[Chem. Pharm. Bull. 34(5)2100—2104(1986)]

Pharmacological Study on *Panax ginseng* C. A. MEYER. IV.[1] Effects of Red Ginseng on Experimental Disseminated Intravascular Coagulation. (3). Effect of Ginsenoside-Ro on the Blood Coagulative and Fibrinolytic System

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(Received October 30, 1985)

The antithrombic activity of ginsenoside-Ro isolated from the roots of *Panax ginseng* C. A. MEYER was evaluated in experimental models of disseminated intravascular coagulation (DIC) induced by infusion of endotoxin or thrombin in rats. Ginsenoside-Ro (50 mg/kg, *p.o.*) prevented the decrease of fibrinogen in endotoxin-induced DIC. Ginsenoside-Ro also inhibited the formation of fibrin thrombi in the renal glomeruli in thrombin-induced DIC.

Ginsenoside-Ro showed a promotive effect on the activation of the fibrinolytic system as determined by the euglobulin lysis time (ELT) assay. The promotive effect of ginsenoside-Ro on the activation of the fibrinolytic system was inhibited by pretreatment with ε-aminocaproic acid (an anti-plasmin agent), but not by pretreatment with promethazine or cyproheptadine (anti-chemical agents).

Keywords——*Panax ginseng*; ginsenoside-Ro; disseminated intravascular coagulation; endotoxin; thrombin; fibrinolysis; plasmin

In a series of pharmacological studies on *Panax ginseng* C. A. MEYER, we previously demonstrated that the 70% methanolic extract inhibited endotoxin- and thrombin-induced disseminated intravascular coagulation (DIC)[2] and further showed that the ginsenosides from Red Ginseng exhibited an anti-thrombic effect, an anti-blood platelet aggregation activity and a promotive effect on activation of the fibrinolytic system.[1]

On the other hand, it was reported that ginsenoside-Rb_1 administered orally was not absorbed from the digestive tract in rats.[3] However, there is no reported on the effectiveness of orally administered ginsenosides on *in vivo* experimental DIC. The purpose of the present investigation was therefore to study the effect of ginsenoside-Ro on *in vivo* experimental DIC in rats.

Materials and Methods

Materials——Ginsenoside-Ro was isolated from the roots of *Panax ginseng* C. A. MEYER as reported previously.[1] The sources of materials were as follows: endotoxin (*Escherichia coli* 055:B5, Difco Lab., U.S.A.), thrombin (Mochida Ltd., Japan), dextran sulfate (Pharmacia Fine Chemicals, Sweden), ε-aminocaproic acid (Nihon Rikagaku, Japan), promethazine hydrochloride (Shionogi, Japan), cyproheptadine hydrochloride (Japan Merck Banyu, Japan) and compound 48/80 (Sigma Chemical Co., U.S.A.).

Animals——Male Wistar-King strain rats weighing 150—200 g were used for the experiments. They were fed a standard diet (Nihon Clea, Japan) for a minimum period of 7 d and then fasted for 24 h before the start of the experiments.

Endotoxin-Induced DIC——Experimental DIC was induced by a modification of the method of Schoendorf *et al.*[4] Ginsenoside-Ro (10 or 50 mg/kg) was administered orally to the rats 1 h before the injection of endotoxin

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(0.1 mg/kg) into the tail vein. Blood samples were withdrawn from the heart into plastic syringes at 4 h after the injection of endotoxin, while the rats were anesthetized with pentobarbital. As anticoagulants, 0.01 M sodium ethylenediaminetetraacetic acid (EDTA) was used for platelet counts and a 1:9 volume of 3.8% sodium citrate for prothrombin time and fibrinogen determination.

Blood platelets were counted with an automatic blood cell counter (Coulter counter, model S-Plus, Coulter Co., U.S.A.). Fibrinogen was determined according to the method of Quick.[5] The prothrombin time was measured with a COAG-A-Mate dual-channel device (Generaldiagnostic, Warner-Lambert Co., U.S.A.). Fibrin degradation product (FDP) was determined by means of the latex aggregation test (FDPL test U, Teikoku Zoki, Japan).

Thrombin-Induced DIC——Experimental DIC was induced by the method of Margaretten *et al.*[6] Ginsenoside-Ro (10, 50 or 100 mg/kg) was administered orally to the rats 1 h before the intravenous infusion of thrombin (4 U/min/kg, for 30 min) dissolved in saline. Blood samples were withdrawn from the heart into plastic syringes immediately after the final infusion of thrombin while the rats were anesthetized with pentobarbital. Blood platelets were counted and fibrinogen, prothrombin time and FDP were determined as described above. Both kidneys were dissected out immediately after the collection of blood, and cryostat sections were prepared. The sections were stained with phosphotungstic acid–hematoxylin for histological examination. One hundred glomeruli were counted and the percentage of glomeruli having fibrin thrombi was determined.

