Polyopes affinis alleviates airway inflammation in a murine model of allergic asthma

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Marine algae have been utilized in food as well as medicine products for a variety of purposes. The purpose of this study was to determine whether an ethanol extract of *Polyopes affinis* (*P. affinis)* can inhibit the pathogenesis of T helper 2 (Th2)-mediated allergen-induced airway inflammation in a murine model of asthma. Mice that were sensitized and challenged with ovalbumin (OVA) evidenced typical asthmatic reactions such as the following: an increase in the number of eosinophils in the bronchoalveolar lavage (BAL) fluid; a marked influx of inflammatory cells into the lung around blood vessels and airways as well as the narrowing of the airway luminal; the development of airway hyperresponsiveness (AHR); the presence of pulmonary Th2 cytokines; and the presence of allergen-specific immunoglobulin E (IgE) in the serum. The successive intraperitoneal administration of *P. affinis* ethanolic extracts before the last airway OVA-challenge resulted in a significant inhibition of all asthmatic reactions. These data suggest that *P. affinis* ethanolic extracts possess therapeutic potential for the treatment of pulmonary allergic disorders such as allergic asthma.

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Abbreviations used: AHR, airway hyperresponsiveness; BAL, bronchoalveolar lavage; EPO, eosinophil peroxidase; ESR, electron spin resonance; DMPO, 5,5-Dimethyl-1-pyrroline-*N*-oxide; DPPH, 1,1-diphenyl-2-picrylhydrazyl; ELISA, enzyme-linked immunosorbent assay; IgG, immunoglobulin G; IL, interleukin; i.p., intraperitoneally; OVA, ovalbumin; Mch, methacholine; *P. affnis*, *Polyopes affnis*; Penh, enhanced pause

1. Introduction

Asthma is a complex inflammatory disease characterized by airway hyperresponsiveness (AHR), airway obstruction and pulmonary inflammation of the airways. 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 (Barnes 1994; Pawankar 2002). The T helper 2 (Th2)-type cytokines 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, the growth of mast cells and the differentiation and activation of mast cells and eosinophils (Iwamoto *et al*. 1993; Punnonen *et al*. 1994; Saito *et al*. 1998).

Marine algae contain a large amount of vitamins, minerals, dietary fibers, essential fatty acids, enzymes, proteins, polysaccharides and various functional polyphenols considered to be a rich source of antioxidants (Heo *et al*. 2010). Hence, many types of seaweed have been examined for the identification of new and effective antioxidant compounds. Recently, many countries are looking more closely at algae as a potent target for bioactive substances because the possibility of valuable uses and applications of algae in the fields of nutraceutics and pharmaceutics (Yamamoto and Maruyama 1985; Okai *et al*. 1994; Jung *et al*. 2007; Athukorala *et al*. 2008) has demonstrated the pharmaceutical and medicinal importance of seaweeds.

Polyopes affinis (*P. affinis*) is edible red seaweed. Previous reports have shown that the genus *polyopes* has α-glucosidase inhibitory activities (Kim *et al*. 2010). So far, other medicinal effects of the *P. affinis* have not been reported. Therefore, we investigated the effects of *P. affinis* extracts against OVA-induced allergic airway reactions in the experimental murine model of asthma.

2. Materials and methods

2.1 *Reagents*

Methacholine, OVA, and 10% (v/v) paraformaldehyde were purchased from Sigma (St Louis, MO). Diff-Quik was purchased from International reagents Corp (Kobe, Japan). Haematoxylin-2 and eosin-Y were purchased from Richard-Allan Scientific (Kalamazoo, MI). Biotin-conjugated rat anti-mouse IgE and TMB Substrate Reagent Set were purchased from BD Biosciencs (San Diego, CA).

2.2 *Preparation of P. affinis polyphenolic extracts*

The red seaweed *P. affinis* was collected along the Coast of Jeju Island in Korea during the period from October 2005 to February 2006. Fresh *P. affinis* was washed three times with tap water to remove the salt, 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 the extraction. The dried *P. affinis* powder (1 kg) was extracted with 95% EtOH (1:10 w/v) and the yield of *P. affinis* EtOH extracts was measured as 11.4% (% dry weight of alga) after evaporation *in vacuo*. The concentrated *P. affinis* was freshly dissolved in DMSO before use. Phenolic contents of *P. affinis* were determined by the method described by Shetty *et al*. (1995). Each 1 ml of the algal extract was mixed with 1 ml of 95% EtOH, 5 ml of distilled water and 0.5 ml of 50% Folin–Ciocalteu reagent (Sigma Chemical, St. Louis, MO). The mixtures were allowed to react for 5 min before 1 ml of 5% Na_2CO_3 was added and were placed in the dark for 1 h. Absorbance was measured at 725 nm, and a gallic acid standard curve was obtained for the calibration of phenolic content. The total carbohydrate was measured by the phenol–sulphuric acid method described by Dubois *et al*. (1956), using a mixture of galactose and fucose (1:1 weight ratio) as a standard. Three milliliter of sample solution was mixed with 0.5 ml of 5% phenol, and then 2.5 ml of 98% sulphuric acid was added. After standing at 30 min, absorbance was measured at 485 nm. The sulphate content in the total carbohydrate of *P. affinis* was measured by the $BaCl_2$/gelation method (Saito *et al*. 1968).

