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Inhibitory Effect of *Brassica rapa* in Ovalbumin-Stimulated Experimental Asthmic Mice

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Abstract – To evaluate antiallergic effect of *Brassica rapa* Metzg (BR, Family Cruciferae), which is widely used as a food source, its anti-asthmic effect was investigated. BR reduced IgE level in the blood of ovalbumin (OVA)-induced asthmic mice. BR also inhibited IgE and proinflammatory cytokine IL-6 productions in trachea of OVA-induced mice, but did not inhibit IL-4 production. BR did not inhibit mRNA expression of proinflammatory cytokines IL-6 and IL-4 in IgE-induced RBL-2H3 cells. BR and its isolated components 10-undecenoic acid 2-methoxy methyl ester and galactosyl diglyceride potently inhibited the NO production of RAW264.7cells induced by lipopolysaccharide. These findings suggest that the anti-asthmic effect of BR may be due to the inhibition of the biosynthesis of proinflammatory cytokine IL-6 in macrophage and BR can improve IgE-induced allergic disease asthma.

Keywords – *Brassica rapa*, asthma, IgE, inflammation

Introduction

Allergic diseases such as asthma, allergic rhinitis, atopic dermatitis and food allergies are rapidly becoming chronic health problem in most countries (Wuthrich, 1989). Allergic asthma is chronic and complex inflammatory disease of the lung characterized by reversible obstruction of airway, hyperresponiveness, infiltration of inflammatory cells into lung tissues, mucus overproduction and the overexpression of Th2-mediated cytokines including IL-4 and TNF-α in the airways of allergic asthmatics (Nakajima and Takatsu, 2006). The etiology of asthma reactivity is based on IgE-mediated pharmacological processes of a variety of cell populations such as mast cell and basophils (Stevens and Austen, 1989). Anti-histamines, steroids and immunosuppressants have been using against allergic diseases (Sakuma *et al.*, 2001; Schafer-Korting *et al.*, 1996; Simons, 1992). However, improving these diseases is too difficult. Therefore, herbal medicines and foods have been advanced for allergic diseases, and its effectiveness has received increasing attention (Bielory, 2004).

Brassica rapa (Family Cruciferae), which contains volatile isothiocyanates, glycosinolate, daucosterol, 4-hydroxyl cinnamyl alcohol, etc, are widely used in a food source (Park *et al.*, 1999; Kim *et al.*, 2004). Its biological activities except of hypolipidemic and hepatoprotective effects (Kim *et al.*, 1999; Choi *et al.*, 2006) have not been thoroughly studied.

Therefore, in the present study, the antiasthmic effect of BR was investigated in experimental mice induced by ovalbumin (OVA).

Experimental

Materials – Dexamethasone (Dx), lipopolysaccharide (LPS) and OVA were purchased from Sigma Co. (St Louis, MO, U.S.A).

Extraction of BR and isolation of its components – BR cultured in Ganghwado was extracted with 80% ethanol, and then concentrated under vaccuo. The extract was used in BR extract in the present study (yield, 5.6%). In addition, its constituents BRE-10-1 (linoleic acid methyl ester), BRE-10-2 (10-undecenoic acid 2-methoxy methyl ester,), BRE-10-3 (linolenic acid methyl ester), BRE-10-4 (oxiraneoctanoic acid 3-octyl methyl ester) and BRE-11-13-3 (galactosyl diglyceride) were isolated from BR extract

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according to the previously reported methods (Kim *et al.*, 2004).

