EFFECTS OF *PANAX GINSENG* SAPONINS ON CHEMICAL MEDIATOR RELEASE FROM AIRWAY SMOOTH MUSCLE IN ACTIVELY SENSITIZED GUINEA PIG

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ABSTRACT

It has been reported that ginseng is effective in the central nervous system, immune system, and the strong inflammatory responses. However, there has been no research report yet about the effect of ginseng on allergic hypersensitivity reactivity. To confirm the ginseng effects on the release of mediators(histamine, leukotrienes etc.) which cause the hypersensitivity reactivity and inflammatory response, we used actively sensitized guinea pig airway tissues by utilizing the superfusion technique. In this procedure, the contractile response and mediators released after antigen stimulation of sensitized tissues, and IgG and IgE antibody products were measured in sera of immunized animals. Then the results of the controll group were compared to those of ginseng pretreatment groups.

In the total saponin(TS) and panaxatriol(PT) pretreatment, histamine release decreased by 20% in the tracheal tissues after active sensitization by ovalbumin(OVA, 10mg/kg), but in the lung parenchyma, histamine release decreased by 40%. Panaxadiol(PD) significantly decreased histamine release by 40% in the both tissues after active sensitization.

TS, PT and PD of ginseng poorly blocked leukotrienes (LTs) and prostagrandin D_2(PGD$_2$) release(less than 10%). Ginseng TS and PT had no effect on the serum IgG antibody production by ovalbumin, whereas PD significantly increased serum IgG antibody contents(approximately by 2 times). However, IgG_1 antibody products in the serum of guinea pig actively sensitized with ovalbumin after PD pretreatment were decreased, compared to that with ovalbumin alone. IgE antibody production by passive cutaneous anaphylaxis(PCA) titer in the TS pretreatment increased 3 times more than in the absence of TS(PCA titer by PT was not detected).

These studies show that some ginseng saponins can in part act to inhibit mediator release in antigen-induced airway smooth muscle by inducing the IgG antibody production which has been changed in the specificity.

INTRODUCTION

Throughout our history a precious Korean herb medicine, *Panax ginseng*, has been used as a folk-medicine for the weakened physical strength, asthenia, and fatigue.

Systemic isolation, purification, and chemical structure of the biologically active components of ginseng extract have been obtained[1-4], and pharmacological and biochemical properties of ginseng saponins have been reported by the recent scientific research works[5-9].

It has been observed that ginseng saponin is very effective in the central nervous system[10-25] and in the metabolism[26-32].

It has also been reported that ginseng saponin is effective in the immune system, that is, an increase in the rate of synthesis of serum proteins such as albumin, β-, γ-globulin[33-34], an increase in the antibody production against sheep red blood cells[35] and chick γ-globulin[36], an increase in the activity of adenosine deaminase which is an enzyme known to affect the production of interleukin-2[37], an increase in the tumoricidal activity of K562 tumor cell through the tumoricidal activity of the macrophage[38]. It was found that ginseng extracts have protective effect on the infection caused by immunodepression[39]. Ginseng saponins were well known to inhibit the proliferation and viability of tumor cell[40-42]. Ginseng polysaccharide fraction also reduced the immunotoxicity of cyclophosphamide[43].

It has also been reported that ginseng has strong anti-inflammatory component in the chloroform extracts[44-45]. There have been many other reports on ginseng to say that they found anti-inflammatory activity on carrageenin edema[46-47], the effect as a biological response modifier[48], the reduction of inflammatory action related to acne[49], an effect on neutrophil accumulation[50].

A type I hypersensitivity reaction (allergy) is induced by certain types of antigens, referred to as allergens (antigens, cold air, irritable gas, exercise and so on). That is, an allergen induces an humoral antibody response, resulting in production of IgE antibody. This class of antibody binds with high affinity to Fc receptors on the surface of tissue mast cells and blood basophils. Such IgE-coated mast cells or basophils are said to be sensitized. A later exposure to the same allergen cross-links the membrane-bound IgE on sensitized mast cells and basophils, causing degranulation of these cells. The pharma-

cologically active mediators(histamine, protaglandins, leukotrienes, platelet - activating factor, ECF - A, NCF etc.) released from the granules exert biological effects on the surrounding tissues. The principal effects such as vasodilatation, smooth muscle contraction, allergic inflammatory reaction, and asthma may be either systemic or localized.

However, there has been no research report yet about the effect of ginseng on allergic hypersensitivity reactivity and allergic inflammatory response.

Our preliminary study, by using guinea pig which has been known as a good model for hypersensitivity reaction in the tracheal and lung parenchymal tissues, showed that ginseng saponins have anti - allergic effects which inhibit the release of mediator(histamine) induced by special antigen(ovalbumin). Based on the preliminary study this study attempted to confirm the ginseng effects on the release of mediators(histamine, leukotrienes, and prostaglandins) which causes the hypersensitivity reaction and inflammatory responses. We also attempted to confirm the ginseng effects on the production of IgG and IgE antibodies.

