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Research article

Effect of Korean Red Ginseng extracts on drug-drug interactions

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ABSTRACT

Background: Ginseng has been the subject of many experimental and clinical studies to uncover the diverse biological activities of its constituent compounds. It is a traditional medicine that has been used for its immunostimulatory, antithrombotic, antioxidative, anti-inflammatory, and anticancer effects. Ginseng may interact with concomitant medications and alter metabolism and/or drug transport, which may alter the known efficacy and safety of a drug; thus, the role of ginseng may be controversial when taken with other medications.

Methods: We extensively assessed the effects of Korean Red Ginseng (KRG) in rats on the expression of enzymes responsible for drug metabolism [cytochrome p450 (CYP)] and transporters [multiple drug resistance (MDR) and organic anion transporter (OAT)] *in vitro* and on the pharmacokinetics of two probe drugs, midazolam and fexofenadine, after a 2-wk repeated administration of KRG at different doses.

Results: The results showed that 30 mg/kg KRG significantly increased the expression level of CYP3A11 protein in the liver and 100 mg/kg KRG increased both the mRNA and protein expression of OAT1 in the kidney. Additionally, KRG significantly increased the mRNA and protein expression of OAT1, OAT3, and MDR1 in the liver. Although there were no significant changes in the metabolism of midazolam to its major metabolite, 1'-hydroxymidazolam, KRG significantly decreased the systemic exposure of fexofenadine in a dose-dependent manner.

Conclusion: Because KRG is used as a health supplement, there is a risk of KRG overdose; thus, a clinical trial of high doses would be useful. The use of KRG in combination with P-glycoprotein substrate drugs should also be carefully monitored.

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1. Introduction

Ginseng is a well-known herbal medicine with a broad range of medicinal functions and pharmacological activities. Korean Red Ginseng (KRG) is known as one of the most effective natural drugs. The fresh ginseng, which has undergone a steaming and drying process, results in the production of ginsenosides, which are the major components of ginseng that exert beneficial effects on human health in terms of quality, safety, and efficacy [1]. KRG (scientific name *Panax ginseng*) not only reinstates the body's liveliness, decreases stress and fatigue, and elevates blood circulation, but also improves brain function. In addition, it enhances the activity of the immune system, maintains homeostasis, combats aging, and has proven beneficial against diabetes and cancers [2–7].

In general, drug transporters and cytochrome P450s (CYPs) are multispecific and play a vital role in the determination of drug disposition, which is a very important part of pharmacokinetics [8,9]. Drug–drug interactions (DDIs) take place when the pharmacokinetics of an ingested drug or its metabolites is modified by another ingested drug and can be additive or antagonistic in nature [10–13]. DDIs can affect the blood levels of drugs in the body and alter their pharmacological effects by regulating metabolizing enzymes and drug transporters [14].

MDR1, a member of the ATP-binding cassette family, is predominantly expressed in the gastrointestinal (GI) tract. It is a major contributing factor to DDIs and may result in low bioavailability and multidrug resistance [15]. The protein organic anion transporter (OAT), which acts as an exchanger, is involved in the excretion of an

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array of drugs through the cellular uptake of xenobiotic drugs and is mainly expressed in the kidney and liver [16,17]. These drug transporters have a pivotal role in distribution and release of metabolites. In addition, these features allow mediation of DDIs when two or more drugs are taken for the similar transporter [18,19]. These factors implied that MDR1, OAT1, and OAT3 have fundamental roles in drug interaction.

The disposition of effective drugs depends on the inhibition and/or induction of important proteins in DDIs, such as CYPs. Incomplete absorption due to DDIs may trigger negative or positive effects that affect drug metabolism and disposition [15,20,21] and can modulate metabolic clearance by inhibiting or inducing CYP enzymes [22]. St. John's wort extracts administration to rats significantly increased the protein expression level of CYP3A4 [23], which plays a role in over 50% of oxidative metabolism of all therapeutic drugs [24,25].

Despite the increased public interest in KRG, the scientific knowledge of ginseng-drug interactions is still incomplete and inconsistencies have been described in studies of CYP isozymes in *in vitro* and as well as *in vivo* studies. To date, there have been several reports of the effect of ginseng ingredients on CYP isozymes. Henderson et al. [26] reported that ginsenoside Rd, ginsenoside Rc, and ginsenoside Rf did not seem to suppress the metabolism of co-administered drugs, because ginsenoside Rd showed weak inhibition of CYP3A4, CYP2C9, CYP2C19, and CYP2D6, whereas ginsenoside Rc and ginsenoside Rf augmented the induction of CYP2C9 and CYP3A4 [26]. Liu et al. [27] also suggested that ginsenosides did not show strong or light inhibition of the activities of human CYPs; however, the major intestinal metabolites inhibited the metabolism of CYP.

By contrast, it was also shown that ginseng extract significantly increased the expression levels of CYP3A11 and CYP1A1 in rat primary hepatocytes, which indicated that CYP promoted xenobiotic metabolism [28]. Moreover, ginsenoside Rg1 and ginsenoside Rb2 significantly increased the mRNA level of CYP1A1 in HepG2 cells [29] and ginsenoside Rg3 blocked membrane lipid fluidity, which indicated that MDR was decreased by ginsenoside Rg3 *in vitro* [30].

Therefore, the significance of ginseng extract in drug–ginseng interactions should be assessed in human studies. The effect of KRG [31] and fermented KRG [32] on CYP enzymes and P-glycoprotein (P-gp) was recently examined in healthy volunteers. In these studies, the recommended daily dose was administered to healthy individuals for 2 wk and none of the ginseng products significantly affected the metabolism of CYP probe drugs [31]. However, the systemic exposure of fexofenadine, a representative drug of P-gp-related interactions, was significantly increased by fermented KRG [32]. By contrast, a 28-d administration of *P. ginseng* capsules resulted in a parallel downward shift in the time course of plasma midazolam concentration, which indicated the possible induction of CYP3A; conversely, no change was observed in fexofenadine pharmacokinetics [33]. It was reported by Bilgi et al. [34] that ginseng was associated with the occurrence of imatinib-induced hepatotoxicity after concurrent administration in a patient with chronic myeloid leukemia, which suggested the inhibition of the CYP3A4 enzyme, which was mainly responsible for imatinib metabolism. Consequently, the influence of ginseng products on the pharmacokinetics of co-administered drugs also appears controversial in clinical studies. Such inconsistencies may be attributable to not only the qualitative differences of extracts arising from the preparation methods, but also the administered quantities of ginseng in the supplements.

