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Preliminary Investigation of Membrane Modifying Effects of Ginseng Components

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인삼성분 및 제제의 생체막 보호 효과에 대한 연구

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Abstract

Prophylactic and curative behaviors of *Panax ginseng* components (95%, 50% ethanol ext., ginsenoside Re and Ginsana G 115) on the hepatomegaly, lipid peroxidation of the thioacetamide-intoxicated animals *in vivo* and *in vitro* were investigated. Ginsenoside Re and Ginsana G 115 significantly decreased in the lipid peroxide formation; the 95% ethanol extract and ginsenoside Re, in the zinc sulfate turbidity test. Besides these investigations, the preventive effect of ginseng components on the degranulation of mast cells in the guinea pig mesentery by compound 48/80 and venom toxin (*Agkistrodon piscivourus*) was also examined. All ginseng components subjected to this experiment were affected significantly at the different degrees.

Introduction

Multi-angle research on *Panax ginseng* components is being conducted by many researchers from various approaches. Recently, the frequency of preventive applications against diseases and actual application for medication of diseases by isolated constituents, extracts and drug preparation of *Panax ginseng* is on the increase.

However, it seems that researches conducted by many ginseng researchers are heavily concentrated on glycosides contained in ginseng.[1-18] It is an encouraging sign that there are now an increasing number of researches on non-glycosides fraction components of ginseng being undertaken.[19-24]

Some researchers have developed an interest in the solubilizing effect of non-soluble medicine in order to explore the possibility of applying the findings of such research into the applicability in the manufacturing process of medicine. On the other hand a number of researches are attempted in the *in vitro* level on the stabilizing effect of cell membrane.

The presenters have attempted, for the first time in the field of ginseng research, to investigate the prophylactic behavior of ginseng extracts, ginsenosides and G115 on

the *in vivo* and *in vitro* levels (hereafter will be referred to as ginseng components).

This paper summarizes the findings derived from the research on the prophylactic behavior of ginseng components conducted by means of the *in vivo* test. This research reveals the effective containment of the pathological increase in the liver weight. Not only that but 50% ethanol extracts, water extracts, ginsenoside Re, Ginsana G-115 among ginseng components, indicated a meaningful efficacy in the lipid peroxidation. Also in *in vitro* test, 50% ethanol extract, ginsenoside Re and Ginsana G-115 showed the lowering effect in the pathological elevation of s-GPT and s-GOT. As for the formation of lipid peroxide concerning the pathological elevation, the presenters confirmed the efficacy of Ginsana G-115 in this activity.

On the other hand, in the area of curative behavior, it is discovered that ginseng water extract, ginseonside Re, and Ginsana G-115 to have efficacy. In the test of guinea pig mast cell degranulation by compound 48/80 and snake venom toxin, ginseng components, though on varying degrees, showed the protective effects. However, in the animal test of ginseng components, the excessive dosage confirmed quite a substantial affected, in the curative effect especially.

Experiment

Materials

Ginseng components used in this test are ginseng ethanol (95% and 50%) extracts, water extract, ginsenoside Re, and Ginsana G-115. Test animals used are male *Sprague-dawley* rats (b.w. 125-175g) and guinea pig (b.w. 750-900g). Collagenase type IV, thiobarbituric acid, snake venoms (from *Agkistrodon piscivorus,* Leukostoma) and compound 48/80 have been purchased from Sigma Chemical Co.

Methods.

1. Preventive behavior of ginseng components *in vivo.*

Panax ginseng components at oral doses of 50, 100 and 200 mg per kg were respectively administered previously to the test rats daily for 4 days, but not to the control animals. On day 5, thioacetamide was subcutaneously given in a dose of 25 mg per kg.[25] 24 hours after thioacetamide-intoxication, samples of blood and liver were respectively taken under ether anesthesia. Blood samples were taken from the inferior vena cava and serum GOT and GPT activities were measured by the Reitman-Frankel method. Liver fixed in 10% formaldehyde solution and stained hematoxylin-eosin. Lipid peroxides in the liver were measured by the Masugi method[26] as malonedialdehyde.

