solubility study. After adding CMC-Na as a mucoadhesive polymer, MPs of GRN was prepared by simply freeze-drying the mixture, which was characterized by SEM, FT-IR, PXRD, DSC and *in vitro* release study. Cytotoxicity of the optimized MPs was evaluated in primary human nasal epithelial (HNE) cells. Moreover, their permeation-enhancing effect was observed by using the primary HNE cell monolayer system cultured by the air–liquid interface (ALI) method.

2. Materials and methods

2.1. Materials

GRN was kindly gifted from Chong Kun Dang Pharmaceutical Co. (Seoul, Korea). Cyclodextrins (α-CD, β-CD, γ-CD and HP-β-CD) were purchased from TCI Co. (Tokyo, Japan). CMC-Na was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Transwell® (0.4 μm pore size, 12 mm diameter, polyester) insert was obtained from Costar Co. (Cambridge, MA, USA). BEGM bullet kit was purchased from Cambrex Bio Science Inc. (Walkersville, MD, USA) and other cell culture reagents were obtained from Invitrogen Co. (Grand Island, NY, USA). All other chemicals were of analytical grade purchased from commercial sources and used without further purification.

2.2. Phase-solubility study

Excess amount of GRN was added into 1 ml of the various molar solutions of α-CD, γ-CD and HP-β-CD (1–40 mM) and β-CD (1–10 mM), and then stirred for 2 days at 37 °C. Suspensions were centrifuged at 15,000 rpm for 10 min and supernatants were filtered through 0.45 μm membrane filter. GRN was analyzed quantitatively by Waters HPLC system (Waters Co., Milford, MA, USA) equipped with a reverse phase C-18 column (XTerra®, RP-18, 250 mm × 4.6 mm, 5 μm, Waters Co., Milford, MA, USA), a pump (Waters 515), an automatic injector (Waters 717plus) and fluorescence detector (Series 200, PerkinElmer Instrument, Norwalk, CT, USA). The mobile phase was consisted of 25 mM acetate buffer (pH 4.8)/acetonitrile (72:28, v/v) and the eluent was monitored at 305 nm for excitation and 360 nm for emission with a flow rate of 1.0 ml/min. The injection volume for drug analysis was 20 μl. The inter- and intra-day variance of this HPLC method was within the acceptable range.

The equilibrium constant (K_c) was calculated from the slope of the linear region of the phase-solubility regression line, according to Eq. (1) (Higuchi and Connors, 1965):

$$K_c = \frac{\text{slope}}{D_0(1 - \text{slope})} \quad (1)$$

where D_0 is the saturation concentration of drug without CD.

2.3. Preparation of freeze-dried microparticles

For formulation 1 (F1 in Table 1), 5 mM of GRN (as a final concentration) solubilized in 1 ml of ethanol was added to 10 ml of double-distilled water (DDW) containing 15 mM of HP-β-CD solution (1:3 molar ratio between drug and CD). CMC-Na (0.5%, w/v) was added to this solution to prepare formulation 2 (F2 in Table 1). After stirring for 1 day, these solutions were filtered through a 0.2 μm membrane to remove insoluble ingredients, if any, followed by freezing at −20 °C for 2 h and then stored at −80 °C for 24 h. The samples were then lyophilized in freeze dryer (OPERON FDU-8612, Korea) with 40 mbar vacuum for 60 h. The residual water content in the lyophilized MPs was removed by 24 h of desiccation.

2.4. Characterization of microparticles

2.4.1. Drug loading efficiency and particle size

To determine the drug loading efficiency of MPs, 3 mg of sample was added into a volumetric flask containing 10 ml of methanol. And then it was stirred for 24 h and filtered through 0.2 μm of syringe filter (Minisart RC 15, Sartorius, Germany). GRN content was analyzed by HPLC. Product yield, drug content and encapsulation efficiency (EE) were acquired from following equations:

$$\text{Product yield (\%)} = \frac{\text{weight of microparticles after lyophilization}}{\text{total weight of ingredients before lyophilization}} \times 100 \quad (2)$$

$$\text{Drug content (\%)} = \frac{\text{actual amount of granisetron in microparticles}}{\text{amount of microparticles}} \times 100 \quad (3)$$

$$\text{EE (\%)} = \frac{\text{actual amount of granisetron in microparticles}}{\text{theoretical amount of granisetron in microparticles}} \times 100 \quad (4)$$

The EE and drug content were determined in three separately prepared MPs and were expressed as the mean ± standard deviation (S.D.).

