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Effects of *Panax ginseng* on Type I Hypersensitivity

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Abstract : Effects of *Panax ginseng* on allergic reactions were studied using various *in vivo* and *in vitro* experimental models such as 48-hr passive cutaneous anaphylaxis, mediators-induced skin reactions, histamine release from rat peritoneal mast cells, hexosaminidase release from RBL-2H3 cells, and lipoxygenase assay. In all of anti-allergic experiments we conducted, ginseng components (50% ethanol extract or ginseng total saponin or ginsenosides) extracted from Korean red ginseng, did not show significant anti-allergic actions. In 48-hr passive cutaneous anaphylaxis and mediators-induced skin reactions, 50% ethanol extract did not suppress hypersensitivity reactions. Total saponin, 50% ethanol extract, and 8 major ginsenosides did not show inhibitory effects on lipoxygeanse activity. Ginseng total saponin did not inhibit histamine release from rat peritoneal mast cells. All of the ginseng components mentioned above were also tested on RBL-2H3 cells, but none of them inhibited hexosaminidase release from this cell line. These results suggest that *Panax ginseng* does not have effects on allergic reactions at the level of 50% ethanol extract or total saponin used. All of 8 major saponin components tested (Rb_1, Rb_2, Rc, Rd, Re, Rf, Rg_1, Rg_2), did not inhibit lipoxygenase activity and degranulation events.

Key words : *Panax ginseng*, allergy, 48-hr passive cutaneous anaphylaxis, degranulation, lipoxygenase, ginsenosides.

Introduction

Allergy is considered as an abnormal biological defense mechanism. Hay fever, rhinitis, atopic dermatitis, bronchial asthma, and anaphylactic shock are well known allergic diseases.[1] Allergic reactions are classified into several different types,[2] and the most common one is the immediate hypersensitivity reaction.[1]

The immediate hypersensitivity reaction can be described by three stages. The first step is IgE production and sensitization.[3] Both foreign antigens and endogenus antigens provoke IgE production, and these IgEs induce sensitization by binding to high affinity IgE receptor (FcεRI) on the basophil and mast cell surface.[4-8] The second step is the degranulation. On subsequent exposure to the same allergen, IgE molecules which have bound to FcεRI are crosslinked by the allergen[9,10] and then degranulation occurs. Various mediators such as, histamine, serotonin, eosinophil chemotactic factor of anaphylaxis, leukotrienes, and prostaglandins are released.[11-13] In the last step, pathogenic disorders occur by smooth muscle contraction and by vascular permeability increases.

Effects of *Panax ginseng* C. A. Meyer (ginseng) on allergy are not well established. Several studies suggest that ginseng might possess some of anti-allergic actions (for review, see 14). Meanwhile, Koda *et al.* (1982) have reported that ginseng does not have anti-allergic actions.[15]

We conducted more extensive studies to make it clear whether ginseng possesses anti-allergic actions. Various ginseng components were tested for their effects on allergic reactions using both

animal models and *in vitro* studies.

Materials and Methods

1. Experimental Animals and Reagents

Male Sprague-Dawley rats weighing 180~250 g were used. Animals were maintained at 12 hr light/dark cycle (8:00 a.m.~8:00 p.m.), and they were freely accessible to food and water. Histamine, 5-hydroxytryptamine (serotonin), compound 48/80 and Hank's balanced salt solution (HBSS) were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). DNP-BSA (2,4-dinitrophenol-bovine serum albumin), DNP-specific rat monoclonal IgE were kindly provided by Dr. Koda (College of Pharmacy, Gifu Univ., Japan). Ginseng total saponin (GTS) and 50% ethanol extract (50% EtOH extract) from Korean red ginseng were provided by Korea Ginseng & Tobacco Research Institute.

2. Forty Eight Hour Homologous Passive Cutaneous Anaphylaxis in Rats

The DNP-specific monoclonal rat IgE (48-hr homologous PCA titer=2^7) was 100 times diluted in saline, and intradermally injected into the back of rat (100 μl) to sensitize animals. After 48 hours, evans blue solution (20 mg/kg) containing DNP-BSA (4 mg/kg), as an antigen, was intravenously injected. Thirty minutes were allowed for the reaction between antigen and antibody to make cross-links. Fifty percent EtOH ginseng extract was orally administered to the rats once per day for one week at the dose of 50 or 200 mg/kg. At 7th day, 50% ethanol extract was administered 1 hr prior to challenge with antigen. Animals were sacrificed and the amount of the dye leaked into the skin was determined using a spectrophotometer at 620 nm.[16,17]

