Effects of a Hop Water Extract on the Compound 48/80-Stimulated Vascular Permeability in ICR Mice and Histamine Release from OVA-Sensitized BALB/c Mice

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The antiallergic properties of a hop water extract (HWE) were studied by evaluating the Evans blue leakage from ICR mice caused by compound 48/80 stimulation, and the histamine release from ovalbumin (OVA)-sensitized BALB/c mice. An oral administration of HWE significantly inhibited the vascular permeability and histamine release. HWE itself did not have any influence on the total and antigen-specific immunoglobulin E (IgE) production in OVA-sensitized mice. These results indicate that HWE exerted an antiallergic effect by inhibiting the release of chemical mediators from mast cells and basophiles.

Key words: hop; allergy; compound 48/80; immunoglobulin E; histamine

Type I allergic symptoms are caused by the activation of mucosal mast cells and/or basophiles. The cross-linking of immunoglobulin E (IgE) intermediated by the binding of a multivalent antigen at the surface of the mast cells triggers the release of many chemical mediators such as histamine, leukotriens and prostaglandins from these cells.1–3) This released histamine causes sneezing and nasal rubbing by binding to the histamine H1-receptors on the sensory nerve endings.4–7) Accordingly, the inhibition of chemical mediator release from mast cells and basophiles seems to be an effective way to ease and prevent symptoms of the type I allergy.

A hop water extract (HWE) has significantly inhibited antigen-induced nasal rubbing and sneezing in egg albumin-sensitized BALB/c mice.8) An in vitro assay of the histamine release from human basophilic KU812 cells induced by calcium ionophore A23187 stimulation has indicated, that HWE inhibited the histamine release from KU812 cells.9) To clarify the antiallergic mechanism of the hop water extract, we investigated the inhibitory effect of an oral administration of HWE on the increase in vascular permeability induced by the stimulation with compound 48/80 of ICR mice. Stimulation of mast cells by compound 48/80 has led to the release of chemical mediators from these cells, and an intradermal injection of compound 48/80 in mice and rats has caused an increase in the vascular permeability and scratching behavior at the stimulated site.10–13) Such foodstuffs as lactic acid bacteria,14–16) β-carotene,17) and royal jelly18) have been reported to have antiallergic properties by inhibiting IgE production through the improved Th1/Th2 balance toward Th1 dominance. We also investigated the effect of an oral administration of HWE on IgE production and histamine release into the serum from ovalbumin (OVA)-sensitized mice.

HWE was prepared by soaking 5 g of hop (Humulus lupulus L.) pellets in 500 ml of cold water (4 °C) overnight. The hop pellet suspension was passed through Advantec 5B 110-mm filter paper (Toyo Roshi Kaisha, Tokyo, Japan). The resulting aqueous solution was lyophilized. Approximately 600 mg of lyophilized powder was obtained (yield about 12%).

Female 6-week-old ICR mice and BALB/c mice were purchased from Charles River Japan (Yokohama, Japan). The animals were housed in an air-conditioned room maintained at 23 ± 2 °C with a relative humidity of 55 ± 15%. They were given standard laboratory rodent feed (Oriental Yeast, Tokyo, Japan) and water ad libitum. All procedures were performed according to the Guidelines for the Care and Use of Experimental Animals of the Japanese Association for Laboratory Animals.

Each value is expressed as the mean ± standard error of the mean (SEM). A statistical evaluation of the results was performed by a one-way analysis of variance (ANOVA) and then by Fisher’s Least Significant Difference (LSD) or Dunnett’s test. A probability value of less than 0.05 is considered statistically significant.

Eight-week-old female ICR mice had their backs shaved on the day prior to the experiment. In our previous study using egg albumin-sensitized BALB/c mice, the successive oral administration of HWE significantly inhibited nasal rubbing and sneezing at a dose of more than 200 mg/kg.8) Accordingly, in this study, HWE

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Abbreviations: IgE, immunoglobulin E; HWE, hop water extract; OVA, ovalbumin; IL, interleukin
dissolved in distilled water was orally administered once a day for a week at a dose of 100, 200 or 500 mg/kg. On the day of the trial, a test sample solution was orally administered an hour before stimulating with compound 48/80. The mice were intradermally injected with 5 mg of compound 48/80 dissolved in 50 ml of physiological saline into their shaved back skin immediately after an intravenous injection of an 0.5% Evans blue solution (5 ml/kg mouse). The mice were sacrificed 30 minutes after the compound 48/80 injection, and the skin of the reaction locus was removed for a quantitative determination of the extravasated dye. The extravasated Evans blue was extracted from each piece of dissected skin with 1 ml of 6 M KOH at 45°C for 6 h. This extract was neutralized by 1 ml of 6 M HCl, and then 2 ml of acetone was added. The extract was clarified by filtration, its absorbance at 595 nm was measured, and the extracted Evans blue content was then calculated from its calibration curve. Each value represents the mean ± SEM (n = 8). ** Significantly different from the control group with P < 0.01.

As shown in Fig. 1, an intradermal stimulation of 5 μg of compound 48/80 caused a significant leakage of Evans blue that had been intravenously injected before compound 48/80 stimulation. The amount of Evans blue that leaked from the compound 48/80-stimulated site was approximately 20 μg in the control group. The oral administration of HWE significantly inhibited this Evans blue leakage from the compound 48/80-stimulated skin at a dose of 500 mg/kg. Ketotifen fumarate (Wako Pure Chemical Industries, Osaka, Japan), which is a histamine H₁ receptor antagonist and chemical mediator release suppressor, also significantly inhibited Evans blue leakage at a dose of 10 mg/kg.

