Effect of Usuhiratake (Pleurotus pulmonarius) on Sneezing and Nasal Rubbing in BALB/c Mice

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The anti-rhinitis properties of Pleurotus pulmonarius were investigated in BALB/c mice. A single administration of Pleurotus Pulmonarius caused no significant effect on antigen-induced nasal rubbing and sneezing at a dose of 500 mg/kg, but a significant inhibition was observed after 2 weeks of repeated treatment at this dose, and at a dose of 200 mg/kg, it also caused a significant inhibition after repeated administration for 4 weeks. Pleurotus pulmonarius showed no significant inhibitory effect on the production of IgE. In addition, Pleurotus pulmonarius caused no inhibition of histamine-induced nasal rubbing and sneezing at a dose of 500 mg/kg, but in vitro study, it inhibited histamine release from rat mast cells induced by compound 48/80 at the soluble supernatant solution of 30 and 100 μg/ml of Pleurotus pulmonarius suspended in PBS. These results demonstrated that Pleurotus pulmonarius may be effective in the relief of symptoms of allergic rhinitis through inhibition of histamine release.

Key words: usuhiratake; Pleurotus pulmonarius; sneezing; nasal rubbing; histamine; compound 48/80

Allergic rhinitis, the most common atopic disease, is characterized by the major symptoms of sneezing, nasal itching, nasal congestion, and rhinorrhea, which are presumed to be triggered by many mediators released from mast cells and other inflammatory cells. The prevalence of allergic rhinitis in the general population has been reported to be increasing year by year, and recent research suggested that approximately 20% of the world population are affected by allergic rhinitis. In the treatment of allergic rhinitis, anti-histaminics, corticosteroids and anti-allergic drugs are widely used, and some traditional folk medicines have been used; therefore, it is meaningful to develop new compounds from natural products for the treatment of allergic rhinitis.

The number of mushrooms on Earth is estimated at 140000, but only 10% (approximately 14000 named species) of mushrooms are known. Mushrooms comprise a vast and largely untapped source of powerful new pharmaceutical products, and some mushrooms such as Ganoderma lucidum, Lentinus edodes, Inontus obliquus have been harvested and used as medicinal fungi for hundreds of years in China, Japan, and Korean. Recent interests have focused on the search for new drugs from mushrooms, and a number of bioactive molecules have been identified from various mushroom species, including several anti-tumor substances. This very interesting, as recently, there have been some reports about the anti-allergic effects of some mushroom species. In previous research, we have demonstrated that cyclooctasulfur extracted from Ganoderma lucidum shows an inhibitory effect on histamine release, and extract of Bulgaria inquinans exhibits an inhibitory effect on scratching behavior and vascular permeability induced by compound 48/80 that occurred via histamine release from mast cells. Also reported that Agaricus blazei, an edible important medicinal mushroom can inhibit mast cell-mediated anaphylaxis-like reactions.

Pleurotus pulmonarius, usuhiratake in Japanese, is a tasty edible fungus, first found by an Indian scholar, and then distributed to China through India. In Japan, Pleurotus pulmonarius has also been cultivated recently. In the phytochemical study of Pleurotus pulmonarius, it has been reported that it contains essentially no lipids or starch, but is high in protein, with eight essential amino acids. It also contains various vitamins especially vitamin D. In China, Pleurotus pulmonarius is used as an ingredient in the Chinese dish Yao Shan, being considered attractive as a health food. Recent studies indicated that Pleurotus pulmonarius contains polysaccharides which are verified to show anti-tumor activity; however, it has not been clarified whether it prevents anti-allergic properties in an experimental model.

Therefore, in this paper, we report the anti-allergic properties of Pleurotus pulmonarius by evaluating the inhibition of sneezing and nasal rubbing induced by antigen in mice as well as the inhibition of histamine release from rat peritoneal mast cells.

Materials and Methods

Animals

Female BALB/c mice (6—11 weeks old) and male Wistar rats (7 weeks 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.

Pleurotus pulmonarius Sample Preparation

Cultivated Pleurotus pulmonarius fruit bodies were supplied from Okayama Prefectural Forest Experimental Station. This sample was deposited at Department of Medicinal Pharmacology, Okayama University Graduate School of Medicine, Okayama, Japan. The fruit bodies were lyophilized and ground by a mail (ULTRA CENTRIFUGAL MILL EM-1, screen 0.5 mm, MRK-RETSCH), the resulted powder was...
preserved in a desiccator at 4 °C until use.