2. Preventive behavior of ginseng components *in vitro.*

1) Dissociation of Hepatocytes

Dissociated hepatocytes were taken by the Firend method.[27] Under ether anesthesia, the portal and superior caval veins were cannulated with soft plastic tubes and the inferior caval vein was tied above the insertion of the renal vein. The liver was perfused by means of a peristaltic pump at a rate of 50 m*l*/min with the warm Ca^{++}-

free Ringer phosphate buffer solution (pH. 7.4), continuously gassed with carbogen (95% O_2 and 5% CO_2). After 5 min, the washing was interrupted and perfusion continued using the Ringer phosphate buffer solution containing collagenase (0.02%). Within 10-15 min the surface of the liver began to leak out perfusion fluid, and then the liver was washed with the Ca^{++}-free Ringer phosphate buffer solution, and liver cells were liberated from the connective vascular tissue by careful raking with a stainless-steel comb. The connective vascular tissue remnant was lifted out and the cell suspension was filtered through 250 and 62 μM stainless-steel filters. The cell suspension at a low temperature (0-4°C) was routinely measured with the hepatocytes by hemocytometer.

2) Stability test of hepatocytes[28]

100 μl of cell suspension was diluted with 300 μl of isotonic 0.6% trypan blue in the Ca^{++}-free Ringer phosphate solution and counted by hemocytometer. 100 cells were counted in each sample under the optical microscope at 100 x magnification. Intact cells appear as pale, refringent structures, containing vascular nucleus and bearing small, granular inclusions; broken cells appear larger and less refraingent. Their nucleus and cytoplasm were stained blue by the dye.

3) Effect of ginseng components on dissociated hepatocytes[29]

One m*l* of cell suspension (1×10^6/m*l*) and 10 m*l* of *Panax ginseng* components at a concentration of 1, 10, 100 and 1,000 μg/m*l* were previously incubated at 37°C in the plastic tubes. 30 min after incubation, 10 μl of the thioacetamide solution (6 mg/m*l*) was added and incubated at 37°C for 2 hrs. 10 μl of the tested cell suspension was stained for stability test. This cell suspension was centrifuged at 750 rpm. The upper layer was used for the test of s-GOT and s-GPT, the lower layer for lipid peroxides.

4) Effect of ginseng components on the stabilitzation of mast cell[30] to venom toxin and compound 48/80

Mast cell in mesentery is a very suitable tissue for the stabilization test of cell. Mesentery in the guinea pig was dissected from the small intestine and cut into several small pieces that were in the cold Tyrode's solution until used. The pieces were incubated with ginseng components at the concentration of 1, 10, 100 and 1,000 μg per m*l*. 15 min after incubation, the compound 48/80 (10 μg/m*l*) and venom toxin (200 μg/m*l*) were added and incubated at 37°C for 10 min in a 10% formaldehyde solution containing 0.1% toluidine blue and 0.1% acetic acid.[31] Mast cell damage was assessed by measuring the percentage of cells with extruded granules.

3. Curative effect of ginseng components *in vivo*

Thioacetamide at a dose of 25 mg per kg was subcutaneously administered on Day 1 and 5. Ginseng components at a doses of 50, 100 and 200 mg per kg were respectively given orally from Day 1 to 5 daily for 5 days to the test animals, but not to the con-

trol animals. On day 1 and 5 Ginseng Components were given 30 minutes after the thioacetamide-intoxication. 24 hrs after the thioacetamide-intoxication, sampls of blood and liver were respectively taken by ether anesthesis, and then the liver function test and histological studies were performed in the same manner as the above experiment of protective effects.

Results and Discussion

The result of the *in vivo* test on the preventive behavior of ginseng components to the thioacetamide-induced liver damage was summarized in Table 1. In the ratio of the liver weight and body weight of the rat intoxicated by thioacetamide, the test groups of 95% ethanol extract, 50% ethanol extract, water extract, ginsenoside Re, and Ginsana G-115 registered.$p<0.05$, $p<0.01$ indicating efficacy as compared with control. As for the lipid peroxidation, the liver in control group indicated 18.0 nM/g. However 50% ethanol extract (50mg/kg), water extract (100mg/kg), ginsenoside Re (5mg/kg), Ginsana G-115 (50mg/kg) groups indicated respectively 12.0, 13.8, 7.3, 14.3 reflecting significantly the prevented lipid peroxidation.

