Inhibition of Experimental Allergic Rhinitis by the n-Butanol Fraction from the Anomalous Fruits of Gleditsia sinensis

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This study was conducted to clarify the effect of the n-butanol fraction from the anomalous fruits of Gleditsia sinensis Lam. (NBGS) on experimental allergic rhinitis. NBGS (100, 200, 400 mg/kg, p.o.) dose-dependently inhibited nasal symptoms (sneezing and nasal rubbing) and dye leakage induced by antigen challenge into the nasal cavity of actively sensitized rats. Significant effects were observed at doses of 200 and 400 mg/kg. NBGS (200, 400 mg/kg) also showed a clear inhibition of sneezing and an inhibitory tendency on nasal rubbing induced by histamine in normal rats. At 400 mg/kg, it significantly reduced dye leakage induced by histamine into the nasal cavity of rats. Terfenadine (10 mg/kg, p.o.), an antihistaminic drug, clearly inhibited the nasal symptoms and the amount of dye leakage induced by antigen or histamine. Furthermore, NBGS significantly reduced in vitro histamine release from rat peritoneal mast cells triggered by compound 48/80 at concentrations of 30 and 100 μg/ml. These results suggest that NBGS may be clinically effective in alleviating the nasal symptoms of allergic rhinitis, probably by inhibiting both histamine release from mast cells and nasal vascular permeability.

Key words Gleditsia sinensis; allergic rhinitis; nasal symptom; histamine release; nasal vascular permeability

Allergic rhinitis, the most common atopic disease, is characterized by the major symptoms of sneezing, itching, nasal congestion and rhinorrhea, which are presumed to be triggered by multiple mediators released from mast cells and other inflammatory cells.1,2) The frequency of allergic rhinitis in the general population has been reported to be increasing year by year. Because of the high prevalence of allergic rhinitis, impaired quality of life, cost of treatment, and presence of comorbidities such as asthma, sinusitis, and otitis media, allergic rhinitis has a tremendous impact on society. Antihistamines, corticosteroids and anti-allergic drugs are used frequently as remedies for the treatment of allergic rhinitis. On the other hand, there has been an enormous interest in the search for new drugs from traditional folk medicines.3) Gleditsia sinensis Lam. is a perennial shrub widely distributed throughout China. The anomalous fruits of G. sinensis have long been known in traditional Chinese medicine as a saponin-rich herbal medicine for treating various diseases.4) Recently, we have demonstrated that the 70% ethanol extract from the anomalous fruits of G. sinensis possesses inhibitory effects on mast cell dependent, immediate allergic reactions such as homologous passive cutaneous anaphylaxis in rats.5) Zhang et al.6)—(9) have isolated and identified over ten kinds of oleane-type triterpenoidal saponins from the n-butanol fraction of the anomalous fruits of G. sinensis (NBGS). In an attempt to develop the fraction into a therapeutic drug for allergic rhinitis, the present study was undertaken to evaluate the effect of NBGS on experimental allergic rhinitis in rats.

MATERIALS AND METHODS

Animals Male SD rats (weighing 180—220 g) from the animal center of China Pharmaceutical University were used. They were allowed food and water ad libitum, and housed in an air-conditioned room at 23 ± 2 °C with lighting from 8:00 to 20:00.

Preparation of NBGS The anomalous fruits of G. sinensis were purchased from a herbal market in Nanjing, China. A voucher specimen (Dai 020301) is deposited in the Department of Pharmacology of Chinese Materia Medica, China Pharmaceutical University. The preparation procedure of NBGS was almost same as that reported by Zhang et al.6) In brief, five hundred grams of G. sinensis fruits were ground and refluxed with 70% ethanol (3.5 l) three times for 2 h each time. The alcoholic extract was concentrated (247 g), suspended in water, and then partitioned successively with chloroform (12 g) and n-butanol (91 g). The n-butanol fraction (NBGS) was freshly prepared in distilled water or physiological buffer saline (PBS) just before use.

