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Formulation and evaluation of ondansetron nasal delivery systems

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Abstract

This study aimed to formulate and evaluate nasal delivery systems containing ondansetron hydrochloride. In the *in vitro* study, the permeation rate with the addition of 10% polyethylene glycol 300 (PEG 300) to aqueous solution containing 0.01% benzalkonium chloride (BC) and 10% sulfobutylether ß-cyclodextrin sodium salt (SBCD) was somewhat more rapid up to 1.5 h compared to the addition of 10% PG. The permeation flux increased as the drug concentration increased regardless of the vehicles used. The addition of nicotinamide or chitosan to aqueous drug solution (40 mg/ml) with 10% PEG 300 and 0.01% BC rather decreased permeation rate and delayed lag time. Even though cyclodextrins including SBCD or dimethyl-ß-cyclodextrin failed to show permeation enhancing effects of ondansetron hydrochloride, the addition of 10% SBCD to aqueous solution containing 10% PEG 300 and 0.01% BC could be a good candidate for ondansetron nasal delivery systems because of its safety profile, stable storage in refrigerator and solubilizing effect. With the above formulation, the nasal delivery system increased $AUC_{0-2\,h}$ and C_{max} by 2.1 and 1.7 times compared to those of oral delivery, respectively while there was no difference found in $AUC_{0-2\,h}$ with intravenous administration. Therefore, the nasal delivery system of ondansetron hydrochloride formulated in this study was feasible for nasal administration.

Keywords: Ondansetron hydrochloride; Vehicles; Permeation rate; Lag time; Pharmacokinetic parameters

1. Introduction

Ondansetron is a serotonin (5-hydroxytryptamine) subtype 3 (5-HT_3) receptor antagonist used in the management of nausea and vomiting (Butcher, 1993; McKenzie et al., 1993; Scuderi et al., 1993). 5-HT_3 receptors, located centrally in the chemoreceptor trigger zone of the area postrema as well as peripherally on vagal nerve terminals, are key receptors in the nausea and vomiting response (Hesketh and Gandara, 1991). Ondansetron has been used to prevent and control nausea and vomiting after cancer chemotherapy, radiotherapy and surgery (Butcher, 1993; Hesketh and Gandara, 1991; McKenzie et al., 1993; Scuderi et al., 1993). Unlike metoclopramide, ondansetron is known not to block dopamine subtype-2 receptors, and therefore not to induce the undesirable side effect such as extrapyramidal reactions. The most commonly reported adverse events with ondansetron are headache, constipation and diarrhea, which are mild to moderate in severity and rarely necessitate treatment withdrawal (Blackwell and Harding, 1989).

Ondansetron hydrochloride has been used by oral and injectable administration. Ondansetron hydrochloride is rapidly absorbed orally, but extensively metabolized by the liver (Figg et al., 1996). It should be administered 30 min before chemotherapy, and the orally administered antiemetic drug tends to be discharged by vomiting (Rolia and Del Favero, 1995). On the contrary, intravenous administration renders rapid effects to a patient, but the onset of effects is too rapid to cause undesirable effects. In addition, it gives a local pain, and may cause an unexpected accident when it is not perfectly prepared.

Nasal delivery has been paid attention as an alternative dosage form. The advantages of nasal route have been suggested as follows: rapid absorption, higher bioavailability allowing lower doses, fast onset of therapeutic action, avoidance of liver or gastrointestinal metabolism, avoidance of irritation of the gastrointestinal membrane, reduced risk of overdose, non-invasive administration, ease of convenience and self-medication, improved patient compliance, feasibility of beneficial adjunct product to an existing product and reduced risk

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of infectious disease transmission (Behl et al., 1998). Although transdermal ondansetron formulation has been reported (Gwak et al., 2003, 2004), the lag time for transdermal permeation is long and the flux is low. Considering that the dose of ondansetron hydrochloride is low (4–24 mg) and rapid onset of action is required, however, it is valuable to develop ondansetron nasal delivery system.

Although the nasal administration has such many advantages, in the case of solution formulation, it should not form precipitates even during longer period of storage. In addition, the higher permeation across the nasal mucosa can be easily achieved when the concentration of active ingredient is relatively high. However, it is difficult to meet the above two requirements at the same time.