MATERIALS AND METHODS

Animals

Hartley albino female guinea pigs weighing about 250 - 350g were used.

Drugs

Total saponin(TS), protopanaxadiol(PD) and protopanaxatriol(PT) were supplied from Korea Ginseng & Tobacco Research Institute.

The following substances were used : Histamine diphosphate, MES, HEPES, indomethacine, ovalbumin(OVA, fraction V), anti - rabbit IgG_1 alkaline phosphate conjugate, p - nitrophenyl phosphate (Sigma Chemical Co., St. Louis, MO) ; Oxazolone(Gallard Schlesinger, Carle Place, NY) ; cyclophosphamide(Mead Johnson, Evansville, IN) ; protein A Sepharose CL - 4B(Pharmacia Fine Chemicals, Pistataway, NJ) ; carbamyl choline chloride(carbachol, Aldrich Chemical, Inc., Milwaukee, WI) ; barium chloride(J.T. Baker Chemical Co., Phillipsburg, NY) ; gelatin(Difco Labs, Ditroit, MI) ; leukotriene (LT) antibody for radioimmunoassay(Stuart Pharmaceuticals, Divison of ICI Americas, Inc., Wilmington, DE) ; ^3H - LTD_4 (specific activity - 39Ci/mmol, New England Nuclear, Boston, MA). All drug solutions were prepared on the day of each experiment. Indomethacin was dissolved in 95% ethanol and diluted 10,000 fold in the physiological salt solution used for tissue superfusion. All other drugs were prepared in 0.9% sodium chloride.

Active Sensitization Protocol

Seven outbred female Hartley albino guinea pig(Sam Yook Experimental Animal, Osan, Korea), weighing approximately 250g, received intraperitoneal(i.p.) injections of 50mg/kg of TS, PD, and PT, respectively, every other day for 2 weeks.

Control group received saline of the same volume as ginseng injection volume in the experimental group. After that, all animals received 10 mg/kg of OVA three times(1st, 3rd, 5th day)[52]. Animals were sacrificed 3 weeks later and tracheal and lung parenchymal tissues were used.

Tissue contraction and mediator release

Actively sensitized trachea were trimmed of excess tissue and cut in a spiral fashion[53-54]. A parenchymal strip (approximately 3×3×15㎜) was obtained from the right lower lobe of the same animals. The tracheal and parenchymal strips were suspended in air - filled, water - jacketed tissue chambers and superfused with a Krebs bicarbonate solution of the following composition(mM) : NaCl, 118 ; KCl, 4.7 ; $CaCl_2$, 2.5 ; $MgCl_2$, 0.5 ; NaH_2PO_4, 1 ; $NaHCO_3$, 25 ; glucose, 11. The solution was gassed with a mixture of oxygen(95%) and carbon dioxide(5%) and pumped from a heated(37.5℃) reservoir through tygon tubing to a water - jacketed coiled glass tube(heat exchanger) using a Gilson Miniplus II peristaltic pump(Gilson Medical Electronics, Middleton, WI). From the heat exchanger, the physiological salt solution superfused the tissues at a rate of 1.5㎖/min. The tracheal spirals and parenchymal strips were maintained at initial tensions of 5g and 1g, respectively, for a 90min equilibration period before antigen challenge. Changes in tension were recorded via force transducers(FT - 03) on Grass model 50 or 79B polygarphs (Grass Instruments, Quincy, MA).

After equilibration, all tissues were challenged with 0.1 mg/㎖ OVA dissolved in the superfusion solution. Superfusate samples were collected at the base of the tissue chamber and each collection tube contained an appropriate amount of gelatin to make 0.1% at the end of the collection period. Superfusate samples were collected for a 2min period for spontaneous release before the challenge with OVA and continuously from the beginning of challenge with OVA. Histamine and LTs were not found in spontaneous release samples. During antigen challenge, consecutive time intervals of superfusate collection were for 5min. If necessary, additional 5min collection interval was given.

The maximum duration for antigen challenge and superfusate collection was 35min. After collection, the superfusate samples were placed in an ice bath and stored at 4℃ until analysis of histamine and LTs(see below).

Tracheal and parenchymal strip tensions were monitored throughout the experiment and quantified at the maximal peak during antigen challenge. Peak changes in tension were determined independent of time interval. At the end of antigen challenge, tracheas were superfused with carbachol,

10^{-3}M, and parenchymal strips with barium chloride, 10^{-2}M, in order to elicit the maximum contractile response from each tissue. Responses to OVA were expressed as a percentage of the maximum contractile response in each tissue. OVA did not cause contraction of tissues taken from unsensitized animals.