2.3 *Measurement of free radical scavenging activity by electron spin resonance spectroscopy*

The direct free radical scavenging effects of *P. affinis* were investigated using an electron spin resonance (ESR) spin-trapping technique. Because of its convenience, high sensitivity and short time consumption, this ESR spin-trapping technique has been widely used as a powerful method to determine different types of radicals (Sachindra *et al*. 2007). Therefore, antioxidant activities were first determined using the ESR spectrometer to scavenge free radicals, such as 1,1-diphenyl-2-picrylhydrazyl (DPPH), superoxide, hydroxyl and alkyl radicals.

Diverse radicals were generated according to the procedures described below, and spin adducts were recorded using a JES-FA ESR spectrometer (JEOL Ltd, Tokyo, Japan). Radical scavenging ability was calculated with the following equation in which H and H_o are the

relative peak heights of the radical signals with and without sample, respectively.

$$\text{Radical scavenging activity}(\%) = \left[\frac{1 - \mathrm{H}}{\mathrm{H}_o}\right] \times 100$$

2.3.1 *DPPH radical scavenging activity:* DPPH radical scavenging activity was measured using the method described by Nanjo *et al.* (1995).

2.3.2 *Hydroxyl radical scavenging activity:* Hydroxyl radicals were generated by the ironcatalysed Fenton Haber-Weiss reaction, and the generated hydroxyl radicals were rapidly reacted with nitrone spin traps (5,5-dimethyl-1-pyrroline-*N*-oxide, DMPO) (Rosen and Rauckman 1984). The resultant DMPO-OH adducts were detectable with an ESR spectrometer.

2.3.3 *Superoxide anion radical scavenging activity:* Superoxide anion radicals were generated by a UV-irradiated riboflavin/EDTA system (Guo *et al*. 1999).

2.3.4 *Peroxyl radical scavenging activity:* Alkyl radicals were generated according to the method described by Hiramoto *et al.* (1993).

2.4 *Animals*

Female BALB/c 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 mice were at 6–8 weeks (20–25 g) of age. 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.

2.5 *Immunization and challenge*

Mice were immunized intraperitoneally (i.p.) with 20 μg of OVA and 1.0 mg of aluminium hydroxide adjuvant on days 1 and 15, as described previously (Kim *et al.* 2004). Mice were challenged via the airway with OVA (50 mg/ml of saline) each day from days 22 to 24 on consecutive days. The control mice were exposed to aerosolized saline. Aerosolization was performed for 20 min by placing the mice in a chamber (15×25×15 cm) connected to the ultrasonic nebulizer (NE-U12, Omron, Tokyo, Japan). Mice were injected i.p. with 200 μl of 20 mg/kg *P. affinis* on consecutive days from days 16 to 20.

2.6 *Bronchoalveolar lavage and cell counting*

Mice were anaesthetized and the trachea was cannulated while gently massaging the thorax. The lungs were lavaged with 0.7 ml of PBS. The bronchoalveolar lavage (BAL) fluid samples were collected and the number of cells in a 50 μl aliquot was determined using a haemocytometer. The remaining sample was centrifuged, and the supernatant was stored at −70°C until the assay of cytokines. The pellet was resuspended in PBS and cytospin preparations of BAL cells were stained with Diff-Quik. Two independent blinded investigators counted the cells using a microscope. Approximately 400 cells were counted in each of four different random locations. The BAL of each animal was processed separately and this fluid was not concentrated before use.

2.7 *Determination of airway hyperresponsiveness*

Airway hyperresponsiveness (AHR) was measured at 3 days after the last OVA challenge in mice in an unrestrained conscious state, according to the method of Hamelmann *et al*. (1997). Mice were placed in a barometric plethysmographic chamber (All Medicus Co, Seoul, Korea), and baseline readings were taken and averaged for 3 min. Airway responsiveness was assessed as the percent increase of Penh in response to increasing doses of methacholine (Mch). Aerosolized Mch was then nebulized in increasing concentrations (from 2.5 to 50 mg/ml) 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 are expressed as an enhanced pause (Penh) and were calculated as follows: [expiratory time (Te)/relaxation time (RT)−1] × [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%.

2.8 *Histological studies*

At 48 h after the last challenge, the lungs were removed from the mice after they had been sacrificed. 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 haematoxylin-2 and eosin-Y. An inflammation score was

graded by three independent investigators who were not associated with this study.