This study aimed to formulate the most suitable nasal delivery system of ondansetron hydrochloride and evaluate it. For this, the effects of vehicles and penetration enhancers on the permeation of ondansetron across excised rabbit nasal mucosa were examined. Based on the *in vitro* results, the most suitable formulation was constructed and administered to the rat by nasal route, and pharmacokinetic parameters were compared with those by oral and intravenous route.

2. Materials and methods

2.1. Materials and animals

Ondansetron hydrochloride was purchased from Zunan Commerce & Industrial Co., Ltd. (China). Isopropyl myristate and benzalkonium chloride (BC) were purchased from Sigma Chemical Co. (USA). Dimethyl-ß-cyclodextrin (DMCD, Sigma Chem. Co. Ltd., USA), sulfobutyl ether ß-cyclodextrin sodium salt (SBCD, CyDex Inc., USA), 2-hydroxypropyl-ß-cyclodextrin (2-HPßCD, Cargill Inc., USA) and ß-cyclodextrin (Kimura Sangyo co., Japan) were used. Nicotinamide (Janssen Chimica, Belgium) and chitosan (M.W. = 300,000, Jakwang, Korea) were purchased. Polyethylene glycol 300 (PEG 300), polyethylene glycol 400 (PEG 400, Hayashi Pure Chemical Ind., Japan), *n*-octanol, propylene glycol (PG, Daejung Chemicals Co., Korea) and povidone K 30 (Kollidon 30, BASF Corp., USA) were used. Acetonitrile and methanol used were of HPLC grade. Other reagents were of analytical grade.

Male New Zealand white rabbits weighing 3.0 kg and Sprague-Dawley female rats weighing 250–300 g were purchased from Samtako Bio Korea Co., Ltd. (Korea).

2.2. Analysis

Samples from permeation and stability studies were analyzed by high-performance liquid chromatography (HPLC). The HPLC system consisted of a pump (Series 410, Perkin-Elmer, USA) with a detector (Model LC 90 UV, Perkin-Elmer, USA) set at 302 nm and an integrator (Model 4290, Varian, USA). An ODS column (μBondapak C18, 3.9 mm × 300 mm, 10 μm, Waters, USA) equipped with a C18 Radial Pak insert was used. The mobile phase was composed of acetonitrile, methanol, water and triethylamine (25:10:65:0.1, v/v), whose pH was adjusted to 4.0 by phosphoric acid, and delivered at a flow rate of 1.2 ml/min. The injection volume was 20 μl. The internal standard (IS) used was terazosin hydrochloride (30 μg/ml). A calibration curve was constructed based on peak area ratio measurements.

2.3. Partition coefficient determination

Water and oil solutions including isopropyl myristate and *n*-octanol were saturated with each other before the experiment. Ondansetron hydrochloride solution (100 μg/ml) was prepared with water saturated with oil phase. One milliliter of this solution was then transferred to 10 ml centrifuge tube containing 1 ml of oil phase saturated with water. The tube was vortex-mixed for 3 min and centrifuged at 3000 × *g* for 5 min. After centrifugation, 100 μl was withdrawn from water phase and oil phase, respectively, and the intrinsic partition coefficient was determined by HPLC.

2.4. Solubility determination

An excess amount of ondansetron hydrochloride was added to various vehicles and shaken in a water bath set at 37 °C for more than 48 h. The solutions were centrifuged at 3000 × *g* for 5 min, and the supernatant was assayed by HPLC after appropriate dilution.

2.5. Observation of precipitation of ondansetron hydrochloride in vehicles

Ondansetron hydrochloride solutions of 10, 20, 30 and 40 mg/ml were prepared using various vehicles with or without additives, and stored at refrigerator for 3 weeks. Precipitate formation was observed by naked eyes.

2.6. Stability test of ondansetron hydrochloride in nasal mucosa extracts of rabbits

Freshly excised nasal mucosa was mounted to a side-by-side permeation system, and 3.5 ml of normal saline was filled in both sides of cells. After 4 h extraction while stirring, extract solutions were collected respectively, from the both sides. Ondansetron hydrochloride was then added at the concentration of 200 μg/ml to the nasal mucosa extract, and incubated at 37 °C up to 4 h. The amount remaining at predetermined time interval (1, 2, 3 and 4 h) was analyzed by HPLC.

