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Physicochemical characteristics and bioavailability of a novel intestinal metabolite of ginseng saponin (IH901) complexed with β-cyclodextrin

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Abstract

In an effort to improve the bioavailability (BA) of the insoluble compound 20-*O*-(β-D-glucopyranosyl)-20(*S*)-protopanaxadiol (IH901), we prepared β-cyclodextrin (βCD) and hydroxypropyl-β-cyclodextrin (HPβCD) inclusion complexes containing IH901. IH901 is a major metabolite formed by intestinal bacteria from protopanaxadiol ginseng saponins. We developed and validated an HPLC-based method to measure IH901 levels from samples prepared in vitro. The phase solubility profiles with both cyclodextrins (CDs) were classified as A_L-type, indicating the formation of a 1:1 stoichiometric inclusion complex. Stability constants (K_s) calculated from the phase solubility diagrams showed that the βCD complex was more stable than the HPβCD complex. Consequently, complexes of IH901 and βCD were prepared by a freeze-drying method and were analyzed by fourier transformation-infrared spectroscopy (FT-IR), X-ray diffraction, differential scanning calorimetry (DSC), and scanning electron microscopy (SEM). From these physicochemical characterizations, we confirmed the presence of a new solid phase in the freeze-dried samples. The IH901 released from the complex in a pH 1.2 solution, the pH range of gastric fluids, was considerably lower than the amount released in the other solutions. The IH901 released from the complex in pH 6.8 solution, the range of intestinal fluids, was 9.0-fold greater than pure IH901 powder. However, the amount of IH901 released from the complex in pH 4.0–8.0 was less than 20%. After oral administration of the IH901–βCD inclusion complex (30 mg/kg IH901) into rats, plasma concentrations were determined by LC/MS/MS. The peak concentration (C_{max}) for the inclusion complex was 2.8-fold higher than that for pure IH901 powder. The BA, calculated from the ratio of the AUC_{oral} to the $AUC_{i.v.}$, for the pure IH901 powder, the IH901–βCD physical mixture, and the inclusion complex was 3.52, 4.34, and 6.57%, respectively. These results indicate that the BA for the inclusion complex was 1.9-fold higher than that for the pure IH901 powder.

Keywords: Ginseng saponin metabolite (IH901); Cyclodextrin; Inclusion complex; Phase solubility; Physicochemical characteristics; Pharmacokinetics; HPLC; LC/MS/MS

1. Introduction

Ginseng, the root of *Panax ginseng* C.A. Meyer, has been taken orally as an important traditional medicine for the treatment of many diseases including diabetes mellitus and neurological disorders. Ginsenosides are regarded as the principal active components of ginseng. Many reports have shown pharmacological activities of the ginsenosides, including immunomodulatory effects, anti-inflammatory effects, effects on the CNS, antiallergic effects, and antitumor effects (Mizuno et al., 1994; Kim et al., 1998; Ro et al., 1998; Surh et al., 2001).

It was recently observed that when humans or rats receive ginseng extract orally, intestinal bacteria generate ginseng saponin metabolites (Hasegawa et al., 1996). One of the major metabolites is 20-*O*-(β-D-glucopyranosyl)-20(*S*)-protopanaxadiol, also known as IH901 (Fig. 1). IH901 appears in the plasma after oral administration of the ginsenoside Rb_1. Hasegawa et al. (1997) showed that the ginsenoside Rb1 was transformed to IH901 by bacteria in the large intestine, and that expression of the antimetastatic and anticarcinogenic effect of ginseno-

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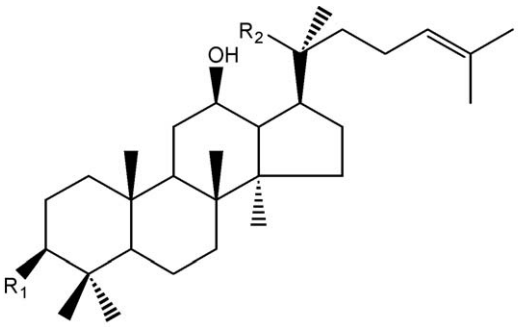


	R_1	R_2
IH901	OH	O- Glc
Rb1	O- Glc(2→1)Glc	O- Glc(6→1)Glc

Fig. 1. Structural formula of IH901, the novel ginseng saponin metabolite 20-*O*-(β-D-glucopyranosyl)-20(*S*)-protopanaxadiol. Glc: β-D-glucopyranose.

sides was based primarily on the intestinal bacterial metabolites (Hasegawa et al., 1997; Wakabayashi et al., 1997).

Akao et al. (1998) have shown that IH901 appears in rat plasma after the oral administration of ginsenoside Rb1, which is transformed in the intestine into IH901 via the ginsenosides Rd1 and F2. This study also found that after oral administration of ginsenoside Rb1, IH901 appeared late in the plasma, and was retained for as long as 48 h. In addition, when IH901 was administered orally it was rapidly absorbed from the intestinal tract, after which its levels in the plasma slowly decreased. Orally administered IH901 was detected in the gastrointestinal tract for a long time, when levels in the blood were already very low (Hasegawa et al., 2000).