3. Vascular Permeability Changes Induced by Chemical Mediators in Rats

Saline, histamine (5×10^{-6} g/ml), serotonin (10^{-6} g/ml) and compound 48/80 (3×10^{-6} g/ml) were intradermally injected at each specific site in the back and evans blue saline solution was intravenously injected. Fifty percent ethanol ginseng extract was orally administered to the rats once per day for one week at the dose of 50 or 200 mg/kg. Thirty minutes after injection of chemical mediators, animals were sacrificed and the amount of extravasated dye was determined using a spectrophotometer at 620 nm.[16,17]

4. Histamine Release from Sensitized Rat Peritoneal Mast Cells

A suspension of peritoneal mast cells was prepared according to the method described by Foreman and Mongar[18] with a slight modification. Rats were sensitized with DNP-specific rat IgE (titer=2^7, 1:10 dilution, 0.5 ml) injected intraperitoneally. Forty eight hours later, animals were sacrificed by decapitation. Ten milliliter of heparin solution (1 U/ml in HBSS) was injected into the abdominal cavity and the suspension of peritoneal cells was collected. Cell suspensions (6×10^5 viable cells, purity of mast cells is 5 to 10% according to original report[18]) were pre-incubated for 10 min at 37℃. GTS was added at concentration of 10~1,000 μg/ml and incubated for 10 min at 37℃, then, DNP-BSA (100 μg/ml, final concentration) was added to the test tubes and incubated for 10 min at 37℃. For the total histamine content, cells were boiled for 10 min. The amount of histamine was spectrofluorometrically measured by the method of May *et al.*[19]

5. Hexosaminidase Assay

Hexosaminidase assay was conducted according to Tanaka *et al.*[20] Briefly, RBL-2H3 cells maintained in EMEM containing 3% fetal bovine serum, were treated with IgE overnight. Next morning cells were treated with an antigen (DNP-BSA, 1 μg/ml) for 15 min at 37℃. Supernatant was used for enzyme assay using p-nitrophenyl-N-acetyl-β-glucosaminide as a substrate. To monitor the percent of release of granular

contents, total hexosaminidase was measured. For this cells were lysed with 0.1% of triton X-100. The absorbance was measured at 405 nm by ELISA reader.

6. Lipoxygenase Assay

Low ethanol method was used (Sigma protocol). The reaction was started by adding enzyme solution (lipoxygenase, final 500 U/3 m*l*) to substrate solution (linoleic acid in 0.2 M borate buffer, pH 9.0). Enzyme activity was calculated from the absorbance measured at 234 nm for the first 30 seconds using a double-beam spectrophotometer (UVIKON 930, Kontron, Switzerland).

7. Statistics

All of statistical significances were determined using Student' *t*-test.

Results

1. Effects of 50% EtOH Ginseng Extract on 48-hr Homologous Passive Cutaneous Anaphylaxis and Skin Vascular Permeability

Passive cutaneous anaphylaxis (PCA) reaction possesses almost all fundamental characteristics of systemic anaphylaxis, and has been used to evaluate anti-allergic actions of test compounds.[17, 21] In PCA reaction, various chemical mediators are released by antigen-antibody reaction, and these mediators increase the skin vascular permeability.

Test on chemical mediator-induced vascular permeability changes is also one of experimental allergy models which utilize characteristics of anaphylactic or inflammatory reaction.[21] Histamine and serotonin released by mast cell degranulation are representative chemical mediators which increase the permeability of the post-capillary venules.[17, 21]

Effects of 50% ethanol ginseng extract on both PCA and chemical mediators-induced vascular permeability are shown in Table 1. Fifty percent ethanol ginseng extract was chosen because it would contain most of ginseng components.[14] As shown in Table 1, 50% ethanol ginseng extract did not significantly inhibit PCA and mediators-induced vascular permeability changes. These results are in agreement with similar findings by Koda *et al.*[15]

2. Effects of Ginseng Total Saponin on Histamine Release from Rat Peritoneal Mast Cells

Mast cell, a mediator releasing cell, plays an important role in type I hypersensitivity.[1] Mast cells possess high affinity IgE receptors on their surface,[16, 18, 19] and degranulation is induced when IgEs are cross-linked by antigen, and chemical mediators are released to produce various allergic symptoms.

