

Effect of *Ganoderma lucidum* on Pollen-induced Biphasic Nasal Blockage in a Guinea Pig Model of Allergic Rhinitis

Nobuaki Mizutani,^{1*} Takeshi Nabe,² Masaji Shimazu,³ Shin Yoshino¹ and Shigekatsu Kohno²

¹Department of Pharmacology, Kobe Pharmaceutical University, 4-19-1 Motoyamakita, Higashinada, Kobe 658-8558, Japan

²Department of Pharmacology, Kyoto Pharmaceutical University, 5 Nakauchi, Misasagi, Yamashina, Kyoto 607-8414, Japan

³Health Products Division, Hamari Chemicals, Ltd, 1-4-29, Kunijima, Higashiyodogawa, Osaka 533-0024, Japan

Ganoderma lucidum (GL), an oriental medical mushroom, has been used in Asia for the prevention and treatment of a variety of diseases. However, the effect of GL on allergic rhinitis has not been well defined. The current study describes the inhibitory effect of GL on the biphasic nasal blockage and nasal hyperresponsiveness induced by repeated antigen challenge in a guinea pig model of allergic rhinitis. Intranasally sensitized guinea pigs were repeatedly challenged by inhalation of Japanese cedar pollen once every week. *Ganoderma lucidum* was orally administered once daily for 8 weeks from the time before the first challenge. The treatment with GL dose-dependently inhibited the early and late phase nasal blockage at the fifth to ninth antigen challenges. Furthermore, nasal hyperresponsiveness to intranasally applied leukotriene D₄ on 2 days after the eighth antigen challenge was also inhibited by the treatment with GL. However, Cry j 1-specific IgE antibody production was not affected by the treatment. In conclusion, we demonstrated that the pollen-induced biphasic nasal blockage and nasal hyperresponsiveness were suppressed by the daily treatment with GL in the guinea pig model of allergic rhinitis. These results suggest that GL may be a useful therapeutic drug for treating patients with allergic rhinitis. Copyright © 2011 John Wiley & Sons, Ltd.

Keywords: *Ganoderma lucidum*; allergic rhinitis; nasal blockage; nasal hyperresponsiveness; guinea pig; cedar pollen.

INTRODUCTION

Patients with allergic rhinitis have serum IgE antibody against specific allergens including pollens of trees, grasses or weeds, and characteristic symptoms of the condition are sneezing, rhinorrhea and nasal blockage (Naclerio, 1991). When specific allergens are applied to the nasal cavities of patients, over 90% immediately respond by sneezing, rhinorrhea and nasal blockage (Iliopoulos *et al.*, 1990). In addition, about 50% of these patients further develop a late phase reaction with the predominant symptom being nasal blockage, which results mainly from sustained nasal congestion (Pelikan, 1978; Iliopoulos *et al.*, 1990). Furthermore, the nasal responsiveness of allergic rhinitis patients to stimuli other than a specific allergen increases compared with those of healthy individuals (Naclerio, 1991; Kanthawatana *et al.*, 1997).

We have established an experimental allergic rhinitis model using cedar pollen as antigen, demonstrating the development of biphasic nasal blockages with peaks at 1 and 4 h by repeated pollen inhalation challenges, immediate sneezing at the challenge and the increase of Cry j 1-specific IgE levels in the sera (Nabe *et al.*, 1998, 2005). Furthermore, marked nasal hyperresponsiveness to histamine and leukotriene (LT) D₄ was observed after repetitive antigen challenges in this model (Mizutani *et al.*, 1999, 2001). These events are

similar to clinical situations, indicating that the model is suitable for analysing the pathogenesis of allergic rhinitis and the evaluation of new types of therapeutic drugs.

