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Effect of Ginsenoside Rc on the Pharmacokinetics of Mycophenolic Acid, a UGT1A9 Substrate, and its Glucuronide Metabolite in Rats

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Abstract : Previous *in vitro* studies have demonstrated that ginsenoside Rc inhibits UGT1A9, but there are no available data to indicate that ginsenoside Rc inhibits UGT1A9 *in vivo*. The effect of single and repeated intravenous injection of ginsenoside Rc was evaluated on the pharmacokinetics of mycophenolic acid. After injection of ginsenoside Rc (5 mg/kg for one day or 3 mg/kg for five days), 2-mg mycophenolic acid was intravenously injected, and the pharmacokinetics of mycophenolic acid and mycophenolic acid-β-glucuronide were determined. Concentrations of mycophenolic acid and its metabolite from rat plasma were analyzed using a liquid chromatography-triple quadrupole mass spectrometry. Single or repeated pretreatment with ginsenoside Rc had no significant effects on the pharmacokinetics of mycophenolic acid ($P > 0.05$): The mean difference in maximum plasma concentration (C_{max}) and area under the concentration-time curve (AUC_{inf}) were within 0.83- and 0.62-fold, respectively, compared with those in the absence of the ginsenoside Rc. These results indicate that ginsenoside Rc has a negligible effect on the disposition of mycophenolic acid *in vivo* despite *in vitro* findings indicating that ginsenoside Rc is a selective UGT1A9 inhibitor. As a result, ginsenoside Rc has little possibility of interacting with drugs that are metabolized by UGT1A9, including mycophenolic acid.

Keywords : Ginsenoside Rc, herb-drug interaction, mycophenolic acid, UGT1A9, uridine 5'-diphosphoglucuronosyltransferase

Introduction

Ginseng is a commonly used herbal medicine in Korea and other East Asian countries mainly due to its vitality restoration and immune-stimulating effect.[1-3] It has been increasingly consumed as a dietary supplement in many countries.[4-6] Formulations of ginseng extract are commonly used over-the-counter preparation in several countries, including Korea and the U.S.A. Processed ginseng products are estimated to be approximat ely US $2,085 million in the world market in 2009.[7] Many studies have shown that most of the pharmacological effects of ginseng are attributable to ginsenosides.[8] Ginsenosides have been known to have beneficial effects in diabetes,[9] dyslipidemia,[10] inflammatory diseases,[11] and cancer.[12]

Among various ginsenosides, ginsenoside Rc has antidiabetic, antiallergic, anticancer, and sedative effects.[13,14] Ginsenoside Rc is the second most abundant ginsenoside in commercially available red ginseng products.[15] Our previous study reported that ginsenoside Rc (Figure 1a) can inhibit UGT1A9 noncompetitively.[16] Ginsenoside Rc selectively inhibited UGT1A9-mediated mycophenolic acid and propofol glucuronidation with K_i values of 3.31 and 2.83 mM, respectively, in human liver microsomes. This suggests the possibility of pharmacokinetic interactions between ginsenoside Rc and drugs mainly metabolized by UGT1A9. The change in active drug exposure can also change drug efficacy. In spite of possible interactions in *in vitro* model, there has been no data yet in animals or humans investigating a drug interaction between ginsenoside Rc and mycophenolic acid, UGT1A9 probe substrate.[16]

Mycophenolic acid (Figure 1b) was developed as an immunosuppressant to complement existing immunosuppressive agents, including calcineurin inhibitor, azathioprine, and corticosteroids.[17] It has been used effectively after organ transplantation.[18] UGT1A9 is the major enzyme involved in the metabolism of mycophenolic acid to its glucuronide conjugate.[19] Drug interaction between mycophenolic acid as a probe substrate of UGT1A9 and other medications was