At the end of each experiment, the tracheal and parenchymal strips were weighed, placed in 5mℓ of 0.4N perchloric acid and the tubes placed in a boiling water bath for 15min before homogenization. After centrifugation, the supernatant was analyzed for histamine content. Total histamine contents for each tissue were not statistically different between the experimental and control group and, for 60 paired tissues, averaged 13.5 ± 0.43 and 33.9 ± 1.6 ㎎/g wet weight for tracheal and parenchymal strips, respectively.

Histamine assay

Histamine content of each sample was determined by an automated continuous flow-extraction (with dialyzer) and fluorometric analyzer as described by Siraganian[35]. The sensitivity of the assay is approximately 5ng/mℓ histamine. The amount of histamine in each superfusate sample was expressed as a percentage of the total tissue histamine content remaining after the preceeding collection period. In preliminary studies, it was found that the antigen and drugs did not give nonspecific fluorescence or quenching of histamine standard solutions.

Leukotriene radioimmunoassay

The LTs content of each superfusate sample was determined by radioimmunoassay as described previously[36]. Antibody(#332, % cross reactivity : LTC_4, 145 ; LTD_4, 100 ; LTE_4, 41) was diluted in buffered saline (5mM MES, HEPES adjusted to pH 7.4 with 1N NaOH) containing 0.1% gelatin. Antibody, 50μℓ of the appropriate dilution(1 : 1000), was added to 100μℓ of sample and mixed with 50μℓ(2,500-3,000 cpm) of ^3H-LTD_4 diluted with bufferred saline. Incubations were carried out at 4℃ for 2h and reactions terminated by addition of 0.5mℓ dextran-coated charcoal(200㎎ charcoal and 20㎎ dextran mixed with 100mℓ of buffered saline). After 5min, the mixture was centrifuged at 2,000 ×g for 5min at 4℃. A volume of 0.4mℓ of the supernatant was added to 5mℓ of Aquasol(NEN Research Products) and counted by liquid scintillation spectrometry(Packard, Model 3255). Standard curves were constructed in the presence of the drugs and antigen using LTD_4 (4.5×10^{-10} to 1×10^{-7}M). The detection limit was 0.045 pmole LTD_4.

The effects of the ginseng saponin on the active sensitzed giunea pig airway tissues were also determined by indirect method. Actively sensitized tracheal and lung parenchymal strip were suspended in air-filled, water-jacketed tissue chamber with a Krebs bicarbonate solution in the presence of 5×10^{-6}M of indomethacin which inhibits the cyclooxygenase pathway. This pathway is prostagrandin D_2 (PGD_2) formation from arachidonic acid.

Preparation of hapten-protein conjugates

Preparation of hapten-protein conjugates was performed as detailed previously[30-31]. Briefly, in the preparation of the hapten-protein conjugate oxazolone-ascaris(Ox-Asc), 10mℓ of an ascaris protein extract(10 ㎎/mℓ) was adjusted to pH 9.0 at 25℃ by addition of 5% Na_2CO_3. Then 0.5mℓ of 10% oxazolone in dioxane(Fischer Scientific Company, Pittsburgh, PA) was added dropwise while the pH was maintained at 9.0 by addition of 5% Na_2CO_3. After 2h of stirring, the mixture was dialyzed extensively against phosphate buffered saline, pH 7.4 (PBS). The dialyzed material was concentrated by negative pressure filtration. The protein quantity was determined by the Micro-Kjeldahl method. The Ox-Asc conjugate contained 5×10^{-7}moles of hapten/㎎ of protein.

Immunization procedures(IgE antibody)

Serum rich in IgE antibody to the hapten-protein conjugate Ox-Asc was obtained using techniques described previously[30]. Briefly, 6-8 outbred female Hartley albino guinea pigs, weighing approximately 250 g, first received intrapenitoneal(i. p.) injection of 50 ㎎/㎏ of TS or saline (control group) every other day for 2weeks, received i.p. injections of 250㎎/㎏ of cyclophosphamide 2 days prior to primary i.p. immunization with 10 ㎍ of conjugate(Ox-Asc) adsorbed to 1㎎ of $Al(OH)_3$ (alum) in 1mℓ of normal saline. Every month thereafter for 5 months, a similar dose of antigen in alum was given i.p. Seven days after antigen injection in 5 months, the animals were exsanguinated. The sera were stored in aliquots at -90℃ until time of use.

Quantitation of IgG_1 and IgE antibody

A quantitative estimation of IgG_1 anti-OVA and IgE anti-Ox-Asc antibody purified by affinity column chromatography from guinea pig serum was obtained by passive cutaneous anaphylaxis(PCA) and enzyme-linked immunosorbent assay(ELISA) method(see below)[30].