On the other hand, the medical mushroom *Ganoderma lucidum* (GL) has been used traditionally in East Asia for the prevention and treatment of various diseases, such as cancer, hypertension, hepatitis, hyperglycemia and bronchitis (Kimura *et al.*, 2002; Maruyama *et al.*, 1989; Min *et al.*, 2000). Furthermore, experimental studies have shown the inhibitory effects of GL on carrageenan- or formalin-induced paw edema (Lakshmi *et al.*, 2003) and mosquito allergy-induced itch in mice (Andoh *et al.*, 2010), suggesting that GL may have anti-inflammatory and antiallergic effects. However, the effects of GL on the nasal symptoms induced by antigen challenge in experimental allergic rhinitis have not yet been elucidated.

In the present study, we investigated the effects of the long-term treatment with GL on sneezing, biphasic nasal blockage, nasal hyperresponsiveness to leukotriene D₄ (LTD₄), and Cry j 1-specific IgE production induced by the antigen challenges in the experimental allergic rhinitis model.

MATERIALS AND METHODS

Animals. Male, 3-week-old Hartley guinea pigs weighing 201–250 g were purchased from Japan SLC, Hamamatsu, Japan. The animals were housed in an air-conditioned room at a temperature of 23 ± 1 °C and 60 ± 1% humidity

* Correspondence to: N. Mizutani, Department of Pharmacology, Kobe Pharmaceutical University, 4-19-1 Motoyamakita, Higashinada, Kobe 658-8558, Japan.

E-mail: mizutani@kobepharm-u.ac.jp

with lights on from 8:00 a.m. to 8:00 p.m.; they were fed a standard laboratory diet and given water *ad libitum*. The sensitization period was started 1 week after the purchase. Animal experiments were approved by the Experimental Animal Research Committee at Kyoto Pharmaceutical University.

Commercial preparation of GL. *Ganoderma lucidum* powder (100%, preparation for human use) containing a guaranteed potency of 7.5% chitin and 40% β -glucans was donated by Hamari Chemicals, Ltd. (Osaka, Japan). In the present study, the powder was suspended in distilled water and administered in a volume of 5 mL/kg body weight.

Reagents. Japanese cedar pollen (*Cryptomeria japonica*) was harvested in Gifu and Shiga Prefecture (Japan) in 1998. Lidocaine hydrochloride was obtained from Fujisawa Pharmaceutical (Osaka, Japan). Leukotriene D₄ was obtained from Cayman Chemical (Ann Arbor, MI, USA).

Al(OH)₃ gel was prepared using 0.25 N NaOH and 0.25 N Al₂(SO₄), as described elsewhere (Nabe *et al.*, 1997a).

The cedar pollen extract used for sensitization was prepared as described elsewhere (Nabe *et al.*, 1998). Briefly, pollen was suspended in phosphate-buffered saline at 100 mg/mL and kept at 4°C for 18 h with mild stirring. The suspension was centrifuged (1700 g, 15 min), and the supernatant was stored at -80°C until used as the sensitization antigen.

Sensitization and challenge. As previously described (Nabe *et al.*, 1998), twice a day for 7 days, guinea pigs were sensitized by bilateral intranasal instillation of cedar pollen extracts adsorbed onto Al(OH)₃ gel (0.3 μ g protein/0.3 mg Al(OH)₃) at a volume of 3 μ L in each nostril. To prevent the rapid elimination of the antigen by ciliary movement, prior to each sensitization, the upper airway mucosal surface was anesthetized by using a 5-min inhalation of a mist of 4% lidocaine hydrochloride solution that was generated by an ultrasonic nebulizer (NE-U12; Omron, Osaka). The sensitized animals were then intranasally challenged once every week until the eleventh challenge by inhalation of 1.8 mg of cedar pollen (3.6 mg/animal) using a hand-made inhalation apparatus (Nabe *et al.*, 1997b), which was designed to allow quantitative inhalation of pollen.

In this study, we evaluated both biphasic nasal blockage and nasal hyperresponsiveness to LTD₄ at the eighth pollen challenge in this model because we have previously reported that such cedar pollen challenge induces reproducible responses from the fourth to

subsequent challenges (Nabe *et al.*, 1998; Mizutani *et al.*, 2001). For a negative control, a group of nonsensitized-challenged animals, which were not sensitized but exposed to the pollen once on the day corresponding to the eighth challenge, was prepared (NS-C group).