2.9 *Cytokine assays*

The level of cytokines in the BAL fluids was determined by ELISA. ELISA kits from R&D Systems (Minneapolis, MN) were employed for the measurement of IL-4, and a kit from eBioscience (San Diego, CA) was employed for the measurement of IL-5.

2.10 *Measurement of OVA-specific serum levels of IgE*

OVA-specific serum IgE levels were determined by the ELISA using samples collected 12 h after the last OVA challenge. In brief, a 96-well microtiter plate was coated with OVA (10 mg/ml) and was then treated with mouse sera, followed by biotin-conjugated rat anti-mouse IgE. Then, avidin-horseradish peroxidase (HRP) solution was added to each well. The plates were 100 µl of TMB substrate solution (BD Biosciencs, San Diego, CA) was added to each well. The optical density of these units was measured at 405 nm.

2.11 *Measurement of eosinophil peroxidase*

The BAL fluid was centrifuged at 4°C for 10 min and serially diluted in a 96-well plate (75 µl/well) followed by the addition of 150 µl of substrate (1.5 mM *o*-phenylenediamine and 6.6 mM H_2O_2 in 0.05 M Tris–HCl, pH 8.0). After 30 min at room temperature, the reaction was stopped by the addition of 75 µl of 30% H_2SO_4, and the absorbance of the samples was determined at 492 nm on an ELISA reader.

2.12 *Statistical analysis*

Data values are presented as the mean ± SEM. The statistical significance of all data was evaluated using the Student's *t*-test. Differences of $p<0.05$ were considered statistically significant.

3. Results

3.1 *Yield of Polyopes affinis EtOH extracts*

After extraction with 95% EtOH, total yield of *P. affinis* EtOH extracts was measured as 11.4% (% dry weight of alga). In the chemical analysis for *P. affinis* EtOH extracts, the total phenolic content of *P. affinis* EtOH extracts was determined as 46.7±0.4 mg/g (n=3). However, low amounts of carbohydrate (1.6±0.1 mg/g, n=3) were detected in *P. affinis*. The sulphate content of *P. affinis* was determined to be 0.09% (weight of total sugar).

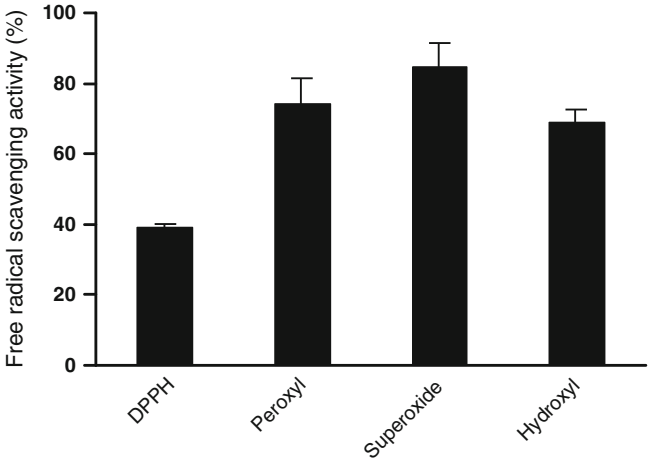

Figure 1. Free radical scavenging activity of *P. affinis* extracts using an ESR spectrometer. Hydroxyl radical was generated by Fenton reaction and trapped with 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO). Superoxide radical the UV irradiation of a riboflavin/EDTA solution and trapped with DMPO. Peroxyl radical was generated by 2,2′-azobis(2-amidinopropane) hydrochloride (AAPH) and trapped with alpha-(4-pyridyl-1-oxide)-N-tert-butylnitrone (4-POBN). Each value was expressed as the mean±SEM (n=3).

3.2 *Antioxidant activity of Polyopes affinis EtOH extracts*

Figure 1 shows the free radical scavenging activities of the four groups against different radical sources. The results show that *P. affinis* exhibited high free radical scavenging activity, and its radical scavenging activity was significantly increased up to 200 µg/ml. At 200 µg/ml, *P. affinis* exhibited considerable scavenging activity of 39.17±0.95%, 74.12±6.71%, 84.65±6.11% and 68.89±2.69% of DPPH, peroxyl, superoxide and hydroxyl radicals, respectively.

3.3 *In vivo study for anti-asthmatic effects of Polyopes affinis extracts*

3.3.1 *Numbers of inflammatory cells in BAL fluids of OVA-sensitized and OVA-challenged mice:* Compared with those in the control group ($5.2\pm0.72\times10^4$/ml), the total cell numbers in the BAL fluids were significantly increased 2 days after the last OVA challenge by approximately 6-fold ($31.4\pm4.91\times10^4$/ml) (figure 2). OVA sensitization and challenge induced marked increases in eosinophils within the BAL fluid ($18.5\pm4.19\times10^4$/ml). However, in the *P. affinis* extract-treated group ($4.9\pm1.44\times10^4$/ml), the numbers were reduced significantly in comparison with the OVA-exposed group. This effect was associated with some degree of inhibition in lung eosinophilia.