After redispersing the lyophilized MPs (6 mg) in 2 ml of $CHCl_3$, their particle sizes were analyzed using a submicron particle size analyzer NICOMP 370 (CA, USA) by volume-weighted Gaussian method.

2.4.2. SEM

The morphology of MPs was examined by SEM. Sample was spread on the specimen stub using a double sided sticky tape, which was coated by gold sputtering at 100 mTorr in SEM chamber (Hitachi S-4100, Japan). Photographs were taken at an acceleration voltages of 15 kV electron beam.

2.4.3. FT-IR spectroscopy

FT-IR spectra of GRN, HP-β-CD, CMC-Na, F1 and F2 were obtained by using JASCO FT/IR-4200 type A (JASCO Co., Tokyo, Japan) with KBr. The spectra were scanned in the range of 400–4000 cm^{-1}.

2.4.4. XRD

Powder X-ray diffraction (PXRD) analysis was carried out with an D5005 model diffractometer (Brucker, Germany) at room temperature using monochromatic CuK_α-radiation (λ = 1.5406 Å) at 40 mA and at 40 kV over a range of 2θ angles from 3° to 40° with an angular increment of 0.02°/s and scan speed of 1°/min.

2.4.5. DSC

Differential scanning calorimetry (DSC) of GRN, HP-β-CD, CMC-Na, F1 and F2 were obtained using DSC-Q100 model (TA Instrument, UK). The samples were analyzed using aluminum sealed pans

Table 1
The composition and characterization of microparticles ($n = 3$).

Formulation	Composition	Product yield (%)	Drug content (%)	Encapsulation efficiency (%)	Mean diameter (μm)
F1	GRN/HP-β-CD (1:3)	85.54	5.78 ± 0.01	83.43 ± 0.12	7.79 ± 2.05
F2	GRN/HP-β-CD/CMC-Na (1:3:0.5%)	73.65	3.59 ± 0.01	63.34 ± 0.10	15.59 ± 3.05

and scanned at 10 °C/min from 50 to 300 °C under the supply of 50 ml/min of nitrogen atmosphere.

2.5. In vitro release study

In vitro release study of the prepared GRN MPs and GRN powder was performed as reported earlier (Huh et al., 2010). The samples (3 mg) were loaded in Midi-GeBAflex-tubes, which had molecular weight cut-off of 3.5 kDa (Gene Bio-Application L.T.D., Israel). Then, these tubes were floated using floating rack in phosphate buffered saline (PBS, pH 6.4 adjusted with phosphoric acid) in a shaking bath maintained at 37 °C. The dissolution medium (100 ml) was rotated at 100 rpm. Aliquot of release medium (1 ml) was collected at predetermined time (5, 15, 30, 60, 90 and 120 min) and equivalent volume of fresh medium (37 °C) was replaced to maintain sink condition. The samples were analyzed by HPLC for the quantitative analysis of GRN.

2.6. In vitro cytotoxicity test

The primary HNE cells were isolated and cultured as described earlier (Lee et al., 2005). After HNE cells of passage 1 and 2 attained 70–80% confluency, the cells were trypsinized and seeded on a 48-well plate at a density of 1.0×10^5 per well. After 24 h, the growth media were replaced with 500 μl of fresh media containing MP formulations (1, 2 or 3 mg), and incubated for 1, 2, or 3 h at 37 °C in an atmosphere of 5% CO_2 and 95% relative humidity. After incubation period, the HNE cells were treated with 80 μl of MTS-based CellTiter 96® AQ_{ueous} One Solution Cell Proliferation Assay Reagent (Promega Corp., WI, USA) at 37 °C for 4 h. The absorbance was read at 490 nm wavelength by EMax precision microplate reader (Molecular Devices, CA, USA).

