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

Jong Wha Lee, Man Hee Rhee and Ki Hyun Park

Department of Biochemical Pharmacology, Korea Ginseng & Tobacco Research Institute, Taejon 305-345, Korea

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The antigenicity of the aqueous extract of red ginseng (ARG) was evaluated using the following assay procedures: 1. active systemic anaphylaxis (ASA) in guinea pigs, 2. active cutaneous anaphylaxis (ACA) in guinea pigs, 3. passive cutaneous anaphylaxis (PCA) in guinea pigs with serum from guinea pigs sensitized with ARG and 4. passive hemagglutination (PHA) with serum from guinea pigs sensitized with ARG.

1. ASA: No anaphylaxis reaction was observed in any of the sensitized guinea pigs by elicitation with ARG.

2. ACA: No skin reaction was observed in sensitized guinea pigs after intradermal injection of ARG.

3. PCA in guinea pigs: PCA titer of sera from all the sensitized animals was less than 10 in elicitation with ARG.

4. PHA reaction: When erythrocytes coated with challenge antigen were added to sensitized sera, the hemagglutination titer was less than 1.

These results suggest that ARG has no antigenicity under the conditions used. And the dose levels of ARG employed in the present experiment were confirmed not to suppress immune reactions.

Key words: *Panax ginseng*, Antigenicity, Active systemic anaphylaxis, Active cutaneous anaphylaxis, Passive cutaneous anaphylaxis, Passive hemagglutination, Guinea pig

INTRODUCTION

Ginseng, *Panax ginseng* C.A. Meyer, has been reported to have a wide range of pharmacological and therapeutical actions; it acts on the central nervous system, cardiovascular system and endocrine secretion, promotes immune function and metabolism, and possesses biomodulation action, anti-stress and anti-aging activities, and so on (Himi *et al.*, 1989; Teng *et al.*, 1989; Lee *et al.*, 1990; Matsunaga *et al.*, 1990; Liu and Xiao, 1992).

Although innumerable research papers have described the therapeutic and pharmacological effects of ginseng, its safety assessment has not been studied extensively. Acute, subacute and chronic toxicity, teratogenicity, carcinogenicity and mutagenicity of some ginseng extracts have been investigated as to the safety assessment of ginseng (Lee *et al.*, 1984; Soldati, 1984). However, there is little information on the antigenicity of ginseng (Lee *et al.*, 1994).

Antigenicity assessment is to evaluate the potential of chemicals and drugs to induce and elicit allergic (hypersensitivity) responses as a part of immunotoxicity assessment. With the introduction of the Gell and Coombs classification of hypersensitivity reactions, the term 'allergy' was restricted further, becoming almost synonymous with hypersensitivity of the immediate IgE type (type I hypersensitivity). Although examples of various types of chemical-induced hypersensitivity exist, immediate response (type I hypersensitivity) occurs most frequently in hypersensitive individuals. Moreover, immediate responses may be life-threatening in severe cases (Dean *et al.*, 1991).

In the present study, therefore, the antigenic potential of red ginseng was evaluated in guinea pigs focusing on type I hypersensitivity.

Correspondence to: Jong Wha Lee, Department of Biochemical Pharmacology, Korea Ginseng & Tobacco Research Institute, #302 Shinsung-dong, Yusung-ku, Taejon 305-345, Korea

MATERIALS AND METHODS

Test and Control Substances

The aqueous extract of red ginseng (ARG) was prepared from the root of red ginseng (Korea Tobacco & Ginseng Corp.) according to the method of Ko and collegues (Ko *et al.*, 1992) and was used as test substance. Clinical dose of ARG employed was 0.1 g/kg as red ginseng.

As positive and negative controls, ovalbumin (OVA, Lot No. 51H7175, Sigma Chemical Co.) and physiological saline were used, respectively.

Formulation of the Test Substances

ARG and OVA were dissolved in physiological saline.

A mixture of equal volumes of ARG and complete Freund's adjuvant (CFA, Lot No. 51H8925, Sigma Chemical Co.) and a mixture of equal volumes of OVA and CFA were prepared. Evans blue (Lot No. 31H0562, Sigma Chemical Co.) was dissolved in physiological saline.

Animals

Hartley male guinea pigs, 5 weeks of age, from Samyuk Experimental Animal Breeding Center (Osan, Kyunggi Do, Korea) were used in these studies after 2 weeks of acclimation. The animal room was maintained at a temperature of 23± 3℃, with a relative humidity of 60± 10 % and 12-h-lighting period. The animals were allowed free access to pellet form food (Purina Korea Co.) and tap water supplemented with vitamin C (1 g/l).

