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Antigenicity Studies of the Aqueous Extract of Fresh Ginseng in Guinea Pigs

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Abstract—Aqueous extract of fresh ginseng (AFG) was examined for the antigenicity in Hartley guinea pigs in comparision with ovalbumin (OVA). When guinea pigs were sensitized with AFG emulsified with complete Freund's adjuvant (CFA), these animals showed negative reactions in active systemic anaphylaxis (ASA), active cutaneous anaphylaxis (ACA) and passive cutaneous anaphylaxis (PCA) tests and passive hemagglutination (PHA) reaction. On the contrary, when guinea pigs were sensitized with OVA emulsified with CFA as positive controls, these animals disclosed positive reactions in ASA, ACA and PCA tests and PHA reaction. From these results, AFG was considered not to possess antigenic properties in guinea pigs. In addition, the dose levels of AFG empolyed in the present experiment were confirmed not to suppress immune reactions.

Keywords—*Panax ginseng* • Araliaceae • antigenicity • active systemic anaphylaxis • active cutaneous anaphylaxis • passive cutaneous anaphylaxis • passive hemagglutination • guinea pig

Ginseng, *Panax ginseng* C.A. Meyer, is a traditional herbal medicine which has been widely used in the treatment of various pathologic states including general complaints such as fatigue, chilly constitution and anorexia. The medicinal value of ginseng has now been clarified by laboratory studies showing a wide spectrum of biological effects including sedative and nerve calming effect, tonic effect for visceral and neural functions and hypoglycemic effect; it also has a property to promote immune function[1~4].

Although a large number of researchers have described the medicinal basis and application of ginseng, only a few have been interested in the safety research of ginseng[5,6]. Moreover, there were several publications affirming that ginseng could be toxic and have harmful effects on the organism[7~10], even though ginseng has been described as a safe medicine in the Oriental medicine literatures. Therefore, safety assessment on the potential toxic actions or adverse side effects of ginseng need to be carried out to eliminate any doubt still in existence.

The present study was carried out to evaluate the antigenicity of the aqueous extract of fresh ginseng (AFG), the most commonly used dosage form for herbal medicine, in guinea pigs.

Experimental Methods

Materials—Fresh ginseng (6-year-old root from Jungpyong Experimental Station, Korea Ginseng & Tobacco Research Institute) was used. AFG used as a test substance was obtained by refluxing the root of fresh ginseng with distilled water at 80°C for 3 hr, 4 times. The pooled infusion was filtered and then concentrated under reduced pressure at 60±1°C. The yield of AFG was 11% for the weight of the root. The moisture content of AFG was 35%. Ovalbumin (OVA, Lot No. 51 H 7175, Sigma Chemical Co.) was used as a positive control, and physiological saline was used as a negative control. Complete Freund's adjuvant (CFA, Difco Lab.) was used as an adjuvant. All other chemicals were of analytical grade.

Animals and environmental conditions—Male Hartley guinea pigs, 5-week-old, were from Samyuk Experimental Animal Breeding Center (Osan, Kyunggi Do, Korea). They were used after 2 weeks of acclimation. The animals were kept in a 12 hr light/dark cycle animal room. The room temperature was maintained at 23±3°C with a relative humidity of 60±10%. The animals were allowed free access to laboratory chow (Purina Korea Co.) and tap water supplemented with vitamin C (1 g/l).

Sensitization of animals—AFG and OVA were dissolved in physiological saline. In AFG sensitized groups, guinea pigs were administered orally or subcutaneously with 11 mg/kg and 110 mg/kg of AFG. Sensitization were repeated 9 times at intervals of every other day. For the sensitization with test and control substances and CFA, solutions of AFG, OVA and saline were emulsified with an equal volume of CFA before subcutaneous administration, respectively. In these groups, sensitization were repeated 3 times once in 3 weeks (Table I). All animals were administered in a volume of 1 ml/kg body weight.

Active systemic anaphylaxis (ASA) test in guinea pigs—At 2 weeks after the final sensitization, AFG (11 mg/kg) or OVA (1.67 mg/kg) was injected intravenously into the leg vein of the animals in a volume of 1 ml/kg body weight. Clinical signs of anaphylaxis were observed for 30 min and evaluated according to the modified method of Kouchi and collegues[11]: [−], asymptoms; [±], urination, evacuation; [+], coughing, sneezing; [#], piloerection, nostril discharge, lacrimation, salivation, nasal bleeding, convulsion, dyspnea, staggering gait, rhonchus, cyanosis, side position, flattening; [##], death.

