Effects of Hop Extracts on Nasal Rubbing and Sneezing in BALB/c Mice

Miho TAKUBO, a Toshio INOUE, a Shuishi JIANG, a Tae TSUMURO, a Yuuki UEDA, a Ric YATSUZUKA, a Shuichi SEGAWA, a Junji WATARU, a and Chiaki KAMEI a, *

a Department of Medicinal Pharmacology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences; Okayama 700–8530, Japan; and b Frontier Laboratories of Value Creation, Sapporo Breweries Ltd.; Shizuoka 425–0013, Japan. Received October 3, 2005; accepted December 26, 2005

The effects of hop extracts (Humulus lupulus L.) on histamine release from rat peritoneal mast cells and human basophilic KU812 cells were studied. Hop water extract (HWE) and XAD-4 50% methanol fraction of HWE (MFH) inhibited histamine release from rat mast cells induced by compound 48/80 at concentrations of 100 and 10 μg/ml, respectively. Almost the same findings were observed with A23187-induced histamine release from KU812 cells. Next, we studied the effects of hop extracts on antigen-induced nasal rubbing and sneezing in sensitized BALB/c mice. HWE caused a significant inhibition of nasal rubbing and sneezing at a dose of 500 mg/kg. MFH also inhibited nasal rubbing and sneezing dose-dependently. A significant difference was observed from 100 mg/kg in nasal rubbing and 200 mg/kg in sneezing. The effects of both extracts became clear after repeated administration. HWE and MFH significantly inhibited both nasal rubbing and sneezing, respectively, after consecutive treatment for 15 d at smaller doses compared with single administration. This finding indicates that the active component of hop is included in MFH, which was absorbed to Amberlite XAD-4 and eluted with 50% methanol. These results clearly demonstrated that hop extracts may be effective in the relief of symptoms of allergic rhinitis.

Key words  hop extract; histamine release; compound 48/80; A23187; nasal rubbing; sneezing

The female flowers of hop (Humulus lupulus L.) have been used in the brewing industry to add bitterness and aroma to beer. Hop is also used in folk medicine as a tranquilizer, and in Japan, it is formulated in over the counter (OTC) drugs for depression of the central nervous system or the activation of gastric function. Moreover, it has been reported that the hop shows a wide range of the physiological roles, such as an inhibitory effect on the bone resorption,1) cancer chemopreventive activity,2,3) inhibition of tumor promotion by 12-O-tetradecanoylphorbol-13-acetate in mouse skin,4) an estrogenic property of the phyto-estrogen, 8-prenylnaringenin,5) and the inhibition of nitric acid production.6) Allergic diseases such as hay fever, atopic dermatitis, food allergy and bronchial asthma are increasing in prevalence in most countries of the developed world. These allergic diseases are classified as type I allergies. Basophils and mast cells play a crucial role in the initiation of such types of allergies. On the other hand, it has been reported that tea,7) peppermint8) and tomato skin9) showed antiallergic properties by inhibiting the degranulation of basophils and mast cells. Antiallergic properties of these plant extracts are attributable to their flavonoids and flavonoid glycosides. It is well known that hop contains polyphenols, including flavonoids and flavonoid glycosides.10) However, relatively little is known about the antiallergic properties of hop extracts.

Therefore, we investigated the antiallergic properties of hop extracts by evaluating the inhibition of histamine release from rat peritoneal mast cells and human basophilic KU812 cells. The effects of hop extracts on the nasal rubbing and the sneezing in allergic rhinitis models were also studied in mice.

MATERIALS AND METHODS

Animals  Female BALB/c mice (6—10 week-old) were obtained from Japan SLC, Inc., Shizuoka. The animals were housed in an air-conditioned room maintained at 24±2°C with a relative humidity of 55±15%. They were given standard laboratory rodent chow (Oriental Yeast, Tokyo) and water ad libitum. All procedures involving animals were conducted in accordance with the Guidelines for Animal Experiments at Okayama University Advanced Research Center.

Hop Sample Preparation  Hop water extract (HWE): 500 g hop (Humulus lupulus L.) pellets were soaked in 9.5 l of distilled cold (4°C) water overnight. The hop pellets suspension was filtered through filter paper. The aqueous solution was concentrated under reduced pressure and then lyophilized. The yield was ca. 22%. XAD-4 50% methanol fraction of HWE (MFH): the HWE that dissolved in distilled water was applied to Amberlite XAD-4 (Organon; Tokyo, Japan). The column was washed with distilled water and then eluted with 50% methanol. The fraction eluted with 50% methanol was evaporated and lyophilized. The yield was ca. 4.5%.