Table 1. Prophylactic behavior of *Panax ginseng* components on the hepatomegaly and lipid peroxidation in the thioacetamide-induced liver damage *in vivo*

Extracts[1)]	Dose (mg/kg, p.o.)	No. of rats	Body wt. increased for 5 days(%)	Liver wt. (%)	$\frac{Liver\ wt.}{Body\ wt.} \times 100$	Lipid peroxide (malonedialdehyde nM/g)[2)]
Control	—	10	8.4 ± 1.1	6.48 ± 0.92	5.40 ± 0.72	18.0 ± 1.4
95% EtOH ext.	50	10	11.6 ± 1.4	5.98 ± 1.12	4.98 ± 0.65*	17.8 ± 1.4
	100	10	6.0 ± 0.8	5.95 ± 0.99	4.66 ± 0.90*	16.3 ± 1.3
	200	10	1.4 ± 0.7**	5.84 ± 1.33	3.99 ± 1.10**	18.8 ± 1.4
50% EtOH ext.	50	10	8.0 ± 1.1	5.97 ± 0.89	4.67 ± 0.76*	12.0 ± 1.5*
	100	10	7.2 ± 1.3	5.84 ± 0.95	4.51 ± 0.65*	12.3 ± 1.3*
	200	10	5.2 ± 0.9	5.46 ± 1.12	4.40 ± 0.55*	13.3 ± 1.4*
Water ext.	50	10	2.0 ± 0.2**	5.96 ± 1.05	4.38 ± 0.62*	15.5 ± 1.8
	100	10	2.8 ± 0.5*	5.93 ± 1.24	4.40 ± 0.62*	13.8 ± 1.1*
	200	10	4.2 ± 0.9*	5.69 ± 1.06	4.35 ± 0.88*	11.5 ± 1.3**
Ginsenoside Re	5	10	5.8 ± 1.2	5.68 ± 0.97	4.47 ± 0.92*	7.3 ± 1.1**
	10	10	7.6 ± 1,4	5.82 ± 1.11	4.53 ± 0.79*	6.3 ± 0.9**
	20	10	8.6 ± 1.6	6.50 ± 1.34	4.56 ± 0.78*	5.4 ± 0.8**
Ginsana G115	50	10	7.2 ± 1.4	5.39 ± 1.25	4.321 ± 0.72*	14.3 ± 1.3*
	100	10	6.6 ± 1.2	5.48 ± 1.34	4.26 ± 0.79**	8.0 ± 0.9**
	200	10	5.8 ± 1.6	5.41 ± 1.37	4.23 ± 0.85**	5.8 ± 0.8**

1) *Panax ginseng* components were previously administered orally to the test animals daily for 4 days before intoxication of thioacetamide (25 mg/kg, s.c.).

2) Lipid peroxides as malonedialdehyde in liver were determined 24 hours after intoxication of thioacetamide.

Significantly different from control, *($P<0.05$) and **($P<0.01$).

When the above test was conducted *in vitro,* ginsenoside Re and Ginsana G-115 at the concentration of 1 μg/m*l.* meaningful efficacy decreased significantly the transaminase activities. In the inhibition of lipid peroxide formation, the components generally indicated the tendency to decrease it, however, Ginsana G115 alone indicated a statistically meaningful behavior. In the stability of hepatocytes test, when the control gruopd was placed at 0%; all ginseng components dosing groups reflected efficacy as indicated in Table II.

