PHARMACO-BIOLOGICAL ACTION OF GINSENOSIDE Rb_1, Rg_1 AND Re

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Introduction

Recently much research has been undertaken on the pharmacological efficacy of ginseng and on its other biological activities. Much of this research deals with ginseng whole extract so far. However, with the advancement of chemical investigation of respective glycosides of ginseng recently, along with separation methods of respective components of dammarane glycosides, pharmacological efficacy of these ginsenosides and their biological activities are in the process of gradual identification. High expectation is being placed particularly on, and its validity has been established, adaptogenic activity and other biological activities possessed by ginseng or ginsenosides.

A number of research have been in progress on these adaptogenic activities and anti-fatigue effects[1-3], protein anabolic effects[4-8], pharmacological functions and coordinative role on the central nervous system[9-11], pharmacological activities on ginsenoside Rg_1 and Rb_1[12], clinical findings[13-15] and analytical chemical approaches[16-23].

Among ginseng efficacies, the improvement of biochemical function to the animal liver damaged by poison, the excretion promotion of retained pigment, and histochemical repair effect on the induced pathologic change of the liver have began to be recognized. Such effects of ginseng are also recognized to be found not only in all ginseng glycosides but also in ginsenoside Rg complex. This group of matters is also recognized to possess significance in the promotion of drug metabolism of the intoxicated animals[24-25]. Besides these biological activities, the role of ginseng glycosides is also beginning to be considered of significance.

Ginseng glycosides separated by Elyakov *et al.*, which were used by I. I. Brekhman in his experiments did not cover all varieties of ginseng glycosides. Therefore, I. I. Brekhman's research and his resultant contention leave much to be reinforced when the remaining varieties of ginseng glycosides are taken into consideration. It is, therefore, consequently regarded significant to attempt comparison of pharmacological and biological activities by employing other methods of investigation of biological activities of various properties of ginseng glycosides. In this respect, from among intoxicated animals, particularly, the liver functions as an index, of course, inclusive of relative comparison between serum GPT and serum GOT, the measurement of retained BSP along with the influence on the metabolic rate of medicines. After having induced a toxic state (by administration of a lethal dose of ephedrine hydrochloride and γ-ray radiation exposure) the effect

of glycoside on the survival rate and protective activities of the test animals. As the author has gained statistically meaningful findings in these spheres and wishes to report these results.

Materials and Methods

Test materials are various kinds of ginseng glycosides.

Ginsenoside Rb_1 supplied by the Korea Ginseng Research Institute is used as the test material. Ginsenoside Rg_1 and Re have been separated, purified and identified through special methods at the author's laboratory.

Experiments

1. Biochemical survey

A) Measurement of activities of serum glutamic oxaloacetic transaminase (s-GOT) and serum glutamic pyruvic transaminase (s-GPT)

Healthy, matured white rabbits of both sexes weighing over 1.8 kg, fed for more than 7 days with the prescribed feed are used as test animals. 95% carbon tetrachloride (0.1 mg/kg) was injected intraperitoneally to the test animals. To the test group animals, treated same as the intoxicated control group, 5 mg/kg of ginsenoside Rb_1, Rg_1, and Re has been orally administered respectively three hours previously. From this control group and ginsenoside Rb_1 group, Rg_1 group and Re group, the blood was extracted 24 hours later by the heart puncture method. Carmen unit of s-GOT and s-GPT has been measured by the normal method from the extracted blood. Within 30 minutes after the extraction of blood from rabbits, serum has been centrifugally separated and the activity rate is measured by Reitman-Frankel modified method. To insure the accuracy of measuring the activity rate, serum with hemolysis de.ects is rejected from use.

B) Measuring bromosulfalein retention

The same method as 1) above is used in the selection of test and control animals, however, the animals were placed under intoxication by carbon tetrachloride and 5 mg/kg of bromosulfalein (BSP) was injected to the ear vein of the rabbits. 30 minutes later the blood was extracted and centrifugally separated at 3,000 rpm. Following the BSP test, BSP retained in the serum was measured by the Beck. Model Du Spectrophotometer at a 575 nm for the determination of retention equivalent %. The test groups were divided into the ginsenoside Rb_1, Rg_1, and Re groups. To each of these test groups, corresponding amounts of natural material was administered on the 5 mg/kg basis.

