



Development of udenafil-loaded microemulsions for intranasal delivery: *In vitro* and *in vivo* evaluations

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ABSTRACT

To achieve rapid onset of action and improved bioavailability of udenafil, a microemulsion system was developed for its intranasal delivery. Phase behavior, particle size, transmission electron microscope (TEM) images, and the drug solubilization capacity of the microemulsion were investigated. A single isotropic region was found in pseudo-ternary phase diagrams developed at various ratios with Cap-Mul MCM L8 as an oil, Labrasol as a surfactant, and Transcutol or its mixture with ethanol (1:0.25, v/v) as a cosurfactant. Optimized microemulsion formulations with a mean diameter of 120–154 nm achieved enhanced solubility of udenafil (>10 mg/ml) compared with its aqueous solubility (0.02 mg/ml). An *in vitro* permeation study was performed in human nasal epithelial (HNE) cell monolayers cultured by the air–liquid interface (ALI) method, and the permeated amounts of udenafil increased up to 3.41-fold versus that of pure udenafil. According to the results of an *in vivo* pharmacokinetic study in rats, intranasal administration of udenafil-loaded microemulsion had a shorter T_{max} value (1 min) compared with oral administration and improved bioavailability (85.71%) compared with oral and intranasal (solution) administration. The microemulsion system developed for intranasal administration may be a promising delivery system of udenafil, with a rapid onset of action and improved bioavailability.

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1. Introduction

Erectile dysfunction (ED) is a common disorder that increases in incidence with age (Prins et al., 2002); indeed, up to 50% of men between 40 and 70 years old could be affected (Feldman et al., 1994; Johannes et al., 2000). ED is defined as the inability to induce or maintain an erection sufficient for satisfactory sexual performance. Relaxation of the smooth muscles of the corpus cavernosum is required for an erection. Cyclic guanosine monophosphate (cGMP) levels are important for the maintenance of an erection because, together with nitric oxide (NO), cGMP is involved in the relaxation of the smooth muscles of the corpus cavernosum. It is known that phosphodiesterase type 5 (PDE5) has a catalytic action on cGMP. The structure of PDE5 inhibitors are similar to the cGMP molecule and compete with cGMP at the catalytic site of PDE5, thus increasing cGMP levels and the erectile process (Salem et al., 2006).

Udenafil, (5-[2-propyloxy-5-(1-methyl-2-pyrrolidinylethylamidosulphonyl)phenyl]-1-methyl-3-propyl-1,6-dihydro-7H-pyrazolo(4,3-d)pyrimidin-7-one), is a potent and selective

inhibitor of PDE5, an enzyme that catabolizes cyclic guanosine monophosphate (cGMP) in the corpus cavernosum (Oh et al., 2000; Doggrell, 2007). Udenafil (Zydena[®]) was developed by Dong-A Pharmaceutical Company (Yongin, Republic of Korea) for oral administration to male patients suffering from ED. Although it has not yet been approved by the U.S. Food and Drug Administration (FDA) nor the European Medicines Agency (EMA), it has been approved by the Korean Food and Drug Administration (KFDA) and is currently used in the Republic of Korea. The absolute oral bioavailability of udenafil at a dose of 30 mg/kg was 38.0% in rats (Shim et al., 2003), but to our knowledge, the value in humans has not been reported. Absorption of udenafil from rat intestinal tract is known to be complete (about 99% of an oral dose of 30 mg/kg; Shim et al., 2003). Thus, the relatively low bioavailability (38%) in rats may be related to the hepatic, gastric, and intestinal first-pass effects (Shim et al., 2003). It was also reported that the bioavailability of udenafil can be altered by P-glycoprotein (P-gp) and/or CYP3A4 and they may induce changes in the pharmacokinetics of udenafil in patients (Ji et al., 2007). T_{max} of udenafil in rats has been reported to be 35.5 ± 12.1 min at a dose of 30 mg/kg (Shim et al., 2003), whereas that in humans is 1.5 h (1–3 h) at a 100-mg dose in healthy males (Bae et al., 2008). However, this T_{max} value needs to be shortened to satisfy patient demand in the erectogenic market, not least because avanafil, a competitor, is a short-acting drug that

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exerts its pharmacological effect within 30 min (Hatzimouratidis and Hatzichristou, 2008).

In comparison with oral administration, intranasal drug delivery may provide an improvement in bioavailability while shortening T_{\max} (Costantino et al., 2007; Ugwoke et al., 2001). This has already been demonstrated in a study comparing the nasal and oral administration of sildenafil citrate, another PDE5 inhibitor (Elshafeey et al., 2009). Thus, it is expected that intranasal delivery of udenafil may also achieve rapid onset of action and improved bioavailability by avoiding the first-pass effect in the liver and intestine (Romeo et al., 1998).

Enhancement of drug solubility is necessary for the intranasal delivery of udenafil. Because its aqueous solubility is only 0.02 mg/ml, a microemulsion system may be an effective strategy to overcome this low aqueous solubility. A microemulsion is defined as a thermodynamically stable and transient dispersion consisting of oil, surfactant, cosurfactant, and aqueous phases (Eccleston, 1992). The advantages of microemulsion as a drug delivery system are the enhancement of drug solubilization and absorption across mucosal membranes (Gao et al., 1998). Here, we report the development of an udenafil-loaded microemulsion system for intranasal delivery and characterization of its physicochemical properties. Its permeation-enhancing effect and improved bioavailability were also evaluated *in vitro* in cell monolayers and *in vivo* in an animal model.