Here, our aim was to elucidate the dose-dependent effects of KRG extract on: (1) the systemic exposure of fexofenadine and midazolam following a 2-wk repeated oral administration in rats; (2) the CYP family members, including CYP3A11, CYP2C29, CYP2C37, CYP2B13, CYP2C40, CYP1A2, CYP2D9, CYP2B6, and CYP2B10; and (3) drug transporters, including MDR1, OAT1, and OAT3 in mice.

2. Methods

2.1. Materials

Korea Ginseng Corporation (Seoul, Korea) donated the KRG extract. Roots from 6-yr-old *P. ginseng* Meyer were processed by steaming and drying to produce the KRG extracts. The extract contains 13 mg/g as a sum of major ginsenosides, ginsenoside Rb1, ginsenoside Rg1, and ginsenoside Rg3, which was provided by the quality control team of Korea Ginseng Corporation. Fexofenadine hydrochloride and midazolam were purchased from Tokyo Chemical Industry Co. (Tokyo, Japan) and Bukwang Pharm. Co., Ltd (Seoul, Korea), respectively. 1'-Hydroxymidazolam, itraconazole, *N,N*-dimethylacetamide, and ammonium acetate were purchased from Sigma-Aldrich (Seoul, Korea). All the remaining reagents used in this study were of the highest available analytical grade.

2.2. Cell lines and animals

Human liver cells HepG2 (KCLB 88065, Korea) and human kidney cells HEK293 (KCLB 21573) were bought from Korean Cell Line Bank (KCLB; Seoul, Korea) and Dulbecco's Modified Eagle's medium (Lonza; Walkersville, MD, USA) containing 10% fetal bovine serum and 1% penicillin-streptomycin (10,000 U penicillin/mL and 10,000 µg streptomycin/mL) was used as culture medium. OAT1- and OAT3-expressing human recombinant CHO-K1 cells and MDR1-expressing Mardin-Darby canine kidney type II (MDR1-MDCKII) cells were cultured in Hanks' balanced salt solution (HBSS)-4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer (pH 7.4).

Four-wk-old mice (C57BL/6N, male) were purchased from Orient Bio, Seongnam, Korea. Animal experiments were performed according to the guidelines of the Korean Animal Protection Law.

2.3. *In vitro* cytotoxicity assays

Cell cytotoxicity was performed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT, Sigma Aldrich, St. Louis, USA). The HepG2 cells and HEK293 cells were seeded in 96-well plates at 5×10^4 cells/well, and incubated for 24 h. KRG treatment of the cells were carried out at various concentrations and incubated for another 48 h. Cells were then treated with MTT reagent (5 mg/mL) and incubated for 1 h at 37°C in the dark. After incubation, the supernatant was aspirated and 100 µL dimethyl sulfoxide was added to each well to solubilize the resultant formazan crystals. To measure the absorbance of each well at 540 nm, a microplate reader (Molecular Devices, CA, USA) was used. All of the experiments were repeated at least three times and the data were examined and normalized to the absorbance of only Dulbecco's Modified Eagle's medium-containing wells (0%) and KRG-untreated cells (100%).

2.4. Animal treatment

Mice were orally administered KRG extract (0 mg/kg, 30 mg/kg, 100 mg/kg, and 300 mg/kg) twice daily for 15 d and the livers and kidneys were collected 1 d after the final administration.

2.5. mRNA analysis

Total RNA from homogenized mice livers and kidneys was isolated using RNAiso Plus reagent (Takara, Kusatsu, Japan) in accordance with the manufacturer's suggestion. cDNA was prepared by a reverse transcriptase kit (cDNA EcoDry kit; Clontech, Takara, Kusatsu, Japan) using 2 µg of total RNA. A final volume of 20 µL containing 100 ng cDNA, 5 pmol of each primer (listed in Table 1), and 2 × RT-qPCR reaction mix (Applied Biosystems, CA, USA) was

used for real-time PCR with a Step One Plus real-time PCR system (Applied Biosystems, CA, USA). The PCR conditions were 95°C for 10 min, 40 cycles of 95°C for 15 s, 55°C for 30 s, and 72°C for 30 s. The relative quantification of the target genes from triplicate reactions was determined by using Step One software (version 2.1). The internal control gene Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was amplified simultaneously in different reaction tubes under similar reaction conditions.

2.6. Western blot analysis

Tissue extracts from homogenized mice livers and kidneys were resolved by 10% reducing sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride membranes (Millipore, MA, USA). After blocking the membrane for 2 h at room temperature with 5% skim milk solution, the membrane was incubated overnight at 4°C in a 1:4,000 dilution of the specific primary antibodies in 3% skim milk solution. The next day, it was washed three times with Tris-buffered saline with 0.1% Tween 20 (TBST), and incubated with secondary antibody (1:2,000) for 1 h, then the band size was detected by using the enhanced chemiluminescence (ECL) buffer (Gen DEPT, Barker, USA) diluted in WEST-ZOL Plus (iNtRON, Seongnam, Korea). Mouse reactive anti-OAT1, anti-OAT3, anti-MDR1, anti-CYP3A11, anti-CYP2C29, anti-CYP2C37, and anti-CYP2C40 antibodies were purchased from Santa Cruz Biotechnology (TX, USA).