Table 2. Prophylactic behavior of ***Panax ginseng*** components on the serum transaminase activities and zinc sulfate turbidity in the thioacetamide-induced liver damage ***in vivo***

Extracts[1)]	Dose (mg/kg, p.o.)	No. of rats	S-GPT(u/*l*)[2)] Reitman	S-GOT(u/*l*)[2)] Frankel units	ZTT(u/*l*)[2)] Kunkel units
Control	—	10	132.3 ± 11.2	182.3 ± 16.2	16.9 ± 1.5
95% EtOH ext.	50	10	144.1 ± 12.2	177.0 ± 14.2	12.5 ± 1.2
	100	10	140.3 ± 7.9	178.0 ± 13.3	11.0 ± 1.7*
	200	10	131.7 ± 15.4	175.0 ± 12.6	9.1 ± 1.3**
50% EtOH ext.	50	10	114.0 ± 8.9*	168.3 ± 11.1	15.6 ± 1.5
	100	10	121.7 ± 11.2	168.7 ± 14.3	15.0 ± 1.7
	200	10	123.0 ± 13.3	168.0 ± 12.5	14.3 ± 1.1
Water ext.	50	10	118.7 ± 9.6*	170.8 ± 13.4	18.7 ± 1.4
	100	10	122.0 ± 10.4	177.7 ± 15.5	15.8 ± 1.5
	200	10	136.7 ± 9.2	176.7 ± 11.2	17.5 ± 2.1
Ginsenoside Re	5	10	114.0 ± 20.4*	151.3 ± 12.4*	4.7 ± 1.6**
	10	10	130.3 ± 16.6	166.7 ± 13.5	4.9 ± 1.2**
	20	10	135.3 ± 13.4	176.7 ± 18.1	5.7 ± 1.7**
Ginsana G115	50	10	110.7 ± 8.9*	154.0 ± 9.8*	15.7 ± 1.4
	100	10	139.7 ± 12.5	169.3 ± 15.5	11.0 ± 1.2*
	200	10	142.7 ± 13.3	176.7 ± 16.2	13.0 ± 1.5*

1) ***Panax ginseng*** components were previously administered orally to the test animals daily for 4 days before intoxication of thioacetamide (25 mg/kg, S.C.)
2) Serum GPT and GOT activities and zinc turbidity were determined 24 hours after intoxication of thioacetamide.
Significantly different from control, *($P<0.05$) and **($P<0.01$)

On the other hand, in the *in vivo* test of the thioacetamide-induced liver damage, among various ginseng components groups, the ginsenoside Re group indicated the suppression of the pathological elevation of s-GPT statistically. And in the lipid peroxide formation, 50% ethanol extract, water extract, ginsenoside Re, and ginsana G115 inhibited statistically. (Table III, IV).

As you can see in Table V, the presenters have been able to make the following conclusion: Ginseng components at the concentration of 1 μg/m*l.* significantly pro-

Table 3. Prophylactic behavior of *Panax ginseng* components on the transaminase activities and stability of dissociated rat hepatocytes intoxication of thioacetamide *in vitro*

Extracts[1)]	Dose (μg/ml)	No. of test	Stability increased(%)[2)]	GPT (u/l) Reitman-Frankel units	GOT (u/l) Reitman-Frankel units
Control	—	10	—	113.5± 7.5	178.3±15.2
95% EtOH ext.	1	10	12.9±1.2	104.4± 8.2	187.7±14.5
	10	10	11.4±1.5	109.4±10.6	173.3±12.2
	100	10	11.6±1.6	101.1± 9.8	165.2±11.5
	1,000	10	11.5±1.2	86.0± 7.6*	151.3±17.2
50% EtOH ext.	1	10	12.0±1.8	116.9±11.4	163.3±14.2
	10	10	11.9±1.4	119.5± 9.9*	151.6±13.6
	100	10	10.7±1.5	102.8± 9.7	140.6±12.2*
	1,000	10	10.2±1.6	101.9±11.5	150.1±13.4
Water ext.	1	10	10.8±1.2	108.8±10.8	166.3±15.2
	10	10	11.6±1.0	94.0±12.5	164.4±14.5
	100	10	12.3±1.5	108.5± 8.8	159.7±15.6
	1,000	10	10.8±1.1	101.1± 9.2	171.6±13.4
Ginsenoside Re	1	10	10.1±1.5	86.9± 8.9*	158.3±14.5
	10	10	10.7±2.2	85.8± 9.5*	142.3±11.5*
	100	10	3.3±0.2	85.3± 7.9*	146.6±12.7
	1,000	10	1.3±0.1	84.1± 8.7*	169.8±16.5
Ginsana G115	1	10	10.5±0.9	89.5± 7.6*	176.8±16.2
	10	10	11.9±0.6	75.9± 8.5*	162.4±14.4
	100	10	11.2±1.0	95.6± 9.2	145.8±11.5*
	1,000	10	5.3±0.5	92.8± 6.9	136.9±12.6**