2. Materials and methods

2.1. Materials

Udenafil (DA-8159) and sildenafil citrate (internal standard, IS) were provided by Dong-A Pharmaceutical Company (Yongin, Korea). Polyethylene glycol (PEG)-8 caprylic/capric glycerides (Labrasol) and oleoyl macroglycerides (Labrafil M 1944 CS) were provided by Gattefossé (Saint-Priest, France). Glycerol monocaprylate (CapMul MCM L8) was also provided by ABITEC (Columbus, OH, USA). Polyethylene glycol 400 (PEG 400) was purchased from Duksan Pure Chemical, Ltd. (Ansan, Korea). Polyoxyethylene sorbitan monooleate (Tween 80) was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). All other reagents were analytical grade or better and were obtained from Sigma–Aldrich (St. Louis, MO, USA).

2.2. Solubility studies

The solubility of udenafil was determined in various oils and surfactants, such as isopropyl palmitate, isopropyl myristate, Labrafil M 1944CS, CapMul MCM L8, oleic acid, Labrasol, Tween 80, PEG 400, polyethylene glycol (PG), Cremophore EL, and Transcutol. An excess amount of udenafil was added to these oils and surfactants. After shaking at 25 °C for 24 h, the samples were centrifuged (16,100 × g, 10 min). The supernatant was diluted with methanol. The concentration of udenafil was then determined using high-performance liquid chromatography (HPLC) to select the oil and surfactant components that had a high solubilizing capacity for udenafil.

Udenafil was quantitatively analyzed using a HPLC system (Waters Co., Milford, MA, USA) equipped with a reversed-phase C-18 column (Fortis Universil C18, 150 × 4.6 mm, 5 μm, Fortis, UK), a pump (Waters 515 HPLC pump), an automatic injector (Waters 717plus auto sampler), and UV/vis detector (Waters 2487). A mixture of 20 mM KH_2PO_4 :acetonitrile (70:30, v/v) was used as the mobile phase at a flow rate of 1.0 ml/min. Precision and accuracy values were within acceptable ranges (<10%).

2.3. Construction of phase diagram

Pseudo-ternary phase diagrams were constructed using the water titration method. Surfactant (Labrasol) and cosurfactant (Transcutol or ethanol) were mixed (S_{mix}) in different weight ratios (1:1 and 2:1). Oil (CapMul MCM L8) and S_{mix} (Labrasol and Transcutol) were mixed thoroughly in different weight ratios, from 1:9 to 9:1, in different glass vials. As more and more water was added, the color changed from transparent to opaque. By drawing the color-change points, the boundaries of phases formed were obtained in the phase diagrams. All samples exhibiting a transparent and homogeneous state were assigned to a microemulsion region, a monophasic area, in the phase diagram (Gatri et al., 2000; Hathout et al., 2010). After acquiring the microemulsion region in the phase diagrams, the desired component ratio was determined. Udenafil-loaded microemulsions were prepared by dissolving udenafil into the microemulsion system.

2.4. Characterization of the microemulsion

The mean diameter, polydispersity index, and zeta potential of microemulsions were measured with a light-scattering spectrophotometer ELS-Z (Otsuka Electronics, Tokyo, Japan) according to the manufacturer's operation manual. The viscosity of the microemulsions was measured with a DV-E Viscometer (Brookfield Engineering Laboratories, Middleboro, MA, USA) using a 61 spindle at a speed of 100 rpm, room temperature, and over 10% torque. The morphological shape of the lipid droplets was observed by TEM. Microemulsions were placed on copper grids with films and dried in air for 10 min. They were then observed using a transmission electron microscope (JEM 1010, Jeol, Japan).

2.5. *In vitro* permeation study in HNE cell monolayers cultured by the ALI method

HNE cells were isolated and cultured for the transport study by the ALI culture method, as described in our previous reports (Cho et al., 2010, 2011; Lee et al., 2005; H. Lin et al., 2007). When HNE cells reached about 70–80% confluency, cells were detached and seeded on Transwells at a density of 1.5×10^5 cells/cm². The apical (0.5 ml) and basolateral side (1.5 ml) of the Transwell were filled with BEGM:DME/F12 (50:50), supplemented with a BEGM bullet kit (Cambrex Bio Science, Inc., Walkersville, MD, USA). Cell-culture media were replaced with fresh media after 1 day, and the cell culture medium in the basolateral side was changed every other day. The incubator was maintained at 37 °C in 5% CO₂ atmosphere at 95% relative humidity. An *in vitro* permeation study was performed on the HNE cell monolayers cultured by the ALI method for 10–14 days. The transepithelial electrical resistance (TEER) value was measured using an EVOM voltohmmeter device (WPI, Sarasota, FL), and Transwell inserts with TEER values higher than 500 Ω cm² were chosen for the drug-permeation study. Udenafil (1 mg) with 0.1 ml of transport media as a control group, and 0.1 ml of F1 and F2 (udenafil concentration of 10 mg/ml) were loaded in the apical side of inserts to evaluate the permeation of udenafil in the apical to basolateral direction. A permeation study was performed in a shaking bath (37 °C, 50 rpm); aliquots (1 ml) were withdrawn from the basolateral compartment at determined times (30, 60, 90, 120 min), and they were analyzed quantitatively by the HPLC method described above.