3.3.2 *Histological changes of OVA-induced asthma:* The results of the histological examination of lung tissues

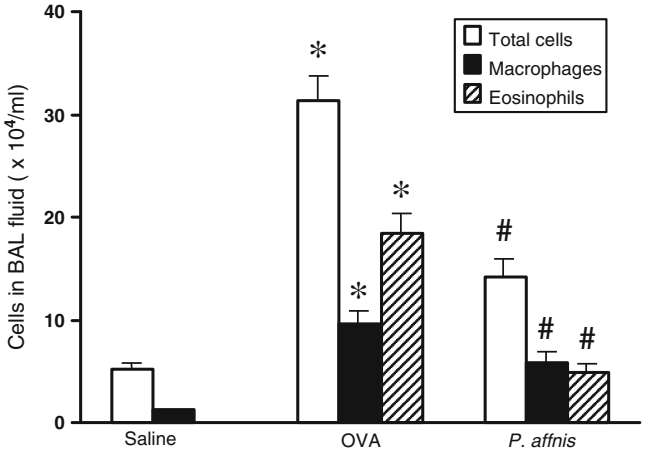

Figure 2. Effects of *P. affinis* extracts on the recruitment of inflammatory cells into BAL in OVA-induced allergic asthmatic mice, 2 days after the last OVA-challenge. The three columns show – mice administered with the saline, OVA-inhaled mice administered saline (OVA), and OVA-inhaled mice administered *P. affinis* extracts 20 mg/kg (OVA+*P. affinis*), respectively. BAL cells were separated using a Cytospin and then stained with Diff-Quik. Differential cell counting was performed using standard morphological criteria. Each value indicates the mean±SEM from five separate experiments (n=6 per group). $^{*}p<0.05$ *vs* saline-treated mice; $^{\#}p<0.05$ *vs* OVA-treated mice.

paralleled the cell numbers in the BAL fluids. Marked influxes of inflammatory cells into the airway and around the blood vessels were detected in the OVA-sensitized/challenged mice (figure 3B), but not in the saline-treated control mice (figure 3A). *P. affinis* extract-treated mice evidenced marked reductions in the infiltration of inflammatory cells within the peribronchiolar and perivascular regions (figure 3C). Mucus cell hypertrophy and airway luminal narrowing caused by the secreted mucus were observed in the OVA-sensitized/challenged mice (figure 3B). The administration of *P. affinis* extracts induced a marked improvement of luminal narrowing in the airway (figure 3C).

3.3.3 *Levels of bronchoalveolar lavage fluid cytokines of OVA-sensitized and OVA-challenged mice:* The levels of IL-4 (69.49±4.76 pg/ml) and IL-5 (107.62±4.11 pg/ml) in the BAL fluids were significantly increased by the airway challenge with OVA when compared with that of the control. The administration of *P. affinis* extracts reduced the concentrations of IL-4 and IL-5 by 10.01±1.24 and 15.87±4.36, respectively (figure 4).

3.3.4 *Effect on airway hyperresponsiveness:* In the OVA-sensitized and OVA-challenged mice, the dose–response curve of percent Penh was shifted to the left compared with that of the control mice (figure 5). In addition, the percentage of Penh produced by methacholine administration (at doses

