



Systemic and Local Anaphylaxis is Not Induced by Korean Red Ginseng Mixture in Guinea Pigs

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Abstract

Currently, injuries to customers due to health functional foods are annually increasing. To evaluate the antigenicity of Korean red ginseng mixture (KRGGM), we tested for systemic anaphylactic shock and passive cutaneous anaphylaxis in guinea pigs. Based on a comparison of measured body weights, there were no changes in body weight for the KRGGM treatment group compared with the control group. In the ovalbumin treated group, however, there was a statistically significant decrease in body weight. For the active systemic anaphylaxis test, after the induction, there were no symptoms that suggested anaphylactic shock in the control and KRGGM treatment group. In the ovalbumin treated group, there were symptoms that suggested severe anaphylaxis, and those symptoms included restlessness, piloerection, tremor, rubbing or licking the nose, sneezing, coughing, hyperpnea, dyspnea, staggering gait, jumping, gasping and writhing, convulsion, side position and Cheyne-stokes respiration. All animals died within thirty minutes in the ovalbumin treated group. For the passive cutaneous anaphylaxis test in guinea pigs sensitized to KRGGM, each anti-serum was diluted in a stepwise manner. This was followed by an intravenous injection of a mixture of KRGGM and Evans blue. The results of the test showed that all the responses were negative in the control and the low-dose and high-dose administration groups. However, in the ovalbumin treated group, all the responses were positive. Based on the above results, there were no anaphylactic responses for up to 12 times the amount of human intake of KRGGM in Hartley Guinea-pigs. The results suggest that KRGGM is safe as measured by the systemic and local antigenicity in guinea pigs.

Key words: Korean red ginseng mixture, Active systemic anaphylaxis, Passive cutaneous anaphylaxis, Guinea pig

INTRODUCTION

The immune system protects the host against foreign substances, and its immune responses to foreign substances cause tissue damage. Thus, it has a detrimental effect on the host. In severe cases, it also produces fatal outcomes. This type of immune response is termed as hypersensitivity reactions or allergic ones. A hypersensitivity reaction is characterized by the occurrence of a response to specific antigens that the host has previously been exposed to from a specific type of organism. Hypersensitivity reactions are

generally divided into four types. These are Type I hypersensitivity reactions that occur due to the involvement of IgE antibodies, Type II and III hypersensitivity reactions that occur due to the involvement of IgG and IgM antibodies and Type IV hypersensitivity reactions that occur due to the involvement of lymphocytes (1). Of these, Type I hypersensitivity reactions are characterized by the occurrence of anaphylaxis responses due to the degranulation of mast cells, which is also associated with the occurrence of diseases such as asthma or urticaria. In particular, an anaphylactic reaction, which causes the most severe responses in an *in vivo* setting, is severe over a short duration. Because of this, vascular collapse occurs following respiratory difficulty. Without the precursor symptoms of respiratory difficulty, a shock phenomenon may also occur. In addition, there are also dermatologic symptoms in which have no relation to the concurrent presence of vascular edema. This is the typical finding of systemic anaphylaxis (2-4). On the other hand, the secretion of chemical mediators from mast cells may also be induced when antigens bind

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to specific receptors on the cell surface after interacting with IgEs. This anti-IgE antibody is a typical *in vivo* model that induces passive cutaneous anaphylaxis (PCA) (5,6).

To date, many studies have reported the effects of ginseng on immune modulation and allergies. Ginseng saponin stimulates and suppresses factors associated with the immune response; thus, it regulates the immune system (7,8). Ginseng saponin has been reported to have a role in suppressing pro-inflammatory cytokines (9-11) and increasing the activation of Th cells, NK cells and macrophages (12-14). In addition, ginseng saponin suppresses the release of histamines and leukotrienes from peritoneal mast cells; thus, it has an anti-allergic effect (15,16). It has also been reported to suppress the PCA responses in rodents (16-18). Caruso *et al.* (19) provided a diet containing red ginseng to patients with an air allergy and reported that the factors associated with the allergy were reduced.

