



Ethnopharmacological communication

Pharmacokinetic comparison of ginsenoside metabolite IH-901 from fermented and non-fermented ginseng in healthy Korean volunteers

Hui Jin^a, Ji-Hyung Seo^b, Yoon-Kyung Uhm^a, Chae-Young Jung^a, Seung-Kwon Lee^c, Sung-Vin Yim^{a,*}^a Department of Clinical Pharmacology, College of Medicine, Kyung Hee University, Seoul 130-701, Republic of Korea^b Department of Pharmaceutical Biochemistry, College of Pharmacy, Kyung Hee University, Hoegi-Dong, Dongdaemun-Gu, Seoul 130-701, Republic of Korea^c Central Research Institute, Ilhwa Co., Ltd., 437 Suteak-Dong Guri-si Gyeonggi-Do, Republic of Korea

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ABSTRACT

Ethnopharmacological relevance: IH-901 (20-O-beta-D-glucopyranosyl-20(S)-protopanaxadiol) is a novel ginseng saponin metabolite formed by human intestinal bacteria and is known to have antitumor and antimetastatic effects. However, there has been no pharmacokinetic study of IH-901 in human beings.

Aim of the study: The aim of this study was to investigate the pharmacokinetic differences of IH-901 from fermented and non-fermented ginseng.

Materials and methods: To investigate whether the pharmacokinetics of IH-901 differ between fermented and non-fermented ginseng, an open label, randomized, single dose, fasting, two-period, cross-over, pharmacokinetic study was conducted. A total of 24 healthy Korean male volunteers participated in this study. All subjects were allocated into two equal groups and administered 3 g of fermented or non-fermented Panax ginseng. Serial blood samples for pharmacokinetic analysis were collected in the 24 h after dosing. Plasma IH-901 concentration was measured by a validated high-performance liquid chromatography–tandem mass spectrometry (LC–MS/MS) method. Pharmacokinetic parameters including AUC_t , C_{max} , and T_{max} were calculated by noncompartmental models in the BA-CALC program (KFDA, 2008, 1.0.0, Korea).

Results: After oral administration of fermented ginseng, 5 subjects experienced diarrhea. The means of AUC_t and C_{max} were significantly different between the two groups. In the fermented ginseng group, AUC_t was 2083.09 ± 91.97 ng h/mL, a 15.5-fold increase over that of IH-901 from the non-fermented group (134.50 ± 63.10 ng h/mL), and the mean C_{max} was 325.00 ± 91.97 ng/mL in the fermented ginseng group, a 27-fold higher value than that in the non-fermented group (13.88 ± 7.24 ng/mL). T_{max} was 3.29 ± 1.00 and 12.04 ± 4.96 h in the fermented and non-fermented group, respectively.

Conclusions: The results of this study showed that the pharmacokinetic parameters of IH-901 from fermented *Panax ginseng* are different from those of non-fermented ginseng, from which IH-901 is formed by intestinal fermentation.

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1. Introduction

IH-901 (also named compound K) is novel ginseng saponin metabolites formed by intestinal bacteria and was identified in fecal specimens. IH-901 was also found in blood and urine of human and rats (Hasegawa et al., 1996).

IH-901 is known to have antimetastatic and anticancer effects. Anticancer effects of IH-901 were reported by Lee et al. (1999). In their study, IH-901 was effective even in the CDDP-resistant PC/DDP cell lines (Lee et al., 1999). The mechanism of the anticancer effects of IH-901 was also investigated by Lee et al. (2000).

They proposed that IH-901 activated caspase-3 through release of mitochondrial cytochrome c, rather than by expression of Bcl-2 (Lee et al., 2000).

Pharmacokinetic study of IH-901 in rats showed that IH-901 was absorbed as IH-901 and from ginsenoside Rb1 (Akao et al., 1998). Tawab et al. (2003) showed that IH-901 was detected in the blood and urine of subjects administered ginseng that did not contain IH-901. Several studies showed that fermentation of ginsenosides by human intestinal bacteria (Hasegawa et al., 1996, 1997) and commercial enzyme preparation (Noh et al., 2009) produced IH-901 *in vitro*.