The antitumor properties of IH901 are currently being tested pre-clinically to determine their suitability for clinical trials (Hasegawa et al., 1997; Lee et al., 1998). The characterization of IH901 pharmacokinetics in a pre-clinical study would be very useful in this respect. However, it has been reported that orally administered ginsenosides are poorly absorbed through the intestines, as they have a bioavailability of approximately 4% (Akao et al., 1998; Hasegawa et al., 2000; Xu et al., 2003). Consequently, we prepared IH901-β-cyclodextrin inclusion complexes to improve the solubility of IH901 in aqueous solutions and to enhance the BA of IH901. The IH901-β-cyclodextrin inclusion complexes were prepared by freeze-drying and the in vitro physicochemical characteristics and in vivo pharmacokinetics of the complex were evaluated. We developed and validated the HPLC-based and LC/MS/MS-based methods to analyze IH901 levels from in vitro samples and in vivo biological samples, respectively.

2. Materials and methods

2.1. Chemicals

20-*O*-(β-D-Glucopyranosyl)-20(*S*)-protopanaxadiol (purity, 99.0–101.0%), which was biosynthesized by incubating ginseng saponins and intestinal bacteria as described by Hasegawa et al. (1996), was kindly donated by IL HWA Co. Ltd. (Kyunggi, South Korea). β-Cyclodextrin (βCD) and hydroxypropyl-β-cyclodextrin (HPβCD) were purchased from Sigma (St. Louis, MO, USA). HPβCD was substituted by 0.67 hydroxypropyl groups per glucose unit. Cremophor® EL was purchased from BASF (Aktiengesellschaft 67056 Ludwig-sharfen., Germany). Solvents used in the IH901 analysis were HPLC grade and were filtered and degassed just prior to use. All other chemicals used in this study were analytical reagent grade.

2.2. Animals

Animals were handled in accordance with the guidelines of the Korea Food and Drug Administration (KFDA). The experimental and surgical protocols were approved by the Chungbuk National University Animal Welfare Committee. Adult male Sprague–Dawley rats (230–250 g; Sam Tac Co. Ltd., Kyunggi, South Korea) were used for the pharmacokinetic studies. The animals were housed in individual metabolic cages during and after administration of the drug. Prior to oral dosing, the rats were fasted overnight and for 4 h after the drug was administered. The animals were maintained under a 12 h light/dark cycle with free access to water at all times.

2.3. Phase solubility studies

Phase solubility studies were carried out in water according to the method previously reported by Higuchi and Connors (1965). Briefly, excess amount of IH901 were added to 10 mL of aqueous solution containing various concentrations of βCD (0–30 mM) or HPβCD (0–60 mM). The suspensions were vigorously shaken in a 50 °C water bath for 7 days. After attaining equilibrium, the samples were filtered through a 0.45 nm membrane filter, and diluted for HPLC analysis. The IH901 concentration was determined by HPLC at 205 nm, as described below. The apparent stability constants (K_s) were calculated from the phase solubility diagrams, according to the following equation

$$K_s = \frac{\text{slope}}{S_0(1 - \text{slope})}$$

where S_0 is the IH901 solubility in the absence of βCD or HPβCD.

2.4. Preparation of IH901-β-cyclodextrin inclusion complex

The inclusion complexes were prepared with the following modifications of the previously described method (Kurozumi et al., 1975). IH901 (50 mM) dissolved in ethanol was added to an equal molar ratio of βCD in aqueous solution, and the mixture was stirred thoroughly for 24 h at 50 °C. Filtration (0.45 μm membrane filter, Whatman) was performed to remove undissolved IH901. The resulting solution was frozen at −80 °C (Model: MDF-U50V, Sanyo Inc., Osaka, Japan) and

then lyophilized in a freeze-dryer (Model: FD-5N, EYELA, Tokyo, Japan) for 2 days. The freeze-dried powder was washed with ether and the residue was dried at 50 °C.

2.5. Physicochemical characterization of the inclusion complexes

2.5.1. Fourier transformation-infrared spectroscopy (FT-IR)

Infrared spectra were obtained using a spectrometer (Model: FTIR-8400S, Shimadzu Co., Kyoto, Japan). The samples were ground and mixed with potassium bromide (KBr), an infrared transparent matrix, using 1% dilution. The KBr disks were prepared by compressing the powder.

2.5.2. X-ray diffractometry

The X-ray diffraction patterns of the powders were recorded using an instrument equipped with a high-temperature attachment (Model: XDS 2000, Scintag Inc., Sunnyvale, CA, USA). The samples were analyzed in the 2 theta (θ) range of 3–50°.

2.5.3. Differential scanning calorimetry (DSC)

Differential scanning calorimetry measurements were performed on a thermal analyzer (Model: TA4100) equipped with a differential scanning calorimeter (Model: MDSC2910, TA Instrument, New Castle, Delaware, USA). Alumina was used as a reference material and the scanning rate was 10 °C/min, with a scanning temperature range of 25–300 °C.

2.5.4. Scanning electron microscopy (SEM)

Shapes and surface characteristics of the raw materials and the binary complexes were investigated and photographed using a scanning electron microscopy (Model: Leo1530, Carl Zeiss SMT AG, Oberkochen, German).

