



Anti-asthmatic effect of marine red alga (*Laurencia undulata*) polyphenolic extracts in a murine model of asthma

Won-Kyo Jung^{a,1}, Inhak Choi^{b,1}, Sangtaek Oh^c, Sae-Gwang Park^b, Su-Kil Seo^b, Soo-Woong Lee^b, Dae-Sung Lee^d, Soo-Jin Heo^e, You-Jin Jeon^e, Jae-Young Je^f, Chang-Bum Ahn^f, Jin Soo Kim^g, Kwang Soo Oh^g, Young-Mog Kim^h, Chisook Moonⁱ, Il-Whan Choi^{b,*}

^a Department of Marine Life Science and Marine Life Research Center, Chosun University, Gwangju 501-759, Republic of Korea

^b Department of Microbiology and Bio-Marker Research Center for Personalized Therapy, College of Medicine, Inje University, Busan 614-735, Republic of Korea

^c Pharmacogenomics Research Center, Inje University, Busan 614-735, Republic of Korea

^d Department of Microbiology, Pukyong National University, Busan 608-737, Republic of Korea

^e Faculty of Applied Marine Science, Cheju National University, Jeju 690-756, Republic of Korea

^f Department of Food Science and Aquaculture Medicine, Chonnam National University, Yeosu 550-749, Republic of Korea

^g Division of Marine Life Science, Gyeongsang National University, Tongyeong 650-160, Republic of Korea

^h Faculty of Food Science and Biotechnology, Pukyong National University, Busan 608-737, Republic of Korea

ⁱ Department of Internal Medicine, Busan-Paik Hospital, Inje University, Busan 614-735, Republic of Korea

ARTICLE INFO

Article history:

Received 13 June 2008

Accepted 3 November 2008

Keywords:

Red alga

Laurencia undulata

Asthma

Cytokines

Airway hyperresponsiveness

ABSTRACT

The aim of the present work is focused on protective effects of an edible red alga, *Laurencia undulata* ethanolic (EtOH) extracts (LU) containing a large amount of polyphenols against OVA-induced murine allergic airway reactions using in vivo histological and cytokine assay. Mice sensitized and challenged with ovalbumin (OVA) showed typical asthmatic reactions as follows: an increase in the number of eosinophil in bronchoalveolar lavage fluid; a marked influx of inflammatory cells into the lung around blood vessels and airways, and airway luminal narrowing; the development of airway hyperresponsiveness; the detection of TNF- α and Th2 cytokines, such as IL-4 and IL-5 in the bronchoalveolar lavage (BAL) fluid; and detection of allergen-specific IgE in the serum. The successive intraperitoneal administration of LU before the last airway OVA-challenge resulted in a significant inhibition of all asthmatic reactions. These results suggest that *L. undulata* polyphenolic extracts possess therapeutic potential for combating bronchial asthma associated with allergic diseases.

© 2008 Elsevier Ltd. All rights reserved.

1. Introduction

Marine algae have been consumed in Asia since ancient times, which are rich in vitamins, minerals, dietary fibers, proteins, polysaccharides, and various functional polyphenols. Moreover, seaweeds are considered to be a rich source of antioxidant. Hence, many types of seaweed have been examined to identify new and effective antioxidant compounds, as well as to elucidate the mechanisms of cell proliferation, anti-inflammation and apoptosis (Yuan and Walsh, 2006; Heo et al., 2006; Nahas et al., 2007; Kim et al., 2008). Recently, many countries are more closely looking at algae as a potent target for bioactive substances because they have showed some possibility for valuable uses and applications in the fields of nutraceuticals and pharmaceuticals (Yamamoto and Maruyama, 1985; Okai et al., 1994). The previous studies (Athukorala et al., 2008; Jung et al., 2007) had supported some proof on phar-

maceutical and medicinal importance of seaweeds. Currently, to elucidate their medicinal benefits on immune system, our present studies are focused on protective activity against allergic diseases such as asthma, increased in both developing and developed countries.

Among various allergic diseases, especially, asthma is a complex inflammatory disease of the lung characterized by variable airflow obstruction, airway hyperresponsiveness (AHR), and airway inflammation. The inflammatory response in the asthmatic lung is characterized by infiltration of the airway wall by mast cells, lymphocytes and eosinophils (Elias et al., 2003) and is associated with the increased expression of several inflammatory proteins, including cytokines, enzymes (Silverman et al., 1998), and adhesion molecules in the airways (Barnes, 1994; Pawankar, 2002). The T helper 2 (Th2)-type cytokines such as interleukins (IL)-4, IL-5, and IL-13, produced by activated CD4⁺ T cells play a central role in the pathogenesis of asthma (Corrigan and Kay, 1992; Renz et al., 1993) by controlling the key process of immunoglobulin E (IgE) production, growth of mast cells and the differentiation and activation of mast cells and eosinophils (Punnonen et al., 1994; Saito et al., 1998;

* Corresponding author. Tel.: +82 51 890 6461; fax: +82 51 891 6004.

E-mail address: cihima@inje.ac.kr (I.-W. Choi).

¹ These authors contributed equally to this work.