Effects of GTS on histamine release are shown in Table 2. GTS showed biphasic actions on histamine release from sensitized mast cells. When cells were sensitized with IgE, GTS slightly inhibited histamine release at low concentration

Table 1. Effects of 50% ethanol ginseng extract on PCA and vascular permeability changes in rats

Drug (mg/kg)	Amount of dye (μg/spot)			
	PCA	Vascular permeability changes		
		Histamine	Serotonin	Compound 48/80
Control	79.7± 5.0	23.3±2.9	17.3±2.1	36.7±7.1
50% EtOH extract 50	84.8±28.2	21.8±4.8	14.1±3.6	29.4±3.3
50% EtOH extract 200	74.5±28.2	24.7±2.9	23.8±3.0	29.3±3.6

Fifty percent EtOH ginseng extract was orally administered to rats once per day for one week (50 and 200 mg/kg). At 7th day, 50% EtOH extract was administered 1 hr prior to challenge with antigen. The control rats received saline instead of 50% EtOH extract. The amount of dye is shown as the mean±S.E. from 5 animals.

(not statistically significant), but as concentration is increased (higher than 100 μg/m*l*), GTS significantly increased histamine release.

To further study whether these unexpected effects of GTS on mast cells involve the antigen-antibody reaction or they are just non-specific effects on cell membrane structure, we repeated the same experiment on non-sensitized mast cells. For this, antigen was removed from incubation mixture to exclude its action on IgE pathway. As shown in Table 2, similar results were obtained as those where antigen was added. GTS alone, even though not statistically significant, decreased histamine release from mast cells at concentration lower than 10 μg/m*l*. However, at concentration higher than 30 μg/m*l*, GTS alone increased the histamine release from mast cell in a dose dependent way, suggesting that GTS non-specifically acts on cell membrane to release histamine from mast cells.

3. Effects of Various Ginsenosides on Hexosaminidase Release from RBL-2H3 Cells

Our results in Table 2 show that, even though statistically not significant, ginseng total saponin has some inhibitory actions on histamine release at low concentrations but it rather increases histamine release at high concentrations. It is possible that different components might have opposite actions on degranulation events and their effects be canceled each other at high concentrations.

To test this possibility, effects of single components of ginseng saponin (Rb_1, Rb_2, Rc, Rd, Re, Rf, Rg_1, Rg_2) on degranulation events were studied using more reliable experimental method (hexosaminidase assay). Hexosaminidase is an enzyme which is packed in the same granules as histamine is stored and is released when degranulation occurs upon stimulation. Therefore, hexosaminidase is one of the granule marker and hesosaminidase assay can be used instead of histamine release.[20] Hexosaminidase assay has advantages over histamine measurements. It exists in a larger quantity than histamine, and the measurement involves just an enzymes assay. These together make it far more reliable than histamine measurement.

Table 3 shows effects of various ginsenosides on hexosaminidase release. None of ginsenosides we tested, inhibited hexosaminidase release from

Table 2. Effects of ginseng total saponin on histamine release from rat peritoneal mast cells

Drug	μg/m*l*	Histamine released			
		Sensitized		Non-sensitized	
		ng/m*l*	%	ng/m*l*	%
Total		3187.0± 0.0	100	1544.5±14.4	100
Control		493.6±67.6	15.5±2.1	39.9±15.5	2.6±1.0
GTS	1	604.8±48.8	18.9±1.5	18.4± 9.0	1.2±0.6
	3	403.9±30.4	12.7±1.0	17.6±13.3	1.1±0.8
	10	443.8±44.8	13.9±1.4	24.3±15.2	1.6±1.0
	30	541.0±40.1	17.0±1.3	88.1±16.1**	5.7±1.1
	100	662.5±72.0*	20.8±2.3	263.7±40.3***	17.1±2.6
	300	1383.8±50.0***	43.4±1.6	448.1±75.2***	29.0±4.9
	1,000	2106.3±75.8***	66.1±2.4	1105.8±48.2***	71.5±3.1

GTS was added to the incubation mixture to see its effects on histamine release (1~1,000 μg/m*l*). The amount of histamine is shown as the mean±S.E. from 3 to 4 measurements. For the control (sensitized) and GTS-treated groups, the values were calculated by subtracting the spontaneously released histamine (not challenged with antigen). *$p < 0.1$, **$p < 0.05$, ***$p < 0.025$.

Table 3. Effect of ginseng components on hexosaminidase release from RBL-2H3 cells

	Dose (μg/ml)	Absorbance (405 nm)
Total		2.308±0.028
Control		1.400±0.022
50% EtOH extract	100	1.277±0.071
GTS	100	1.386±0.020
Ginsenosides Rb_1	50	1.329±0.017
Rb_2	50	1.260±0.028
Rc	50	1.264±0.024
Rd	50	1.290±0.014
Re	50	1.381±0.017
Rf	50	1.342±0.037
Rg_1	50	1.429±0.016
Rg_2	50	1.373±0.055

Each absorbance value represent the mean±S.D. of duplicates.

