Natural Product Sciences
13(3) : 263-267 (2007)

Small Black Soybean (*Glycine max* Merr.) Inhibits Mast Cell-mediated Allergic Reaction and Inflammatory Cytokine Secretion

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Abstract – The mast cell-mediated immediate-type allergic reaction is involved in many allergic diseases such as asthma, allergic rhinitis, and sinusitis. The discovery of drugs for the treatment of mast cell-mediated immediate-type allergic diseases is a very important subject in human health. In this study, we investigated the effect of small black soybean (*Glycine max* Merr.) (Leguminosae) on mast cell-mediated allergic reaction and pro-inflammatory cytokine secretion. Small black soybean (SBS) inhibited compound 48/80-induced systemic reaction. SBS attenuated immunoglobulin (Ig) E-mediated local allergic reaction. In addition, SBS decreased the phorbol 12-myristate 13-acetate plus calcium ionophore A23187-stimulated tumor necrosis factor (TNF)-α and interleukin (IL)-8 secretion in human mast cells. These results indicate that SBS may be beneficial in the treatment of mast cell-mediated immediate-type allergic reactions.
Keywords – Small black soybean (*Glycine max* Merr.), Allergic reaction, Mast cells, Tumor necrosis factor (TNF)-α, Interleukin (IL)-8

Introduction

Small black soybean (*Glycine max* Merr.) (Leguminosae) is a cultivar in Korea. Small black soybean has been used as a health food in Korea, and it also has medicinal uses due to as its physiological functions. It has been reported that small black soybean has physiological activities, including antioxidant activity (Sa *et al*., 2003) and hypoglycemic effect (Lee *et al*., 2004). The primary effector cell in immediate-type allergic reactions is the mast cell. Mast cells are important mediators of inflammatory responses, such as allergy and anaphylaxis. Anaphylaxis, an acute systemic allergic reaction, is mediated by histamine released in response to the antigen cross-linking of immunoglobulin E (IgE) bound to FceRI on mast cells. Mast cell activation causes the process of degranulation that result in releasing of mediators, such as histamine and an array of inflammatory cytokines (Metacalf *et al*., 1981; Church *et al*., 1997; Miyajima *et al*., 1997). Among the inflammatory substances released from mast cells, histamine remains the best-characterized and most potent vasoactive mediator implicated in the acute phase of immediate hypersensitivity (Petersen *et al*., 1996). Mast cell degranulation also can be elicited by the synthetic compound 48/80, and it has been used as a direct and convenient reagent to study the mechanism of anaphylaxis (Ennis *et al*., 1980). Activated mast cells can produce histamine, as well as a wide variety of other inflammatory mediators such as eicosanoids, proteoglycans, proteases, and several pro-inflammatory and chemotactic cytokines such as TNF-α, IL-6, IL-4, IL-8, IL-13, and transforming growth factor-β (Burd *et al*., 1989; Plaut *et al*., 1989; Galli *et al*., 1991; Bradding *et al*., 1993). Therefore, modulation of secretion of these cytokines from mast cells can provide a useful therapeutic strategy for allergic inflammatory disease. In this study, we evaluated the effect of small black soybean (SBS) on compound 48/80-induced systemic reaction and anti-dinitrophenyl (DNP) IgE antibody-induced local allergic reaction. Additionally, the effect of SBS on phorbol 12-myristate 13-acetate (PMA) plus calcium ionophore

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A23187 (A23187)-induced TNF-α and IL-8 secretion in a human mast cell line (HMC-1) was also investigated.

Experimental

Culture of HMC-1 Cells – HMC-1 cells, a human mast cell line were grown in Iscove's media supplemented with 10% FCS and 2 mM glutamine.

Animals – The original stock of male ICR mice were purchased from Dae-Han Biolink Co. Ltd., and the animals were maintained in the College of Pharmacy, Woosuk University. The animals were housed five per cage in a laminar air flow room maintained at a temperature of 22 ± 2 °C and relative humidity of 55 ± 5% throughout the study. The care and treatment of the mice were in accordance with the guidelines established by the Public Health Service Policy on the Humane Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee.