Affinity column chromatography

In the current study, all IgG anti-OVA and IgE anti-Ox-Asc antibody were separated from guinea pig serum by passage through a column of protein A Sepharose CL-4B.

Protein A Sepharose was equilibrated in citrate (0.1M)-phosphate buffer(0.2M), pH 7.4 and 10-20mℓ of the mate rial was packed into a column. Serum to be adsorbed of IgG antibody or non-adsorbed of IgE antibody was applied(under-loaded according to predetermined conditions) to this column and allowed to incubate in the column beads for 30-45min at 4℃. At the end of this time period, the column

was washed with the equilibrating buffer(pure IgE antibody). The eluted serum was concentrated using negative pressure filtration and dialysed against PBS. IgG antibody was desorbed from the protein A affinity column using 0.1M citric acid pH 2.0(pure IgG_1 antibody). This material was concentrated and dialyzed against PBS.

Passive cutaneous anaphylaxis method

PCA antibody titrations were performed as described by Ovary[57-58]. In this procedure, ten-fold dilutions of the serum(in PBS) to be tested were made in test tubes, and 0.1 mℓ of each dilution was injected intradermally into flank skin of 300 g guinea pigs(done in triplicate). A sensitization period of 4h or 10 days was employed, and PCA reactions were developed by injecting i.v. 1mℓ of 0.5% Evans Blue containing 1mg of antigen(OVA or Ox-Asc). The PCA titer was taken as the highest dilution of antiserum giving a 6×6 mmblue reaction on the reflected skin surface in at least 2 of 3 recipients. Heat sensitivity of the PCA antibody activity was determined by incubating antisera at 56℃ for 4h prior to making dilutions for the PCA titration.

Enzyme-linked immunosorbent assay

IgG_1 anti-OVA was determined by ELISA method[61-62]. Rabbit anti-guinea pig IgG_1(heavy chain specific antibody) are diluted in coating buffer(1.59g of Na_2CO_3 2.93g of $NaHCO_3$, and 0.2g of NaN_3 made up to 1 liter, pH 9.6). This diluted antibody is added to polystyrene microplate well(plate), incubated at 4℃ in humid chamber for 18h. Plate is washed with PBS-Tween(0.2%), added with IgG_1 obtained from protein A column or normal IgG with rabbit serum for control, incubated at 36℃ in CO_2 incubator for 2h, added with goat anti-guinea pig IgG alkaline phosphatase(1 : 1000 dilution with PBS-Tween), incubated for 2h at dark room temperature, washed and added with substrate(p-nitrophenyl phosphate), incubated for 30min at room temperature and then reaction is stopted by adding 3N NaOH. The color change in each well is estimated spectrophotometrically at 415nm. IgG_1 anti-OVA is confirmed by immunoelectrophoresis methods.

RESULTS

Effects of ginseng saponins on the mediator release and antibody production

In order to confirm whether the ginseng saponins themselves have the mediator-releasing effect on hypersensitivity, 50 mg/kg of TS, PD, and PT, respectively, were injected into guinea pigs every other day for 6 weeks. When isolated tracheal and lung parenchymal tissues were challenged by 0.1mg/mℓ of ovalbumin(OVA), all ginseng saponins did not have any effect on the histamine and LT release(not shown data).

We examined contractile response and mediator release in the actively sensitized pulmonary tissues by utilizing superfusion technique, and examined IgG antibody products. compared to that of ginseng saponin pretreatment(Fig. 1, 2, 3, 4 ; Table 1). In addition to these, we also examined IgE antibody products(Table 5).

All ginseng saponins did not have any effect on the contractile response in the antigen-induced tracheal and parenchymal tissues after active sensitization(Fig.1). In the TS and PT pretreatment, histamine release decreased by 20% in the tracheal tissues. But in the lung parenchyma, histamine release decreased by 40%. PD significantly decreased histamine release in the both tissues(by 40%) after active sensitization(Fig. 2, 3 ; Table 1). All ginseng saponins did not decrease LT release in the antigen-induced tracheal and parenchymal tissues after active sensitization(Fig. 2, 3, Table 1).

Effects of ginseng saponins on prostaglandin D_2(PGD_2) release

The effects of ginseng saponins on the release of PGD_2, which is one of the newly formed mediators in antigen-antibody reaction of actively sensitized tissues, were indirectly observed in the presence of indomethacin(5×10^{-6}M).

In the presence of indomethacin, all ginseng saponin pretreatment did not have any effect on the contractile responses of both tissue strips obtained from actively sensitized guinea pig(Table 2). But the contractility in the presence of indomethacin was prolongated in ginseng saponin treated tissues and control group tissues(not shown data).