Active cutaneous anaphylaxis (ACA) test in guinea pigs—At 2 weeks after the final sensitization, the challenge antigen was intradermally injected into the shaved backs of the

Table I. Sensitization schedule of guinea pigs

Group	Sensitization antigen	Dose	Route	No. of administration	No. of animals
Gp-Ⅰ	AFG	11 mg/kg	p.o.	9	10
Gp-Ⅱ	AFG	11 mg/kg	s.c.	9	10
Gp-Ⅲ	AFG	110 mg/kg	p.o.	9	10
Gp-Ⅳ	AFG	110 mg/kg	s.c.	9	10
Gp-Ⅴ	AFG+CFA	110 mg/kg	s.c.	3	10
Gp-Ⅵ	OVA+CFA	2.5 mg/kg	s.c.	3	10
Gp-Ⅶ	Saline+CFA	1 ml/kg	s.c.	3	10

guinea pigs. The volume administered was 0.1 ml per site. At 2, 4, 8, 24 and 48 hr after challenge, the degree of the changes at the serum-injected site was observed for the occurrence of positive skin reactions such as papula, hemorrhage, redness, induration and necrosis[12].

Homologous passive cutaneous anaphylaxis (PCA) test in guinea gigs—At 12 days after the final sensitization, blood was collected from the retro-orbital venous plexus of the animals under ether anesthesia, and sera were obtained from blood and stored frozen at −80°C. Each 0.05 ml of the guinea pig serum diluted from 10- to 5120-fold with saline was injected intradermally into the shaved backs of the non-treated guinea pigs. At 4 hr after the intradermal passive sensitization with sera, 1 : 1 mixture of AFG (11 mg/kg) or OVA (1.67 mg/kg) solution and a 1% solution of Evans blue dye was injected intravenously into the leg vein in a volume of 1 ml/kg body wight. Thirty min later, the guinea pigs were bled to death and the extent of leakage of the dye at the serum-injected site was examined to determine the PCA titer. The PCA reaction was judged as positive by a mean diameter of 5 mm or more[13].

Passive hemagglutination (PHA) reaction—The sensitized sera obtained from blood collected on 12 days after the final sensitization were inactivated at 56°C for 30 min, and were diluted from 2^0 to 2^{11} with a microtiter method. Sheep red blood cell (SRBC) suspensions (Korea Media Co.) washed with phosphate buffered saline (PBS) were treated with tannic acid at 37°C for 15 min. SRBC sediments were prepared by washing tanned SRBC with PBS. Each antigen solution (10 ml) was challenged by adding slowly 0.4 ml of SRBC sediments and 1.2 ml of 2.5% glutaraldehyde, and the mixture was stirred for approximately 1 hr at 37°C. After washing coated erythrocyte with PBS, the coated erythrocytes were suspended in PBS containing 1% normal rabbit serum to obtain a 1.5% erythrocyte concentration. To each original or diluted serum solution (25 μl) the coated erythrocyte suspension (25 μl) were added, and the mixture was well mixed. The mixture was then incubated at 37°C for 16 hr. Thereafter, the hemagglutination condition was examined at the bottom of the plate[14].

Immunosuppressive effect—To confirm whether the dose levels of AFG employed in the present experiment suppress immune reactions or not, the animals were sensitized by subcutaneous administration of a mixture of OVA (2.5 mg/kg) and CFA or a mixture of OVA, AFG and CFA 3 times once in 3 weeks. At 12 days after the final sensitization, the sera were obtained from the sensitized guinea pigs. The PCA titer of the serum sensitized with OVA after OVA challenge was compared with that of the serum sensitized with OVA and AFG[12].

Results and Discussion

The antigenicity of AFG was evaluated using the following type I hypersensitivity assay systems: ASA and ACA tests in guinea pigs, PCA test in guinea pigs with sera of sensitized guinea pigs and PHA reaction in guinea pigs.