C) Histochemical survey

As the result derived from the biochemical survey is normally in parallel to the histochemical survey, the destruction of liver function was deliberately conducted for the purpose of validating the protective effect of the natural material by means of the histochemical test.

Health Sprague Dowley albino rats have been fed by the prescribed feed for more than 7 days, after which time rats weighing 100–160 g have been selected. To these animals 25 mg of thioacetamide was injected and used as the first group (control group). The test groups are divided into the ginsenoside Rb_1 group, Rg_1 group and Re group. For the classification purpose these test groups are referred to as the second group. To these test groups, which are classified as the second group, are administered both the natural test material and thioacetamide simultaneously; the third group was administered with the natural test material 3 hours prior to the injection of thioacetamide for the purpose of investigating the preventive effect of ginsenosides against thioacetamide.

2. Anti-toxic state test

A) Survival test

This experiment was conducted on the control and test groups of mice weighing from 13 to 15g.

Test groups were composed of three groups 1) the ginsenoside Rb_1, 2) the ginsenoside Rg_1, and 3) the ginsenoside Re, with 10 test mice in each group. These four groups were intoxicated with the lethal dose (390 mg/kg) of ephedrine hydrochloride by means of I.P. injection. Three hours prior to the injection of the lethal dose of ephedrine-HCl, ginsenoside Rb_1, Rg_1, and Re, 5 mg/kg each, had been orally administered to each test group respectively for the purpose of measuring the possibility of their resistance to the lethal dose and their degree of resistance was calculated as respective survival rates. The number of mice survival was counted within 24 hours following the injection of the lethal dose of ephedrine-HCl.

B) Anti-radiation test

Protective effect of three ginsenosides Rb_1, Rg_1 and Re have been studied through the γ-ray irradiation-induced increase in the capillary permeability of the rat intestine.

Albino female rats 160–270 g were portioned into five groups of 6 animals each. The rats were lightly anesthetized with 25 mg/kg of nembutal given intraperitoneally. γ-ray irradiation was carried out with Co 60 source, Picker, Co 60 Fele therapy unit, exposed to the field size of 5 $\times$ 10 cm over the abdomen from xiphisternum to pubis.

A dose of 500R was given by vertical irradiation. 0.5 ml/100 g of Evans blue (E. Merck) 1 % (wt/vol) dissolved in 0.9% (wt/vol) sodium chloride solution was I.V. injected into the test animals 30 minutes before the animals were killed.

The degree of the increase in capillary permeability was estimated by spectrophotometric determination of the Evans blue leakage in the intestinal tissue using the K. Shimomura method[26]. Ginsenosides were given by oral and I.P. route three times each. First dosing was administered orally at 40 mg/kg before 12 hours of irradiation; second, intraperitoneally the dose of 20 mg/kg after 3 hours of irradiation; and third, orally at 20 mg/kg after 6 hours of irradiation.

A graded increase in the permeability of the intestinal vessel was observed following 48 hours of γ-ray irradiation as demonstrated by the increased Evans blue content of the intestine.

C) Metabolic test

Ginsenoside Rb_1, Rg_1 and Re are supposed to expedite the metabolic rate of the artificially intoxicated animals. 5 white rabbits were used in each group subjected to this test. 10 mg/kg of aminophylline only has been injected to the control group, while 5 mg/kg of respective ginsenosides were administered to the test groups 3 hours prior to the injection of aminophylline. At the intervals of 1,2,3, and 4 hours after the injection of aminophylline, 3 ml each of blood was extracted by the heart puncture method. Extracted blood was filtered twice by 30 ml mixture of chloroform: isopropyl, alcohol (20:1), 40 ml of filtrate was again filtered through 6 ml of 0.1 N-NaOH. The resultant was then used as the agent for the measurement of the absorbance rate at 277 nm, and evaluated at the reference curve.