Park *et al.* (18) and Bae *et al.* (20) confirmed that the PCA responses were suppressed by the ginsenoside Rg3, Rf and Rh2 in an animal model using mice. Accordingly, in the current study, the effects of Korean red ginseng mixture, a type of health functional food, were evaluated for its autogenous PCA reaction, which is induced by systemic anaphylaxis, and anti-IgE antibodies using guinea pigs.

Currently, injuries to customers due to health functional foods are annually increasing. The number of side effects of health functional foods, which have been reported to the consultation center of the Consumers Union of Korea, was 1,733 in 2014, 502 in 2015 and 696 in 2016. These results indicate that it is annually increasing. In addition, most of the products causing side effects were found to be those for which there was no KFDA (Korea Food & Drug Administration) approval for health functional foods. This is because the distribution and safety of health functional foods remain problematic although their sales and consumption are increased due to an increased mean life span and improvement in quality of life. Moreover, regarding the KFDA regulatory system for health functional foods, there are no regulations against other mixed constituents; only the active constituents are of primary concern. The resulting side effects have not been demonstrated to an appropriate extent. In the product that was used in the current study, there are mixed constituents including red ginseng, deer antlers, Korean angelica, calcium and vitamins.

Given the above background, we conducted this study to examine whether the current product would trigger the occurrence of allergic symptoms from immune responses. Thus, we attempted to obtain safety data based on scientific evidence.

MATERIALS AND METHODS

Materials. This experiment was performed using 6-year-old *Panax ginseng* C. A. Meyer. The red ginseng

manufacturing process was registered with the International Organization for Standardization (ISO) and internationally certified in April 2017 (ISO 19610). This extract was concentrated at 50~60°C until 70~73 degrees Brix were reached. Red ginseng water extract was a blackish brown viscous liquid with approximately 36% moisture, pH of 4.6 or below, and 70~72 degrees Brix, with water-insoluble materials amounting to 2% or below. The samples used in this study were Korean red ginseng mixture (KRGM) containing 7.5% red ginseng extracts (Rg1 + Rb1 + Rg3 = 5.5 mg/kg) and less than 1% each of deer antlers and Korean angelica. The analytical method for each component was as follows. Ginsenoside standards (Rg1, Rb1, Rg3) were purchased from Chromadex Co. (Irvine, CA, USA) for ginsenoside analysis. 2 g of red ginseng extract were weighed in a beaker, and 25 mL of deionized water was added. After sitting at room temperature for 1 hr, the diluted sample was transferred in a 50 mL volumetric flask where the volume was brought up to 50 mL by adding MeOH. Extraction was performed in an ultrasonic cleaner (60 Hz, Wiseclean, Busan, Korea) for 30 min. Then, the solution was filtered (0.2 µM, Acrodisc, St. Louis, MO, USA) and injected into the HPLC system (Waters, Milford, MA, USA). 7-Ketocholesterol (Sigma, St. Louis, MO, USA) was used as a standard for deer antler analysis. Approximately 0.5 g of deer antlers was precisely weighed and 30 mL of a chloroform : methanol (2 : 1) solution was added and extracted with ultrasonic cleaner (Wiseclean). Then centrifuged and extracted two more times. After concentration, the filtrate was resuspended in 2 mL, filtered through a 0.2 µM (syringe filter, Sigma-Aldrich, St. Louis, MO, USA), and analyzed by UPLC/ UVD (Acquity UPLC system, Waters, Milford, MA, USA). Decursin (Chromadex Co.) was used as a standard for Korean angelica analysis. 1 g of Korean angelica was dissolved in the saponin buffer solution, and the extract was loaded onto a C18 cartridge activated with MeOH and H₂O. After washing the cartridge with H₂O, the washed cartridges were eluted with MeOH and analyzed by HPLC after syringe filter (0.2 µM, Nylon; Sigma-Aldrich) was applied to the flask.