2.6. *In vivo* toxicity testing by histological staining

Toxicity of the prepared microemulsion (F2) to the rat nasal epithelium was evaluated by hematoxylin and eosin (H&E) staining. Histological examination was performed by a slight modification of

a reported method (Young, 1981). Briefly, the head of the rat was amputated by guillotine 6 h after instillation of the microemulsion (F2) and normal saline into the right and left nostrils, respectively. The eyes, integument, lower jaw, brain, and pituitary were removed from the head. Then, the nasal cavity was gently washed with 10 ml of phosphate-buffered saline (PBS, pH 7.4) by means of a 10 ml syringe fitted with a 15 Gauge needle inserted into the posterior opening of the nasopharynx. After washing, the head was placed and fixed in 10% formalin for 5 days. Decalcification was performed in formic acid–sodium citrate for 7–10 days with frequent changes of solution. The decalcified head was cut perpendicular to the plane of the nasal septum with sharp razor blade. Embedding, sectioning, and staining with H&E were conducted using standard methods.

2.7. In vivo pharmacokinetic studies in rats

2.7.1. In vivo pharmacokinetic study

Male Sprague–Dawley (SD) rats (body weight, 250–300 g) were purchased from Orient Bio, Inc. (Seongnam, Korea). Rats were maintained in a light-controlled room kept at a temperature of $22 \pm 2^\circ\text{C}$ and relative humidity of $55 \pm 5\%$ (Animal Center for Pharmaceutical Research, College of Pharmacy, Seoul National University, Korea). The experimental protocols for the animal study were approved by the Animal Care and Use Committee of the College of Pharmacy, Seoul National University. The femoral artery was cannulated with a polyethylene tube (PE-50, Clay Adams, Parsippany, NJ) under anesthesia, and blood was collected from the cannula. F2 (10 mg/ml of udenafil in the microemulsion) was instilled into both nostrils of the rat at a dose of 0.83 mg/kg for the intranasal pharmacokinetic study. Blood samples were collected at predetermined times (1, 5, 15, 30, 60, 120, 180, 240, 360, 480 min) after intranasal administration. After each sampling, heparinized blood samples were centrifuged ($16,100 \times g$, 5 min). Then, the plasma samples were collected and stored at -70°C until analyzed. Pharmacokinetic parameters were calculated using the WinNonlin software (ver. 2.1; Scientific Consulting, Lexington, KY, USA).

2.7.2. LC–MS/MS analysis of udenafil in plasma

The udenafil concentration in rat plasma was analyzed by a previously reported method (Ku et al., 2011). Briefly, 20 μl of internal standard (IS, sildenafil) solution (1 $\mu\text{g}/\text{ml}$) and 100 μl plasma were mixed. After adding 1 ml of dichloromethane, the mixture was vortex-mixed for 10 min, followed by centrifugation ($16,100 \times g$, 10 min). The organic phase (0.9 ml) was transferred to a new tube and evaporated at 50°C under a gentle stream of nitrogen gas. The residue was reconstituted in 100 μl of acetonitrile and double distilled water (DDW) mixture (90:10, v/v) and centrifuged ($16,100 \times g$, 1 min). Supernatant (5 μl) was injected into the analytical column for quantitative analysis. The HPLC Agilent 1200 Series equipped with a binary pump, a degasser, and high-performance auto-sampler with a thermostatted column compartment were used. Chromatographic separations were performed using a mobile phase composed of acetonitrile and 10 mM ammonium acetate (90:10, v/v) at a flow rate of 0.2 ml/min. A Pursuit XRS C₁₈ column (50 mm \times 2.1 mm, 3 μm , Varian, Inc., Lake Forest, CA, USA) was used, and the column temperature was maintained at 35°C . An API 3200 triple quadrupole mass spectrometer (Applied Biosystems MDS SCIEX, Toronto, Canada) was operated with an electrospray ionization (ESI) interface in positive ionization mode. The working parameters of the mass spectrometer were as follows: 5500 V ion spray voltage, 91 V declustering potential for the analyte and 76 V for internal standard (IS), 550°C source temperature, 8.5 V entrance potential, 35 psi of ion source gas (gas 1), and 50 psi of ion source gas (gas 2). Quantification was performed in multiple-reaction monitoring (MRM) mode with specific ion transitions of protonated precursor ions to product ions at m/z 517.4 \rightarrow 283.1 for

Table 1

Solubility of udenafil in various vehicles at 25°C after incubating for 24 h.

Type	Vehicle	Solubility (mg/ml)
Oil	Isopropyl palmitate	0.50 ± 0.01
Oil	Isopropyl myristate	0.56 ± 0.02
Oil	Labrafil M 1944CS	1.30 ± 0.14
Oil	Capmul MCM L8	12.91 ± 1.97
Oil	Oleic acid	4.24 ± 6.48
Surfactant	Labrasol	2.53 ± 0.69
Surfactant	Tween 80	0.85 ± 0.89
Surfactant	PEG 400	6.98 ± 0.40
Surfactant	PG	4.50 ± 0.70
Surfactant	Cremonophore EL	0.55 ± 0.20
Surfactant	Transcutol	39.83 ± 4.29

Each value represents mean \pm SD ($n = 3$).

the analyte and at m/z 475.3 \rightarrow 100.0 for the IS. The optimized collision energy was 59 eV for udenafil and 43 eV for IS. The lower limit of quantification (LLOQ) was 0.5 ng/ml, and the mean correlation coefficient (r^2) for the calibration curve was over 0.999. Precision and accuracy values were within acceptable ranges, as reported previously (Ku et al., 2011).

