Drug Metabolism and Disposition

Supplement Materials

Inhibition of P-glycoprotein leads to improved oral bioavailability of Compound K, an anti-cancer metabolite of red ginseng extract produced by gut microflora

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Table S1. The ingredients of oral suspending vehicle used in pharmacokinetic studies.

Ingredients
Purified water
Lambda Carrageenan
Simethicone
Xanthan Gun
Microcrystalline Cellulose/Sodium CMC
Sodium Phosphate, Dibasic
Potassium Sorbate
Methylparaben
Propylparaben

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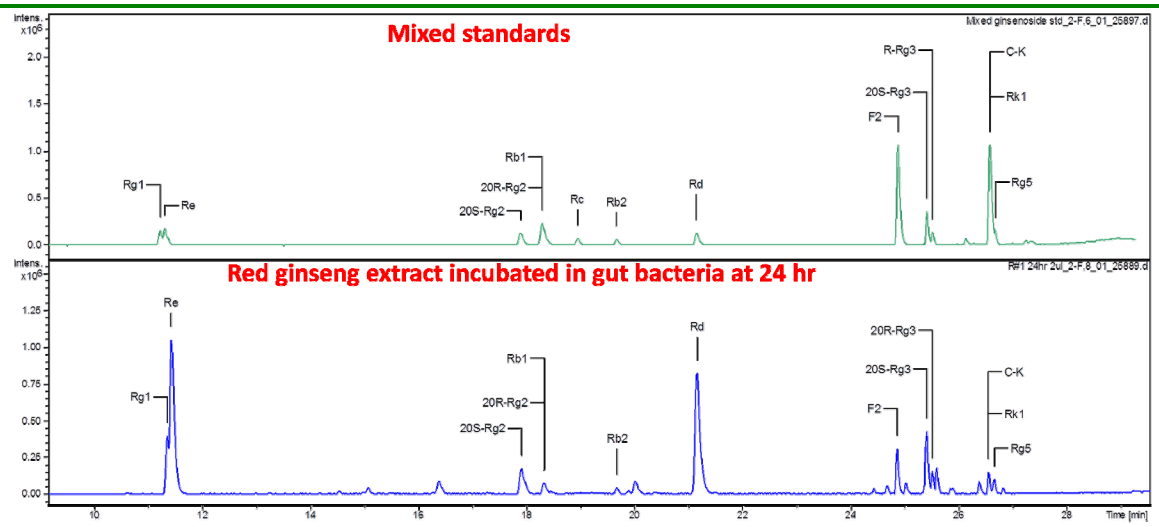
Table S2.

The chromatograph and UPLC/MS parameters of multiple ginsenosides Rg1, Re, F2 and C-K Analysis in Bruker MicroTOF mass spectrometer.

Ginsenosides	$[M+HCOO]^-$ *(m/z)*	Fragment ion (*m/z*)
Rg1	845.4892	637, 475
Re	991.5504	783, 637, 475
F2	829.4913	621, 499
C-K	667.4396	499



The chromatography was performed on an Acquity BEH C_{18} column (2.1 × 100 mm, 1.7 ㎛). The mobile phase consisted of 0.1% formic acid in distilled water (A) and acetonitrile containing 0.1% formic acid (B), starting with linear gradient from 20 to 40% B within 20 min, then from 40 to 100% B at 20 - 29 min, and maintained at 100% B for 3 min at flow rate of 0.35 mL/min. Sodium formate solution was prepared by mixing 0.05 mmol NaOH solution with 0.05% formic acid in 90:10 proportion of 2-propanol/distilled water.