Chemicals and Reagents Ovalbumin (chicken egg, Grade V), compound 48/80 and bovine serum albumin were purchased from Sigma; histamine diphosphate monohydrate was purchased from the Biochemical Research Institute of Shanghai, China; terfenadine was purchased from Jiangsu Lianhuan Pharmaceutical Company, China; Triton X-100 was purchased from Amresco; o-phthalaldehyde (OPT) was obtained from Wako; and, disodium cromoglycate (DSCG) was obtained from Biomol. Other reagents used were of analytical grade. Just before use, all reagents were dissolved in distilled water for oral administration or in PBS for injection.

Nasal Symptoms Induced by Antigen The experiment was carried out according to the method of An et al.10) Briefly, rats were actively sensitized by a peritoneal injection of 1 ml of physiological saline containing ovalbumin (1 mg) and alum (10 mg) on the first day. They were immunized every other day for seven times following the same procedure. Then, local sensitization was performed by dripping ovalbumin in saline solution (10 μg/10 μl/nostril) into the bilateral nasal cavities using a micropipette each day from day 14 to day 21. Before the experiment, rats were placed in an observation cage (32×20×12 cm) for about 10 min for acclimatization. After nasal instillation of ovalbumin, the rats were placed back into the observation cage (one rat/cage). The number of sneezes and nasal rubbings were counted for 30 min. Test drugs were administered orally 1 h before antigen challenge.

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Nasal Symptoms Induced by Histamine  As previously described, histamine was administered orally 1 h before histamine challenge. Each column and vertical bar represents the mean ± S.D. for 8 rats. *: Significantly different from the control group ($p<0.05$ and $p<0.01$, respectively).

Histamine Release from Rat Peritoneal Mast Cells

The procedure for examining histamine release from rat peritoneal mast cells was same as reported previously. In brief, mixed rat peritoneal cells were collected by peritoneal lavage and were purified by centrifugation through Ficoll density gradient. Purified mast cells were washed and resuspended in PBS (NaCl 154 mM, KCl 2.7 mM, CaCl2 0.9 mM, Na2HPO4 4 mM, KH2PO4 2.7 mM, glucose 5.6 mM and 0.1% bovine serum albumin). Mast cell preparations were about 92% pure as assessed by toluidine blue staining. Cell viability was confirmed to be around 90% before and after experiments by the trypan blue exclusion test.

Purified mast cells ($2\times10^6$ cells/ml) were preincubated at 37°C for 10 min. Then, NBGS and DSCG dissolved in PBS were added 5 min before activation by compound 48/80 (0.5 µg/ml). The reaction was stopped 10 min later by chilling the test tubes in ice water. The supernatants and cell pellets were then separated by centrifugation, and 0.05% Triton X-100 was added to the cell pellets to liberate the residual histamine. After addition of 0.036% OPT methanol solution, histamine content in supernatants (Supernatant) and cell pellets (Cell pellet) was determined spectrophotometrically (Em 360 nm, Ex 450 nm). To estimate the spontaneous release of histamine (Spontaneous), exactly the same procedure was followed but without adding samples or compound 48/80. The release percentage of histamine was calculated by the following equation.

$$\text{Histamine release} (\%) = \left( \frac{\text{Supernatant} - \text{Spontaneous}}{\text{Supernatant} + \text{Cell pellet}} \right) \times 100%$$

Statistical Analysis The data are presented as means ± S.D. Statistical significance was tested by ANOVA and Dunnett’s test. A probability value less than 0.05 was considered as significant.

RESULTS

Effects of NBGS and Terfenadine on the Sneezes and Nasal Rubbings Induced by Antigen  As shown in Fig. 1, NBGS (100, 200, 400 mg/kg) caused a dose-related inhibition of the sneezes and nasal rubbings of rats; significant effects were observed at doses of 200 and 400 mg/kg. Terfenadine (10 mg/kg) significantly inhibited the sneezes and nasal rubbings.