Experimental animals. Five-week-old male Hartley Guinea-pigs were purchased from the Samtako Bio Korea (Gyeonggi-do, Korea) and acclimatized to the facility for at least 1 week before the experiment. Rodents were housed individually in stainless steel wire-mesh cages or group housed in accordance with the individual laboratory Institutional Animal Care and Use Committee (IACUC) requirements and provided food and tap water ad libitum and kept at a constant temperature 22 ± 2°C, the relative humidity 55 ± 5%, the frequency of ventilation 10~15 times/hr, the light/dark cycle 12 hr and the illumination 150~300 Lux.

The determination of administration dose. During the sensitized administration, the concentration of experimental substance was set at three times of the amount of human intake in the low-dose administration group. In the high-dose administration group, it was set at 12 times of the amount of human intake. During the induced administration, the dose of experimental materials was set at the maximal concentration at which it could be dissolved in the solution.

The examination of general symptoms. The general symptoms were examined more than once a day in all the experimental animals.

The measurement of body weight. The body weight was measured once a week in dividing the experimental animals into the groups and prior to and following the initiation of the administration of the experimental materials.

Active systemic anaphylaxis (ASA) test. Guinea pigs were divided into four groups comprising five animals each. The negative control (Control) and positive control (OVA) groups were orally administered saline for 2 weeks. KRGM was orally administered at 500 and 1,000 mg/kg for 2 weeks. In the OVA group, the OVA was mixed with an immune supplement, Freund's Complete Adjuvant (FCA, Sigma), at the same amount. This was followed by the sensitized administration a total of three times via a subcutaneous route at a 6 day interval. In the control group and the KRGM administration group, a saline was subcutaneously administered. Following an 18 day resting period after the final sensitized administration, the causative antigen (KRGM 500 mg/kg) was administered in the vein of lower extremities. After 30 min, the occurrence of anaphylactic shock was examined.

Homologous passive cutaneous anaphylaxis (PCA) test. Following an 18 day resting period after the final sensitized administration, the anti-serum was obtained from guinea pigs for the ASA experiment. The anti-serum was isolated from each species and then diluted at a sequential 2-fold dilution ratio until the appropriate ratios from $\times 10$ ($\times 10$, 20, 40, 80, 160, 320, 640, 1280, 2560 and 5120). Then, the anti-serum was intradermally administered to two guinea pigs at a volume of 50 μ L. After four hours following the intradermal administration, a mixture of the causative antigen (KRGM 500 mg/kg) and a 2% Evans blue (Sigma) (1 : 1) was administered to the vein of lower extremities at a volume of 0.5 mL. Thirty minutes later, guinea pigs were euthanized. Following the extraction of dorsal skin, the blue spot was confirmed.

Determination of the experimental results.

• **Active systemic anaphylaxis:** 18 day later since the final date of sensitization (on 35 day following the

initial administration), the experimental substance was administered to the vein. Thus, the occurrence of anaphylactic shock was examined. The presence of anaphylaxis was evaluated based on the severity of shock by examining the Restlessness, Piloerection, Tremor, Rubbing or licking nose, Sneezing, Coughing, Hyperpnea, Urination, Evacuation, Lacrimation, Dyspnea, Rhonchus, Cyanosis, Staggering gait, Jumping, Gasping and writhing, Convulsion, Side position, Cheyne-Stokes respiration and Death. The determination was made based on the following four criteria: negative (–) is referred to as cases in which no symptoms were observed; mild (\pm) is referred to as cases in which the restlessness, piloerection, tremor and rubbing or licking the nose were observed; moderate (+) is referred to as cases in which sneezing, coughing, hyperpnea, urination, evacuation and lacrimation were observed; severe (++) is referred to as cases in which dyspnea, rhonchus, cyanosis, staggering gait, jumping, gasping and writhing, convulsion, side position and Cheyne-Stokes respiration were observed; and the death (+++).

• **Passive cutaneous anaphylaxis:** Any cases in which the diameter of blue spot appearing at the sites of injection [(long diameter + short diameter)/2] exceeded 5 mm were determined to be positive. The final coefficient of the dilution of serum (the maximal dilution ratio) was determined to be the final titer (the antibody titer), based on which it was determined that the IgE associated with the anaphylaxis was formed (21).