Euglobulin Lysis Time (ELT) in Normal Rats——Whole blood samples were collected into plastic syringes from the heart of rats anesthetized with pentobarbital at 1 h after the oral administration of ginsenoside-Ro (10 or 50 mg/kg). One-tenth volume of 3.8% sodium citrate was added to the blood sample and the mixture was centrifuged at 4000 rpm at 4 °C for 10 min. Using the plasma thus obtained, ELT was measured in the manner reported by Kaulla and Schultz.[7] After addition of 9.8 ml of precooled water, the plasma was incubated at 4 °C for 5 min in a stream of CO_2 gas, then centrifuged at 4000 rpm for 10 min. The resulting precipitates were dissolved in 0.7 ml of 1/15 N phosphate buffer solution, then 40 μl of thrombin solution (125 U/ml) was added. The coagulating plasma was incubated at 37 °C, and the ELT was measured.

Whole blood samples obtined from the heart at 30 min after intravenous injection of dextran sulfate (1 or 10 mg/kg), were treated in the same manner as mentioned above and the ELT was measured.

ELT in Rats Treated with ε-Aminocaproic Acid, Promethazine or Cyproheptadine——ε-Aminocaproic acid (200 mg/kg) was administered intraperitoneally to rats 5 min before the administration of ginsenoside-Ro (50 mg/kg, *p.o.*) or dextran sulfate (10 mg/kg, i.v.), followed by collection of whole blood samples from the heart. ELT was measured after treatment of the samples as described in the case of normal rats.

Promethazine (40 mg/kg, *p.o.*) or cyproheptadine (10 mg/kg, *p.o.*) was administered to the rats 2 h before the administration of ginsenoside-Ro (50 mg/kg, *p.o.*) or dextran sulfate (10 mg/kg, i.v.), followed by collection of whole blood samples from the hearts. Each sample was treated as described above for the determination of ELT.

Edema Formation in Rat Hind Paw——The experiments to test edema formation in rat hind paw were performed by the method of Winter *et al.*[8] The volume of the right hind paw was measured 30, 60 or 120 min after the subcutaneous injection of ginsenoside-Ro (1 mg/kg) or compound 48/80 (50 μg/kg). The swelling percentage was calculated as compared with the volume of the right hind paw before the subcutaneous injection of the test substance.

Results

Endotoxin-Induced DIC

It was shown that DIC could be induced by injection of endotoxin (0.1 mg/kg) into the tail vein, resulting in a decrease of blood platelets and fibrinogen, prolongation of the prothrombin time and an increase of FDP. Before the injection of endotoxin, 10 or 50 mg/kg of ginsenoside-Ro was administered orally, and its preventive effect against the endotoxin-induced DIC was examined (Table I).

The blood platelet count was $92 \pm 5 \times 10^4/mm^3$ in normal rats injected with saline only. It was reduced to $56 \pm 9 \times 10^4/mm^3$ in rats injected with 0.1 mg/kg of endotoxin. When rats were orally given 50 mg/kg of ginsenoside-Ro, the reduction of the blood platelet count by endotoxin was smaller.

The level of fibrinogen was 188 ± 9 mg/dl in normal rats given saline only. The level decreased to 112 ± 17 mg/dl in DIC rats. The decrease of fibrinogen level was significantly less in rats orally given 10 or 50 mg/kg of ginsenoside-Ro.

Prothrombin time was 12.2 ± 0.7 s in the normal rats, while it was prolonged to 14.4 ± 0.7 s in the DIC rats. Some shortening of prothrombin time was observed in rats orally given 10 or 50 mg/kg of ginsenoside-Ro, as compared with the control.

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TABLE I. Effects of Ginsenoside-Ro and Aspirin on Endotoxin-Induced Experimental DIC in Rats Injected with 0.1 mg/kg of Endotoxin

Treatment	Dose (mg/kg)	Route	No. of rats	Blood platelets ($\times 10^4$/mm^3)	Fibrinogen (mg/dl)	Prothrombin time (s)	FDP (μg/ml)
Normal		*p.o.*	8	92±5	188±9	12.2±0.2	0.2±0.1
Control		*p.o.*	8	56±9	112±17	14.8±0.7	7.6±1.5
Ginsenoside-Ro	10	*p.o.*	8	63±8	141±9[a]	14.4±0.6	5.8±0.8
Ginsenoside-Ro	50	*p.o.*	8	66±4	153±5[b]	13.8±0.7	6.0±1.0
Normal		*p.o.*	8	82±12	180±17	12.6±0.3	0.2±0.1
Control		*p.o.*	8	32±5	77±12	26.7±3.2	6.0±1.7
Aspirin	50	*p.o.*	8	44±4[a]	141±17	24.4±1.7	4.3±1.0
Aspirin	200	*p.o.*	8	50±4[a]	131±26[a]	25.3±3.3	4.6±2.3

Each value represents the mean±S.E. Significantly different from the control, *a*) $p<0.05$, *b*) $p<0.01$.