2.7. In vitro permeation study

2.7.1. In vitro permeation study through HNE cell monolayers cultured by ALI method

Primary HNE cells culture for transport study by ALI culture method was performed as reported earlier (Lin et al., 2007; Huh et al., 2010). Briefly, when HNE cells attained 70–80% confluency, the cells were detached and seeded on Transwell® insert (12-well plate) at a density of 1.5×10^5 cells per well. The apical and basolateral side were filled with BEGM:DME/F12 (50:50) supplemented with BEGM bullet kit (all supplied by Cambrex Bio Science Inc., Walkersville, MD, USA) and then was incubated at 37 °C in an atmosphere of 5% CO_2 and 95% relative humidity. The growth media in both sides were changed after 24 h of seeding, and then the apical surface of the monolayer was exposed to air after the HNE cells reached confluence on day 3, after which only the media in the basolateral side were changed every 2 days.

In vitro permeation study was performed with Transwell® inserts of which transepithelial electrical resistance (TEER) value was higher than 500 Ω cm^2. TEER value was measured by EVOM voltohmmeter (WPI, Sarasota, FL, USA). The growth media of HNE cell monolayers were removed and incubated with transport media (HBSS supplemented with 10 mM HEPES and 10 mM D-(+)-glucose) for 30 min at 37 °C to get steady state for transport study. The prepared MPs or GRN powder (equivalent to 2 mg GRN) was loaded in the apical side and 0.4 ml of transport medium was added. To calculate the permeated amount of GRN from the apical side to the basolateral side (A to B), 1 ml of medium was collected at determined time (15, 30, 45, 60, 90 and 120 min) from basolateral side and equivalent volume of fresh medium was added. The GRN content in the samples was analyzed by HPLC method.

2.7.2. In vitro cytotoxicity test followed by in vitro permeation study

TEER values of the cell monolayers 1 day after transport study were measured by recording the percent change to the initial value before transport study. After washing the HNE cell monolayers on the Transwell® inserts at least 3 times with PBS (pH 7.4) to remove the MPs remained on the apical side, the cell monolayers were incubated with cell culture media for 24 h and then TEER value was measured again. HNE cell viability 1 day after permeation study was measured and HNE cells without treatment of GRN-loaded MPs were used as the control group for calculating the change of cell viability. Then, 80 μl of MTS-based CellTiter 96® AQ_{ueous} One Solution Cell Proliferation Assay Reagent (Promega Corp., WI, USA) was added on the apical side of Transwell® inserts and incubated for 4 h at 37 °C. The absorbance was determined at 490 nm wavelength by EMax precision microplate reader (Molecular Devices, CA, USA).

3. Results and discussion

3.1. Phase-solubility study

The phase-solubility diagrams at 37 °C were obtained by plotting the apparent equilibrium concentrations of the GRN against various CDs (Fig. 1). It can be observed that the apparent solubility of GRN (0.163 ± 0.011 mg/ml in double-distilled water) increased as a function of β-CD and HP-β-CD concentration over the entire concentration range studied linearly ($r^2 > 0.99$). Linearity was a characteristic of A_L-type system (Higuchi and Connors, 1965), suggesting that water-soluble complex was formed in the solution of β-CD and HP-β-CD. However, in the case of α-CD and γ-CD, GRN solubility did not significantly change with the increase of CD con-

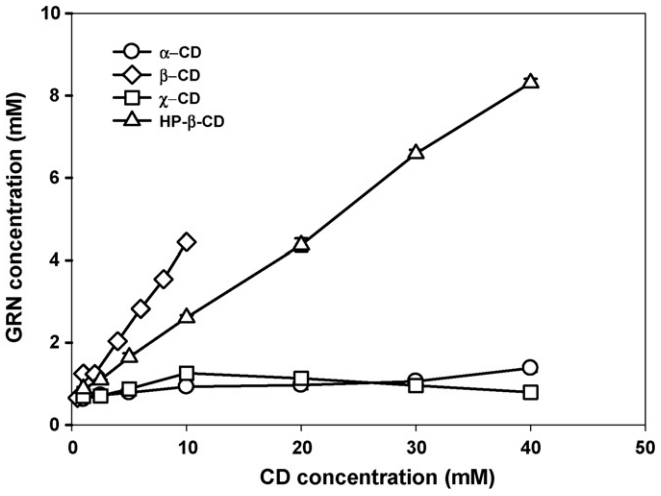

Fig. 1. Phase-solubility study of GRN in α-CD, β-CD, γ-CD and HP-β-CD solutions. ($n = 3$).