Results

When 95% carbon tetrachloride is administered to rabbits, their S-GOT or S-GPT values increase drastically as compared with the normal values. Carbon tetrachloride alone is dosed to the rabbits, S-GOT value increases to 351 $\pm$8.1. However, in the case of ginsenoside Rb_1, S-GOT value stands improvingly at 149.3 $\pm$ 14.6; ginsenoside Rg_1, 174.5 $\pm$ 17.2. In the case of ginsenoside Rg_1, S-GPT value registered at 270 $\pm$ 9.5 with p-value over 0.004, thus validating their efficacy. The relevant data are shown in Table 1. Results of BSP retention rate test indicated that ginsenoside Rb_1, Rg_1 and Re to be significantly effective. The retention equivalents of the control group intoxicated with 95% carbon tetrachloride registered at 83.6 $\pm$ 2.5 while Rb_1, 25.8 $\pm$ 6.8, Rg_1, 11.7 $\pm$ 1.7, and Re, 23.5 $\pm$ 1. 2; validating the exceedingly superior efficacy of these natural matters. Relative data are shown in Table 2. The experimental findings pertaining to the survival rate indicated the survival rate of 60% in the case of ginsenoside Rb_1; Rg_1, 50%; and Re,

Table 1. The effect of ginsenosides on the S-GOT and S-GPT activities of rabbits intoxicated with 95% CCl_4 at the time of 24 hours

(Karman unit)

	Control*	Rb_1**	Rg_1**	Re**
S-GOT	351.0 ± 8.5	149.3 ± 14.6	174.5 ± 17.2	165.0 ± 11.9
S-GPT	325.8 ± 8.1	252.5 ± 23.5	270.0 ± 9.5	248.0 ± 12.6
			($p < 0.004$)***	

Rb_1 : Ginsenoside-Rb_1 Rg_1 : Ginsenoside-Rg_1 Re : Ginsenoside-Re

* Only CCl_4 (0.1 ml/kg) i.p. inj.

** Ginsenoside: 5 mg/kg orally before 3 hrs from CCl_4 inj.

*** According to Mann-Whitney U test. Animal number of each groups were five.

Table 2. ESP retention equivalents of rabbit affected by ginsenoside

(Unit : %)

	$Control_1$**	Rb_1***	Rg_1	Re
ESP retention equivalent*	83.6 ± 2.5	25.8 ± 6.8	22.7 ± 1.7	23.8 ± 1.6

Rb_1 : Ginsenoside-Rb_1 Rg_1 : Ginsenoside-Rg_1 Re: Ginsenoside-Re

* Measured at the time of 48 hours after intoxicated with 95 % CCl_4 inj.

** Only CCl_4 (0.1 ml/kg) i.p. inj.

*** Glycosides: 5 mg/kg orally before 3 hours from CCl_4 inj. respectively.

Animal number of each groups were five.

Table 3. Survival rates of mice affected by ginsenoside

Group	Number of animal	Number of death	Survival* rate (%)
Rb_1**	10	4	60
Rg_1	10	5	50
Re	10	3	70
Control***	10	7	30

Rb_1 : Ginsenoside-Rb_1 Rg_1 : Ginsenoside-Rg_1 Re : Ginsenoside-Re

* Measured at the time of 24 hrs after intoxicated with ephedrine HCl (390 mg/kg: lethal dose).

** Glycosides: 5 mg/kg orally before 3 hrs from ephedrine HCl intoxication.

*** Only ephedrine HCl i.p. inj.

70%. These rates are exceedingly significant compared with the 30% survival rate of the control group. Detailed data concerning this test are indicated in Table 3.

Also in the γ-ray radiation test, the efficacy of ginsenoside Rb_1, Rg_1 and Re test groups has been validated in comparison with that of the control group.