ASA test in guinea pigs—In the AFG sensitized groups by challenging with AFG, urination or evacuation was observed in some animals. The same symptoms, however, were also observed in the negative control group and non-sensitized group. These clinical signs, therefore, are considered to be negative reactions. In contrast to the animals challenged with AFG, all animals in the group challenged with OVA showed anaphylactic signs which were characterized by coughing, sneezing, piloerection, nostril discharge, lacrimation, nasal bleeding, convulsion, staggering gait, rhonchus and side position

Table II. Active systemic anaphylaxis test in guinea pigs

Group	Sensitization antigen	Challenge antigen	No. of animals	Severity of anaphylaxis[a]				
				[−]	[±]	[+]	[++]	[+++]
Gp-Ⅰ	AFG (11 mg/kg, p.o.)	AFG (11 mg/kg)	5	3	2	0	0	0
GP-Ⅱ	AFG (11 mg/kg, s.c.)	AFG (11 mg/kg)	5	4	1	0	0	0
Gp-Ⅲ	AFG (110 mg/kg, p.o.)	AFG (11 mg/kg)	5	3	2	0	0	0
Gp-Ⅳ	AFG (110 mg/kg, s.c.)	AFG (11 mg/kg)	5	2	3	0	0	0
Gp-Ⅴ	AFG+CFA (110 mg/kg)	AFG (11 mg/kg)	5	3	2	0	0	0
Gp-Ⅵ	OVA+CFA (2.5 mg/kg)	OVA (1.67 mg/kg)	5	0	0	0	5	0
Gp-Ⅶ	Saline+CFA (1 ml/kg)	AFG (11 mg/kg)	5	2	3	0	0	0
Gp-Ⅷ	-[b]	AFG (11 mg/kg)	5	4	1	0	0	0

[a] Severity of anaphylaxis was evaluated as described under Experimental Methods.
[b] Not sensitized.

Table III. Active cutaneous anaphylaxis test in guinea pigs

Group	Sensitization antigen	Challenge antigen	Findings[a]				
			2	4	8	24	48 hr
Gp-Ⅰ	AFG (11 mg/kg, p.o.)	AFG (11 mg/kg)	0	0	0	0	0
Gp-Ⅱ	AFG (11 mg/kg, s.c.)	AFG (11 mg/kg)	0	0	0	0	0
Gp-Ⅲ	AFG (110 mg/kg, p.o.)	AFG (11 mg/kg)	0	0	0	0	0
Gp-Ⅳ	AFG (110 mg/kg, s.c.)	AFG (11 mg/kg)	0	0	0	0	0
Gp-Ⅴ	AFG+CFA (110 mg/kg)	AFG (11 mg/kg)	0	0	0	0	0
Gp-Ⅵ	OVA+CFA (2.5 mg/kg)	OVA (1.67 mg/kg)	Ⅱ (5/5)[b]	Ⅱ (5/5)	Ⅱ (5/5) Ⅲ (5/5)	Ⅱ (5/5) Ⅲ (5/5)	Ⅲ (5/5) Ⅳ (5/5) Ⅴ (4/5)
Gp-Ⅶ	Saline+CFA (1 ml/kg)	AFG (11 mg/kg)	0	0	0	0	0
Gp-Ⅷ	-[c]	AFG (11 mg/kg)	0	0	0	0	0

[a] Findings are classified as follows: 0, asymptoms; I, papula; II, hemorrhage; III, redness; IV, induration; and V, necrosis.
[b] These parentheses mean no. of animals showing the finding/no. of animals tested.
[c] Not sensitized.

(Table II).

ACA test in guinea pigs—In the AFG sensitized groups, no skin reaction was observed in any animal at any observation time following challenge with AFG. In the OVA sensitized groups, hemorrhage of OVA-injected site was observed in all animals from 2 hr observation time after OVA challenge and redness from

8 hr observation time. Induration was observed in all animals at 48 hr after OVA challenge and necrosis in 4 out of 5 animals (Table III).

Homologous PCA test in guinea pigs—In the AFG sensitized groups, no blue region due to extravasation of Evans blue initiated by interaction of antigen and antibody was observed at any serum-injected site after AFG challenge. Specific antibodies were not detected in 10-fold dilution of original sera. The PCA titer was therefore less than 10 in all the AFG sensitized sera. On the contrary, in group sensitized with OVA emulsified with CFA, antibodies were detected in all 5 sera with PCA titer ranging from 1280 to 2560 (Table IV).