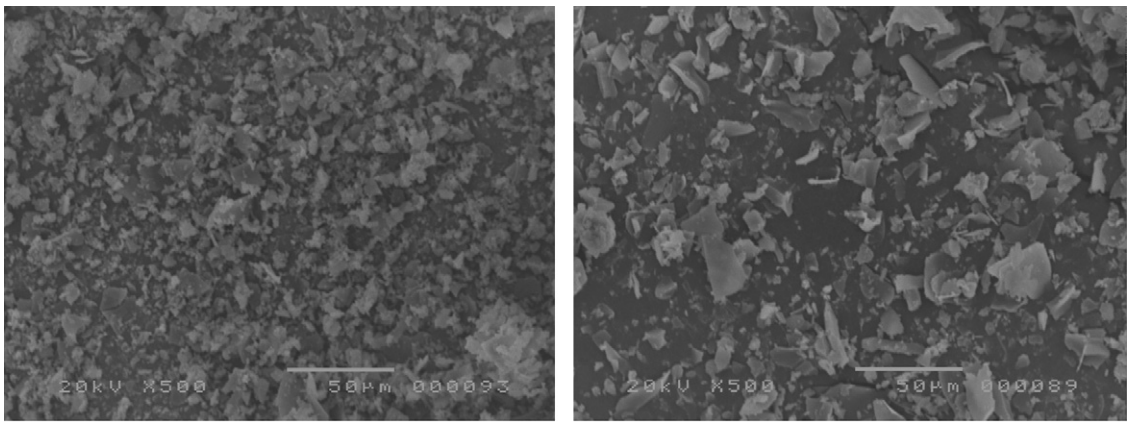

Fig. 2. Scanning electron micrograph of MPs (A) F1 (GRN/HP-β-CD) and (B) F2 (GRN/HP-β-CD/CMC-Na).

centration, implying B_S-type system. The equilibrium constant K_c (Eq. (1)) of the GRN–CD complexes for α-CD, β-CD, HP-β-CD and γ-CD were 31.33 M^{-1}, 1157 M^{-1}, 454.96 M^{-1} and 4.41 M^{-1}, respectively. The K_c value for HP-β-CD complex was smaller than that of β-CD complex, probably due to the steric hindrance of the hydroxypropyl group in HP-β-CD (Liu and Zhu, 2006), whereas that for HP-β-CD complex was greater than that of α-CD and γ-CD. Since α-CD, β-CD and γ-CD consist of six, seven and eight glucose units, respectively, this also could lead to the differences in the hydrophobic cavity size (Challa et al., 2005). Thus, it is expected that they have different host–guest interactions and the complex conformations, resulting in difference in subsequent K_c values. Additionally, HP-β-CD is known to have higher aqueous solubility (>75%, w/v) compared to β-CD (<2%, w/v), and higher safety than the aforementioned CDs in nasal formulations (Merkus et al., 1999). Thus, it was selected as a solubilizer of GRN in this study.

3.2. Preparation and characterization of microparticles

In preliminary studies, GRN base showed relatively high permeability in Caco-2 cell monolayer ($>1.0 \times 10^{-5}$ cm/s) with relatively low aqueous solubility (about 163 μg/ml) (data not shown). Thus, solubilization of GRN would be a major factor determining the absorption through nasal mucosal membrane. Moreover, since the administration volume to the nostril is limited to 150 μl in human and 13 μl in rat (Ugwoke et al., 2001), an increase in solubility was considered crucial for the nasal delivery of GRN. Hence, to obtain a higher solubility with the formulation, the HP-β-CD was added at 1:3 molar ratios (GRN:CD) in the prepared nasal formulations (Table 1). The MPs containing GRN–CD complex were successfully prepared by the freeze-drying method. Preparing MPs by freeze-drying method is relatively simple, cost-effective and easy for scale-up. The product yield (Eq. (2)) of F1 and F2 was 85.54% and 73.65%, the drug content (Eq. (3)) assayed by HPLC was 5.78% and 3.59%, and encapsulation efficiency (Eq. (4)) of F1 and F2 was 83.43% and 63.34%, respectively (Table 1). The mean diameter of F2 was significantly higher ($p < 0.05$) than that of F1 (Table 1), probably since the adhesion nature of CMC-Na lead to the bigger particle size. Morphological shapes observed by SEM were irregular shape, but was consistent with particle size measurement (Fig. 2).