2.6. Dissolution studies

IH901, the IH901–βCD physical mixture, or the IH901–βCD inclusion complex (5 mg as IH901) was inserted into a Spectra/por™ dialysis membrane, containing 5 mL of buffer solutions (pH 1.2, 4.0, 6.8, or 8.0) or water. The membrane bag was sealed, and then immersed in 45 mL of the same solution and shaken at 100 rpm at 37 °C in the water bath. An aliquot of the solution (1 mL) was withdrawn at each time interval and an equal volume of the same solution was replaced. The IH901 concentration in the solution was determined by HPLC at 205 nm as described below.

2.7. Analysis of IH901

2.7.1. HPLC analysis of IH901 levels from in vitro samples

The sample solutions from the solubility and dissolution studies were analyzed by HPLC (Hasegawa et al., 2000). IH901 levels were assayed by reverse phase HPLC on a Inertsil ODS-2 C_{18} column (UG120A, 4.6 mm × 250 mm, 5 μm, GL Sciences Inc., Tokyo, Japan) that was interfaced with a Jasco HPLC system. This system consisted of a model PU-980 pump, a model AS-950-10 autoinjector, a UV–vis detector, and a LC-Net II control borwin integrator (Jasco Co. Ltd., Tokyo, Japan). The mobile phase was a mixture of acetonitrile and doubly deionized water (50:50, v/v). The flow rate was 1 mL/min. The IH901 in the eluate was monitored spectrophotometrically at 205 nm.

Immediately after collection of the samples (200 μL), ginsenoside-Rb3 (internal standard; 50 μL, 200 μg/mL) and methanol (3 mL) were added. These mixtures were vortexed for 15 min and centrifuged for 15 min at 1500 × *g*. The supernatants were withdrawn, dried under a stream of dry nitrogen and reconstituted in 150 μL methanol for quantitative HPLC analyses. Control samples taken prior to drug treatment show that there were no peaks that interfered with the IH901 or IS signals. The mean regression equation was $y = 0.0482x + 0.0042$ ($r^2 = 0.998$, $n = 5$), where y is the peak area ratio of IH901 to internal standard and x is the concentration. These equations show that there is significant linearity over the concentration range of 0.1–1000 μg/mL. The diluted solution was used for samples over the concentration of 1000 μg/mL. Variations for both precision and accuracy of the inter- and intra-day results never exceeded 15%. The lower limit of quantitation (LOQ) was therefore defined as 0.1 μg/mL.

2.7.2. LC/MS/MS analysis of IH901 levels in biological samples

The plasma concentrations of IH901 were determined by liquid chromatography–mass spectrometry with a triple quadrupole mass spectrometer (Model: Sciex API 3000, Applied Biosystems, MDS Sciex, Concord, Canada) equipped with a turbo-ion-spray interface. The positive ionization mode was used to generate the molecular ion for mass detection. The multiple reaction monitoring (MRM) mode was used to quantify IH901. The product ion at m/z 425.0 from the most intensive precursor ion m/z 640.8 for $[IH901 + NH4]^+$ was detected. The HP1100 series (Agilent Technologies, Palo Alto, USA) LC system equipped with degasser, pump, and autosampler was used. The analytical column was an Xterra RP18 column (5 μm, 2.1 mm × 150 mm, Waters Co., USA) and a mixture of acetonitrile: 20 mM ammonium acetate (70:30, v/v) was used as the mobile phase at a flow rate of 0.2 mL/min.

Typical chromatograms of IH901 and the internal standard (IS) in plasma are shown in Fig. 2. The retention time of IH901 and the IS (prednisolone) were 4.3 and 2.0 min, respectively. Control plasma samples taken prior to drug treatment showed that there are no peaks that interfere with the IH901 or IS signals. The mean regression equation for IH901 in plasma was $y = 0.0121x + 0.0024$ ($r^2 = 0.999$, $n = 10$), where y is the peak area ratio of each compound to IS and x is the concentration. These equations show that there is significant linearity over the concentration range of 5–500 ng/mL. Variations for both precision and accuracy of the inter- and intra-day results never exceeded 15%. The lower limit of quantitation was therefore defined as 5 ng/mL. The mean absolute recovery of IH901 in plasma was over 96%.