Sensitization Procedure

For sensitization, group of guinea pigs received either oral dose of ARG, subcutaneous dose of ARG or subcutaneous dose of OVA as shown in Table I. In addition to these groups, a non-treated control group was also employed. The animals were sensitized by oral or subcutaneous administration of ARG (0.1 g/kg or 1 g/kg as red ginseng) 9 times every other day, or by subcutaneous administration of a mixture of ARG (1 g/kg as red ginseng) and CFA or a mixture of OVA (2.5 mg/kg) and CFA 3 times once in 3 weeks (Park *et al.*, 1992). The dosing volume administered was 1 ml/kg.

ASA in Guinea Pigs

Two weeks after the final sensitizing dose, the challenge antigen (1 ml/kg) was injected into the leg vein and animals were observed for 30 min for the occurrence of an active systemic anaphylaxis reaction. The signs observed were scored according to the modified method of Kouchi and collegues (Kouchi *et al.*, 1989): [−], asymptoms; [±], urination, evacuation; [+], coughing, sneezing; [++], piloerection, nostril discharge, lacrimation, salivation, nasal bleeding, convulsion, dyspnea, staggering gait, rhonchus, cyanosis, side position, flattening; [+++], death.

ACA in Guinea Pigs

Two weeks after the final sensitizing dose, the challenge antigen was intradermally injected into the shaved backs of the guinea pigs. The volume administered was 0.1 ml per site. At 2, 4, 8, 24 and 48 h after intradermal injection of the challenge antigen, the changes of the injection site were observed for the occurrence of cutaneous allergic reaction such as papula, hemorrhage, redness, induration and necrosis. The degree of the changes observed was recorded according to the method of Kawano and collegues (Kawano *et al.*, 1988).

PCA in Guinea Pigs

Twelve days after the final sensitizing dose, blood samples were obtained from the retro-orbital venous plexus of the sensitized guinea pigs under light ether anesthesia and were centrifuged to obtain the sensiti-

Table I. Sensitization schedule of guinea pigs

Group	Sensitizing antigen	Dose	Route	No. of administration	No. of animals
Gp-I	ARG	0.1 g/kg[a]	p.o.	9[b]	5
Gp-II	ARG	0.1 g/kg	s.c.	9	5
Gp-III	ARG	1 g/kg	p.o.	9	5
Gp-IV	ARG	1 g/kg	s.c.	9	5
Gp-V	ARG+CFA	1 g/kg	s.c.	3[c]	5
Gp-VI	OVA+CFA	2.5 mg/kg	s.c.	3	5
Gp-VII	Saline+CFA	1 ml/kg	s.c.	3	5

[a]The dose of ARG is the dose calculated as red ginseng.
[b]Sensitization was repeated 9 times at intervals of every other day.
[c]Sensitization was repeated 3 times once in three weeks.

zed sera.

Each 0.05 ml of the sensitized serum diluted from 10- to 5120-fold with saline was injected intradermally into the backs of the non-treated male guinea pigs which had been clipped their back hair short. Four hours later, the challenge antigen was injected into the leg vein in a volume of 1 ml/kg.

Mixtures of equal volumes of Evans blue (10 mg/ml) and each antigen were prepared as described previously for ASA, and were used as the challenge antigen.

Approximately 30 min after challenge, the guinea pig were sacrificed by exsanguination following a blow to the head. The dorsal skin was removed and the reaction diameter of the blue region on the inverted skin surface was measured.

When the macula cerulean showed more than 5 mm in diameter [(longest+shortest)/2], the reaction was judged to be positive. PCA titers were expressed as the number of the highest serum dilution levels producing a blue coloration of 5 mm or more (Ovary, 1958).

PHA Reaction

The sensitized sera obtained from blood collected on 12 days after the final sensitizing dose were inactivated at 56℃ for 30 min, and were diluted from 2^0 to 2^{11} with a microtiter method (Kouchi *et al.*, 1989; Shibata *et al.*, 1991). For each dilution 25 μl were prepared.

Sheep red blood cell (SRBC) suspensions (Korea Media Co.) were washed with phosphate buffered saline (PBS) and were treated with tannic acid at 37℃ 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 h at 37℃. After washing coated erythrocyte with PBS, the coated erythrocytes were suspended in PBS containing 1% normal guinea pig serum to obtain a 1.5% erythrocyte concentration. To each diluted serum solution 25 μl of the coated erythrocyte suspension were added, and the mixture was well mixed. The mixture was then incubated at 37℃ for 16 h. Thereafter, the hemagglutination condition was examined at the bottom of the plate (Kawano *et al.*, 1988).