Total histamine release was poorly increased, but was not significantly altered by indomethacin alone and ginseng saponin pretreatment after active sensitization(Table 2).

In the all indomethacin, total measurable LT release from both superfused tissues(trachea and parenchyma) was not significantly decreased by ginseng saponin pretreatment. That is, ginseng saponins did not have any effect on the PGD_2 formation. But amounts of LTs released in all indomethacin treatment tissues after sensitization increased more than in absence of indomethacin(Table 2).

Effects of ginseng saponin on the IgG antibody formation in the guinea pigs

IgG antibody responses produced in the guinea pigs actively immunized by 10mg/kg of OVA after ginseng saponin pretreatment was measured. IgG antibody products were measured by PCA method and protein A column(Table 3, 4). IgG antibody products by both methods did not increase in the TS pretreatment(OVA alone titer, 1,600 ; OVA+TS titer, 2,400 ; IgG anti-OVA, 2.32 ± 0.41 ; IgG anti-OVA with TS, 2.58 ± 0.29), but in the PT pretreatment, poorly increased(OVA+PT titer, 3,200 ; IgG anti-OVA with PT, 3.523

± 0.38). IgG antibody products by PD pretreatment significantly increased in both methods(OVA+PD titer, 6,400 ; IgG anti-OVA with PD, 4.75 ± 0.57).

IgG_1 antibody was detected by ELISA(Fig. 4) and immunoelectrophoresis method. The guinea pigs actively sensitized with OVA after PD and PT pretreatment consistantly produced IgG_1 antibodies to titer that were 2-4 fold lower than those sensitized with OVA alone.

Effects of total saponin on the IgE antibody formation in the guinea pigs

IgE antibody titers were determined from animal serum sensitized by 10㎍ of Ox-Asc in the 1㎎ of Alum after total saponin pretreatment(Table 5). PCA titer in the total sa ponin pretreatment increased 3 times more than in the absence of total saponin(Table 5).

DISCUSSION

It has been well known that several chemical mediators (which are stored to granules in the mast cells and basophils, and which are newly formed by the stimulation of mast cell and basophil membrane) are released when antigen-antibody reaction is induced by antigens.

This study examined the effect of ginseng saponins on the histamine and LT release, and on the antibody formation.

When the guinea pig tracheal and lung parenchymal tissues taken from animal(which received ginseng saponins alone every other day for 6weeks) were challenged by anti gen(OVA), all ginseng saponins did not have effects on the histamine and LT release. Therefore, we suggest that ginseng saponins themselves does not induce effect on antigen-antibody reaction of hypersensitivity.

The contractility of actively sensitized tracheal and parenchymal tissues was not altered by ginseng saponins (Fig 1). But as table 1 shows, the variation in the contractility of tissues treated with the ginseng saponins was due to the reduction of maximum contractility of carbachol and barium chloride which are used to elicit the maximum contractile response from each tissue. This implies that ginseng saponins have some effects on the action of both carbachol and barium chloride (Table 1).

Histamine release in the tracheal and parenchymal tissues taken from actively sensitized guinea pig after ginseng saponin pretreatment decreased significantly. But LT release did not have effect on the both tissues. In the PD treatment, the remarkable reduction of histamine release was observed. Mast cells in the lung tissues showed more reduction of histamine release than in the tracheal tissues(Fig. 2, 3 ; Table 1).

These results support the report that 20(S)-G-Rg_3 inhibits histamine release induced by the antigen-antibody reaction in the rat peritoneal mast cells[59]. Thus, it is found that there is no difference in the inhibition of histamine release between two species whereas there is heterogeneity of mast cell between species.

LT release in the both tissues sensitized actively were not inhibited by pretreatments of ginseng saponins. It suggests that ginseng saponins do not inhibit the 5-lipoxygenase which is related to the metabolism of the arachidonic acid. When newly fromed mediator, PGD_2, were observed indirectly in the actively sensitized tissues after ginseng saponin pretreatment, ginseng saponins did not have effect on the PGD_2 production. But ginseng saponins used in the experiments are complex component material. If we seperate further, we may find some components which have effects inhibiting newly formed mediator release. The amounts of histamine released from tissues by the indomethacin treatment increased a little than in the absence of indomethacin(Table 2). Therefore, it is suggested that several biochemical reactions caused by the activation of mast cell membrane may be effective on the degranulation of mast cells.

Serum IgG antibody products were increased by active immunization after ginseng saponin pretreatment, especially by PD pretreatment(Table 3, 4). This increase may support that ginseng extracts increased amounts of serum antibody in the several species[34, 35, 37, 39].