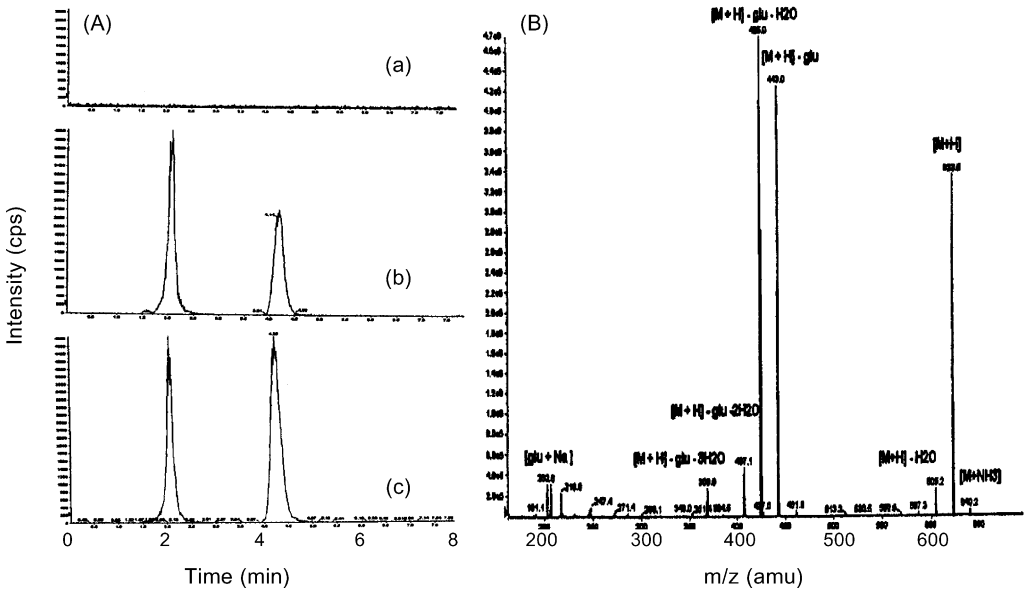


Fig. 2. Representative LC/MS/MS chromatograms of IH901 (A), and positively ionized mass spectrum of IH901 (B). The upper, middle and lower panel of chromatograms represent (a) blank plasma, (b) plasma spiked with IH901 (10 ng/mL) and internal standard (IS), and (c) plasma sample 2 h after oral administration of IH901 (30 mg/kg), respectively. The retention times of IH901 and IS (prednisolone) were 4.3 min and 2.0 min, respectively. IH901 was detected at *m/z* 640 $[IH901 + NH_4]^+ \rightarrow$ *m/z* 460 $[IH901 + NH_4]$-glu^+ using MRM.

2.8. Bioavailability studies

2.8.1. Administration of IH901 and analysis of IH901 levels in the plasma

Under light ether anesthesia, the femoral vein and artery were cannulated with PE-50 polyethylene tubing (Intramedic, Clay Adams, U.S.A.) for IH901 administration and blood sampling, respectively. IH901, dissolved in 7% Cremophor® EL solution (Kwon and Chung, 2004), was administered into the femoral vein at a dose of 30 mg/kg. Oral administration with pure IH901, physical mixture, or βCD inclusion complex at a dose of 30 mg/kg was performed after suspending the materials in water just prior to use. Blood (200 μL) was collected into heparinized tubes from the femoral artery at 0.167, 0.333, 0.5, 0.75, 1, 1.5, 2, 4, 8, 12, 24, 36, and 48 h after i.v. bolus or oral dose. The blood samples were centrifuged for 15 min at 1500 × *g* and the plasma was harvested. Immediately after the collection of the plasma samples (100 μL), prednisolone (10 μL, 100 ng/mL) was added to each plasma test tube as an internal standard. Acetonitrile (3 mL) was then added to precipitate the proteins and extract the compounds of interest. These mixtures were vortexed for 15 min and centrifuged for 15 min at 1500 × *g*. The supernatants were withdrawn, dried under a stream of dry nitrogen and reconstituted in 100 μL acetonitrile for quantitative analysis of IH901 by LC/MS/MS.

2.8.2. Pharmacokinetic analysis

Non-compartment methods (Yamaoka et al., 1981) were used to determine the pharmacokinetic parameters of IH901 following i.v. bolus or oral administration. The area under the plasma concentration–time curve from time zero to infinity (AUC) was calculated from the equation $AUC = AUC_t + C_t/k$, where C_t is the last quantifiable concentration, and k was calculated from the slope of a straight-line in the terminal phase of plasma disappearance. The terminal phase half-life ($t_{1/2}$) was calculated as: $t_{1/2} = 0.693/k$. The area under the plasma concentration–time curve from time zero to the time of the last quantifiable concentration (AUC_t) was calculated by linear trapezoidal approximation. The following parameters were calculated using standard methods: the total plasma clearance (CL_t) = F·Dose/AUC; the steady-state volume of distribution (Vd_{ss}) = CL_t·MRT; the mean residence time (MRT) = AUMC/AUC, where AUMC represents the area under the moment curve. The BA% value for oral doses of IH901 was calculated from the dose-adjusted ratio of AUC_{oral} to $AUC_{i.v.}$.

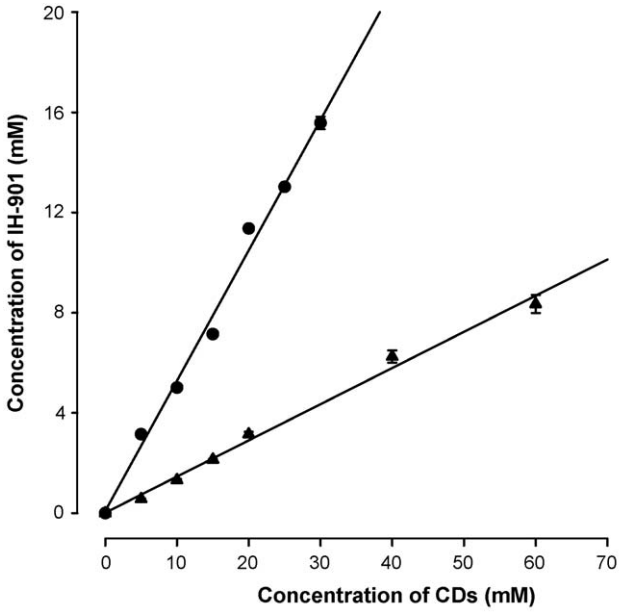
2.9. Statistical analysis

The unpaired Student's *t*-test was used to compare two groups. One-way analysis of variance was used to test for significant differences between multiple groups. Statistical significance was defined as $P < 0.05$.

