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Pharmacological Study on *Panax ginseng* C.A. MEYER V.[1] Effects of Red Ginseng on the Experimental Disseminated Intravascular Coagulation (4). On Ginsenoside-Rg3, Rh1 and Rh2

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20S, 20R ginsenoside-Rg3, 20S, 20R ginsenoside-Rh1 and ginsenoside-Rh2 isolated from Red Ginseng were investigated for their effect on blood platelet aggregation and thrombin-induced conversion of fibrinogen to fibrin *in vitro*.

20S, 20R ginsenoside-Rg3 inhibited collagen- and ADP-induced blood platelet aggregation. 20S ginsenoside-Rg3, 20S, 20R ginsenoside-Rh1 inhibited the thrombin-induced conversion of fibrinogen to fibrin.

Keywords——*Panax ginseng;* red ginseng; ginsenoside-Rg3; ginsenoside-Rh1; ginsenoside-Rh2; blood platelet aggregation; thrombin; fibrin

In the previous paper,[2] we reported the effect of major components of *Panax ginseng*, ginsenoside-Ro, Rb1, Rb2, Rc, Re, Rg1 and Rg2 obtained from *n*-BuOH soluble fraction of *Panax ginseng*, on blood coagulative and fibrinolytic system *in vitro*.

Recently, Kitagawa *et al.*[3] isolated 20S ginsenoside-Rg3, 20R ginsenoside-Rh1, ginsenoside-Rh2 from Red Ginseng. Odajima *et al.*[4] reported that ginsenoside-Rh2 inhibited the multiplication of tumor cells.

We reported that ethylacetate-soluble fraction of *Panax ginseng* extract, containing these saponins inhibited blood platelet aggregation and thrombin-induced conversion of fibrinogen to fibrin.

The present paper deals with a study on the effect of 20S, 20R ginsenoside-Rg3, 20S, 20R ginsenoside-Rh1 and ginsenoside-Rh2 on blood platelet aggregation and conversion of fibrinogen to fibrin.

Materials and Methods

Materials——20S, 20R ginsenoside-Rg3, 20S, 20R ginsenoside-Rh1 and ginsenoside-Rh2 were obtained from Red Ginseng as reported previously.[3] Collagen and ADP disodium salt: Sigma Chemical Co., USA., thrombin: Mochida Ltd., Japan.

Animals——Male rats of Wistar-King strain, weighing 150–200 g were used for the present experiment. They were fed on a standard diet (Nihon Clea, Japan) for at least 7 days and were deprived of food for 24 hr before the start of the experiments.

Blood platelet aggregation——Rats were anesthetized with pentobarbital and blood samples were collected by cardiac puncture with plastic syringes. Nine ml of blood and 1 ml of heparin solution (10 U/ml) were transfered into a plastic tube and centrifuged at 1,000 rpm for 10 min. The supernatant platelet-rich plasma (PRP) was removed with a siliconized pipet and stored in sealed plastic test tubes, which was gently stirred at 5–10°C for 30 min prior to use. The red cell precipitate was recentrifuged at 3,000 rpm for 30 min to obtain platelet-poor plasma

(PPP), which was used as a maximal transmittance standard.

Blood platelet aggregation test was performed according to the method of Born *et al.*[5] The aggregating agents used were collagen (500 μg/ml) and ADP (0.5 μM). A 0.2 ml aliquot of PRP was placed in a tube and stirred at 1200 rpm, at 37°C, for 1 min. Then to the reaction mixture was added a 10 μl aliquot of a test solution and 1 min later, an aggregating agent. Blood platelet aggregation was monitored by continuous recording of light transmittance in a Husm System Platelet Aggregometer (Rika Electric Co., Japan). The extent of aggregation was estimated from the transmission maxima obtained after the addition of aggregating agents and expressed as the % increase in transmission as compared with the control. An anti-blood platelet aggregating drug, aspirin was used as a standard drug.

Conversion of fibrinogen to fibrin——Fibrinogen (500 mg) was dissolved in 100 ml of 0.05 M tris acetate buffer (pH 7.4) containing 0.15 M NaCl. A test solution (0.1 ml) was added to 1.8 ml of the fibrinogen solution with stirring. After 1 min, 0.1 ml of thrombin solution (0.2 U/ml) was added and the whole was gently stirred until fibrin appeared. The time required for clotting was recorded. Anti-thrombin drug, heparin was used as a standard drug.