Experimental asthmic mice – The BALB/c mice were sensitized by an intraperitoneal injection of the mixture of 1 mL of 0.005% ovalbumin (OVA) and 1 mL of aluminum hydroxide gel (alum, Rehydragel; Reheis, Berkeley Heights, NJ, USA). The non-sensitized mice received an intraperitoneal injection of alum alone. On the 10^{th} day, the mice were given an intraperitoneal booster injection of the same antigen or alum. On the 17^{th} day after sensitization, the mice were challenged with aerosolized 5% OVA, which was generated by an ultrasonic nebulizer (Ultra-Neb 99; DeVilbiss, Somerset, PA, USA). The aerosol was circulated through a large acrylic cylindrical chamber, and mice were placed into the chamber for 1 h in a single day. The OVA aerosol challenge was repeated for 5 days. On the next (6^{th}) day, the mice were sacrificed. The levels of IgE and cytokines in the blood and trachea were measured by ELISA assay (Mastuda *et al.*, 2002).

Enzyme-linked immunosorbent assay (ELISA) – Trachea prepared from experimental asthmic mice was homogenated in 50 mM Hepes buffer. The supernatant (50 μL) and serum prepared the blood were transferred into 96-well ELISA plates, and the IgE, IL-4 and IL-6 concentrations then determined using commercial ELISA Kits (Pierce Biotechnology, Inc., Rockford, IL, USA).

Reverse transcription – polymerase chain reaction (RT-PCR) – The test agents were treated, after RBL-2H3 cells pretreated with IgE was stimulated with antigen (DNP-HSA) for 1 h, incubated for 20 min at the CO_2 incubator and then DNP-HSA treated for 40 min. RT-PCR analysis for RBL-2H3 cells treated with test agents and/or IgE with DNP-HSA was performed by the modified method of Shin *et al.* (2005). Briefly, the cells were collected and total RNA was extracted by RNeasy Mini kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instruc-tions. RT-PCR was used to analyze the expression of mRNA for IL-4, IL-6 and b-actin. The primers were designed as described by UniSTS database: IL-4, forward primer 5'-ACCTTGCTGTCACCCTGTT CTGC-3' and reverse primer 5'-GTTGTGAGCGTGGAC TCATTC ACG-3' (product size 352 bp); IL-6, forward primer 5'-CTCCGCAAGAG ACTTCCAGC-3' and reverse primer 5'-ACTCCAGGTAGAAACGGAACT-3' (product size 356 bp); GAPDH, forward primer 5'-ACCACAGTC CATGCCATCAC-3' and reverse primer 5'-TCCACCAC CCTGTTGCTGTA-3' (product size 452 bp). Optimization of cycle number was performed to ensure that production accumulation was in the linear range. Amplified products were separated by electro-phoresis on 2% agarose gel containing ethidium bromide. The gels were photographed under UV light. The GAPDH gene was used as an internal control.

Statistics – All the data were expressed as the mean ± standard deviation, and statistical significance was analyzed by one way ANOVA followed by Student-Newman-Keuls test.

Results and Discussion

To evaluate the anti-asthmic effect of BR in an experimental animal, we investigated its inhibitory effect against experimental asthmic mice induced by OVA. OVA increased blood IgE level in the mice (Fig. 1). However, the treatment of BR with OVA inhibited the increment of IgE production in the blood of asthmic mice. Therefore, we also investigated its inhibitory effect against proinflam-matory cytokine IL - 6 production in the trachea of the asthmic mice by ELISA assay (Fig. 2). BR potently inhibited it. However, BR did not inhibit the production of IgE-inducing cytokine IL4.

To understand antiasthmic mechanism of BR, when the degranulation-inhibitory activities of BR and its

