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Formulation of film-coated tablets bioequivalent to soft gelatin capsules: Case studies on dutasteride and choline alfoscerate

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ABSTRACT

Formulation of film-coated tablets bioequivalent to soft gelatin capsules: Case studies on dutasteride and choline alfoscerate

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Formulation study of dutasteride (BCS class II) and choline alfoscerate (BCS class III) was conducted to develop film-coated tablets which are bioequivalent to commercially available gelatin capsules. Due to the solubility issue, commercial soft capsule (Avodart®) was dissolved it in oil phase. In the case of choline alfoscerate, it was very hygroscopic and was expected to cause diverse tablet processing problems. It was launched as the name of Gliatilin® soft capsule in which choline alfoscerate was dissolved in glycerin. Generally, the physical strength of gelatin shell is known to be weaken in high

temperature or can be damaged by external impact such as high pressure. Drug contained in soft capsule might leak out of the gelatin shell. To overcome the disadvantage of soft capsule, film-coated tablets were developed for dutasteride and choline alfoscerate. Cyclodextrin complexation technology was applied to enhance the solubility of dutasteride. The appropriate solubilizing agents were subsidiarily selected to increase the solubility. *In vitro* dissolution pattern for tablet preparation containing dutasteride-cyclodextin complex was shown to be similar to the soft capsules. AUC value was comparable to Avodart® in the *in vivo* pharmacokinetic study in beagle dogs. The hygroscopicity of choline alfoscerate could be controlled by the addition of Neusilin (magnesium aluminometasilicate) in tablet preparations. The amount and adding process of Neusilin to tablet were examined to confirm the physical stability and rapid disintegration of tablet. Choline alfoscerate film-coated tablet with optimized formulation of Neusilin was proved to be stable for 3 months under the accelerated condition. *In vivo* pharmacokinetic study in healthy Korean male volunteers was performed for choline alfoscerate tablet. The mean plasma concentration profile of choline was corrected by subtracting the endogenous choline level. The bioequivalence between the test tablet and the reference soft capsule of choline alfoscerate was confirmed. These results suggested that each tablet formulations of dutasteride and choline alfoscerate might be substituted for the soft capsule.

Keywords: Tablet; Soft capsule; Dutasteride; Choline alfoscerate; Formulation; Bioequivalent

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Part I. Formulation of a film-coated dutasteride tablet bioequivalent to a soft gelatin capsule (Avodart®): Effect of γ-cyclodextrin and solubilizers

Background

1.1. Pros and Cons of soft gelatin capsules

A soft gelatin capsule is usually consisted of outer gelatin shell and inner liquid core with active ingredient. It has developed as an effective pharmaceutical dosage form for especially poorly soluble drug and lots of them are commercially available. However, there are some advantages and disadvantage of soft gelatin capsules as follows [1].

Advantage)

- Improved bioavailability, as the drug is presented as a solubilized form in soft gelatin capsule
- Enhanced drug solubility. Protection from light and oxidation for active pharmaceutical ingredient (API)
- Consumer preference, masking odors and unpleasant tastes
- Offer opportunities for product differentiation to product line extension

Disadvantage)

- Highly sensitive to heat and humidity, stick together or even break open
- More costly, necessary for the special equipment to fill soft gelatin capsule
- Dietary restrictions, animal-free substitute gelatin capsule

1.2. Selection for solubilization method of dutasteride

Various solubilization technologies for poorly soluble drug are well known and applied to drug product. The low solubility of the pooly soluble drug often leads to poor bioavailability due to the insufficient exposure of dissolved drug portion in the small intestine. A commonly used simple technology to improve the solubility of drug is size reduction by milling. And also chemical modifications of the compound as salts, cocrystal and amorphous are tried for solubilization. Especially solid dispersion with polymer using solvent could be considered to enhance the drug solubility but it had some issues for physical stability of amorphous drug. It might be difficult for the solid dispersion with only small amounts of surfactant to expect the dutasteride solubility to increase very high. Emulsion type formulation for dutasteride could provide good content uniformity and high bioavailability, but it could need large amounts of excipients to adsorb oil portion to solid carrier. For this reason, its tablet size would be too large that elderly patients might feel difficult to swallow. On the other hand, cyclodextrin could solubilize insoluble compound by means of inclusion complexation and drug-cyclodextrin aggregates. Cyclodextrin was selected as an excipient for solubilization of dutasteride in this paper because it could make a function of a good diluent for the solidification process at the same time. α-, β-, γ-cyclodextrin were introduced into the GRAS list of the FDA, respectively. The water soluble polymer is known to have the inhibitory effect on drug nucleation and crystal growth. When cyclodextrin and water soluble

polymer were used together, synergic effect was expected on drug solubilization. The addition of surfactant could influence on the solubility of free drug dissociated with the dilution and degradation of drug-cyclodextrin complex in physiological condition. Thus, dutasteride-cyclodextrin complex was mixed with water soluble polymer and surfactant to enhance the drug solubility.

1.3. Surface coverage of hygroscopic drug particle by Neusilin

Most lubricants are fine powder enough to cover of drug particle surfaces with small amounts that they get rid of sticking, picking and even capping during tableting process. But over-lubrication of drug with lubricant results in weakening bonding between drug particles and causes to significantly reduce hardness of tablet. It also leads to the prolongation of disintegration time and decrease of the dissolution rate. From this point of view, it is very important to select the proper excipient that could efficiently cover the surface of hygroscopic drug particle without influencing on disintegration time. And an excipient should not negatively effect on the hardness of the tablet to avoid any issues during tableting process. Neusilin is a synthetic, amorphous form of magnesium aluminometasilicate and used in both direct compression and wet granulation as glidant. Neusilin has porous structure to protect sensitive API from moisture or adsorb high oily formulation to remain flowable.

Neusilin UFL2 is fine powder with submicron diameter and could be used more than 30% of tablet weight as excipient. It is expected to effectively cover the surface of hygroscopic drug due to its small particle size and high contents in tablet. Neusilin was selected as an excipient to understand the effect on the moisture stability of choline alfoscerate.

1.4. Pharmacokinetic study for endogenous compound

Particular attention should be paid to the investigation of pharmacokinetics of endogenous substance, which could already include the endogenous synthesis (homeostatic equilibrium) and supply by dietary route. Baseline concentration could be stable or could vary with age, diet, or could have a specific rhythm [2]. The correction of pharmacokinetic profile should be performed to determine the true concentration added by an exogenous drug dosing. Bioequivalence studies of endogenous studies were described in EMEA guideline on the investigation of bioequivalence [3].

- If the substance being studied is endogenous, the calculation of pharmacokinetic parameters should be performed using baseline correction so that the calculated pharmacokinetic parameters refer to the additional concentrations provided by the treatment.
- Factors that may influence the endogenous baseline levels should be controlled if possible (e.g. strict control of dietary intake)
- For endogenous substances, the sampling schedule should allow

characterisation of the endogenous baseline profile for each subject in each period.

- Often, a baseline is determined from 2-3 samples taken before the drug products are administered. In other cases, sampling at regular intervals throughout 1-2 day(s) prior to administration may be necessary in order to account for fluctuations in the endogenous baseline due to circadian rhythms.
- The additional concentrations over baseline provided by the treatment may be reliably determined.
- The exact method for baseline correction should be pre-specified and justified in the study protocol.
- In general, the standard subtractive baseline correction method, meaning either subtraction of the mean of individual endogenous pre-dose concentrations or subtraction of the individual endogenous predose AUC, is preferred.
- In rare cases where substantial increases over baseline endogenous levels are seen, baseline correction may not be needed.

Part I. Formulation of a film-coated dutasteride tablet bioequivalent to soft gelatin capsules (Avodart®): Effect of γ-cyclodextrin and solubilizers

1. Introduction

Dutasteride is a competitive inhibitor of type I and type II 5-α-reductases and is used to treat benign prostatic hyperplasia (BPH) and hair loss [4]. Studies have revealed that dutasteride can reduce fetal adrenal and prostate weight and can increase fetal ovarian and testis weight. It has been classified as pregnancy category X by the FDA; thus, women who are pregnant or may become pregnant must avoid taking and handling dutasteride.

Dutasteride is classified as Biopharmaceutics Classification System (BCS) class II and is commercially available in the market only as a soft gelatin capsule formulation due to its low aqueous solubility [4]. However, the physical strength of the gelatin shell could become weaker under high temperature, which might break the seam-line or deform the shape of the capsule. Additionally, the active ingredient could migrate into the gelatin shell [5]. Because dutasteride is readily absorbed through the skin, these issues can lead to various health problems. Therefore, developing a tablet form of dutasteride is required to enhance the safety of the drug. Additionally, improved patient compliance is expected with a smaller solid tablet than a soft gelatin capsule. Moreover, because dutasteride is commonly co-prescribed with other BPH medicines such as tamsulosin, it would be more convenient to formulate solid dosage forms for fixed-dose combinations with other drugs.

Previous studies on solubilization of dutasteride have been mainly focused on self-emulsifying drug delivery system (SMEDDS) technology [6-8], which

is an oil formulation suitable for soft capsule. To increase the bioavailability of various hydrophobic and poorly water-soluble drugs, the drugs can be formulated to form a complex with cyclodextrin (CD) as a solid dosage form, thereby enhancing their solubility and/or dissolution rate [9-15]. Because no covalent bonds are involved in the drug-CD complex formation, the complex can be easily dissociated in aqueous solution [16]. Moreover, diverse approaches have been attempted to further enhance the complexation efficacy, which include the addition of polymers [17], organic salts [18], and buffer [19] to the complexation media. Addition of a small amount of a water-soluble polymer to an aqueous complexation medium increases the complexation efficiency, which consequently can decrease the formulation bulk by reducing the amount of CD required [16]. Moreover, water-soluble polymers form complexes with various compounds and stabilize micelles and other types of aggregates in aqueous solutions [16, 20]. They are additionally capable of increasing the aqueous solubility of cyclodextrins without decreasing their complexing abilities [21]. Pharmaceutical polymers such as methylcellulose, hydroxypropylmethylcellulose and polyvinylpyrrolidone have traditionally been used to prevent drug nucleation and crystal growth by creating a polymeric network around growing crystals [22]. Thus, their addition leads to a decrease in drug crystallization and generates a synergetic effect on the solubilizing effect of CDs [11].

Additionally, we assume that the addition of surfactants would further enhance the solubilization of free drug dissociated from the drug-CD complex. The objective of this study was to investigate the effect of the CD complex on

enhancing the aqueous solubility and dissolution of dutasteride, after which the formulation was further optimized with diverse polymers and/or surfactants. After a film-coated tablet formulation was finalized, its pharmacokinetics in beagle dogs was compared to that of Avodart® soft capsule.

2. Materials and Methods

2.1. Materials

Dutasteride was purchased from Cipla Ltd (Mumbai, India). α-Cyclodextrin (α-CD), β-Cyclodextrin (β-CD), γ-Cyclodextrin (γ-CD) and hydroxypropyl-β-Cyclodextrin (HP-β-CD) were obtained from Wacker Chemie AG (München, Germany). Polyvinylpyrrolidone K30 (PVP) (BASF, Germany), d-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) (Isochem, France), stearoyl polyoxylglycerides (Gelucire 50/13) (Gattefosse, France), polyethyleneglycol (PEG400) (Yakuri Pure Chem, Japan) and polyethyleneoxide-polypropylene oxide copolymer (Poloxamer 407) (BASF, Germany) were used as solubilizers. Lactose (SuperTab 11SD) (DFE pharma, Japan), microcrystalline cellulose (Avicel PH102) (FMC, USA), crospovidone (Polyplasone XL) (Ashland, Netherland), magnesium stearate (Faci, Italy), Opadry® (Colorcon, Singapore) and ethylcellulose (Ethocel 10) (Colorcon, Korea) were used as excipients for the tablets. Avodart® soft capsules (GlaxoSmithKline, United Kingdom) were purchased from a local pharmacy.

2.2. Preparation of dutasteride-cyclodextrin complex and solubility study

Dutasteride-loaded CD complexes were prepared by the oven-drying method. Briefly, dutasteride was first dissolved in ethanol at 2 mg/ml concentration. Various types of cyclodextrins (α-CD, β-CD, γ-CD, HP-β-CD) were separately dissolved in distilled water (DW) at a concentration of 100 mg/ml. The dutasteride solution and CD solution were homogeneously mixed at a 1:1 volume ratio, followed by drying in an oven at 60°C (SANYO, Japan), to determine the aqueous solubility of dutasteride complexed with various CDs at a 1:50 weight ratio. Dried dutasteride-cyclodextrin (DuCD) complexes (equivalent to approximately 0.5 mg of dutasteride) were dispersed in 1.0 ml of DW. After gentle stirring for 1 h, undissolved dutasteride was removed through filtration (0.45-μm PVDF filter), followed by appropriate dilution with a mixture of acetonitrile and water (60/40, v/v). The concentration of dutasteride was analyzed using high-performance liquid chromatography (HPLC), equipped with a reverse phase C_{18} column (Zorbax SB-phenyl, 150 x 3 mm, 3.5 um, Agilent) and UV detector at 240 nm. The mobile phase was a mixture of acetonitrile and water (55/45, v/v) at a flow rate of 0.5 ml/min. The injection volume was 50 μl [23].

Because the γ-CD complex exhibited the highest solubility among the complexes tested, complexes were prepared at various weight ratios (1:10~1:70) of dutasteride to γ-CD (DuγCD) to optimize the solubility of dutasteride. Next, a 0.4 or 1.0 weight ratio of polymer and/or surfactant was added to the dutasteride-γ-Cyclodextrin complex (DuγCD-PS) as a solubility auxiliary additive to further enhance the aqueous solubility of dutasteride. The aqueous solubility of dutasteride in the DuγCD and DuγCD-PS solutions was

determined after filtration as described above.

2.3. Pharmacokinetics after oral administration of DuγCD-PS complex in rats

The pharmacokinetics of dutasteride after oral administration of diverse DuγCD-PS complexes was compared with that of the reference (Avodart®, GlaxoSmithKline) in rats. The animal studies were approved by the WhanIn Pharmaceutical Company Animal Ethics Committee. Male Sprague-Dawley rats (8 weeks old, 230-270 g) were purchased from DBL Co., Ltd (Chungcheongbuk-do, Korea). All rats were habituated for 1 week before the experiment and randomly divided into groups of 4~6 animals each. The rats were subjected to fasting 12 h prior to the study, and the carotid arteries were cannulated with polyethylene tubing PE-50 under isoflurane (I-FRAN LIQUID, Hana Pharm Co., Ltd., Seoul, Korea). Each group of animals was administered either the reference drug (interior oil content of Avodart® soft capsule) or DuγCD-PS complex (suspended in DW) via oral gavage at a dose of 2.39 mg/kg of dutasteride, and each rat was orally administered 10 ml/kg of DW. Blood samples (approximately 0.3 ml) were collected from the carotid artery into heparinized tubes at 0, 0.5, 1, 2, 4, 8, and 24 h after the administration. The plasma was obtained by centrifuging the samples at 13,000 rpm for 5 min and stored at -70°C until analysis.