2.7. Procedure for mucosa permeation in vitro

After sacrificing rabbit by injecting air into the marginal ear vein, the nasal, duodenal, colonic and rectal mucosae were carefully excised and mounted onto the cell opening (0.64 cm^2) of a side-by-side permeation system. The mucosal side of excised mucosa was faced to donor compartment and its serosal side was faced to receptor compartment. Donor half-cell was filled with 3.5 ml of ondansetron hydrochloride solution in normal saline and the receptor half-cell was filled with 3.5 ml of 40% PEG 400

in saline. To maintain the freshness of the specimen as far as possible, permeation study was initiated right after the mucosa was excised, and performed only for 4 h. Both media were stirred by a Teflon-coated magnetic bar to keep them well mixed. The permeation media were maintained at 37 °C. At predetermined time intervals, 100 μl of receiver solutions were withdrawn, and mixed with 10 μl of IS solution. The amount of ondansetron hydrochloride permeated was determined by HPLC.

2.8. Procedures for pharmacokinetic studies in vivo

Ondansetron hydrochloride was dissolved in the composite solution (aqueous solution containing 10% PEG 300, 0.01% BC and 10% SBCD) obtained from *in vitro* study for nasal administration, in 0.9% saline for intravenous administration, and in purified water for oral administration as vehicles, respectively. For the oral, intravenous and nasal administration, the rat was anesthetized with 50 mg/kg of sodium thiopental (Yuhan Corp., Korea), fixed on the surgical die illuminated with light, and the right femoral artery was cannulated using a polyethylene tubes (0.58 mm i.d. × 0.96 mm o.d.; Naume Corp., Japan). For oral administration, the animals were fasted overnight and until the end of the experiment. The *in vivo* experiment for nasal administration was conducted as suggested by Hirai et al. (1981). Drug was administered to each group at the dose of 2 mg/kg.

The blood samples (0.4 ml) were collected at the predetermined time intervals to analyze the concentration of ondansetron in the plasma. The pharmacokinetic studies of ondansetron nasal spray solutions were carried out according to the Principles for Biomedical Research Involving Animals developed by the Council for International Organizations of Medical Sciences.

2.9. Procedure for plasma sample preparation

Plasma sample (0.1 ml) was mixed with 10 μl of the working IS solution and alkalized with 20 μl of 2 mol/l sodium hydroxide solution. The mixture was added with 5.5 ml of *tert*-butylmethylether, vortex-mixed for 7 min, and centrifuged for 5 min. Five milliliter of organic phase was back-extracted into 100 μl of 0.05% phosphoric acid by vortex-mixing for 3 min. And then 50 μl of aqueous phase was injected directly into the above HPLC system.

2.10. Pharmacokinetic parameters

The first-order terminal elimination rate constant (k_e) was estimated by linear regression from the points describing the elimination phase on a log-linear plot. Half-life ($t_{1/2}$) was derived from the rate constant ($t_{1/2} = \ln(2)/k_e$). The maximum observed plasma concentration (C_{max}) and the time taken to achieve this concentration (T_{max}) were obtained directly from the curves. The $AUC_{0-2\,h}$ was calculated using the trapezoidal formula.

2.11. Statistical analysis

The pharmacokinetic variables from three dosage forms were compared with a one-way ANOVA, which followed by a posterior testing with an unpaired *t*-test using the Bonferroni correction. A *P*-value of less than 0.05 was considered significant.

3. Results and discussion

3.1. Partition coefficient, solubility and precipitate formation of ondansetron hydrochloride

To predict ondansetron hydrochloride permeability through nasal mucosa, the partition coefficient was determined using isopropyl myristate and *n*-octanol; the partition coefficients were calculated to be 0.007 ± 0.001 and 1.60 ± 0.12 and, respectively, which was not high. Even though lipophilicity is one of the determinants affecting permeability of drug through membranes, and partition coefficient is closely related to the lipophilicity, it was reported that some drugs with low lipophilicity showed good permeation profile through nasal mucosa due to its relatively large surface area and thin thickness (Chien et al., 1989).

To design nasal delivery systems, it is necessary to have vehicles to solubilize drug amount enough to administer for therapeutic effect. Considering that the intravenous dose of ondansetron hydrochloride is 4 mg and optimal spray volume is 100 μl, vehicles which can dissolve 4 mg with 100 μl are required. The solubility (mg/ml) of ondansetron hydrochloride at 37 °C in various vehicles decreased in the rank order of PG (282.9 ± 3.4) ≫ water (36.1 ± 1.9) > PEG 300 (31.7 ± 1.4) > ethanol (20.9 ± 1.8) > DGME (18.1 ± 3.8) > PEG 400 (13.0 ± 0.6) > 0.9% saline (11.2 ± 0.5).