Statistical analysis. Results are expressed as the mean \pm SD. Statistical analysis was performed using SAS Program (version 9.2, SAS Cary, NC, USA). Fisher's exact test was used for comparison of the negative control group to the test substance groups or the positive control group (significance level: 0.05).

Table 1. Mortality and clinical signs in guinea pigs treated orally with Korean red ginseng mixture during experiment periods

Group/ Dose (mg/kg)	Dose (mg/kg)	No.	Signs	Mortality (dead/total)
Control	Saline	5	NAD	0% (0/5)*
KRGM 500	KRGM (500 mg/kg)	5	NAD	0% (0/5)
KRGM 2000	KRGM (2,000 mg/kg)	5	NAD	0% (0/5)
OVA	OVA + FCA (5 mg/kg)	5	NAD	0% (0/5)

*No. of dead animals/No. of tested animals.

No, Number of Animal; NAD, No Abnormality Detected; KRGM, Korean red ginseng mixture; OVA, ovalbumin; FCA, Freund's complete adjuvant.

RESULTS

General symptoms and the mortality. There were no general symptoms due to the administration during the sensitization period where the KRGM was administered (Table 1).

Changes in the body weight. Following the measurement of body weight, there were no changes in the body weight in the KRGM treatment group as compared with the negative control group. In the positive control group, however, there was a statistically significant decrease in the body weight (Table 2).

Active systemic anaphylaxis. Following the induction, there were no symptoms that are suggestive of anaphylactic shock in the negative control group, in the low-dose administration group (500 mg/kg) and the high-dose administration group (2,000 mg/kg). In the positive control group, there were findings that are suggestive of severe anaphylactic shock and these include restlessness, piloerection, tremor, rubbing or licking the nose, sneezing, coughing, hyperpnea, dyspnea, staggering gait, jumping, gasping and writhing, convulsion, side position and Cheyne-stokes respiration. In the positive control group, 2 out of 5 animals died within 10 min, and the remaining animals died within 30 min (Table 3).

Passive cutaneous anaphylaxis. After an intradermal injection following a stepwise dilution of the anti-serum which was obtained from guinea pigs which were sensitized using the KRGM, followed by an intravenous injection of a mixture of the antigen with Evans blue solution, there were negative responses in the negative control group, the low-dose KRGM treatment group (500 mg/kg) and the low-dose treatment group (2,000 mg/kg). There were all the positive responses in the positive control group. The antibody titer was 5,120 times (Table 4).

DISCUSSION

To date, various pharmacological effects of red ginseng have been shown based on scientific evidence. Ongoing studies even now are currently underway evaluating the pharmacological effects of red ginseng. However, there are few studies on its anti-allergic effects. According to studies that have been done up to now, red ginseng has been reported to have an anti-allergic effect (18-20). However, there are also controversial reports on the anti-allergic effect (22). Accordingly, in the current study, guinea pigs were orally administered KRGM to examine its antigenicity. Thus, to determine whether this leads to the formation of IgEs which induce anaphylactic shock and passive cutaneous anaphylaxis (PCA), autogenous passive cutaneous anaphylaxis was induced in an animal model using

Table 2. Body weights of guinea pigs during experiment periods

Group/ Dose (mg/kg)	Body weight (g)						
	1	6	12	16	21	28	35
Control	376.2 ± 6.38	440.6 ± 15.95	480.2 ± 18.19	535.0 ± 28.71	566.8 ± 27.50	610.8 ± 31.49	655.0 ± 39.27
KRGM 500	374.6 ± 13.13	427.8 ± 15.35	472.2 ± 17.25	513.8 ± 16.90	543.4 ± 14.05	584.6 ± 12.58	626.2 ± 15.75
KRGM 2000	373.8 ± 11.03	423.4 ± 14.55	479.6 ± 20.28	521.2 ± 26.57	544.0 ± 28.89	576.2 ± 34.16	608.2 ± 40.65
OVA	363.8 ± 8.41	417.0 ± 8.43*	461.8 ± 9.63	500.4 ± 11.61	525.2 ± 12.76**	550.6 ± 19.14**	577.7 ± 43.14*

Each value represents the mean ± SD.