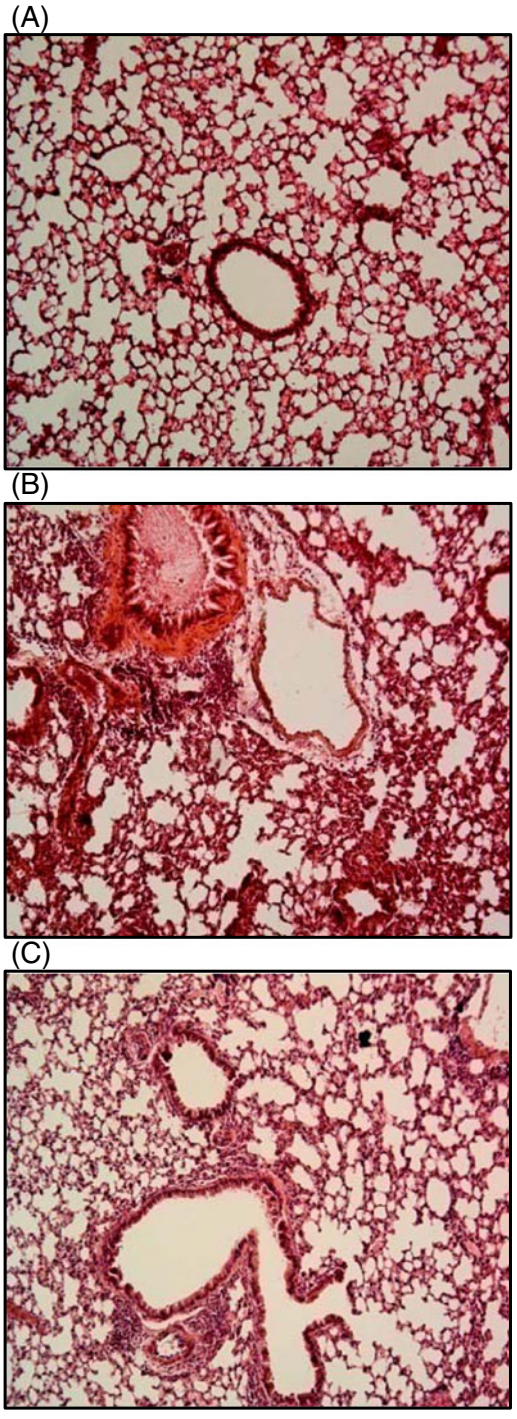

Figure 3. Inhibition of pathological changes in lung tissues of OVA-sensitized and -challenged mice by *P. affinis* extracts. Sections were obtained from the lungs of mice that received saline, OVA-inhaled mice that were administered saline (OVA), and OVA-inhaled mice that were administered *P. affinis* extracts (20 mg/kg, OVA+*P. affinis*), respectively. Lungs were removed 2 days after the last airway challenge. Sections were stained by haematoxylin and eosin staining (×200). Six animals were assigned to each group.

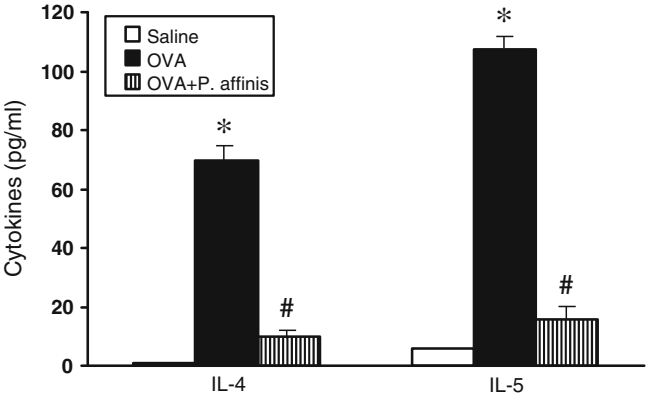

Figure 4. The effect of *P. affinis* extracts (20 mg/kg) on IL-4 and IL-5 cytokine levels in BAL fluid 4 h after the last airway challenge. IL-4 and IL-5 cytokine levels in the BAL fluids were measured by an ELISA Kit. Each value indicates the mean±SEM from five separate experiments (n=6 per group). $^{*}p<0.05$ *vs* saline-treated mice; $^{\#}p<0.05$ *vs* OVA-treated mice.

from 2.5 mg/ml to 50 mg/ml) increased significantly in the OVA-sensitized and OVA-challenged mice compared with the controls. OVA-sensitized and OVA-challenged mice that were treated with *P. affinis* extracts showed a dose–response curve of percentage of Penh that shifted to the right compared with that of untreated mice. The shift was dose-dependent.

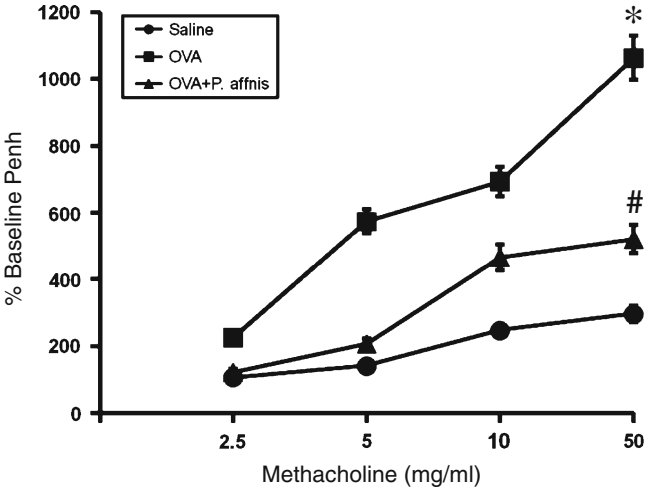

Figure 5. Effect of *P. affinis* extracts on airway responsiveness in OVA-sensitized unrestrained, conscious mice. Airway responsiveness was measured at 72 h after the last airway challenge. Mice were injected intraperitoneally with 20 mg/kg/day in 200 µl of *P. affinis* extracts each day from days 16 to 20 for five consecutive days. 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±SEM from five separate experiments (n=6 per group). $^{*}p<0.05$ *vs* saline-treated mice; $^{\#}p<0.05$ *vs* OVA-treated mice.

3.3.5 *Effect on OVA-specific serum IgE levels:* We determined that sensitization and challenge with OVA resulted in increased serum levels of OVA-specific IgE when compared with the saline-treated mice. The treatment of sensitized mice with *P. affinis* extract resulted in a 72% reduction in OVA-specific IgE (figure 6).