The concentration of dutasteride in the plasma samples was analyzed

using LC/MS/MS, as previously described [24]. Briefly, 100 μl of plasma samples was vortex mixed with 900 μL of acetonitrile containing finasteride (10 ng/ml) as an internal standard and centrifuged at 13000 rpm. Next, 5 μl of supernatant was injected into the LC/MS/MS system. LC separation was performed by an Acquity H class UPLC (Waters, USA), and the mass spectrometric detection was performed on a TQ Detector (Waters, USA) using MRM. A turbo electrospray interface was used in positive ionization mode. The major working parameters of LC and the mass spectrometer are summarized in Supplement Table S1. The pharmacokinetic parameters (T_{max}, C_{max}, and $AUC_{0\text{-}24\ h}$) of dutasteride were analyzed using WinNonlin® (ver. 6.2, Pharsight) based on the linear trapezoidal rule. The relative bioavailability (BA) of the DuγCD-PS complexes was calculated as follows:

$$\text{Relative BA}\,(\%) = \frac{AUC_{test}}{AUC_{reference}} \times 100$$

2.4. Characterization of dutasteride and γ-cyclodextrin complexes

The surface morphology was observed using field emission scanning electron microscope (FESEM) (JSM-6700F, JEOL, Japan) at an accelerating voltage of 5 kV. Samples were spread onto carbon tabs (double-adhesive carbon-coated tape) adhered to aluminum stubs, which were then coated with a thin layer of platinum. Thermal analysis of DuγCD and DuγCD-PS

complexes were conducted by using a differential scanning calorimeter (DSC 200 F3 Mala, Netzsch). Analyses were performed in an aluminum pan under a heating rate of 10°C/min over a temperature range of 20-280°C. XRD Ultima III (Rigaku) was used to perform the powder X-ray diffraction (pXRD) analyses. The measurement conditions were as follows: scanning speed of 3°/min and step width of 0.02°. FTIR was observed using Nicolet IR Spectrometer (iS50, Thermo, USA).

2.5. Preparation of dutasteride tablet

Tablets of DuγCD-PS complexes (F4 and F5) were prepared by the compression method. Briefly, the DuγCD-PS complexes were granulated using the fluid-bed granulator (WBF-II, Enger, Taiwan) with a mixture of lactose (Super Tab 11SD) and microcrystalline cellulose (Avicel PH 102) as a powder bed. The formulation was designed for each tablet (240 mg total weight) to contain 0.5 mg of dutasteride. Carr's index for the granules before tablet compression was 18, indicating fair flowability. The granules were compressed on a rotary tablet compressor using an 8.5-mm round shape punch, and the hardness of the tablet was adjusted between 12 and 13 kp. Next, the tablet was film-coated with HPMC-based Opadry®. The dimension of the film-coated tablet after 3% weight coating (diameter 8.5 mm, thickness 4.2 mm, round shape) was smaller compared with the marketed soft gelatin capsule Avodart® (length 19 mm, thickness 6.7 mm, rod shape) (Fig. 2).

2.6. Dissolution test of the dutasteride tablet

In vitro dissolution profiles of dutasteride from the DuγCD-PS complex tablet were evaluated by the USP dissolution method (Tier I and Tier II) and compared with that of the reference (Avodart®). In the Tier I method, the dissolution rates of dutasteride were measured using apparatus 2 in which the dissolution medium was 900 ml of 0.1 N HCl solution with 2% (w/v) sodium lauryl sulfate (SLS) at 37°C and stirred at 50 rpm. In the Tier II method, the dissolution medium was 450 ml of 0.1 N HCl solution with pepsin (1.6 g/L, label activity 1:3000) for the first 25 min, followed by the addition of 450 ml of 0.1 N HCl solution with SLS (4%, w/v) for the remaining dissolution test. The samples (5 ml) were obtained at fixed time intervals and were analyzed by HPLC with a UV detector, as described above, after filtering through a 0.45-μm PVDF filter.

Additional dissolution test for dutasteride tablet was performed by modification of Tier I method. Considering the low surfactant level in physiological fluid, the dissolution of F4 and F5 tablets were evaluated in various dissolution media containing SLS in the range of 0.1 – 2 w/v%.

2.7. Pharmacokinetic study of the dutasteride tablet in beagle dogs

An *in vivo* cross-over pharmacokinetic study of dutasteride was performed

after oral administration of the DuγCD-PS complex tablet or the reference (Avodart®) in beagle dogs. The animal studies were approved by the Institutional Animal Care and Use Committee of Korea Animal Medical Science Institute. Six male beagle dogs (10 kg, 10 months old) were subjected to fasting overnight before the experiment. Each dog was administered either one capsule of the reference (Avodart®, 0.5 mg as dutasteride) or one tablet of DuγCD-PS (F5) (0.5 mg as dutasteride), followed by 10 ml of water. Blood samples were taken from the cephalic vein and collected (3 ml) into heparinized tubes at 0, 0.5, 1, 2, 4, 8, 12, 24, and 48 h after the administration. The plasma was obtained by centrifuging the samples at 3,000 rpm for 5 min and stored at -70 °C until analysis. The wash-out period between treatments was 4 weeks. The treatment of the plasma samples and the LC/MS/MS analysis conditions were the same as that used above for the rat study. The C_{max} and T_{max} were determined from the experimental data. The calculated dutasteride concentrations were used to obtain the area under the plasma concentration-time profile from time zero to the last concentration time point (AUC_{0-t}) by the linear trapezoidal method. Statistical analysis was performed with the unpaired *t*-test where appropriate. Significance was set at $p < 0.05$. All data were presented as mean ± standard deviation.

3. Results

3.1. Effect of cyclodextrin complex on the aqueous solubility of dutasteride

Table 1 presents the aqueous solubility of dutasteride when complexed with various cyclodextrins at a 1:50 weight ratio, together with their binding affinity obtained by the computer docking simulation tool Glide (Schrödinger, New York, USA). Among the CDs tested, the γ-CD complex resulted in the highest aqueous solubility of dutasteride and showed the lowest binding affinity value, indicating stable complex formation. Thus, γ-CD complexes with various weight ratios (1:10~1:70) of DuγCD were prepared, and the aqueous solubility of dutasteride was determined. The aqueous solubility of dutasteride increased up to a 1:70 weight ratio (Table 2), and this value was thus selected for further evaluation. It is interesting to note that the solubility of dutasteride synergistically increased with the addition of a solubilizing polymer at a 0.4 weight ratio (*i.e.*, PVP and PEG) and a surfactant (*i.e.*, Gelucire, TPGS and Poloxamer) to the DuγCD complex (dutasteride : γ-Cyclodextrin = 1:70) (Table 3). The highest solubility of dutasteride achieved with the addition of the 0.4 weight ratio of PVP and Gelucire was 147 μg/ml, which is 1.5 times higher than the solubility of DuγCD (1:70) (93 μg/ml). In a previous report, the highest solubility of dutasteride was only 47.1 μg/ml when dutasteride was complexed with HP-β-CD and HPMC at a weight ratio

of 1:26.6:13.3 [25]. Moreover, the study prepared the complex by the supercritical fluid manufacturing method, which is environmentally friendly but not widely equipped in pharmaceutical companies. Thus, it is notable that DuγCD-PS prepared by the simple drying method achieved an aqueous solubility of dutasteride that was higher than the reported solubility.

Based on the preliminary solubility study, DuγCD-PS complexes were further optimized by changing the weight ratio of dutasteride to γ-CD (1:10 to 1:70) and surfactant. Table 4 presents the composition of DuγCD-PS complexes selected for further evaluation and the aqueous solubility of dutasteride. When the 0.4 weight ratio of PVP was selected as a solubilizing polymer, the solubility of dutasteride increased up to 170 μg/ml as the content of the surfactant (Gelucire:TPGS=1:1) increased to 2 weight ratio (F5). These results are consistent with previous reports that the addition of a water-soluble polymer synergistically enhances the solubilizing effect of CDs by preventing drug nucleation and/or crystal growth [11]. Moreover, it is notable that the surfactant further increased the solubility of dutasteride, which supports our assumption that surfactants would further enhance the drug solubility by solubilizing the free drug dissociated from the drug-CD complex.

3.2. Pharmacokinetic study of DuγCD-PS complex in rats

The plasma concentration profiles of dutasteride after oral administration of Avodart® or the DuγCD-PS complex at a dose of 2.39 mg/kg of dutasteride in rats are presented in Fig. 3, and the pharmacokinetic parameters are

summarized in Table 5. DuγCD-PS complexes (F1) with 10 and 0.4 weight ratios of γCD and surfactant, respectively, resulted in only 29.6% relative BA compared to that of the reference (Avodart®). However, when the weight ratio of γCD increased to 30 and 50 (F2 and F3, respectively), the relative BA of dutasteride increased up to 74% of the reference. Moreover, the addition of surfactant at a weight ratio of 2 (F4 and F5) further increased the relative BA up to 93.6% of the reference. To understand the effect of solubilization by DuγCD-PS complexes on oral absorption in rat, the correlation solubility with AUC_{0-t} in rat was plotted (r^2 = 9763, Fig. 4)

For BCS class II drugs including dutasteride, improving the aqueous solubility is the most practical strategy to increase its oral bioavailability by enhancing the dissolution of the drug in the gastrointestinal (GI) tract [26]. As the solubility of dutasteride increased by increasing the drug to γ-CD weight ratio and by adding surfactant (Table 4), the relative BA of dutasteride increased proportionally (Table 5). In addition to the solubilizing effect of γ-CD, surfactant appears to synergistically enhance the bioavailability (F3 *vs*. F4) by inhibiting precipitation and solubilizing the free drug dissociated from the CD complex, as we assumed. However, it is interesting to note that the increase in the γ-CD weight ratio up to 70 (F5) could not further increase the relative BA of dutasteride, despite its higher solubility than F4 formulation. It is known that γ-CD solubilizes poorly water-soluble drugs by inclusion of insoluble drug into its hydrophobic cavity and formation of γ-CD aggregates [18]. However, the drug could additionally be embedded among γ-CD aggregates at a high CD ratio, resulting in supersaturation of the drug. The

drug is easily released by the dissociation of γ-CD aggregates (by dilution in the gastrointestinal fluid and/or by the ring opening of γ-CD with the attack of digestive enzyme), after which the drug may be precipitated. Surfactant might not be able to sufficiently inhibit the precipitation of supersaturated dutasteride in F5 *in vivo*; thus, the higher solubility of dutasteride compared to F4 could not proportionally increase the bioavailability.

3.3. Characterization of dutasteride and γ-cyclodextrin complexes

Surface morphology of dutasteride observed by the FESEM was irregular in shape, while γCD was a parallelogram shape. However, the morphology of complexes was similar to an aggregate (Fig. 5). In FTIR study, characteristic peaks of dutasteride in the range of 1650-1700 cm^{-1} (carbonyl stretching), 1600 cm^{-1} (N-H bend) were markedly decreased in *γ-CD complexes* (Fig. 6). DSC and pXRD are useful techniques to determine the occurrence of inclusion of drug crystals. The thermograms of dutasteride and the complexes are presented in Fig. 7(A). Dutasteride showed a very sharp endothermic peak at 251°C, which corresponds to its melting temperature. It is notable that the endothermic peak of dutasteride was almost negligible when the weight ratio of γCD was higher than 1:30 in the DuγCD complexes. Moreover, as shown in Fig. 7(B), the endothermic peak of dutasteride completely disappeared in DuγCD-PS complexes (F3, F4 and F5) containing solubilizing polymer (*i.e.*,

PVP) and surfactant (*i.e.*, Gelucire and TPGS), indicating that dutasteride is present in an amorphous form in DuγCD-PS complexes. Moreover, the pXRD results of the DuγCD-PS complexes were consistent with those of DSC and did not exhibit the specific pattern for dutasteride crystal (Fig. 8). Because both F4 and F5 formulations could achieve high bioavailability (93.6%) compared to the reference, they were selected for preparing the tablet formulation.

3.4. Dissolution study of DuγCD-PS tablet

Fig. 9 presents the *in vitro* dissolution profiles of dutasteride from the tablets of DuγCD-PS complexes (F4 and F5) coated with HPMC-based Opadry®, which were compared with that of the reference (Avodart®, soft gelatin capsule). In the Tier I method (Fig. 9A), dissolution of F5 was more rapid than that of F4 and the reference for the first 15 min. However, both F4 and F5 tablets showed a complete release of dutasteride within 45 min, which is similar to the reference. Notably, dutasteride was not dissolved for the first 25 min until SLS was added in the Tier II method (Fig. 9B), indicating the importance of surfactants in the release medium to mimic the physiological condition. Moreover, it was previously reported that poorly water-soluble drugs can be solubilized in the gastrointestinal tract by endogenous surfactants including bile acids, bile salts and lecithin [27].

Considering the low surfactant level in physiological fluid, the dissolution profiles of F4 and F5 tablets were shown in Fig 10. F4 tablet with a relatively

low solubility showed a low dissolution rate at 15min in 0.7w/v% SLS containing dissolution media. Whereas F5 tablet showed rapid dissolution rate at 10 min regardless of the concentration of SLS in media (0.1-2w/v %) as well as the reference. The DuγCD-PS complex (1:70:0.4:2) used in F5 tablet contained higher amounts of γ-cyclodextrin than that(1:50:0.4:2) in F4 tablet. High solubility of F5 tablet was seemed to maintain a high dissolution rate even though a small amount of SLS was contained in dissolution media.

Dutasteride is classified as a BCS class II drug, which implies that it is highly membrane permeable and lipophilic with a log P value of 5.09. Its terminal elimination half-life is known to be very long (3-5 weeks) at steady-state in humans [24]. Thus, rapid initial dissolution of dutasteride from the tablet followed by gastrointestinal absorption would be critical to achieve a similar pharmacokinetic profile after oral administration. Based on the *in vitro* dissolution study, the F5 tablet was selected for the *in vivo* animal study.

3.5. Pharmacokinetic study of DuγCD-PS tablet in beagle dogs

Fig. 11 shows the plasma concentration profiles of dutasteride after oral administration of Avodart® or the tablet of DuγCD-PS complexes (F5) in beagle dogs. Their pharmacokinetic parameters summarized in Table 6 indicate that the C_{max} and AUC_{0-t} values were not significantly different. P-values were greater than 0.05 when T-test was applied to the pharmacokinetic

data. Notably, the T_{max} value of F5 was shorter than that of the reference, which is consistent with the *in vitro* dissolution study (Fig. 7). Moreover, the relative bioavailability of F5 was 92.4% of that of the reference. Thus, further investigation would be necessary with a larger number of animals and/or humans to evaluate the bioequivalence of the F5 tablet to the reference soft capsule.