The solubility of ondansetron hydrochloride was affected by buffers and buffer concentrations. Compared to citric acid buffer, phosphoric acid buffer showed high solubility, and the solubility of ondansetron hydrochloride decreased as the molar concentration of buffers increased; those in phosphate and citrate buffers at the molar concentrations of 0.02, 0.05 and 0.10 were 33.4 ± 0.9, 25.7 ± 1.2 and 9.4 ± 0.8, and 23.9 ± 1.3, 12.4 ± 2.1 and 8.2 ± 1.4 mg/ml, respectively.

PG was considered as a good candidate of vehicle for the formulation of ondansetron nasal delivery systems due to the viscosity, which could prevent nasal mucosa dryness as well as its relatively high solubility. In water-PG mixture, as the concentration of PG increased, the solubility of ondansetron hydrochloride markedly increased; the solubilities of ondansetron hydrochloride in aqueous solutions containing 20, 40, 60, 80 and 100% PG were 44.5 ± 0.5, 59.9 ± 0.8, 70.6 ± 1.9, 81.3 ± 0.2 and 282.9 ± 3.4, respectively. PEG 300 was also evaluated as a candidate vehicle because of the property of moisturizing the nasal mucosal surface. As the composition of nasal delivery systems were preferably adjusted to a weak acidity of pH 4.0–6.0 so as to increase the chemical stability of the active ingredient and aid to prevent a growth of microorganism, PEG 300 was added to 0.02 M phosphate buffer (pH 6.0). The solubilities of ondansetron hydrochloride in phosphate buffers containing 0, 5, 10, 20 and 100% PEG 300 were calculated to be 33.4 ± 2.8, 38.9 ± 1.4, 40.7 ± 0.3, 39.4 ± 2.1 and 31.7 ± 1.4, respectively.

We further examined the effects of nicotinamide (Sanghvi et al., 2007), Kollidon 30 and cyclodextrins (Loftsson and

Duchêne, 2007) as solubilizers on the solubility of ondansetron hydrochloride. The solubility of ondansetron hydrochloride increased 1.9, 2.2, 2.1 and 4.8 times by the addition of 15% of 2HPßCD (115 mM), SBCD (69.4 mM), DMCD (112.7 mM) and nicotinamide (1227.6 mM), compared to that in water.

Even though drug dose enough to achieve the effective drug concentration is required, high drug concentration can cause precipitate formation; precipitates formed during storage can plug the orifice of the nozzel, and delay the dissolution and absorption. No precipitation was observed in aqueous solution with 10% PEG 300 and 0.01% BC at the drug concentration of 10 mg/ml al least up to 21 days. However, at the drug concentration of 20 mg/ml, precipitation started to be formed at least 1 day after storage, and the formation of precipitates was dependent on the drug concentrations. The addition of SBCD at the concentration of 5% inhibited the formation of precipitates at the drug concentration of 20 mg/ml and delayed precipitation at the higher drug concentrations (30 and 40 mg/ml), however, failed to completely prevent precipitation for the study period. By adding 10% of SBCD or DMCD, precipitation could be prevented at least up to 3 weeks at 40 mg/ml of drug concentration.

3.2. In vitro permeation profiles

After applying drug to mucosa, it is critical to be stable physicochemically and enzymatically for a certain period of time. It was found that ondansetron hydrochloride was stable up to 4 h in nasal mucosa extract; with the starting concentration of 200 μg/ml, the amounts remaining at 0, 1, 2, 3, and 4 h were 198.6 ± 1.3, 199.0 ± 5.2, 203.5 ± 3.0, 196.9 ± 0.6 and 197.9 ± 9.6 μg/ml, respectively.