Drug administration. The protocol followed in this study is shown in Fig. 1. Guinea pigs that had been sensitized with pollen extract plus Al(OH)₃ were repeatedly challenged with the cedar pollen once every week. For a long-term treatment protocol, oral administration of GL (100 and 1000 mg/5 mL/kg) was started 2 days before the first challenge, followed by once daily treatment until the day of the ninth challenge. For a short-term treatment protocol, oral GL administration (1000 mg/kg) was started 1 day after the seventh antigen challenge until the day of the eighth challenge. On days of the pollen inhalation challenge, GL was administered 2 h before the respective pollen challenges.

During the daily long-term treatment with GL, there was no significant difference in body weight among the three groups: mean $\pm$ SE of body weight values on the day of the ninth antigen challenge in sensitized-challenged-vehicle, sensitized-challenged-GL (100 mg/kg) and sensitized-challenged-GL (1000 mg/kg) were 730.5 $\pm$ 24.3, 714.0 $\pm$ 19.5 and 740.2 $\pm$ 10.5 g, respectively.

Measurement of nasal blockage. To evaluate the degree of nasal blockage, specific airway resistance (sRaw) was measured in conscious guinea pigs before 1 and 4 h after the fifth, sixth, seventh, ninth, tenth, and eleventh antigen challenges using a two chambered, double-flow plethysmograph system (Pulmos-I; MIPS, Osaka, Japan) according to the method of Pennock *et al.* (1979). Changes in sRaw after challenges were calculated by subtracting individual baseline sRaw values. At the eighth challenge, sRaw was measured before and 1 to 8 h after the challenge. The effect of GL on early and late phase nasal blockage was expressed as the area under the response curve (AUC) for the changes in sRaw at 0–3 h (early phase) and 3–8 h (late phase) after the eighth challenge.

Because the guinea pig functionally respire through the nose but not through the mouth, sRaw can be taken as the total resistance of the upper and lower airways in the animal. However, in a previous study, when spontaneously breathing conscious guinea pigs were forced to inhale pollen as described above, almost all of the inhaled pollen was trapped in the upper airways, with less than 0.001% reaching the lower airways (Nabe *et al.*, 1997b). Furthermore, although antigen inhalation-induced early bronchoconstrictor response is characterized by rapid and shallow breathing in another guinea pig model of

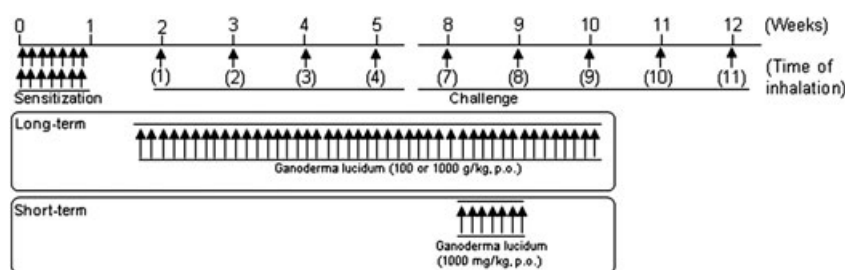


Figure 1. Schedules for sensitization with cedar pollen extracts and subsequent nasal challenge by inhalation with cedar pollen in the guinea pig, and for long- and short-term treatment with *Ganoderma lucidum*.

asthma (Iijima *et al.*, 1987), previous findings show that in the present model, the pollen-inhalation challenge-induced elevation of sRaw correlated well with the decrease in respiratory frequency (Nabe *et al.*, 1998). In addition, eosinophil accumulation in the lung, which is a characteristic feature of asthmatic response, was not observed at 5 h after the pollen-inhalation challenge in this model (Yamasaki *et al.*, 2001a), although eosinophils were markedly increased in the nasal cavity lavage fluid at 5 h after the challenge (Yamasaki *et al.*, 2001b). These findings indicate that the change in sRaw induced by pollen challenge in the present model almost exclusively reflects upper airway obstruction.