2.7. Inhibition assays

OAT1- and OAT3-expressing human recombinant CHO-K1 cells and MDR1-overexpressing Mardin-Darby canine kidney type II (MDR1-MDCKII) cells were preseeded in 96 well plates at 2×10^4 cells/well for 2 d. The HBSS-HEPES buffer (pH 7.4) that contained different concentrations of KRG extract (0.001 mg/mL, 0.005 mg/mL, 0.01 mg/mL, 0.05 mg/mL, 0.1 mg/mL, 0.5 mg/mL, 1 mg/mL, and 2 mg/mL) was added to the plate and then incubated for 15 min at 37°C. Subsequently, to each well, the substrates of OAT1 and OAT3, 6-carboxyfluorescein (10 µM), and the substrate of MDR1, calcein AM (2 µM), were added, and then incubated for 20 min at 37°C. Cold

assay buffer was used to wash the plates and the fluorescence assay was subsequently performed with fluorogenic substrates. The cells were lysed using 0.2N NaOH solution followed by liquid scintillation counting for assays with radioactive substrates. The percentages of control were determined by the following formula. The IC₅₀ value (the concentration at which half-maximal inhibition of the control value occurs) was measured by nonlinear regression analysis of the concentration-response curve using the Hill equation.

$$\text{Control(\%)} = \frac{\text{Compound} - \text{Background}}{T1 - \text{Background}} \times 100 \quad (1)$$

where *Compound* is the individual reading in the presence of the test compound and *T1* is the mean reading in the absence of the test compound. *Background*: for MDR1, this was the mean reading in the presence of the highest effective concentration of the reference inhibitor and for OAT1 and OAT3, this was the mean reading in the absence of both the test compound and the substrate.

2.8. Effect of KRG on the systemic exposure of midazolam and fexofenadine in rats

Male Sprague-Dawley rats (216–234 g) purchased from Samtako Bio Co. (Osan, Korea) were given access to tap water and food *ad libitum*. The animals were housed at $20 \pm 2^\circ\text{C}$ with a humidity of $45 \pm 10\%$ under a 12-h light/dark cycle. This study was approved by the Institutional Animal Care and Use Committees at Chung-Ang University, Seoul, Korea. The rats were divided into four groups: control group (tap water, 1 mL/kg/d; $n = 5$); low-dose group (KRG, 100 mg/kg/d; $n = 5$); middle-dose group (KRG, 200 mg/kg/d; $n = 5$); high-dose group (KRG, 400 mg/kg/d; $n = 5$). KRG was dissolved in tap water and orally administered once daily for 14 consecutive days. Subsequently, rats were fasted overnight but allowed free access to tap water for at least 12 h. On Day 15, the rats received a single oral dose of 10 mg/kg midazolam followed by 10 mg/kg fexofenadine. Heparinized blood samples were collected from the subclavian vein at 0.25 h, 0.5 h, 0.75 h, 1 h, 1.5 h, 2 h, 4 h, 6 h, 8 h, 12 h, and 24 h after the drug administration. After centrifugation (3000g, 10 min, 4°C), the plasma samples were stored until analysis.

Plasma samples (30 µL) were prepared using a simple protein precipitation method by the addition of 90 µL acetonitrile containing 100 ng/mL itraconazole for the internal standard. After centrifugation at 3000g for 10 min, 5 µL of supernatant was injected onto the LC-MS/MS system. Fexofenadine, midazolam, 1'-hydroxymidazolam, and itraconazole were quantified simultaneously using an API 4000 LC-MS/MS system (AB SCIEX, Framingham, MA, USA) equipped with an electrospray ionization source. The compounds were separated on a reversed-phase column (Atlantis T3, 50 × 2.1 mm internal diameter, 3 µm particle size; Agilent, Cork, Ireland) at 30°C. The mobile phase consisted of acetonitrile and 20mM ammonium acetate aqueous solution (70:30, v/v), which was pumped at a constant rate of 0.2 mL/min; the total run time was 5 min. The mass spectrometer was set to positive ionization mode. Quantitative analysis was performed using multiple reaction monitoring with the precursor-to-daughter ion transitions of m/z 502.4 o-daughter ion transitionve analysis was performed using multiple reaction monito1'-hydroxymidazolam, and m/z 705.2 amhter ion itraconazole. The accuracy and precision of the assay were in accordance with Food and Drug Administration regulations for the validation of bioanalytical methods, and the lower limit of quantification was 10 ng/mL of the analytes.

The pharmacokinetic parameters were calculated by non-compartmental analysis using WinNonlin version 2.1 (Pharsight Corporation, CA, USA). The maximum plasma concentration ($C_{\max}$) and time to $C_{\max}$ ($T_{\max}$) were obtained directly from the

Table 1
Primers used in this study

Gene	Forward primer	Reverse primer
GAPDH	TGC ATC CTG CAC CAC CAA	TCC ACG ATG CCA AAG TTG TC
CYP3a11	GAC AAA CAA GCA GGG ATG G	AAT GTG GGG GAC AGC AAA G
CYP2c29	GGG CTC AAA GCC TAC TGT CA	AAC GCC AAA ACC TTT AA
CYP2c37	ATA CTC TAT ATT TGG GCA GG	GTT CCT CCA CAA GGC AAC
CYP2b13	CTG CAT CAG TGT ATG GCA TTT T	TTT GCT GGA ACT GAG ACT ACC A
CYP2c40	CAT TGA ACA CTG GCA ACA TTG	GTC ACA GGT TAC TTC ATG CAC
CYP1a2	AAG ATC CAT GAG GAG CTG GA	TCC CCA ATG CAC CGG CGC TTT CC
CYP2d9	TGG CAC AGA TAG AGA AGG CCA	TCA CGC ACC ACC ATG AGC
CYP2b6	TGA AGC TTT TCT GCCC CTT CT	TGG AGA CAT GCA ATA GGA GG
CYP2b10	CAG GTG ATC GGC TCA CAC C	TGA CTG CAT CTG AGT ATG GCA TT
OAT1	ATG CCT ATC CAC ACC CGT GC	GGC AAA GCT AGT GGC AAA CC
OAT3	CAG TCT TCA TGG CAG GTA TAC TGG	CTG TAG CCA GCG CCA GTG AG
MDR1	GCA GGT TGG CTA GAC AGG TTG T	GAG CGC CAC TCC ATG GAT AA

concentration-time curves. The area under the plasma concentration versus time curve (AUC) was calculated using the linear trapezoidal rule.