We compared the permeability profiles using various mucosae including nasal, duodenal, colonic and rectal mucosa. The permeation flux through the nasal mucosa was almost double those through duodenal and colonic mucosae, and four times higher than that through rectal mucosa; the permeation rates (μg/cm^2/h) of ondansetron hydrochloride at the concentration of 20 mg/ml was in the rank of nasal (134.85 ± 12.24) > duodenal (77.97 ± 19.10) ≥ colonic (76.77 ± 10.65) > rectal mucosa (36.23 ± 4.38). Drug permeation through nasal mucosa was so immediate that the lag time could not be calculated, and those through duodenal, colonic and rectal mucosa were 0.37 ± 0.04, 0.30 ± 0.03 and 1.81 ± 0.08 h, respectively. The dramatically short lag time from nasal mucosa indicated that the nasal delivery can exert its action very rapidly after administration. The high permeation rate and short lag time were thought to be due to the nasal cavity property in which it has a relatively high surface area and rich vasculature (Turker et al., 2004).

It has been suggested that approximately 160 cm^2 of nasal mucosa available for absorption, each cell in this area has about 120–200 cilia and there are microvilli between the cilia which greatly increase the surface area for absorption (Quraishi et al., 1997). In addition, the nasal cavity is highly vascularized, in which a lot of arteries are involved in supplying blood to the nasal cavity including external and internal carotid arteries, maxillary artery, ophthalmic artery, facial artery and palate artery (Behl et al., 1998).

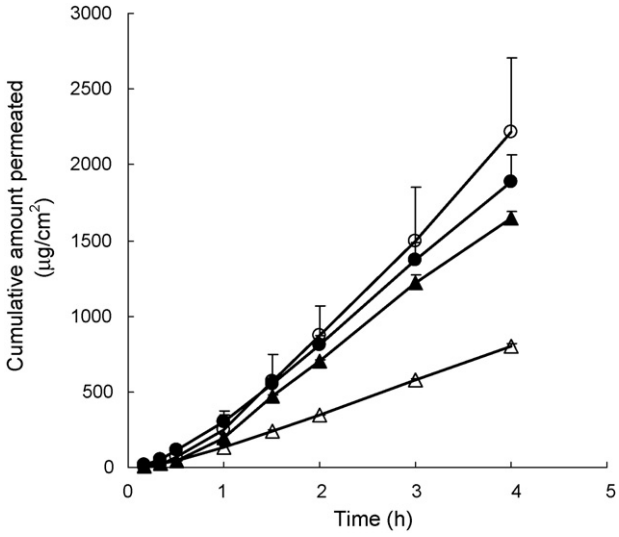

Fig. 1. Effect of 10% polyethylene glycol 300 and propylene glycol content (%) in aqueous solution containing 0.01% benzalkonium chloride and 10% sulfobutylether ß-cyclodextrin sodium salt on the permeation of ondansetron hydrochloride ($n = 3$). Key: ●, 10% polyethylene glycol 300; ○, 10% propylene glycol; ▲, 60% propylene glycol; △, 80% propylene glycol.

To examine the effects of various solvents with/without additives on the permeation of ondansetron hydrochloride, PG and PEG 300 were used to maintain the surface of the nasal mucosa to be wet so as to facilitate permeation of ingredient and deliver the effective dose of drug. Cyclodextrins including SBCD and DMCD, and nicotinamide for enhancing solubility, chitosan for improving drug attachment to nasal mucosa and BC as a preservative to prevent the composition from the microbial contamination were also employed. As depicted in Fig. 1, when PG or PEG 300 was added at the concentration of 10% to ondansetron hydrochloride (40 mg/ml) aqueous solution containing 0.01% BC and 10% SBCD, the permeation rate with the addition of PEG 300 was somewhat more rapid up to 1.5 h. The higher initial permeation rate with PEG 300 was attributable to low solubility of ondansetron hydrochloride in PEG 300 (31.65 ± 1.41 mg/ml) compared to that in PG (282.94 ± 3.37 mg/ml), indicating that thermodynamic activity could be one of the factors affecting permeation. As the PG concentrations increased such as 10, 60 and 80%, the permeation flux decreased suggesting that thermodynamic activity decreased due to higher solubility.