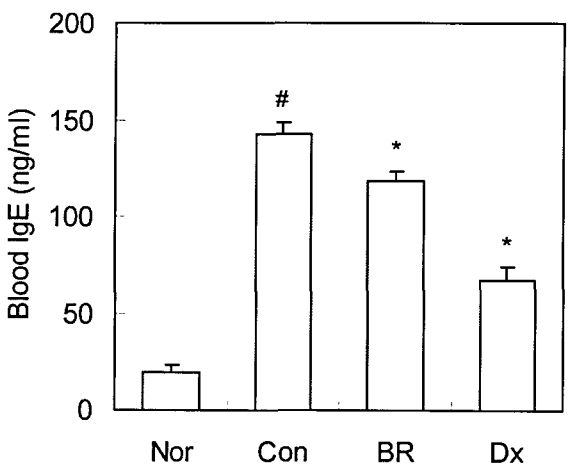

Fig. 1. Effect of BR on the IgE level in the blood of asthmic mice induced by OVA. The BALB/c mice were sensitized with an intraperitoneal injection of OVA mixed with alum and given a booster injection. The mice were challenged with aerosolized 5% OVA for 5 days after sensitization. The OVA was not treated in the mice of normal group (Nor). Test agents [Con, saline alone; BR, 50 m/kg BR; and Dx, dexamethasone (10 mg/kg)] were orally administered 1 h before the OVA aerosol challenge. On the next day, the mice were sacrificed and the level of IgE in the blood was measured using an ELISA kit (BD Biosciences, San Diago, CA, USA). The IgE values indicate mean ± S.D. (n = 5).

The control group is significantly different ($p < 0.05$) compared with the normal group.

*Significantly different ($p < 0.05$), compared with the control group.

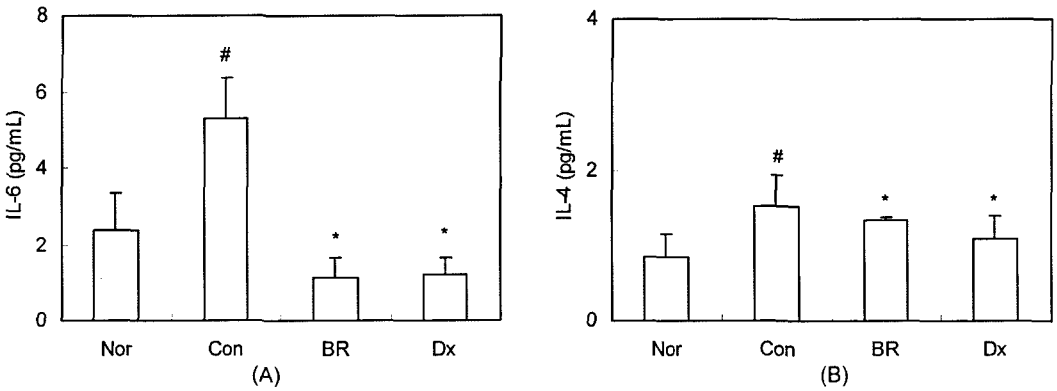

Fig. 2. Effect of BR on the levels of IgE (A) and cytokines IL-4 (B) and IL-6 (C) in the trachea and lung of asthmic mice induced by OVA. The sensitization to mice was performed like Fig. 1. Nor, normal group (saline alone); Con, saline alone in OVA-induced mice; BR, 50 mg/kg BR; and Dx, dexamethasone (10 mg/kg). The cytokine values indicate mean ± S.D. (n = 5).
[#] The control group is significantly different ($p < 0.05$) compared with the normal group.
*Significantly different ($p < 0.05$), compared with the control group.

Table 1. Inhibitory effect of BR and its components on degranulation of RBL-2H3 cells induced by IgE-antigen complex and NO production of RAW264.7 cells and their DPPH radical scavenging activities

Agent	IC_{50} (g/mL)		
	Degranulation	NO production	DPPH radical
BR extract	> 50	> 50 (35)[a)]	> 200
BRE 10-1	> 50	> 50	> 200
BRE 10-2	> 50	6.8	> 200
BRE 10-3	> 50	> 50	> 200
BRE 10-4	> 50	44.1	> 200
BRE 11-13-3	> 50	8.7	121
Azelastine	36.4	–	–
Dexamethasone	–	10.5	–
Caffeic acid	–	–	4.8

[a)] The value of parenthesis indicated in inhibition percent against the NO production of RAW264.7 cells.