Guinea pig IgG_1 antibody induces allergic reaction. IgG antibody produced by our laboratory is IgG_1 antibody. In order to confirm how ginseng saponins decrease the chemical mediators, IgG_1 antibody products were assayed by ELISA method. This result shows that IgG_1 antibody was decreased by PD pretreatment(Fig. 4). This tells that IgG_1 antibody may change into IgG subclass antibody when IgG antibody is produced by antigen(OVA) after PD pretreatment. Therefore, it is suggested that the degranulation of activated mast cell is inhibited by IgG antibody production which has been changed in the specificity. But this suggestion requires more studies.

IgE antibody titers were increased by Ox-Asc and alum after TS pretreatment(Table 5). This result may support Wu et al[60] who reported that saponins from Q. Saponaria augment antibody responses as a adjuvant. This result suggest that one of components of Korean ginseng saponins may induce antibody responses as a adjuvant, but this suggestion requires more studies.

These studies show that some ginseng saponin can in part act to inhibit mediator release in antigen-induced airway smooth muscle by inducing the IgG antibody production which has been changed in the specificity.

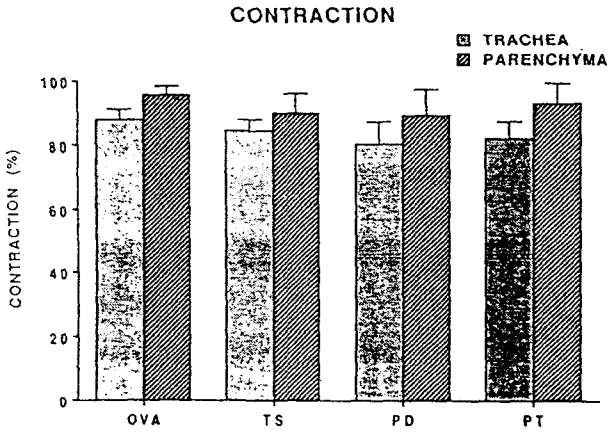

Fig. 1. Contraction in absence and presence of ginseng saponins(50 mg/kg) after challenge with OVA, 0.1 mg/mℓ, in superfused guinea pig airway smooth muscle strips. Animals were actively sensitized with OVA(10 mg/kg) every other day for 3 times after ginseng saponin pretreatment, respectively, and then tracheal and parenchymal tissues were isolated, superfused with OVA (0.1 mg/mℓ). Vertical lines in dicate SEM.

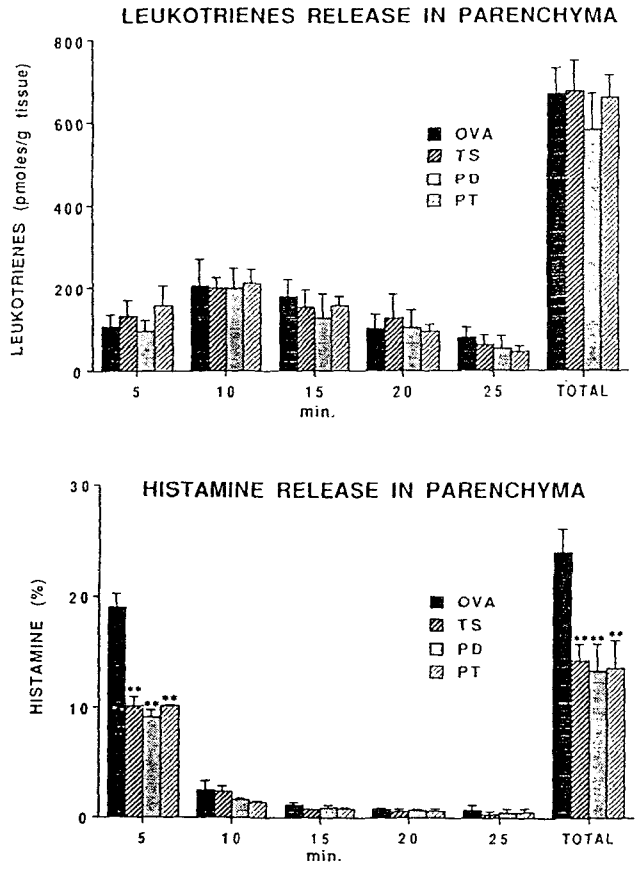

Fig. 3. Time course for histamine and leukotriene contents in absence and presence of ginseng saponins(50 mg/kg) after challenge with OVA, 0.1 mg/mℓ, in superfused guinea pig parenchymal strips.
All precedure and pattern are the same as Figure 2 except using the parenchymal tissues instead of tracheal tissues. The data are summarized in Table 2.