3.3.6 *Changes in eosinophil peroxidase levels in BAL fluids:* Levels of eosinophil peroxidase (EPO) showed a good correlation with the numbers of eosinophils at the inflammation sites. In accordance with the numbers of eosinophils measured in the BALFs of OVA-challenged mice (figure 7), we determined that the EPO levels in BAL fluids increased significantly 48 h after OVA inhalation (0.271±0.018), as compared with the EPO levels after saline inhalation (0.045±0.002). The increased levels of EPO were reduced significantly by approximately 35% upon the administration of the *P. affinis* extract (0.094±0.015).

4. Discussion

The administration of *P. affinis* extracts prior to the final airway OVA challenge resulted in a significant inhibition of all asthmatic reactions. This study is the first to provide experimental evidence demonstrating that *P. affinis* extracts inhibit OVA-induced airway inflammation in a murine model of asthma.

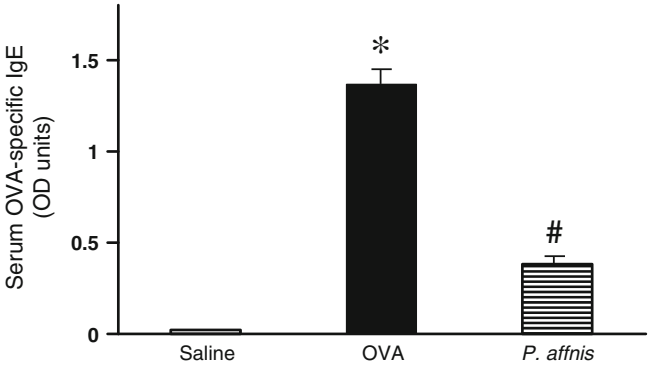

Figure 6. Effects of *P. affinis* extracts on serum OVA-specific IgE antibody levels in mice 12 h after the last airway challenge. Blood was collected by cardiac puncture for the measurement of OVA-specific IgE with ELISA. Mice were injected intraperitoneally with 20 mg/kg/day in 200 µl of *P. affinis* extracts each day from days 16 to 20 for five consecutive days. Each value indicates the mean±SEM from five separate experiments (n=6 per group). $^{*}p<0.05$ *vs* saline-treated mice; $^{\#}p<0.05$ *vs* OVA-treated mice.

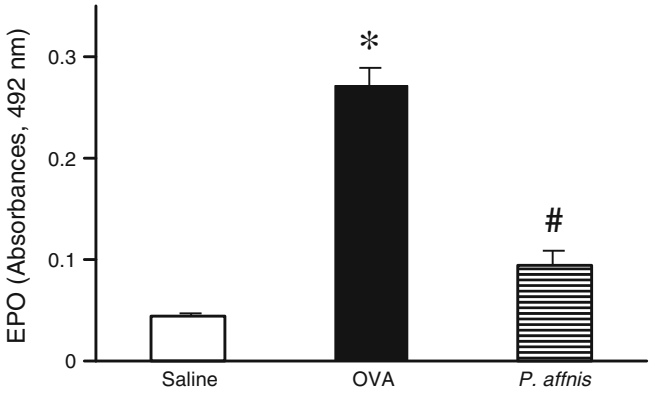

Figure 7. Effects of *P. affinis* extracts on the level of EPO content in the BAL fluid of OVA-sensitized mice and OVA-challenged mice. EPO activity was measured in BALF 48 h after the last airway challenge. *P. affinis* extracts was administered i.p. from the last sensitization to 1 day before the first airway challenge. Data represent mean±SEM from 6 independent experiments. *$p<0.05$ *vs* saline-treated mice; and #$p<0.05$ *vs* OVA-treated mice.

We assayed the polyphenol contents from the *P. affinis* extract. Polyphenols from marine algae called phlorotannins are abundant in terrestrial plants, such as fruits, vegetables, medicinal plants and marine plants, such as seaweeds. These substances have received the greatest attention and have been investigated extensively because they are high free radical scavengers, less toxic and have fewer side-effects. Phlorotannins have anti-oxidant, anti-allergic, anti-bacterial and anti-tumour functions, and studies about phlorotannins are of great interest among researchers (Tada *et al*. 1992; Singh and Etoh 1997; Nagayama *et al*. 2002). In the chemical analysis, total phenolic content are abundant in the EtOH extracts of *P. affinis*. Although red algae are an abundant source of sulphated polysaccharides such as sulphated galactans that have many 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 amounts of polysaccharides with large molecular size.

At first, we evaluated the radical scavenging activity of *P. affinis* polyphenolic extracts against DPPH, hydroxyl and alkyl radicals using an electron spin trapping technique (figure 1). In the result of these experiments, we found that the ethanol fraction from the *P. affinis* extract displayed potent anti-oxidative activities. Collectively, these results suggest that the *P. affinis* extract might be proposed as a drug for the prevention or treatment of inflammatory diseases including allergic asthma.

