Deglycosylated Ginsenosides Are More Potent Inducers of CYP1A1, CYP1A2 and CYP3A4 Expression in HepG2 Cells than Glycosylated Ginsenosides

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Summary: Ginseng is one of the most commonly used herbal medicines worldwide. Ginsenosides are believed to be responsible for the therapeutic activities of ginseng; however, co-administration of prescription drugs with ginseng products may give rise to ginseng-drug interactions. Cytochrome P450 enzymes are major phase I enzymes involved in the metabolism of most currently used drugs. Inhibition or induction of P450 enzymes can lead to pharmacokinetic drug interactions. Previous reports on ginseng-drug interactions have been controversial and confusing. In the present study, we examined the effects of thirteen ginsenosides on the expression of CYP1A1, CYP1A2 and CYP3A4 in HepG2 cells. We found that eight ginsenosides and aglycones potently induced CYP1A1 expression, and that structure-activity relationships existed for these effects. Moreover, we discovered that deglycosylated ginsenosides, some of which are putative ginsenoside metabolites, were more potent inducers of CYP1A1, CYP1A2 and CYP3A4 than glycosylated ginsenosides. This finding indicates that ginsenoside metabolites may partially account for ginseng-drug interactions, and that differences in the composition of intestinal bacteria and the extent of deglycosylation of the ginsenosides could be a contributing factor to the inconsistencies observed in previous clinical and pre-clinical studies with regard to ginseng-drug interactions.

Keywords: ginseng; drug interactions; P450; induction; HepG2

Introduction

Ginseng (genus, *Panax*) has been used as a therapeutic herbal medicine in Asian countries for thousands of years, and its use has been increasing in the West as a complementary and alternative medicine.1) In fact, ginseng is the fifth most commonly used natural product in the U.S.3) Ginsenosides, a class of steroid glycosides and triterpene saponins found in ginseng, are believed to account for most of the pharmacological activities of ginseng, including its anti-neoplastic, antioxidant, and immunomodulatory effects.3) Despite the clinical benefits that have been demonstrated for ginseng and the ginsenosides, administration of ginseng or ginseng products may also lead to side effects, and taking ginseng with other xenobiotic compounds can lead to ginseng-drug interactions (GDIs).4,5)

The cytochrome P450 (CYP or P450) family is a superfamily of enzymes involved in the metabolism of a variety of endogenous substances, such as steroid hormones, and exogenous compounds, such as drugs and toxicants.6) Due to the importance of P450 enzymes in the detoxification or bioactivation of xenobiotics, inhibition or activation of P450 enzymes or induction of P450 enzyme expression is a major source of drug-drug interactions (DDIs), including those leading to changes in the pharmacokinetics of drugs.7,8) While there have been many studies examining the effects of ginseng extracts or individual ginsenosides on P450 enzymes, the results have often been conflicting and remain
controversial. For example, Chang et al. found that standardized ginseng extracts inhibited CYP1 catalytic activities in an enzyme- and extract-dependent manner.9 Ginsenoside Rd was shown to have significant inhibitory effects on CYP2C9- and CYP3A4-mediated reactions, with IC₅₀ values of 105 and 62 µM, respectively.10 Ginsenosides Rb₂ and Rb₁ were found to inhibit CYP2C9 activity, while Rb₂ inhibited CYP2D6.11 In another study, however, ginsenosides Rb₁, Rb₂, Re, and Rg₁ did not have any significant effect on five major drug-metabolizing P450 enzymes.12 With regard to the effects of ginseng products on P450 expression, ginsenosides Rg₁ and Rb₁ were found to induce CYP1A1 mRNA levels in HepG2 cells in one study;13 however, another study found that even at a concentration of 0.5 mg/ml, ginseng did not enhance the CYP3A4 mRNA level in hepatocytes.14 In the clinical setting, co-administration of Panax ginseng with probe-drug cocktails was found to have no significant effect on the activities of CYP1A2, CYP2D6, CYP2E1 or CYP3A4 in a single time point metabolic assay.15 The inconsistencies in these reported effects of ginseng products on P450 activities may be due to the probe drug(s) used for each P450 enzyme16 and variability in the concentrations of ginsenosides present in commercial ginseng preparations.17

Previously, we studied the effects of fifteen ginsenosides and sapogenins on the activity of five major drug-metabolizing P450 enzymes and found that there were structure-activity relationships for these effects.18 In the present study, we explored the effects of ginsenosides and sapogenins on the expression of several P450 enzymes. Due to the limited availability of primary human hepatocytes, HepG2 cells were employed for the study. These cells represent a suitable surrogate of primary human hepatocytes for studying changes in the expression of CYP1A1, CYP1A2 and CYP3A4.19,20 CYP1A1 and CYP1A2 are not only involved in drug metabolism but are also associated with bioactivation of many pro-carcinogens.20,21 CYP3A4 is the most abundantly expressed P450 enzyme in the human liver and is involved in the metabolism of approximately half of all currently used prescription drugs.22,23 Thus, any alteration in the expression of the P450 enzymes, in particular CYP3A4, is likely to have a major impact on the metabolism of other drugs.