* and ** indicate the weight significantly different from controls at $p < 0.05$ and $p < 0.01$, respectively.

KRGM, Korean red ginseng mixture; OVA, ovalbumin.

Table 3. Results of the active systemic anaphylaxis in guinea pigs

Group/ Dose (mg/kg)	Sensitizing antigen	Challenging antigen	No. of animals	Severity of anaphylaxis					Positive ratio
				[-]	[±]	[+]	[++]	[+++]	
Control	Saline	Saline	5	5	-	-	-	-	0/5
KRGM 500	KRGM (500 mg/kg)	KRGM (500 mg/kg)	5	5	-	-	-	-	0/5
KRGM 2000	KRGM (2,000 mg/kg)	KRGM (500 mg/kg)	5	5	-	-	-	-	0/5
OVA	OVA + FCA	OVA (5 mg/kg)	5	-	-	-	-	5	5/5*

KRGM, Korean red ginseng mixture; OVA, ovalbumin; FCA, Freund's complete adjuvant.

[-], Asymptomatic; [±], Mild; [+], Moderate; [++], Severe; [+++], Death.

* indicates significantly different from controls at $p < 0.05$.

Table 4. Results of the homologous passive cutaneous anaphylaxis test

Sensitized antigen (50 µL, id)	Challenged antigen (0.5 mL, iv)	No. of animals Guinea pig	Dilution of antiserum in sensitized guinea pigs										
			10	20	40	80	160	320	640	1,280	2,560	5,120	Saline
Guinea pig anti-saline	Saline	1	-	-	-	-	-	-	-	-	-	-	-
		2	-	-	-	-	-	-	-	-	-	-	-
		3	-	-	-	-	-	-	-	-	-	-	-
		4	-	-	-	-	-	-	-	-	-	-	-
	+ Evans blue (1%)	5	-	-	-	-	-	-	-	-	-	-	-
		6	-	-	-	-	-	-	-	-	-	-	-
		7	-	-	-	-	-	-	-	-	-	-	-
		8	-	-	-	-	-	-	-	-	-	-	-
		9	-	-	-	-	-	-	-	-	-	-	-
		10	-	-	-	-	-	-	-	-	-	-	-
Guinea pig anti-KRGM (500 mg/kg)	KRGM (500 mg/kg)	1	-	-	-	-	-	-	-	-	-	-	
		2	-	-	-	-	-	-	-	-	-	-	
		3	-	-	-	-	-	-	-	-	-	-	
		4	-	-	-	-	-	-	-	-	-	-	
	+ Evans blue (1%)	5	-	-	-	-	-	-	-	-	-	-	
		6	-	-	-	-	-	-	-	-	-	-	
		7	-	-	-	-	-	-	-	-	-	-	
		8	-	-	-	-	-	-	-	-	-	-	
		9	-	-	-	-	-	-	-	-	-	-	
		10	-	-	-	-	-	-	-	-	-	-	
Guinea pig anti-KRGM (2,000 mg/kg)	KRGM (500 mg/kg)	1	-	-	-	-	-	-	-	-	-	-	
		2	-	-	-	-	-	-	-	-	-	-	
		3	-	-	-	-	-	-	-	-	-	-	
		4	-	-	-	-	-	-	-	-	-	-	
	+ Evans blue (1%)	5	-	-	-	-	-	-	-	-	-	-	
		6	-	-	-	-	-	-	-	-	-	-	
		7	-	-	-	-	-	-	-	-	-	-	
		8	-	-	-	-	-	-	-	-	-	-	
		9	-	-	-	-	-	-	-	-	-	-	
		10	-	-	-	-	-	-	-	-	-	-	
Guinea pig anti-OVA (5 mg/kg)	OVA (5 mg/kg)	1	-	-	-	-	-	-	-	-	-	-	
		2	-	-	-	-	-	-	-	-	-	-	
		3	-	-	-	-	-	-	-	-	-	-	
		4	-	-	-	-	-	-	-	-	-	-	
	+ Evans blue (1%)	5	-	-	-	-	-	-	-	-	-	-	
		6	-	-	-	-	-	-	-	-	-	-	
		7	-	-	-	-	-	-	-	-	-	-	
		8	-	-	-	-	-	-	-	-	-	-	
		9	-	-	-	-	-	-	-	-	-	-	
		10	-	-	-	-	-	-	-	-	-	-	