4. Discussion

The present study provided the development possibility of dutasteride tablet. Mono-dosing of dutasteride was only 0.5mg per tablet but its water solubility was very low. To prepare the solid dosage form, cyclodextrin was selected as a proper main solubilizer due to the ease of powdering and the increase of drug solubility by the interaction drug molecule and hydrophobic interior cavity of CD. The γ-cyclodextrin was determined as the suitable cyclodextrin by drug solubility test and computer docking simulation for complexation. The inclusion complex formation depended on the chemical structures and physicochemical properties of both guest and CD molecules [28]. Dutasteride had a larger aromatic ring like the steroidal structure, which seemed to be easier to pose into γ-cyclodextrin with larger cavity size. It coincided to the results of the lowest binding energy in computer simulation and the highest drug solubility to γ-cyclodextrin among CDs (Table 1). In the preparation of DuγCD complex by the change of the weight ratio (1:10~1:70), the higher the ratio of γ-cyclodextrin was, the higher the solubility of the drug was (Table 2). Maybe, cyclodextrin was presumed to solubilize dutasteride by making drug-cyclodextrin solid dispersion/aggregate in addition to inclusion complexation. Additionally, polyvinylpyrrolidone and gelucire/TPGS added to DuγCD complex were shown to act as solubility adjuvant to increase the drug solubility (Table 3). F4 and F5 composition(Drug : γ-CD : polymer : surfactant = 1:50:0.4:2, 1:70:0.4:2, respectively) exhibited comparatively high

solubility of drug. Their solubilities were 118.9ug/mL, 170.6ug/mL respectively (Table 4), which were markedly higher in comparison with that of HP-β-CD complex for dutasteride in the previous study, 47.1ug/mL [25]. The crystallinity of drug was proved to disappear after preparation of cyclodextrin complex, considering from the data of DSC and pXRD (Fig. 7–Fig. 8). To confirm the level of solubilization by CD complex, *in vivo* oral absorption study in rat was tested compared with the soft capsule. To understand the IVIVC of DuγCD-PS complex, the correlation between *in vitro* solubility data and *in vivo* AUC_{0-t} in rat was plotted(r^2 = 0.9763, Fig. 4). The absorption rate of dutasteride increased by the increase of the drug solubility, which was consistent with the fact that dutasteride was a BCS class II drug. As a result, the complex with solubility more than about 120ug/mL was expected to be bioequivalent to the reference drug in rat.

The fluid-bed granulation method was applied as the solidification process for DuγCD-PS complex. The flowability of final mixture was fair and any issues for tableting and coating process were not occurred. It was judged to manufacture the dutasteride tablets commercially through the check for the preparation unit process and inter process control for granules. 8.5mm round type core tablets using DuγCD-PS complexes (F4 and F5) were prepared with a weight of 240mg. Dutasteride tablet was expected to enhance the patient compliance compared with the Avodart® soft capsule of 18mm length (Fig. 2).

The dissolution for dutasteride tablet was performed whether enhancement of drug solubilization by cyclodextrin and solubility adjuvants properly effected on drug release from tablet. The dissolution conditions

followed the dissolution method for soft capsule of dutasteride listed on US FDA site. The both F4 and F5 tablets showed the equivalent dissolution profiles to soft capsule under Tier I and Tier II dissolution conditions (Fig. 9). In aspect that complete dissolution was observed in dutasteride tablet within 45min in dissolution media, solubilization of dutasteride by DuγCD-PS complex was estimated to be effective. According to Tier II method, dissolution was performed in 450mL of 0.1N HCl solution with pepsin for the first 25min, followed by the addition of 450mL of 0.1N HCl solution with 4w/v% SLS for the remaining dissolution test. Tier II method was guessed to show the effect of SLS on gelatin capsule shell because SLS was well known protein solubilizer and denaturant and might retard the dissolution of drug from gelatin capsule [29]. So, The Tier II condition was seemed to be suitable to evaluate the dissolution for gelatin capsule. For that reason, the dissolution of dutasteride tablet was carried out according to Tier I method in which SLS was already added in the beginning of dissolution. The F5 composition with higher solubility than F4 was selected as a candidate for pharmacokinetic study in beagle dogs. Prior to animal testing, Tier I dissolution condition was carefully reviewed for the test tablet and also partially modified to get the meaningful correlation between *in vitro* dissolution and *in vivo* PK profile, considering of low surfactant level in physiological fluid. SLS could accelerate dissolution rate and extent of poorly soluble drug by the increase of wetting through reduction of the interfacial tension and micellar solubilization of drug. Low level of surfactants in dissolution medium recommended to give a better correlation between *in vitro* and *in vivo* data [30]. In the case of F4

tablet, the concentration of SLS in dissolution media was affected to the dissolution rate of dutasteride. The dissolution profile for F4 was low compared with that of reference tablet in dissolution media with low level of SLS (0.7 w/v %). On the other hand, F5 tablet showed the similar dissolution profiles regardless of SLS contents(0.1~2 w/v %) in dissolution media. With this result, F5 tablet was administered into beagle dogs. Its pharmacokinetic parameters, C_{max} and AUC_{0-t} values were not significantly different from those of reference when statistical analysis was performed with the t-test ($P>0.05$). T-test analysis was performed between the two groups because the number of six animals was not enough for statistical analysis using ANOVA. T_{max} value of F5 was shorter than that of the reference, which is consistent with the in vitro dissolution study. The relative bioavailability of F5 tablet was 92.4% compared with soft capsule and judged to be bioequivalent to soft capsule in beagle dog. But further investigation would be necessary with a larger number of animals to prove clearly the bioequivalence of F5 tablet to soft capsule. And also, additional formulation study might be needed for more solubilization of drug to pass the bioequivalence in human because the human GI tract is longer than beagle dog, considering the oral absorption difference between rat and beagle dog.

Thus, it could be confirmed that the formulation strategy of introducing the concept of cyclodextrin complex was working for solubility enhancement of dutasteride. The manufacturing process was established using fluid bed granulator and considered to apply successfully for commercial production. Finally, dutasteride tablet was expected to avoid safety issues for children and

women by percutaneous absorption because there was not worried about the leakage of solubilized drug by the damage of gelatin shell. And dutasteride tablet could provide good patient compliance by medication with small sized of tablet.

5. Conclusion

DuγCD-PS complexes were successfully prepared by the simple oven-drying method. The amorphous form of dutasteride was confirmed via DSC and pXRD studies. *In vitro* dissolution of dutasteride from the tablets of DuγCD-PS complexes was comparable with that from the reference (Avodart®, soft gelatin capsule). Moreover, *in vivo* pharmacokinetic parameters of the DuγCD-PS complex tablet after oral administration in beagle dogs were not significantly different from that of the reference. These results suggest the feasibility of developing a tablet formulation of dutasteride with bioequivalence to the commercial soft gelatin capsule, which requires further evaluation in a larger number of animals and/or humans.

Table 1 Aqueous solubility of dutasteride complexed with various cyclodextrins at a 1:50 weight ratio and the average binding affinity as obtained by the computer docking simulation tool Glide (Schrödinger, New York, USA).

Cyclodextrin	**Solubility (μg/ml)***	**Average binding affinity (ΔG_{bind}) (kcal/mol)**
α-cyclodextrin	1.3±0.3	-55.14
β-cyclodextrin	23.8±1.8	-89.58
γ-cyclodextrin	61.8±3.1	-98.69
HP-β-cyclodextrin	25.5±2.0	ND

*Each value is the mean ± SD (n=3).
ND: not determined.

Table 2 Effect of the weight ratio of dutasteride:γ-cyclodextrin (DuγCD) on the aqueous solubility of dutasteride.

Weight Ratio (Dutasteride:γ-cyclodextrin)	Solubility (µg/ml)*
1:10	5.5±1.2
1:30	24.7±2.0
1:50	61.8±3.1
1:70	93.9±2.2

*Each value is the mean ± SD (n=3).

Table 3 Effect of the solubilizing polymer and surfactant on the aqueous solubility of dutasteride (μg/ml) added to the DuγCD (1:70) complex at weight ratios of 0.4 and 1.0, respectively.

Complex	Polymer	Surfactant	Solubility (μg/ml)*
DuγCD (1:70)	-	-	93.9±2.2
	PVP	-	118.4±3.3
		Gelucire	147.0±4.0
		TPGS	138.7±1.2
		Poloxamer	134.1±1.7
	PEG	-	109.6±2.2
		Gelucire	127.0±3.7
		TPGS	118.6±0.9
		Poloxamer	139.7±2.0

*Each value is the mean ± SD (n=3).

Table 4 Composition of DuγCD-PS complexes and the aqueous solubility of dutasteride.

Rx	Composition (weight ratio)				Solubility (μg/ml)*
	Dutasteride	γ- CD	Polymer [a]	Surfactant	
F1	1	10	0.4	0.4 [b]	33.8±1.5
F2	1	30	0.4	0.4 [b]	97.3±3.1
F3	1	50	0.4	0.4 [b]	104.5±3.0
F4	1	50	0.4	2 [c]	118.9±3.4
F5	1	70	0.4	2 [c]	170.6±4.9

[a]Polymer: PVP

[b]Gelucire

[c]Gelucire/TPGS (1:1)

*Each value is the mean ± SD (n=3).

Table 5 Pharmacokinetic parameters of dutasteride after oral administration of the reference (Avodart®) or DuγCD-PS complex at a dose of 2.39 mg/kg of dutasteride in rats.

Rx	T_{max} (h)	C_{max} (ng/ml)	$AUC_{0\text{-}24\ h}$ (ng·h/ml)	Relative BA% (to the reference)
Reference (Avodart®)	10.4 ± 7.8	253.4 ± 22.8	4336.9 ± 497.4	-
F1	12.0 ± 8.0	63.7 ± 23.5	1282.4 ± 334.9	29.6
F2	8.9 ± 9.0	148.8 ± 31.9	3147.3 ± 689.1	72.6
F3	10.4 ± 7.8	170.6 ± 43.4	3228.2 ± 459.9	74.4
F4	3.0 ± 1.2	215.2 ± 51.3	4061.1 ± 588.9	93.6
F5	11.0 ± 8.9	195.8 ± 21.3	4060.2 ± 295.3	93.6

Each value is the mean ± SD (n=4~6).

Table 6 Pharmacokinetic parameters of the reference or F5 tablet after oral administration in beagle dogs (n=6, crossover study).

Composition	T_{max} (h)	C_{max} (ng/ml)	$AUC_{0\text{-}24\ h}$ (ng·h/ml)	Relative BA% (to the reference)
Reference	1.7 ± 0.5	67.3 ± 17.9	1964.7 ± 546.1	-
F5	1.2 ± 0.7	61.2 ± 16.9	1815.6 ± 532.4	92.4
P-value	N/A	0.5990	0.5593	-

N/A : Not assessed

Table S1 Working parameters of LC and the tandem mass spectrometer for analysis of dutasteride in the plasma.

<table>
<tr><th>Parameter</th><th>Value</th></tr>
<tr><td>< LC system></td><td></td></tr>
<tr><td>Column</td><td>ACQUITY UPLC® BEH C18 (2.1 x 50 mm, 1.7 µm)</td></tr>
<tr><td>Column Mobile Phase</td><td>Solvent A: 100% water with 0.1% formic acid
Solvent B: 100% acetonitrile with 0.1% formic acid
<table><tr><td>Time (min)</td><td>%A</td><td>%B</td></tr><tr><td>Initial</td><td>70</td><td>30</td></tr><tr><td>0.20</td><td>70</td><td>30</td></tr><tr><td>2.20</td><td>10</td><td>90</td></tr><tr><td>3.00</td><td>10</td><td>90</td></tr><tr><td>3.10</td><td>70</td><td>30</td></tr><tr><td>5.50</td><td>70</td><td>30</td></tr></table></td></tr>
<tr><td>Injection volume</td><td>5 µl</td></tr>
<tr><td>Flow rate</td><td>0.4 ml/min</td></tr>
<tr><td>Temperature</td><td>Sample 4 °C, Column 30 °C</td></tr>
<tr><td><MS/MS conditions></td><td></td></tr>
<tr><td>Mass spectrometer</td><td>TQ Detector (Waters, USA)</td></tr>
<tr><td>Ion source</td><td>ES^+</td></tr>
<tr><td>Temperatures</td><td>Source Temp: 120 °C, Desolvation Temp: 350 °C</td></tr>
<tr><td>Gas flow</td><td>Desolvation: 800 L/h, Cone: 50 L/h</td></tr>
<tr><td>Compound information</td><td>MRM
<table><tr><th>Analyte</th><th>Parent (m/z)</th><th>Daughter (m/z)</th><th>Dwell (s)</th><th>Cone (V)</th><th>Collision (V)</th></tr><tr><td>Dutasteride</td><td>529.33</td><td>461.27</td><td>0.161</td><td>62</td><td>35</td></tr><tr><td>Finasteride</td><td>373.2</td><td>305.17</td><td>0.161</td><td>56</td><td>28</td></tr></table></td></tr>
</table>

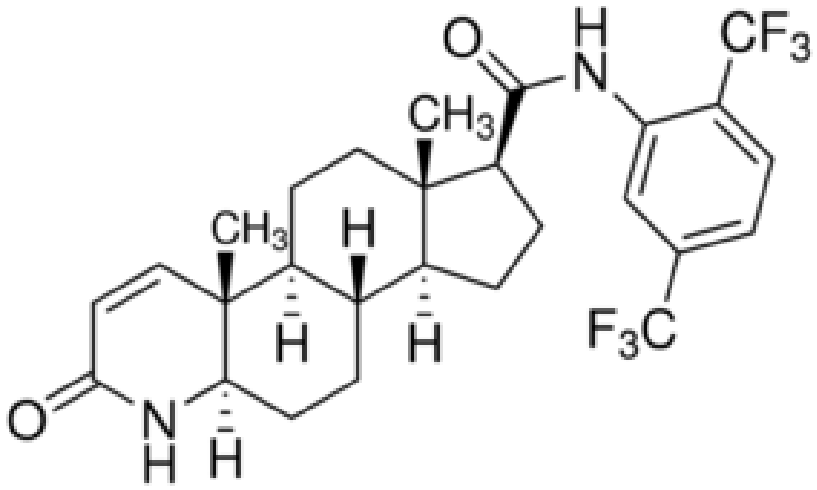


Figure 1 Chemical structure of dutasteride

Figure 2 Comparison of the size of Avodart® soft gelatin capsule (left) and the dutasteride tablet (right).