PHA reaction—When the AFG sensitized

Table IV. Passive cutaneous anaphylaxis test in guinea pigs with sera of sensitized guinea pigs

Group	Sensitization antigen	Challenge antigen	Positive ratio[a]	PCA titer[b]
Gp-I	AFG 11 mg/kg (p.o.)	AFG 11 mg/kg	0/5	<10
Gp-II	AFG 11 mg/kg (s.c.)	AFG 11 mg/kg	0/5	<10
Gp-III	AFG 110 mg/kg (p.o.)	AFG 11 mg/kg	0/5	<10
Gp-IV	AFG 110 mg/kg (s.c.)	AFG 11 mg/kg	0/5	<10
Gp-V	AFG 110 mg/kg+CFA	AFG 11 mg/kg	0/5	<10
Gp-VI	OVA 2.5 mg/kg+CFA	OVA 1.67 mg/kg	5/5	1280~2560
Gp-VII	Saline+CFA	AFG 11 mg/kg	0/5	<10
Gp-VIII	-[c]	AFG 11 mg/kg	0.5	<10

[a] Positive ratio is no. of sera showing positive reaction/no. of sera tested.
[b] PCA titer represents the maximum dilution factor of original serum showing positive reaction.
[c] Not sensitized.

Table V. Passive hemagglutination reaction in guinea pigs

Group	Sensitization antigen	Challenge antigen	Positive ratio[a]	PHA titer[b]
Gp-I	AFG 11 mg/kg (p.o.)	AFG 11 mg/kg	0/5	<1
Gp-II	AFG 11 mg/kg (s.c.)	AFG 11 mg/kg	0/5	<1
Gp-III	AFG 110 mg/kg (p.o.)	AFG 11 mg/kg	0/5	<1
Gp-IV	AFG 110 mg/kg (s.c.)	AFG 11 mg/kg	0/5	<1
Gp-V	AFG 110 mg/kg+CFA	AFG 11 mg/kg	0/5	<1
Gp-VI	OVA 2.5 mg/kg+CFA	OVA 1.67 mg/kg	5/5	2^6~2^8
Gp-VII	Saline+CFA	AFG 11 mg/kg	0/5	<1
Gp-VIII	-[c]	AFG 11 mg//kg	0/5	<1

[a] Positive ratio is no. of sera showing positive reaction/no. of sera tested.
[b] PHA titer represents the maximum dilution factor of original serum showing positive reaction.
[c] Not sensitized.

Table VI. Immunosuppressive effect of aqueous extract of fresh ginseng on homologous passive cutaneous anaphylaxis test in guinea pigs

Group	Sensitization antigen	Challenge antigen	Positive ratio[a]	PCA titer[b]
Gp-I	OVA 2.5 mg/kg	OVA 1.67 mg/kg	5/5	1280~2560
Gp-II	OVA 2.5 mg/kg +AFG 110 mg/kg	OVA 1.67 mg/kg	5/5	640~2560

[a] Positive ratio is no. of sera showing positive reaction/no. of sera tested.
[b] PCA titer represents the maximum dilution factor of original serum showing positive reaction.

serum was mixed with erythyrocytes coated with AFG, no hemagglutination was observed in all cases indicating specific antibodies were not detected in undiluted original sera. The PHA reaction was negative in all AFG sensitized sera by the challenge of AFG. The hemagglutination titer was therefore less than 1 in all AFG sensitized sera. When the OVA sensitized serum was mixed with erythrocytes coated with OVA, hemagglutination was observed in all cases with a hemagglutination titer ranging from 2^6 to 2^8 (Table V).

Immunosupressive effect—All sera from the OVA and AFG sensitized group, as well as from the OVA sensitized group, showed positive PCA reaction by the challenge of OVA. The PCA titer was more than 640 in all sera sensitized with OVA and ARG and more than 1280 in all sera sensitized with OVA (Table VI). However, the PCA titer of the serum sensitized with OVA after OVA challenge was not different significantly from that of the serum sensitized with OVA and AFG.

These results were summarized that AFG showed no antigenicity in the studies of ASA, ACA and PCA tests and PHA reaction in guinea pigs under the conditions used in the present studies. And also, the dose levels of AFG employed were confirmed not to suppress immune reactions.

Even though the test substance for these antigenicity studies was AFG, fresh ginseng can be used in itself for its clinical application, and also, can be used as a raw material for all other processed ginseng products including red ginseng and white ginseng products. These safety assessment studies on the antigenic potential of fresh ginseng, therefore, can be applied as one of the basic research for understanding of immunotoxicological basis of all other ginseng products.

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