Id, intradermal injection; iv, intravenous injection; KRGM, Korean red ginseng mixture; OVA, ovalbumin.
[-], less than 5 mm of PCA titer; [+], more 5 mm of PCA titer.

guinea pigs (23). To confirm whether guinea pigs present with specific symptoms from the administration of KRGM, the general symptoms and mortality were examined during

the study period. The results showed that there were no abnormal symptoms due to the administration of KRGM in all the experimental animals. Based on the measured

body weights, there were no changes in body weight in the KRGM treatment group compared with the negative control group. In the positive control group, however, there was a statistically significant decrease in the body weight. These results indicate that body weight decreased because of the allergic responses to ovalbumin and immune supplements. Male guinea pigs were orally given KRGM at a concentration of 3 times or 12 times higher the amount of human intake. Thus, the experimental animals were sensitized. Following an 18 day rest period, KRGM 500 mg/kg/b.w. were administered to the vein of the lower extremities. The results showed that there were no reactions that suggested anaphylaxis in the negative and KRGM treatment groups. However, in the positive control group in which OVA was administered, the experimental animals died within 30 min. Of these, one experimental animal presented with findings that suggested severe anaphylactic shock (restlessness, piloerection, tremor, rubbing or licking the nose, sneezing, coughing, hyperpnea, dyspnea, staggering gait, jumping, gasping and writhing, convulsion, side position and Cheyne-stokes respiration). However, these symptoms were resolved over time.

In the PCA test in the guinea pigs, each anti-serum was diluted in a stepwise manner and then intradermally injected at a volume of 50 μ L. Four hours later, this was followed by an intravenous injection of a mixture of antigens (KRGM 500 mg/kg/b.w.) with 2% Evans blue at a ratio of 1 : 1. Thirty minutes later, the skin was dissected. Thus, the blue spots were confirmed to be present at the sites of injection. In all the experimental animals in the negative control and KRGM treatment groups, there were no blue spots. In the positive control group in which OVA was administered, the anti-serum was found to be positive when the dilution was done at 5120 folds. It is therefore presumed that not only does KRGM cause no anaphylactic shock at a concentration of 3 and 12 times the amount of human intake but also that it does not form IgEs, which are biocompatible immunoglobulins. The repeated oral administration of either 50~2,000 mg/kg of red ginseng water extract or 500, 1,000, or 2,000 mg/kg of red ginseng extract to female and male mice or rats for 28 day did not lead to: animal deaths or abnormalities in general symptoms caused by the administration of the test specimens during the test period; significant differences in body weight changes, feed/water intake, biochemical blood tests, or organ weight measurements; or unusual findings in the results of gross autopsies (24,25). In doses of 20~2,000 mg/kg, red ginseng water extract was orally administered every day to male mice for 63 day before mating, to female mice from 14 day before mating to the last stage of pregnancy, and to female mice undergoing combined fecundity and maternal function tests from 2 weeks before cohabitation to pregnancy, delivery, and lactation. When generations F0 and F1 were observed for clinical symp-

toms, body weight changes, water/feed intake, estrus, sex hormones in the blood, and organ weight, there were no unusual changes, and the production, motility, and denaturation rates of spermatozoa as well as the number of spermatozoa in the epididymides did not change (26). In conclusion, KRGM, the main ingredient of red ginseng extracts does not induce systemic and local anaphylaxis in guinea pigs indicating that KRGM is safe as measured by its antigenicity in guinea pigs.

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