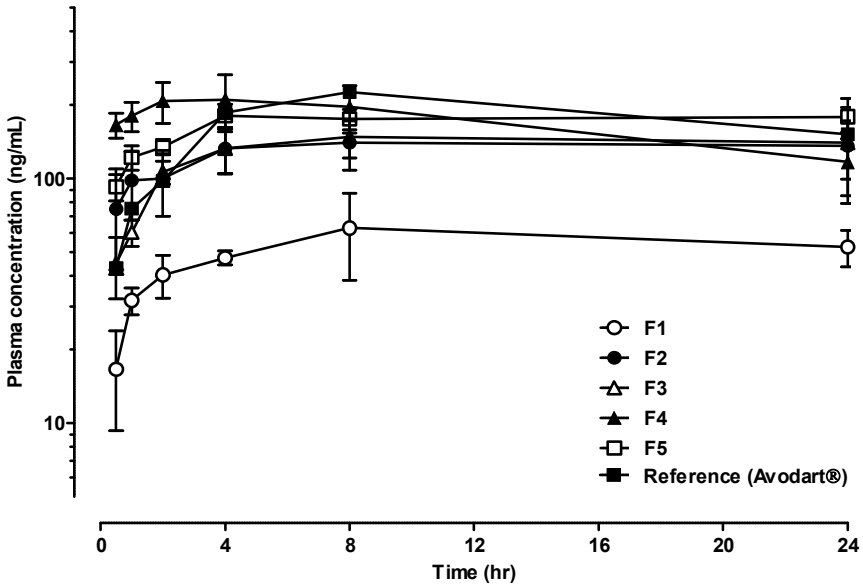


Figure 3 Mean plasma concentration-time profiles of dutasteride after oral administration of the DuγCD-PS complex at a dose of 2.39 mg/kg of dutasteride in rats (n=4~6). Each point and vertical bar represent the mean and standard deviation, respectively.

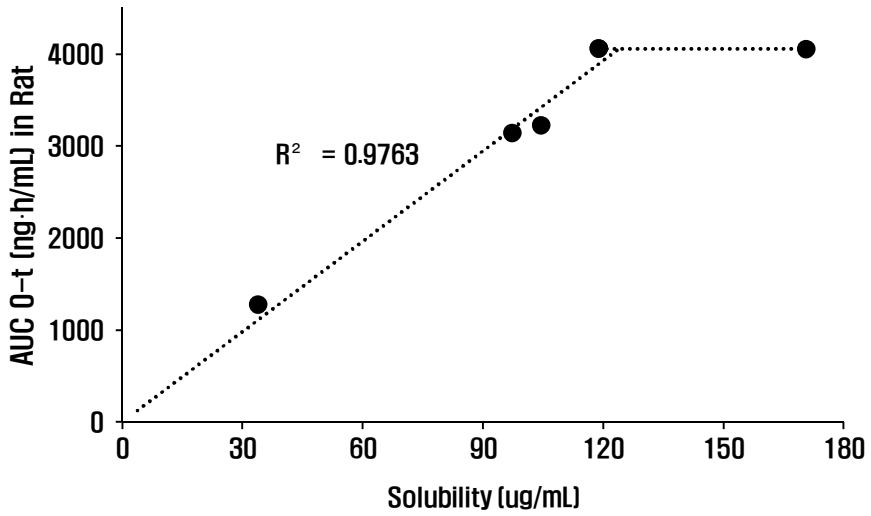


Figure 4 Correlation between the solubility of DuγCD-PS complexes(F1-F5) and oral absorption(AUC_{0-t}) in rat.

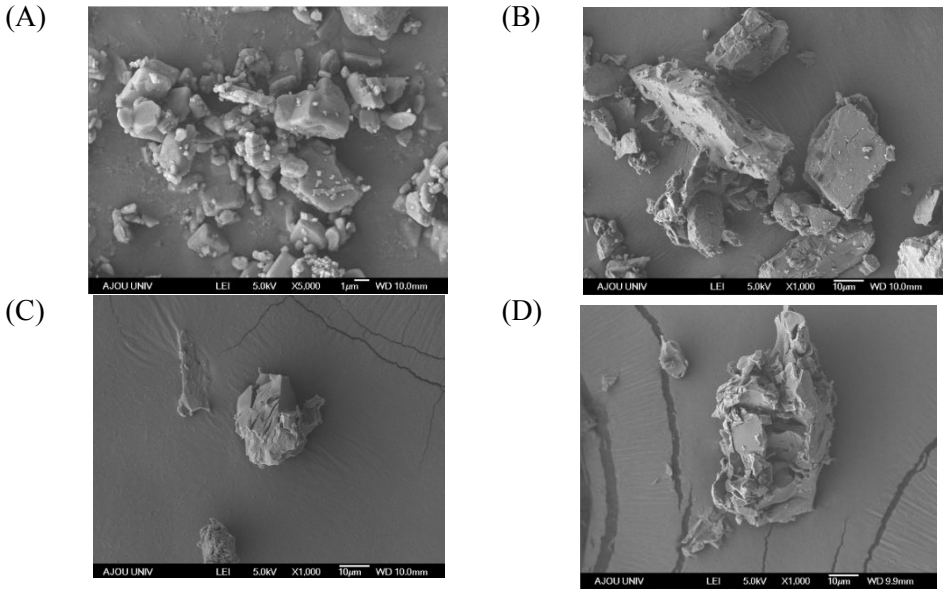


Figure 5 Scanning electron microscope of (A) dutasteride (X5000), (B) γ-cyclodextrin (X1000), (C) DuγCD complex (1:70) (X1000), and (D) DuγCD-PS complex (1:70:0.4:2, F5) (X1000).

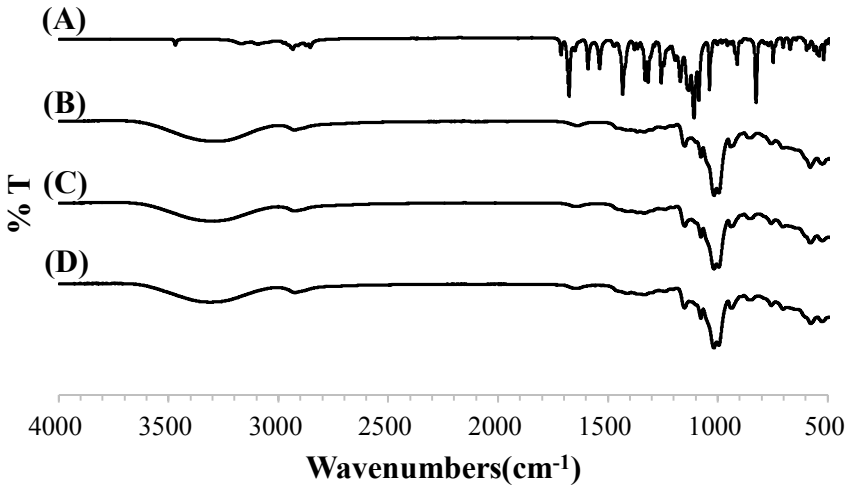
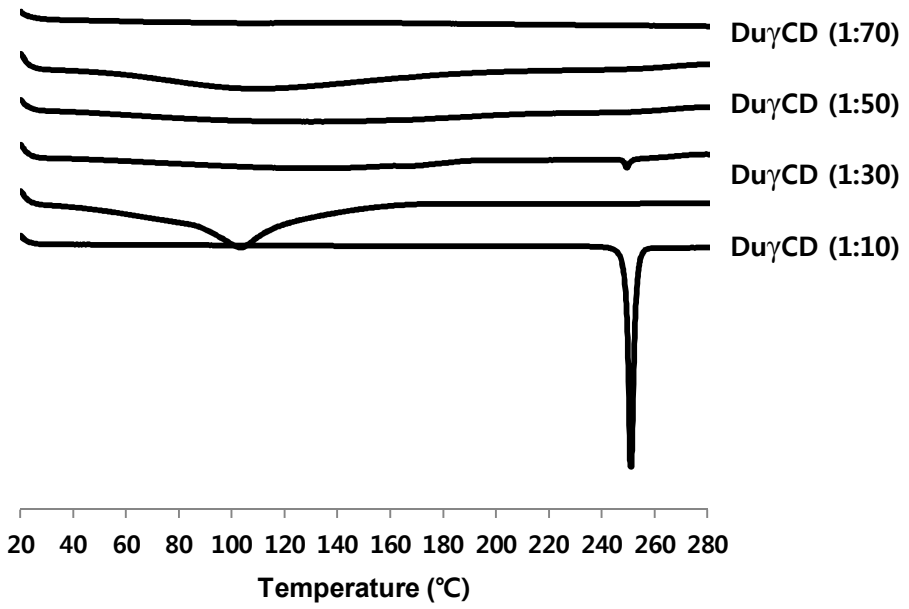


Figure 6 FTIR Spectra of (A) dutasteride, (B) γ-cyclodextrin, (C) DuγCD complex (1:70), and (D) DuγCD-PS complex (1:70:0.4:2, F5).

(A)



(B)

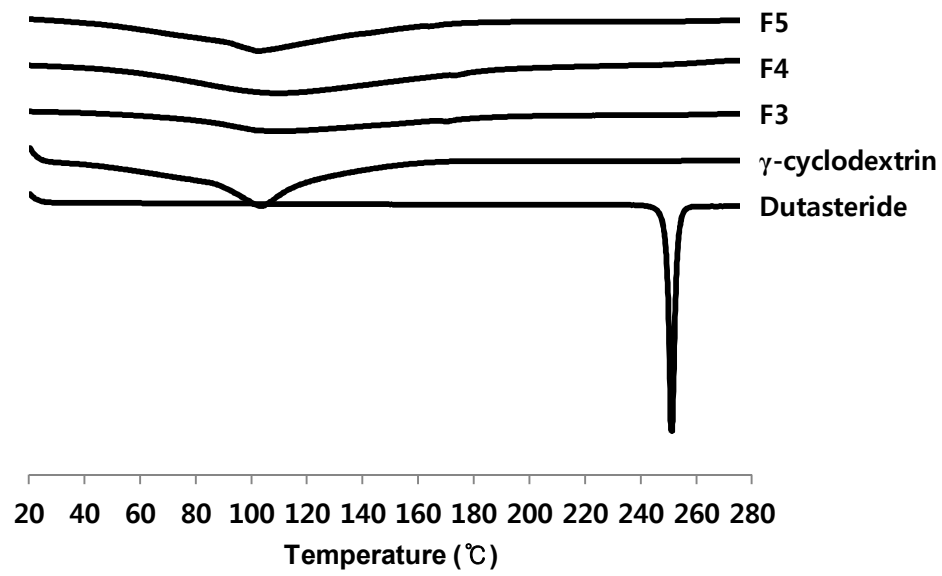


Figure 7 DSC thermograms of the (A) DuγCD complexes and (B) DuγCD-PS complexes.

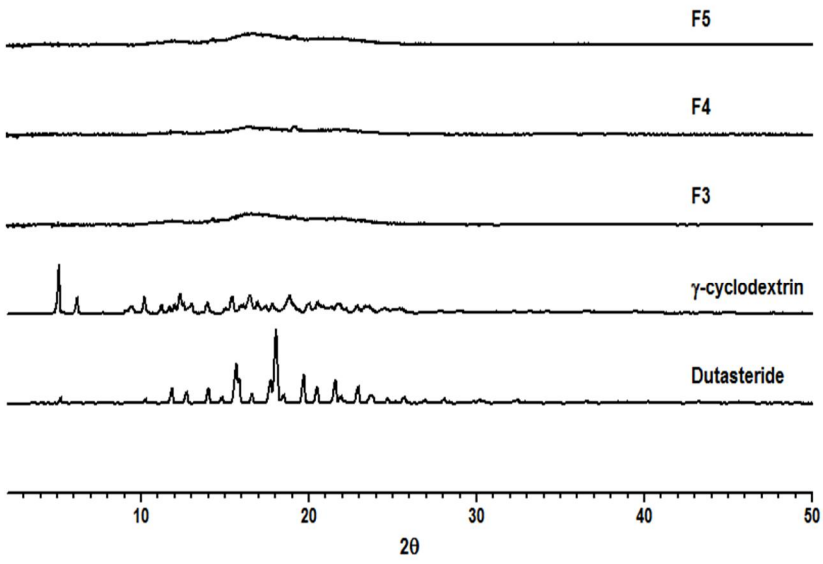
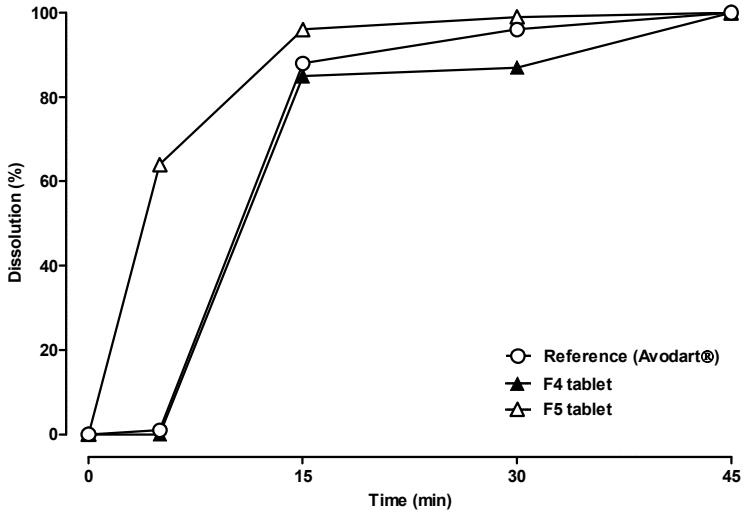


Figure 8 Powder X-ray diffraction pattern of the DuγCD-PS complexes.

(A)



(B)

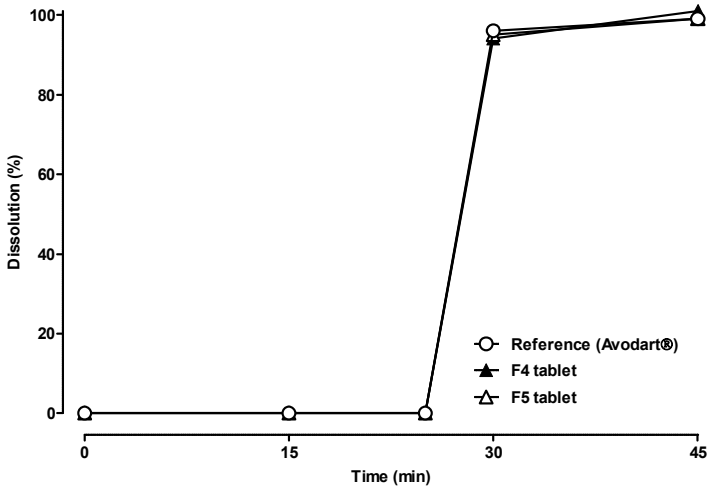


Figure 9 *In vitro* dissolution profiles of dutasteride from the reference soft gelatin capsule (Avodart®) and the film-coated tablets of DuγCD-PS complexes determined following the USP dissolution method (A) Tier I and (B) Tier II by using apparatus 2.