TABLE II. Effects of Ginsenoside-Ro and Heparin on Thrombin-Induced Experimental DIC in Rats Infused with 4 U/min of Thrombin for 30 min

Treatment	Dose (mg/kg)	Route	No. of rats	Blood platelets ($\times 10^4$/mm^3)	Fibrinogen (mg/dl)	Prothrombin time (s)	FDP (μg/ml)
Normal			10	101±9	252±33	12.7±0.7	0.4±0.1
Control			10	61±4	40±11	17.3±0.7	6.7±1.8
Ginsenoside-Ro	10	*p.o.*	10	48±4	44±14	18.5±3.0	6.6±1.5
Ginsenoside-Ro	50	*p.o.*	10	53±7	68±9[a]	18.0±0.5	7.2±0.8
Ginsenoside-Ro	100	*p.o.*	10	52±4	87±11[b]	18.7±1.4	7.5±1.4
Normal			10	115±7	240±20	11.5±1.8	0.2±0.1
Control			10	52±6	52±6	32.1±6.2	3.5±0.9
Heparin	3 U/min/kg	i.v.	10	72±5[a]	134±21[b]	19.6±3.4	3.2±0.7

Each value represents the mean±S.E. Significantly different from the normal, *a*) $p<0.05$, *b*) $p<0.01$.

The fibrin degradation product (FDP) level was 0.2±0.1 μg/ml in normal rats injected with saline only. The level increased to 7.6±1.5 μg/ml in the DIC rats. When 10 or 50 mg/kg of ginsenoside-Ro was administered to rats 1 h before the injection of endotoxin, the FDP level was not reduced.

A clear preventive effect of aspirin (used as a standard drug) was recognized in terms of blood platelets and fibrinogen, but not prothrombin time or FDP.

Thrombin-Induced DIC

It was shown that DIC could be induced by infusion of thrombin (4 U/min/kg, for 30 min), resulting in a decrease of blood platelets and fibrinogen, prolongation of the prothrombin time and an increase of FDP. Before the infusion of thrombin, 10 or 50 mg/kg of ginsenoside-Ro was administered orally, and the preventive effect against the thrombin-induced DIC was examined (Table II). The decrease of fibrinogen level was significantly less in rats orally given 50 or 100 mg/kg of ginsenoside-Ro. However, the FDP level increased above that in the DIC rats.

A clear preventive effect of heparin (used as a standard drug) was recognized in terms of blood platelets and fibrinogen, but not prothrombin time or FDP.

Histological examination of the kidneys collected from rats given intravenous infusion of thrombin (4 U/min/kg, for 30 min) was carried out. One hundred glomeruli were checked, and the number containing fibrin thrombi is given as a percentage in Table III.

In the kidneys of the normal rats infused with saline for only 30 min, 53±10% of thrombi-containing glomeruli were detected. In the control rats pretreated with thrombin

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TABLE III. Effects of Ginsenoside-Ro and Heparin on Thrombin-Induced Experimental DIC in Rats Infused with 4 U/min of Thrombin for 30 min

Treatment	Dose (mg/kg)	Route	No. of rats	Percentage of fibrin deposits on glomeruli
Control (water)		*p.o.*	10	87±5
Ginsenoside-Ro	10	*p.o.*	10	75±4
Ginsenoside-Ro	50	*p.o.*	10	62±9[a]
Ginsenoside-Ro	100	*p.o.*	10	43±9[b]
Control (saline)		i.v.	10	53±10
Heparin	3 U/min/kg	i.v.	10	4±3[b]

Each value represents the mean±S.E. Significantly different from the control, *a*) $p<0.05$, *b*) $p<0.01$.

TABLE IV. Effects of Ginsenoside-Ro and Dextran Sulfate on Euglobulin Lysis Time in Rats

Treatment	Dose (mg/kg)	Route	No. of rats	ELT (min)
Normal (water)		*p.o.*	10	223±21
Ginsenoside-Ro	10	*p.o.*	10	213±29
Ginsenoside-Ro	50	*p.o.*	10	138±14[a]
Normal (saline)		i.v.	10	188±13
Dextran sulfate	1	i.v.	10	148±20
Dextran sulfate	10	i.v.	10	88±9[b]

Each value represents the mean±S.E. Significantly different from the normal, *a*) $p<0.05$, *b*) $p<0.01$.