2.8. Statistical analyses

Statistical analyses were performed using analysis of variance (ANOVA). All experiments were performed at least three times, and data are presented as means \pm standard deviations (SD).

3. Results and discussion

3.1. Solubility study

Determining the optimized microemulsion composition is necessary to achieve maximum udenafil solubility. Several kinds of oils and surfactants for the development of intranasal microemulsion systems such as isopropyl myristate, CapMul MCM L8, oleic acid, Tween 80, and Labrasol have been reported (Li et al., 2002; Zhang et al., 2004; Sharma et al., 2009; Vyas et al., 2005, 2006; Elshafeey et al., 2009). Solubilities of udenafil in these oils and surfactants are presented in Table 1. As shown in Table 1, the solubility of udenafil was high in CapMul MCM L8 (12.91 ± 1.97 mg/ml), which was previously used as the oil phase for the intranasal delivery of cabergoline (Sharma et al., 2009). Thus, CapMul MCM L8 was selected as the oil phase for the preparation of the microemulsion system. Labrasol has been used as a surfactant in various reports (Zhang et al., 2004; Vyas et al., 2005; Elshafeey et al., 2009) and also exhibits a permeation-enhancing effect in nasal drug delivery (Zhang et al., 2004); thus, it was selected as a surfactant. Transcutol, the commercial name for diethylene glycol monoethyl ether, was also added as a cosurfactant because it exhibited the highest solubility for udenafil.

3.2. Phase diagram study

Construction of pseudo-phase diagrams was performed to determine the region of microemulsion (Fig. 1). In Fig. 1, DDW, CapMul MCM L8, Labrasol, and Transcutol are referred to as H₂O, Oil, S, and CoS, respectively. The weight ratios of surfactant and cosurfactant were 2:1 and 1:1, respectively. Fig. 1(C) and (D) were drawn using ethanol as another cosurfactant. The cosurfactant consisted of Transcutol and ethanol (1:0.25, w/w), whereas the ratio between surfactant and cosurfactant was (C) 2:1 and (D) 1:1. It has been reported that a cosurfactant can reduce the bending stress at an interface and make the interfacial film sufficiently flexible to form nano-sized droplets from different curvatures (Kawakami et al.,