Iwamoto et al., 1993). The direction of Th cell differentiation is determined by the cytokine environment at the site of initial antigenic activation. It has been well documented that the presence of IL-4 during the induction phase accounts for a predominance of Th2 cells (Kopf et al., 1993), which subsequently determine the allergic inflammatory responses. Th2 cells are the predominant lymphocyte population that infiltrates the airways of asthmatics, and the cytokine products of Th2 cells perform essential roles in airway eosinophilia, AHR, and serum IgE in animal models (Wills-Karp, 1999).

The red algal genus *Laurencia* is known to produce a wide array of natural products exhibiting some kind of biological activities, due to produce natural bioactive materials of polysaccharides, polyphenols, terpenes and the other halogenated secondary metabolites. They have been shown to possess antioxidant, anticancer and antibacterial activities against marine bacteria as reported in the previous studies (Liang et al., 2007; Pec et al., 1999; Vairappan et al., 2001). Taken together, these results, especially antioxidant activity, lead to the hypothesis that *Laurencia* might be associated with a reduced incidence of allergic asthma. Asthma in both adults and children is associated with increased oxidative stress. However, to our knowledge, no previous report has described the anti-allergic effects of *Laurencia*. During our screening for anti-allergic activity, potent antioxidant effect was detected from an ethanolic extracts of *Laurencia undulata* (*L. undulata*) containing a large amount of polyphenols. The anti-asthmatic activity of *L. undulata* has never been reported. Therefore, the objective of the present work is focused on identification of anti-asthmatic effect of *L. undulata* polyphenolic extracts (LU) against OVA-induced allergic airway reactions in experimental murine model of asthma.

2. Materials and methods

2.1. Preparation of *L. undulata* polyphenolic extracts

L. undulata polyphenolic extracts (LU) were prepared according to the method of Heo et al. (2006). The red seaweed, *L. undulata* was collected along Jeju Island coast of Korea during the period from October 2005 to February 2006. Fresh *L. undulata* was washed three times with tap water to remove salts, epiphytes and sand attached to the surface of the samples and stored at -20°C . The frozen samples were lyophilized and homogenized using a grinder before extraction. The dried *L. undulata* powder (1 kg) was extracted with 95% EtOH (1:10 w/v) at 20°C for 3 days and evaporated in vacuo. The concentrated LU was freshly dissolved in DMSO before use.

Phenolic contents of LU were determined by the method of Shetty et al. (1995). Each 1 ml of the algal extract, 1 ml of 95% EtOH, 5 ml of distilled water, and 0.5 ml of 50% Folin–Ciocalteu reagent (Sigma Chemical, St. Louis, MO) were mixed. The mixtures were allowed to react for 5 min, and then 1 ml of 5% Na_2CO_3 was added, and placed in the dark for 1 h. Absorbance was measured at 725 nm and gallic acid standard curve was obtained for the calibration of phenolic content. Total carbohydrate was measured by the phenol–sulfuric acid method of Dubois et al. (1956), using a mixture of galactose and fucose (1:1 weight ratio) as a standard. The sulfate content in the total carbohydrate of LU was measured by the BaCl_2 /gelation method (Saito et al., 1968).

2.2. Animals

Female C57BL/6 mice were obtained from the Charles River Laboratories (Yokohama, Japan), and were kept in our animal facility for at least 1 week before use. All experimental animals used in this study were maintained under a protocol approved by the Institutional Animal Care and Use Committee of the Inje University Medical School. All mice were at 6–8 weeks of age.

2.3. Immunization and challenge

Mice were immunized on days 1 and 15 by intraperitoneal injection of 20 μg ovalbumin (OVA) emulsified in 1.0 mg aluminium hydroxide adjuvant in a total volume of 200 μl . Mice were challenged via the airway with OVA (50 mg/ml of saline) each day from days 22–24 on consecutive days. Control mice were exposed to aerosolized saline. Aerosolization was performed for 20 min by placing the mice in a chamber (15 $\times$ 25 $\times$ 15 cm) connected to the ultrasonic nebulizer (NE-U12, Omron, Tokyo, Japan). Mice were injected intraperitoneally with 20 mg/kg/day in 200 μl of *L. undulata* each day from days 16 to 20 on consecutive days.

2.4. Bronchoalveolar lavage and cell counting

The mice were sacrificed with a lethal dose of pentobarbital, the trachea of mice was cannulated while gently massaging the throat. Lungs were lavaged with 0.7 ml of PBS. The BAL fluid samples collected and the number of cells in a 50 μl aliquot were determined using a haemocytometer. The remaining sample was centrifuged, and the supernatant was stored at -70° until assay of cytokines. The pellet was resuspended in PBS and cytospin preparations of BAL cells were stained with Diff-Quik (International reagents Corp., Kobe, Japan). Two independent, blinded investigators counted the cells, using a microscope. Approximately, 400 cells were counted in each of four different random locations.

2.5. Cytokine assays

The levels of cytokines in bronchoalveolar lavage (BAL) fluid were determined by enzyme-linked immunosorbent assay (ELISA). ELISA kits from R&D Systems (Minneapolis, MN) were employed for the measurement of IL-4, IL-5, and TNF- α .