3.3. Solid state studies of microparticle

FT-IR is a useful tool to establish the presence of both host and guest components in the inclusion complex. The FT-IR spectra of GRN, HP-β-CD, CMC-Na, F1 and F2 in the region from 400 to 4000 cm^{-1} are presented in Fig. 3. The formation of inclusion complex was identified by the shift and intensity changes on the peaks of inclusion complex compared to those of individual component. In GRN spectrum, absorption bands at 1531.2 and 1657.5 cm^{-1} generally indicate C=O bond, which is located in 3-carboxamide group in the chemical structure of GRN. It was assumed that the band at 2925.5 cm^{-1} was related to C–H stretching. In HP-β-CD spectrum, the vibration of free OH group was presented in the range of 3100–3800 cm^{-1} and CO stretching glucosidic bond and OH group were found in the range of 1000–1200 cm^{-1}. Some bands of the host and guest are affected by the formation of the inclusion complex, resulting in a change of position and relative intensities. In the spectrum of both freeze-dried MPs, band at 2925.5 cm^{-1} was almost disappeared and absorption bands between 1500 and 1700 cm^{-1} were also unseen. Only weaken bands of CO stretching bond in the range of 1000–1200 cm^{-1} of CD were observed. These changes imply the embedding of GRN into the internal cavity of CD and the formation of drug–CD complex.

The XRD pattern of pure drug presented several diffraction peaks indicating the crystalline nature of GRN (Fig. 4). HP-β-CD and CMC-Na did not exhibit crystalline diffraction patterns compared to GRN itself in this study. The freeze-dried MPs showed a single very broad band, in which the sharp diffraction peaks of GRN (diffraction angle of 2θ 13.98° and 15.40°) were disappeared. This phenomenon indicated that drug amorphization and the formation of drug–CD inclusion complex.

The DSC curves for raw materials and the freeze-dried MPs studied are shown in Fig. 5. GRN base exhibited endothermic melting peak at 153 °C. No polymorph of GRN was observed in this DSC study. In the temperature range observed, DSC curve of HP-β-CD

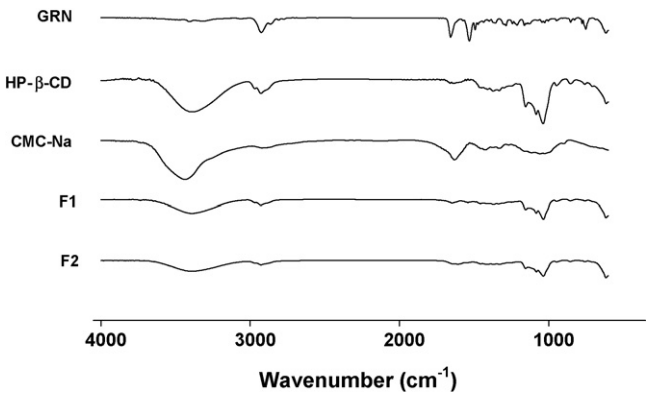

Fig. 3. FT-IR spectra of GRN, HP-β-CD, CMC-Na, F1 and F2.

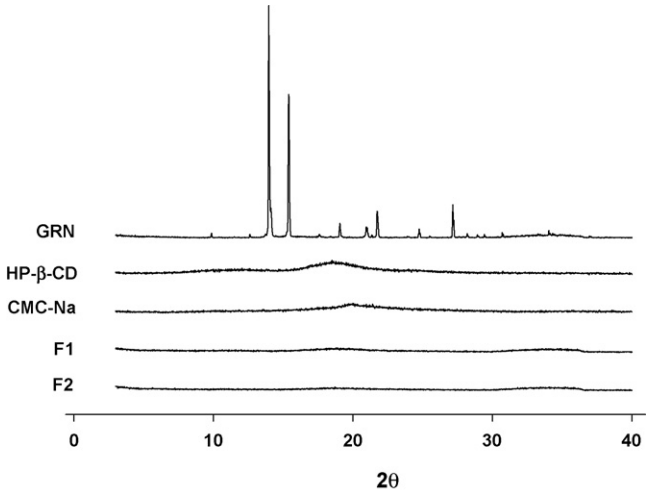

Fig. 4. PXRD patterns of GRN, HP-β-CD, CMC-Na, F1 and F2.