Effects of NBGS and Terfenadine on Sneezes and Nasal Rubbings Induced by Histamine  The effects of NBGS and terfenadine on the sneezes and nasal rubbings induced by histamine are shown in Fig. 2. NBGS (100, 200, 400 mg/kg) dose-dependently reduced the sneezes and nasal rubbings of rats. At doses of 200 and 400 mg/kg, NBGS showed a significant inhibition of the sneezes and an inhibitory tendency on nasal rubbings. Terfenadine (10 mg/kg) clearly reduced the sneezes and nasal rubbings.

Effects of NBGS and Terfenadine on Nasal Vascular Permeability Induced by Antigen  As shown in Fig. 2, NBGS (100, 200, 400 mg/kg) showed a clear reduction of the amount of dye leakage induced by antigen in actively sensitized rats. Terfenadine also significantly inhibited the dye leakage induced by antigen.
leakage at a dose of 10 mg/kg (Fig. 3).

**Effects of NBGS and Terfenadine on Nasal Vascular Permeability Induced by Histamine in Normal Rats**

As shown in Fig. 4, NBGS (400 mg/kg) clearly reduced the amount of dye leakage induced by histamine in normal rats, and terfenadine (10 mg/kg) also did.

**Effects of NBGS and DSCG on Histamine Release from Rat Peritoneal Mast Cells Induced by Compound 48/80**

Mast cell histamine content was approximately 12.5 μg/million cells, and the spontaneous release of histamine from rat peritoneal mast cells was 8.5%. Compound 48/80 (0.5 μg/ml) elicited about 86% histamine release from mast cells. NBGS (30, 100 μg/ml) pretreatment significantly reduced the release by 50.9% and 31.3%, respectively. DSCG clearly inhibited histamine release at a concentration of 500 μg/ml (Fig. 5).

**DISCUSSION**

Immune responses in allergic rhinitis can be divided into two phases, the immediate or early-phase response and the late-phase response. Sneezing, itching, and copious clear rhinorrhea are characteristic symptoms during the early phase response, which is mediated by the actions of preformed and newly generated chemical mediators released after IgE-dependent activation of nasal mucosal mast cells and other inflammatory cells. Among these mediators, histamine plays a pivotal role in both humans and rats. On the other hand, nasal congestion, fatigue, malaise, and neurocognitive deficits are characteristic symptoms during the late-phase response. Eosinophils, neutrophils, basophils, T lymphocytes and macrophages infiltrate into the nasal mucosa. The inflammatory mediators derived from these cells lead ultimately to the clinical and histological pictures of chronic al-
ergic diseases. Antihistaminics, extensively used for the treatment of allergic rhinitis, are capable of relieving immediate sneezing but not nasal blockage. In the present study, ovalbumin-induced nasal symptoms and nasal vascular permeability in actively sensitized rats were clearly inhibited by terfenadine, suggesting that the animal model of allergic rhinitis is mast cell- and histamine-dependent.

NBGS (100, 200, 400 mg/kg) dose-dependently inhibited nasal symptoms such as sneezes and nasal rubbing as well as dye leakage into the nasal cavity induced by ovalbumin challenge in actively sensitized rats. In vitro, it significantly reduced histamine release from rat peritoneal mast cells caused by compound 48/80 at concentrations of 30 and 100 μg/ml. Furthermore, NBGS (200, 400 mg/kg) reduced both nasal symptoms and increase of nasal vascular permeability induced by histamine in rats. These findings indicate that the inhibitory effect of NBGS on experimental allergic rhinitis is probably contributed by inhibiting both the release of mediators such as histamine from mast cells and the increase of nasal vascular permeability induced by these mediators.

NBGS is the saponin-rich fraction isolated from the anomalous fruits of *Gleditsia sinensis*. The results of the present study suggest that NBGS and single saponins that make up NBGS are potential remedies for alleviating the symptoms of allergic rhinitis and other allergy-related diseases. Further investigations are being made to clarify the precise mechanism and effective components responsible for the anti-allergic activities of NBGS.

REFERENCES