2.6. Determination of airway hyperresponsiveness

Airway hyperresponsiveness (AHR) was measured at 3 days after the last OVA-challenge in mice in an unrestrained conscious state. Mice were placed in a barometric plethysmographic chamber (All Medicus Co., Seoul, Korea) and baseline readings were taken and averaged for 3 min. Aerosolized methacholine (Mch) in increasing concentrations (from 2.5 to 50 mg/ml) was then nebulized through an inlet of the main chamber for 3 min, readings were taken and were averaged for 3 min after each nebulization. The bronchopulmonary resistances is expressed as enhanced pause (Penh), calculated as: [expiratory time (Te)/relaxation time (RT) – 1] $\times$ [peak expiratory flow (PEF)/peak inspiratory flow (PIF)], according to the manufacturer's protocol. Results are expressed as the percentage increase of Penh following challenge with each concentration of Mch, where the baseline Penh (after saline challenge) is expressed as 100%.

2.7. Histological studies

At 48 h after the last challenge, lungs were removed from the mice after they had been sacrificed. Prior to the removal of the lungs, the lungs and trachea were filled intratracheally with a fixative (4% paraformaldehyde) using a ligature around the trachea. Lung tissues were fixed with 10% (v/v) paraformaldehyde. The specimens were dehydrated and embedded in paraffin. For histological examination, 4 μm sections of fixed embedded tissues were cut on a Leica model 2165 rotary microtome (Leica, Nussloch, Germany), placed on glass slides, deparaffinized, and sequentially stained with hematoxylin 2 and eosin-Y (Richard-Allan Scientific, Kalamazoo, MI). An inflammation score was graded by three independent investigators who were not associated with this study.

2.8. Measurement of OVA-specific serum levels of IgE

OVA-specific serum IgE levels were determined by Enzyme-Linked Immuno Sorbent Assay (ELISA) using samples collected 12 h after the last OVA-challenge. In brief, a 96 well microtitre plate was coated with OVA (10 mg/ml), and then treated with mouse sera followed by biotin-conjugated rat anti-mouse IgE (pharmin-gen, San Diego, CA). Then, avidin-horseradish peroxidase (HRP) solution was added to each well. Units are optical density readings at 405 nm.

2.9. Statistical analysis

Data are expressed as means $\pm$ S.E.M. Student's *t*-test was used to analyse data between the groups and analysis of variance (ANOVA) among groups followed by Dunnett's *t*-test for multiple comparisons. The statistical significance was set at $P < 0.05$.

3. Results and discussion

3.1. Preparation of *L. undulata* polyphenolic extracts (LU)

To identify anti-asthmatic effect of *L. undulata* polyphenolic compounds against OVA-induced murine allergic airway reactions, the dried alga was extracted by 95% EtOH. The yield of *L. undulata* EtOH extracts (LU) was 8.9% (% dry wt. of alga). In the chemical analysis, total phenolic content of *L. undulata* EtOH extracts (LU) was determined as 43.6 ± 0.3 mg/g ($n = 3$). However, low amounts of carbohydrate (1.2 ± 0.1 mg/g, $n = 3$) were detected in LU. The sulfate content of LU was determined as 0.07 (sulfate/total sugar). As reported by Sugiura et al. (2006), methanol extracts of brown alga (*E. arborea*) mainly include phenolic compounds (10.5 ± 0.1 mg/g).

They mentioned that the anti-histamine effects of the *E. arborea* extract were likely to be attributed to phenolic compounds like phlorotannins, due to low yield of polysaccharides by alcohol extraction. The previous study (Shibata et al., 2003) had identified the inhibitory effects of brown algal phlorotannins on phospholipase A2s, lipoxygenases and cyclooxygenases. Although red algae are an abundant source of sulfated polysaccharides like sulfated galactans with several bioactivities (Pereira et al., 2005), macromolecular polysaccharides such as alginate and fucoidan are poorly extracted in ethanol. Hence, Athukorala et al. (2008) performed enzymatic hydrolysis of marine algae with several carbohydrases to extract higher amount of polysaccharides with large molecular size.

3.2. In vivo study for anti-asthmatic effects of *L. undulata* extracts (LU)

In the murine asthma model, OVA-induced asthma has been recognized as a disease characterized by increased levels of influx of inflammatory cells into the lung, mucus hypersecretion, and airway occlusion, and the thicknesses of the bronchial wall and the area of the smooth muscle. In addition, in many allergic inflammatory diseases, elevations in the plasma level of IgE, inducing the degranulation of mast cells via the cross-linking of allergen-specific IgE.