Because of the known effects of IH-901, increasing the bioavailability of IH-901 will aid its use for medicinal purposes. However, there is no study about the pharmacokinetic properties of IH-901 from ginseng in humans. In this study, we investigated the

* Corresponding author. Tel.: +82 2 9589567; fax: +82 2 9589559.

E-mail addresses: yvin@khu.ac.kr, yvin@yahoo.co.kr (S.-V. Yim).

pharmacokinetic characteristics of IH-901 from fermented and non-fermented ginseng. Fermented ginseng named as GINST was prepared in order to increase the amount of IH-901. Fermented ginseng contains 6.3 mg IH-901/g, while non-fermented ginseng does not contain IH-901. For this purpose, a randomized, open-labeled, cross-over clinical study was conducted to compare the pharmacokinetic characteristics of IH-901 in fermented and non-fermented ginseng in healthy volunteers.

2. Materials and methods

2.1. Preparation and characterization of fermented ginseng and non-fermented ginseng

Fermented ginseng was kindly supplied by the Central Research Center, Ilhwa Pharmaceutical Co. (Guri-Si, Korea). Briefly, dried ginseng (1 kg) was extracted in 5 L of 50% ethanol in water and concentrated with a vacuum concentrator. The dry ginseng extract was incubated with an enzyme solution containing 2.4% Pectinase (DSM food specialties (ZAE La Baume, Servian, France)) at 55 °C for 24 h. Diol ginsenosides such as Rb1, Rc, Rb2, and Rd of ginseng extract were changed to IH-901. The contents of ginsenosides were analyzed with UPLC (Waters, Co. Ltd.). The fermented ginseng extract contains 4.96 mg/g of Rg1, 9.16 mg/g of Re, 24.55 mg/g of Rb1, 25.10 mg/g of Rc, 17.72 mg/g of Rb2, and 10.76 mg/g of Rd, respectively. The non-fermented ginseng extract contains 7.54 mg/g of Rg1, 1.87 mg/g of Re, 5.42 mg/g of Rb1, 0.29 mg/g of Rc, 0.36 mg/g of Rb2, and 0.70 mg/g of Rd, respectively. The IH-901 content in fermented ginseng was 6.3 mg/g. Non-fermented ginseng was also kindly supplied by the Ilhwa Pharmaceutical Co. (Guri-Si, Korea) and contained no IH-901.

2.2. Subjects

Healthy Korean male volunteers aged 20–45 years were enrolled in the study. Subjects underwent screening examinations and the written informed consent was obtained from all subjects.

2.3. Study design and sample collection

The study had an open-label, randomized-sequence, single-dose, 2-period crossover design, with a 1-week washout between study days. The study protocol was reviewed by the institutional review board of Kyung Hee University Hospital. All study procedures were conducted in accordance with the principles of the Declaration of Helsinki and the Korean Good Clinical Practice guidelines.

Subjects were randomly assigned to receive fermented ginseng or non-fermented ginseng in the first period; in the second period, they received the alternative formulation. In both periods, subjects were admitted to the Kyung Hee University Clinical Research Center at 5 PM on the day before administration of ginseng. They received a standardized dinner, and no food was permitted after 8 PM. The next day, subjects received 3 g of fermented ginseng (test) or non-fermented ginseng (reference) along with 240 mL of tap water. Food and water were prohibited during the first 4 h after dosing. At 4 h after the oral administration, all subjects were given standardized meals. The subjects were not allowed to remain in a supine position or to sleep until 8 h after the oral administration.

Blood samples (7 mL) for IH-901 concentration determination were obtained before dosing and at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 10, 12 and 24 h after dosing in each period. Each sample was collected in heparinized tubes. The blood samples were centrifuged immediately (3000 rpm, 10 min), and were frozen at –80 °C until LC–MS/MS analysis.

2.4. Determination of IH-901 concentrations

2.4.1. Drugs and reagents

IH-901 was kindly supplied by the Ilhwa Pharmaceutical Co. (Guri-Si, Korea). Felodipine was used as internal standard and purchased from Sigma–Aldrich (St. Louis, MO, USA). All other chemicals including acetonitrile, formic acid, and ammonium acetate were purchased from Fisher Scientific (Fair Lawn, NJ, USA). A Milli-Q® (Millipore Co., Bedford, MA, USA) water purification system was used to obtain the purified water for the HPLC analysis.