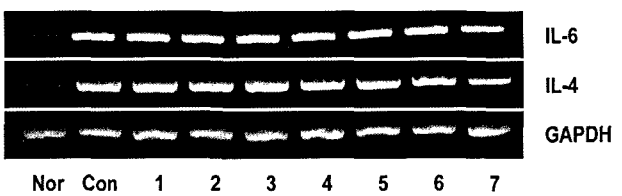

Fig. 3. Effect of BR on mRNA expression of IL-6 and IL-4 in RBL-2H3 cells induced by IgE-antigen compex. RBL-2H3 cells (5×10^5 cells) were treated with 0.5 μg/ml of mouse monoclonal IgE, exposed to 0.2 mL of agents [Nor, normal; Con, control treated with vehicle alone; 1, 10-undecenoic acid 2-methoxy methyl ester (UM) 5 μg/mL; 2, UM 20 μg/mL; 3, UM 50 μg/mL; 4, galactosyl diglyceride (GD) 5 μg/mL; 5, GD 20 μg/mL; 6, GD 50 μg/mL; and 7, Dexamethasone 10 μM] for 4 h, followed by the treatment with 0.2 mL of dinitrophenol-human serum albumin (DNP-HSA, 1 μg/mL) for 40 min at 37 °C and then RT-PCR for IL-6 and IL-4 was performed. Normal was treated with vehicle alone instead of agents and IgE-antigen. Values represent the mean ± S.D. for duplicate experiments. [#]Significantly different from the normal control group ($^{\#}P < 0.05$). *Significantly different from the control group ($^{*}P < 0.05$).

isolated components against RBL-2H3 cells were measured (Table 1), BR and its components did not inhibit the degranulation of RBL-2H3 cells induced by IgE-antigen complex. However, BR inhibited the NO production of RAW264.7 cells. Of the components isolated from BR, 10 - undecenoic acid 2-methoxy methyl ester and galactosyl diglyceride potently inhibited it. Therefore, their inhibitory effects in mRNA expression of proinflammatory cytokines IL-4 and IL-6 in RBL-2H3 cells induced by IgE-antigen complex were measured by RT-PCR assay (Fig. 3). BR and its components except galactosyl diglyceride did not inhibit the mRNA expression of these cytokines. Galactosyl digly-ceride alone weakly inhibit the production of these cytokines. Betamethasone, a representative, anti-allergic medicine (Schafer- Korting *et al.*, 1996), was also found to reduce blood IgE level in mice induced by OVA. BR did not only inhibit the increment of blood IgE level in the experimental asthmic mice. However, BR reduced proinflammatory cytokine IL-6 and regenic antibody IgE in the trachea of experimental asthmic mice, although it did not inhibit the IL-4 production. The inhibition of proinflammatory cytokine expression from mast cells must be one of the key indicators of reduced allergic symptoms (Wuthrich, 1989). Mast cells and basophils are well-known as critical participants in various biologic processes of allergic diseases (Bruhns *et al.*, 2005; Plaut *et al.*, 1989). These cells express surface membrane receptors with high affinity and specificity for IgE. The interaction

of antigen-bound IgE in surface membrane receptors releases histamine, prostaglandins, leukotrienes and cytokines (Mican *et al.*, 1992; Plaut *et al.*, 1989; Zhao *et al.*, 2005). These cytokines activate chemotaxis and phagocytosis of neutrophils and macrophages. Finally cytokine-induced reactions cause tissue inflammation. However, BR and its components did not inhibit the production of these cytokines in RBL-2H3 cells induced by IgE-antigen complex. These results suggest that BR may not directly inhibit the degranulation of mast cells and the biosynthesis of proinflammatory cytokines in RBL-2H3 cells. However, BR potently inhibited the production of IL-6, not IL-4, in experimental asthmic mice. BR and its components potently inhibited the NO production in LPS-stimulated RBL-2H3 cells. It suggests that the anti-asthmic effect of BR may be due to the inhibition of IL-6 biosynthesis in macrophages.

Based on these findings, BR may be useful in the protecting against IgE-mediated allergic diseases.

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