2.9. Statistical analysis

Experimental data were expressed as mean $\pm$ standard deviation. Statistical analysis was conducted using a one-way analysis of variance followed by *a posteriori* testing with Tukey's or Holm-Sidak procedures. A *p* value < 0.05 was considered significant.

3. Results

3.1. Induction of CYPs and transporters by KRG in the mouse liver

In order to check the effect of KRG on the expression of enzymes and transporters in the liver, 30 mg/kg, 100 mg/kg, and 300 mg/kg of KRG were administered orally to mice for 15 d and the mRNA and protein expression levels of CYP3A11, CYP2c29, CYP2c37, CYP2b13, CYP2c40, CYP1A2, CYP2d9, CYP2B6, and CYP2b10 were measured. The mRNA levels of CYP3A11, CYP2c37, and CYP2c40 markedly increased in the liver at 100 mg/kg (Fig. 1A). In agreement with this, mRNA analysis also showed that OAT1, OAT3, and MDR1 expression levels significantly increased when KRG was administered at 300 mg/kg (Fig. 1B). To see the effect of KRG on the protein expression of CYPs, western blotting analysis was used (Figs. 1C and 1D). The results showed that 30 mg/kg KRG significantly increased CYP3A11 level in the liver (Fig. 1E). We also found that OAT1, OAT3, and MDR1 protein levels were significantly increased by KRG in a dose-dependent manner (Fig. 1F).

3.2. Induction of transporters by KRG in the kidney

To determine how KRG affected drug transporter expression in the kidney, total RNA was extracted and qPCR was used for mRNA

quantitation. The results revealed that only 100 mg/kg KRG significantly increased OAT1 mRNA expression (Fig. 2A). To confirm these results, the effect of KRG on protein expression was determined by western blotting analysis. Although KRG did not affect MDR1 expression, OAT1 and OAT3 protein expression levels significantly increased (Figs. 2B and 2C).

3.3. Inhibition of drug transport by KRG in kidney cells in vitro

Prior to evaluation of the effect of KRG on cells, the toxicity, if any, of KRG to human kidney HEK293 cells and human liver HepG2 cells was measured. Various doses (0.001 mg/mL, 0.005 mg/mL, 0.01 mg/mL, 0.05 mg/mL, 0.1 mg/mL, 0.5 mg/mL, 1 mg/mL, and 2 mg/mL) of KRG were incubated with the cells for 48 h and then cytotoxicity was determined by MTT assay. KRG was not cytotoxic to HEK293 cells below 0.5 mg/mL (Fig. 3A). Similarly, KRG was not cytotoxic to HepG2 cells below 1 mg/mL (Fig. 3B). After that, to determine the effect of KRG on drug transport *in vitro*, CHO-K1 cells expressing the OAT1 and OAT3 genes and MDR1-MDCKII cells expressing the MDR1 gene were used for drug transport inhibition assays. Different concentrations of KRG extract were used to treat these cells. KRG treatment did not affect drug transport in CHO-K1 cells expressing OAT1 and OAT3 genes over the concentration range tested, and KRG did not significantly affect transport of the OAT1 and OAT3 substrates (Figs. 3C and 3D). However, KRG inhibited MDR1 transporter activity ($IC_{50} = 1.26$ mg/mL; Fig. 3E).

3.4. Effect of KRG on the systemic exposure of fexofenadine, 1'-hydroxymidazolam, and midazolam

The time courses of plasma fexofenadine concentrations after the oral administration of fexofenadine 10 mg/kg in the presence and absence of 2-wk repeated oral administration of KRG 100 mg/kg, 200 mg/kg, and 400 mg/kg in rats are depicted in Figs. 4A and 4D. An increase in the KRG dose lowered the mean maximum