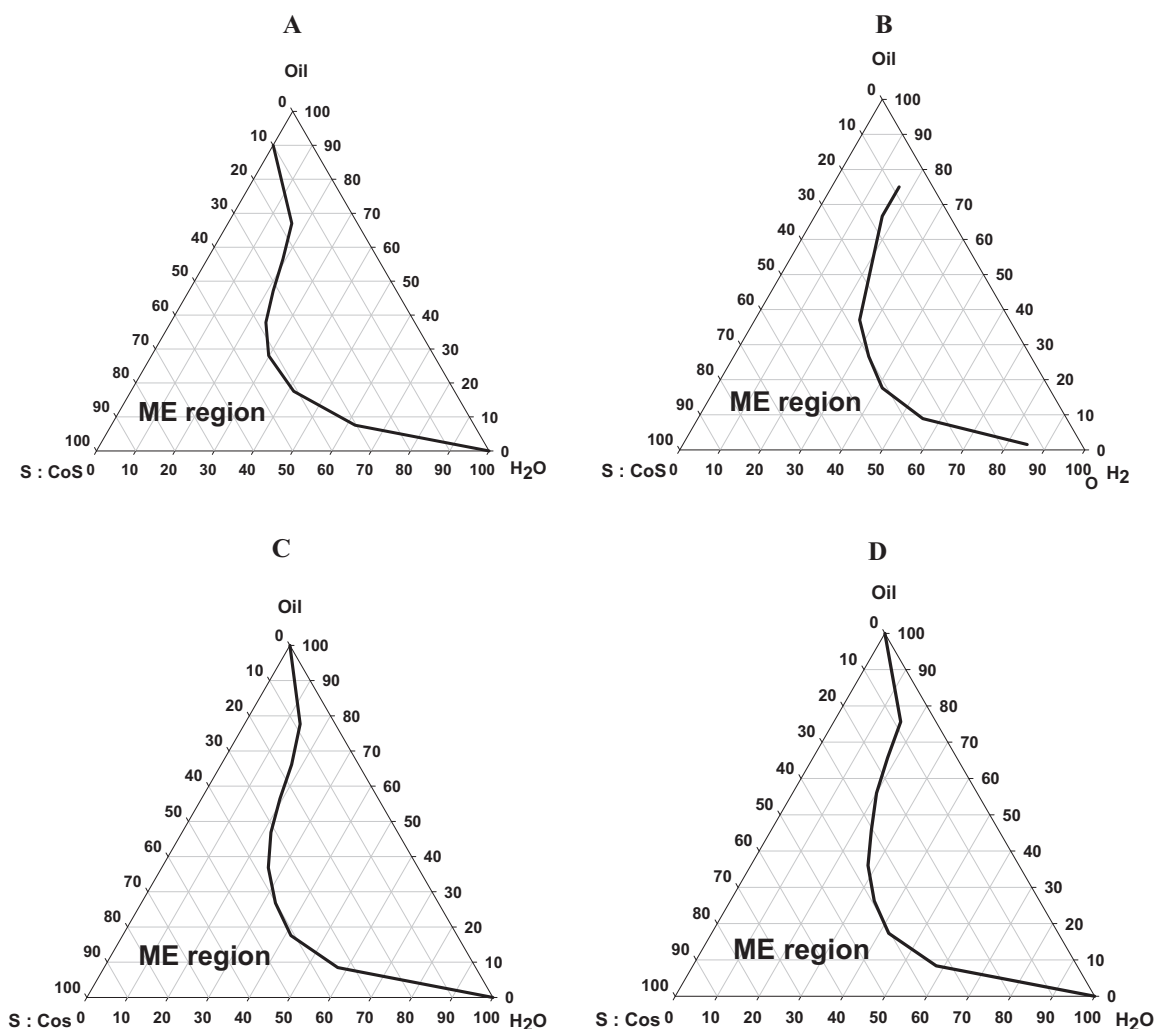


Fig. 1. Pseudo-ternary phase diagrams of microemulsions consisting of Labrasol and Transcutol as the surfactant and cosurfactant, respectively, at ratios of (A) 2:1 (w/w) and (B) 1:1 (w/w). The cosurfactant was composed of Transcutol and ethanol at a ratio of 1:0.25 (w/w) for (C) and (D), whereas the ratio of surfactant to cosurfactant was (C) 2:1 (w/w) and (D) 1:1 (w/w). CapMul MCM L8 was used as the oil phase for all microemulsion systems.

2002a; Shakeel et al., 2007). An optically transparent monophasic microemulsion was formed in a broad region. This area could be separated further into w/o or o/w microemulsion phases by simply considering the composition, that is, whether it was oil rich or water rich (Kawakami et al., 2002b). According to Winsor's report (Winsor, 1948), microemulsions can be classified into four classes. It is generally accepted that surfactants with a high hydrophilic–lipophilic balance (HLB) value (8–18) are preferred for the formation of o/w microemulsion systems (Lawrence and Rees, 2000). Labrasol, a medium-length alkyl chain surfactant with a high HLB value (14), may contribute to the formation of an o/w microemulsion. The other region of phase diagram is the turbid emulsion region, which is not monophasic. Based on the results in Fig. 1, a microemulsion was developed successfully regardless of the difference in the weight ratio between surfactant and cosurfactant. The compositions of F1 and F2 were determined, as shown in Table 2, for further studies.

3.3. Characterization of microemulsions

Because the nasal administration volume is limited (about 150 μ l in human), a high drug-solubilizing capacity is preferred (Gizurarson, 1993; Ugwoke et al., 2001). As shown in Table 3, two formulations achieved udenafil solubilities above 10 mg/ml,

whereas its aqueous solubility is only 0.02 mg/ml. The drug solubilization-enhancing effect of the microemulsion composed of surfactant/cosurfactant (1:1 ratio) was better than that of surfactant/cosurfactant (2:1 ratio; data not shown) because of the high solubility of udenafil in Transcutol among the solvents tested (Table 1). Thus, two microemulsion formulations based on a 1:1 ratio of surfactant/cosurfactant were selected. The formulations developed were stable at room temperature for 1 month, with no phase separation (data not shown). The average mean diameters of microemulsions measured by ELS ranged from 120 to 154 nm (Table 3). Particle sizes and the spherical shape of the lipid droplets were also confirmed by TEM images (Fig. 2). Zeta potential values of microemulsions exhibited neutral charge due to the combination of non-ionic surfactants. The viscosity of F2 was slightly lower than that of F1 because of the different weight ratio between Transcutol

Table 2
The compositions of microemulsions.

Formulation	Water (%)	Oil (%)	S (%)	CoS 1 (%)	CoS 2 (%)
F1	45	15	20	20	–
F2	45	15	17.8	17.8	4.4

O indicates for oil phase (CapMul MCM L8), S for surfactant (Labrasol), CoS 1 for cosurfactant 1 (Transcutol) and CoS 2 for cosurfactant 2 (ethanol).

Table 3
Physicochemical properties of udenafil-loaded microemulsions.

Formulation	Solubility (mg/ml)	Mean diameter (nm)	Polydispersity index	Zeta potential (mV)	Viscosity (cP)
F1	10.84 ± 0.36	153.53 ± 21.45	0.07 ± 0.02	-0.34 ± 0.47	15.06 ± 0.06
F2	13.04 ± 0.61	119.97 ± 8.39	0.09 ± 0.01	-0.26 ± 0.78	10.80 ± 0.06

Each value represents mean ± SD ($n = 3$).

and ethanol. The viscosity of a microemulsion can be affected by the component ratio and concentration of oil, water, and surfactant (Yuan et al., 2008). It is known that increasing the water or surfactant content can lower the viscosity of a microemulsion (El Maghraby, 2008; Hathout et al., 2010). It has also been reported that the viscosity of intranasal formulations can influence drug absorption across the nasal mucosa (Furubayashi et al., 2007). Due to mucociliary clearance in the respiratory epithelium, a high-viscosity formulation would tend to stay longer on the mucosa and increase the mean residence time in the nasal cavity. However, increasing the viscosity of the formulation applied can decrease the drug penetration rate across the mucus layer and lead to a delay in the drug's reaching the cellular surface. According to the reported relationship between the viscosity of formulation and nasal absorption (Furubayashi et al., 2007), it seems that the viscosity values in this investigation (10–15 cp) may contribute to the high nasal-absorption efficiency of the drug.