The effects of drug concentration on the permeation of ondansetron hydrochloride using various vehicles were examined. As shown in Fig. 2, the permeation flux increased as the drug concentration increased regardless of the vehicles used. Compared to PG-containing vehicles, PEG 300-containing vehicles showed higher fluxes at the all drug concentrations tested. In vehicles containing 10% PEG 300 and 0.01% BC, the addition of 1% chitosan and 5% nicotinamide resulted in higher permeation rate at the lower concentrations (10, 20 mg/ml), however, at the concentration of 40 mg/ml, vehicles without 1% chitosan and 5% nicotinamide showed higher permeation rate. Considering solubility difference of ondansetron hydrochloride in aqueous solution with 10% PEG 300 and 0.01% BC,

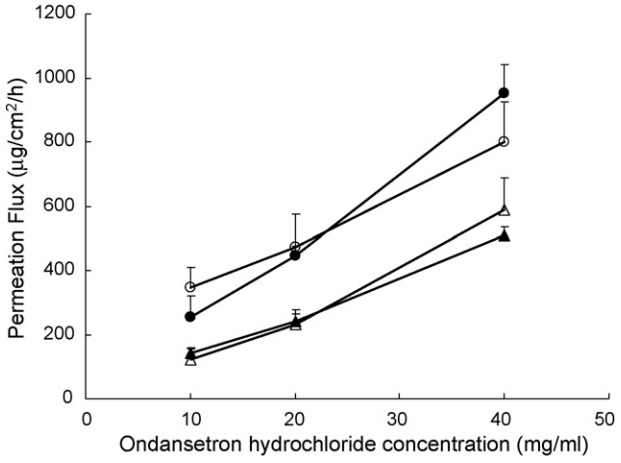

Fig. 2. Dose-dependent permeation flux in various vehicles ($n = 3$). Key: ●, aqueous solution containing 10% polyethylene glycol 300 and 0.01% benzalkonium chloride; ◯, aqueous solution containing 10% polyethylene glycol 300, 0.01% benzalkonium chloride, 1% chitosan and 5% nicotinamide; ▲, aqueous solution containing 60% propylene glycol and 0.01% benzalkonium chloride; △, aqueous solution containing 10% propylene glycol, 0.01% benzalkonium chloride and 10% sulfobutylether ß-cyclodextrin sodium salt.

and aqueous solution with 10% PEG 300, 0.01% BC, 1% chitosan and 10% nicotinamide, which were 38.8 ± 0.6 and 90.9 ± 1.4 mg/ml, respectively, the rapid increase of permeation rate in aqueous solution with 10% PEG 300 and 0.01% BC at the drug concentration of 40 mg/ml was thought to be due to the high thermodynamic activity.

To study the effects of nicotinamide and chitosan on the permeation of ondansetron hydrochloride, 5% nicotinamide or 1% chitosan was added to aqueous solution with 10% PEG 300 and 0.01% BC; drug concentration used for this experiment was 40 mg/ml. The permeation flux of 953.43 ± 87.64 μg/cm^2/h was obtained with aqueous vehicle containing 10% PEG 300 and 0.01% BC. The addition of nicotinamide or chitosan decreased permeation rate to 378.25 ± 34.17 or 537.55 ± 125.39 μg/cm^2/h, respectively. The lag time increased with the addition of nicotinamide or chitosan; it was 0.002, 0.28 and 0.41 without nicotinamide and chitosan, with nicotinamide and with chitosan, respectively. The decreased flux and the increased lag time by the addition of chitosan were possibly because of the increased viscosity. Harris et al. (1989) suggested that the addition of viscous agent to nasal formulation may produce a more sustained effect, delay the onset of activity while no enhancement was achieved in the total bioavailability. On the other hand, the decreased permeation by the addition of nicotinamide was considered to be due to the stacking complexation of drug molecule with nicotinamide (Sanghvi et al., 2007) and decreased concentration of free drug molecule. It was reported that the absorption percent followed an inverse relationship with molecular weight (McMartin et al., 1987). As depicted in Fig. 3, the permeation rate was almost half by the addition of only 1% nicotinamide compared to no nicotinamide. The further decrease by the increased concentration of nicotinamide was minimal indicating that the addition of nicotinamide itself can greatly affect the permeation of ondansetron hydrochloride.

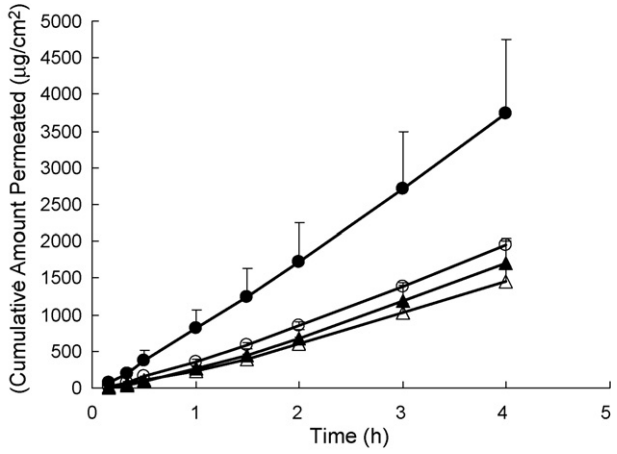

Fig. 3. Effect of various concentrations of nicotinamide in aqueous solution containing 10% polyethylene glycol 300 and 0.01% benzalkonium chloride on the permeation of ondansetron hydrochloride ($n = 3$). Key: ●, 0%; ◯, 1%; ▲, 3%; △, 5%.

