

Inhibitory effect of unicellular green algae (*Chlorella vulgaris*) water extract on allergic immune response

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Abstract

BACKGROUND: *Chlorella* is used as a functional food in East Asia and has been shown to enhance immune system function. However, there has been no direct evidence of the suppressive effect of a hot water extract of *Chlorella vulgaris* (CVE) on histamine-mediated allergic responses.

RESULTS: The antihistamine activity of CVE was analysed using rat peritoneal mast cells (RPMCs) stimulated by compound 48/80. For *in vivo* verification, ovalbumin (OVA)-immunised BALB/c mice were treated with CVE orally. Serum immunoglobulin E (IgE) levels and splenocyte cytokine levels were determined by enzyme-linked immunosorbent assay (ELISA). CVE prevented histamine release through degranulation of mast cells by blocking the uptake of extracellular Ca^{2+} into RPMCs. Moreover, CVE administration inhibited serum IgE overproduction by OVA via induction of T helper 1 (Th1) skewing that was dependent on interferon- γ (IFN- γ) and interleukin 12 (IL-12) secretion.

CONCLUSION: The results of this study clearly demonstrate that CVE acts as an antiallergic dietary agent by suppressing histamine release via its enhance effect on Th1-related responses.

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Keywords: *Chlorella vulgaris* water extract (CVE); IgE; Th1; mast cells; histamine

INTRODUCTION

Mast cells activated by immunoglobulin E (IgE) release inflammatory mediators from granules, such as histamine, that play a critical role in the initiation and progression of allergic inflammatory reactions.¹ The mechanism by which drugs alleviate allergic conditions has been demonstrated to be either via their blocking of histamine release or via their degranulation of mast cells as therapeutic targets.² *Chlorella* is a genus of unicellular and microscopic algae containing carbohydrate, protein, amino acids, nucleic acids, fatty acids, β -carotene and a number of minerals and vitamins. It has been used to improve health as a functional food in East Asia, including Japan.³ It has been previously reported that extracts of *Chlorella* might exert various beneficial effects against cancers as well as antioxidant activities.⁴ Moreover, *Chlorella* extracts are involved in the enhancement of immune system function. Queiroz *et al.*⁵ reported that *Chlorella vulgaris* extract decreases opportunistic infection by increasing interferon (IFN) levels. Pugh *et al.*⁶ demonstrated that *Chlorella pyrenoidosa* has potent immunostimulatory activities via stimulating cytokine expression by activating nuclear factor- κ B (NF- κ B) signalling. However, the suppressive effect of a hot water extract of *C. vulgaris* (CVE) on histamine release still needs to be elucidated.

The aims of this study were to investigate whether CVE prevents histamine release by using rat peritoneal mast cells (RPMCs) and whether it suppresses allergic response *in vivo* by using an ovalbumin (OVA)-induced allergic mouse model.

EXPERIMENTAL

Animals

Five-week-old female BALB/c mice weighing 18–20 g were purchased from OrientBio Inc. (Kyeonggi, Korea). Sprague–Dawley rats (8 weeks old, 230–280 g) were purchased from Dae Han Experimental Animal Center (Daejeon, Korea). The animals were housed in an air-conditioned room ($23 \pm 2^\circ\text{C}$, 12/12 h light/dark cycle) and allowed free access to food and tap water. All animal experiments were performed in accordance with the Guidelines for Animal Use and Care at the Korea Food Research Institute

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Feed manufacturing

The CVE used in this study was manufactured by Daesang Co. (Seoul, Korea). First, 0.5 g of *C. vulgaris* was placed in 50 mL of boiling water and stirred for 30 min until the sample liquid temperature reached 95 °C. Then the supernatant was spray dried to obtain CVE according to the manufacturer's protocols. The feed was manufactured in pellet form by mixing 2 g kg⁻¹ CVE or 0.005 g kg⁻¹ dexamethasone (Dex) as a positive control into regular chow (AIN-93G-based cereal formula, G-Bio Co., Ltd, Gwacheon, Korea) in our laboratory.

Oral administration of CVE in OVA-sensitised mice

Mice were sensitised with 20 µg of OVA adsorbed in 2 mg mL⁻¹ Imject Alum by intraperitoneal injection on days 7 and 21. The naïve control group was injected with saline. The naïve and sham control groups were administered saline throughout the duration of the experiment. In the CVE and Dex groups, 2 g kg⁻¹ CVE and 2.5 mg kg⁻¹ Dex respectively were administered orally. Each sample treatment was administered on days 7–28.

Measurement of OVA-specific IgE and cytokine levels

Twenty-four hours after the last treatment, blood was sampled from the brachial plexus of each mouse to estimate serum IgE levels by enzyme-linked immunosorbent assay (ELISA) as described previously.⁷ Cytokine (IFN-γ, interleukin 12 (IL-12), IL-4 and IL-5) levels in the supernatant of splenocytes from each mouse were measured according to the manufacturer's protocols. The absorbance at 450 nm was measured by means of a microplate reader (Molecular Devices, Sunnyvale, CA, USA).