Reagents  The reagents used in the experiments were obtained from the sources shown in parentheses: compound 48/80 (Sigma, St. Louis, MO, U.S.A.), A23187 (Sigma), ketotifen fumarate (Sankyo, Tokyo, Japan), egg albumin (Sigma), alum (LSL Co., Tokyo, Japan) and B. pertussis (Sigma).

Histamine Release from Rat Peritoneal Mast Cells  Peritoneal mast cells were harvested from peritoneal fluid of Wistar rat (Wistar male strain, obtained from Japan SLC) and purified to greater than 90% purity by percoll density gradient centrifugation. The collected mast cells (2.5×10^4 cells/tube) were then incubated with physiological buffer solution (in mm: NaCl 140, KCl 2.7, CaCl2 0.9, glucose 5.6, HEPES 5, pH 7.4) for 10 min. Histamine release was evoked by adding 0.1 ml of compound 48/80 (final concentration; 0.5 μg/ml) and incubating at 37°C for 10 min. The reaction was stopped 10 min later by cooling the tubes in ice water and the tubes were centrifuged at 200×g for 15 min. Histamine contents were measured by means of a fluorometric
Histamine Release from KU812 Cells

KU812 cells were obtained from the Health Science Research Resources Bank (Osaka, Japan). The cells were cultured in RPMI 1640 medium (Invitrogen Corp., Carlsbad, CA, U.S.A.) supplemented with 10% fetal bovine serum (Equitech-Bio, Kerrville, TX, USA) at 37°C in a humidified atmosphere with 5% CO₂. The KU812 cells were washed twice with Tyrode buffer (in mM: NaCl 137, KCl 2.7, NaH₂PO₄·2H₂O 0.4, MgCl₂·6H₂O 1, NaHCO₃ 12, CaCl₂·2H₂O 1.8). Then, the cells were suspended in 0.5 ml Tyrode buffer to be 2 × 10⁶ cells/ml. CaCl₂ (1 mM), calcium ionophore A23187 (final concentration: 0.5 mM) and test sample solution (10% v/v) were added into the cell suspension. The cell suspension was incubated at 37°C for 20 min and the reaction was stopped by cooling in an ice bath for 5 min. After centrifugation at 2000 g for 5 min at 4°C histamine concentration of the supernatant was determined using a fluorometric method.¹¹

Sensitization

The mice were sensitized by the injection of 0.2 ml of physiological saline containing egg albumin (0.1 mg), alum (1 mg) and B. pertussis (300 ng) into the peritoneal cavity on the first day. Five days later, they were boosted by the subcutaneous injection of 1 ml of physiological saline containing egg albumin (0.05 mg) in the back. Then, local sensitization was performed every day from day 18 by instilling egg albumin in physiological saline (50 mg/ml/nose) into the bilateral noses using a micropipette.

Nasal Rubbing and Sneezing Behavior

To evaluate nasal rubbing, each time the animal rubbed or touched the area near the nose with its forepaws, it was counted as one event. Touches around the eyes and the mouth were disregarded. Before the experiment, the animals were placed into an observation cage (32 × 22 × 20 cm) for about 10 min for acclimatization. The extracts of hop were administered orally. One hour later, 1 μl of antigen (50 μg) was instilled into the bilateral nasal cavities. The animals were placed into the observation cage (one animal/cage), and nasal rubbing and sneezing were counted for 30 min. Nasal rubbing and sneezing behavior were observed on day 39. HWE and FMH were administered orally every day from day 25 to day 39.

Statistical Analysis

All values are expressed as the mean ± standard error of the mean (S.E.M.). Statistical evaluation of the results was performed by one-way analysis of variance (ANOVA) followed by the Bonferroni or Dunnnett test. A probability value of less than 0.05 was considered statistically significant.

RESULTS

Histamine Release from Rat Peritoneal Mast Cells

Figure 1 shows the effects of the HWE, MFH and ketotifen on histamine release from rat peritoneal mast cells. In nontreated cells with compound 48/80, the intracellular histamine concentration of rat peritoneal mast cells was 923.5 ± 10.3 ng/2.5 × 10⁴ cells. Both extracts caused a concentration-related inhibition of the histamine release induced by compound 48/80, and a significant effect was observed at 100 μg/ml in HWE and 10 μg/ml in MFH. Ketotifen also inhibited the histamine release, and the significant inhibition was observed at a concentration of 100 μg/ml.