Immunosuppressive Effect

To confirm whether the dose levels of ARG used in the present studies 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, ARG (1 g/kg as red ginseng) and CFA 3 times once in 3 weeks. Twelve days after the final sensitizing dose, blood was obtained from the retro-orbital venous plexus of the sensitized guinea pigs and centrifuged to obtain the sensitized serum. The PCA titer of the serum sensitized with OVA after OVA challenge was compared with that of the serum sensitized with OVA and ARG (Kawano *et al.*, 1988).

RESULTS

ASA in Guinea Pigs

In the ARG sensitized groups, only urination and evacuation of feces were observed following dosing with the challenge antigens: no other symptoms nor death were noted. In the OVA sensitized group, the signs of anaphylaxis such as urination, evacuation of feces, coughing, sneezing, piloerection, nostril discharge, lacrimation, salivation, nasal bleeding, convulsion, dyspnea, staggering gait, rhonchus, cyanosis, side position or flattening were observed following challenge with OVA (Table II).

ACA in Guinea Pigs

In the ARG sensitized groups, no skin reaction was observed in any animals at any observation times following dosing with the challenge antigens. In the OVA sensitized group, hemorrhage of injection site was observed in all animals from 2-h observation time after OVA challenge and redness from 8-h observation time. Induration was observed in all animals at 48 h after OVA challenge and necrosis in 4 out of 5 animals (Table III).

PCA in Guinea Pigs

In the ARG sensitized groups, no blue region due to extravasation of Evans blue were observed at any site treated with sensitized serum following ARG challenge. PCA reaction was therefore negative in all ARG sensitized groups. In the OVA sensitized group, blue regions were observed at injection sites of all animals treated with sensitized serum indicating a PCA titer of more than 1280 (Fig. 1, Table IV).

PHA

When the ARG sensitized serum was mixed with erythrocytes coated with ARG, no hemagglutination was observed. The hemagglutination titer was therefore less than 1 in all ARG sensitized groups. When the OVA sensitized serum was mixed with erythrocytes coated with OVA, hemagglutination was observed in all cases indicating a hemagglutination titer of between 2^6 and 2^8 (Table V).

Immunosupressive Effect

Table II. Active systemic anaphylaxis in guinea pigs

Group	Sensitizing antigen	Challenging antigen	No. of animals	Severity of anaphylaxis[a]				
				[−]	[±]	[+]	[++]	[+++]
Gp-I	ARG (0.1 g/kg[b], p.o.)	ARG (0.1 g/kg)	5	3	2			
Gp-II	ARG (0.1 g/kg, s.c.)	ARG (0.1 g/kg)	5	4	1			
Gp-III	ARG (1 g/kg, p.o.)	ARG (0.1 g/kg)	5	3	2			
Gp-IV	ARG (1 g/kg, s.c.)	ARG (0.1 g/kg)	5	2	3			
Gp-V	ARG+CFA (1 g/kg)	ARG (0.1 g/kg)	5	1	4			
Gp-VI	OVA+CFA (2.5 mg/kg)	OVA (1.67 mg/kg)	5				5	
Gp-VII	Saline+CFA (1 ml/kg)	ARG (0.1 g/kg)	5	1	4			
Gp-VIII	−[c]	ARG (0.1 g/kg)	5	4	1			

[a] Severity of anaphylaxis was expressed as follows: [−], asymptoms; [±], urination, evacuation; [+], coughing, sneezing; [++], piloerection, nostril discharge, lacrimation, salivation, nasal bleeding, convulsion, dyspnea, staggering gait, Rhonchus, cyanosis, side position, flattening; [+++], death.
[b] The dose of ARG is the dose calculated as red ginseng.
[c] Not sensitized.

Table III. Active cutaneous anaphylaxis in guinea pigs

Group	Sensitizing antigen	Challenging antigen	Findings[a]				
			Hours after challenge				
			2	4	8	24	48
Gp-I	ARG (0.1 g/kg[b], p.o.)	ARG (0.1 g/kg)	0	0	0	0	0
Gp-II	ARG (0.1 g/kg, s.c.)	ARG (0.1 g/kg)	0	0	0	0	0
Gp-III	ARG (1 g/kg, p.o.)	ARG (0.1 g/kg)	0	0	0	0	0
Gp-IV	ARG (1 g/kg, s.c.)	ARG (0.1 g/kg)	0	0	0	0	0
Gp-V	ARG+CFA (1 g/kg)	ARG (0.1 g/kg)	0	0	0	0	0
Gp-VI	OVA+CFA (2.5 mg/kg)	OVA (1.67 mg/kg)	II(5/5)[c]	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)	ARG (0.1 g/kg)	0	0	0	0	0
Gp-VIII	−[d]	ARG (0.1 g/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] The dose of ARG is the dose calculated as red ginseng.
[c] These parentheses mean no. of animals showing the finding/no. of animals tested.
[d] Not sensitized.