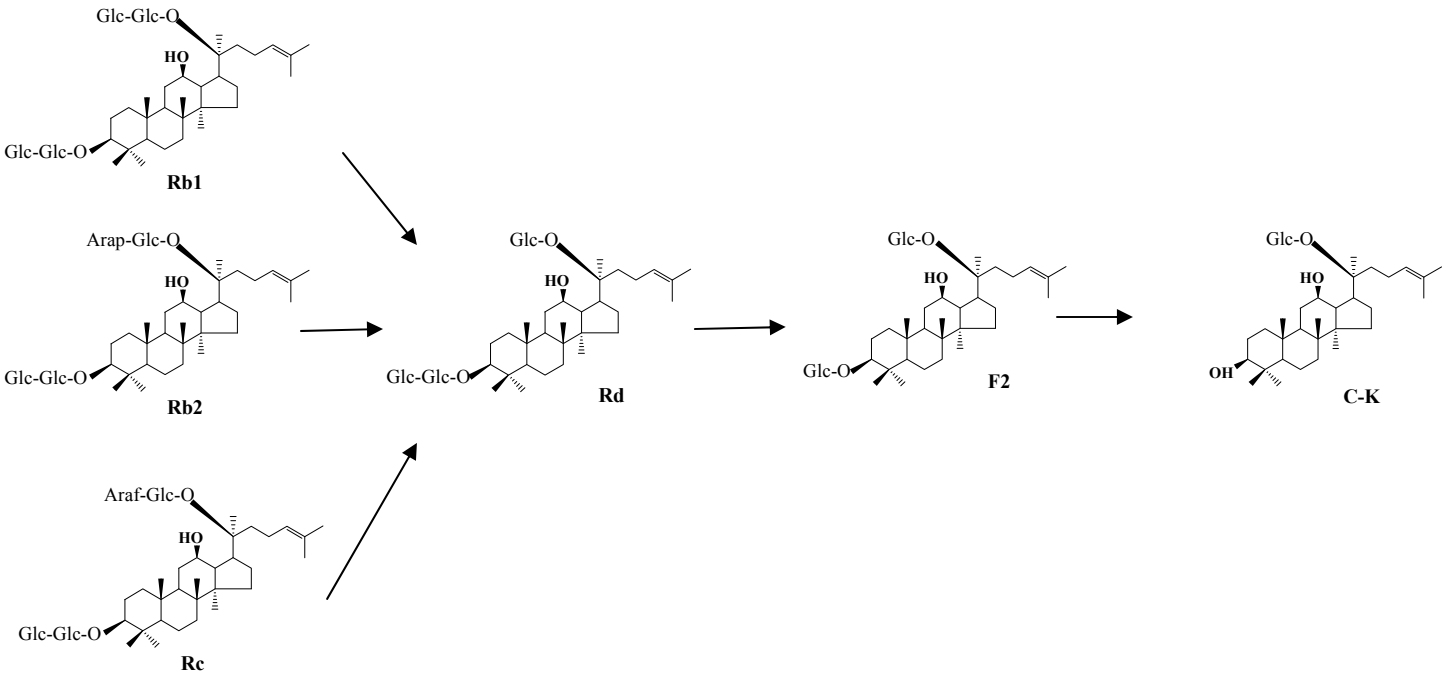
Supplement Table S3.

Instrument-dependent parameters for C-K and F2 in MRM mode for UPLC–MS/MS analysis in AB SCIEX 3200 Qtrap mass spectrometer.

ESI(+)	Q1	Q3	Time (ms)	DP (v)	CEP (v)	CE (v)	CXP (v)
CK	645.0	203.0	100	96	32	46	3
F2	807.6	627.5	100	125	50	53	5

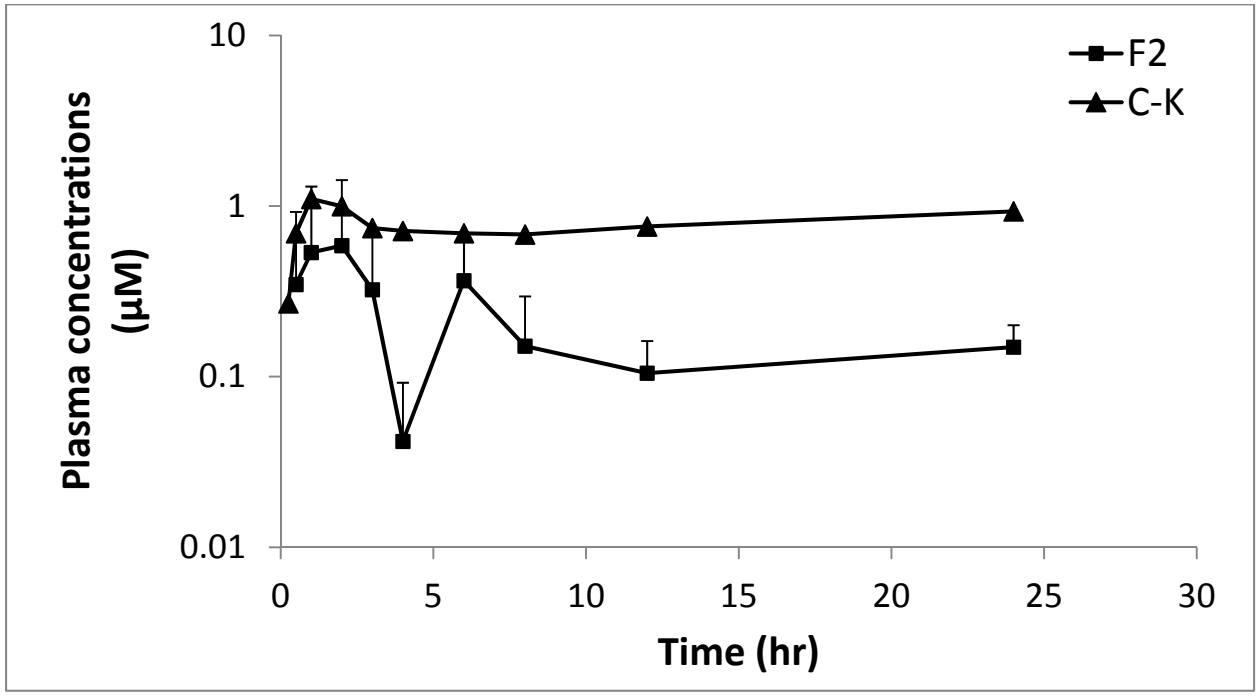
UPLC gradient for C-K and F2 analysis were: gradient, 0-0.5 min, 0% B, 0.5-1 min, 0-40% B, 1-1.9 min, 40-90% B, 1.9-2.3 min, 90-95% B, 2.3-2.5 min, 95% B, 2.5-32.8 min, 95-0% B. The other UPLC conditions includes: Flow rate, 0.55 ml/min, column temperature, 45 degree; injection volume, 10 μl. System, Waters Acquity™ (Milford, MA, USA) with DAD detector; column, Acquity UPLC BEH C18 column (50×2.1mm I.D., 1.7μm, Waters); mobile phase A, 0.1% formic acid; mobile phase B, 100%, acetonitrile;