Reagent  The reagents used in the experiments were obtained from the source shown in parentheses: histamine dihydrochloride (Sigma, St. Louis, MO, U.S.A.), compound 48/80 (Sigma), disodium cromoglycate (DSCG; Sigma), egg albumin (Sigma), alum (LSL Co., Tokyo, Japan) and B. pertussis (Sigma). The powder of Pleurotus pulmonarius was suspended in distilled water and administered orally.

Sensitization  The mice were sensitized by an 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 a 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/nostril) into the bilateral noses using a micropipette.

Sneezing and Nasal Rubbing Behavior Induced by Antigen in Sensitized Mice To evaluate nasal rubbing, each time the animal rubbed or touched the area near the nose with its forepaws was counted as one event. Touches around the eyes and 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 suspension of Pleurotus pulmonarius was administered orally. One hour later, 2 μl of antigen (50 μg) was instilled into the bilateral nasal cavities. The animals were placed into the observation cage (one animal/cage), and sneezing and nasal rubbing were counted for 30 min. Sneezing and nasal rubbing behavior were observed on day 32, 39, 46 and 53. The suspended solution of Pleurotus pulmonarius was administered orally every day for four weeks (from day 25 to day 53).

Determination of Total IgE in Mouse Serum  Blood was collected from the tail vein of sensitized mice on day 0, 25, 32 (after one week), 39 (after two weeks), 46 (after three weeks) and 53 (after four weeks). The serum was obtained by centrifugation at 500×g for 10 min at 4 °C and stored at −20 °C until use. Total IgE levels in serum were measured using an enzyme immunoassay (Bethyl Laboratories Inc., Montgomery, TX, U.S.A.).

Sneezing and Nasal Rubbing Behavior Induced by Histamine in Non-sensitized Mice  The suspension of Pleurotus pulmonarius was administered orally. One hour later, 2 μl of histamine (500 nmol/nostril) was instilled into the bilateral nasal cavities, and sneezing and rubbing behavior was observed as for the previous methods.

Histamine Release from Rat Peritoneal Mast Cells  Peritoneal mast cells were harvested from the peritoneal fluid of Wistar rats and purified to greater than 90% purity by Percoll density gradient centrifugation. The collected mast cells (2.5×10⁶ cells/tube) were then incubated with physiological buffer solution: PBS (in mM: NaCl 140, KCl 2.7, CaCl₂ 0.9, glucose 5.6, HEPES 5, pH 7.4) for 10 min at 37 °C. Pleurotus pulmonarius was suspended in PBS, separated by centrifugation and the soluble supernatant solution was obtained. The test drugs dissolved in PBS[1%] were added (0.1 ml) 10 min and DSCG were added (0.1 ml) 30 s before compound 48/80 (final concentration: 0.5 μg/ml), respectively. The reactions were stopped 10 min later by cooling the tubes in ice water followed by centrifugation at 200×g for 15 min. Histamine contents were measured by fluorometric assay. The histamine release was expressed as a percentage of the total histamine content (i.e., of histamine in the supernatant plus the cell-associated histamine).

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 Dunnett’s test. A probability value of less than 0.05 was considered statistically significant.

RESULTS  Effect of Pleurotus pulmonarius on Nasal Rubbing and Sneezing Induced by Antigen  Pleurotus pulmonarius caused no significant effect at a high dose of 500 mg/kg (date not shown); however, repeated administration of Pleurotus pulmonarius showed gradual inhibition of sneezing (Fig. 1A) and nasal rubbing (Fig. 1B). Pleurotus pulmonarius at a dose of 500 mg/kg caused significant inhibition of both sneezing and nasal rubbing after two weeks of repeated treatment. At a dose of 200 mg/kg it also caused a significant inhibition after repeated administration for four weeks.

Effect of Pleurotus pulmonarius on Total Serum IgE  Total serum IgE level in the mice after treatment of Pleurotus pulmonarius was slightly decreased, but no significant difference was observed even at a high dose of 500 mg/kg for four weeks, administration (date not shown).

Effect of Pleurotus pulmonarius on Sneezing and Nasal Rubbing Induced by Histamine  Pleurotus pulmonarius caused no significant effect even at a high dose of 500 mg/kg (date not shown).