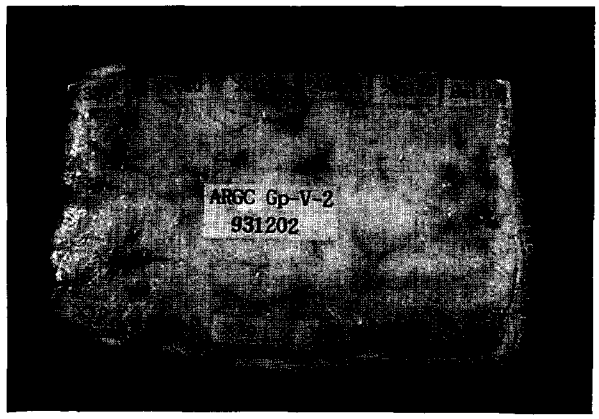
Blue regions were observed at injection sites treated with sensitized serum following challenge with same antigen, OVA, in OVA and ARG sensitized group, as in OVA sensitized group. The PCA titer was more than 320 in all sera sensitized with OVA and ARG and more than 1280 in all sera sensitized with OVA (Table VI).

Table IV. Four-hour homologous passive cutaneous anaphylaxis in guinea pigs with sera of sensitized guinea pigs

Group	Sensitizing antigen	Challenging[a] antigen	PCA[b] titer	Positive[c] ratio
Gp-I	ARG 0.1 g/kg[d] (p.o.)	ARG 0.1 g/kg	−[e]	0/5
Gp-II	ARG 0.1 g/kg (s.c.)	ARG 0.1 g/kg	−	0/5
Gp-III	ARG 1 g/kg (p.o.)	ARG 0.1 g/kg	−	0/5
Gp-IV	ARG 1 g/kg (s.c.)	ARG 0.1 g/kg	−	0/5
Gp-V	ARG 1 g/kg+CFA	ARG 0.1 g/kg	−	0/5
Gp-VI	OVA 2.5 mg/kg+CFA	OVA 1.67 mg/kg	1280-2560	5/5
Gp-VII	Saline+CFA	ARG 0.1 g/kg	−	0/5
Gp-VIII	−[f]	ARG 0.1 g/kg	−	0/5

[a]Challenging antigen was intravenously injected 4 hours after sensitization of guinea pigs with sera.
[b]PCA titer represents the maximum dilution factor of original serum which gives positive reaction.
[c]Positive ratio is no. of animals judged as positive/no. of animals tested in PCA reaction.
[d]The dose of ARG is the dose calculated as red ginseng.
[e]Specific antibodies were not detected in 10-fold dilution of original sera.
[f]Not sensitized.

(a) Sensitizing Antigen: ARG 1 g/kg as red ginseng
Challenging Antigen: ARG 0.1 g/kg as red ginseng



(b) Sensitizing Antigen: OVA 2.5 mg/kg
Challenging Antigen: OVA 1.67 mg/kg

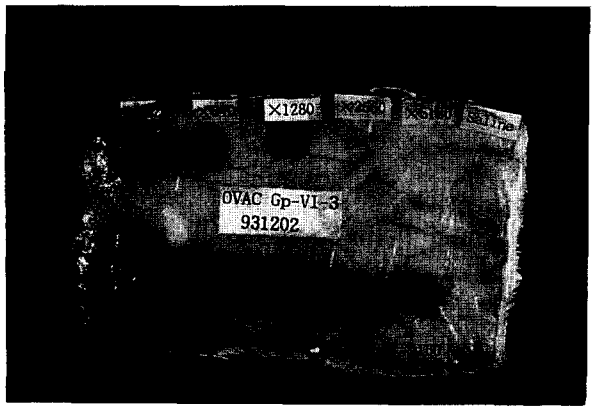


Fig. 1. Four-hour homologous passive cutaneous anaphylaxis in guinea pigs with sera of sensitized guinea pigs.
a. Injection site of antiserum sensitized with 1 g/kg of ARG as red ginseng; b. Injection site of antiserum sensitized with 2.5 mg/kg of OVA.