Preparation of RPMCs and histamine assay

RPMCs were isolated as described previously.⁸ RPMCs (2 × 10⁵ cells per well) were preincubated with CVE (0–1 mg mL⁻¹) and then incubated with compound 48/80 (0.25 µg mL⁻¹). The released histamine was measured as described previously.⁸ % inhibition = [(histamine release without CVE – histamine release with CVE)/histamine release without CVE] × 100.

Measurement of ⁴⁵Ca uptake

Mast cell suspensions with ⁴⁵Ca at 1.5 mCi mL⁻¹ (1 Ci = 3.7 × 10¹⁰ Bq) (PerkinElmer Life Sciences, Boston, MA, USA) were preincubated with CVE (0–1 mg mL⁻¹) and then incubated

with compound 48/80 (0.25 µg mL⁻¹). ⁴⁵Ca uptake into RPMCs was measured using a scintillation β-counter (Liquid Scintillation Analyzer, CANBERRA, Canberra, Australia) as described previously.⁸

Statistical analysis

Results are expressed as mean ± standard deviation (SD) of triplicate determinations. Differences among experimental data were assessed by analysis of variance (ANOVA) followed by Tukey's *post hoc* test or Dunnett's multiple range tests.

RESULTS AND DISCUSSION

To investigate whether CVE inhibited histamine release from mast cells, we used compound 48/80-stimulated RPMCs. Compound 48/80 is a mixed polymer of phenethylamine crosslinked by formaldehyde that has been used to induce the degranulation of mast cells through perturbation of the membrane in experimental studies.⁸ In this study, treatment with CVE at concentrations of 0–1 µg mL⁻¹ inhibited degranulation and histamine release of RPMCs stimulated with compound 48/80 (Figs 1(A) and 1(B)). Moreover, CVE attenuated the compound 48/80-triggered vascular permeability by approximately 30%, as documented by Evans blue extravasation (data not shown). The intracellular Ca²⁺ induces the movement of granules to the plasma membrane and the degranulation of mast cells.⁹ In the present study, CVE inhibited compound 48/80-induced calcium uptake into RPMCs in a dose-dependent manner (Fig. 1(C)). These results suggest that CVE could prevent the release of allergic mediators by suppressing calcium uptake into mast cells, resulting in the inhibition of immediate-type allergic reactions.

IgE binds to the high-affinity receptor on mast cells and provokes allergic inflammation.⁹ To prove the antiallergic effect of CVE *in vivo*, we examined the effects of CVE using an OVA-sensitised allergic mouse model. In the present study, total IgE levels were reduced by approximately 76.3% in mice fed CVE compared with those in sham animals, and OVA-specific IgE levels were reduced by approximately 71.4% (Fig. 2). IFN-γ and IL-12 strongly suppress IgE production by B cells.¹⁰ In the present study, levels of IFN-γ and IL-12 in CVE-fed mice were significantly increased compared with those in sham mice, but not levels of IL-4 and IL-5 (Table 1). These results are similar to those found in earlier studies. Hasegawa *et al.*¹¹ reported that CVE enhanced the production of IFN-γ by T cells in response to casein by augmenting IL-12 production. An *et al.*¹² also revealed that CVE strongly increased the production of IFN-γ but did not affect the production of IL-4 in the human T cell leukaemia cell line. These results indicate that CVE administration exerts a potent inhibitory effect on IgE overproduction by inducing

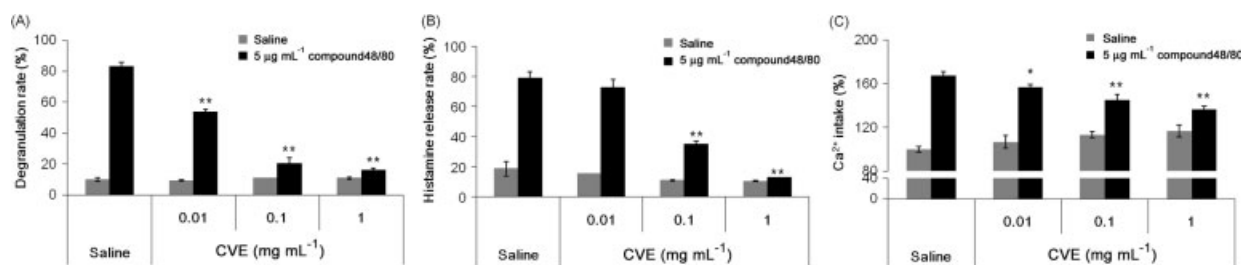


Figure 1. Inhibitory effect of CVE on compound 48/80-induced RPMC response. RPMCs were preincubated with CVE (0–1 mg mL⁻¹) at 37 °C for 5 min before incubation with compound 48/80. The inhibitory effect of CVE was measured by (A) compound 48/80-induced degranulation, (B) histamine release and (C) calcium uptake. Values are presented as mean ± SD (n = 5). Data were assessed by ANOVA followed by Dunnett's multiple range tests. Statistical differences *versus* the saline control are indicated as follows: *P < 0.05; **P < 0.01.