2.4.2. Calibration curve and quality control (QC) samples

The stock solution (58,800 ng/mL) of IH-901 was prepared in acetonitrile and further diluted with acetonitrile to a final concentration of 9800 ng/mL. This solution was used for the working standard. The IH-901 stock solution was diluted in plasma to obtain a concentration of 9.8, 24.5, 49, 98, 245, 490 and 980 ng/mL. The calibration curve was constructed from a blank sample (a plasma sample processed without IS), a zero sample (plasma processed with IS) and seven non-zero samples covering the total range (9.8–980 ng/mL), including the lower limit of quantitation (LLOQ). This calibration curve was generated on two consecutive days. The calibration curve had to have a correlation coefficient (r^2) of 0.99 or better. The quality control (QC) samples were prepared in a pool for each concentration, 24.5 ng/mL (low), 490 ng/mL (medium), and 980 ng/mL (high), and then divided into aliquots that were stored in the freezer at –70 °C. The internal standard (felodipine) stock solution was prepared in acetonitrile (20,000 ng/mL) and diluted with acetonitrile for the working standard at a final concentration of 200 ng/mL.

2.4.3. Preparation of plasma samples

After thawing at room temperature, an aliquot of each sample (200 μ L) was pipetted into an Eppendorf tube, and felodipine (IS) solution (700 μ L, 200 ng/mL) was added. After vortexing briefly, samples were centrifuged for 8 min at 12,000 rpm. After centrifuging, 1 μ L of the sample was injected into the LC–MS/MS system.

2.4.4. Instrumentation and chromatographic conditions

The Agilent 1200 HPLC system and the API 4000 MS/MS (Applied Biosystems/MDS SCIEX, Foster City, CA, USA) were used for the LC/MS/MS system. The most abundant product ions were at m/z 425.5 from the parent m/z 623.5 ion of IH-901 and at m/z 338.2 from the parent m/z 384.4 ion of felodipine (IS). The desolvation temperature was 300 °C and ion spray capillary voltage was 5500 V. The analytical column used was a Gemini 3 μ m C18 110A (50 mm \times 2.0 mm, i.d., 3 μ m). The mobile phase consisted of 80% acetonitrile and 20% 10 mM ammonium acetate (adjusted to pH 4.5 with formic acid), and was filtered and degassed before use. A flow rate of 0.2 mL/min was used for sample analysis. The analytical data were processed using Analytical software (version 1.4.1).

2.4.5. Pharmacokinetic analysis

Individual pharmacokinetic parameters were assessed by BA-CALC program supplied by the Korean Food and Drug Administration. Pharmacokinetic parameters included the area under the plasma concentration versus time curve from 0 h to the last measurable concentration (AUC_t), maximum plasma concentration (C_{max}), and time required to reach maximum plasma concentration (T_{max}).

2.4.6. Tolerability

Adverse effects were monitored throughout the study based on spontaneous reports by volunteers, questioning by investigators, and clinical examinations. The investigators assessed all clinical

Table 1
Pharmacokinetic (PK) parameters of IH-901 in plasma from healthy Korean volunteers.

PK parameter	Fermented ginseng (mean \pm S.D)	Non-fermented ginseng (mean \pm S.D)
AUC_t (ng h/mL)	2083.00 \pm 524.68	134.05 \pm 63.10
C_{max} (ng/mL)	325.00 \pm 91.97	13.88 \pm 7.24
T_{max} (h)	3.29 \pm 1.00	12.04 \pm 4.96

adverse effects in terms of intensity (mild, moderate, or severe), duration, outcome and relationship to the study drug.

3. Results

3.1. Subjects

Twenty-four healthy Korean male subjects were enrolled. The mean (S.D.) age, height, and weight of these subjects were 25.50 \pm 3.76 years, 176.95 \pm 5.27 cm, and 71.18 \pm 9.70 kg, respectively. There were no significant differences in demographic characteristics between the groups.

3.2. Pharmacokinetics

The means of AUC_t , C_{max} , and T_{max} for fermented and non-fermented ginseng were summarized in Table 1 and the time–concentration curve of IH-901 was shown in Fig. 1.

3.3. Tolerability

No serious adverse events (AEs) were reported, and no subjects discontinued the study due to AEs. But five subjects reported AEs of a mild single episode of diarrhea after administration of fermented ginseng.