Determination of sneezing frequency. Sneezing frequencies in intervals 0–10 min and 10 min to 1 h after the eighth pollen challenge were counted.

Measurement of nasal responsiveness to LTD₄. To estimate the nasal responsiveness to LTD₄, sRaw was measured before and 10 min after intranasal instillation of a LTD₄ solution. Briefly, 2 days after the eighth inhalation challenge, 10 µL of LTD₄ (10⁻⁸ and 10⁻⁶ M) in solution was consecutively applied to each nasal cavity of conscious guinea pigs.

Changes in sRaw induced by the intranasal instillation of LTD₄ solution can also be considered to reflect upper airway obstruction, because the LTD₄ challenge decreased respiratory frequency in our allergic model (Mizutani *et al.*, 1999).

Measurement of NO₂⁻ and NO₃⁻ in nasal cavity lavage fluid. The nasal cavity lavage fluid (NCLF) was measured according to the previously described method (Nabe *et al.*, 1997b; Mizutani *et al.*, 2001) in sensitized-challenged and sensitized-challenged-GL (1000 mg/kg)-treated groups 2 days after the eighth inhalation challenge. Briefly, after anesthetization with sodium pentobarbital (40 mg/kg, i.p.), one end of the silicone tubing, the other end of which was connected to an air pump, was properly positioned in the right nostril under reduced pressure by the air pump; then 1 mL saline pre-warmed at 37°C was aspirated from the left nostril via a tube.

Three initial NCLFs were performed to remove pre-existing NO₂⁻ and NO₃⁻ at 20-min intervals to sensitized-challenged or sensitized-challenged-GL-treated guinea pigs, and the third NCLF served as a baseline. The LTD₄ (10⁻⁸ M) was intranasally instilled 10 min after the third NCLF and then NCLF was performed 10 min after the LTD₄ instillation.

The NCLF recovered was centrifuged at 10000 r.p.m. for 40 min at 4°C. The resultant supernatant was used for the NO₂⁻ and NO₃⁻ assay. The amounts of NO₂⁻ and NO₃⁻ were measured by NO₂⁻ and NO₃⁻ assay kit-F (fluorometric) (Dojindo Lab. Co., Ltd, Kumamoto).

Measurement of Cry j 1-specific IgE antibody in sera. As previously described (Nabe *et al.*, 2005; Nagata *et al.*, 2008), Cry j 1-specific IgE antibody was determined by an enzyme-linked immunosorbent assay (ELISA) kit (Guinea pig IgE ELISA MARUPI; Dainippon Pharmaceutical Co., Osaka) using blood samples collected 3 days after the ninth inhalation challenge. Because this kit is for the measurement of guinea pig total IgE, we modified the method provided by the manufacturer to

measure Cry j 1-specific IgE. Diluted sera was added into the wells of a microtitre plate pre-coated with anti-guinea pig IgE antibody, and then the plate was incubated for 1 h at 15–25°C. After washing, 100 ng/mL of biotinylated Cry j 1 (Asahi Food & Healthcare Co., Tokyo) was added at a volume of 100 µL/well, followed by incubation for 1 h at 15–25°C. The plate was washed, and then conjugated avidin-horseradish peroxidase (BD Pharmingen, San Diego, CA, USA) was added. Following incubation for 30 min and a subsequent washing, substrate solution was added, and the enzyme reaction was developed for 30 min at 15–25°C. The reaction was stopped with stop solution and absorbance values were measured at 450 nm.

Values for Cry j 1-specific IgE level in tested sera were expressed in arbitrary units relative to the value of a pooled standard serum from the sensitized-challenged guinea pig. The standard serum was prepared by i.p. injection of the pollen extract adsorbed onto Al(OH)₃ (20 µg pollen extract/20 µg Al(OH)₃/mL/time/animal) once every week for a total of nine times in naïve guinea pigs. The sera were harvested 2 weeks after the last sensitization and combined. Cry j 1-specific IgE titre of the serum was regarded as 1000 AU/mL.