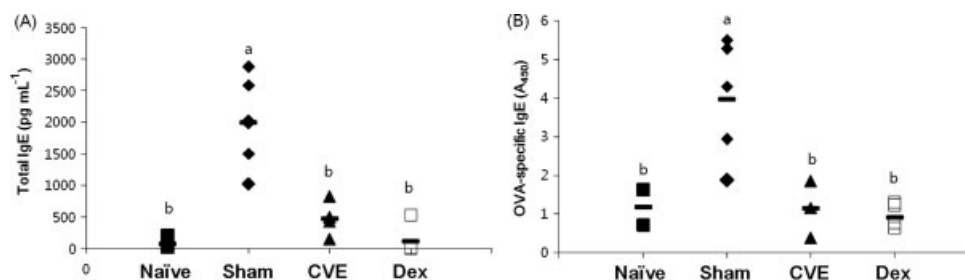


Figure 2. Suppressive effect of CVE on IgE production in serum of OVA-sensitised BALB/c mice. (A) Total IgE and (B) OVA-specific IgE levels in serum were measured by ELISA. Collected sera were diluted 1:50 (v/v) for total IgE and 1:5 (v/v) for OVA-specific IgE detection. Values are presented as mean $\pm$ SD ($n = 16$). Data were assessed by ANOVA followed by Tukey's *post hoc* test. Data are statistically different ($P < 0.05$) between columns with different letters.

Table 1. Enhance effect of CVE on Th1 secretion in OVA-induced allergic mice. Secreted cytokines were quantified by ELISA of splenocytes of OVA-sensitised BALB/c mice after 72 h of culture

Group	IFN- γ	IL-12	IL-5	IL-4
Naïve	2.05 $\pm$ 0.38b	437.64 $\pm$ 30.82b	7.51 $\pm$ 0.89b	9.64 $\pm$ 5.22
Sham	2.35 $\pm$ 0.18b	310.07 $\pm$ 18.17c	28.67 $\pm$ 13.04a	10.24 $\pm$ 6.17
CVE	55.74 $\pm$ 16.57a	487.09 $\pm$ 17.80a	30.61 $\pm$ 4.01a	5.29 $\pm$ 0.12
Dex	23.32 $\pm$ 15.21b	124.58 $\pm$ 49.54d	24.16 $\pm$ 2.82a	6.75 $\pm$ 1.71

Values (pg/mL) are presented as mean $\pm$ SD ($n = 5$). Data were assessed by ANOVA followed by Tukey's *post hoc* test. Means with different letters in a column are significantly different ($P < 0.05$).

T helper 1 (Th1) secretion, resulting in the suppression of histamine release from mast cells.

The main chemical constituents of CVE are known to be nucleic acids, polysaccharides (β -1,3-glucan) and glycoproteins. These compounds can be recognised by antigen-presenting cells (APCs) such as macrophages that lead to IL-2, IL-6, IL-12 or IFN- γ production, whose cytokines serve to activate B, T and natural killer (NK) cells.^{13,14} Queiroz *et al.*⁵ showed that oral administration of *C. vulgaris* affected the NK cell activity of bacterium- or virus-infected mice. Hasegawa *et al.*¹⁵ found that glycoproteins from *C. vulgaris* produced IL-12p40 through Toll-like receptor 2 (TLR-2). Hsu *et al.*¹⁶ reported that hot water-soluble polysaccharides from *C. pyrenoidosa* regulated cytokine secretion in macrophages via TLR-4. Thus glycoproteins and polysaccharides derived from CVE could impact immune cells in circulating blood, lymph nodes, the spleen and the gastrointestinal tract (e.g. Peyer's patch, lamina propria). Sakurai *et al.*¹⁷ reported that polysaccharides administered orally might be absorbed into M cells, which take up and transfer soluble antigens and pathogens from the intestinal lumen and deliver them to the lymphoid tissue, enabling the assessment of immune cells. Therefore it was deduced that glycoproteins and polysaccharides of CVE might be absorbed into the gastrointestinal tract via M cells and affect immune cells in Peyer's patch. Moreover, nucleic acids, glycoproteins or polysaccharides present in CVE might help to suppress serum IgE production and to stimulate splenocyte APCs to produce IFN- γ and IL-12, resulting in the suppression of histamine release from mast cells.

CONCLUSIONS

Our results revealed that CVE inhibited compound 48/80-induced histamine release by blocking the uptake of calcium in RPMCs.

Furthermore, CVE suppressed the allergic response through Th1 skewing, which is dependent on the secretion of IFN- γ and IL-12, in an OVA-induced allergic mouse model. To the best of our knowledge, this is the first observation of the inhibitory effect of CVE on excessive histamine secretion and on an OVA-induced allergic state. These results provide scientific proof of the prevention of allergic diseases by exploiting the suppressive effects of CVE on histamine release from mast cells.

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