Cyclodextrins have been employed as the permeation enhancers and drug solubilizers of many drugs including peptide and protein drugs (Merkus et al., 1999; Choi and Chun, 2001; Loftsson and Duchêne, 2007). Among cyclodextrins, SBCD is safety-proved material, which is contained in Sporanox® tablet produced by Janssen. Also, methylated ß-cyclodextrins were proved to be the effective permeation enhancers of the peptide and protein drugs and suitable to human use (Hermens et al., 1991; Matsubara et al., 1995; Merkus et al., 1991; Schipper et al., 1995). Especially, DMCD was studied as the effective permeation enhancer of 17-ß-estradiol and insulin (Hermens et al., 1991; Merkus et al., 1991).

We employed SBCD and DMCD at the concentration of 10% as the enhancers for both of permeation and solubility of ondansetron hydrochloride, which revealed to inhibit precipitate formation at drug concentration of 40 mg/ml and up to 3 weeks. As shown in Fig. 4, to the aqueous solution composed of 10% PEG 300 and 0.01% BC containing 40 mg/ml ondansetron hydrochloride, the addition of cyclodextrins including DMCD or SBCD did not increase the permeation rate of ondansetron,

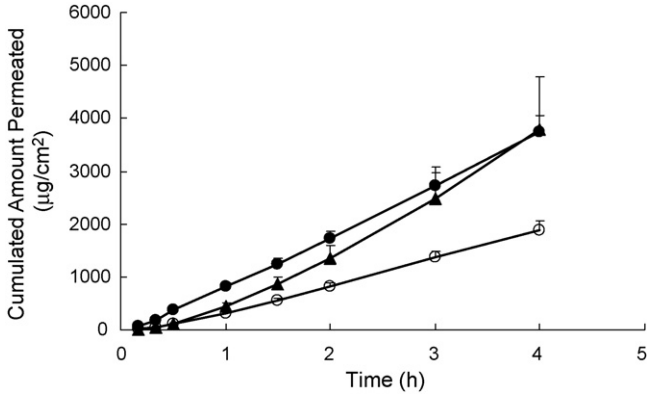

Fig. 4. Effect of cyclodextrins in aqueous solution containing 10% polyethylene glycol 300 and 0.01% benzalkonium chloride on the permeation of ondansetron hydrochloride ($n = 3$). Key: ●, without cyclodextrin; ◯, sulfobutylether ß-cyclodextrin sodium salt; ▲, dimethyl-ß-cyclodextrin.

Table 1
Residual concentration (%) of ondansetron hydrochloride in an aqueous solution containing polyethylene glycol 300 (10%), benzalkonium chloride (0.01%) and sulfobutyl ether ß-cyclodextrin sodium salt (10%) at various temperatures

Time (day)	Percent remaining		
	35 °C	45 °C	60 °C
0	100	100	100
4	97.7 ± 1.2	97.6 ± 1.2	97.1 ± 1.9
10	95.3 ± 2.6	93.9 ± 1.9	93.5 ± 0.3
18	91.5 ± 0.6	87.5 ± 4.0	86.8 ± 0.1

The initial drug concentration was 40 mg/ml. Data were expressed as the mean ± S.D. ($n = 3$).

and lag time was prolonged by the addition of cyclodextrins. Permeation fluxes were calculated to be 953.43, 469.91 and 906.30 μg/cm^2/h and lag times were 0.002, 0.28 and 0.82 with no cyclodextrins, SBCD and DMCD, respectively. Even though cyclodextrins failed to show permeation enhancing effects of the ondansetron hydrochloride, the addition of 10% SBCD to aqueous solution containing 10% PEG 300 and 0.01% BC could be a good candidate for ondansetron nasal delivery systems because of its safety profile, excellent solubilizing effect, safe storage in refrigerator, and, compared to the DMCD, rapid onset of action.