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 ARG ($P<0.05$).

DISCUSSION

The antigenicity of ARG was evaluated using the following type I hypersensitivity assay systems: ASA and ACA in guinea pigs, PCA in guinea pigs with serum isolated from guinea pigs and PHA with serum from guinea pigs.

In the ASA test in guinea pigs, evacuation of feces and urination were observed following administration of the challenge antigens in some animals sensitized with ARG by the oral and subcutaneous route. The same symptoms, however, were also observed in the non-treated guinea pigs. These symptoms therefore are not considered to be attributable to anaphylactic reaction initiated by interaction of antigen and antibody (Shibata, 1991). Other anaphylactic reactions were not observed. From these results, it is concluded that ARG is negative in ASA in guinea pigs.

In the ACA test in guinea pigs, no skin reaction was observed in any animals sensitized with ARG following ARG challenge, indicating that ARG is negative in the ACA test in guinea pigs.

In the PCA test in guinea pigs, no blue regions were observed at any site treated with serum from animals sensitized by oral and subcutaneous administration of ARG after challenge with ARG. The PCA titer was less than 10 in all the sensitized sera. From these results, it is concluded that ARG is negative in the PCA test in guinea pigs.

When the ARG sensitized serum was mixed with erythrocytes coated with ARG, hemagglutination titer was less than 1, indicating that ARG is negative in

Table V. Passive hemagglutination on sera from guinea pigs

Sensitizing antigen	Adsorbed antigen	Animal no.	Titer[a] $x2^0$	$x2^1$	$x2^2$	$x2^3$	$x2^4$	$x2^5$	$x2^6$	$x2^7$	$x2^8$	$x2^9$	$x2^{10}$	$x2^{11}$
		1												
		2												
ARG	ARG	3	−	−	−	−	−	−	−	−	−	−	−	−
(0.1 g/kg[b], p.o.)	(0.1 g/ml)	4												
		5												
		1												
		2												
ARG	ARG	3	−	−	−	−	−	−	−	−	−	−	−	−
(0.1 g/kg, s.c.)	(0.1 g/ml)	4												
		5												
		1												
		2												
ARG	ARG	3	−	−	−	−	−	−	−	−	−	−	−	−
(1 g/kg, p.o.)	(0.1 g/ml)	4												
		5												
		1												
		2												
ARG	ARG	3	−	−	−	−	−	−	−	−	−	−	−	−
(1 g/kg, s.c.)	(0.1 g/ml)	4												
		5												
		1												
		2												
ARG+CFA	ARG	3	−	−	−	−	−	−	−	−	−	−	−	−
(1 g/kg)	(0.1 g/ml)	4												
		5												
		1	+	+	+	+	+	+	+	+	±	−	−	−
		2	+	+	+	+	+	+	+	+	±	−	−	−
OVA+CFA	OVA	3	±	±	±	±	+	+	+	±	±	−	−	−
(2.5 mg/kg)	(2.5 mg/ml)	4	+	+	+	+	+	+	+	±	−	−	−	−
		5	+	+	+	+	+	+	+	+	+	±	−	−
		1												
		2												
Saline+CFA	ARG	3	−	−	−	−	−	−	−	−	−	−	−	−
(1 ml/kg)	(0.1 g/ml)	4												
		5												

[a] −, Not agglutinated: ±, Partially agglutinated; +, Completely agglutinated.
[b] The dose of ARG is the dose calculated as red ginseng.

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

Sensitizing antigen	Challenging[a] antigen	PCA[b] titer	Positive[c] ratio
OVA 2.5 mg/kg	OVA 1.67 mg/kg	1280-2560	5/5
OVA 2.5 mg/kg +ARG 1 g/kg[d]	OVA 1.67 mg/kg	320-2560	5/5

[a] Challenging antigen was intravenously injected 4 hours after sensitization of guinea pigs with sera.
[b] PCA titer represents the maximum dilution factor of original serum which gives positive reaction.
[c] Positive ratio is no. of animals judged as positive/no. of animals tested in PCA reaction.
[d] The dose of ARG is the dose calculated as red ginseng.

PHA.

These results indicate that ARG has no antigenicity under the conditions used in the present studies.

And also the anti-OVA antibody titer of serum sensitized with OVA was compared with that of serum sensitized with OVA and ARG using PCA in guinea pigs to confirm whether the dose levels of ARG used in the present studies suppress immune reactions. From the results of PCA, it is considered that the dose levels of ARG used in the present studies has no immunosuppressive effects.

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