Histamine Release from KU812 Cells

Figure 2 shows the effects of Hop Water Extract and XAD-4 50% Methanol Fraction of Hop Water Extract on Histamine Release from Human Basophilic KU812 Cells Induced by A23187. HWE: hop water extract, MFH: XAD-4 50% methanol fraction of hop water extract, S: spontaneous release, C: control. Each value represents the mean ± S.E.M. (n=4). *, ** Significantly different from the control group with p<0.05 and p<0.01, respectively.

Effect of HWE on Nasal Rubbing and Sneezing

Variances in nasal rubbing and sneezing behavior were observed on day 39. HWE and FMH inhibited the histamine release, and the significant inhibition was observed at a concentration of 100 μg/ml.

Fig. 1. Effects of Hop Water Extract, XAD-4 50% Methanol Fraction of Hop Water Extract and Ketotifen on Histamine Release from Rat Peritoneal Mast Cells Induced by Compound 48/80

HWE: hop water extract, MFH: XAD-4 50% methanol fraction of hop water extract, S: spontaneous release, C: control. Each value represents the mean ± S.E.M. (n=4). *, ** Significantly different from the control group with p<0.05 and p<0.01, respectively.

Fig. 2. Effects of Hop Water Extract and XAD-4 50% Methanol Fraction of Hop Water Extract on Histamine Release from Human Basophilic KU812 Cells Induced by A23187

HWE: hop water extract, MFH: XAD-4 50% methanol fraction of hop water extract, S: spontaneous release, C: control. Each value represents the mean ± S.E.M. (n=3). *, ** Significantly different from the control group with p<0.05 and p<0.01, respectively.
Figure 3 shows the effect of HWE on nasal rubbing and sneezing induced by antigen. HWE caused no inhibitory effect on nasal rubbing at doses of 100 and 200 mg/kg; however, at a dose of 500 mg/kg, it caused a significant inhibition. Almost the same results were obtained with sneezing. When HWE was administered once a day for 15 d, the inhibitory effect became clear; and a significant effect was observed, even at a dose of 200 mg/kg (Fig. 4).

**DISCUSSION**

In the present study, it was demonstrated that HWE and MFH significantly inhibited the histamine release from rat peritoneal mast cells induced by compound 48/80 and from human basophilic KU812 cells induced by A23187. Although intracellular histamine concentration of rat peritoneal mast cells was relatively higher than that of KU812 cells, WHE and MFH inhibited the histamine release from both cells in the same manner. In addition to HWE and MFH, ketotifen which was able to prevent mast cell degranulation also inhibited the histamine release from both cells. The effect of MFH was more potent than HWE in histamine release from both mast cells and KU812 cells. This finding indicates that the active component of hop is included in MFH, which was absorbed to Amberlite XAD-4 and eluted with 50% methanol. It is well recognized that hop contains flavonoids, such as quercetin and kaempferol, and their glycosides.10) Moreover, it was reported that biologically active substances such as flavonoid and flavonoid glycosides were easily separated from other hydrophilic constituents by using highly polus polymer column.12) We speculate therefore, that flavonoids and flavonoid glycosides in hop may contribute to the inhibition of histamine release from mast cells or basophilic KU812, although the detailed chemical structure of active components in MFH are under investigation. In association with these findings, it was reported that tea,7) peppermint8) and tomato skin9) have antiallergic properties by inhibiting the degranulation from basophilic and mast cells, and their flavonoids and/or flavonoid glycosides are its active components.
its binding to the histamine H₁-receptors on the sensory nerve endings. ¹³⁻¹⁶ As a matter of course, there are some findings that histamine H₁-receptor antagonists inhibited the symptoms of sneezing and nasal rubbing of the allergic rhinitis model in mice and rats. ¹⁷,¹⁸

The oral administration of HWE and MFH to the allergic rhinitis model mice, which are sensitized to egg albumin, significantly suppressed the sneezing and the nasal rubbing of these mice. Both extracts showed inhibition of the sneezing and nasal rubbing at a single dose of 100—500 mg/kg. We have reported that natural products, such as Brazilian propolis¹⁹ and Lo Han Kuo,¹¹ exert a stronger effect by consecutive administration; therefore, we studied the effect of hop extract by consecutive treatment for 15 d on both behaviors. As a result, it was found that repeated oral administration of both extracts resulted in more potent inhibitory effect than the single administration. As stated previously, both hop extracts inhibited histamine release from rat mast cells and basophilic KU812 cells. Therefore, it seems likely that the inhibitions of sneezing and nasal rubbing induced by the antigen occurred through histamine release inhibition from basophilic and/or mast cells.

From these results, it may be concluded that hop extract is effective in the relief of allergic symptoms when used in a clinical setting.

REFERENCES

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