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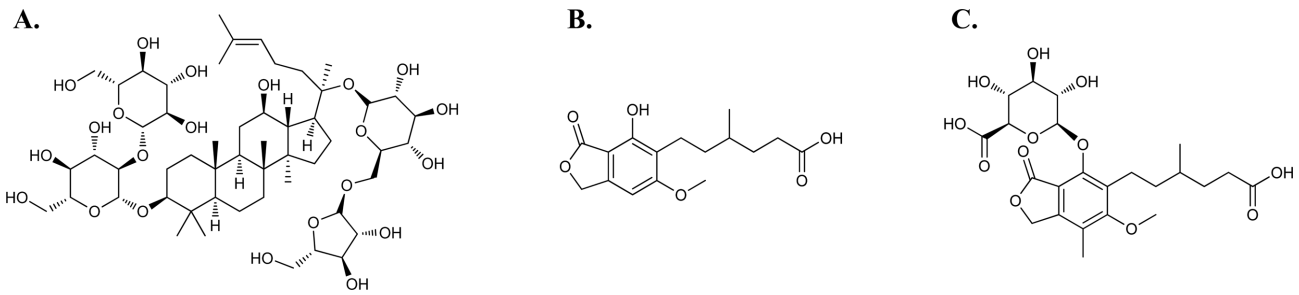

Figure 1. Chemical structures of ginsenoside Rc (A), mycophenolic acid (B), and mycophenolic acid-β-D-glucuronide (C).

performed. Rifampin induced systemic clearance of mycophenolic acid by inducing UGT1A9-mediated mycophenolic acid glucuronidation.[20] Failure to recognize this drug interaction might lead to mycophenolic acid underexposure and loss of clinical efficacy because mycophenolic acid has a narrow therapeutic window,[20] whereas increased plasma levels of toxic glucuronide metabolites could lead to side effects.

Although ginseng, particularly red ginseng, is one of the most commonly used herbal medicines in U.S.A. and Europe, no *in vivo* studies have been conducted to determine the effect of ginsenoside Rc, one of the most abundant ginsenosides, on UGT1A9 activity or interactions with other drugs. This study aimed to assess the effect of ginsenoside Rc on UGT1A9 activity in rats using mycophenolic acid glucuronidation as a UGT1A9 probe.

Experimental

Materials

Mycophenolic acid (MPA, purity > 98%) and estrone-β-D-glucuronide (EG) were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A). Mycophenolic acid-β-D-glucuronide (MPAG, Figure 1c) was obtained from Toronto Research Chemicals (Toronto, ON, Canada). Ginsenoside Rc (GRc, purity > 98%) was obtained from Ambo Institute (Daejeon, Korea). Solvents were LC-MS grade (Fisher Scientific Co., Pittsburgh, PA, U.S.A).

Animal study

Male Sprague–Dawley rats (6–7 weeks old, 220–250 g) were purchased from Samtako Co. (Osan, Korea). The animals were acclimatized for one week in an animal facility at Kyungpook National University (Daegu, Korea). Food and water were provided ad libitum. The study protocol was approved by the Animal Care and Use Committee of Kyungpook National University (Approval No. KNU 2019-83). To calculate and compare the pharmacokinetic parameters of MPA and GRc, we conducted repeated blood sampling through the retro-orbital puncture under isoflurane anesthesia.[21]

Rats were randomized into three groups: a control group, single dose group, and multiple dose group, each consisting of four animals. The single dose group was intravenously injected once with GRc solution (5 mg/mL/kg, dissolved in saline) via the tail vein, while the multiple dose group was intravenously injected with GRc solution (3 mg/mL/kg, dissolved in saline) via the tail vein for five consecutive days. The control group received saline (1 mL/kg) via the tail vein. After 1 h following the last GRc treatment, MPA was intravenously injected into all groups via the tail vein at 2 mg/kg (dissolved in DMSO-saline = 2:8, *v*/*v*). Heparinized blood samples were taken at 0.17, 0.33, 0.67, 1.5, 2, 4, 8, 24, and 48 h following mycophenolic acid dosing via the retro-orbital vein. After centrifugation (16,000 g, 10 min, 4°C), aliquots (50 μL each) of plasma samples were stored at -80°C until the analysis of MPA and MPAG.