Next, from the basis of the *in vitro* results, we assessed the anti-inflammatory effects of *P. affinis* in a murine model of asthma. OVA-induced asthma has been recognized as a disease characterized by increased levels of influx of inflammatory cells into the lung, mucus hyper-secretion and airway occlusion as well as by the thicknesses of the bronchial wall and the area of the smooth muscle. In the current experiment, the administration of *P. affinis* extracts prior to the final airway OVA challenge resulted in a significant inhibition of characteristic asthmatic reactions. 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). Using the present model, the number of eosinophils in BAL fluids was increased in the control group at 2 days after the OVA sensitization/challenge. Interestingly, the number of eosinophils decreased in BAL fluids in the *P. affinis* pre-treated group of mice. The results of histological examination of the lung tissues paralleled the cell numbers in the BAL fluids (figure 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 *P. affinis* 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 *P. affinis* resulted in a marked improvement of luminal narrowing in the airway. These results suggest that *P. affinis* is highly capable of inhibiting the development of the allergic status induced by OVA in mice.

Numerous studies have established that T cells from the lungs of asthmatics express a Th2 pattern of cytokines. 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 are responsible for IgE production by B cells and eosinophil activation and recruitment (Romagnani 1994; Akdis *et al*. 2004). Of these, IL-4 especially induces isotype switching in B cells, from IgG to IgE production (Fish *et al*. 2005). IL-5 is most specific to eosinophils and is the major hematopoietin responsible for eosinophil growth, differentiation, mobilization, recruitment, activation and survival (Sanderson 1992; Simon *et al*. 2004). IL-5 acts through the IL-5 receptor complex expressed on the eosinophil cell surface. Tissue levels of eosinophil peroxidase (EPO) correlated well with the numbers of eosinophils at the inflammation sites. In addition, the *P. affinis* extracts mediated the inhibition of asthmatic reactions and appears to be attributable to the initial suppression of an allergen-specific

IgE response. In many allergic inflammatory diseases, elevations in the plasma level of IgE induce the degranulation of mast cells via the cross-linking of allergen-specific IgE. Therefore, a novel therapeutic approach to asthma and other allergic respiratory diseases involves interference in the action of IgE, and this immunoglobulin has been viewed as a target for novel immunological drug development in asthma. Administration of *P. affinis* extracts reduced the serum levels of OVA-specific IgE. These results support the conclusion that *P. affinis* extracts suppressed the generation of a Th2-type immune response in this animal asthma model. Thus, interventions that inhibit Th2 cytokines may be useful in the treatment of allergic asthma.

In conclusion, in this study it was found that *P. affinis* extracts have antioxidant properties. Based on this information, we examined the anti-asthmatic effects of *P. affinis* extracts against the OVA-induced allergic airway reaction in animals and the administration of *P. affinis* extracts before the last airway OVA challenge resulted in a significant inhibition of all asthmatic reactions. The results showed that Th2 cytokine production that leads to reduced pulmonary eosinophilia, and EPO levels and IgE serum levels was inhibited. The chemical properties and structures of the polyphenolic components derived from the red alga are yet to be determined and the use of ethanolic extract of *P. affinis* in humans is too speculative as yet. Thus, these results support that *P. affinis* extracts may prove to be a useful therapeutic approach to the treatment of allergic airway diseases.

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References

Akdis CA, Blaser K and Akdis M 2004 Genes of tolerance. *Allergy* **59** 897–913

Athukorala Y, Jung WK, Park PJ, Lee YJ, Kim SK, Vasanthan T, No HK and Jeon YJ 2008 Evaluation of biomolecular interactions of sulfated polysaccharide isolated from Grateloupia filicina on blood coagulation factors. *J. Microbiol. Biotechnol.* **18** 503–511

Barnes PJ 1994 Cytokines as mediators of chronic asthma. *Am. J. Respir. Crit. Care. Med.* **150** 42–49.

Corrigan CJ and Kay AB 1992 T cells and eosinophils in the pathogenesis of asthma. *Immunol. Today* **13** 501–507

Dubois M, Gilles KA, Hamilton JK, Rebers PA and Smith F 1956 Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **28** 350–356

Elias JA, Lee CG, Zheng T, Ma B, Homer RJ and Zhu Z 2003 New insights into the pathogenesis of asthma. *J. Clin. Invest.* **111** 291–297

Fish SC, Donaldson DD, Goldman SJ, Williams CM and Kasaian MT 2005 IgE generation and mast cell effector function in mice deficient in IL-4 and IL-13. *J. Immunol.* **174** 7716–7724

Guo Q, Zhao B, Shen S, Hou J, Hu J and Xin W 1999 ESR study on the structure-antioxidant activity relationship of tea catechins and their epimers. *Biochem. Biophys. Acta* **1427** 13–23