3. Results and discussion

3.1. Phase solubility studies

The phase solubility diagrams for IH901–βCD and IH901–HPβCD inclusion complexes at 37 °C were obtained by plotting the apparent equilibrium concentrations of IH901 against the CD concentrations (Fig. 3). The apparent solubility of IH901 increased linearly as a function of CD concentration, over the entire concentration range studied. Linearity was charac-



Cyclodextrin	Medium	S_0 (×10^{-3}M)	Slope (×10^{-2})	K_s (×10^{-3}M^{-1})	1/slope	r^2
βCD	water	0.245±0.007	51.58±0.534	4.52±0.340	1.94±0.020	0.9914
HPβCD	water	0.235±0.007	14.2±0.310	0.68±0.037	7.02±0.152	0.9917

Fig. 3. Phase solubility diagrams of IH901–βCD (●) and IH901–HPβCD (▲) in water. IH901 solubility in the absence of CDs. The S_0, slope, K_s, 1/slope, and r^2 correlation coefficient parameters are derived from IH901-cyclosporin (CD) phase solubility diagrams. Each point represents the mean ± S.D. ($n = 4$).

teristic of the A_L-type system (Higuchi and Connors, 1965) and suggested that water soluble complexes formed in solution. Furthermore, the slope values were always lower than 1.0, indicating that inclusion complexes in a molar ratio of 1:1 between the guest (IH901) and host (CDs) molecules were formed.

The apparent stability constants (K_s) of the 1:1 complexes were calculated from the slopes of the phase solubility diagrams and the respective S_0 values. The values of K_s, S_0, the corresponding slopes, and the correlation coefficients of the phase solubility diagrams are summarized in Fig. 3. The K_s value of βCD was greater than that of HPβCD, suggesting that βCD formed more stable inclusion complexes with IH901 than did HPβCD. This phenomenon could be due to steric hindrance of the hydroxypropyl groups of HPβCD, which can hamper the inclusion of guest molecules within the CD cavity (Prankerd et al., 1992). Consequently, we prepared IH901–βCD inclusion complexes for the physicochemical characterization and the BA studies of the complexes.

3.2. Drug content in the IH901–βCD physical mixture and inclusion complex

The actual IH901 content in each binary mixture was determined by an HPLC-based method. Equal amounts of IH901 and CDs mixed by shaking at 50 °C for 30 min, referred to

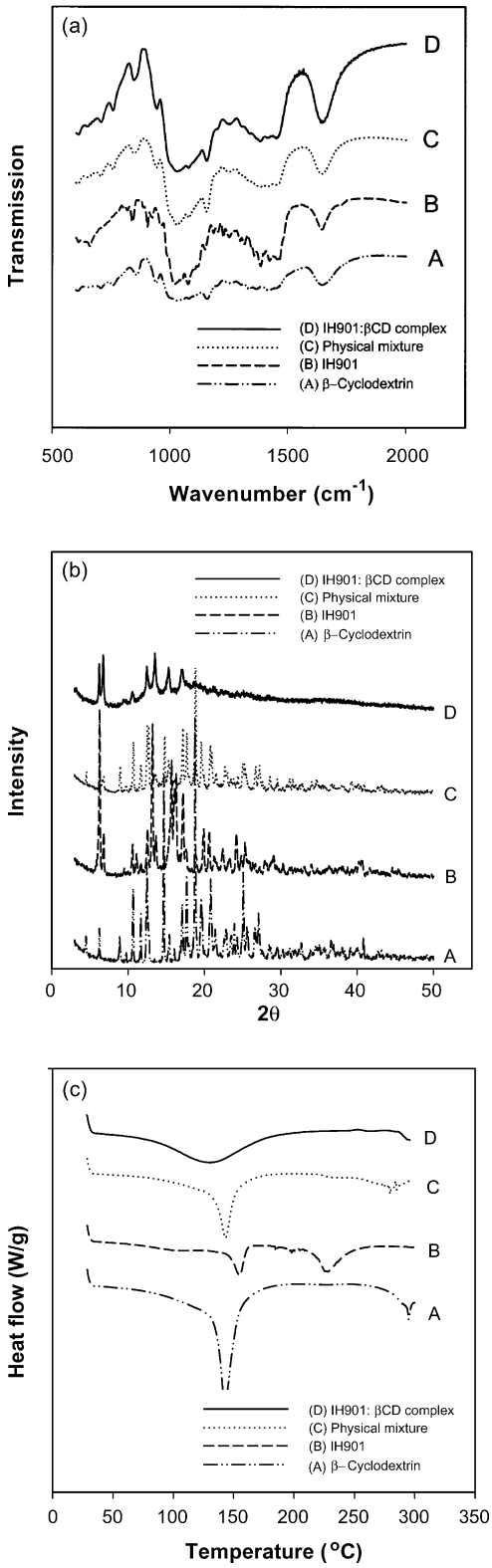


Fig. 4. FR-IR spectra (a), X-ray diffractograms (b), and DSC thermograms (c) of βCD (A), IH901 (B), IH901–βCD physical mixture (C), and IH901–βCD inclusion complex (D).