Methods

Chemicals: The ginsenosides studied (Fig. 1), which were isolated as described previously (24), were at least 95% pure. β-Naphthoflavone (β-NF) and DMSO were purchased from Sigma-Aldrich (St. Louis, MO, USA), while rifampicin (RIF) was purchased from AppliChem GmbH (Darmstadt, Germany).

Cell culture and enzyme induction study: The human hepatocellular carcinoma cell line HepG2 was cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 units/ml streptomycin, and cells were maintained in an incubator with a humidified atmosphere of 5% CO₂ at 37°C. For P450 induction studies, 3×10⁵ cells were seeded in each well of 6-well plates. After 24 h of incubation, the cells were treated with test compounds for 6 h at a final concentration of 50 µM. All compounds were dissolved in sterile DMSO at a concentration of 30 mM to make stock solutions, and these were added to the culture medium with a final concentration of 0.1%. DMSO was used as a vehicle control.

RNA isolation and cDNA synthesis: Total RNA was isolated from cells using the TRizol reagent (Invitrogen) following the manufacturer’s protocol. Reverse transcription of 1.0 µg total RNA was performed in a total volume of 10 µl using a ReverTra Ace qPCR RT Kit (ToyoBo, Japan). The resulting cDNA samples were diluted 25 times, and 4.2, 2.5, and 7.5 µl of the diluted cDNA was used as the template for amplification of the CYP1A1, CYP1A2, and CYP3A4 genes, respectively, in real-time PCR assays.

Quantitative real-time PCR analysis: Quantitative real-time PCR for all three genes was performed using the SYBR Green fluorescent dye methodology. Amplification reactions were performed with a total volume of 20 µl containing the Real-time PCR Master Mix (Toyobo, Japan) and 10 pmol of each pair of primers (Table 1). PCR was performed for 40 cycles of 95°C for 10 s, 60°C for 30 s and 72°C for 30 s using a CFX96 real-time system (Bio-Rad, USA). All samples were analyzed in triplicate and normalized to β-actin mRNA levels. Furthermore, a dissociation curve was obtained after the PCR to verify the specificity of the amplification. The results are expressed as the relative levels compared to the control.

Statistical analysis: All data were expressed as the mean ± SD. The statistical significance of differences between the control and treated groups was examined using Student’s t test. Differences between groups were considered to be significant when p < 0.05.

Results and Discussion

It was previously reported that a 6-h exposure to ginsenosides Rg₁ and Rb₁ caused the greatest increase in the CYP1A1 mRNA level in HepG2 cells for various time points examined (0–48 h).13 Thus, we chose to incubate the HepG2 cells with the tested ginsenosides for 6 h and then determined the mRNA levels of CYP1A1, CYP1A2 and CYP3A4 by quantitative real-time PCR analysis.

CYP1A1: Among the thirteen ginsenosides and sapogenins (50 µM) tested, eight significantly increased the mRNA level of CYP1A1: Rg₁ (2.5-fold that of the control), Rh₂ (2.2-fold), C-K (5.8-fold), Rg₁ (7.2-fold), protopanaxadiol (PPD) (2.4-fold), 20(R)-dammarane-3β,12β,20,25-tetrol (25-OH-PPD) (13.9-fold), 20(S)-25-methoxy-dammarane-3β,12β,20-triol (25-OCH₃-PPD) (9.6-fold) and 25-OH-protopanaxatriol (25-OH-PPT) (17.2-fold). The 25-OH-PPD (13.9-fold) and 25-OH-PPT (17.2-fold) compounds were more potent than the positive control compound, β-NF (10.1-fold) (Fig. 2A). Moreover, there
were obvious structure-activity relationships with respect to the induction of CYP1A1 by the ginsenosides/sapogenins. For example, an increase in the number of glycosyl groups covalently linked to the dammarane scaffold was associated with decreased induction of the enzyme, while compounds with a methoxyl or hydroxyl group at the C25 site showed increased induction of CYP1A1.

CYP1A2: Ginsenoside C-K and sapogenin PPD were also found to induce the expression of CYP1A2 in HepG2 cells; however, the effects were not as potent as those on CYP1A1. C-K increased the CYP1A2 mRNA level by 107%, while Rd, PPD, 25-OH-PPD, and 25-OH-PPT increased CYP1A2 expression by 35–48%. However, these compounds were still relatively active, as the positive control induced CYP1A2 by only 29% (Fig. 2B).

CYP3A4: The effects of the ginsenosides on CYP3A4 were similar to those on CYP1A2, except that 25-OCH3-PPD was also found to induce the expression of the enzyme. Compounds Rd, Compound K (C-K), PPD, 25-OH-PPD, and 25-OH-PPT increased CYP3A4 expression by 35–48%. However, these compounds were still relatively active, as the positive control induced CYP3A4 by only 29% (Fig. 2B).