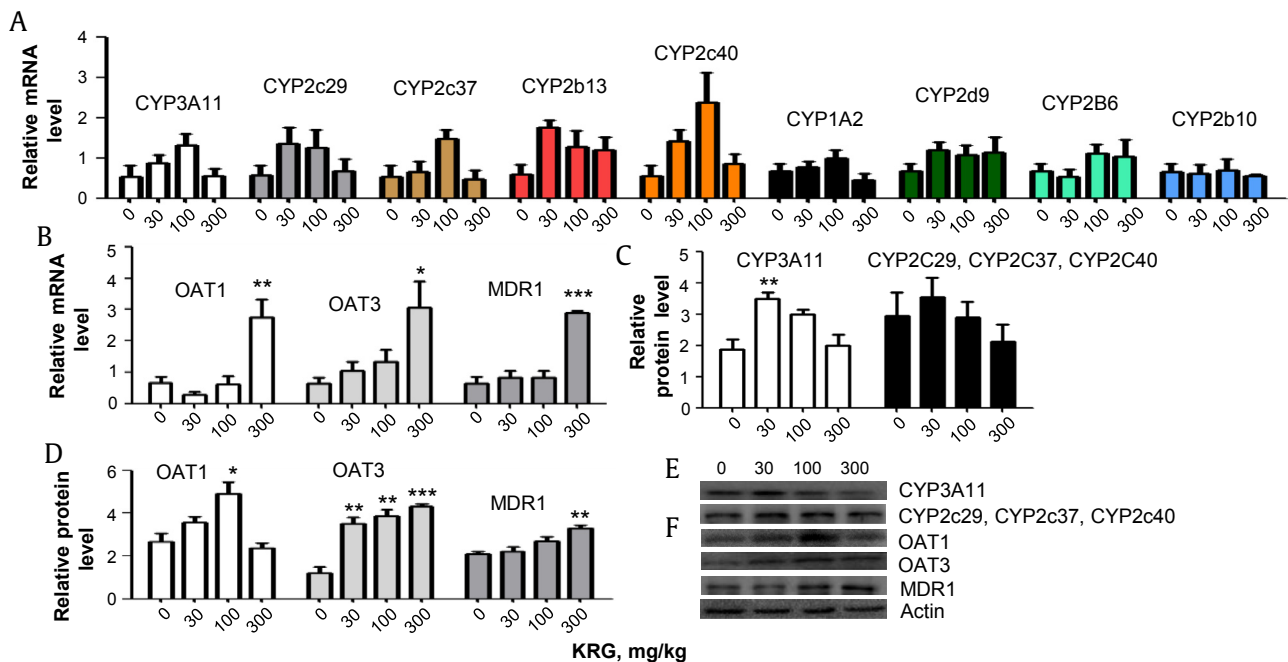


Fig. 1. Induction of cytochrome P450s (CYPs) and transporters by Korean Red Ginseng (KRG) in the liver. (A–D) The KRG extract (0 mg/kg, 30 mg/kg, 100 mg/kg, and 300 mg/kg) was administered orally to mice ($n = 4$ /group) for 15 d. Total RNA (A, B) and protein (C, D) were extracted from the liver for expression analysis of the CYP family (A, C) and transporters (B, D). (A, B) mRNA level was quantitated by real time polymerase chain reaction (PCR). (C, D) Quantitation of protein levels were determined by Western blotting analysis. (E, F) Representative data of three independent experiments of (C) and (D), respectively. Statistical significance was analyzed by analysis of variance. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ compared with the 0 mg/kg group.

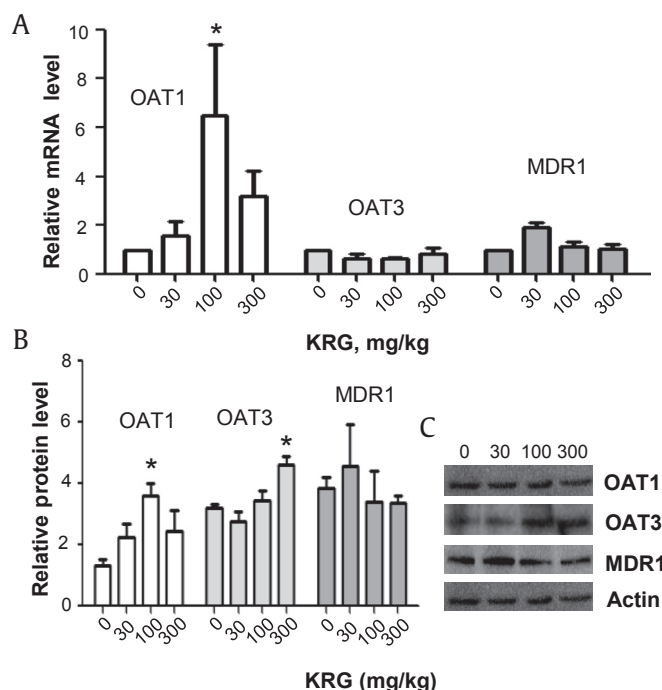


Fig. 2. Induction of transporters by Korean Red Ginseng (KRG) in the kidney. (A, B) The KRG extract (0 mg/kg, 30 mg/kg, 100 mg/kg, and 300 mg/kg) was orally administered to mice ($n = 4/\text{group}$) for 15 d. Total RNA (A) and protein (B) were extracted from the kidney for the determination of transporter expression. (A) mRNA level was quantitated by real time polymerase chain reaction (PCR). (B) Quantitation of protein levels was determined by Western blotting analysis. (C) Representative data of three independent experiments from (B). Statistical significance was analyzed by analysis of variance. * $p < 0.05$ compared with the 0 mg/kg group.

concentrations of fexofenadine by 19%, 44%, and 63% compared with that of the control (32 ng/mL), although no statistical significance was observed owing to high interindividual variations. After the first peak at 0.5 h, a second peak (18 ng/mL) at approximately 2–3 h was observed in the control group, whereas the double peak phenomenon was not present in the KRG-treated groups. The mean $\text{AUC}_{24\text{h}}$ (138 ng·h/mL) in the control group was significantly decreased by 32% ($p < 0.05$), 43% ($p < 0.01$), and 58% ($p < 0.001$) with the increasing doses of KRG, respectively (Figs. 4A and 4D).

The plasma concentrations of midazolam and 1'-hydroxymidazolam versus time are illustrated in Figs. 4B and 4C, respectively. In contrast to that for fexofenadine, KRG treatment did not produce statistically significant differences in the systemic exposure of midazolam and the generation of 1Is expressing OAT1 (Figs. 4B and 4E, and 4C and 4F).

4. Discussion

It is purported that ginseng is a composite chemical mixture which improves vitality, immune function, cognitive function, and enhances overall wellbeing [35,36]. The use of plant-based medicines has increased due to their safety, effectiveness, and lower side effects in comparison with conventional chemical drugs. However, as there is a lack of evidence from clinical studies to confirm these perceptions, patients should always be cautious while taking herbal medicines and chemical drugs together. In recent days, it has been reported that the interaction of herbal medicines-drugs may result in some serious disease outcomes [37,38], which is why the extensive knowledge of herbal medicines-drug interaction, as well as communication with people regarding the usage of these products, is essential.

The phytochemical compounds from medicinal herbs may elevate or suppress the activity of metabolic enzymes of mechanistic pathways, such as CYPs, and result in null, beneficial, or toxic responses [39,40]. It is essential to carry out the study regarding the interaction of ginseng and CYP enzymes, not solely because these herbal medicines show protective efficacy against chemical carcinogens, but also due to the resultant herb-drug interactions as a result of combined ingestion of ginseng and prescribed drugs. It is already well established that the majority of conventional drugs undergo metabolism by the CYP enzymes [25], and that is why the alteration in CYP functionality caused by herbal medicines will lead to potential changes in the pharmacokinetics of drugs that are taken in combination with herbal medicines. Therefore, analysis of CYP is required to make sure of the safe use of usual drugs and herbal medicines in combination.