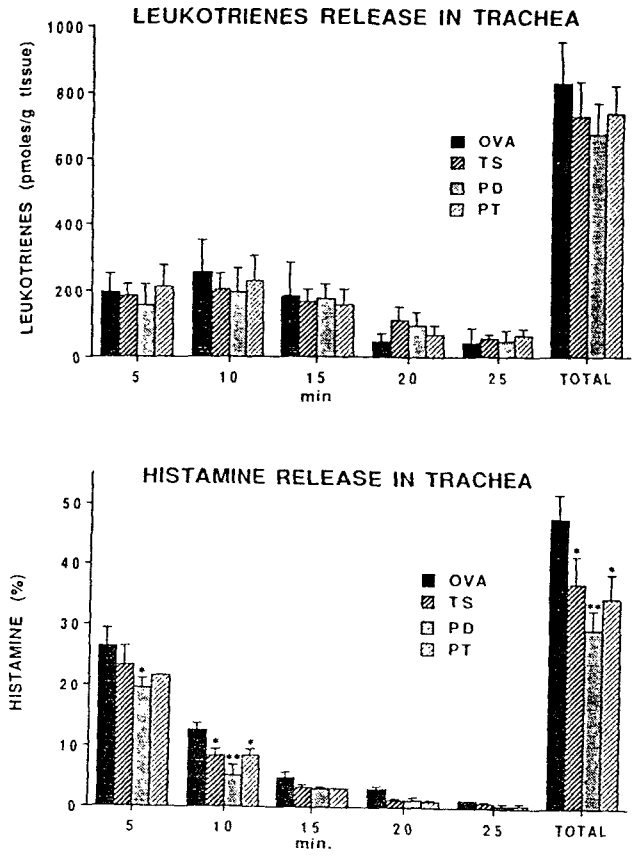

Fig. 2. Time course for histamine and leukotriene contents in absence and presence of ginseng saponins (50mg/kg) after challenge with OVA, 0.1 mg/mℓ, in superfused guinea pig tracheal strips.
Animals were actively sensitized with OVA(10 mg/kg) every other day for 3 times after ginseng saponins pretreatment, respectively, and then tracheal tissues were isolated, superfused OVA(0.1mg/mℓ).
Superfusates were collected, and then histamine (lower panel) and LT contents (upper panel) were determined from superfusate samples. Vertical lines indicate SEM and are not shown where they lie in the limits of the symbol. An asterisk denotes a value obtained in the ginseng saponin pretreatment is statistically different ($p<0.05$) from that obtained in the control(saline pretreatment) during same collection interval or time point after antigen challenge. The data summarized in Table 1.

Table 1. Effects of ginseng saponins on the smooth muscle contraction and the release of mediators evoked from the superfused trachea and lung parenchyma after active sensitization of guinea pig ⓐ

	Tracheal strip			Parenchymal strip		
	Contraction (%)	Histamine # (%)	Leukotrienes (pmol/g tissue)	Contraction (%)	Histamine # (%)	Leukotrienes (pmol/g tissue)
OVA	88.1 ± 3.0	48.0 ± 3.8	829 ± 125	95.5 ± 2.8	24.1 ± 2.1	673 ± 61
TS + OVA	84.5 ± 3.6	37.2 ± 4.4*	727 ± 103	91.1 ± 5.8	14.3 ± 1.5**	677 ± 74
PD + OVA	80.4 ± 6.7	29.7 ± 3.0**	676 ± 91	89.3 ± 7.9	13.4 ± 2.4**	585 ± 88
PT + OVA	81.9 ± 5.5	34.6 ± 4.1*	733 ± 85	92.7 ± 6.4	13.6 ± 2.6**	663 ± 54

⊙ Ginseng saponins(50 mg/kg) were injected 4 hrs before active sensitization. Animals were actively sensitized by three i.p. injections of OVA(10 mg/kg) on days 1, 3 and 5. After that, ginseng saponins(50 mg/kg) were injected by i.p. on every other day for 3 weeks. On 21 day after last OVA injection, tracheal and lung parenchymal tissues were isolated and challenged with OVA, 0.1mg/mℓ.

Values represent total histamine found in superfusate after OVA challenge expressed as a percentage of the total tissue histamine content.

* A value that is statistically different($P<0.05$) from the value obtained after active sensitization with OVA(10 mg/kg).

** $P<0.01$.

Table 2. Effects of ginseng saponin on the release of leukotrienes evoked from the superfused trachea and parenchyma by indomethacin pretreatment after active sensitization of guinea pig #.

