College of Pharmacy and Research Institute of Pharmaceutical Sciences[1], Natural Products Research Institute[2], Seoul National University, Seoul, Korea

Platelet antiaggregating activity of ginsenosides isolated from processed ginseng

Jin Gyun Lee[1], Yong Yook Lee[1], Sun Young Kim[2], Jae Sung Pyo[1], Hye Sook Yun-Choi[2], Jeong Hill Park[1]

Received March 24, 2009, accepted April 24, 2009

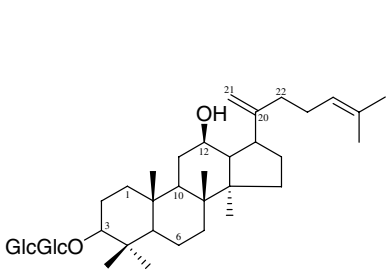
Prof. Jeong Hill Park, College of Pharmacy, Seoul National University, Seoul 151-742, Korea
hillpark@snu.ac.kr

Pharmazie 64: 602–604 (2009) *doi: 10.1691/ph.2009.9577*

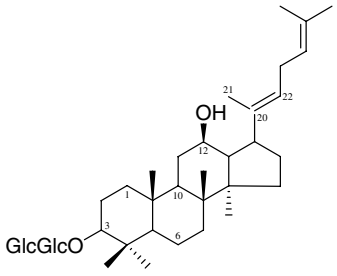
Four dammarane glycosides, namely ginsenosides Rk_1 (**1**), Rg_5 (**2**), 20(*S*)-Rg_3 (**3**), and 20(*R*)-Rg_3 (**4**), isolated from a new processed ginseng, were evaluated for their inhibitory activity against platelet aggregation induced by adenosine diphosphate (ADP), collagen, arachidonic acid (AA) and U46619 (thromboxane A_2 mimetic agent). Ginsenoside Rk_1 and Rg_5 inhibited AA-induced platelet aggregation in a dose dependent manner. Their activity against AA-induced platelet aggregation were found to be 8–22 fold higher than that of a known antiplatelet drug acetylsalicylic acid (ASA). They also inhibited U46619-induced platelet aggregation. Ginsenoside 20(*S*)-Rg_3 and 20(*R*)-Rg_3 showed mild inhibitory activity against AA and U46619-induced aggregation.

1. Introduction

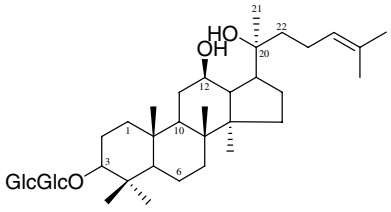
As a part of ongoing search for antithrombotic agents from natural plants, the antiplatelet aggregatory activity of four ginsenosides isolated from processed ginseng was evaluated. Ginseng has been known to possess mild antithrombotic activity, and several ginsenosides were examined to explain the antithrombotic activity of ginseng. Antiplatelet aggregatory effects of various ginsenosides have been investigated earlier (Matsuda et al. 1986; Kuo et al. 1990; Park et al. 1993; Lee et al. 1997). Among them, ginsenosides Rg_2 and Rg_3 were reported to have inhibitory effects comparable to that of acetylsalicylic acid (ASA) which is widely used as antiplatelet drug. However, most of the other ginsenosides show only negligible effects on platelet aggregation. Steaming of ginseng at a higher temperature and pressure than those applied to the conventional preparation of red ginseng provided a new processed