3.2.1. Effect of LU extracts on cellular changes in BAL fluid and pathological changes in lung tissues

First, we examined how LU made effect on the development of OVA-induced eosinophilia in murine model of asthma. Airway allergen challenge causes cellular inflammation in the airways, which is dominated by eosinophils in both humans and mice (Kroegel et al., 1994). It has been suggested that eosinophils contribute to several of the clinical features of allergic asthma, including tissue damage and AHR (Lotvall et al., 1998). OVA sensitization and challenge remarkably increased the number of inflammatory cells, containing eosinophils and macrophages in the BAL fluid compared to the control group. Pathological processes that result in lung eosinophilia may involve antigen-induced T cell activation through macrophages or other antigen presenting cells, T cell cytokine release, specific sensitization of mast cells, and release of activating mediators by macrophages (Drazen et al., 1996). The total cell numbers in BAL fluids were significantly increased, about 24-fold, compared to those in the control group 2 days after the last OVA-challenge. The pattern of aeroantigen-induced airway eosinophilia and AHR in the murine models described by several investigators (Takeda et al., 1997; Corry et al., 1996) may differ depending on the mouse strains, the immunization regimen, and the frequency and duration of the aerolized antigen challenge. Using the present model, we have observed that the number of eosinophils occupied 60% more of the total cells in BAL fluid. The number of eosinophils in BAL fluids was increased about 16-fold, compared to those in the control group at 2 days after the OVA-challenge. Interestingly, the number of eosinophils observed in BAL fluids in the LU treated group of mice decreased to the level of 0.22-fold of OVA-challenge group (Fig. 1). The results of histological examination of the lung tissues paralleled the cell numbers in the BAL fluids (Fig. 2). Marked influxes of inflammatory cells into the airway and around the blood vessels were observed in the OVA-sensitized/challenged mice, but not in the saline-treated control mice. Mice treated with LU showed marked reductions in the infiltration of inflammatory cells in the peribronchiolar and perivascular regions. Mucus hypersecretion and occlusion of the airways were the prominent histopathologic features of the murine asthmatic lung. Mucus cell hypertrophy and airway luminal narrowing caused by secreted mucus were observed in the OVA-sensitized/challenged mice. Administration of LU resulted in a marked

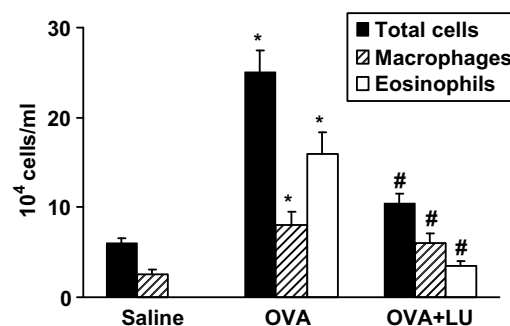


Fig. 1. Effects of *L. undulata* extracts (LU) on the recruitment of inflammatory cells into BAL in OVA-induced allergic asthmatic mice. Mice were treated with the Saline, OVA-inhaled mice administered saline (OVA), and OVA-inhaled mice administered *L. undulata* extract 20 mg/kg (OVA + LU), respectively. BAL cells were separated using a Cytospin, and then stained with Diff-Quik. Differential cell counting was performed using standard morphological criteria. The BAL cells were collected 2 days after the last OVA-challenge. Each value indicates the mean $\pm$ S.E.M. from five separate experiments ($n = 6$ per group). * $p < 0.05$ vs. saline-treated mice; # $p < 0.05$ vs. OVA-treated mice.

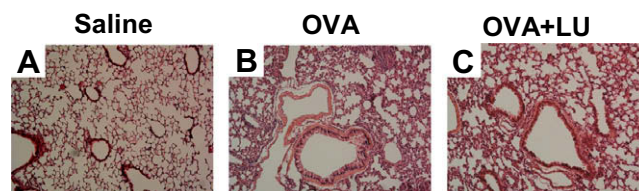


Fig. 2. *L. undulata* extracts (LU) inhibits pathological changes in lung tissues of OVA-sensitized and -challenged mice. Mice were sensitized and challenged as described in methods. Sections were obtained from the lungs of mice receiving saline, OVA-inhaled mice administered saline (OVA), and OVA-inhaled mice administered *L. undulata* extract (20 mg/kg, OVA + LU), respectively. Lungs were removed 2 days after the last airway challenge. Sections were stained by haematoxylin and eosin staining ($\times 200$). Six animals were assigned to each group.

improvement of luminal narrowing in the airway. These results suggest that LU is highly capable of inhibiting the development of allergic status induced by OVA in mice.

3.2.2. Effect of LU extracts on BAL fluid cytokines

To study whether LU was related to inflammatory cytokine production, BALF supernatants were collected, and the productions of IL-4 and IL-5 were analyzed by ELISA. Initiation of allergic response appears to occur with presentation of the allergen by antigen-presenting cells to CD4⁺ T cells. Antigen-activated CD4⁺ T cells have been shown to induce many of the characteristic features of asthma, including the secretion of cytokines such as IL-4 and IL-5, which regulate mucus production, inflammation, and adhesion molecules (Hogan et al., 2003; Taube et al., 2004). Numerous studies have established that T cells from the lungs of asthmatics express a Th2 pattern of cytokines. Thus, interventions that inhibit Th2 cytokines, may be useful in the treatment of allergic asthma. Also, several studies have suggested that TNF- α plays an important role in the pathogenesis of asthma. TNF- α mRNA and protein levels are increased in the sputum or serum of patients with asthma (Bradding et al., 1994; Keatings et al., 1997) and TNF- α induces aggravation of AHR in animals (Kips et al., 1992; Zuany-Amorim et al., 1995) and human beings (Thomas et al., 1995) as well as recruitment of inflammatory cells into the airways (Zuany-Amorim et al., 1995; Lukacs et al., 1995). Preliminary studies have demonstrated an improvement in lung function, AHR, and exacerbation rate in patients treated with anti-TNF therapy. To determine if pretreatment with LU affects the secretion of cytokines in the BAL fluid, the levels of IL-4, IL-5 and TNF- α were determined by ELISA