RBL-2H3 cells, suggesting that major ginsenosides we tested do not have effects on degranulation events.

4. Effects of Ginseng on Lipoxygenase Activity

Lipoxygenase pathway is very important in allergic reaction in that it produces leukotrienes which are known to play important roles in asthma.[12,13] Therefore, substance which has inhibitory action on lipoxygenase would have anti-allergic actions.

Effects of ginseng on lipoxygenase activity are shown in Table 4. Neither GTS nor 50% ethanol extract inhibited lipoxygenase activity. Also all of ginsenosides we tested (Rb_1, Rb_2, Rc, Rd, Re, Rf, Rg_1, Rg_2) did not inhibit lipoxygenase activity.

Table 4. Effect of ginseng components on lipoxygenase activity

	Drug (μg/ml)	Relative activity (%)
Control		100.0±3.0
GTS	500	91.2±2.6
	300	89.4±0.9
	100	106.1±3.5
50% EtOH extract	500	108.3±1.7
	300	113.7±1.0
	100	115.7±2.5
Ginsenosides Rb_1	50	120.0±6.2
	10	107.0±4.8
Rb_2	50	120.0±7.0
	10	107.0±5.2
Rc	50	115.0±6.3
	10	114.0±5.2
Rd	50	109.0±4.4
Re	50	109.0±4.6
Rf	50	105.0±4.7
Rg_1	50	111.0±4.9
Rg_2	250	117.0±3.9
	50	119.0±6.2
	10	111.0±4.7

Each data value represent mean±S.E. from three experiments. For the better comparison between control and ginseng-treated groups, lipoxygenase activity for the control was normalized to 100%.

Discussion

As mentioned above, effects of ginseng on allergic reaction are not well established. In this study, we tested the anti-allergic actions of ginseng using animal models and *in vitro* studies.

Our results from PCA experiments show that giseng does not have anti-allergic action at the 50% ethanol extract level. Since most essential components of anaphylaxis are contained in PCA, our results would suggest that administration of whole ginseng is not beneficial for allergic states. This was further confirmed by its effects on mediator-induced vascular permeability changes, histamine release, and lipoxygenase activity.

Effects of GTS on histamine release was biphasic in dose-dependent way, especially when cells were not challenged with antigen. These results might suggest that GTS could stabilize cell membrane at low concentration. However, this was not clearly observed when cells were challenged with antigen, and it is hard to judge whether this could be beneficial for allergic reactions at this point.

Effects of single saponin components on lipox-

ygenase activity and hexosaminidase release suggest that ginseng components other than major saponin components should be further investigated to find out active components on these targets.

요 약

본 실험에서는 인삼이 알레르기에 미치는 효과를 알아보기 위하여, 홍삼에서 추출된 여러 가지의 인삼 성분을 이용하여 실험하였다. 인삼의 항알레르기 효과를 검토하기 위하여 48시간 수동 피부 아나필락시, 화학적 전달물질에 의한 피부 반응, 흰쥐의 복강 비만세포로부터의 히스타민 유리, RBL-2H3 cells에서 hexosaminidase 유리 및 lipoxygenase 활성 측정 실험을 하였다.

인삼 50% 에탄올엑스는 48시간 수동 피부 아나필락시와 화학적 전달물질에 의한 피부반응 실험에서 야기된 과민반응을 억제하지 못했다. 또한 인삼총사포닌과 50% 에탄올엑스, 그리고 몇 가지 인삼 사포닌의 단일 성분들도 모두 lipoxygenase 활성을 억제하지 못했다. 한편 인삼총사포닌은 흰쥐의 복강 비만세포로부터의 히스타민 유리를 고농도에서 유의성 있게 증가시켰다. 감작을 시키지 않은 비만세포의 경우 인삼총사포닌은 낮은 농도에서는 히스타민의 유리를 약간 억제하였으나 농도가 증가함에 따라 용량의존적으로 증가시켰다. 또한, 몇 가지 사포닌 단일성분들 역시 hexosaminidase 유리를 억제하지 못했다.

이러한 결과들은 인삼이 에탄올 추출물이나 총사포닌 수준에서는 알레르기 질환에 효과가 없으며, 주요 사포닌 성분들(Rb_1, Rb_2, Rc, Rd, Re, Rf, Rg_1, Rg_2) 역시 lipoxygenase나 degranulation 단계에는 아무런 효과를 지니지 않음을 제시한다.

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