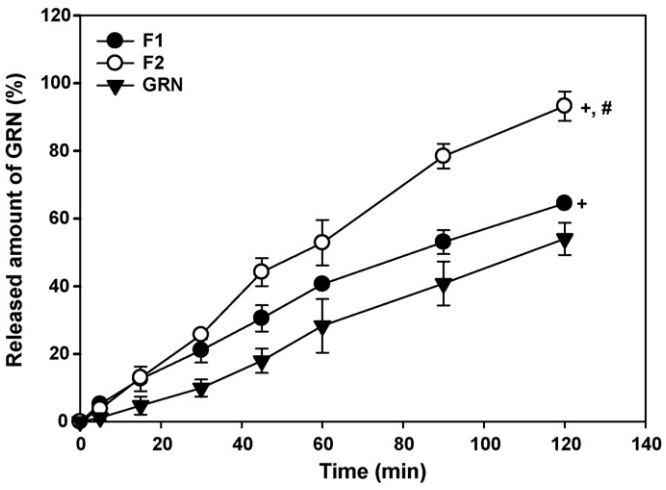

Fig. 6. *In vitro* release profiles of freeze-dried microparticles (F1 and F2) and GRN powder. Each point represents the mean ± S.D. ($n = 3$). $^{+}p < 0.005$ and $^{\#}p < 0.005$ compared to pure GRN and F1, respectively.

exhibited no peak and CMC-Na showed a broad exothermal phenomenon around 240 °C. No endothermal peak of GRN was noted in the freeze-dried products (F1 and F2), suggesting that the complete inclusion complex without free GRN was formed.

3.4. In vitro release study

In vitro drug release test was performed to predict the *in vivo* performance of the GRN-loaded MPs. The aim of this study was to develop MPs which is able to release higher amounts of the GRN at the pH of the nasal cavity (*i.e.*, pH 6.4). Fig. 6 shows the dissolution profiles of the pure GRN as well as the *in vitro* drug release from the prepared MP formulations. It has been reported that the presence of other excipients, including polymers, is known to possibly influence on the interaction between drug and CD. The polymers such as water-soluble cellulose derivatives can form complexes with CD and they can show different physicochemical properties compared to that of the CD alone (Carrier et al., 2007). In the classical Noyes–Whitney dissolution expression for a drug particle in the absence of CD, dissolution rate is proportional to the concentration gradient across the unstirred boundary layer surrounding a dissolving particle in a well-mixed compartment. When CD is existed, the additional driving force, *i.e.*, the difference in the concentration of complex at the particle surface and in the fluid of mucosal membrane, would enhance the dissolution of drug (Carrier et al., 2007). In the current study, F1 and F2 exhibited significantly higher drug release rate than that of pure GRN ($p < 0.05$). Moreover, F2 showed higher drug release rate (75%) for 2 h compared to F1 (45%) ($p < 0.05$). This might be due to the enhanced solubilization by the presence of CMC-Na in F2 (Carrier et al., 2007).

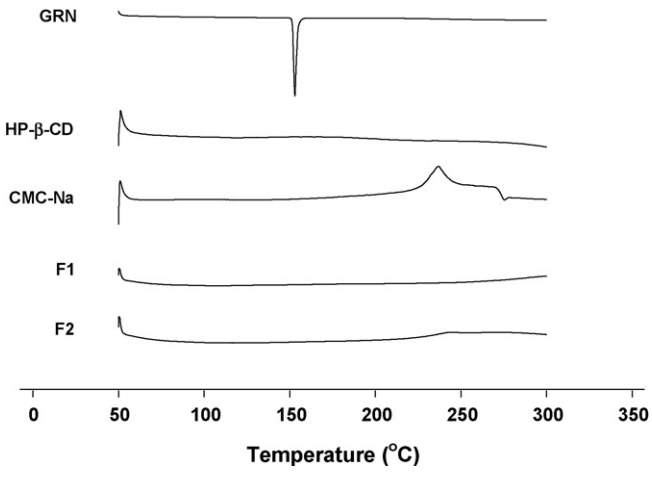

Fig. 5. DSC thermograms of GRN, HP-β-CD, CMC-Na, F1 and F2.