Statistical analyses. Statistical analysis was performed by one-way analysis of variance. If a significant difference was detected, the individual group difference was determined by Bonferroni's multiple test. Comparisons of the amounts of NO₂⁻ and NO₃⁻ before and after LTD₄ instillation were performed by the paired *t*-test. A probability value (*p*) of less than 0.05 was considered to be statistically significant.

RESULTS

Effect of GL on the antigen-induced biphasic nasal blockage

We investigated effects of the long-term treatment with GL on early and late phase nasal blockages by measuring sRaw at 1 and 4 h, respectively, after antigen challenges. *Ganoderma lucidum* (100 and 1000 mg/kg) was found to inhibit both early and late phase nasal blockage at the fifth to ninth antigen challenges (Fig. 2). The inhibitions were not always statistically significant at the challenging times observed, whereas dose-dependent inhibitory effects, or their tendency, were consistently produced by the long-term GL treatment (Fig. 2). As shown in Fig. 3A, time-course changes of sRaw of respective groups following the eighth antigen challenge were assessed: GL at both 100 and 1000 mg/kg significantly inhibited not only early but also late phase nasal blockage. When expressed as AUCs, significant inhibition by GL on the early and late phase nasal blockage was recognized in a dose-dependent fashion (Table 1). In contrast, although short-term treatment with GL (1000 mg/kg) showed a tendency to inhibit the biphasic nasal blockage, the difference was not significant (Table 2).

During the long-term treatment study, there was no significant difference in baseline sRaw at times before respective challenges among the three groups: mean ± SE of baseline sRaw value 2 h before the eighth challenge in

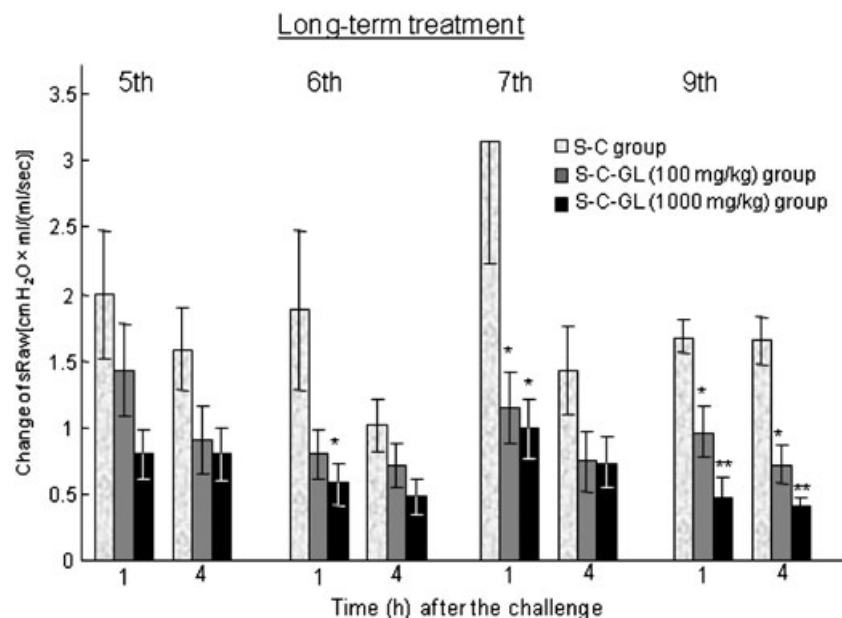


Figure 2. Effect of long-term treatment with *Ganoderma lucidum* on the biphasic nasal blockage induced by the fifth to ninth antigen challenges in sensitized guinea pigs. Data represents the mean $\pm$ SE of 12 animals. * p < 0.05, ** p < 0.01: significantly different from the S-C group. S-C, sensitized–challenged; sRaw, specific airway resistance; GL, *Ganoderma lucidum*.