3.4. *In vitro* permeation study

The permeation-enhancing effect on udenafil of the microemulsion system developed was evaluated in HNE cell monolayers cultured by the ALI method. In our previous studies, this cell monolayer system was used for the evaluation of *in vitro* drug permeability with various formulations such as microspheres,

microparticles, and thermoreversible gels (Cho et al., 2010, 2011; Huh et al., 2010). It is expected that this system can be used as a screening system before any *in vivo* pharmacokinetic study, thereby reducing the financial cost and evaluation period. The amounts of udenafil that permeated were measured for a 2-h transport study with pure udenafil, F1, and F2. As shown in Fig. 3, the permeated amounts of udenafil from F1 and F2 were 2.95- and 3.41-fold higher than that of the pure udenafil group ($p < 0.05$). We suggest that ingredients in the microemulsion can be one of principal factors causing permeation-enhancing effects. The CapMul MCM concentration- and incubation-time-dependent permeability-enhancing effects of small chemicals and macromolecules in the intestine and colon have been reported previously (Yeh et al., 1995). It was reported that Labrasol can enhance drug absorption in various sites in the intestinal tract in rats (Y.L. Lin et al., 2007). Additionally, the apparent permeability coefficients (P_{app}) of poorly water-soluble drugs loaded with Transcutol were also increased in Caco-2 cell monolayers in several reports (Takahashi et al., 2002; Yin et al., 2009). It seems that the combination of these ingredients exerted a permeation-enhancing effect on udenafil in this investigation, although their individual effects have not yet been characterized in the nasal epithelium. In particular, the permeability of udenafil from F2 was higher than from F1 ($p < 0.05$), and this may be due to the permeation-enhancing effect of ethanol present only in F2. It has been reported that low

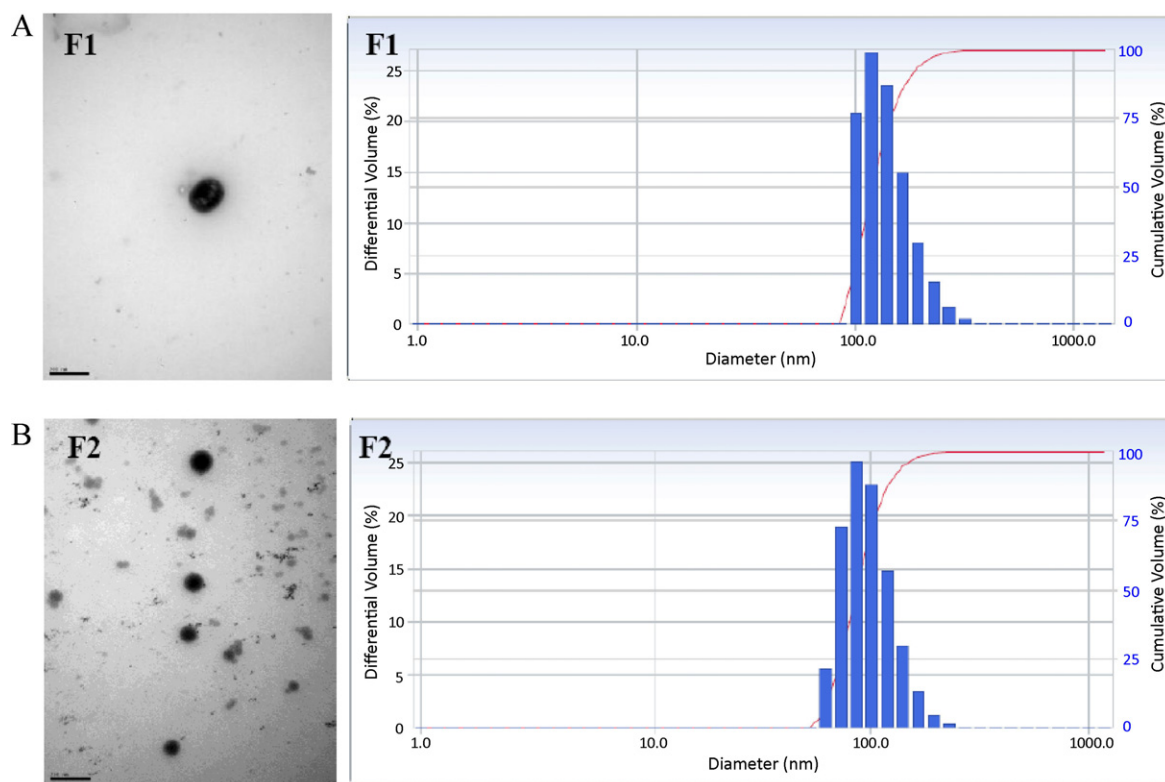


Fig. 2. Transmission electron microscopy (TEM) images (left panel) and volume-distribution diagrams of mean diameter (right panel) of (A) F1 and (B) F2, respectively. A 200-nm bar in the TEM images indicates 100,000-fold magnification.