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Table 1. Primers used for gene expression analysis by real-time PCR

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<td>NM_000499</td>
<td>GGGCCTGTTGCTTTGTAAACCA</td>
<td>AGGTAGGAACTCAGATGGGTTGAC</td>
</tr>
<tr>
<td>CYP1A2</td>
<td>NM_000761</td>
<td>GATGCTGTTTGGCATGGGCA</td>
<td>CGTAGATGGGGGTGAGTCG</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>NM_017460</td>
<td>TGCTTCTCACGGGACTTTTC</td>
<td>CTCTATCAGAGCTAGGAGTAA</td>
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Fig. 1. Structures of tested ginsenosides and aglycones

a. The C-20 configuration of the test ginsenosides was 20(S), except when indicated behind the substituent groups. b. Numerical superscripts indicate the carbons at the glycosidic bonds.

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25-OH-PPD and 25-OCH3-PPD are two novel ginsenoside aglycones which have recently been demonstrated to have promising anti-neoplastic effects against several types of cancer.24–27 It is hoped that these agents will eventually be used in the clinical setting. Due to the common practice of using combination chemotherapy, it is worthwhile to determine the effects of 25-OH-PPD and 25-OCH3-PPD on major P450 enzymes to predict whether they have the potential to induce adverse drug interactions. Previously, we found that both 25-OH-PPD and 25-OCH3-PPD could potently inhibit or activate the catalytic activity of CYP3A4, but that the findings depended on which probe substrate was used.18 In the present study, we showed that both compounds dramatically induced the expression of CYP1A1 and CYP3A4. These findings suggest that particular attention should be paid to pharmacokinetic drug interactions during the preclinical and clinical development of these two novel anticancer compounds. Nevertheless, further studies on the induction of P450 protein expression and enzyme activities are needed to make this observation more clinically relevant. Moreover, information about the concentrations of various components in ginseng would be helpful to understand the effect of ginseng extracts on P450 and the potential for in vivo ginseng-drug interactions. Ginseng contains approximately 2–4% total ginsenosides,28,29 of which Rg1, Rd and Rb1 are the most abundant (not less than 0.1–0.3% of ginseng). In a preliminary assay, we found that...
only 1–2% 25-OH-PPD and 25-OCH3-PPD can be obtained from total ginsenosides (data not shown). One concern may arise from the tested concentration of all ginsenosides (50 µM), which may not be physiologically relevant in the liver. Most reported serum or plasma concentrations of ginsenosides are much lower than 50 µM in humans and experimental animals, likely due to the low dose administered. However, this concentration (50 µM) of ginsenoside is achievable in vivo, at least in experimental animals, if a high dose is given. After a single oral dose of a commercial Panax notoginseng product (600 mg/kg), the peak concentrations of Rb1 and Rg1 in rat serum were approximately 42 µM and 9 µM, respectively.30 After rats were treated with a low (5 mg/kg) intravenous dose of 25-OH-PPD, the peak plasma concentration was approximately 30 µM.31 Moreover, we observed that the 25-OH-PPD concentration in mouse liver is higher than that in plasma and other analyzed tissues (data not shown), suggesting that the induction of P450 enzymes by ginsenosides/ginseng products could be underestimated based on the serum or plasma concentrations of ginsenosides. Nevertheless, further investigations of the dose dependence of the induction of P450 enzymes by ginsenosides are still needed to help better determine the clinical relevance of these effects.

The metabolism of ginsenosides in vivo may add complexity to estimation of ginseng-drug interactions based on data for individual ginsenosides. In a previous report, C-K and PPD, which are believed to be the main metabolites of 20S-protopanaxadiol-type ginsenosides,32 were found to have more potent inhibitory effects on the catalytic activities of P450 enzymes than intact ginsenosides in an incubation system using human liver microsomes.33,34 In our present study, we found that deglycosylated ginsenosides, some of which are putative ginsenoside metabolites (C-K and PPD), were more likely to induce CYP1A1, CYP1A2 and CYP3A4 expression than glycosylated compounds. One possible reason may be that the relatively large sizes of the glycosylated ginsenosides may hinder their binding with the ligand-binding domains of the aryl hydrocarbon receptor (AhR) and pregnane X receptor (PXR), resulting in less induction of CYP1A1/2 and CYP3A4, respectively, by the compounds. Another possibility is that glycosylated ginsenosides are more hydrophilic and so are less likely to permeate the cell membrane, resulting in lower cellular bioavailability, and subsequently, less P450 induction. Interestingly, although CYP1A1/2 and CYP3A4 were induced by different mechanisms, they showed similar responses to ginsenosides. This is an unusual result. As far as we know, a similar phenomenon has been observed previously only with modafinil.35 Nevertheless, the reason why these compounds could induce both AhR-mediated and PXR-mediated transcriptions is still not clear and needs further research.

Our discovery indicates that ginsenoside metabolites may be responsible for GDIs because the ginsenosides are deglycosylated by enterobacteria before they enter the circulation.32 In addition, the induction of P450 enzymes by ginsenosides/ginseng products may depend on the composition of the
intestinal bacteria present in different individuals and populations. This may at least partially explain the controversy that currently exists with regard to ginseng-drug interactions.

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References