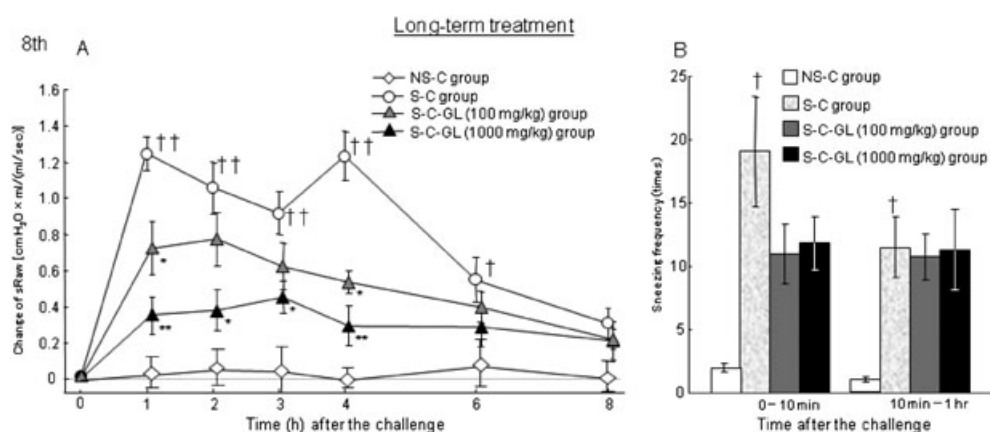


Figure 3. Effect of long-term treatment with *Ganoderma lucidum* on (A) biphasic nasal blockage and (B) sneezing frequency induced by the eighth antigen challenge in the sensitized guinea pig. Data represents the mean $\pm$ SE of 12 animals. † p < 0.05, †† p < 0.01: significantly different from the NS-C group. * p < 0.05, ** p < 0.01: significantly different from the S-C group. NS-C, non-sensitized–challenged; S-C, sensitized–challenged; sRaw, specific airway resistance; GL, *Ganoderma lucidum*.

Table 1. Effect of long-term treatment with *Ganoderma lucidum* on the area under the curve (AUC) for the increase in specific airway resistance (sRaw) at the early (0–3 h) and late (3–10 h) phases after the eighth antigen challenge

	Increase in sRaw	
	Early phase [AUC _{0–3 h}]	Late phase [AUC _{3–10 h}]
NS-C group	0.14 $\pm$ 0.07	0.12 $\pm$ 0.05
S-C group	2.76 $\pm$ 0.24 ^{††}	4.01 $\pm$ 0.52 ^{††}
S-C-GL (100 mg/kg) group	1.81 $\pm$ 0.34 (34)	2.35 $\pm$ 0.28* (41)
S-C-GL (1000 mg/kg) group	0.96 $\pm$ 0.24** (65)	1.67 $\pm$ 0.53* (58)

Data represent the mean $\pm$ SE of 12 animals. Values in parentheses represent the percentage inhibition.

^{††} p < 0.01: significantly different from the NS-C group.

* p < 0.05 and; ** p < 0.01: significantly different from the S-C group.

NS-C, non-sensitized–challenged; S-C, sensitized–challenged; GL, *Ganoderma lucidum*.

Table 2. Effect of short-term treatment with *Ganoderma lucidum* on the area under the curve (AUC) for the increase in specific airway resistance (sRaw) at the early (0–3 h) and late (3–10 h) phases after the eighth antigen challenge

	Increase in sRaw	
	Early phase [AUC _{0-3 h}]	Late phase [AUC _{3-10 h}]
NS-C group	0.13 ± 0.05	0.17 ± 0.09
S-C group	2.31 ± 0.45 ^{††}	3.51 ± 0.68 ^{††}
S-C-GL (1000 mg/kg) group	1.94 ± 0.39 (16)	2.96 ± 0.55 (16)

Data represent the mean ± SE of 12 animals. Values in parentheses represent the percentage inhibition.

^{††} $p < 0.01$: significantly different from the NS-C group.

NS-C, non-sensitized-challenged; S-C, sensitized-challenged; GL, *Ganoderma lucidum*.

sensitized-challenged-vehicle, sensitized-challenged-GL (100 mg/kg), and sensitized-challenged-GL (1000 mg/kg) were 1.026 ± 0.087 , 1.172 ± 0.057 , and 1.135 ± 0.072 ($n = 12$) $\text{cmH}_2\text{O} \times \text{mL}/(\text{mL}/\text{s})$, respectively.