Reagents – Compound 48/80, anti-DNP IgE, DNP-human serum albumin (HSA), phorbol 12-myristate 13-acetate, calcium ionophore A23187 were purchased from Sigma Chemical Co. (St Louis, MO). rTNF-α and rIL-8 were purchased from R & D Systems Inc. (Minneapolis, MN).

Preparation of SBS – Small black soybean of *G. max* (the 2003 product) were purchased at Imsil, Jeonbuk, Korea. A voucher specimen was deposited at the Herbarium of the College of Pharmacy, Woosuk University. The plant sample was extracted with purified water at 70 °C for 5 h. The extract was filtered, and lyophilized. The dried extract was dissolved in saline or Tyrode buffer A (10 mM HEPES, 130 mM NaCl, 5 mM KCl, 1.4 mM $CaCl_2$, 1 mM $MgCl_2$, 5.6 mM glucose, 0.1% bovine serum albumin) before use.

Compound 48/80-induced systemic reaction – Mice were given an intraperitoneal injection of 0.008 g/kg body weight (BW) of the mast cell degranulator, compound 48/80. SBS was dissolved in saline and administered intraperitoneally ranging from 0.01 to 1 g/kg BW 1 h before the injection of compound 48/80 (n = 10/group). In the time dependent experiment, SBS (1 g/kg) was administered intraperitoneally at 5, 10, 15, and 20 min after injection of compound 48/80 (n = 10/group). Mortality was monitored for 1 h after induction of anaphylactic shock.

PCA reaction – The mice were injected intradermally with 0.5 μg of anti-DNP IgE into each of 2 dorsal skin sites that had been shaved 48 h earlier. The sites were outlined with a water-insoluble red marker. After 48 h, each mouse was received an injection of 1 μg of DNP-HSA in PBS containing 4% Evans blue (1 : 4) via the tail vein. SBS (0.001 to 1 g/kg BW) was intraperitoneally administered 1 h before the challenge. Thirty minutes after the challenge, the mice were killed and the dorsal skin was removed for measurement of the pigment area. The amount of dye was determined colorimetrically after extraction with 1 mL of 1 M KOH and 9 mL of a mixture of acetone and phosphoric acid (5 : 13) based on the previous report (Katayama *et al*., 1978). The absorbent intensity of the extraction was measured at 620 nm by using a spectrophotometer, and the amount of dye was calculated with Evans blue measuring-line.

Assay of TNF-a and IL-8 secretion – TNF-α and IL-8 secretion was measured by modification of an enzyme-linked immunosorbent assay (ELISA) as described previously (Scuderi *et al*., 1986). HMC-1 cells were cultured with a-MEM plus 10% FBS and resuspended in Tyrode buffer A. The cells were sensitized with PMA (20 nM) plus A23187 (1 μM) for 8 h in the absence or presence of SBS. The ELISA was performed by coating 96-well plates with 6.25 ng/well of monoclonal antibody with specificity for TNF-α and IL-8 respectively. Before use and between subsequent steps in the assay, the coated plates were washed twice with PBS containing 0.05% Tween-20 and twice with PBS alone. For the standard curve, rTNF-α and rIL-8 were added to serum previously determined to be negative to endogenous TNF-α and IL-8. After exposure to the medium, the assay plates were exposed sequentially to biotinylated anti-human TNF-α and IL-8, 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) tablets substrates. Optical density readings were made within 10 min of the addition of the substrate with a 405 nm filter.

Statistical analysis – Statistical analyses were performed using SAS statistical software (SAS Institute, Cary, NC). Treatment effects were analyzed using one-way ANOVA, followed by Duncan's multiple range tests. $P < 0.05$ was used to indicate significance.