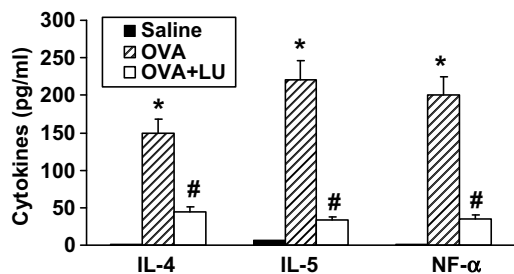


Fig. 3. The effect of *L. undulata* extracts (LU, 20 mg/kg) on IL-4, IL-5, and TNF- α cytokine levels. OVA-sensitized mice were treated as described in methods. Bronchoalveolar lavage (BAL) was performed 2 h after the last airway challenge. IL-4, IL-5, and TNF- α cytokine levels in the BAL fluids were measured by ELISA Kit. Each value indicates the mean $\pm$ S.E.M. from five separate experiments ($n = 6$ per group). * $p < 0.05$ vs. saline-treated mice; # $p < 0.05$ vs. OVA-treated mice.

(Fig. 3). BAL fluids were obtained 4 h after the last airway challenge. The levels of IL-4, IL-5, and TNF- α in the BAL fluids were significantly increased by airway challenge with OVA when compared with that of the control. The administration of LU reduced the concentration of IL-4 and IL-5 by 70% and 85%, respectively, and led to an 83.5% reduction in TNF- α secretion. It has been suggested that LU may have inhibitory activities of CD4⁺ T cells and eosinophils including asthmatic inflammation.

3.2.3. LU extracts decreases the AHR

OVA-induced asthma is characterized by AHR and inflammation of the airways (Mappe et al., 1987). This inflammation is associated with the infiltration of eosinophils, neutrophils, and lymphocytes into the bronchial lumen and lung tissues (Mappe et al., 1987; Boschetto et al., 1987). These cellular infiltrates release various chemical mediators that can cause AHR (Bousquet et al., 1990; Busse et al., 1993; Kay, 1991). Airway responsiveness was assessed as a percent increase of Penh in response to increasing doses of methacholine. In the OVA-sensitized and -challenged mice, the dose-response curve of percent Penh shifted to the left compared with that of control mice (Fig. 4). In addition, the percent Penh

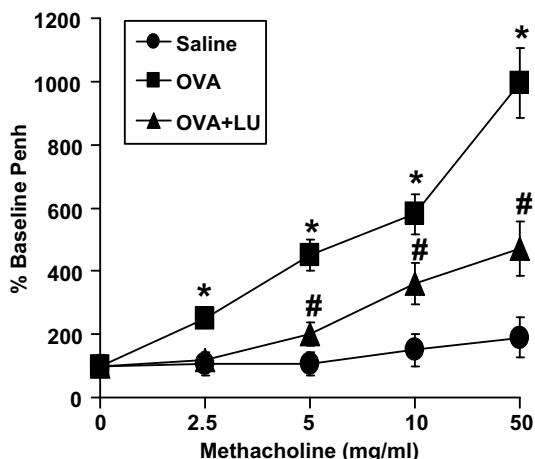


Fig. 4. Effects of *L. undulata* extracts on airway responsiveness in OVA-sensitized mice and OVA-challenged mice. Airway responsiveness was measured at 72 h after the last airway challenge. Mice were injected intraperitoneally with 20 mg/kg/day in 200 μ l of *L. undulata* each day from days 16 to 20 on consecutive days. Airway responsiveness to aerosolized methacholine was measured in unrestrained, conscious mice. Mice were placed into the main chamber and were nebulized first with saline, then with increasing doses (2.5–50 mg/ml) of methacholine for 3 min for each nebulization. Readings of breathing parameters were taken for 3 min after each nebulization during which Penh values were determined. Each value indicates the mean $\pm$ S.E.M. from five separate experiments ($n = 6$ per group). * $p < 0.05$ vs. saline-treated mice; # $p < 0.05$ vs. OVA-treated mice.