To determine the probable modulatory effects of KRG extract on each CYP enzyme, experiments were conducted on the CYP family members which take part in drug metabolism (Fig. 1) in which the effect of KRG was evaluated after oral administration of 30 mg/kg, 100 mg/kg, and 300 mg/kg to mice. The relative mRNA expression level of the CYP family members (CYP3A11, CYP2c29, CYP2c37, CYP2b13, CYP2c40, CYP1A2, CYP2d9, CYP2b6, CYP2b10) increased in the liver at all concentrations without a statistical significance, but only CYP2c40 showed maximum activity (Fig. 1A). However, the protein level of CYP3A11 significantly increased in the liver (Fig. 1C). These results suggested that increased CYPs may cause both the metabolism and clearance of drugs; thus, the ingested drugs can be efficiently distributed to other organs. In particular, CYP3A11 has a vital role in the catalysis of toxicant bioactivation and the metabolism of clinically useful therapeutic agents through the elimination of accumulated liver toxins [41,42]. Similarly [43,44], it was reported that eight ginsenosides and also their aglycones have the potential to induce the expression of CYP1A1; additionally, it was also revealed that the CYP3A promoter activation was slightly effected by Rf ginsenoside in the HepG2 cell line. However, other contrasting results [45] showed that Rd ginsenoside feebly inhibited the function of CYP3A and also of CYP2D6 in cDNA-expressed CYP enzymes and that regular extract of ginseng reduced the exerted effects of the CYP1 family on the functionalities of the human recombinant CYP1A1, CYP1A2, and CYP1B1 *in vitro*. Several factors may be responsible for these inconsistent results, including: (1) the complicated nature and variations in the active constituents derived from the different cultivation areas, harvest times, storage conditions, and processing cycles of the plant; (2) different responses and interspecies variability of the various *in vitro* models (cell lines, transgenic cell lines, and primary or cryopreserved hepatocytes, liver/kidney slices, and perfused liver/kidney; for example, it was reported that tanshinone IIA isolated from *Salvia miltiorrhizae* Radix only induced CYP1A in C57BL/6J mice, but not DBA/2J mice [46]; and (3) different study designs greatly influence the outcomes of *in vivo* interaction studies.

CYP enzymes as well as drug transporters affect drug disposition such as absorption, distribution, metabolism, and excretion [47]. In recent days, the transporter-mediated DDI has been considered as a vital factor while dealing with drug kinetics. It is therefore imperative to characterize the role of transporters such as OAT and MDR families involved in DDIs, which can lead to marked changes in the systemic drug exposure level, prolonged plasma elimination half-lives, and consequent accretion of the drug in body as a result of concomitant administration; these factors may induce pharmacological or hostile effects [48]. The extent of activity of drug transporters, like MDR1, mainly showed its expression in the GI tract, as well as in tumor cells, and may cause a lower oral bioavailability and multidrug resistance [49]. The OAT family mediates the uptake of structurally diverse substrates: endogenous compounds