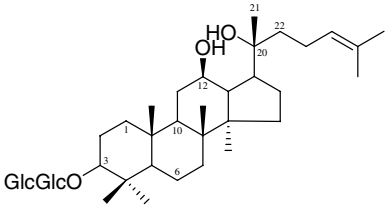
Ginsenoside Rk_1



Ginsenoside Rg_5



20(S)-Ginsenoside Rg_3



20(R)-Ginsenoside Rg_3

ginseng with enhanced cytotoxic activity against various cancer cell line, radical scavenging activity, and vasodilation activity (Keum et al. 2000; Kim et al. 2000; Park et al. 2002c). Ginsenosides are well known to undergo structural changes during heat processing. This new processed ginseng contains ginsenosides Rg_3, Rg_5, Rk_1, Rk_2, Rk_3, Rs_4, Rs_5, Rs_6 and Rs_7 which are produced by deglycosylation and dehydration at the position C_{20} in dammarane backbone (Kwon et al. 2001; Park et al. 2002a, b). These components, not usually found in white ginseng, are thought to be responsible for enhanced pharmacological effects of processed ginseng. Especially, there is evidence about the beneficial effect of red ginseng unique ginsenoside such as ginsenoside Rg_3 on antiplatelet aggregatory activity (Lee et al. 1997). However, until now there is no available literature reporting investigation on the inhibitory effect of new processed ginseng on platelet aggregation. In the present study, we examined the antiplatelet aggregating activity of four ginsenosides isolated from the processed ginseng.

2. Investigations, results and discussion

Most of the tested compounds showed dose dependent inhibitory effect to collagen, AA and U46619 (thromboxane A_2 mimetic drug)-induced platelet aggregation. However, they showed a negligible effect on adenosine diphosphate (ADP)-induced aggregation (Table). Among the four tested compounds, ginsenoside Rk_1 exhibited the strongest inhibitory effect on collagen, AA and U46619-induced platelet aggregation. In particular, it showed the 22-fold activity of that of ASA on AA-induced aggregation. Ginsenoside Rg_5 also showed 8-times stronger inhibitory activity against AA-induced aggregation than ASA. 20(*S*)-Ginsenoside Rg_3 showed effects similar to ASA on the inhibition of AA-induced platelet aggregation, whereas, 20(*R*)-Rg_3 showed only poor platelet antiaggregating activity. While ginsenoside Rk_1 exhibited a 3-fold higher activity than ASA on collagen-induced aggregation, activities of ginsenosides Rg_5 and 20(*R*)-Rg_3 were comparable to those of ASA. Ginsenosides Rk_1 and Rg_5 showed 5–6 times stronger inhibition than ASA on U46619-induced aggregation. According to Lee et al. (1997), racemic ginsenoside Rg_3 which has deglycosylated structure at C_{20} exhibits inhibitory effect on AA-induced platelet aggregation.

In the present study, 20(*S*)-ginsenoside Rg_3 and 20(*R*)-ginsenoside Rg_3, the isomers of ginsenoside Rg_3 were isolated and their antiplatelet activity was examined. Interestingly, they seem to have different activity in stereospecific manner. 20(*S*)-Ginsenoside Rg_3 showed a relatively strong inhibitory effect on AA-induced aggregation, while 20(*R*)-ginsenoside Rg_3 inhibited collagen and U46619-induced aggregation.

In conclusion, 20(*S*)-ginsenoside Rg_3 and 20(*R*)-ginsenoside Rg_3 showed antiplatelet aggregatory activity comparable to that of ASA. However, their dehydrated forms (at C_{20} position, obtained because of heat processing), namely ginsenosides Rk_1 and Rg_5 were found to be potent inhibitors of platelet aggregation induced by AA and U46619.

Table: Anti-aggregatory activity of ginsenoside Rk_1, Rg_5, 20(*S*)-Rg_3 and 20(*R*)-Rg_3

Ginsenoside	IC_{50} (μM)			
	ADP[a]	Collagen[b]	AA[c]	U46619[d]
Rk_1	555	197	3	78
Rg_5	>1000	409	8	102
20(S)-Rg_3	>1000	>700	53	>700
20(R)-Rg_3	650	492	>300	357
ASA[e]	>1000	588	66	508

[a] ADP: 3–4 μM
[b] Collagen: 3–4 μg/mL
[c] AA: 40–50 μM in presence of a threshold concentration of collagen (1.0–1.4 μg/mL
[d] U46619: 3–4 μM in presence of a threshold concentration of collagen (0.8–1.2 μg/mL)
[e] ASA = acetylsalicylic acid, used as a positive control
Experiments were performed in triplicate, and data were expressed as IC_{50}