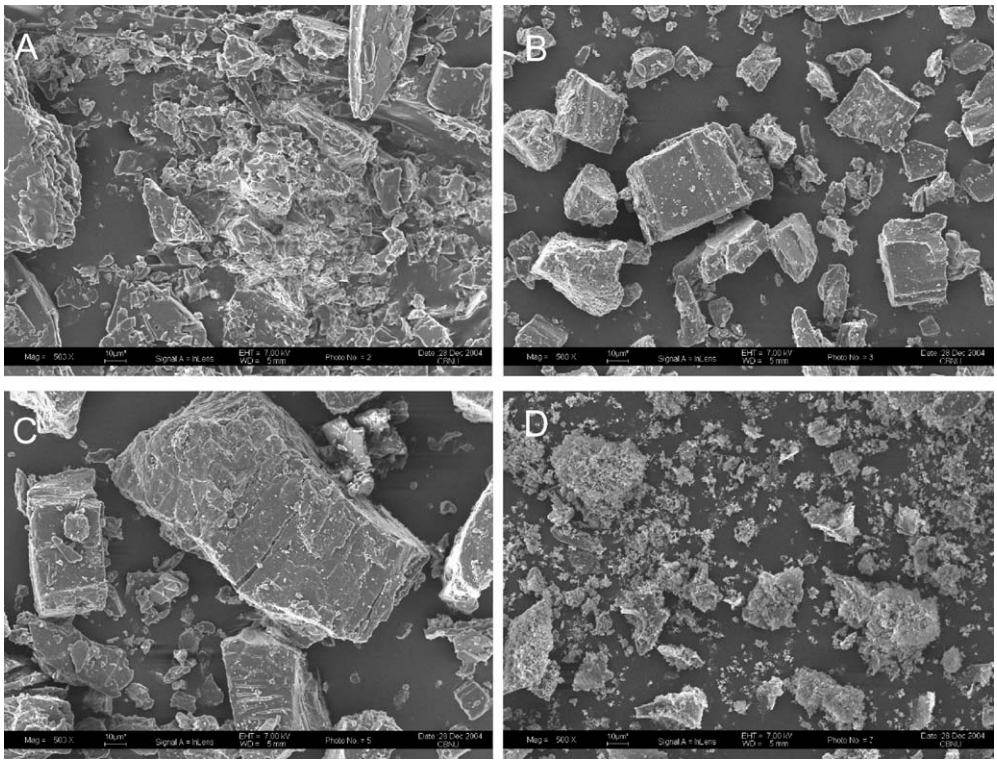


Fig. 5. Scanning electron microphotographs of IH901 (A), βCD (B), IH901–βCD physical mixture (C), and IH901–βCD inclusion complex (D).

as a physical mixture, were used for comparison to the inclusion complexes. IH901 content for the physical mixture was 49.8 ± 0.51 (% molar ratio ± S.D.). In the freeze-dried inclusion complexes, IH901 was not completely dissolved in water due to poor solubility, and the actual drug content (41.3 ± 0.67%) was consequently lower than the theoretical value (50%).

3.3. Physicochemical characterization of IH901–βCD inclusion complexes

3.3.1. Fourier transformation-infrared spectroscopy

Infrared spectra obtained from FT-IR, showed no significant differences among IH901, βCD and freeze-dried powder