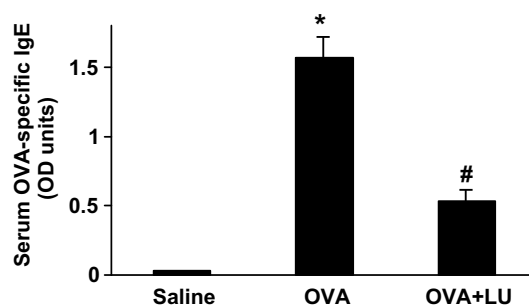


Fig. 5. Effects of *L. undulata* extracts (LU) on serum OVA-specific IgE antibody levels. Mice were sensitized and challenged as described in methods. The levels of OVA-specific IgE were measured at 12 h after the last airway challenge. Blood was collected by cardiac puncture for measurement of OVA-specific IgE with ELISA. Mice were injected intraperitoneally with 20 mg/kg/day in 200 μ l of *L. undulata* each day from days 16 to 20 on consecutive days. Each value indicates the mean $\pm$ S.E.M. from five separate experiments ($n = 6$ per group). * $p < 0.05$ vs. saline-treated mice; # $p < 0.05$ vs. OVA-treated mice.

produced by methacholine administration (at doses from 2.5 to 50 mg/ml) increased significantly in the OVA-sensitized and OVA-challenged mice compared with the controls. OVA-sensitized and OVA-challenged mice treated with LU showed a dose-response curve of percent Penh that shifted to the right compared with that of untreated mice. The shift was dose-dependent. These results indicate that LU has inhibitory effects on OVA-induced AHR.

3.2.4. LU extracts reduces the levels of OVA-specific serum IgE

Finally, we sought LU could modify OVA-specific IgE response in vivo. Because IgE levels in BALF are dependent upon IL-4, IL-5, IL-13 and may be considered an additional index of Th2 cytokine secretion in the pathogenesis of asthma, we measured IgE levels in BALF from mice in all groups. Cross-linking of allergen-specific IgE on the mast cell surface upon allergen challenge is an important key to initiate the early asthmatic reaction. Therefore, a novel therapeutic approach to asthma and other allergic respiratory diseases involves interference in the action of IgE, and this antibody has been viewed as a target for novel immunological drug development in asthma. In several clinical controlled trials anti-IgE antibodies resulted effective in reducing asthma-related symptoms, decreasing corticosteroid use and improving quality of life of asthmatic patients. The serum levels of OVA-specific immunoglobulins were measured 12 h after the last airway challenge. Sensitization and challenge with OVA resulted in increased serum levels of OVA-specific IgE when compared with the saline-treated mice. Treatment of sensitized mice with LU resulted in a 66.05% reduction in OVA-specific IgE (Fig. 5). Our data showed that serum levels of OVA-specific IgE were significantly reduced by LU pretreatment. These results support the conclusion that LU suppressed the generation of a Th2-type immune response and activity of mast cells in this animal model of asthma.

4. Conclusion

This study is the first to provide experimental evidence demonstrating that a kind of red alga, *L. undulata* EtOH extract (LU) containing a large amount of polyphenols could inhibit OVA-induced airway hyperresponsiveness and inflammation in a murine model of asthma. The administration of LU before the last airway OVA-challenge resulted in a significant inhibition of all asthmatic reactions. Although it still remains to identify chemical property and structure of polyphenolic components derived from the red alga, these results support that LU could be used as adjuvant therapeutic materials for patients with bronchial asthma. In addition, the results

can give the motive of research on the other type I allergic reactions such as allergic rhinitis, atopic dermatitis, and anaphylaxis.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Acknowledgements

This work was supported by the Korea Science and Engineering Foundation (KOSEF) grant funded by the Korean government (MOST) (No. R13-2007-023-00000-0 to IC), and by the 2005 Inje University research grant (to CM), also from the Korea Institute of Science & Technology Evaluation and Planning (KISTEP) and Ministry of Science & Technology (MOST), Korean government, through its National Nuclear Technology Program (to SO).