Results

SBS inhibits compound 48/80-induced systemic reaction – Compound 48/80 (0.008 g/kg) was used as a model of induction of systemic fatal allergic reaction. After the intraperitoneal injection of compound 48/80, the mice were monitored for 1 h, after which the mortality rate was determined. Compound 48/80 induced fatal shock in 100% of animals. When SBS was administered intraperitoneally at a concentrations ranging from 0.01 to 1 g/kg BW for 1 h, the mortality with compound 48/80 was dose-dependently reduced (Table 1). In addition, the mortality of mice administered with SBS (1 g/kg) 5, 10,

Table 1. Effect of SBS on compound 48/80-induced systemic allergic reaction

SBS treatment (g/kg BW)	compound 48/80 (0.008 g/kg BW)	mortality (%)
none (saline)	+	100
0.01	+	100
0.05	+	60
0.1	+	30
0.5	+	0
1	+	0
1	–	0

Groups of mice (n = 10/group) were intraperitoneally pretreated with 200 μL of saline or SBS at various doses 1 h before the intraperitoneal injection of compound 48/80. Mortality (%) within 1 h following compound 48/80 injection is represented as the number of dead mice × 100/total number of experimental mice.

Table 2. Time-dependent effect of SBS on compound 48/80-induced systemic allergic reaction

SBS treatment (g/kg, BW)	time (min)	compound 48/80 (0.008 g/kg BW)	mortality (%)
none (saline)	0	+	100
1	5	+	0
	10	+	70
	15	+	80
	20	+	100

Groups of mice (n = 10/group) were intraperitoneally pretreated with 200 μL of saline or SBS. SBS (1 g/kg) was given at 5, 10, 15, and 20 min after the intraperitoneal injection of compound 48/80. Mortality (%) within 1 h following compound 48/80 injection is represented as the number of dead mice × 100/total number of experimental mice.

15, and 20 min after compound 48/80 injection increased time-dependently (Table 2).

SBS inhibits the IgE-mediated local allergic reaction – PCA is one of the most important *in vivo* models of anaphylaxis in local allergic reactions and, in part, mediated by histamine in the blood stream. Local extravasation is induced by a local injection of anti-DNP IgE followed by an intravenous antigenic challenge. SBS was administered intraperitoneally 1 h prior to the challenge with antigen. The administration of SBS showed a dose-dependent inhibition in the PCA reaction (Fig. 1).

SBS inhibits TNF-a and IL-8 secretion in HMC-1 cells – TNF-α and IL-8 are important inflammatory cytokines released from mast cells. The HMC-1 cell line is a useful cell for studying the cytokine activation pathway (Kim *et al*., 2006). Therefore, we tested the effects of SBS on the secretion of TNF-α and IL-8 from HMC-1 cells. Culture supernatants were assayed for each cytokine level by ELISA. Stimulation of HMC-1 cells

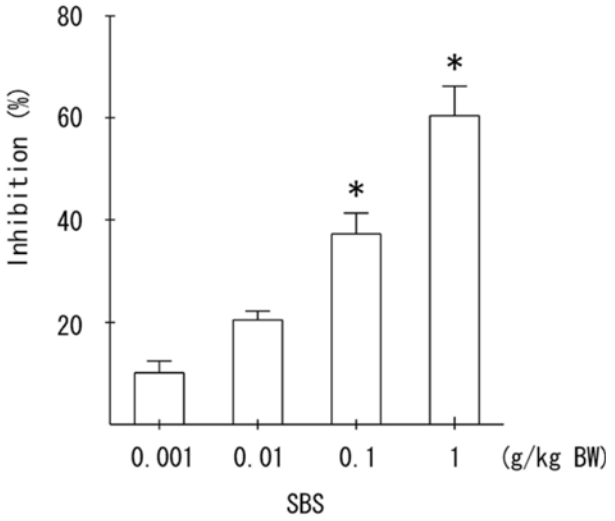

Fig. 1. Effect of SBS on the PCA reaction. SBS was intraperitonellay administered 1 h prior to the challenge with antigen. Each date represents the mean ± SEM of three independent experiments. *$p < 0.05$; significantly different from the saline value.