The effect of ginsenosides on the increase in the capillary permeability of rat intestine after 48 hours γ-ray irradiation as demonstrated by the increased Evans blue contents of the intestine is:

Normal (Evans blue dose only) 107.49 ± 1.3 μg/g of evacuated fresh intestine;

Control (γ-ray 500R + Evans blue) 185.07 ± 1.6 μg/g;

Test group 1 (γ-ray + Evans blue + Rb_1 80 mg/kg) 155.12 ± 1.3 μg/g

Test group 2 (γ-ray + Evans blue + Re 80 mg/kg) 127.78 ± 0.8 μg/g

Test group 3 (γ-ray + Evans blue + Rg_1 80 mg/kg) 110.86 ± 1.6 μg/g

Therefore inhibition of the dye leakage (%) is 16.18, 30.96, 40.10, and protective activity against γ-irradiation is 0.39, 0.74, 0.96 respectively, referring with 1.00 of normal value Rg_1 indicates the strongest effect then followed by Re and Rb_1. The detailed data are shown in Table 4.

Table 4. Protective effect of ginsenosides on the increased in the capillary permeability of rat intestine after 24 hrs. of γ-irradiation

Group	Evans blue content of intestine (μg/g of Evacuated flesh intestine)	Inhibition of the dye leakage (%)	Protective activity against γ-irradiation
Normal	107.49 ± 11.9	41.92	1.00
γ-irradiation alone	185.07 ± 20.6	—	—
γ-irradiation + Ginsenoside Rb_1	155.12 ± 12.4	16.18	0.39
γ-irradiation + Ginsenoside Re	127.78 ± 18.6	30.96	0.74
γ-irradiation + Ginsenoside Rg_1	110.86 ± 12.6	40.10	0.96

Six rats were used in each group. All values are significant.

1) Ginsenosides were administered orally the dose of 40 mg/kg before 12 hours of irradiation
2) intraperitoneally the dose of 20 mg/kg after 3 hours of irradiation and
3) orally the dose of 20 mg/kg after 6 hours of irradiation.

Evans blue 1 % (wt/vol) in 0.85% (wt/vol) NaCl solution had been injected at 0.5 ml/100g (IV) 30 minutes before the animals were sacrificed.

The findings from the histochemical survey are shown in slide. When 10 mg/kg of aminophylline was injected to the control group 5 mg/kg of ginsenoside Rb_1, Rg_1, and Re had been administered to the respective ginsenoside test groups 3 hours prior to the injection of aminophylline to the control group, 805 mcg of aminophylline remained in the 100 ml of blood at one hour after the injection; two hours later, 628 mcg; 3 hours later, 520 mcg; 4 hours later, 430 mcg. However, in the cases of ginsenoside Rb_1, they were 476, 350, 309, and 250; those of Rg_1, 509, 346, 240, and 170; and Re, 490, 350, 250 and 190, thus indicating the escalation of metabolic rates in the cases of ginsenosides.

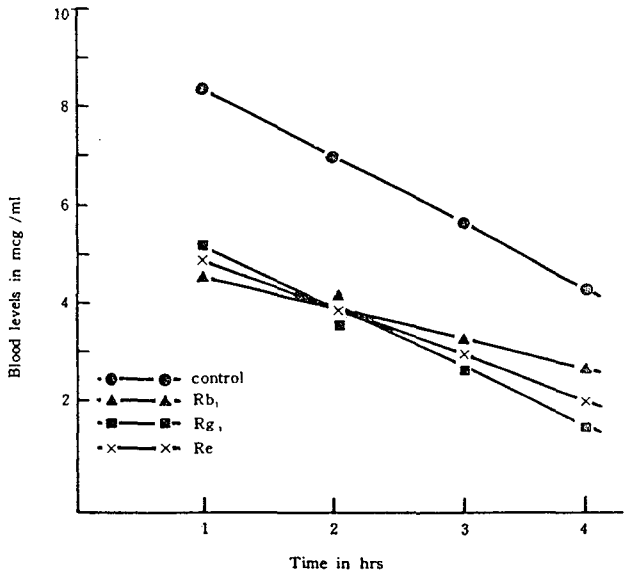

Fig. 1. Aminophylline blood level curves following oral administration of ginsenosides of 5 mg/kg dose. Control group were given 10 mg/kg of aminophylline only i.p. injection. Glycosides were given 5 mg/kg 3 hrs before aminophylline administration by i.p. injection.

Therefore, the test medicine indicates the efficacy in the speeding of the metabolism. This was remarkably extraordinary in the cases of ginsenosides Rb_1, Rg_1 and Re. These data are shown in Fig. 1.

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