Results

Collagen-induced blood platelet aggregation——As shown in TABLE I, aspirin (as a standard drug) inhibited the collagen-induced blood platelet aggregation at a concentration of 1.0 mM. The inhibitory effect on collagen-induced blood platelet aggregation of 20S ginsenoside-Rg3, 20R ginsenoside-Rg3 and 20R ginsenoside-Rh1 at a concentration of 1.0 mM were 21.4±4.2%, 27.4±3.4% and 10.3±2.1% and ginsenoside-Rh2 did not inhibited the collagen-induced blood platelet aggregation.

ADP-induced blood platelet aggregation——As shown in TABLE II, only 20R ginsenoside-Rg3 inhibited the ADP-induced blood platelet aggregation.

Conversion to fibrinogen to fibrin——As shown in TABLE III, the clotting time of the control with no test solution was 201±10 sec. The clotting time of a mixture containing heparin at a concentration of 10 U/ml was 247±13 sec. Addition of 20S ginsenoside-Rg3, 20S or 20R ginsenoside-Rh1 produced dose-dependent extension of the clotting time. 20R ginsenoside-Rg3 and ginsenoside-Rh2 did not extend the clotting time.

TABLE I. Effect of Ginsenosides and Aspirin on Collagen-induced Blood Platelet Aggregation

Treatment	Inhibitory percentage	
	0.5	1.0 (mM)
20S Ginsenoside-Rg3	8.7±2.0	21.4±4.2
20R Ginsenoside-Rg3	14.2±2.2	27.4±3.4
20S Ginsenoside-Rh1		4.3±3.0
20R Ginsenoside-Rh1		10.3±2.1
Ginsenoside-Rh2		3.8±1.6
Aspirin	8.0±2.7	21.6±3.2

Each value represents the mean±S.E. of 5 experiments.

TABLE II. Effects of Ginsenosides and Aspirin on ADP-induced Blood Platelet Aggregation

Treatment	Inhibitory percentage	
	0.5	1.0 (mM)
20S Ginsenoside-Rg3	4.0±1.2	10.3±2.5
20R Ginsenoside-Rg3	12.3±3.2	23.4±3.4
20S Ginsenoside-Rh1		3.2±1.2
20S Ginsenoside-Rh1		7.2±0.9
Ginsenoside-Rh2		2.1±0.7
Aspirin		2.2±0.9

Each value represents the mean±S.E. of 5 experiments.

TABLE III. Effects of Ginsenosides and Heparin on Conversion of Fibrinogen to Fibrin Induced by Thrombin

Treatment	Clotting time of fibrinogen (sec)		
	0	0.5	1.0 (mM)
Control	201±10		
20S Ginsenoside-Rg3		210±7	248±8**
20R Ginsenoside-Rg3		205±10	217±8
20S Ginsenoside-Rh1		243±11**	294±7**
20R Ginsenoside-Rh1		223±8*	258±4**
Ginsenoside-Rh2		198±7	203±6
Heparin (10 U/ml)	247±13**		

Each value represents the mean±S.E. of 5 experiments.
Significantly different from contorl, $*p<0.05$, $**p<0.01$.

Discussion

It has already been reported that fat-soluble fraction of Red Ginseng shows platelet aggregation inhibition and anti-thrombin action. In this paper were studied platelet aggregation inhibition and anti-thrombin action of 20S ginsenoside-Rg3, 20R ginsenoside-Rh1, 20R ginsenoside-Rh1 and ginsenoside-Rh2 obtained from the fat-soluble fraction of Red Ginseng.

20R ginsenoside-Rg3 was demonstrated to inhibit collagen- and ADP-induced platelet aggregation. However the effect of 20S (natural type) was found to be weaker than that of 20R (not-natural type or artifact). The same trend was noted in ginsenoside-Rh1.

20S ginsenoside-Rg3, 20S ginsenoside-Rh1 and 20R ginsenoside-Rh1 were effective on thrombin-induced conversion of fibrinogen. In this system, 20S was more active than 20R.

A number of works are available on the physiological activity of *Panax ginseng*. However most works are on the saponins contained in the *n*-BuOH soluble fraction and scarcely any are on the saponins in the fat-soluble fraction. Fat-soluble fraction, is contained in larger quantity in Red Ginseng than in White Ginseng. The present work showed that 20S ginsenoside-Rg3 and 20R ginsenoside-Rh1 which are the particular components of Red Ginseng, possessed anti-thrombin action. It is interesting that different preparation procedures of crude drugs result in the production of crude drugs of different pharmacological activities.

References and Notes

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