TABLE V. Effects of Various Antagonists on the Shortening of Euglobulin Lysis Time Induced by Ginsenoside-Ro and Dextran Sulfate in Rats

Treatment	Euglobulin lysis time (min)
Normal (water, *p.o.*)	233±15
Ginsenoside-Ro (50 mg/kg, *p.o.*)	168±19
Ginsenoside-Ro (50 mg/kg, *p.o.*)+ε-aminocaproic acid (200 mg/kg, i.p., 5 min[a])	240±13[b]
Ginsenoside-Ro (50 mg/kg, *p.o.*)+promethazine (40 mg/kg, *p.o.*, 2 h)	175±20
Ginsenoside-Ro (50 mg/kg, *p.o.*)+cyproheptadine (10 mg/kg, *p.o.*, 2 h)	182±18
Normal (saline, i.v.)	190±12
Dextran sulfate (10 mg/kg, i.v.)	142±11
Dextran sulfate (10 mg/kg, i.v.)+ε-aminocaproic acid (200 mg/kg, i.p., 5 min)	182±10[b]
Dextran sulfate (10 mg/kg, i.v.)+promethazine (40 mg/kg, *p.o.*, 2 h)	142±10
Dextran sulfate (10 mg/kg, i.v.)+cyproheptadine (10 mg/kg, *p.o.*, 2 h)	156±14

a) Time of administration of agonist (ginsenoside-Ro or dextran sulfate) after antagonist. Each value represents the mean±S.E. Significantly different from ginsenoside-Ro or dextran sulfate, *b*) $p<0.05$.

(4 U/min/kg, for 30 min), as many as 87±5% of the glomeruli contained thrombi. However, when rats were orally given 50 or 100 mg/kg of ginsenoside-Ro, the formation of fibrin thrombi in glomeruli was prevented significantly as compared with that in the control rats.

ELT in Normal Rats

As shown in Table IV, ELT was 223±21 min in normal rats given with water only. When 10 mg/kg of dextran sulfate (as a standard drug) was injected into rats. The ELT was significantly shortened to 88±9 min. It was significantly shortened to 138±14 min when 50 mg/kg of ginsenoside-Ro was given orally.

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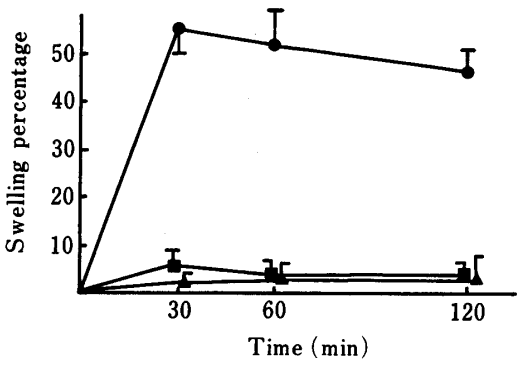

Fig. 1. Time Course of Rate Paw Edema
—▲—: Control (saline). —■—: Ginsenoside-Ro 1 mg/kg. —●—: Compound 48/80 50 μg/kg.

ELT in Rats Treated with ε-Aminocaproic Acid, Promethazine or Cyproheptadine

As shown in Table V, ELT was 233 ± 15 min in the normal rats. It was shortened to 168 ± 19 min in rats given 50 mg/kg of ginsenoside-Ro. When 200 mg/kg of ε-aminocaproic acid was injected into rats 5 min before the administration of ginsenoside-Ro, the ELT was significantly prolonged to 240 ± 13 min. However, when promethazine (40 mg/kg) or cyproheptadine (10 mg/kg) was administered orally to rats 2 h before the administration of ginsenoside-Ro, the ELT was not prolonged.

Among the three antagonists, only ε-aminocaproic acid abolished the shortening of ELT by dextran sulfate.

Formation of Edema in Rat Hind Paw

The time courses of edema in rat hind paw induced by ginsenoside-Ro (1 mg/kg) or compound 48/80 (50 μg/kg, as a standard drug) are shown in Fig. 1. Compound 48/80 (used as a chemical mediator-releasing agent from mast cells) caused edema in rat hind paw. However, ginsenoside-Ro did not cause edema.

Discussion

Ginsenoside-Ro, when orally given to rats at 50 mg/kg, inhibited the decrease of fibrinogen and the formation of fibrin thrombi in the renal glomeruli in thrombin-induced DIC. Similar inhibitory activity on the decrease of fibrinogen was also observed in endotoxin-induced DIC. These inhibitory activities of ginsenoside-Ro against *in vivo* experimental DIC were similar to those observed *in vitro*.[1] It seems likely that ginsenoside-Ro is absorbed from the digestive tract in rats.

Ginsenoside-Ro at 50 mg/kg (*p.o.*) showed a promotive effect on the activation of the fibrinolytic system in the ELT assay. Since the effect disappeared on pretreatment with an anti-plasmin agent, ε-aminocaproic acid, but not with chemical mediators, promethazine and cyproheptadine, the mode of the promotive effect of ginsenoside-Ro seems to be similar to that of urokinase, enhancing the conversion of plasminogen into plasmin.

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