Effect of GL on the antigen-induced sneezing

The number of sneezes at 0–10 min and 10 min to 1 h after the eighth pollen challenge was 20 for the former and 10 for the latter in the sensitized, vehicle-treated guinea pigs (Fig. 3B). *Ganoderma lucidum* (100 and 1000 mg/kg) tended to reduce the number of sneezes at 0–10 min but not for 10 min to 1 h (Fig. 3B). The short-term treatment did not show any inhibitory effect (data not shown).

Effect of GL on the development of nasal hyperresponsiveness to LTD₄

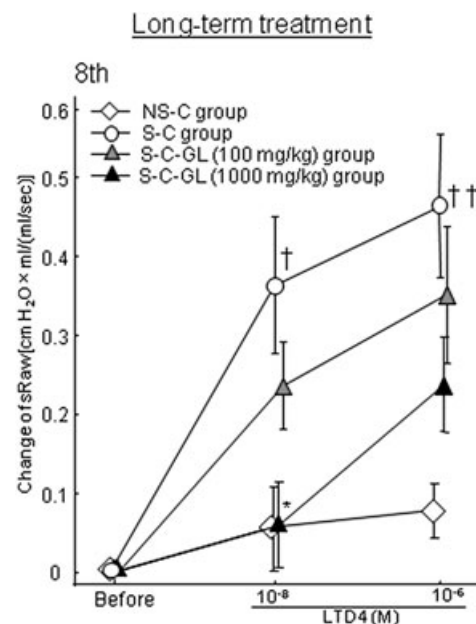
Consistent with previous findings (Mizutani *et al.*, 2001), nasal responsiveness to LTD₄ in the sensitized-challenged guinea pig on 2 days after the eighth challenge was considerably more marked compared with that of the non-sensitized-challenged guinea pig (NS-C group). The development of the nasal hyperresponsiveness to LTD₄ was significantly suppressed by the long-term treatment with GL (1000 mg/kg) (Fig. 4).

Effect of GL on the increase in NO₂[−] and NO₃[−] in NCLF induced by LTD₄

In the sensitized-challenged group, LTD₄ application caused a significant increase in NO₂[−] and NO₃[−] in NCLF compared with before treatment. In contrast, levels of NO₂[−] and NO₃[−] in NCLF in the sensitized-challenged-GL (1000 mg/kg)-treated group showed only a slight tendency to increase in response to LTD₄ (Table 3).

Effect of GL on Cry j 1-specific IgE production

An increased level of Cry j 1-specific IgE antibody in the serum was recognized after the ninth antigen challenge.

**Figure 4.** Effect of long-term treatment with *Ganoderma lucidum* on the development of the nasal hyperresponsiveness to LTD₄ induced by the eighth antigen challenge in the sensitized guinea pig. Data represent the mean ± SE of 12 animals. [†] $p < 0.05$, ^{††} $p < 0.01$: significantly different from the NS-C group. ^{*} $p < 0.05$: significantly different from the S-C group. NS-C, non-sensitized-challenged; S-C, sensitized-challenged; sRaw, specific airway resistance; GL, *Ganoderma lucidum*.**Table 3.** Effect of long-term treatment with *Ganoderma lucidum* on amounts of NO₂[−] and NO₃[−] in nasal cavity lavage fluid (NCLF) induced by LTD₄ 2 days after the eighth antigen challenge in sensitized guinea pigs

	Amount of NO ₂ [−] and NO ₃ [−] (nmol/animal)
S-C-before group	4.01 ± 0.29
S-C-LTD ₄ group	5.23 ± 0.36 [†]
S-C-GL-before group	4.11 ± 0.21
S-C-GL-LTD ₄ group	4.48 ± 0.35

Data represent the mean ± S.E. of eight animals. The amounts of NO₂[−] and NO₃[−] before and after LTD₄ instillation were compared in S-C and S-C-GL groups.