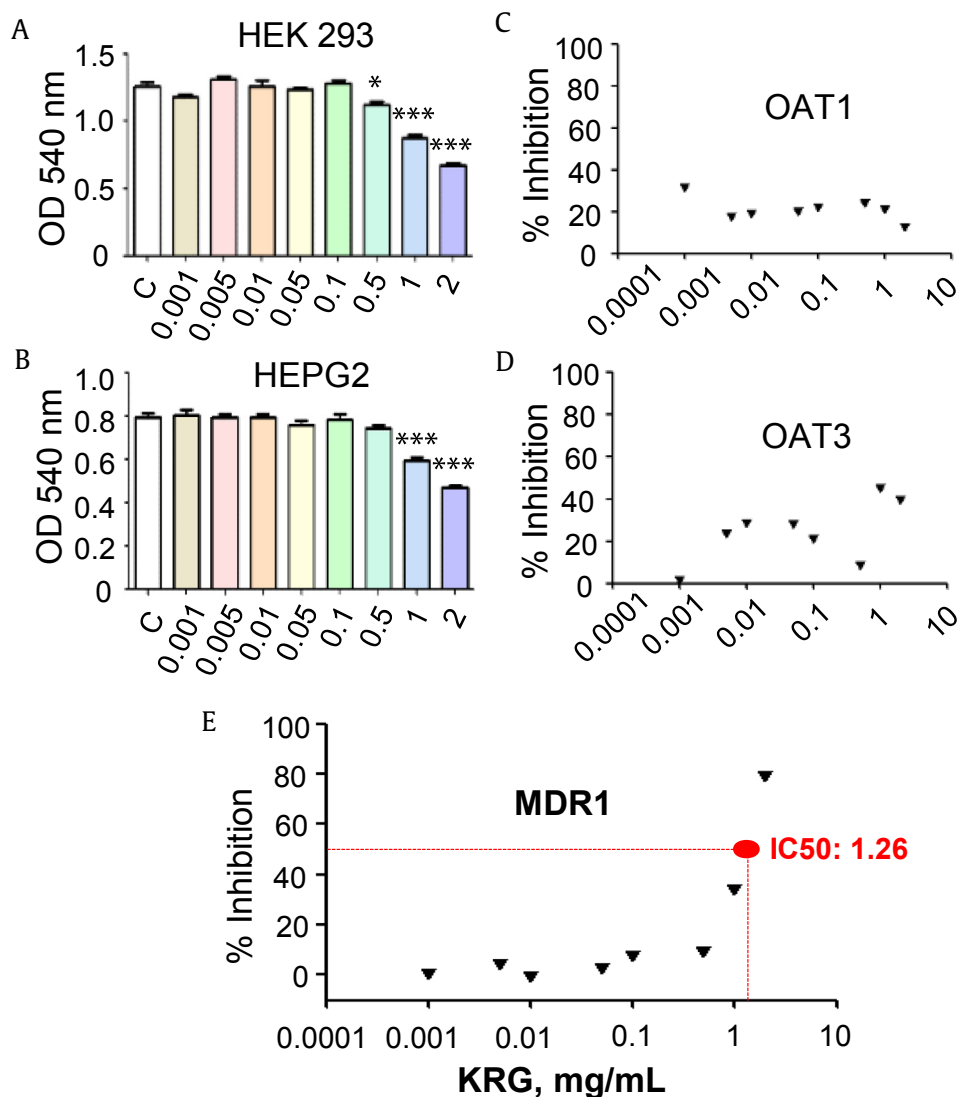


Fig. 3. Inhibition of drug transport by Korean Red Ginseng (KRG) in kidney cells *in vitro*. To optimize the KRG concentration for *in vitro* drug transport assay (A, B), 5×10^4 HEK 293 (A) and HepG2 cells (B) were treated with KRG extracts (0 mg/mL, 0.001 mg/mL, 0.005 mg/mL, 0.01 mg/mL, 0.05 mg/mL, 0.1 mg/mL, 0.5 mg/mL, 1 mg/mL, 2 mg/mL) for 48 h. Subsequently, a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl- tetrazolium bromide (MTT) assay was performed for cytotoxicity determination. Once optimum concentration of KRG was determined, then hOAT1/3-expressing CHO-K1 cells and hMDR1-expressing MDCKII cells were preseeded in a 96 well plate at 2×10^4 cells/well for 2 d. The cells were preincubated with KRG extracts with various concentrations as described in the Methods. Subsequently, a fluorescence assay was conducted using fluorogenic substrates. The percentages related to the control were determined by the following formula. The IC₅₀ value (the concentration at which the half-maximal inhibition of the control value) was measured by nonlinear regression analysis of the concentration-response curve using the Hill equation (C–E). Experiments were performed in triplicate. Statistical significance was analyzed by analysis of variance. * $p < 0.05$; *** $p < 0.001$ compared with the control group.

(steroids, cyclic nucleotides, and neurotransmitters), drugs (nonsteroidal antiinflammatory drugs, diuretics, and antibiotics), environmental toxins, and other organic waste [50]. OATs facilitate substrate drugs to be endorsed properly into the cells and allow the tubule cells of the nephron to excrete the drugs through the urine, thus mediating excretion of diverse drug via kidney. Thus, OAT suppression results in decreased renal clearance [51].

OAT1 and OAT3, which play a role in drug uptake in organs, including the liver, kidney, and brain, have been identified as well-known drug transporters. MDR1 participates in the excretion of drugs and toxins in several tissues. To achieve the maximum effect of the drug, it is very important that the drug should be absorbed, metabolized, and distributed to other targets by the liver. Therefore, drug transporters are an important protein in this respect. After the administration of KRG extracts, mRNA and protein levels of OAT1, OAT3, and MDR1 significantly increased (Figs. 1B and 1D). This

indicates that transporters do not cause drug toxin accumulation in the liver and efficiently distribute the drug to target cells and tissues.

Moreover, mRNA and protein OAT1 expression significantly increased in the kidney, and the OAT3 protein level significantly increased. However, MDR1 was not found to be significantly altered (Fig. 2). Similar results were observed in our *in vitro* study. P-gp, a protein encoded by the human MDR1 gene, was inhibited by KRG extracts in MDR1-MDCK II cells (Fig. 4). The efficacy of ingested drugs is often not obtained because they are excreted before the drugs reach the target *in vivo*. However, DDI, which is involved in the drugs absorption and excretion, can have the potential to extend elimination half-lives from plasma and cause gradual build-up of the drugs in the body as a result of concomitant administration; thus, in our data, the inhibition of P-gp indicated that KRG would increase drug concentration in the cell via the inhibition of