Figure S1. Possible Metabolic pathway of PPD-type ginsenosides Rb1, Rb2 and Rc in gut bacteria collected from A/J mice



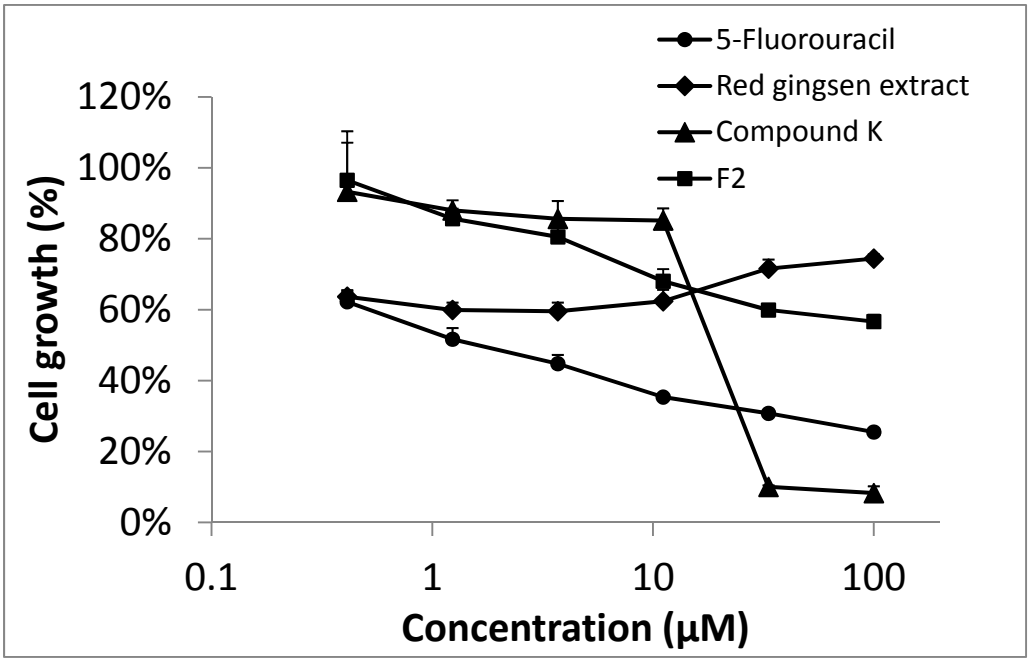
These results suggested the tendency of stepwise de-glycosylation was terminal sugar > inner sugar (C-3) > inner sugar (C-20).

Figure S2. Pharmacokinetic studies of red ginseng extract in A/J mice



Pharmacokinetic study of red ginseng extract was performed in A/J mice to confirm the in vivo hydrolysis of ginsenosides by gut microflora. Red ginseng extract was dissolved in distilled water and was orally gavaged to A/J mice at 200 mg/kg (n=5). The blood sample collection and processing procedures used for A/J mouse samples were the same as those described for the FVB mouse pharmacokinetic study.

Figure S3. Cytotoxicity of ginsenosides in LM1 cell line



The cell growth inhibitory effects of ginseng powder and various metabolites generated in gut bacteria were determined using MTT assay in LM1 cell line. Cells were suspended in fresh medium at a concentration of 5×10^3 cells/ml and seeded in a 96-well flat bottomed plate in a volume of 100 μl/well. Cells were stabilized by incubation for 24 hrs at 37°C and 100 μl aliquots of each drug with different concentrations (0.5% DMSO) ranging from 0.41 to 100 μM were added to wells. The plate was incubated at 37°C for 48 hrs. For assay, 50 μl of MTT were added to each well and the plate was incubated for 4 hrs at 37°C. The optical density was measured at 570 nm on a microplate reader (Bio-Rad, California). Each experiment was performed in triplicate and repeated at least twice. Antitumor activity was evaluated using IC_{50} determined by non-linear regression analysis using the GraphPad PRISM 5 (San Diego, CA).