LC-MS/MS analysis of mycophenolic acid and its glucuronide

The concentration of MPA and MPAG was analyzed using a liquid chromatography coupled to a triple quadrupole mass spectrometry (LC-MS/MS) as previously reported by Wiesen et al.[22] with some modification. Briefly, acetonitrile (100 μL) including EG (IS) was added to the plasma samples (50 μL). After vortexing, the samples were centrifuged (16,000 g, 10 min, 4°C). A 5 μL sample of the supernatants was injected onto the LC-MS/MS.

MPA, MPAG, and the internal standard were quantified in a single run using a Shimadzu LCMS 8060 triple-quadrupole mass spectrometer coupled with a Nexera X2 ultra high performance liquid chromatography system (Shimadzu, Kyoto, Japan) equipped with an electrospray ionization interface. MPA, MPAG, and IS were separated on a Kinetex XB-C18 column (100 × 2.1 mm, 2.6 μm, Phenomenex, Torrance, CA, U.S.A). The mobile phase consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B), and was set as 0%→30% B (0–1 min), 30%→50% B (1–5 min), 50%→0% B (5–5.1 min), and 0% B (5.1–8 min). The flow rate was 0.2 mL/min. Electrospray ionization was performed in positive ionization mode at 4000 V or negative ionization mode at -3500 V. The optimum operating conditions were determined as follows: vaporizer temperature, 300°C; capillary temperature, 350°C; collision gas (argon) pressure, 1.5 m Torr. Quantification was conducted in the selected reaction

Table 1. Selected reaction monitoring (SRM) condition for mycophenolic acid, mycophenolic acid-β-D-glucuronide, and internal standard (IS)

Compound	SRM Transition (*m/z*)	Polarity	Collision Energe (eV)
Mycophenolic acid	321.2 > 207.0	+	-19
Mycophenolic acid-β-D-glucuronide	495.0 > 319.0	-	25
Estrone-β-D-glucuronide	455.0 > 269.0	-	35