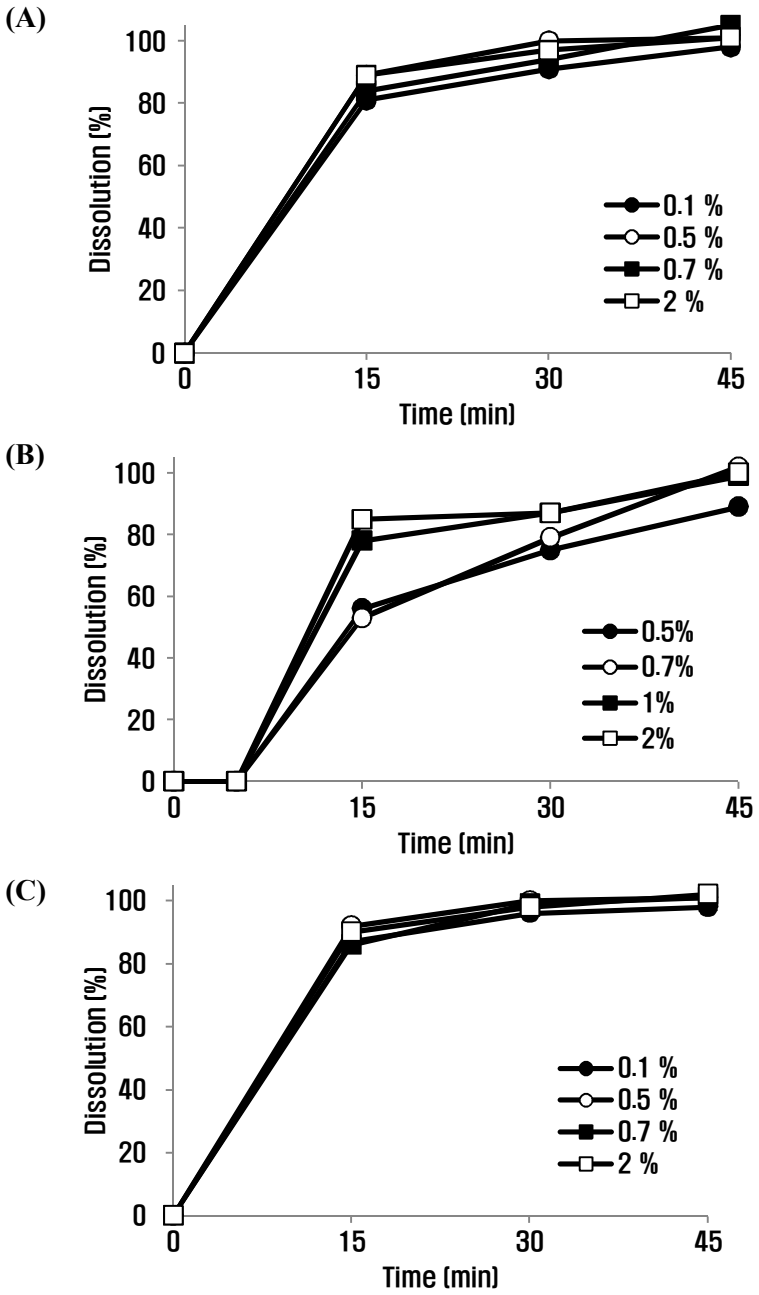


Figure 10 *In vitro* dissolution profiles of dutasteride from (A) the reference soft gelatin capsule (Avodart®), (B) the film-coated tablet (F4), and (C) the film-coated tablet (F5) in dissolution media with the various SLS contents (Tier I modified).

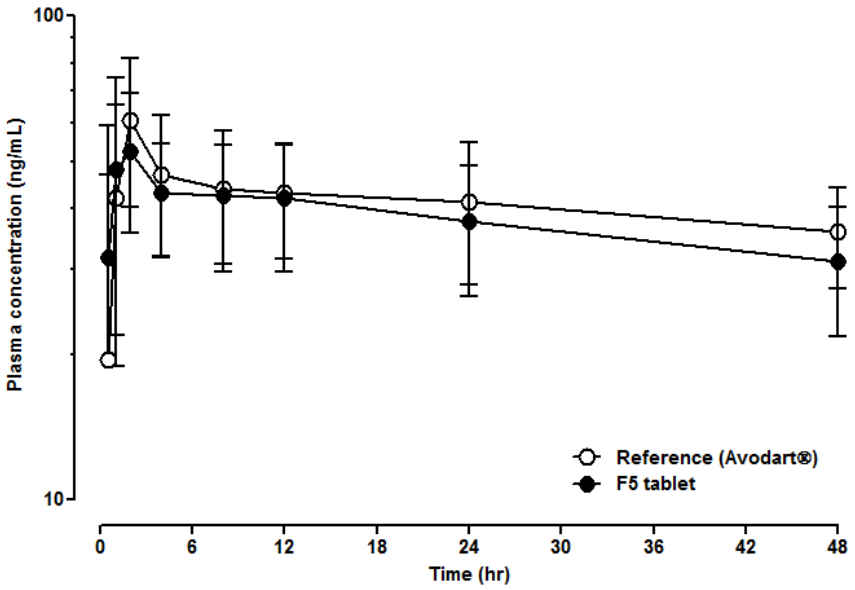


Figure 11 Mean plasma concentration-time profiles of dutasteride after oral administration of the reference soft gelatin capsule (Avodart®) or the F5 tablet in beagle dogs (n=6, crossover). Each point and vertical bar represent the mean and standard deviation, respectively.

Part II. Formulation of a film-coated choline alfoscerate tablet bioequivalent to soft gelatin capsules (Gliatilin®): Effect of Neusilin

1. Introduction

Choline alfoscerate (Fig. 1) is hydrolyzed to choline which is the precursor for the neurotransmitter acetylcholine, and is used for the improvement of cognitive dysfunction in dementia of neurodegenerative and vascular origin [31, 32]. It has an elimination half-life of 0.5~6.2 hr [33] and is completely absorbed following oral administration [34]. Choline alfoscerate is commercially available as soft capsule and administered 400 mg each three times a day. The physical strength of the gelatin shell could become weaker under high temperature and the gelatin shell can deform at high temperature. The drug dissolved in soft gelatin can also migrate to the gelatin shell over time [35]. Thus, a tablet dosage form of choline alfoscerate was developed to overcome these disadvantages of soft capsule. Unfortunately, however, choline alfoscerate is highly hygroscopic, and thus choline alfoscerate powder is apt to turn to be sticky when it is exposed to humid air during manufacturing process. This could be the main reason that the first commercial preparation (Gliatilin®, Reference) of choline alfoscerate was launched in the market as a soft capsule, in which choline alfoscerate was dissolved in glycerin. Formulation strategy of developing a choline alfoscerate tablet in this study was to select suitable excipients that could efficiently surround the surface of the drug to inhibit the water absorption. However, the amount of hydrophobic excipients needed to be minimized since they could retard the dissolution [36], thereby negatively influencing the result of the

bioequivalence of tablet. Based on the formulation studies, magnesium aluminosilicate (Neusilin) was selected as a proper excipient, and tablet formulation was designed to include minimum amount of Neusilin.

Choline alfoscerate is readily hydrolyzed by phosphodiesterases in the gut mucosa to form free choline [37]. The active major metabolite, choline can be measured in plasma following oral administration of choline alfoscerate and the increased plasma levels of choline reflects the absorption of choline alfoscerate [34]. Choline in plasma can be measured using liquid chromatography with tandem mass spectrometry (LC/MS/MS). However, choline is an endogenous material which comes from one of two sources, the dietary intake and synthesis by *de novo* pathway from phosphatidylcholine et al [38]. The difference level of endogenous choline concentrations may cause subject variability in drug absorption and failure in bioequivalence studies. Thus, the absorption of choline by drug administration should be checked under choline-limited diet control of healthy volunteers and the removal of individual interference by endogenous choline.

The aim of the present study was to investigate the optimum tablet formulation of choline alfoscerate and to compare the bioequivalence between a newly formulated tablet (Alfocetine®, test drug) and soft capsule(Gliatilin®, reference drug) according to the KFDA guidelines [39] in healthy Korean male volunteers. The absorbed plasma concentration of choline after drug administration was determined, and was calculated by correcting with the baseline values determined before dosing at the same plasma sampling time [3, 39].

2. Materials and Methods

2.1. Materials

Choline alfoscerate was purchased from HanseoChem (Kyeonggi-do, Republic of Korea). Magnesium aluminometasilicate (Neusilin, Fuji Chemical, Japan) was used to control the water absorption of drug and to improve the granule fluidity. Polyvinylpyrrolidone K30 (PVP) (BASF, Germany) was added as binder. Microcrystalline cellulose (Mingtai chemical, Taiwan), lactose monohydrate and lactose anhydrous (DFE Pharma, Germany) and dicalcium phosphate anhydrous(DCP A-TAB, Innophos, USA) were used as excipients for the tablets. Croscarmellose sodium(DFE Pharma, Japan), Sodium starch glycolate (Yung zip, Taiwan), crospovidone (Polyplasdone XL, Ashland, Netherland), magnesium stearate(Faci, Italy) and sodium stearyl fumarate (Pruv, JRS Pharma, Spain) were tested as superdisintegrant and lubricant, respectively. Opadry I® and Opadry AMB® were obtained from Colorcon(Shanghai, China). Gliatilin® soft capsules (Daewoong Pharmaceutical, Seoul, Republic of Korea) were purchased from a local pharmacy.

2.2. Selection of excipient to block the water absorption of drug

Choline alfoscerate was a powder form with a little good flowability. But it was highly hygroscopic and was apt to be sticky under the exposure to the air. It caused sticking and picking problem during tablet manufacturing process, in which choline alfoscerate adhered to the surface of a tablet-punch face. The commonly used excipients for tablet were listed as Table 1 and tested for their ability to prevent water uptake of choline alfoscerate. 1g of choline alfoscerate was mixed with each excipient at the same weight and relative moisture uptake (%) was calculated by comparing the weight gain before and after left in an 80% RH condition for 1day. The excipients with relatively low moisture uptake were selected for the tablet formulation of choline alfoscerate.

2.3. Formulation study of choline alfoscerate tablet

Wet granulation method was applied in preparing the choline alfoscerate tablet. Table 2 shows the composition of core tablets to observe the effect of Neusilin on the protection of moisture uptake and disintegration time. Neusilin (5%-30%) was added both in and out of the granules in order to surround the drug particles more efficiently [40]. Briefly, after preblending choline alfoscerate with Neusilin and microcrystalline cellulose, the

granulation process was performed using high speed mixer (PharmaConnect™, GEA, Germany) with 70% ethanol binder solution containing PVP K-30. The binder solution was sprayed through a 0.3 mm spray nozzle at 1.5 bar, followed by drying at 50℃ in the oven (MOV-212S, Sanyo, Japan). Then, the granules were mixed with Neusilin, dicalcium phosphate, and croscarmellose sodium. After adding lubricant (sodium stearyl fumarate), oblong-shaped core tablets were compressed using the tableting machine (Rimek MINI II SF, Karnavati Engineering, India). Tablet processing problems including sticking, picking, laminating and punch-filling issue for each composition were recorded.

The core tablets were subcoated with HPMC based Opadry I® using organic solvent for 1% weight gain and followed by coating with PVA based Opadry AMB® using aqueous system for 3% weight gain to improve the water stability during storage of tablet [40].

2.4. Effect of Neusilin on the water stability and the disintegration time of tablet

2.4.1. Moisture uptake of core tablet

Weight gain by moisture uptake was measured to evaluate the effect of Neusilin at various contents (F1-F4, Table 2) and ratios of inter/intragranules (F5-F8, Table 2). Core tablets were put in the petri dish and left for 1 day in

the desiccator with 80% RH, equilibrium with the saturated aqueous solution of ammonium sulfate [41]. The weight gain(%) was determined by measuring the weight of tablets before and after storage in desiccator.

2.4.2. Disintegration time of core tablet

Disintegration time for core tablets was determined in water using disintegrator (DIT-200, Fine Scientific, Republic of Korea) by the disintegration method, USP. Soft capsule was also tested for disintegration as control.

2.4.3. Appearance change of film coated tablet

To evaluate the water stability for film coated tablet under various RH conditions, the desiccators with 22%, 33%, 60% and 80% RH were prepared using different saturated salts solution with potassium acetate, magnesium chloride, sodium bromide and ammonium sulfate, respectively [41]. Film-coated tablet containing of 15% Neusilin with 1:2 ratio of inter/intragranules was tested for the appearance stability. Film coated tablet was put on petri dishes as open state in desiccators for up to 30days. The time point at which the appearance began to change due to the uptake of moisture was recorded.

2.5. In vitro evaluation of test drug and reference drug

2.5.1. Test drug and Reference drug

Test drug (Alfocetine® tablet) was manufactured with Neusilin at a suitable ratio in Whanin Pharmaceutical company. Based on the formulation study of choline alfoscerate tablet, less than 18% of Neusilin to tablet weight was used. Not less than 50% of this was added into granules and the residual Neusilin was mixed with choline alfoscerate granules. And reference drug (Gliatilin® soft capsule) was produced by Daewoong Pharmaceutical company.

2.5.2. *In vitro* dissolution test

The dissolution of choline alfoscerate was measured using USP apparatus 2 (paddle). The dissolution medium was 900 mL of distilled water at 37 ± 0.5°C and stirred at 50 rpm. Dissolution study was conducted on 12 individual film coated test tablets or reference soft capsules. At the predetermined intervals (0, 5, 10, 15 and 30 min), 5 mL of the medium was sampled and filtered through a membrane filter (0.45 μm). Then, the concentration of choline alfoscerate was analyzed by using the high-performance liquid chromatography (HPLC) system with refractive index (RI) detector (Waters 410, Waters**,** MA, USA) [42]. Zorbax SB-CN column (250 x 4.6 mm, 5um, Agilent) filled with porous silica particles chemically bonded with nitrile

groups was applied for analytical assay and maintained at 38℃. The mobile phase was a mixture of acetonitrile and water (60/40, v/v) at a flow rate of 1.5 ml/min. The injection volume was 20 μL.

2.5.3. Stability test in the accelerated condition

Stability of the film coated tablets packaged with Zymax blister film (Bilcare, Singapore) was tested after keeping in the accelerated chamber (40℃ /75% RH) for 3months (Table 3). Appearance, assay and disintegration were compared with those of the reference soft capsule. The content of choline alfoscerate was analyzed by HPLC system, as described above. Hardness of test tablet was also checked using the hardness tester (MT50, Sotax, Switzerland), which is apt to be lowered by the moisture uptake. Dissolution test in water was also performed for the test tablets.

2.6. Bioequivalence study

2.6.1. Subjects

The bioequivalence study was conducted at Yangji Hospital (Seoul, Republic of Korea) with 19-46 aged healthy Korean male volunteers. All subjects were determined to be in good health based on medical history, physical examination and hematological examination. Subjects were excluded

if they had hypersensitivity to any ingredient in the choline alfoscerate tablets, took other drugs that could interfere with the study results within 10 days before this trial, had taken alcohol or medications that induce or inhibit drug-metabolizing enzymes (ex. barbitals) within 1 month before this study. All subjects signed a written informed consent after explained the purpose, the methods and the adverse drug reactions of this study in accordance with the regulatory guideline [39]. Subjects were monitored by hospital staff during the study period using interview, vital sign measurement, adverse event collection and physical examination.