4. Discussion

IH-901 is produced in the intestine by intestinal bacteria and by enzymatic digestion of ginsenosides *in vivo* and *in vitro* (Hasegawa et al., 1996; Tawab et al., 2003; Zhou et al., 2008). Because of its various pharmacological effects, the possibilities for its development as a new agent have been explored.

To develop a new agent from IH-901, increasing the amount of bioavailable IH-901 and examining the pharmacokinetic properties

of IH-901 is important. Our aim was to compare the pharmacokinetic properties and tolerability of IH-901 from fermented and non-fermented ginseng.

The pharmacokinetics and metabolism of IH-901 after oral administration of a large dose of IH-901 to rat and mice have been assessed (Akao et al., 1998; Hasegawa et al., 2000). Akao et al. (1998) compared the pharmacokinetics of compound K (IH-901) in a ginsenoside Rb1-treated (200 mg/kg, equal mole to 112.4 mg of compound K) and compound K-treated rats (56.2 mg/kg). In their study, compound K was more rapidly absorbed (T_{max} = 30 min) than IH-901 synthesized from intestinal bacteria (ginsenoside Rb1 group, T_{max} = 7 h). The AUC_{0-24} was 3120 ng h/mL in the compound K-treated group and 1160 ng h/mL in the ginsenoside Rb1-treated group, respectively. The C_{max} was 520 ng/mL in the compound K-treated group and below 100 ng/mL in ginsenoside Rb1-treated group. These results correspond well with our results. In our study, the fermented ginseng group showed a more rapid T_{max} (3.29 h) than the non-fermented ginseng group (12.04 h). The amount of absorbed IH-901 (AUC_t) was also greater in the fermented ginseng group (2083.00 ng h/mL) than in the non-fermented ginseng group (134.05 ng h/mL).

In human studies, IH-901 was detected in plasma and urine of subjects administered ginseng that did not contain IH-901 (Hasegawa et al., 1996; Tawab et al., 2003). Tawab et al. (2003) screened ginsenoside and the degradation products from two human subjects administered Ginsana G115 capsules that contained various ginsenosides. In this study, they analyzed the plasma and urine of two subjects. With regard to compound K (IH-901), compound K and its hydrated form were detectable 7–8 h after the intake of Ginsana G115. In our clinical trials, the T_{max} of IH-901 from non-fermented ginseng was 12.04 h. Considering the two previous studies, although there are some differences, it is clear that IH-901 detected in subjects given non-fermented ginseng might be a degradation product of ginsenoside by bacteria in the large intestine.

During the trial, no serious AEs were reported, but five subjects experienced diarrhea. No other AEs were observed.

In this study, we first studied pharmacokinetics of IH-901 from fermented and non-fermented ginseng in humans. There were great differences in AUC_t , C_{max} , and T_{max} between fermented and non-fermented ginseng. These results will be valuable information about pharmacokinetic properties of IH-901 in humans.

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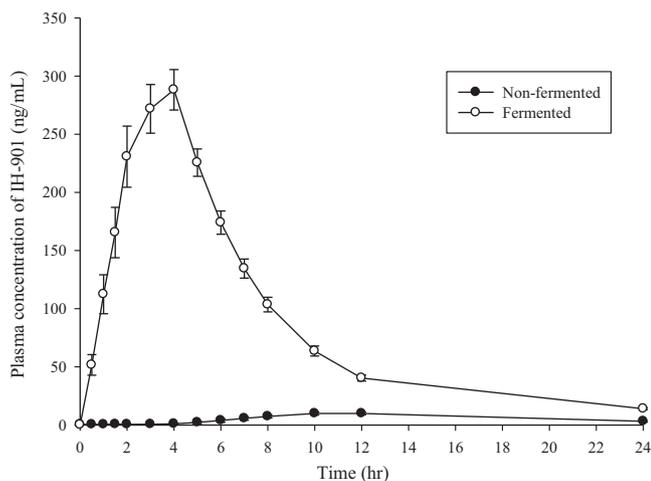


Fig. 1. Plasma concentration–time profile of IH-901 after administered fermented and non-fermented ginseng in healthy Korean volunteers. Data are shown as mean \pm SEM.

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