3.5. In vitro cytotoxicity test in HNE cells

The cytotoxicity of the GRN-loaded MPs was evaluated by assessing cell viability using the MTS-based assay on primary HNE cells. For this current investigation, we separately performed cytotoxicity of pure CDs on HNE cells for 3 h and found that the percentage of HNE cell viability (%) for the studied CDs up to 50 mM concentration was in the order of γ-CD > β-CD = HP-β-CD ≫ α-CD (data not shown). Though the γ-CD showed low cytotoxicity compared to HP-β-CD, the solubility profile for GRN obtained with γ-CD was poor, thus we selected HP-β-CD. CMC-Na was reported that it was considerably safe for short term intranasal administration (Ugwoke et al., 2000a). However, to facilitate the basis for comparison, primary HNE cells were incubated for 1–3 h with various amounts of MPs. The cytotoxicity of the GRN-loaded MPs is shown in Fig. 7. Although both F1 and F2 showed significantly ($p < 0.05$) increased cytotoxicity at 3 h compared to 1 h (Fig. 7A), the change of survival percentage was almost negligible. Moreover, the different amounts of MPs showed no significant difference in the cytotoxicity among them for the entire time (2 h) course tested (Fig. 7B). Furthermore, regardless of incubation time (1, 2 and 3 h) and loading amount (1, 2 and 3 mg) of MPs, the lowest cell viability observed was around 80%. Thus, it can be concluded that these formulations did not induce severe toxicity to primary HNE cells.

3.6. In vitro permeation study

One of the greatest limitations of nasal drug delivery is inadequate nasal drug absorption. Therefore, to evaluate the freeze-dried MPs, *in vitro* permeation studies were performed using HNE cell monolayers cultured by ALI method. In our previous studies, HNE cell monolayer system cultured by ALI method was established (Lee et al., 2005; Lin et al., 2007). This *in vitro* system was successfully applied to evaluate the permeability of drug released from hyaluronic acid-based microspheres for intranasal delivery (Huh et al., 2010). The cultured HNE monolayer integrity in this investigation was assayed by means of TEER value measurement, which was 734.3 ± 84.1 Ω cm^2. In order to assess the damage and recov-

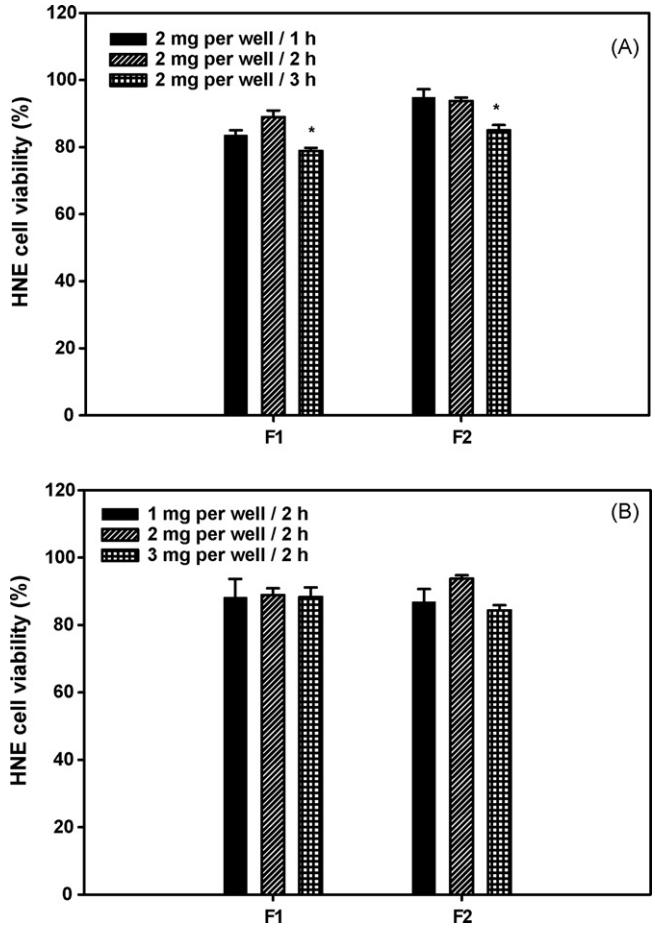

Fig. 7. *In vitro* cytotoxicity of MPs (F1 and F2) in primary HNE cells was determined by MTT assay. (A) Effect of incubation time (1, 2 and 3 h) when the amount of MPs was fixed as 2 mg and (B) effect of the amount of MPs (1, 2 and 3 mg) when the incubation time was fixed as 2 h. Each point represents the mean ± S.D. ($n = 3$). $^{*}p < 0.05$ compared to 1 h data.