2.6.2. Study design

This study was performed under fasted conditions with a randomized, single-dose, 2-period crossover design [39]. All subjects randomized in this crossover study received a single dose of choline alfoscerate of test tablet and reference soft capsule, separated by 7 days of washout between treatments. The protocol was approved by the Yangji Hospital institutional review board (IRB). Subjects were hospitalized for 5 days before the study and exercise, meal, smoking and consumption of grapefruit juice were restricted from 10hr before the beginning of the trial to the end of blood collecting. During the trial, consumption of food and drink, except water, were controlled and choline-free meals were provided.

Subjects were fasted for 10 hours before and 4 hours after drug administration to exclude the effects of diet. Two groups were treated with

1200 mg (choline alfoscerate 400 mg x 3 doses) of the reference capsule or the test tablet orally with 150 mL of water at 8 A.M. Subjects were not allowed to drink water for 1 hour before and after drug administration. Choline restricted standard meals were provided for lunch and dinner at 4 and 10 hours after dosing. After the Period I blood collection, subjects returned home and were advised to avoid excessive drinking, taking drugs, drinking grapefruit and to prohibit excessive intake of choline-containing foods (*e.g.*, eggs, beans). After 7 days for washout period, all subjects were called up to hospitalize for 5 days before the study and administered in the same manner as in the Period I.

A total of 24 blood samples were collected at predetermined time points (0, 0.5, 0.75, 1, 1.33, 1.67, 2, 3, 4, 6, 8 and 12 hours) on one day before drug dosing and on the day of drug administration. Before collecting each blood sample, 1 mL of blood was drawn and discarded to completely remove any remaining saline in the catheter. Aliquot (8 mL) of blood was collected into vaccutainer with sodium heparin and then 1 mL of heparinized normal saline was injected into the catheter to prevent blood clotting. Blood samples were centrifuged at 3,000 rpm for 10 minutes. The plasma was transferred to Eppendorf tube and stored at -70°C until analysis.

2.6.3. Determination of plasma choline concentrations

Choline concentration in each plasma sample was determined by a validated liquid chromatography-tandem mass spectrometry (LC-MS-MS)

assay for choline [43]. The plasma samples were placed at room temperature to thaw. Aliquot *(*1 mL) of metformin (20 ng/mL in methanol) was added as internal standard (IS) to 50 µL of plasma. Each sample was vortexed *and* centrifuged at 12,000 rpm for 5 min. The supernatant (2 µL) of the mixture was taken and chromatically analyzed by Shiseido Nanospace SI-2 (Osaka Soda, Japan) with an Luna 3µm HILIC (3 µm, 2.0 mm I.D.×150 mm L., Phenomenex, CA, USA). The mobile phase consisted of 1 mM ammonium formate and acetonitrile (45 : 55, v/v%). The flow rate was 0.3 mL/min. Column and sample tray temperatures were set at 45°C and 4°C, respectively. Detection and quantification were measured by Triple Quadruple Mass Spectrometer System, API 4000 (AB SCIEX, Canada) in positive ion electrospray ionization (ESI+) with the multiple reaction monitoring (MRM) mode. The m/z value of the precursor to product for choline and IS were 104.2 → 60.1 and 113.3 → 69.1, respectively. The LC-MS-MS system was controlled by using Analyst software (version 1.4, AB SCIEX, Canada) and the results were processed by using Microsoft Office Excel 2007 (Microsoft Corp., Washington, USA). The validation of this chromatographic analytical method was performed in order to evaluate its specificity, linearity, precision, accuracy and stability in solution. The calibration curve from the standard choline samples was constructed based on the peak area measurements, which was linear in 0.05 -10 µg/mL range.

2.6.4. Pharmacokinetic and statistical analysis

The concentration of choline in the plasma before and after drug administration was calculated with the peak area ratio of choline to the internal standard, metformin. The choline concentration after drug absorption at each time point was calculated by subtracting the endogenous choline level of the same blood collection point of each subject before the drug administration. When negative value was obtained after correction, it was considered as zero [44].

The pharmacokinetic parameters of choline were determined for both test tablet and reference soft capsule using a noncompartmental model with BA Calc 2007 program (version 1.0.0., MFDS, Seoul, Korea) [45]. The C_{max} and T_{max} were determined from the experimental data. The elimination rate constant (k_e) was calculated from the least-squares regression slope of the terminal plasma concentration, and then the t1/2 value was calculated as $0.693/k_e$. The calculated choline plasma concentrations were used to obtain the area under the plasma concentration-time profile from time zero to the last concentration time point (AUC_{0-t}). The AUC_{0-12} was calculated by the linear trapezoidal method [46]. $AUC_{0-\infty}$ was calculated as $AUC_{0-12} + C_{12}/ke$, where C_{12} was the choline concentration at the last time point (12 hr). Comparative bioavailability was measured by 90% confidence intervals (CIs) of the geometric mean ratios of test to reference which were determined using log-transformed data of AUC_{0-t} and C_{max}. All statistical calculations were performed using K-BE Test 2007 program (version 1.1.0., MFDS, Seoul, Korea) for bioequivalence analysis program recommended by the MFDS [47]. The KFDA regulatory range of bioequivalence for 90% CIs of geometric

mean ratios is 0.8-1.25 [39].

3. Results

3.1. Effect of Neusilin on the moisture uptake and the disintegration time of tablet

3.1.1. Moisture uptake and disintegration time of core tablet

Proper excipients with low moisture uptake rates were selected by category for the formulation of choline alfoscerate tablet. Table 1 summarized the list of excipients tested for the formulation of choline alfoscerate tablets and the weight gain (%) due to moisture uptake when each mixture was left in 80% RH condition for 1 day. Choline alfoscerate powder had good flowability, but was highly hygroscopic and was apt to be sticky under the exposure to air. It would cause sticking and picking problem during tablet manufacturing process by adhering to the surface of a tablet-punch face. When each excipient was mixed with choline alfoscerate, the amount of moisture uptake decreased because the surface area of drug exposed to the air decreased. Based on the moisture uptake measurement, the excipients with low moisture uptake were selected and marked in Table 1. It was notable that Neusilin showed lowest moisture uptake among tested. It is known to have ultrafine particle size of 15nm and high porosity [48]. Large surface area of Neusilin was expected to improve the stability of the drug against moisture by surrounding it with a small amount, thereby minimizing the retardation effect on the dissolution

rate.

Core tablets containing Neusilin with various contents and ratios of inter/intragranules were evaluated in terms of the tablet processing problems (Table 2). As shown in Table 2, the average weight of core tablets was in the range of 530~780 mg depending on the compositions and the hardness of the tablets was maintained in the range of 18~20 kp. F1 composition containing 5% Neusilin showed sticking and picking phenomenon during tableting process due to the insufficient amounts of it. Although higher content (30%) of Neusilin (F4) did not cause the tablet processing problem, the disintegration of tablet was retarded up to 19 min. Since the disintegration time of the reference soft capsule was 10 min, 15% Neusilin was selected, and was added in and out of granules at various ratio (F5~F8). When Neusilin was added only in granules (F5), sticking and picking were observed during the tableting process and weight gain by moisture uptake was relatively high. Moreover, when Neusilin was added out of the granules only (F8), filling of final mixture into the punch was not smooth enough. Thus, F7 composition was selected for film coating since the disintegration time was less than 10 min without tablet processing problem and the lowest moisture uptake. Neusilin was not added into intergranules but also was placed between granules. These core tablets were shown the similar disintegration time to reference drug without any tablet process problems. The proper distribution of Neusilin to inter/intragranules was needed to efficiently enclose the drug.

3.1.2. Appearance stability of film coated tablet

Fig. 2 shows the time point when the appearance of tablet began to change due to the uptake of moisture at various RH conditions. When the tablets took moisture, the film coating layer was broken by the swelling of core or water droplets were formed on the surface of tablets. It was noteworthy that the film-coated tablet of F7 maintained the appearance for up to 30 days in 60% RH condition.

3.2. In vitro evaluation of test drug and reference drug

3.2.1. *In vitro* dissolution test

Fig. 3 shows the dissolution profiles of choline alfoscerate (400 mg) from the film-coated test tablet and the reference soft capsules in distilled water. It was notable that the dissolution rate of choline alfoscerate from the test tablet was higher than that of the reference soft capsule for 10 min. It was probably due to the slower disintegration of gelatin shell than that of the tablet, and also indicated that Neusilin in tablet formulation did not affect the dissolution of choline alfoscerate. Moreover, the average dissolution rate of both test and reference reached not less than 85% within 15 minutes and dissolution profiles were accepted as similar, according to the KFDA guidelines [39]. In soft capsules, the deviation of dissolution was bigger than the test tablet,

which was seemed to be caused by the individual difference of disintegration of gelatin shell.

3.2.2. Stability test in the accelerated condition

The stability of the film-coated test tablet and the reference soft capsule stored under the accelerated condition for 3 months was summarized in Table 3. There was no change in appearance, disintegration time, content and harness of the test tablet during the storage period. Moreover, the dissolution profiles of choline alfoscerate in water were within the acceptance level of similarity with the initial for 3 months (Fig. 4). Based on the results of the stability studies under the accelerated condition, it was concluded that the hygroscopicity of choline alfoscerate was effectively controlled in the film-coated tablet, and thus the bioequivalence between the tablet and the soft capsule was tested in human.

3.3. Bioequivalence study

3.3.1. Subjects

Age 19-46 years old (mean ± SD: 27.7±6.5), height 158-188 cm (mean ± SD: 173.3 ± 6.6), weight 55-96 kg (mean ± SD: 69.4 ± 8.3) of fifty healthy Korean male volunteers were enrolled in this study. All of the subjects

completed the study in period 1, but 2 subjects withdrew in period 2. One subject withdrew because of acute upper respiratory infections, and the other subject withdrew because of a bruised leg. Thus, 48 subjects completed the study and were included in the pharmacokinetic analysis.

3.3.2. Pharmacokinetic analysis

Figure 5 showed the mean plasma concentration profiles of choline before and after the administration of test tablet and reference soft capsule. The corrected plasma concentration-time curve by subtracting the endogenous choline level was shown in the Figure 6, which suggested that test tablet had similar bioavailability to the reference soft capsule. The major mean pharmacokinetic parameters such as AUC_{0-t}, C_{max} and T_{max} for two preparations were summarized in Table 4. The mean $AUC_{0-\infty}$ for the test tablet was 3.43 ± 2.17 μg·h/mL and was not significantly different from that of the reference soft capsule (3.31 ± 1.80 μg·h/mL). The mean C_{max} of test tablet (0.37 ± 0.16 μg/mL) was also not significantly different from that of the reference soft capsule (0.38 ± 0.11 μg/mL). The mean T_{max} of test tablet and reference soft capsule were 3.51 ± 2.57 and 3.85 ± 3.19 hours, respectively, and was not significantly different, although the initial dissolution rate of choline alfoscerate from the reference soft capsule was slightly slower than that from the test tablet (Figure 3).

3.3.3. Statistical analysis and bioequivalence evaluation

Statistical results of bioequivalence evaluation between two formulations of choline alfoscerate 400 mg in healthy Korean male volunteers were summarized in Table 5. The calculated 90% confidence intervals (CIs) for geometric mean ratios of test to reference of AUC_{0-t} and C_{max} were 0.8451-1.1198 and 0.8331-1.0410, respectively, and were satisfied with the accepted bioequivalence criterial of 0.80-1.25. Moreover, AUC_{0-t} and C_{max} of the test tablet (Alfocetine®) and the reference soft capsule (Gliatilin®) were not significantly different.

3.3.4. Tolerability

Tolerability was recorded by vital sign, adverse event and physical examination, and no adverse events were reported during this study. Two subjects were excluded from the trial because of an acute upper respiratory tract infection (runny nose, cough) and a bruised leg with external impact during washout period and later fully recovered. These findings were considered to have little causal relationship with the drug.

4. Discussion

In this study, the formulation strategy was proposed on the development of tablet dosage form for choline alfoscerate. The functional excipients were screened to prevent the hygroscopicity of drug. Drug was changed to be wet and eventually liquefied within only 1day under 80% RH. Neusilin was selected as a proper excipient to minimize the hygroscopic property of drug because it showed the lowest moisture uptake among various excipients (Table 1). Small amounts of Neusilin could efficiently cover the surface of drug to block the moisture absorption because it had wide surface area and high porosity. A formulation design was required to use small amounts of effective excipient because choline alfoscerate was a drug for elderly person and tablet size should be minimized for patient compliance. Under this viewpoint, formulation strategies as follows were introduced in developing the choline alfoscerate tablet, 1) choline alfoscerate was granulated to reduce surface area of drug exposed to the air; 2) Neusilin was added into choline alfoscerate granules to control hygroscopicity of drug; 3) choline alfoscerate-containing granules were surrounded with Neusilin by simply mixing process to minimize the drug exposure; 4) After tableting, core tablet was subcoated with HPMC using organic solvent and followed by coating with water-dispersible moisture barrier coating system(Opadry AMB). Prototype compositions were prepared in the range of 5% - 30% Neusilin and more than

14% Neusilin could be solved for tablet processing problem due to the hygroscopicity of drug. When Neusilin was applied only into granules or outer granules, it could cause the tableting issues like as sticking or picking due to the insufficient coverage of drug (Table 2). As a result, it was necessary to add Neusilin into intergruanules and intragranules at the proper ratio of 2:1-1:2 to make tablet without any problems during tableting process. The moisture stability for F7 film coated tablet was tested as open state in various RH conditions (Fig. 2). Appearance stability of F7 tablet in 60% RH had been kept for about 1 month. This meant that choline alfoscerate tablet could be circulated in the market for a month without the change of appearance as packaged with medicinal porridge in drugstore, considering the annual average relative humidity in Seoul was 64%. The F7 tablet was packaged with PVDC blister (packaging type for commercialization) and stored at the accelerated condition(40℃/75% RH) for 3 months. There was no change in appearance and hardness during storage period for test tablet (Table 3). From this result, it was turned out that the hygroscopicity of drug might be effectively controlled using Neusilin in film coated tablet.

The bioequivalence study for choline alfoscerate tablet was designed according to the regulatory guideline for endogenous compound. Because choline alfoscerate was hydrolyzed into choline, endogenous substance, oral absorption by drug dosing should be corrected by the baseline of choline. Before drug dosing, endogenous baseline profile was checked for each subject who was managed to have choline restricted standard meal. The *in vivo* oral pharmacokinetic parameter of choline alfoscerate tablet was compared to

reference soft capsule. Statistical results of bioequivalence evaluation in healthy Korean male volunteers (Table 5) showed that AUC_{0-t} and C_{max} were not significantly different between test tablet and reference soft capsule and satisfied with the acceptance criteria of 0.80-1.25.