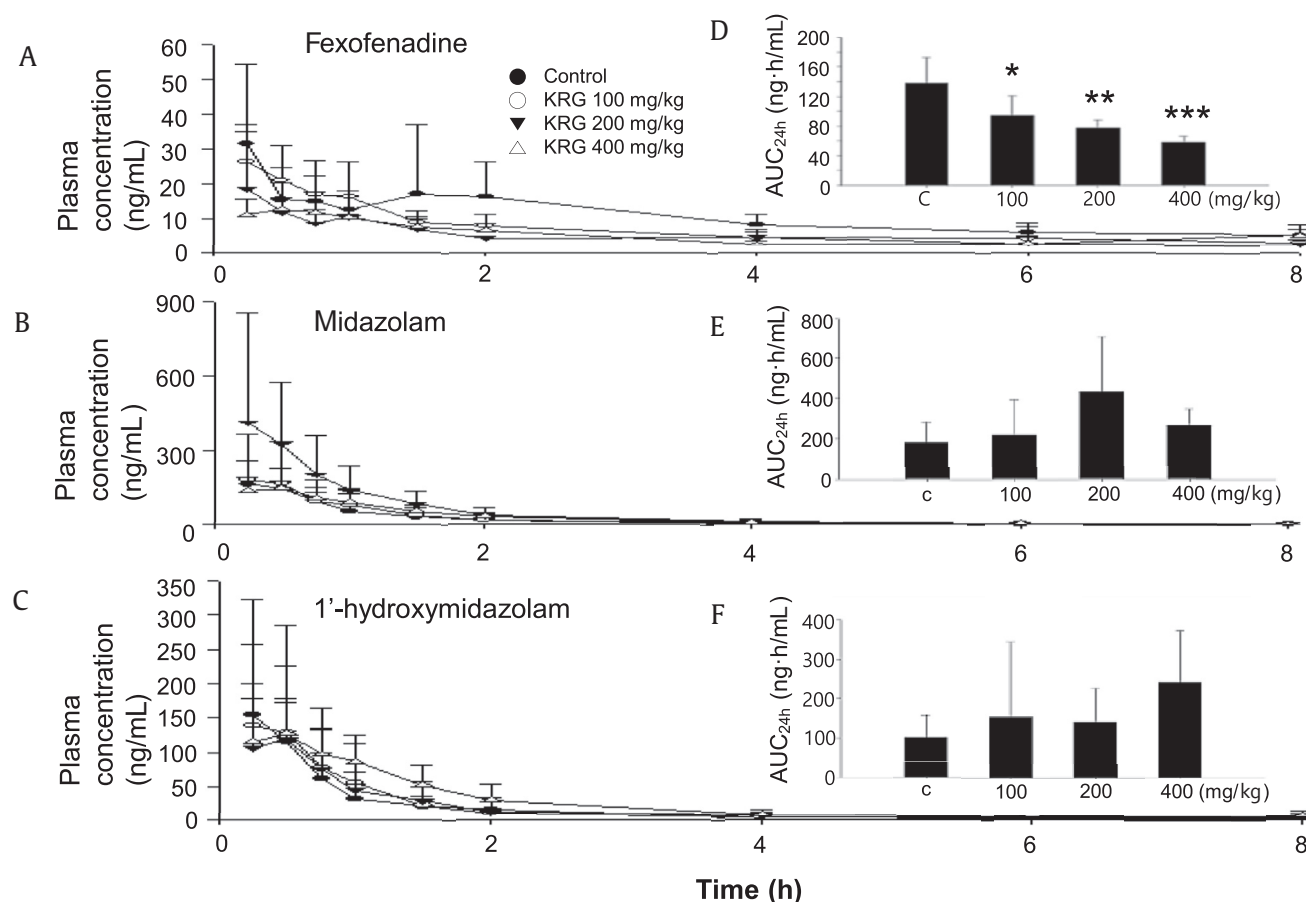


Fig. 4. Effect of Korean Red Ginseng (KRG) on the systemic exposure of fexofenadine, 1'-hydroxymidazolam, and midazolam. Time courses of the plasma concentrations of fexofenadine, 1'-hydroxymidazolam, and midazolam following a single oral administration of midazolam (10 mg/kg) and fexofenadine (10 mg/kg) in rats on Day 15. KRG extracts at doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg were administered orally once/d for 14 d. Data are the mean $\pm$ standard deviation ($n = 5$). Inserts represent the systemic exposure (AUC) of each drug at the different doses of KRG extracts. Statistical significance was analyzed by analysis of variance. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

the efflux pump in the kidney. Previous data showed that ginsenoside Rg3 promoted accumulation in drug-resistant KBV cells, inhibited (^3H) vinblastine efflux, and reversed MDR to doxorubicin [52].

Next, we evaluated the effect of KRG on HEK293 cells and HepG2 cells using the MTT assay with an incubation period of 48 h and determined the percentage cell viability relative to the control. As shown in Fig. 3, the viability of HEK293 cells and HepG2 cells was not affected by KRG at 0.1 mg/mL and 0.5 mg/mL, respectively. The viability of HEK293 cells and HepG2 cell lines was significantly reduced with 1 mg/mL and 2 mg/mL KRG ($p < 0.001$). The cytotoxicity observed with the maximum dose concentration may result from increased oxidative stresses including lipid peroxidation, depletion of glutathione, and generation of reactive oxygen species, which eventually lead to apoptosis and necrosis.

Although it may appear that KRG induced the main drug transporter proteins from our *in vitro* results, the significance must be investigated *in vivo* to provide an accurate prediction of the clinical consequences, because many previous reports have indicated the discrepancy between *in vitro* and *in vivo* data. Two probe drugs, fexofenadine and midazolam, were orally administered to rats to systemically confirm the influence of KRG on drug transportation and drug metabolism, respectively. As fexofenadine is a representative P-gp substrate and only 5% of its dose is known to be metabolized by CYP3A4 [52], the drug has been widely used to evaluate the effect of substances administered concomitantly on its

systemic exposure in terms of drug transportation. Midazolam is a well-known CYP3A probe drug, and is mainly biotransformed to 1'-hydroxymidazolam. However, unlike many other drugs that are metabolized by CYP3A and are also P-gp substrates, midazolam is not transported by P-gp [53].

The pharmacokinetic profiles of midazolam and 1'-hydroxymidazolam did not appear to be significantly altered by KRG despite the induction of CYP3A isozyme observed in our *in vitro* experiments, which indicated that drug-KRG interactions related to drug metabolism could be neglected in clinical settings. However, KRG decreased the systemic exposure of fexofenadine dose-dependently in the range 100–400 mg/kg in rats: the AUCs of fexofenadine were significantly diminished. In addition, the second peak was not observed with the coadministration of KRG. Such double-peak phenomena may be attributed to enterohepatic circulation, however, drug transporters induced by KRG might expel fexofenadine from the GI tract during the absorption. Of course, KRG might also affect the disposition of fexofenadine owing to induction of transporters in the liver and the kidneys that was consistent with *in vitro* results, although this cannot be easily distinguished in the whole body.

Kim et al. [31] reported that KRG affected neither CYP- nor P-gp-related drug interactions in healthy individuals. The discrepancy with our results was probably attributable to the KRG dose. KRG was previously administered at 11.6 mg (0.2 mg/kg in humans) as a sum of major ginsenosides, ginsenoside Rb1,

ginsenoside Rg1, and ginsenoside Rg3, which was equivalent to approximately 1.2 mg/kg in rats considering the conversion factor of animal doses to human equivalent doses based on body surface area or weight [54]. The KRG extract used in this study contained 13 mg/g of ginsenoside Rb1, ginsenoside Rg1, and ginsenoside Rg3, so the extract doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg can be regarded as concentrations of 1.3 mg/kg, 2.6 mg/kg, and 5.2 mg/kg of the marker substances, respectively. Finally, one may speculate that the effect of KRG extract on the transportation of fexofenadine was even greater at two- and fourfold (200 mg/kg and 400 mg/kg, respectively) doses. As the KRG extract has been sold as a health supplement and is known to have a wide safety margin, overdose is more likely. Therefore, clinical trials at high doses of KRG extract are required to confirm the present findings.

5. Conclusion

In conclusion, a 2-wk repeated treatment of KRG extract resulted in the induction of enzymes responsible for drug metabolism and transportation, but the only significance in rats was found in the dose-dependent decrease in the systemic exposure of fexofenadine, a representative P-gp substrate. As a health supplement, safe overdose of KRG can occur; thus, a clinical trial of high doses is recommended. Additionally, caution should be exercised when KRG is used in combination with P-gp substrate drugs.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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