The tight junctions of the nasal epithelium are known to be located at the apical side of the respiratory epithelium. At the location of the tight junction the membranes are in close proximity. In a study of the effects of cyclodextrins on the structural change in tight junction of the nasal epithelium, no structural change was found after administration of 2% randomly methylated ß-cyclodextrins (Marttin et al., 1999). From the results of a study using water-soluble cyclodextrins at various concentrations, it was found that less than 20% (w/v) solutions of 2-HPßCD and 10% randomly methylated ß-cyclodextrins did not induce gross tissue damage, and *in vivo* study, repeated randomly methylated ß-cyclodextrins did not cause irritation to the nasal mucosa (Asai et al., 2002).

Table 1 shows the stability profile of ondansetron hydrochloride in aqueous solution containing 10% PEG 300, 0.01% BC and 10% SBCD at various temperatures (35, 45 and 60 °C). Ondansetron hydrochloride was stable for at least 18 and 10 days at 35 °C, and higher temperature (45 and 60 °C), respectively.

3.3. *In vivo* pharmacokinetic results

Based on the *in vitro* results from permeation of ondansetron hydrochloride nasal delivery systems, aqueous solution containing 10% PEG 300, 0.01% BC and 10% SBCD was employed as vehicles for the *in vivo* pharmacokinetic study of ondansetron nasal delivery system.

As shown in Table 2 and Fig. 5, ondansetron was rapidly absorbed through nasal route. The mean C_{max} of ondansetron by nasal route was found to be 1.7 times higher than that by oral route, which was statistically significant ($P < 0.05$). The $AUC_{0-2\,h}$ after nasal administration was significantly higher than that after oral administration ($P < 0.01$) and comparable to that after intravenous administration. The time to reach C_{max} after nasal administration was somewhat faster than that by oral administration even though the difference was not significant.

Table 2
Pharmacokinetic parameters of ondansetron hydrochloride after intravenous, intranasal, and oral administration in rats

Route	Intravenous	Oral	Nasal
N	6	11	11
C_{max} (ng/ml)	–	29.7 ± 10.6	49.4 ± 18.2*
T_{max} (min)	–	11.8 ± 1.82	9.4 ± 1.83
$AUC_{0-2\,h}$ (ng h/ml)	51.1 ± 7.4	24.9 ± 12.6	53.2 ± 9.5**
$AUC_{oral,nasal}/AUC_{iv}$	–	0.49	1.04

Data were expressed as the mean ± S.E. Statistically significant difference from oral administration ($^*p < 0.05$, $^{**}p < 0.01$).

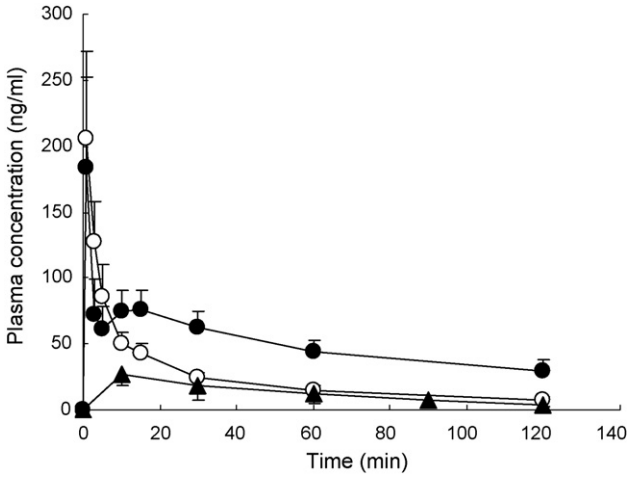

Fig. 5. Mean plasma concentration–time curves of ondansetron in rats. Data points represent as the mean ± S.E. ($n = 11$ for oral and nasal administration, $n = 6$ for intravenous administration).

In conclusion, the nasal delivery system of ondansetron hydrochloride formulated in this study was feasible for nasal administration, and was expected to rapidly exert its antiemetic effect. In addition, since bioavailability of ondansetron as an active ingredient was highly improved, the desired effect would be attained even by administering relatively small amount of active ingredient. Furthermore, the nasal delivery system might completely avoid the discharge of the active ingredient from stomach and the unfavorable effects such as local pain or skin rash.

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