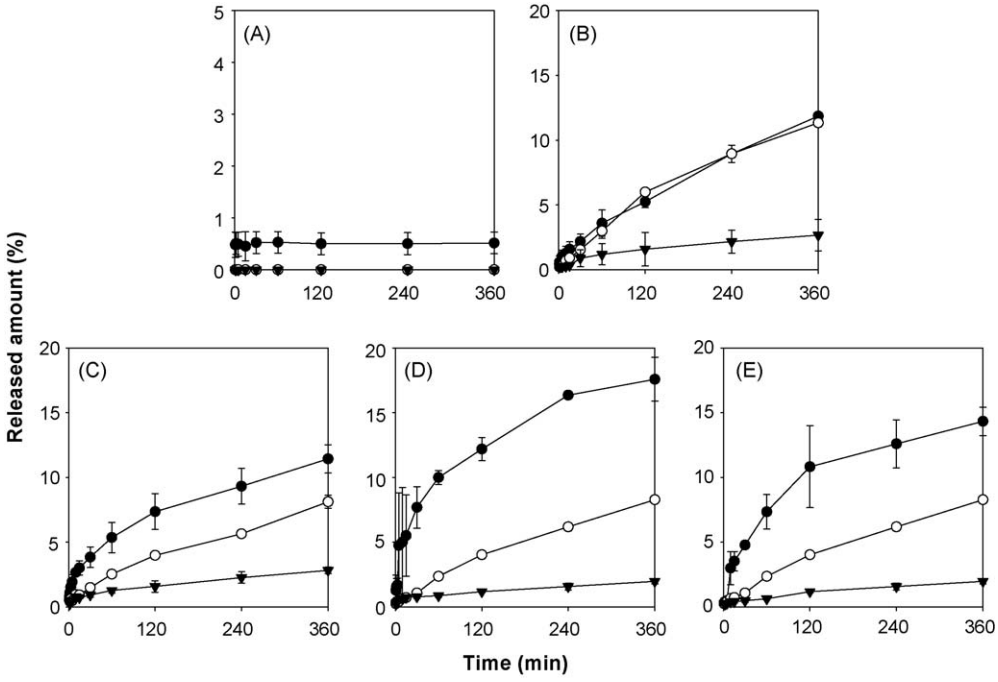


Fig. 6. Dissolution profiles of IH901 (▼), IH901–βCD physical mixture (◯), and IH901–βCD inclusion complex (●) in simulated gastric fluid (pH 1.2) (A), water (B), acetate buffer (pH 4.0) (C), simulated intestinal fluid (pH 6.8) (D), and a phosphate buffered solution (pH 8.0). Each point represents the mean ± S.D. ($n = 4$).

(Fig. 4(a)). This phenomenon might be attributed to the fact that both IH901 and βCD have a sugar moiety, even though IH901 has a steroid structure.

3.3.2. X-ray diffraction

The X-ray diffraction patterns of the IH901–βCD inclusion complexes are shown in Fig. 4(b). The diffractograms of IH901 and βCD exhibited a series of intense peaks, which is indicative of their crystalline character. The X-ray diffraction pattern of the physical mixture was composed of the superposition of the spectra of each single component, indicating that no new structures were formed. In contrast, the freeze-dried powder showed fewer broader and less intense peaks, but the disappearance of the prominent crystalline peak of IH901 situated at 6.2° and 13.2° (2θ) was clearly observed.

3.3.3. Differential scanning calorimetry

Thermal analysis provided additional evidence that inclusion complexes were formed. When guest molecules are embedded in CD cavities or in the crystal lattice, their melting, boiling or sublimation point generally shifts to a different temperature or disappears within the temperature range where CD decomposes (Cabral-Marques et al., 1990). A thermogram of IH901 exhibited two endothermic peaks at 228 and 155 °C, corresponding to the melting point and dehydration point, respectively (Fig. 4(c)). However, the thermogram of βCD showed a broad endothermic peak at 142 °C, possibly due to the release of water molecules (Hassan et al., 1990). The thermal profile of the IH901–βCD product showed a complete disappearance of the IH901 endothermic peaks, indicating the formation of an amorphous solid dispersion or the molecular encapsulation of the drug inside the CD cavity (Esclusa-Diaz et al., 1994; Mura et al., 1999). However, we could not exclude the possibility that the loss of the melting endotherm may also be associated with the conversion of the crystalline material into an amorphrous phase.

3.3.4. Scanning electron microscopy

Scanning electron microphotographs of IH901, βCD, and IH901–βCD inclusion complexes are shown in Fig. 5. IH901 appeared as irregular-shaped crystals and βCD presented a parallelogram shape. However, in the freeze-dried product, the original morphology of the raw materials disappeared and it was impossible to differentiate the two components. This drastic change in particle shape and aspect in the freeze-dried complexes was indicative of the presence of a new solid phase.

3.4. Dissolution studies

The dissolution profiles of IH901, the physical mixture, and the IH901–βCD inclusion complexes in buffer solutions (pH 1.2, 4.0, 6.8, and 8.0) and water are shown in Fig. 6. Although the amount of IH901 released at pH 1.2, the pH of gastric fluids, was considerably lower than that at other pH values, the dissolution profiles of IH901 within the pH range 4.0–8.0, the pH range of intestinal fluids, were pH independent. At pH range 4.0–8.0, the amount of IH901 released by inclusion complexes was considerably greater than that of the physical mixture or the IH901 powder. Unexpectedly, the amount of IH901 released in water did not differ between inclusion complexes and the physical mixture. The amount released by inclusion complexes at pH 6.8 was 2.1-fold and 9.0-fold greater than that for the physical mixture and the IH901 power, respectively. However, the absolute amount of IH901 released from complexes was less than 20%.

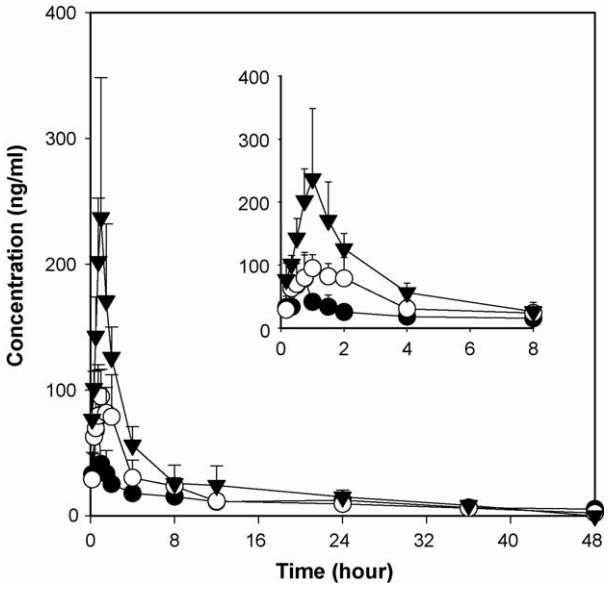


Fig. 7. Plasma concentration–time profiles after oral administration of IH901 (●), IH901–βCD physical mixture (◯) and IH901–βCD inclusion complex (▼) at a dose of 30 mg/kg IH901 to rats. Each point represents mean ± S.D. (n = 4).