Hamelmann E, Schwarze J, Takeda K, Oshiba A, Larsen GL, Irvin CG and Gelfand ER 1997 Noninvasive measurement of airway responsiveness in allergic mice using barometric plethysmography. *Am. J. Respir. Crit. Care. Med*. **156** 766–775

Heo SJ, Yoon WJ, Kim KN, Ahn GN, Kang SM, Kang DH, Affan A, Oh C, Jung WK and Jeon YJ 2010 Evaluation of anti-inflammatory effect of fucoxanthin isolated from brown algae in lipopolysaccharide-stimulated RAW 264.7 macrophages. *Food Chem. Toxicol* **48**, 2045–2051

Hiramoto K, Johkoh H, Sako KI and Kikugawa K 1993 DNA breaking activity of the carbon-centered radical generated from 2,2-azobis-(2-amidinopropane)-hydrochloride (AAPH). *Free Radical Res. Commun.* **19** 323–332

Iwamoto I, Nakajima H, Endo H and 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

Kim YS, Kwon KS, Kim DK, Choi IW and Lee HK 2004. Inhibition of murine allergic airway disease by *Bordetella pertussis*. *Immunology* **112** 624–630

Kim KY, Nguyen TH, Kurihara H and Kim SM 2010 α-Glucosidase Inhibitory Activity of Bromophenol Purified from the Red Alga *Polyopes lancifolia*. *J. Food Sci*. **75** H145-H150

Kroegel C, Liu MC, Hubbard WC., Lichtenstein LM and Bochner BS 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

Lotvall J, Inman M and O'Byrne P 1998 Measurement of airway hyperresponsivenes. New considerations. *Thorax* **53** 419–424

Nagayama K., Iwamura Y, Shibata T, Hirayama1 I and Nakamura T 2002 Bactericidal activity of phlorotannins from the brown alga *Ecklonia kurome*. *J. Antimicrob. Chemother*. **50** 889–893

Nanjo H, Adachi M, Aketa T, Mizoguchi T, Nishihara T and Terada T 1995 The role of cysteine in the alteration of bovine liver dihydrodiol dehydrogenase 3 activity. *Biochem. J.* **310** 101–107

Okai Y, Higashi-Okai K, Nakamura S, Yano Y and 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 promotordependent ornithine decarboxylase induction in BALB/c 3 T3 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

Pereira MG, Benevides NM, Melo MR, Valente AP, Melo FR and Mourão PA 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 BG and de Vries JE 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 GL and Gelfand EW 1993 T cells expressing specific Vβ elements regulate immunoglobulin E production and airways responsiveness *in vivo*. *J. Exp. Med*. **177** 1175–1180

Romagnani S 1994 Lymphokine production by human T cells in disease states. *Annu. Rev. Immunol*. **12** 227–257

Rosen GM and Rauckman EJ 1984 Spin trapping of superoxide and hydroxyl radicals. *Methods Enzymol*. **105** 198–209

Sachindra NM, Sato E, Maeda H, Hosokawa M, Niwano Y, Kohno M and Miyashita K 2007 Radical scavenging and singlet oxygen quenching activity of marine carotenoid fucoxanthin and its metabolites. *J. Agric. Food Chem*. **55** 8516–8522

Saito H, Hatake K, Dvorak AM, Leiferman KM., Donnenberg AD, Arai N, Ishizaka K and Ishizaka T 1998 Selective differentiation and proliferation of hematopoietic cells induced by recombinant human interleukins. *Proc. Natl. Acad. Sci. USA*. **85** 2288–2292

Saito H, Yamagata T and Suzuki S 1968 Enzymatic methods for the determination of small quantities of isomeric Chondroitin Sulfates. *J. Biol. Chem*. **243** 1536–1542

Sanderson CJ 1992 Interleukin-5, eosinophils, and disease. *Blood* **79** 3101–3109

Shetty K, Curtis OF, Levin RE, Witkowsky R and 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

Silverman E, In KH, Yandava C and Drazen JM 1998 Pharmacogenetics of the 5-lipoxygenase pathway in asthma. *Clin. Exp. Allergy* **28** 164–170

Simon D, Braathen LR and Simon H-U 2004 Eosinophils and atopic dermatitis. *Allergy* **59** 561–570

Singh IP and Etoh H 1997 Biological activities of phloroglucinol derivatives from *Eucalyptus* spp. *Nat. Prod. Sci.* **3** 1–7

Tada M, Chiba K, Kawamura T and Kojima E 1992 Analogues of natural phloroglucinols as antagonists against both thromboxane A2 and leukotriene D4. *J. Med. Chem.* **35** 1209–1212

Yamamoto I and Maruyama H 1985 Effect of dietary seaweed preparations on 1,2-dimethylhydrazine-induced intestinal carcinogenesis in rats. *Cancer Lett*. **26** 241–251

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