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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.



## 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 chromic 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* Mayer 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 $5 \times 10^4$ 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

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