Thus, we could confirm that choline alfoscerate tablet was prepared by controlling the contents and inter/intragranular ratio of Neusilin without any tableting issue. The stability data in accelerated condition revealed that choline alfoscerate tablet was stable in appearance and dissolution for 3 months. The result of *in vivo* pharmacokinetic study suggested that tablet formulation containing Neusilin prepared in this study indicated bioequivalent to soft capsule for choline alfoscerate.

5. Conclusion

The hygroscopicity of choline alfoscerate was effectively controlled by using Neusilin in the preparation of film coated tablet. The proper content of Neusilin and its inter/intragranular distribution in tablet allowed to disintegrate of tablet quickly and led to eliminate the tablet process problems. The stability of tablet under the accelerated condition proved that film coated tablet of choline alfoscerate was successfully prepared. In the bioequivalence test of healthy Korean male volunteers, no significant differences in pharmacokinetic parameters were found between the test tablet and the reference soft capsule since 90% confidence intervals for AUC_{0-t} and C_{max} were within the regulatory acceptance criteria. These results indicated that the new tablet formulation (Alfocetine®) can be prescribed as an alternative to choline alfoscerate soft capsule.

Table 1. Selection of excipient for choline alfoscerate tablet (n=3).

Function	Excipient	Moisture uptake (%)*	Selection
Drug	Choline alfoscerate only	18.7±0.4[a]	
Diluent	Dicalcium phosphate	28.2±1.0	√
	Microcrystalline cellulose	32.2±1.0	√
	Lactose hydrate	34.4±0.6	
	Lactose anhydrous	34.4±1.6	
Binder	Polyvinylpyrrolidone K-30	31.8±1.2	√
	Hydroxypropylcellulose	32.8±1.6	
Superdisintegrant	Croscarmellose sodium	33.4±1.0	√
	Sodium starch glycolate	34.0±1.4	
	Crospovidone	33.8±1.6	
Glidant	Neusilin	29.6±1.4	√
	Calcium silicate	33.0±0.8	
	Aerosil	34.0±1.0	
	Talc	30.2±1.4	
Lubricant	Magnesium stearate	34.8±0.4	
	Sodium stearyl fumarate	29.0±1.4	√

* Weight gain (%) to choline alfoscerate (1g) when each mixture was left at 80% RH for 1 day.

[a] Weight gain (%) to choline alfoscerate powder (1g), which changed to be wet and eventually liquefied.

Table 2. Compositions of the Choline alfoscerate core tablets and effect of Neusilin on tablet processing problems and disintegration of tablet

Compositions	F1	F2	F3	F4	F5	F6	F7	F8
Choline alfoscerate (mg)	400	400	400	400	400	400	400	400
Neusilin(%)[a] (in/out of granules)	5 (1:1)	8 (1:1)	14 (1:1)	30 (1:1)	15 (1:0)	15 (2:1)	15 (1:2)	15 (0:1)
DCP/MCC(%)	11	17	17	10	15	15	15	15
PVP K-30(%)	2	2	0.5	1	0.5	0.5	0.5	0.9
Croscarmellose Na(%)	5	5	5	5	5	5	5	5
Sodium Stearyl Fumarate(%)	2	2	2	2	2	2	2	2
Total weight(mg)	530	600	650	780	650	650	650	650
Tablet processing problem	Sticking Picking	Lami-nating	No issue	No issue	Sticking Picking	No issue	No issue	Filling issue
Weight gain(%)[b]	49.0 ±0.4	33.2 ±0.6	29.0 ±0.7	28.0 ±0.3	38.4 ±0.9	26.8 ±0.6	25.6 ±0.8	29.2 ±1.0
Disintegration (min)	13	11	11	19	13	10	9	8

[a] Weight percent to total tablet weight

[b] Weight increase rate(%) to core tablets when it was left as open state at 80% RH for 1day

Table 3. Stability for test drug and reference drug of choline alfoscerate 400mg after 3 months storage under the accelerated condition (40℃/75% RH).

	Test		Reference	
	Initial	3 Months	Initial	3 Months
Appearance	Light yellow Oblong type Film coated tablet	No change 	Pale yellow Oval type Soft capsule	Deform 
Assay [a]	100.1±1.3%	101.9±0.4%	99.9±1.0%	101.2±1.2%
Disintegration, water	9 min	9 min	10 min	12 min
Hardness [a]	21.0±0.8 kp	20.7±1.2 kp	-	-

[a] Values were expressed as mean ± standard deviation.

Table 4. Pharmacokinetic parameters of choline after single oral administration of choline alfoscerate 1200 mg (n=48).

Parameter[a]	Test	Reference
AUC_{0-t} (μg·h/mL)	2.01±0.77	2.00±0.84
$AUC_{0-\infty}$ (μg·h/mL)	3.43±2.17	3.31±1.80
C_{max} (μg/mL)	0.37±0.16	0.38±0.11
T_{max} (h)	3.51±2.57	3.85±3.19
$t_{1/2}$ (h)	6.82±6.80	5.25±3.80

[a] Values were expressed as mean ± standard deviation except median (ranges) for T_{max}.

Table 5. Statistical results of bioequivalence evaluation between test drug and reference drug of choline alfoscerate in healthy Korean male volunteers.

Parameter	Geometric Mean[a]		Geometric Mean Ratio (Test/Ref.)	90% CIs	1-β
	Test (n=48)	Reference (n=48)			
AUC_{0-t} (μg·h/mL)	1.80	1.85	0.97	0.8451-1.1198	> 0.9
C_{max} (μg/mL)	0.34	0.36	0.93	0.8331-1.0410	> 0.9

[a] Values were log-transformed.

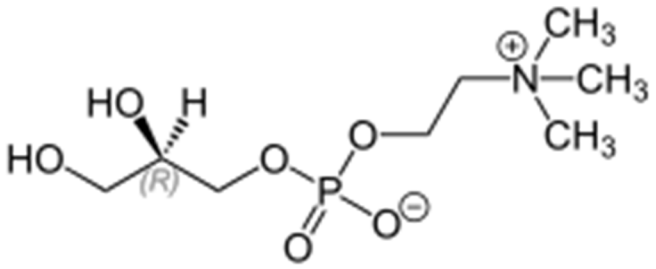


Figure 1. The structure of choline alfoscerate (L-Alpha glycerylphosphoryl choline).

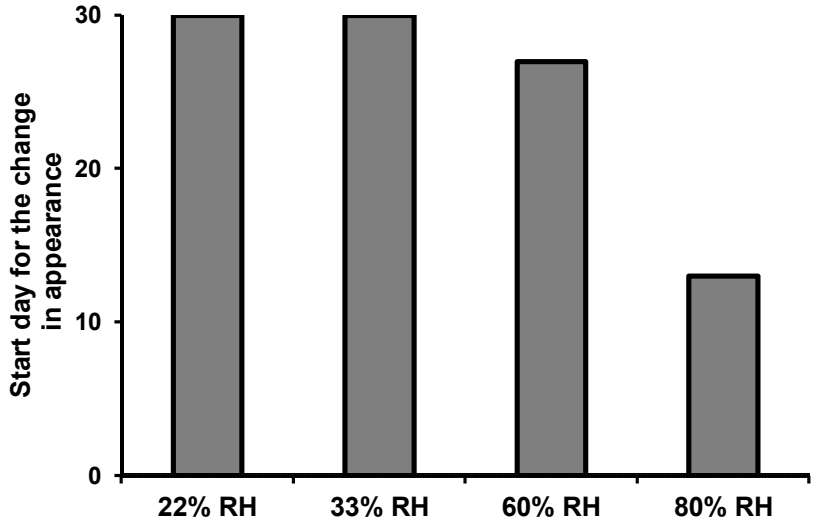


Figure 2. Appearance stability of choline alfoscerate film coated tablet containing 15% Neusilin with 1:2 ratio of inter/intragranules when left as open state under various RH conditions for 30days.

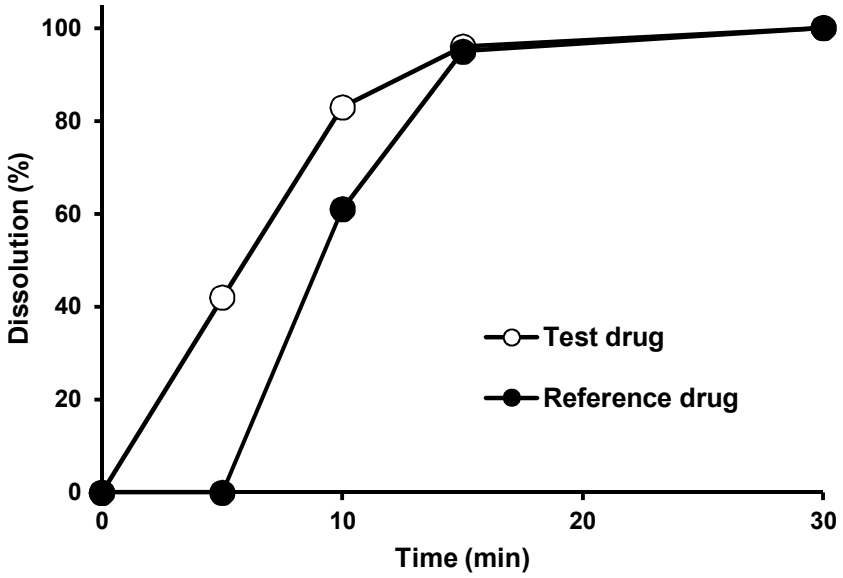


Figure 3. *In vitro* dissolution test for test drug and reference drug of choline alfoscerate 400 mg in distilled water (n=6).

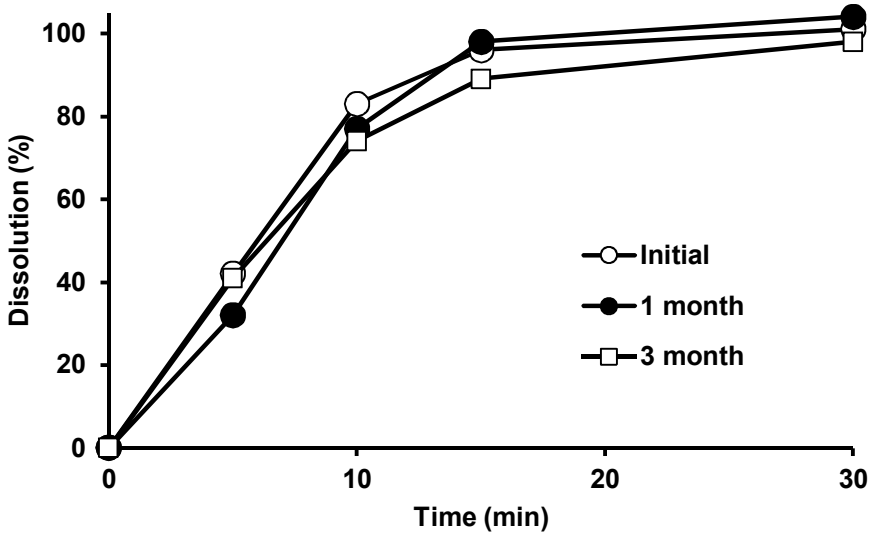
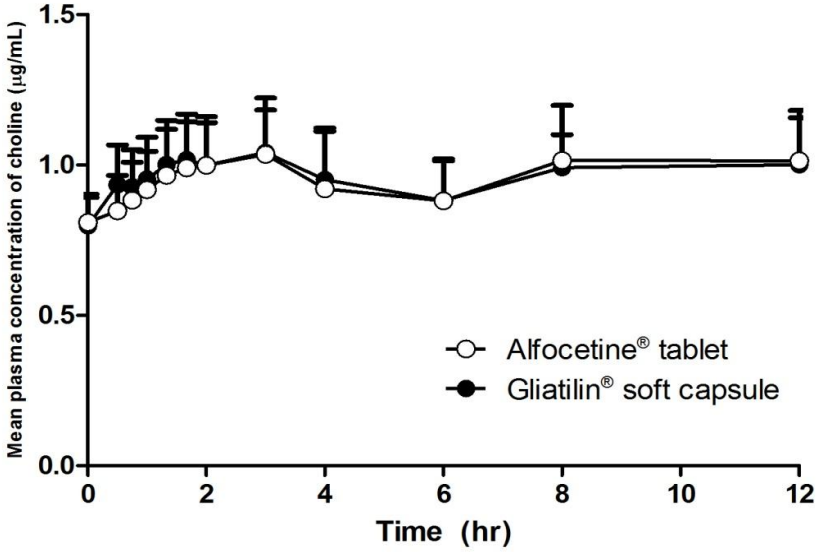


Figure 4. Dissolution profiles of choline alfoscerate from the film coated tablets stored in the accelerated condition (40℃/75% RH) for 3 months.

(A)



(B)

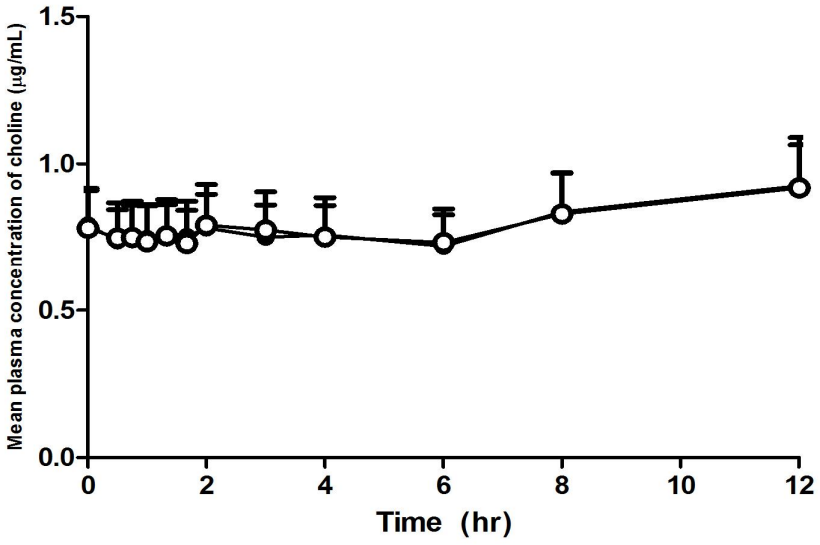


Figure 5. Baseline-uncorrected mean plasma concentration-time curve of choline (A) after oral administration of test tablet (Alfocetine®) or reference soft capsule (Gliatilin®) at the dose of choline alfoscerate 1200 mg, and (B) before drug administration. Vertical bars represent the standard deviation (n=48).