We next investigated the effect of the oral administration of HWE on IgE production by using BALB/c mice, because this strain is regarded as a high IgE responder to OVA. Eight-week-old female BALB/c mice were intraperitoneally injected on days 7 and 21 with 20 μg of OVA and 2 mg of alum in a total volume of 200 μl. The mice (n = 7 per group) were fed on a diet (MF; Oriental Yeast) containing 0%, 0.05% or 0.25% HWE from days 0 to 28. Serum was collected from the mice on day 28, and the concentration of total IgE and OVA-specific IgE was determined by sandwich ELISA. The level of total IgE in the serum was measured by using a mouse IgE ELISA quantification kit (Bethyl Laboratories, Montgomery, TX, USA). The level of the OVA-specific IgE titer in the serum was determined by sandwich ELISA. Biotinylated OVA was used instead of the HRP-conjugated anti-mouse IgE second antibody. The biotinylation of OVA was carried out by using a biotinylation kit (Cygnus Technologies, Southport, NC, USA). The OVA-specific IgE titer was calculated from a comparison with the hyperimmunized mouse serum obtained from mice that had been intraperitoneally immunized with OVA adsorbed to alum three times. This hyperimmunized serum was arbitrarily taken to be 100 units/ml. The OVA-sensitized mice were intraperitoneally challenged with 2 mg of OVA dissolved in 200 μl of PBS a week after the second immunization to induce histamine release into the serum. Eight minutes
after the antigen challenge, the mice were killed and blood was collected to measure the histamine concentration in the serum. This histamine concentration in the serum was determined by using a histamine EIA kit (Spi-bio, Montigny-le-Bretouneux, France).

Figure 2 shows the total and OVA-specific IgE levels in the serum of the OVA-sensitized mice which had been fed with the HWE 0% (control), 0.05% or 0.25% added diet. The oral administration of HWE for 4 weeks did not affect the total IgE and OVA-specific IgE production. The production of interleukin (IL)-4, which promotes B cell proliferation, IgE class switching and then augments IgE secretion, by OVA-sensitized mouse splenocytes was not changed by the oral administration of HWE (data not shown). On the other hand, the histamine release into the serum from OVA-sensitized mice that had been induced by the intraperitoneal antigen challenge was decreased by the oral administration of HWE. In particular, the histamine level in the serum of the mice fed with the 0.25% HWE added diet was significantly lower than that of the control mice (Fig. 3).

One week’s consecutive oral administration of 500 mg/kg HWE in this study significantly inhibited the Evans blue leakage induced by compound 48/80 stimulation. Compound 48/80 is a potent activator of connective tissue-type mast cells and skin mast cells.22) Compound 48/80 stimulation of mast cells leads to the release of chemical mediators and causes an increase in vascular permeability at the stimulated site.10–13) Moreover, the oral administration of HWE significantly inhibited histamine release from the OVA-sensitized mice. In our previous in vitro study, HWE inhibited histamine release from human basophilic KU812 cells, quercetin glycosides contained in HWE being mainly responsible for this inhibition of histamine release.9)

Quercetin glycosides have been reported to be digested and absorbed as aglycones from the gastrointestinal tract when orally administered.23,24) Therefore, the results in this study probably indicate that orally ingested quercetin in HWE had an inhibitory effect on the release of chemical mediators from the mast cells. The absorption

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Fig. 2. Effect of the Oral Administration of HWE on Total IgE and OVA-Specific IgE in the Serum of OVA-Sensitized Mice.

Eight-week-old female BALB/c mice were intraperitoneally injected on days 7 and 21 with 20 μg of OVA and 2 mg of alum in a total volume of 200 μl. The serum was collected from the mice on day 28, and the concentrations of total IgE and OVA-specific IgE were determined by ELISA. The mice were fed on the HWE 0%, 0.05% or 0.5% added MF diet from days 0 to 28. Each value represents the mean ± SEM, (n = 10).

** Significantly different from the OVA-sensitized control group with P < 0.01.

Fig. 3. Serum Histamine Levels in the Serum of OVA-Sensitized Mice 8 min after the Intraperitoneal Antigen Challenge.

BALB/c mice were intraperitoneally injected on days 7 and 21 with 20 μg of OVA and 2 mg of alum in a total volume of 200 μl. A week after the second immunization, the mice were intraperitoneally challenged with 2 mg of OVA dissolved in 200 μl of PBS. Eight minutes after the antigen challenge, the mice were killed, and blood was collected to measure the histamine concentration in the serum. The histamine concentration in the serum was determined by using a histamine EIA kit. The mice were fed on the HWE 0%, 0.05% or 0.25% added diet from days 0 to 28. Each value represents the mean ± SEM, (n = 10). ** Significantly different from the OVA-sensitized control group with P < 0.05 and P < 0.01, respectively.
and metabolism of quercetin glycosides contained in HWE must be investigated in the future.

HWE itself did not affect the total and antigen-specific IgE production with this immunization protocol. Activated mast cells have been shown to produce such cytokines as IL-3, IL-4, IL-5 and TNF-α and such lipid mediators as prostaglandins and leukotrienes. These are mainly responsible for the late-phase inflammatory reaction. IL-3, IL-4, IL-5, and TNF-α have been reported to promote mast cell proliferation, Th2 differentiation, eosinophil activation, and inflammation.1) Therefore, the oral administration of HWE might be effective for preventing chronic inflammatory disease such as eczema and atopic dermatitis by inhibiting the release of these mediators and cytokines from activated mast cells.

In conclusion, the oral administration of HWE inhibited the vascular permeability induced by compound 48/80 stimulation of ICR mice and histamine release into the serum from OVA-sensitized BALB/c mice. These findings indicate that HWE exerted an antiallergic effect by inhibiting the release of chemical mediators from the mast cells and basophiles.

References

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