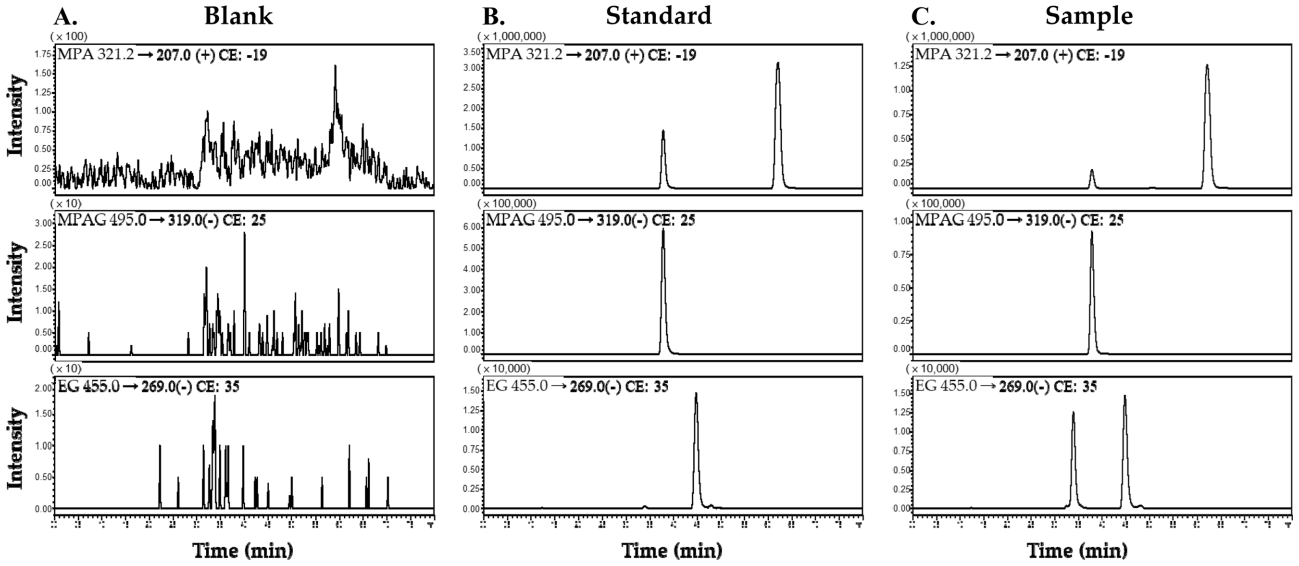

Figure 2. LC-MS/MS selected ion chromatograms of mycophenolic acid (MPA), mycophenolic acid-β-D-glucuronide (MPAG), and estrone-β-D-glucuronide (EG). Left column: blank plasma (A); middle column: plasma spiked with 5000 ng/mL of MPA and MPAG (B); right column: plasma sample equivalent to 2282.55, and 1676.78 ng/mL for MPA and MPAG, respectively, from rats 0.17 h after the intravenously injected dose of 3 mg/kg GRc and 2 mg/kg MPA.

monitoring (SRM) modes at *m/z* 321→207 for MPA (Collision energy, 19 eV), *m/z* 495→319 for MPAG (Collision energy, -25 eV), and *m/z* 455→269 for EG (Collision energy, -35 eV) (Table 1 and Figure 2). Analytical data were processed using a Shimadzu LabSolution LCMS software. Plasma calibration standards for the quantification of MPA and MPAG ranged from 20 to 5000 ng/mL, correlation coefficient (r^2) ranged from 0.995 to 0.998, and the intraday and interday accuracy ranged from 86.8% to 109.7%. The intraday and interday precision ranged from 1.0% to 7.8%.

Data analysis

The pharmacokinetic parameters of MPA and MPAG were calculated from plasma concentration-time profiles using a non-compartment analysis of WinNonlin software (version 5.1; Pharsight, Cary, NC, U.S.A). GraphPad Prism (version 6.0; GraphPad, San Diego, CA, U.S.A) was used for statistical analysis. The estimated parameters obtained from the control and single or multiple dose groups were statistically compared using Student's t-test. Statistical significance was assessed at a level of $p < 0.05$.

Results and Discussion

So far, only one drug interaction study between mycophenolic acid, a UGT1A9 probe drug, and other drugs has been conducted. Rifampin co-administration with mycophenolic acid increased area under the plasma concentration-time curve (AUC) value of mycophenolic acid by induction of UGT1A9 glucuronidation activity.[20] However, there have been no studies on drug interactions based on the inhibition of UGT1A9 enzyme activity. In this study, therefore, we investigated the UGT1A9 inhibitory effects of ginsenoside Rc, a strong and selective UGT1A9 inhibitor, on the pharmacokinetics of mycophenolic acid, a UGT1A9 probe drug. GRc was intravenously injected to maximize the plasma concentration, thus, the UGT1A9 inhibitory potential of GRc.

Co-administration of MPA and GRc resulted in the lack of herb-drug interaction (HDI) between GRc and MPA. Mean plasma concentration-time profiles of MPA and MPAG in the absence or presence of either single dose GRc (5 mg/mL/kg, iv) or repeated dose GRC (3 mg/mL/kg/day, iv for five days) in rats were similar (Figure 3), and relevant pharmacokinetic parameters of MPA and MPAG