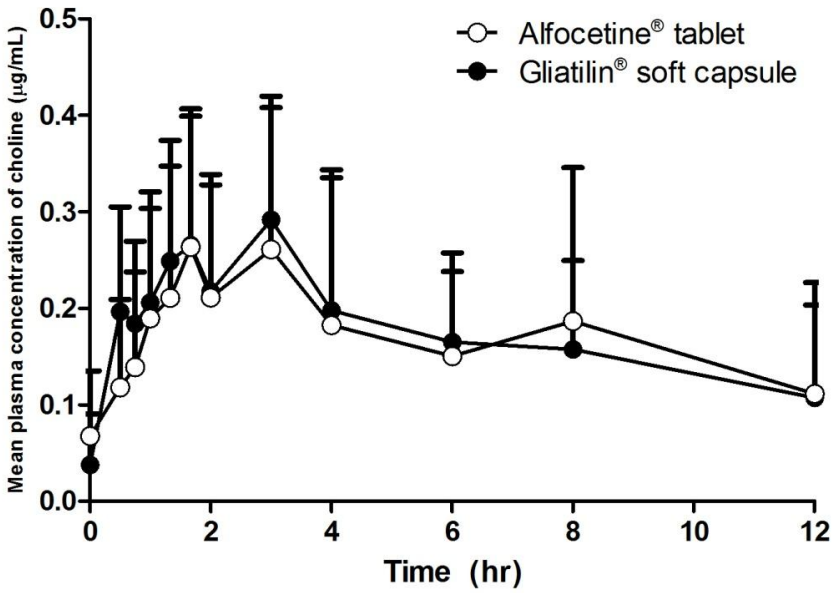


Figure 6. Baseline- corrected mean plasma concentration-time curve of choline after oral administration of test tablet (Alfocetine®) or reference soft capsule (Gliatilin®) at the dose of choline alfoscerate 1200 mg. The choline concentration after drug administration at each time point was calculated by subtracting the endogenous choline level of the same blood collection point of each subject before the drug administration. Vertical bars represent standard deviation (n=48).

References

[1] Advantages and disadvantages of soft gel capsules, 2011;June. Available from: http://formulation.vinensia.com/2011/06/advantages-and-disadvantages-of-soft.html. Accessed November, 2018

[2] Marzo A, Rescigno A. Pharmacokinetics of endogenous substances: some problems and some solutions. *Eur J Drug Metab Pharmacokinet.* 1993;18(1):77-88

[3] EMA, Guideline on the investigation of bioequivalence. January 2010. Doc. Ref.: CPMP/EWP/QWP/1401/98 Rev.1. Available from: https://www.ema.europa.eu/investigation-bioequivalence. Accessed November, 2018

[4] Kang MJ, Cho HR, Lee DH, Yeom DW, Choi YW, Choi YS. A method to monitor dutasteride in rat plasma using liquid-liquid extraction and multiple reaction monitoring: comparisons and validation. *Mass Spectrometry Letters* 2014; 5(3):79-83.

[5] Gullapalli RP. Soft gelatin capsules (softgels). *J Pharm Sci* 2010;99(10):4107-48.

[6] Subramanian P, Siddalingam R. Self-nanoemulsifying drug delivery systems of poorly soluble drug dutasteride: formulation and in-vitro characterization. *J Appl Pharm Sci.* 2017;7(4):11-22.

[7] Lee DH, Yeom DW, Song YS, Cho HR, Choi YS, Kang MJ, Choi YW. Improved oral absorption of dutasteride via Soluplus®-based supersaturable self-emulsifying drug delivery system (S-SEDDS). *Int*

J Pharm. 2015;478(1):341-347.

[8] Ali MS, Alam MS, Alam N, Siddiqui MR. (2014). Preparation, characterization and stability study of dutasteride loaded nanoemulsion for treatment of benign prostatic hypertrophy. *Iran J Pharm Res.* 2014;13(4):1125-1140.

[9] Avdeef A, Bendels S, Tsinman O, Tsinman K, Kansy M. Solubility-excipient classification gradient maps. *Pharm Res.* 2007;24(3):530-545.

[10] Loftsson T, Jarho P, Masson M, Jarvinen T. Cyclodextrins in drug delivery. *Exp Opin Drug Deliv.* 2005;2(2):335-351.

[11] Kurkov SV, Loftsson T. Cyclodextrins. *Int J Pharm.* 2013;453:167-180.

[12] Bonnet V, Gervaise C, Djedaïni-Pilard F, Furlan A, Sarazin C. Cyclodextrin nanoassemblies: a promising tool for drug delivery. *Drug Disc Today.* 2015;20(9):1120-1126.

[13] Miranda JCD, Martins TEA, Veiga F, Ferraz HG. Cyclodextrins and ternary complexes:technology to improve solubility of poorly soluble drugs. *Braz J Pharm Sci.* 2011;47(4):665-681.

[14] Jambhekar SS, Breen P. Cyclodextrins in pharmaceutical formulations I:structure and physicochemical properties, formation of complexes, and types of complex. *Drug Disc Today.* 2016;21(2):356-362.

[15] Mura, P. Analytical techniques for characterization of cyclodextrin complexes in the solid state: A review. *J Pharm Biomed Analysis.* 2015;113:226-238.

[16] Brewster ME, Loftsson T. Cyclodextrins as pharmaceutical

solubilizers. *Adv Drug Deliv Rev.* 2007;59(7):645-666.

[17] Loftsson T, Másson M. The effects of water-soluble polymers on cyclodextrins and cyclodextrin solubilization of drugs. *J Drug Deliv Sci Technol.* 2004;14(1):35-43.

[18] Loftsson T, Matthíasson K, Másson M. The effects of organic salts on the cyclodextrin solubilization of drugs. *Int J Pharm.* 2003;262(1):101-107.

[19] Li P, Tabibi SE, Yalkowsky SH. Combined effect of complexation and pH on solubilization. *J Pharm Sci.* 1998;87(12):1535-1537.

[20] Malmsten M. Surfactants and polymers in drug delivery. CRC Press 2002.

[21] Loftsson T, Brewster ME. Pharmaceutical Applications of Cyclodextrins. 1. Drug Solubilization and Stabilization. *J Pharm Sci.* 1996;85(10):1017-1025

[22] Raghavan SL, Trividic A, Davis AF, Hadgraft J. Crystallization of hydrocortisone acetate: influence of polymers. *Int J Pharm.* 2001;212(2):213-221.

[23] Reddy GSK, Kumar SA, Debnath M, Kumar VR. Analytical method development & validation for simultaneous determination of dutasteride and tamsulosin in bulk as well as in pharmaceutical dosage form by using RP-HPLC. *Int J Pharm Pharmaceut Sci.* 2014;6:77-84.

[24] Ramakrishna NVS, Vishwottam KN, Puran S, Koteshwara M, Manoj S, Santosh M. Selective and rapid liquid chromatography–tandem

mass spectrometry assay of dutasteride in human plasma. *J Chromatogr B*. 2004;809(1):117-124.

[25] Kim MS. Influence of hydrophilic additives on the supersaturation and bioavailability of dutasteride-loaded hydroxypropyl-β-cyclodextrin nanostructures. *Int J Nanomedicine.* 2013; 8:2029-2039.

[26] Taupitz T, Dressman JB, Buchanan CM, Klein S. Cyclodextrin-water soluble polymer ternary complexes enhance the solubility and dissolution behavior of poorly soluble drugs. Case example:Itraconazole. *Eur J Pharm Biopharm.* 2013;83:378-387.

[27] Donato EM, Martins LA, Fröehlich PE, Bergold AM. Development and validation of dissolution test for lopinavir, a poorly water-soluble drug, in soft gel capsules, based on *in vivo* data. *J Pharm Biomed Analysis*. 2008;47(3):547-552.

[28] Muankaew C, Loftson T. Cyclodextrin-based formulations: a non-invasive platform for targeted drug delivery. *Basic Clinic Pharmacol Toxicol*. 2018;122:46-55

[29] Zhao F, Malayev V, Rao V, Hussain M. Effect of sodium lauryl sulfate in dissolution media on dissolution of hard gelatin capsule shells. *Pharm Res*. 2004;21(1):144-148

[30] Shah VP, Konecny JJ, Everett RL, McCullough B, Noorizadeh AC, Skelly JP. In vitro dissolution profile of water-insoluble drug dosage forms in the presence of surfactants. *Pharm Res.* 1989; 6(7): 612-618

[31] Parnetti L, Mignini F, Tomassoni D, Traini E, Amenta F. Cholinergic precursors in the treatment of cognitive impairment of vascular origin:

ineffective approaches or need for re-evaluation?. *J Neurol Sci*. 2007;257:264-269

[32] Moreno MD. Cognitive improvement in mild to moderate Alzheimer's dementia after treatment with the acetylcholine precursor choline alfoscerate: a multicenter, double-blind, randomized, placebo-controlled trial. *Clin Ther*. 2003;25(1):178-193

[33] Gatti G, Barzaqui N, Acuto G, Abbiati G, Fossati T, Perucca E. A comparative study of free plasma choline levels following intramuscular administration of L-alpha-glycerylphosphorylcholine and citicoline in normal volunteers. *Int J Clin Pharmacol Ther Toxicol*. 1992;30(9):331-335

[34] Kang HA, Kim SM, Kang SR, Kang MS, Lee SN, Kwon IH, Yoo HD, Kim YG, Lee YB. Bioequivalence of cholicerin soft capsule to gliatilin soft capsule (choline alphoscerate 400mg). *J Kor Pharm Sci*. 2010;40(2):109-115

[35] Choi SU, Cho SW. Formulation of liquid choline alfoscerate as a solid dosage form. *J Korea Acad Industr Coop Soc*. 2013;14(12):6324-6329

[36] Helmis M, Mohamad B, Kumpugdee-Vollrath M. Influence of several excipients on drug release of tablets containing resveratrol. *M J Phar*. 2016;1(2):1-7

[37] Traini E, Bramanti V, Amenta F. Choline alphoscerate (alpha-glyceryl-phosphoryl-choline) an old choline-containing phospholipid with a still interesting profile as cognition enhancing agent. *Curr Alzheimer Res*. 2013;10(10):1070-1079

[38] Zeisel SH. Phosphatidylcholine:Endogenous precursor of choline. In Lecithin. Springer, Boston, MA. 1987;Chapter 11:107-120

[39] Korea Food & Drug Administration (KFDA). Guideline for managementof bioequivalence test (KFDA Notification No. 2013-115). April 2013. Available from: www.bio-core.com/UpFiles/NewsLetter/ 201308/5.pdf. Accessed November, 2018

[40] An BN, Min MH, Park JH. Tablets containing choline alfoscerate and methods for making the same, Korean patent 10-1628937, filed in Nov. 2014.

[41] Greenspan L, Humadity fixed points of binary saturated aqueous solutions. *J Res Natl Bureau Standards*. 1977;81(1):89-96

[42] The Korean Pharmacopoeia eleventh edition. Choline alfoscerate capsules. P1269, September 2018. Available from: http://drug.mfds.go.kr/html/CLASS/board/download.jsp?boardType=BOARD&boardSeq=154&seq=3321&fileSeq=2305. Accessed November, 2018

[43] Holm PI, Ueland PM, Kvalheim G, Lien EA. Determination of choline, betaine, and dimethylglycine in plasma by a high-throughput method based on normal-phase chromatography-Tandem Mass Spectrometry. *Clin. Chem*. 2003;49(2):286-294

[44] Korea Ministry of Food and Drug Safety. General consideration for bioequivalence studies of endogenous material. February 2013. Available from: http://drug.mfds.go.kr/html/CLASS/board/download.

jsp?boardType=BOARD&boardSeq=104&seq=1372&fileSeq=566. Accessed November, 2018

[45] Lee YJ, Kim YG, Lee MG, Chung SJ, Lee MH, Shim CK. Analysis of bioequivalence study using log-transformed model. *Yakhakhoeji*. 2000;44(4):308-314.

[46] Takka S, Sakr A, Goldberg A. Development and validation of an in vitro-in vivo correlation for buspirone hydrochloride extended release tablets. *J Cont Rel*. 2003;88(1):147-157.

[47] Lee YJ, Choi JH, Song SH, Seo CH, Kim DS, Park IS, Choi KH, Na HK, Chung SJ, Lee MH, Shim CK. Development of K-BE test, a computer program for the analysis of bioequivalence. *J Kor Pharm Sci*. 1998;28:223-229.

[48] Rowe RC, Sheskey PJ, Quinn ME. Handbook of pharmaceutical excipients sixth edition. London:Pharmaceutical press gear

국문초록

본 연구에서는 상업적으로 생산되고 있는 연질캡슐제제와 동등수준의 정제 제형을 개발하기 위해 서로 다른 물성을 보이는 2 종의 주성분을 대상으로 하여 제제화전략을 수립하였고 in vivo 에서의 생체동등성을 확인하였다. 일반적으로 연질캡슐의 경우 고온에서 젤라틴 피막의 성상 변화가 일어나거나 장기간 보관시 내부에 용해되어 있던 약물이 젤라틴 피막으로 이행되는 경우가 있다. 고온 및 외부 충격에 의한 젤라틴피막의 물리적 강도의 약화는 피막의 손상으로 이루어져 내부에 봉입되어 있는 약물이 누출되는 문제점이 있다. 약물의 용해도 이슈로 인해 연질캡슐제로 개발된 두타스테라이드와 약물의 수분안정성 이유로 연질캡슐제로 시판된 콜린알포세레이트, 두 약물에 대해 각각 가용화연구와 인습방지연구를 진행하여 연질캡슐과 동등수준의 품질기준을 만족하는 필름코팅정을 제조하였다. 각 정제는 대조약인 연질캡슐제 대비 동등한 *in vitro* 용출패턴을 나타냄을 확인하였다. BCS class II 약물인 두타스테라이드의 경우 감마사이클로덱스트린과의 포접연구 및 적절한 용해보조제를 선택함으로써 원하는 용출패턴을 얻을 수 있었다. BCS class III 약물인 콜린알포세레이트의 경우 주성분의 함습을 방지하기 위해 노이실린(메타규산알루민산마그네슘)을 과립내외에 적절한 비율로 첨가함으로써 타정장애 없이

안정한 필름코팅정을 제조할 수 있었다. 두타스테라이드정은 대조약인 아보다트연질캡슐보다 작은 크기로 개발되어 노인 환자의 복약편의성을 증진시킬 수 있을 것으로 예상되었으며 연질캡슐과 달리 주성분이 고형상태로 제형화되었기 때문에 연질캡슐과는 달리 약물 누출에 따른 안전성 이슈를 해결할 것으로 기대된다. 두타스테라이드정은 비글견을 이용한 약물동태시험을 통해 연질캡슐과 *in vivo* 에서의 동등성을 확인하였다. 콜린알포세레이트정은 가속조건에서 3 개월간 안정함을 확인하였으며 건강한 성인남자를 대상으로한 생체동등성 평가에서 연질캡슐과의 동등성을 입증하였다.

주요어 : 정제; 연질캡슐제; 두타스테라이드; 콜린알포세레이트; 생체동등성

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