References

- Athukorala, Y., Jung, W.K., Park, P.J., Lee, Y.J., Kim, S.K., Vasanthan, T., No, H.K., Jeon, Y.J., 2008. Evaluation of biomolecular interactions of sulfated polysaccharide isolated from *Grateloupia filicina* on blood coagulation factors. *J. Microbiol. Biotechnol.* 18, 503–511.
- Barnes, P.J., 1994. Cytokines as mediators of chronic asthma. *Am. J. Respir. Crit. Care Med.* 150, 42–49.
- Boschetto, P., Fabbri, L.M., Zocca, E., Milani, G., Pivrotto, F., Dal Vecchio, A., Plebani, M., Mapp, C.E., 1987. Prednisone inhibits late asthmatic reactions and airway inflammation induced by toluene diisocyanate in sensitized subjects. *J. Allergy Clin. Immunol.* 80, 261–267.
- Bousquet, J., Chanez, P., Lacoste, J.Y., Barneon, G., Ghavanian, N., Enander, I., Venge, P., Ahlstedt, S., Simony-Lafontaine, J., Godard, P., 1990. Eosinophilic inflammation in asthma. *New Engl. J. Med.*, 1033–1039.
- Bradding, P., Roberts, J.A., Britten, K.M., Montefort, S., Djukanovic, R., Mueller, R., Heusser, C.H., Howarth, P.H., Holgate, S.T., 1994. Interleukin-4, -5, and -6 and tumor necrosis factor- α in normal and asthmatic airways: evidence for the human mast cell as a source of these cytokines. *Am. J. Respi. Cell. Mol. Biol.* 10, 471–480.
- Busse, W.W., William, F.C., Sedgwick, J.D., 1993. Mechanism of airway inflammation in asthma. *Am. Rev. Respir. Dis.* 147, 20–24.
- Corrigan, C.J., Kay, A.B., 1992. T cells and eosinophils in the pathogenesis of asthma. *Immunol. Today* 13, 501–507.
- Corry, D.B., Folkesson, H.G., Warnock, M.L., Erle, D.J., Matthay, M.A., Wiener-Kronish, J.P., Locksley, R.M., 1996. Interleukin 4, but not interleukin 5 or eosinophils, is required in a murine model of acute airway hyperreactivity. *J. Exp. Med.* 183, 109–117.
- Drazen, J.M., Arm, J.P., Austen, K.F., 1996. Sorting out the cytokines of asthma. *J. Exp. Med.* 183, 1–5.
- Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* 28, 350–356.
- Elias, J.A., Lee, C.G., Zheng, T., Ma, B., Homer, R.J., Zhu, Z., 2003. New insights into the pathogenesis of asthma. *J. Clin. Invest.* 111, 291–297.
- Heo, S.J., Cha, S.H., Lee, K.W., Jeon, Y.J., 2006. Antioxidant activities of red algae from Jeju island. *Algae* 21, 149–156.
- Hogan, M.B., Weissman, D.N., Hubbs, A.F., Gibson, L.F., Piktet, D., Landreth, K.S., 2003. Regulation of eosinophilopoiesis in a murine model of asthma. *J. Immunol.* 171, 2644–2651.
- Iwamoto, I., Nakajima, H., Endo, H., Yoshida, S., 1993. Interferon- γ regulates antigen-induced eosinophil recruitment into the mouse airways by inhibiting the infiltration of CD4⁺ T cells. *J. Exp. Med.* 177, 573–576.
- Jung, W.K., Athukorala, Y., Cha, S.H., Song, C.B., Lee, C.H., Kim, S.K., Jeon, Y.J., 2007. Sulfated polysaccharide purified from *Ecklonia cava* accelerates antithrombin III mediated plasma proteinases inhibition. *J. Appl. Phycol.* 19, 425–430.
- Kay, A.B., 1991. Asthma and inflammation. *J. Allergy Clin. Immunol.* 87, 893–910.
- Keatings, V.M., O'Connor, B.J., Wright, L.G., Huston, D.P., Corrigan, C.J., Barnes, P.J., 1997. Late response to allergen is associated with increased concentrations of tumor necrosis factor- α and IL-5 in induced sputum. *J. Allergy Clin. Immunol.* 99, 693–698.
- Kim, S.K., Lee, D.Y., Jung, W.K., Kim, J.H., Choi, I.H., Park, S.K., Seo, S.K., Lee, S.W., Lee, C.M., Yea, S.S., Choi, Y.H., Choi, I.W., 2008. Effects of *Ecklonia cava* ethanolic extracts on airway hyperresponsiveness and inflammation in a murine asthma model: role of suppressor of cytokine signaling. *Biomed. Pharmacother.* 62, 289–296.
- Kips, J.C., Tavenier, J., Pauwels, R.A., 1992. Tumor necrosis factor (TNF) causes bronchial hyperresponsiveness in rats. *Am. Rev. Respir. Dis.* 145, 332–336.
- Kopf, M., Le Gros, G., Bachmann, M., Lamers, M.C., Bluethmann, H., Kohler, G., 1993. Disruption of the murine IL-4 gene blocks Th2 cytokine responses. *Nature* 362, 245–248.
- Kroegel, C., Liu, M.C., Hubbard, W.C., Lichtenstein, L.M., Bochner, B.S., 1994. Blood and bronchoalveolar eosinophils in allergic subjects after segmental antigen challenge: surface phenotype, density heterogeneity, and prostanoid production. *J. Allergy Clin. Immunol.* 93, 725–734.
- Liang, H., He, J., Ma, A.G., Zhang, P.H., Bi, S.L., Shi, D.Y., 2007. Effect of ethanol extract of alga *Laurencia* supplementation on DNA oxidation and alkylation damage in mice. *Asia Pac J. Clin. Nutr.* 16, 164–168.
- Lotvall, J., Inman, M., O'Byrne, P., 1998. Measurement of airway hyperresponsiveness. New considerations. *Thorax* 53, 419–424.