1) Dissociated hepatocyte suspension (1×10^6/ml) and *Panax ginseng* components were previously incubated at 37°C for 30 minutes and then thioacetamide (6mg/ml) added into them and incubated for 2 hours.
2) Stability of hepatocytes were performed by trypan blue exclusion test. Significantly different from control, *($P<0.05$) and **($P<0.01$)

tected the mast cell degranulation of the guinea pig mesentery *in vitro*.

The presenters have obtained the following findings through a histological survey conducted as part of this study to measure the damaging activity of medicines to the liver cell membrane and the protective behavior of ginseng components to such damage:

The liver in the control gorup showed the necrosis of parenchymal cells around the central area, fatty change of hepatocytes around central and midzonal areas, inflammatory cell infiltration in sinusoids, and central area and cellular swelling around the panlobular area.

The preventive administration of ginseng components have, dose-dependently, improved necrosis and fatty change but not inflammatory cell infiltration in the sinusoid

Table 4. Curative behavior of ***Panax ginseng*** components on the hepatomegaly and lipid peroxidation in the thioacetamide-induced liver damage *in vivo*

Extracts[1)]	Dose (mg/kg, P.O.)	No. of rats	Body wt. increased(%)	Liver wt. (%)	$\frac{\text{Liver wt.}}{\text{Body wt.}} \times 100$	Lipid peroxide (malonedialdehyde nM/g)[2)]
Control		10	2.4±0.6	6.60±1.31	5.46±0.66	21.5±1.6
95% EtOH ext.	50	10	7.8±1.8*	6.30±1.29	5.78±0.45	26.3±1.7
	100	10	4.0±1.1*	6.01±1.18*	5.31±0.42	21.0±1.4
	200	10	3.2±0.8*	6.02±1.11*	5.59±0.55	19.0±1.2
50% EtOH ext.	50	10	7.4±1.2*	6.33±1.15	5.44±0.49	18.9±1.3
	100	10	5.2±1.4*	6.17±1.22	5.31±0.50	21.5±1.9
	200	10	4.8±0.9*	5.77±0.98*	5.18±0.47	15.0±1.2*
Water ext.	50	10	9.5±1.4*	6.50±1.45	5.75±0.51	19.2±1.6
	100	10	4.7±0.5*	6.24±1.21	6.04±0.39	16.0±1.5*
	200	10	1.5±0.2	6.03±1.15*	6.60±0.62	16.2±1.4*
Ginsenoside Re	5	10	1.8±0.2	6.51±1.30	5.45±0.49	12.2±1.5**
	10	10	1.7±0.1	6.55±1.29	5.54±0.52	9.8±0.9**
	20	10	1.0±0.3	5.70±1.10*	5.39±0.48	9.4±0.7**
Ginsana G115	50	10	2.7±0.8	5.55±1.05*	5.61±0.58	12.7±1.6**
	100	10	6.8±1.6*	6.10±1.14	6.93±0.50	11.8±1.1**
	200	10	8.0±1.5*	6.16±1.16	6.27±0.72	0.0±0.7**

1) Thioacetamide (25mg/kg) were subcutaneously administered on day 1 and 5, and ***Panax ginseng*** components were orally given from day 1 to day 5 daily for 5 days after 30 minutes of intoxication.

2) Lipid peroxides as malonedialdehyde in liver were determined 24hours after last intoxication with thioacetamide.

and central areas.