Table 3. Effect of SBS on the secretion of pro-inflammatory cytokines

treatment	concentration (mg/mL)	content (ng/mL)	
		TNF-α	IL-8
none (saline)		0.288 ± 0.05	0.837 ± 0.15
PMA + A23187		1.296 ± 0.14	4.106 ± 0.32
PMA + A23187 + SBS	0.01	1.081 ± 0.23	4.022 ± 1.30
PMA + A23187 + SBS	0.1	0.913 ± 0.14*	3.053 ± 0.73*
PMA + A23187 + SBS	1	0.682 ± 0.05*	2.445 ± 0.48*

PMA plus A23187-stimulated HMC-1 cells were incubated for 8 h in the absence or presence of SBS. TNF-α and IL-8 secreted into the medium are presented as the mean ± SEM of three independent experiments. *$p < 0.05$; significantly different from the PMA + A23187 value.

with PMA plus A23187 induced the secretion of TNF-α and IL-8. However, pretreatment with SBS decreased PMA plus A23187-induced TNF-α and IL-8 secretion (Table 3).

Discussion

Using *in vivo* and *in vitro* model, we show that SBS inhibits mast cell-mediated allergic responses. In the present study, we demonstrated that SBS decreased compound 48/80-induced systemic reaction and anti-DNP IgE-mediated local allergic reaction. These results indicate that mast cell-mediated immediate-type allergic reactions are inhibited by SBS. Numerous reports established that stimulation of mast cells with compound 48/80 or IgE

initiates the activation of signal-transduction pathway, which leads to histamine release. Several recent studies have shown that compound 48/80 and other polybasic compounds are able, apparently directly, to activate G-proteins (Mousli *et al*., 1990a; Mousli *et al*., 1990b; Chahdi *et al*., 2000). Compound 48/80 increases the permeability of the lipid bilayer membrane by causing a perturbation in the membrane. This result indicates that the increase in membrane permeability may be an essential trigger for the release of the mediator from mast cells. In this sense, anti-allergic agents having a membrane-stabilizing action may be desirable. SBS might stabilize the lipid bilayer membrane, thus preventing the perturbation being induced by compound 48/80. Additionally, the SBS administered mice are protected from anti-DNP IgE-mediated PCA, one of the most important *in vivo* models of anaphylaxis in local allergic reaction. This finding suggests that SBS might be useful in the treatment of allergic skin reactions. Pro-inflammatory cytokines, including TNF-α and IL-8, play a major role in triggering and sustaining the allergic inflammatory response in mast cells (Walsh *et al*., 1991; Mullarkey *et al*., 1994). Mast cells are a principal source of TNF-α in human dermis, and degradation of mast cells in the dermal endothelium is abrogated by the anti-TNF-α antibody (Walsh *et al*., 1991). IL-8 from mast cells acts on surrounding cells, such as neutrophils, T lymphocytes, and eosinophils, and plays a role in activation of inflammatory effector cells (Sengupta *et al*., 2004). These reports indicate that the reduction of pro-inflammatory cytokines from mast cells is one of the key indicators of reduced allergic symptom. In our present study, SBS inhibited the secretion of TNF-α and IL-8 in PMA plus A23187-stimulated HMC-1 cells. This result suggests that the anti-allergic effect of SBS results from its reduction of TNF-α and IL-8 release from mast cells.

In conclusion, the results obtained in the present study provide evidence that SBS contributes importantly to the prevention or treatment of mast cell-mediated allergic diseases.

Acknowledgements

"This work was supported by the Korea Research Foundation Grant funded by the Korean Government (MOEHRD)" (The Regional Universities Program/Center for Healthcare Technology Development).

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(Accepted August 22, 2007)