3. Experimental

3.1. Plant material and chemicals

White ginseng (dried root of *Panax ginseng* C.A. Meyer, Araliaceae, 4 years old) was purchased from a local market in Kumsan, Korea. Ginseng was processed by steaming at 120 °C for 3 h in an autoclave as reported previously (Kwon et al. 2001). Voucher specimens of white ginseng (ANALAB-0701) and processed ginseng (ANALAB-0702) were deposited at the herbarium of the College of Pharmacy, Seoul National University. Adenosine 5'-diphosphate dicyclohexylammonium salt, sodium arachidonate, U46619 (9,11-dideoxy-11α,9α-epoxymethanoprostaglandin $F_2\alpha$) and acetylsalicylic acid were purchased from Sigma Aldrich Chem. Co (St. Louis, MO, USA). Collagen was purchased from Chrono-Log Co. (Havertown, PA, USA). Unless stated otherwise, all other reagents were from Sigma Aldrich. All the chemicals including solvents were either of analytical grade or cell culture tested.

3.2. Isolation of the compounds

Ginsenosides Rk_1, Rg_5, 20(*S*)-Rg_3, 20(*R*)-Rg_3 were isolated as described earlier (Park et al. 2002c). The processed ginseng (2 kg) was extracted thrice with methanol (MeOH) (3 l). After solvent evaporation, the residue (380 g) was suspended in H_2O (5 l) and extracted with n-butanol (*n*-BuOH, 5 l). A portion of evaporated *n*-BuOH soluble fraction (30 g) was subjected to silica-gel column chromatography eluting with a $CHCl_3$–MeOH stepwise gradient (40 : 1 → 10 : 1) yielding 10 fractions. Ten fractions were screened for the inhibitory activity on platelet aggregation induced by AA, employing modified smearing method described by Yun-Choi et al. (Yun-Choi et al. 1985). From two active fractions (Fr. 7 and 8), ginsenosides Rk_1, Rg_5, 20(*S*)-Rg_3 and 20(*R*)-Rg_3 were isolated by reversed phase (RP) semi-preparative HPLC/UV (two L-7100 pumps coupled with L-4000 UV detector, Hitachi, Japan) employing isocratic elution by 55% aqueous acetonitrile. The purities of isolated ginsenosides as assessed by RP-HPLC(two L-7100 pumps, Hitachi, Japan) coupled with evaporative light scattering detector (ELSD; Sedex, Sedere, France) were > 95.0%. The isolated compounds (Fig. 1) were identified by comparing their ^{1}H-, ^{13}C NMR spectra [measured on Avance 500 Spectrometer (Bruker, Germany)] and MS spectra [recorded using JMS-700 Mass spectrometer (Jeol, Japan)] with those of authentic compounds already isolated by our group (Park et al. 2002b, c).

3.3. Animal study

Male Sprague-Dawley rats (250 ± 20 g) were purchased from OrientBio (Korea) and the experiment was conducted in accordance with the Guide for the Care and Use of Laboratory Animals by Seoul National University. Whole blood was collected from the heart of the animals using a syringe containing 2.2% sodium citrate. Supernatant platelet rich plasma (PRP) was obtained by centrifugation at 200 × g for 10 min and the residue was centrifuged at 1500 × g for 15 min to obtain the platelet poor plasma (PPP). PRP was diluted with PPP to adjust the final platelet number in PRP to $4.0–4.5 \times 10^8$/ml. Platelet number was counted using a platelet counter (Excell 18 MWI, DANAM Electronics, USA). Platelet antiaggregation activity was monitored turbidimetrically as described by Born and Cross (1963). An aliquot (500 μl) of platelet count adjusted PRP was preincubated for 3 min at 37 °C in an aggregometer (490-X optical aggregometer, Chrono-Log Corp, USA) under constant stirring at 1,000 rpm. Ginsenoside solution (5 μl) was added followed by successive addition of aggregation inducing agent at an intervals of 30 s. Because of poor solubility of ginsenosides in saline, DMSO was used as a vehicle at a concentration (1.0%) which does not affect platelet aggregation. The aggregation induced by AA and U46619 was determined in presence of a near-threshold concentration (1.0–1.4 μg/ml and 0.8–1.2 μg/ml, respectively) of collagen that can only induce change in platelet shape but no aggregation (Pyo et al. 2002). Inhibition (%) was calculated as follows:

Inhibition (%) = (1 – maximal aggregation of sample-treated aPRP/maximal aggregation of vehicle-treated aPRP) × 100

All experiments were performed in triplicate. IC_{50} values were calculated from the linear regression of the plot of mean values (n = 3) of percent inhibition versus concentration of corresponding ginsenoside. Sigma Plot

software was used for graphing. Regression equations were calculated using Regression Wizard from the Sigma Plot equation library.

Acknowledgements: This work was supported by a grant of Health and Medical Technology R & D Program (No. 040021) funded by the Ministry for Health, Welfare and Family Affairs, Republic of Korea.

References

Born GV, Cross MJ (1963) The Aggregation of Blood Platelets. J Physiol (Lond) 168: 178–195.

Keum YS, Park KK, Lee JM, Chun KS, Park JH, Lee SK, Kwon H, Surh YJ (2000) Antioxidant and anti-tumor promoting activities of the methanol extract of heat-processed ginseng. Cancer Lett 150: 41–48.

Kim WY, Kim JM, Han SB, Lee SK, Kim ND, Park MK, Kim CK, Park JH (2000) Steaming of ginseng at high temperature enhances biological activity. J Nat Prod 63: 1702–1704.

Kuo SC, Teng CM, Lee JC, Ko FN, Chen SC, Wu TS (1990) Antiplatelet components in Panax ginseng. Planta Med 56: 164–167.

Kwon SW, Han SB, Park IH, Kim JM, Park MK, Park JH (2001) Liquid chromatographic determination of less polar ginsenosides in processed ginseng. J Chromatogr A 921: 335–339.

Lee SR, Park JH, Choi KJ, Kim ND (1997) Inhibitory effects of ginsenoside Rg_3 on platelet aggregation and its mechanism of action. Korean J Ginseng Sci 21: 132–140.

Matsuda H, Namba K, Fukuda S, Tani T, Kubo M (1986) Pharmacological study on Panax ginseng C. A. Meyer. III. Effects of red ginseng on experimental disseminated intravascular coagulation. (2). Effects of ginsenosides on blood coagulative and fibrinolytic systems. Chem Pharm Bull (Tokyo) 34: 1153–1157.

Park HJ, Rhee MW, Park KM, Nam KY, Park KH (1993) Panaxadiol from Panax ginseng C. A. Meyer inhibits synthesis of thromboxane A_2 in platelet aggregation induced by thrombin. Korean J Ginseng Sci 17: 131–134.

Park IH, Han SB, Kim JM, Piao L, Kwon SW, Kim NY, Kang TL, Park MK, Park JH (2002a) Four new acetylated ginsenosides from processed ginseng (sun ginseng). Arch Pharm Res 25: 837–841.

Park IH, Kim NY, Han SB, Kim JM, Kwon SW, Kim HJ, Park MK, Park JH (2002b) Three new dammarane glycosides from heat processed ginseng. Arch Pharm Res 25: 428–432.

Park IH, Piao LZ, Kwon SW, Lee YJ, Cho SY, Park MK, Park JH (2002c) Cytotoxic dammarane glycosides from processed ginseng. Chem Pharm Bull (Tokyo) 50: 538–540.

Pyo MK, Lee Y, Yun-Choi HS (2002) Anti-platelet effect of the constituents isolated from the barks and fruits of Magnolia obovata. Arch Pharm Res 25: 325–328.

Yun-Choi HS, Kim SO, Kim JH, Lee JR, Cho HI (1985) Modified smear method for screening potential inhibitors of platelet aggregation from plant sources. J Nat Prod 48: 363–370.