ery of HNE cell monolayer after the permeation study, TEER value was measured after culturing for 1 day. The *in vitro* drug permeation profile across the HNE cell monolayer is shown in Fig. 8. It was observed that a significant increase in the permeation profile of F1 compared to GRN itself. At the end of 2 h, permeated amount of GRN from F1 was 2.48-fold higher than that of GRN itself ($p < 0.005$). Moreover, F2 showed significantly higher permeation profile than that of F1. As shown in Fig. 8, F2 showed almost 3.4- and 1.4-fold higher permeated amount of GRN compared to GRN itself and F1 after 2 h of transport study, respectively ($p < 0.005$). Moreover, the TEER value of F1 and F2 treated HNE cell monolayers were recovered to 87.11 ± 9.49% and 86.42 ± 7.68% of their initial values, respectively, after 24 h (Table 2), which indicates low cytotoxicity of F1 and F2. The cell viability (%) of F1 and F2 measured after permeation study in the HNE cell monolayers was 97.78 ± 1.01% and 94.32 ± 5.26%, respectively (Table 2), and this results coincided with high recovery rate of TEER value.

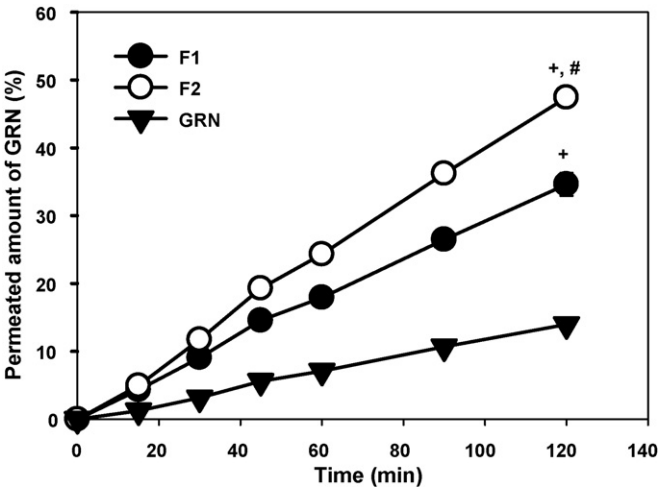

Fig. 8. *In vitro* permeation study of MPs (F1 and F2) and pure GRN across the primary HNE cell monolayers cultured by ALI method. Each point represents the mean ± S.D. ($n = 3$). $^{+}p < 0.005$ and $^{\#}p < 0.005$ compared to GRN and F1, respectively.

Table 2
TEER value changes and HNE cell viability after *in vitro* permeation study ($n = 3$).

Formulation	TEER value change (%)	HNE cell viability (%)
F1	87.11 ± 9.49	97.78 ± 1.01
F2	86.42 ± 7.68	94.32 ± 5.26

All values were measured 24 h after permeation study in HNE cell monolayers.

It is evident from our study that the presence of CMC-Na in F2 enhanced drug permeation compared to F1, which contained HP-β-CD alone. It is well known that CDs enhance the absorption by increased solubilization and dissolution of the drug (Loftsson et al., 2007). The permeation-enhancing effect of CD was also identified *in vitro* cell monolayer system like Caco-2 cell monolayer (Shah et al., 2007). The higher permeation profile of F2 might be due to enhanced dissolution by the synergistic result of CMC-Na and HP-β-CD. Though the bioadhesive effect of CMC-Na could not be evaluated in this *in vitro* permeation study, the bioadhesive nature of CMC-Na by gamma scintigraphic evaluation has already been reported (Ugwoke et al., 2000b). Thus, it is expected that CMC-Na would extend the residence time of the MPs, thereby enhancing the bioavailability of GRN. The result of our *in vitro* permeation study suggest that these freeze-dried MPs can be used as an alternative intranasal formulation for the currently available oral and intravenous formulations of GRN for effective treatment of nausea and vomitting.

4. Conclusions

GRN–CD complex-loaded MPs with CMC-Na were easily prepared by the simple freeze-drying method. Their solid state characterization revealed that GRN in MPs is molecularly dissolved stage. The loading of GRN in the MPs led to a significant increase in its permeation across the HNE cell monolayers. Furthermore, the prepared MPs exhibited low cytotoxicity in HNE cells. Thus, this intranasal formulation could be a potential candidate for nasal delivery of GRN.

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