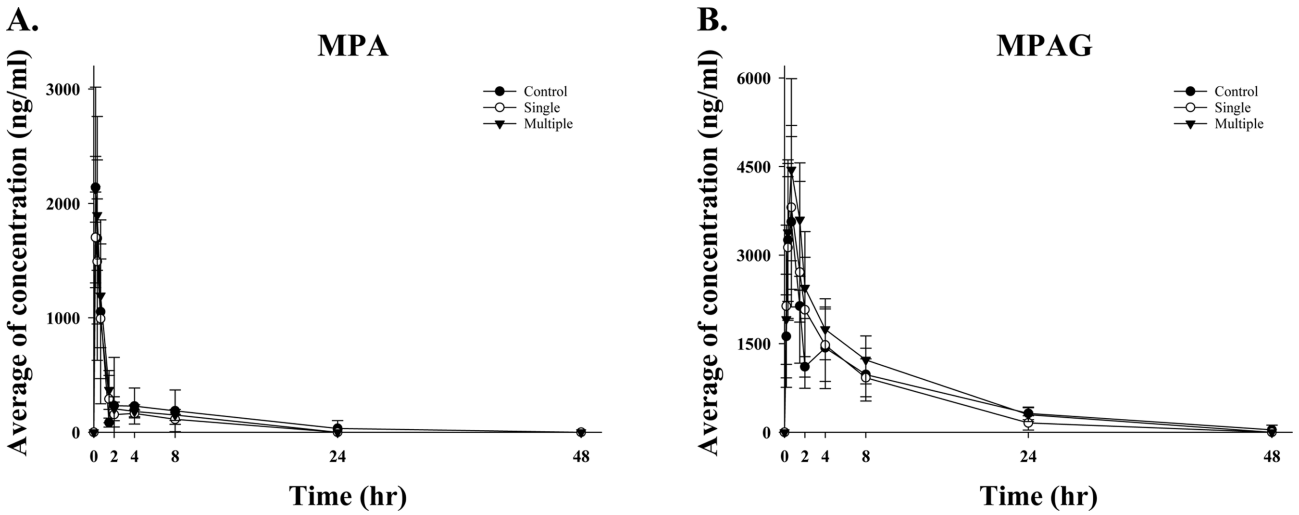

Figure 3. (A) Plasma concentration-time profile of mycophenolic acid (MPA) in the control, single dose (ginsenoside Rc 5 mg/mL/kg), and multiple dose (ginsenoside Rc 3 mg/mL/kg for 5 days) groups following intravenous injection of MPA at a dose of 2 mg/kg in rats (mean ± SD, n = 4). (B) Plasma concentration-time profile of mycophenolic acid-β-D-glucuronide (MPAG) in the control, single dose (ginsenoside Rc 5 mg/mL/kg), and multiple dose (ginsenoside Rc 3 mg/mL/kg for 5 days) groups following intravenous injection of MPA at a dose of 2 mg/kg in rats (mean ± SD, n = 4).

Table 2. Pharmacokinetic parameters of mycophenolic acid in control, single dose (ginsenoside Rc 5 mg/mL/kg), and multiple dose (ginsenoside Rc 3 mg/mL/kg for five days) groups following intravenous injection of MPA at a dose of 2 mg/kg in rats (mean ± SD, n = 4).

Mycophenolic acid			
Parameters	Control	Single Dose	Multiple Dose
$T_{1/2}$ (h)	5.24 ± 4.71	6.20 ± 5.89	3.75 ± 2.82
C_0 (ng/mL)	2141.97 ± 874.61	1766.55 ± 326.61	2135.04 ± 293.88
AUC_{48h} (ng·h/mL)	3975.91 ± 4561.37	2344.61 ± 825.57	2878.11 ± 859.44
AUC_{∞} (ng·h/mL)	5611.19 ± 6338.15	3459.07 ± 1741.85	3618.36 ± 971.81
MRT (h)	3.39 ± 2.18	2.17 ± 0.09	2.16 ± 0.22
CL (mL/h/kg)	0.85 ± 0.47	0.82 ± 0.63	0.62 ± 0.20
Vd (L/kg)	1.04 ± 0.35	1.16 ± 0.20	0.95 ± 0.15

Data represent mean ± SD of four rats per group. $T_{1/2}$: elimination half-life; C_0: initial plasma concentration at 1 h; AUC_{48h} or AUC_{∞}: Area under the plasma concentration-time curve from zero to 24 h or infinity; *MRT*: mean residence time; *CL*: systemic clearance; *Vd*: Volume of distribution.

Table 3. Pharmacokinetic parameters of mycophenolic acid-β-D-glucuronide (MPAG) in control, single dose (ginsenoside Rc 5 mg/mL/kg), and multiple dose (ginsenoside Rc 3 mg/mL/kg for five days) groups following intravenous injection of MPA at a dose of 2 mg/kg in rats (mean ± SD, n = 4).