Table 1
Pharmacokinetic parameters of IH901 after oral administration of IH901, IH901–βCD physical mixture, or IH901–βCD inclusion complexes at a dose of 30 mg/kg IH901[a]

Parameters[b]	IH901	Physical mixture	Inclusion complex
C_{max} (ng/mL)	84.7 ± 20.8	109 ± 18.7	238 ± 149[c]
t_{max} (h)	0.625 ± 0.177	0.917 ± 0.144	0.917 ± 0.144
$t_{1/2}$ (h)	28.2 ± 3.79	28.4 ± 7.31	25.1 ± 9.14
AUC (ng h/mL)	739 ± 24.3	910 ± 56.2[c]	1379 ± 174[c]
MRT (h)	31.3 ± 2.59	23.6 ± 4.29[c]	18.9 ± 4.09[d]
CL_t (L/h)	1.43 ± 0.047	1.43 ± 0.088	1.42 ± 1.97
Vd_{ss} (L/kg)	44.8 ± 2.61	33.7 ± 1.95[d]	26.8 ± 2.74[d]
BA (%)	3.52 ± 0.116	4.34 ± 0.159[d]	6.57 ± 1.43[d]

[a] Each value represents the mean ± S.D. of four rats.
[b] The following parameters were calculated using standard methods: the total plasma clearance, $(CL_t) = F \cdot \text{Dose/AUC}$, where F were 0.0352, 0.0434, 0.0657 for IH901, physical mixture and inclusion complex, respectively; the steady-state volume of distribution, $(Vd_{ss}) = CL_t \cdot MRT$; the mean residence time, (MRT) = AUMC/AUC, where AUMC represents the area under the moment curve. The BA% value for the oral dose was calculated from the dose-adjusted ratio of AUC_{oral} to $AUC_{i.v.}$, where the value of $AUC_{i.v.}$ was calculated to be 20.9 μg h/mL after an i.v. bolus administration of 30 mg/kg IH901. The $t_{1/2}$ was calculated from the slope of a straight-line in the terminal phase of plasma disappearance.
[c] Significantly different from the group of IH901 ($p < 0.05$).
[d] Significantly different from the group of IH901 ($p < 0.01$).

3.5. Pharmacokinetic studies

After oral administration of IH901 powder, the physical mixture, or the IH901–βCD inclusion complex (30 mg/kg as IH901), plasma concentrations were determined by an LC/MS/MS-based method (Fig. 7). The peak concentrations (C_{max}) for the physical mixture and the inclusion complexes were 1.3- and 2.8-fold higher, respectively, than that for the IH901 powder. However, the time to reach C_{max} (t_{max}) did not show a significant difference among the preparations. The pharmacokinetic parameters were determined by non-compartment methods and are summarized in Table 1. The Vd_{ss} and MRT for the physical mixture and inclusion complexes were lower than those for the IH901 powder. In contrast, the CL_t and $t_{1/2}$ values did not differ significantly among the preparations. The BA of orally administered preparations was calculated from the dose-adjusted ratios of the AUC_{oral} to the $AUC_{i.v.}$, where the $AUC_{i.v.}$ was calculated to be 20.9 μg h/mL. Consequently, the BA values for the IH901 powder, the physical mixture, and the inclusion complexes were 3.52, 4.34, and 6.57%, respectively. These results indicated that BA values for the physical mixture and the inclusion complexes were 1.2- and 1.9-fold higher than that for the IH901 powder.

4. Conclusions

A significant finding in this study is that the inclusion complex was successfully prepared and evaluated separately from the insoluble ginseng saponin (IH901) and the water-soluble complexing agent βCD. We prepared IH901–βCD inclusion complex as an oral formulation for future preclinical and clinical studies. Complex formation was evaluated by both physicochemical methods and in vivo BA studies. Compared to pure IH901, the solubility of IH901 in intestinal fluids (pH 4.0–8.0) was improved by 9.0-fold as a result of complex formation with βCD. In vivo studies after oral administration showed higher AUC values for βCD inclusion complexes than the physical mixture or pure IH901. After oral administration of IH901–βCD inclusion complexes at a dose of 30 mg/kg IH901, the peak concentrations (C_{max}) and the BA of IH901 were 2.8- and 1.9-fold higher, respectively, than for the pure IH901 powder. Although complex formation enhanced the BA of IH901, it was less pronounced than the increase in solubility. Considering that IH901 is not further metabolized in the gastro-intestinal tract after oral administration (Lee et al., 1998), poor membrane permeability might be another factor limiting the BA of IH901. However, when administered orally, the IH901–βCD inclusion complex may be advantageous for oral formulations of ginsenosides, including IH901, in future studies.

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