- Lukacs, N.W., Strieter, R.M., Chensue, S.W., Widmer, M., Kunkel, S.L., 1995. TNF- α mediates recruitment of neutrophils and eosinophils during airway inflammation. *J. Immunol.* 154, 5411–5417.
- Mapp, C.E., Boschetto, P., Zocca, E., Milani, G.F., Pivrotto, F., Teggazini, V., Fabbri, L.M., 1987. Pathogenesis of late asthmatic reactions induced by exposure to isocyanates. *Bull. Eur. Physiopathol. Respir.* 23, 583–586.
- Nahas, R., Abatis, D., Anagnostopoulou, M.A., Kefalas, P., Vagias, C., Roussis, V., 2007. Radical-scavenging activity of Aegean Sea marine algae. *Food Chem.* 102, 577–581.
- Okai, Y., Higashi-Okai, K., Nakamura, S., Yano, T., Otani, S., 1994. Suppressive effects of the extracts of Japanese edible seaweeds on mutagen-induced umu C gene expression in *Salmonella typhimurium* (TA 1535/pSK 1002) and tumor promoter-dependent ornithine decarboxylase induction in BALB/c 3T3 fibroblast cells. *Cancer Lett.* 87, 25–32.
- Pawankar, R., 2002. Epithelial cells as immunoregulators in allergic airway diseases. *Curr. Opin. Allergy Clin. Immunol.* 2, 1–5.
- Pec, M.K., Moser-Their, K., Fernández, J.J., Souto, M.L., Kubista, E., 1999. Growth inhibition by dehydrothiarsiferol – a non-Pgp modulator, derived from a marine red alga – in human breast cancer cell lines. *Int. J. Oncol.* 14, 739–743.
- Pereira, M.G., Benevides, N.M., Melo, M.R., Valente, A.P., Melo, F.R., Mourão, P.A., 2005. Structure and anticoagulant activity of a sulfated galactan from the red alga, *Gelidium crinale*. Is there a specific structural requirement for the anticoagulant action? *Carbohydr. Res.* 340, 2015–2023.
- Punnonen, J., Aversa, G., Cocks, B.G., de Vries, J.E., 1994. Role of interleukin-4 and interleukin-13 in synthesis of IgE and expression of CD23 by human B cells. *Allergy* 49, 576–586.
- Renz, H., Bradley, K., Saloga, J., Loader, J., Larsen, G.L., Gelfand, E.W., 1993. T cells expressing specific V β elements regulate immunoglobulin E production and airways responsiveness in vivo. *J. Exp. Med.* 177, 1175–1180.
- Saito, H., Yamagata, T., Suzuki, S., 1968. Enzymatic methods for the determination of small quantities of isomeric Chondroitin Sulfates. *J. Biol. Chem.* 243, 1536–1542.
- Saito, H., Hatake, K., Dvorak, A.M., Leiferman, K.M., Donnenberg, A.D., Arai, N., Ishizaka, K., Ishizaka, T., 1998. Selective differentiation and proliferation of hematopoietic cells induced by recombinant human interleukins. *Proc. Natl. Acad. Sci. USA* 85, 2288–2292.
- Shetty, K., Curtis, O.F., Levin, R.E., Witkowsky, R., Ang, V., 1995. Prevention of vitrification associated with the in vitro shoot culture of oregano (*Origanum vulgare*) by *Pseudomonas* spp.. *J. Plant Physiol.* 147, 447–451.
- Shibata, T., Nagayama, K., Tanaka, R., Yamaguchi, K., Nakamura, T., 2003. Inhibitory effects of brown algal phlorotannins on phospholipase A2s, lipoxygenases and cyclooxygenases. *J. Appl. Phycol.* 15, 61–66.
- Silverman, E., In, K.H., Yandava, C., Drazen, J.M., 1998. Pharmacogenetics of the 5-lipoxygenase pathway in asthma. *Clin. Exp. Allergy* 28, 164–170.
- Sugiura, U., Takeuchi, Y., Kakinuma, M., Amano, H., 2006. Inhibitory effects of seaweeds on histamine release from rat basophil leukemia cells (RBL-2H3). *Fisheries Sci.* 72, 1286–1291.
- Takeda, K., Hamelmann, E., Joetham, A., Shultz, L.D., Larsen, G.L., Irvin, C.G., Gelfand, E.W., 1997. Development of eosinophilic airway inflammation and airway hyperresponsiveness in mast cell-deficient mice. *J. Exp. Med.* 186, 449–454.
- Taube, C., Dakhama, A., Gelfand, E.W., 2004. Insights into the pathogenesis of asthma utilizing murine models. *Int. Arch. Allergy Immunol.* 135, 173–186.
- Thomas, P.S., Yates, D.H., Barnes, P.J., 1995. Tumor necrosis factor- α increases airway responsiveness and sputum neutrophilia in normal human subjects. *Am. J. Respir. Crit. Care Med.* 152, 76–80.
- Vairappan, C.S., Daitoh, M., Suzuki, M., Abe, T., Masuda, M., 2001. Antibacterial halogenated metabolites from the Malaysian *Laurencia* species. *Phytochemistry* 58, 291–297.
- Wills-Karp, M., 1999. Immunologic basis of antigen-induced airway hyperresponsiveness. *Ann. Rev. Immunol.* 17, 255–281.
- Yamamoto, I., Maruyama, H., 1985. Effect of dietary seaweed preparations on 1,2-dimethylhydrazine-induced intestinal carcinogenesis in rats. *Cancer Lett.* 26, 241–251.
- Yuan, Y.V., Walsh, N.A., 2006. Antioxidant and antiproliferative activities of extracts from a variety of edible seaweeds. *Food Chem. Toxicol.* 44, 1144–1150.
- Zuany-Amorim, C., Haile, S., Leduc, D., Dumarey, C., Huerre, M., Vargaftig, B.B., Pretolani, M., 1995. Interleukin-10 inhibits antigen-induced cellular recruitment into the airways of sensitized mice. *J. Clin. Invest.* 95, 2644–2651.