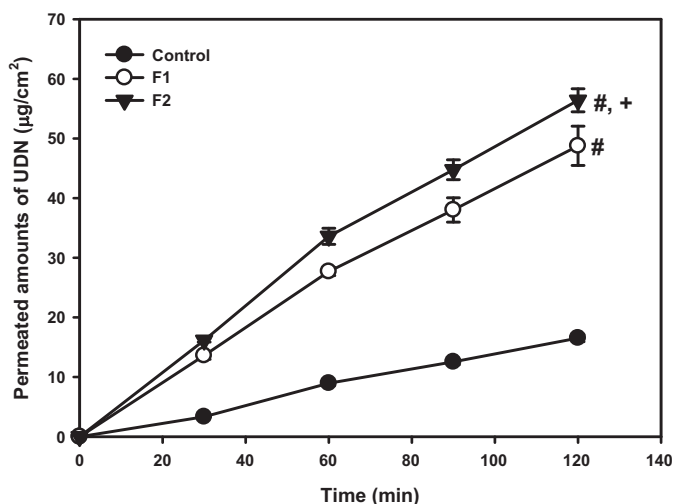


Fig. 3. *In vitro* permeation study of udenafil on HNE cell monolayers cultured by the ALI method. The corresponding weight for 1 mg of udenafil was added in the apical side of the Transwell insert covered with HNE cell monolayers. Each point represents mean \pm SD ($n = 3$). # $p < 0.05$, compared with control; * $p < 0.05$, compared with F1.

and non-toxic doses (<10%) of ethanol can induce the opening of tight junctions, resulting in increased drug permeability across the gastrointestinal epithelium (Volpe et al., 2008). Together with the absorption-enhancing effect of the ingredients of microemulsions, nano-sized oil droplets are also supposed to play a critical role in the enhancement of drug permeation (Koga et al., 2010). F2 was used in further *in vivo* studies because it exhibited a higher *in vitro* absorption-enhancing effect on udenafil.

3.5. *In vivo* toxicity testing

Toxicity of the microemulsion to the nasal epithelium was evaluated by histological staining after intranasal administration of F2. As described in Section 2.6, microemulsion (F2) and normal saline were instilled into right and left nostrils, respectively. An optical image of the nasal septum is presented in Fig. 4. The cilia in the nasal septum are apparently well preserved in the F2-treated nostril group, indicating that F2 has negligible toxicity to the nasal epithelium. In our previous study (Piao et al., 2010), we also reported that microemulsion-induced damage to the nasal epithelium was temporary and reversible. Thus, based on the safety of the microemulsion (F2) in the nasal epithelium in this study, its clinical use may be appropriate.

Table 4

Pharmacokinetic parameters of udenafil in rats.

Pharmacokinetic parameters	Intravenous ^a	Oral ^a	Intranasal ^a (solution)	Intranasal (microemulsion, F2)
T_{max} (min) ^b	–	30 (30)	1 (1–5)	1 (1)
C_{max} (ng/ml)	–	19.90 \pm 7.92	69.90 \pm 10.88	213.67 \pm 36.75
$AUC_{0-\infty}$ (ng min/ml)	21,831.60 \pm 2210.47	1317.20 \pm 273.09	4850.70 \pm 493.51	9355.83 \pm 1027.83
$AUC_{0-\infty}/dose$ (10^{-6} kg min/ml)	13,072.81 \pm 1323.64	788.74 \pm 163.53	2846.00 \pm 349.59*	11,204.58 \pm 1230.93**
Bioavailability (%) ^c	–	6.03	22.22	85.71

All data are presented as the mean \pm SD ($n = 3$ or 4). Dose (D) for intravenous, oral and intranasal (solution) route was 1.67 mg/kg and that for intranasal (microemulsion, F2) administration was 0.83 mg/kg.

^a Results of intravenous, oral and intranasal (solution) administration were cited from our previous report (Ku et al., 2011).

^b T_{max} values are expressed as median (range).

^c Bioavailability can be calculated by following formula:

$$\text{Bioavailability (\%)} = \frac{AUC_{PO(\text{or IN})}/D_{PO(\text{or IN})}}{AUC_{IV}/D_{IV}}$$

* $p < 0.01$, compared to oral administration.

** $p < 0.01$, compared to intranasal (solution) administration.

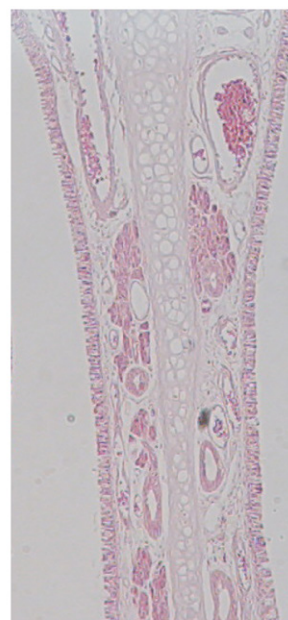


Fig. 4. *In vivo* toxicity testing of udenafil-loaded microemulsion (F2) in rats. Toxicity was evaluated by H&E staining. Normal saline and F2 were instilled into the left and right nostril, respectively.