Mycophenolic acid-β-D-glucuronide			
Parameters	Control	Single Dose	Multiple Dose
$T_{1/2}$ (h)	14.33 ± 10.41	7.04 ± 2.80	7.96 ± 1.31
T_{max} (h)	0.58 ± 0.17	0.50 ± 0.19	0.88 ± 0.42
C_{max} (ng/mL)	3630.31 ± 1475.59	3953.17 ± 1362.24	4574.31 ± 1318.95
AUC_{48h} (ng·h/mL)	23253.83 ± 6861.06	20244.47 ± 7567.91	29087.93 ± 8185.30
AUC_{∞} (ng·h/mL)	28691.08 ± 6209.48	23724.83 ± 7667.03	32610.42 ± 9009.45
MRT (h)	9.58 ± 4.73	5.57 ± 1.82	6.45 ± 0.61

Data represent mean ± SD of four rats per group. $T_{1/2}$: elimination half-life; T_{max}: time to reach C_{max}; C_{max}: maximum plasma concentration; AUC_{48h} or AUC_{∞}: Area under the plasma concentration-time curve from zero to 24 h or infinity; *MRT*: mean residence time.

are listed in Table 2 and Table 3. In the MPA alone group, the mean C_0 and AUC_{inf} were 2.14 mg/mL and 5.61 mg×h/mL, respectively, and *CL* is 0.85 mL/h/kg. Single dose GRc did not affect the pharmacokinetics of MPA (Table 4). To achieve the highest and stable plasma concentration of GRc, GRc was intravenously injected for five days before MPA administration. However, the plasma concentration of MPA was not affected by repeated GRc treatment (Figure 2), and all pharmacokinetic parameters were not statistically different between the two groups (Table 2). We also calculated pharmacokinetic parameters of MPAG, UGT1A9 specific metabolite of MPA (Table 3). Similar to MPA, there was no significant difference in pharmacokinetic parameters of MPAG among three groups (control, single dose GRc, and multiple dose GRc) (Table 3).

Jeon et al. reported that GRc showed high protein binding in rat plasma and liver homogenates (> 99.5%) and was not widely distributed to the liver with a liver-to-plasma concentration ratio of 0.13-0.2.[23] High protein binding and limited liver distribution of GRc might contribute to the lack of pharmacokinetic HDI involving MPA in rats although their plasma concentration was maximized following intravenous injection of GRc. Jiang et al. also reported that the unbound fraction of GRc was low (0.6%) in human plasma.[24] Based on the similarity in the protein binding features between rats and humans[23,24] and inhibitory effect on UGT1A9 of GRc, no HDI between MPA and GRc would be expected in humans.

UGT1A9 is one of the most essential UGT isoforms abundantly expressed in human's liver and kidney.[25] Human UGT1A9 had been regarded as a minor hepatic drug-metabolizing enzyme, with 1.78% hepatic expression. However, it represents above 50% of the total kidney UGT content.[25] UGT1A9 was suggested to be responsible for the hepatic glucuronidation of 16% of 200 top prescribed drugs in the United States in 2002.[26,27] UGT1A9 is essential UGT isoform responsible for the metabolism of endogenous estrogen and various therapeutic drugs such as dapagliflozin, edaravone, entacapone, morinidazole, mycophenolic acid, propofol, and sulfinpyrazone.[28] Therefore, the findings showing the lack of HDI between GRc and mycophenolic acid would provide helpful information for patients taking drugs that are mainly metabolized by UGT1A9 such as mycophenolic acid and propofol.

Conclusions

In conclusion, the present findings suggest that ginsenoside Rc has no statistically significant effects on the pharmacokinetics of mycophenolic acid and its glucuronide metabolites in rats. Additionally, considering high protein binding and limited liver distribution of GRc, no HDI between UGT1A9 substrate drug and GRc would also be expected in humans.

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