Drug pretreatment	Total histamine release(%)		Total LT release(pmole/g)	
	Trachea	Parenchyma	Trachea	Parenchyma
Control				
OVA	48.0 ± 3.8	24.1 ± 2.1	829 ± 125	673 ± 61
TS+OVA	37.2 ± 4.4*	14.3 ± 1.5**	727 ± 103	677 ± 74
PD+OVA	29.7 ± 3.0**	13.4 ± 2.4**	676 ± 91	585 ± 88
PT+OVA	34.6 ± 4.1**	13.6 ± 2.6**	733 ± 85	663 ± 54
Indomethacin				
OVA	53.2 ± 3.7	29.5 ± 2.7	1380 ± 96ⓐ	1280 ± 120ⓐ
TS+OVA	42.7 ± 4.0*	19.0 ± 2.1*	1300 ± 150ⓐ	1150 ± 58ⓐ
PD+OVA	34.5 ± 2.1**	15.3 ± 2.1**	1150 ± 180ⓐ	1006 ± 88ⓐ
PT+OVA	40.2 ± 3.1**	16.5 ± 1.4**	1420 ± 75ⓐ	1210 ± 80ⓐ

All procedure and pattern are the same as Table 1 except using indomethacin in the Kreb bicarbonate buffer solution.

* A value that is statistically different(*, $P<0.05$; **, $P<0.01$) from the value obtained after only OVA injection in the same experimental protocol.

ⓐ A value that is statistically different($P<0.05$) from the value obtained with OVA and the same saponin in the absense of indomethacin pretreatment.

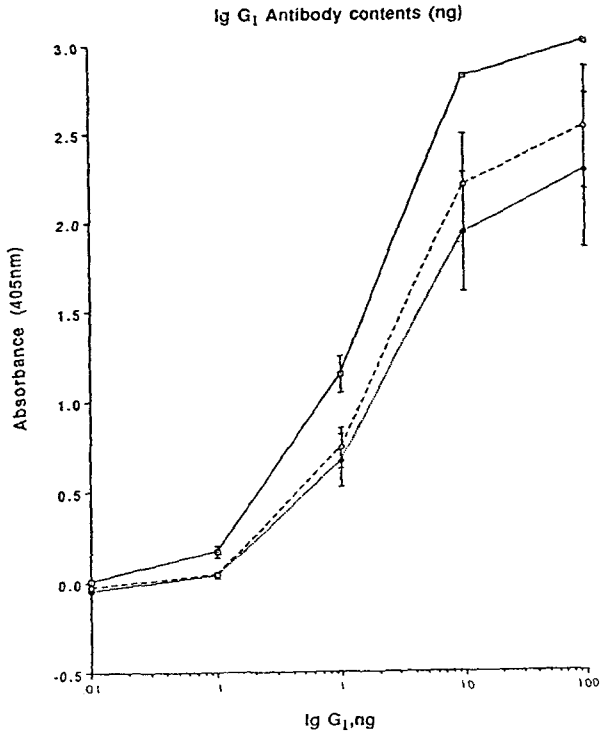

Fig. 4. Measuremet of guinea pig serum antibodies specific to OVA by ELISA after immunization with OVA alone(▣), PD+OVA(◆), and PT+OVA(○). Serum samples from indivaidual guinea pigs were assayed separately by protein A Sepharose column, and then by ELISA method ; the data shown represent the mean ± SEM of absorbance(415nm) reading.

Table 3. Antibody titer of sera obtained after active sensitization of guinea pig with OVA(10㎎/㎏) or OVA with saponin㉠.

	OVA	TS + OVA	PD + OVA	PT + OVA
Ab titer (n=8)	1600	2400	6400	3200

㉠Animals received ip injections of 50㎎/㎏ each saponin every other day for 2 weeks and then 10㎎/㎏ of OVA every other day for 3 times. After 3 weeks, serum was obtained and then IgG titer in serum was quantitated by PCA method.

Table 4. Responses of ginseng saponin on the IgG antibody content secreted into serum after active sensitization of guinea pig using column chromatography㉡

	OVA	TS + OVA	PD + OVA	PT + OVA
IgG content (㎎/㎖ serum) (n=8)	2.32 ± 0.41	2.58 ± 0.19	4.75 ± 0.57**	3.52 ± 0.38

㉡ IgG antibody secreted into serum after active sensitization of guinea pig was separated using protein A Sepharose column. Purified IgG antibody obtained by protein A column was concentrated by negative pressure filteration, read spectrophotometrically at 280nm.

** A value that is statistically different($p<0.01$) from the value obtained after active sensitization with OVA(10㎎/㎏).

Table 5. Antibody titer of sera obtained after active sensitization of guinea pig with Ox-Asc saponin㉢.

	Ox-Asc + Alum	Ox-Asc + Alum + TS
Ab titer # (n=6)	200	900*

㉢ Animals received i.p. injections of 250 ㎎/㎏ of cyclophosphamide 2 days before primary i.p. immunization with 10 ㎍ of conjugate(Ox-Asc) adsorbed to 1㎎ of Alum in 1㎖ of normal saline or in 1 ㎖ of total saponin.

Obtained by PCA method.

* $P<0.01$.

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