Effect of Pleurotus pulmonarius on Histamine Release from Rat Mast Cells Induced by Compound 48/80  Figure 2 shows the effects of Pleurotus pulmonarius and DSCG on histamine release from rat peritoneal mast cells induced by compound 48/80. Spontaneous histamine release was 6.1±1.0% (n=8—10), whereas the addition of compound 48/80 at a concentration of 0.5 μg/ml induced a histamine release of 52.9±2.7% (n=8—10). Administration of Pleurotus pulmonarius resulted in the concentration-related inhibition of histamine release. The soluble supernatant solution of 10 μg/ml of Pleurotus pulmonarius suspended in PBS caused no significant inhibition; however, 30 and 100 μg/ml significantly inhibited compound 48/80-induced histamine release. DSCG used as a positive control at a dose of 300 μmol/ml also caused an inhibition of this response.

DISCUSSION  In the present study, we studied the effect of Pleurotus pulmonarius on sneezing and nasal rubbing induced by antigen–antibody reaction. A single administration of Pleurotus pulmonarius at a dose of 500 mg/kg showed somewhat but not significant inhibition of sneezing and nasal rubbing induced by antigen. Takubo et al.¹⁰ and Inoue et al.¹³ have reported that the pharmacological activity of a natural product might be enhanced by repeated administration. Pleurotus pulmonarius showed gradually inhibition of nasal symptoms by repeated administration. Significant inhibition appeared
after two weeks, administration of *Pleurotus pulmonarius* at a dose of 500 mg/kg, and a dose of 200 mg/kg also exhibited a significant inhibition after four weeks, repeated administration; therefore, it became clear that the enhancing effects observed by consecutive administration of natural products is also applicable to *Pleurotus pulmonarius*.

It is well known that mast cells play an important role in allergic reactions through the release of histamine, proteases, prostaglandins and several multifunctional cytokines. This activation of mast cells is initiated by binding the antigen to IgE. In addition, some investigations indicate that allergic rhinitis is a manifestation of atopy characterized by the formation of IgE in response to environmental conditions, and a significant relationship has been observed between the levels of IgE in the serum and clinical symptoms of allergic patients.14) In the treatment of allergic rhinitis, suplatast tosilate has an inhibitory effect on IgE production,15) and we also measured the serum levels of total IgE in mice. As a result, the serum IgE of mice was slightly decreased, but no significant difference was observed even at a high dose of 500 mg/kg for four weeks, administration (data not shown). From these results, it is reasonable to presume that the inhibition by *Pleurotus pulmonarius* on nasal symptoms is not attributable to IgE production.

In general, the cross-linkage of IgE at the surface of mast cells triggers the release of many chemical mediators, such as histamine, leukotrienes and prostaglandins from these cells.16) Among those chemical mediators, histamine is considered one of the most important chemical mediators and it can cause all the pathological features of allergic rhinitis.17) Several investigations have indicated that sneezing and nasal rubbing are due to the stimulation of histamine H1 receptors on sensory nerve endings.18) In our previous study, we observed that H1 receptor antagonists potently depressed antigen antibody-induced sneezing and nasal rubbing in rats.19) In order to clarify the mechanism of action of *Pleurotus pulmonarius*, we studied the effect of *Pleurotus pulmonarius* on sneezing and nasal rubbing induced by histamine in non-sensitized mice. *Pleurotus pulmonarius* caused no inhibition of the sneezing and nasal rubbing induced by histamine even at a high dose of 500 mg/kg (data not shown); therefore, it can be concluded that the inhibition of sneezing and nasal rubbing induced by *Pleurotus pulmonarius* is not ascribed to an H1-antagonistic effect.

Next, we studied the effect of *Pleurotus pulmonarius* on histamine release using rat peritoneal mast cells. It was demonstrated that *Pleurotus pulmonarius* at both 30 and 100 μg/ml significantly inhibited compound 48/80-induced histamine release. Thus, we assumed that the inhibitory ef-
effects of *Pleurotus pulmonarius* on sneezing and nasal rubbing behavior may be attributable to the inhibition of mast cell degranulation.

In conclusion, *Pleurotus pulmonarius* showed inhibitory effects on immediate allergic reactions, and its mechanism of action is generated probably by reducing the release of chemical mediators such as histamine from mast cells.

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**REFERENCES**