3.6. *In vivo* pharmacokinetic studies

In vivo pharmacokinetic studies were performed in rats that remained conscious during the experiment. This conscious state may support the maintenance of functional mucociliary clearance during the whole process (Piao et al., 2010). The pharmacokinetic profile after intranasal administration of F2 was compared with our previously reported data on intravenous, oral, and intranasal (solution) administration (Ku et al., 2011). The mean plasma-concentration profiles of udenafil are shown in Fig. 5, and corresponding pharmacokinetic parameters and bioavailability values are presented in Table 4. After dose normalization, the bioavailability of F2 (85.71%) was higher than those of oral (6.03%) or intranasal (solution type, 22.22%) administration. The T_{max} of F2 (1 min) was comparable to that of the intranasal solution group (1 min) and shorter than that for oral administration (30 min). The microemulsion system developed demonstrated enhanced

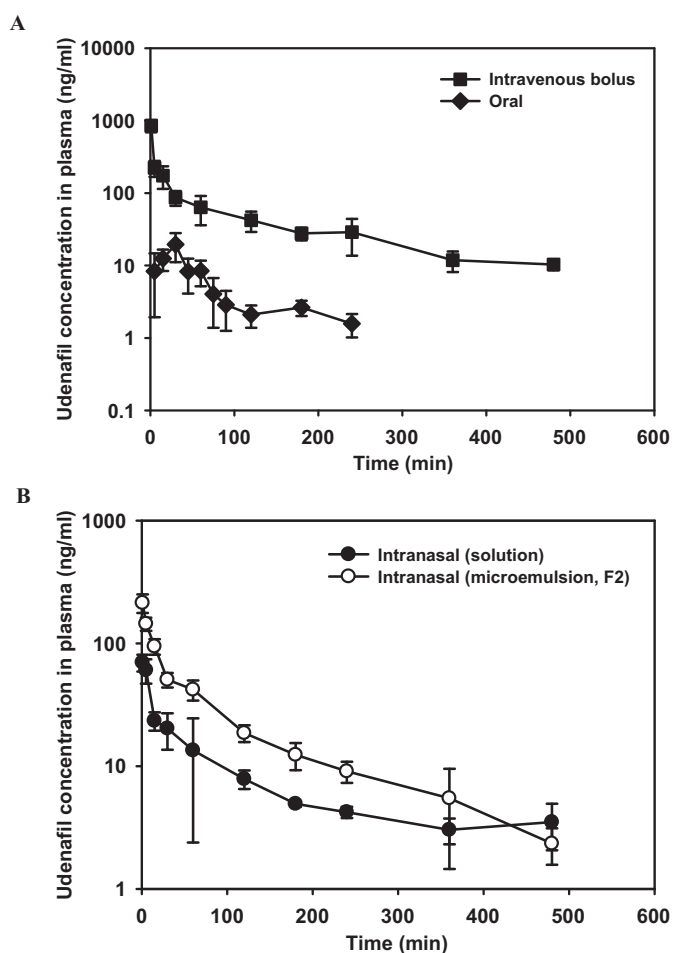


Fig. 5. *In vivo* pharmacokinetic profiles of udenafil in rats. The plasma concentration profiles of udenafil (A) after intravenous (■, 1.67 mg/kg), oral (◆, 1.67 mg/kg) administration and (B) intranasal administration of udenafil solution (●, 1.67 mg/kg) and F2 (○, 0.83 mg/kg) are shown. Each point represents mean \pm SD ($n=3$ or 4). Profiles of intravenous, oral, and intranasal (solution) administration were from our previous report (Ku et al., 2011).

bioavailability compared with the oral and intranasal (solution) groups and significantly rapid onset compared with the oral administration group.

The apical to basolateral P_{app} values of udenafil in Caco-2 cell monolayers in the range of 1–20 μ M were 2.9 – 9.6×10^{-6} cm/s, suggesting that it would be well absorbed in the intestinal tract (Ji et al., 2007). This suggests that the low bioavailability of udenafil after oral administration may be associated with hepatic and/or intestinal first-pass effects, not an absorption problem in the intestinal tract. It has been demonstrated that the low bioavailability of udenafil at a 30-mg/kg dose in rats was due primarily to a considerable intestinal first-pass effect (Shim et al., 2003). Intranasal administration can avoid these first-pass effects and guarantee improved bioavailability compared with oral administration. Moreover, the microemulsion formulation (F2) for intranasal delivery achieved a higher bioavailability of udenafil than did a solution, coinciding with the result of *in vitro* permeation (Fig. 3). It can be concluded that drug absorption-enhancing effect of the microemulsion led to improvement in bioavailability. Enhancement of bioavailability after intranasal administration of a microemulsion formulation has also been reported in other studies (Elshafeey et al., 2009; Li et al., 2002; Lu et al., 2011; Yu et al., 2011). In particular, the microemulsion system developed provided rapid onset of action and improved bioavailability. This udenafil-loaded microemulsion system may be

an effective intranasal dosage form for male patients suffering from ED.

4. Conclusions

Microemulsion systems consisting of CapMul MCM L8, Labrasol, Transcutol, ethanol, and DDW showed high solubilization capacities for udenafil. The development of optimized microemulsion formulations containing udenafil was confirmed in pseudo-ternary phase diagrams, and their physicochemical properties were characterized. A drug permeation-enhancing effect of the microemulsions was identified in a HNE cell monolayer system, cultured by the ALI method. Rapid onset of action and improved bioavailability after intranasal administration of an udenafil-loaded microemulsion were achieved, and negligible toxicity to the nasal epithelium was observed in the histopathological staining. The microemulsion system developed may be a convenient and effective intranasal formulation for the administration of udenafil.

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