On the other hand, the curative administration of ginseng components at lower dosages have improved the pathological change of tissues. However, higher dosages of ginseng components have failed to bring about higher effects that the improved evidence observed in the tests of lower dosages.

In a final analysis, the presenters have come up with the conclusion that ginseng components, even though having differing degrees of biological activities according to their constituents, have, as a whole, preventive and curative effects to the intoxicated liver cell membranes. It is presumed that such positive effects have been attributable to their two behaviors: first is the preventive behavior to the damaging factor of the lipid peroxidation and second is the membrane modifying effect that prevents the cell degranulation. This study is the first of its kind ever conducted by the presenters in this specific area. It is a sincere hope of the authors that the ensuing studies in this new approach would be able to further validate the findings made by the authors.

Table 5. Preventive effect of Ginseng components on the degranulation of mast cell in guinea pig mesentery by compound 48/80, and venom toxin.

Extracts	Concentration (μg/ml)	No. of test	Protection of degranulation	
			Compound 48/80	Venom toxin
Control	—	10	—	—
95% EtOH ext.	1	10	25.6 ± 5.4*	25.4 ± 4.5*
	10	10	19.0 ± 3.2*	27.2 ± 6.2*
	100	10	32.4 ± 5.8*	32.1 ± 7.5*
	1,000	10	38.2 ± 4.5**	49.3 ± 4.5**
50% EtOH ext.	1	10	32.2 ± 3.7*	20.4 ± 5.5*
	10	10	45.1 ± 5.1**	25.6 ± 6.9*
	100	10	59.7 ± 4.9**	31.4 ± 5.8*
	1,000	10	62.1 ± 6.6**	39.5 ± 7.6*
Water ext.	1	10	35.1 ± 4.8*	7.8 ± 2.3
	10	10	44.2 ± 5.4**	20.6 ± 4.9*
	100	10	56.3 ± 4.9**	32.5 ± 7.2*
	1,000	10	65.2 ± 8.9**	45.4 ± 6.5**
Ginsenoside Re	1	10	14.5 ± 2.6	20.8 ± 4.1*
	10	10	19.7 ± 5.0	28.5 ± 3.9*
	100	10	25.4 ± 4.1*	39.4 ± 5.4*
	1,000	10	37.5 ± 3.5*	45.5 ± 6.5**
Ginsana G115	1	10	27.3 ± 2.9*	21.4 ± 4.0*
	10	10	35.4 ± 4.4*	29.5 ± 3.7*
	100	10	42.5 ± 3.2**	35.4 ± 5.1*
	1,000	10	52.9 ± 6.7**	39.7 ± 4.9*

1) Mast cell in mesentery incubated with Ginseng Components at the various concentration. 15 minutes after incubation compound 48/80 (1μg/ml) and venom toxin (200μg/ml) respectively added and incubated for 10 minutes at 37°C.
2) Mast cell Degranulation was assessed by measuring the percentage of cells with extruded granules (200 cells counted, 430 X magnification).
Significantly different from control, *($P<0.05$) and **($P<0.01$)

要 約

人蔘抽出物(95%, 50% 에탄올 및 水浸液) ginsenoside Re 및 ginsana G115를 供試藥物로 해서 thioacetamide로 intoxication 시킨 동물에 대한 *in vitro, in vivo* test를 통해 豫防的 效果 및 治療的인 效果에 대해서 실험하였다.

抗酸化作用에 있어서 ginsenoside Re, 水浸液, Ginsana C 115가 統計的으로 有効하였으며 zine sulfate turbidity test에서는 95% 에탄올 抽出物과 ginsenoside Re가, GOT에 대해서는 Ginsana G 115, GPT에 대해서는 ginsenoside Re가 統計的인 有意性을 나타냈다.

Venom toxin과 compound 48/80에 의한 mast cell의 脫顆粒現像에 대한 防禦試驗에서 50% 에탄올 抽出物, 水浸物, 95% 에탄올 抽出物 및 Ginsana G 115가 有効하였다.

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