[†] $p < 0.05$: significantly different from S-C-before group.

S-C, sensitized-challenged; GL, *Ganoderma lucidum*; NCLF, nasal cavity lavage fluid.

No treatment with GL exerted any influence on IgE production (Table 4).

Resolution of inhibitory effect of GL on biphasic nasal blockage

To evaluate whether the effectiveness on biphasic nasal blockage is sustained after ceasing administration of GL, we stopped the oral administration 1 day after the ninth antigen challenge, and then observed the nasal blockage at the tenth and eleventh challenges. The inhibitory effect on the late phase nasal blockage was consistently sustained at the tenth challenge (Fig. 5).

Table 4. Effect of long-term treatment with *Ganoderma lucidum* on the production of Cry j 1-specific IgE at the ninth antigen challenge in the sensitized guinea pig

	Amount of IgE (AU/mL)
NS-C group	ND
S-C group	4.18 ± 3.2
S-C-GL (100 mg/kg) group	4.68 ± 2.7
S-C-GL (1000 mg/kg) group	4.31 ± 3.5

Data represent the mean ± S.E. of 12 animals.

S-C, sensitized–challenged; GL, *Ganoderma lucidum*; ND, not detected.

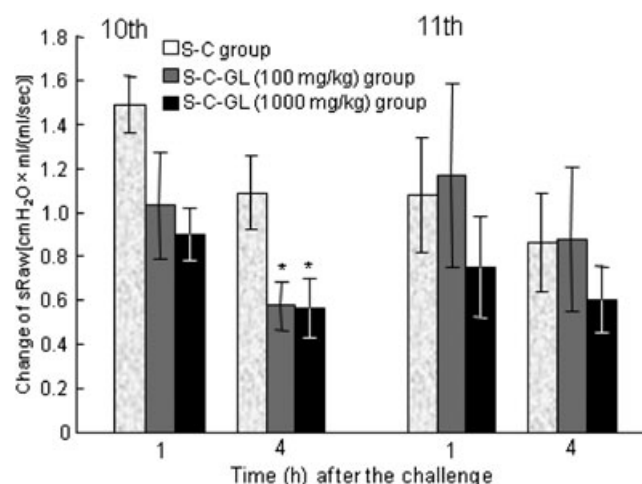


Figure 5. Sustained effect of *Ganoderma lucidum* (GL) on the biphasic nasal blockage after the tenth and eleventh inhalation challenges with Japanese cedar pollen in sensitized guinea pigs. The long-term treatment with GL was stopped at the ninth challenge. Data represent the mean ± SE of 12 animals. * $p < 0.05$: significantly different from the S-C group. S-C, sensitized–challenged; sRaw, specific airway resistance.

However, no inhibitory effect of GL was observed at the eleventh challenge in groups that had been treated with GL.

DISCUSSION

This study has demonstrated that when the sensitized–challenged guinea pig was treated daily with GL during the course of antigen challenge, the antigen-induced nasal blockage was consistently inhibited. In addition, GL also suppressed the development of nasal hyperresponsiveness to LTD₄. As previously reported (Nabe *et al.*, 2005), however, the level of specific IgE was elevated in proportion to the increase in the number of the antigen challenges in this experimental allergic rhinitis. Increase of the Cry j 1-specific IgE level, however, was not altered by the long-term treatment with GL, suggesting that the suppression of nasal symptoms by GL was not related to the level of specific IgE.

To assess whether the inhibitory effect of biphasic nasal blockage is sustained even after the cessation of the treatment with GL, we stopped the treatment at the ninth challenge, and observed the magnitude of pollen-induced nasal blockage at the tenth and eleventh

antigen challenges. The inhibitory effect of nasal blockage was sustained for at least 1 week after stopping the treatment with GL, but was gone by the following week. In contrast, short-term (1 week) treatment with GL (1000 mg/kg) did not significantly affect the antigen-induced nasal blockage. These results mean that the long-term treatment with GL from the initial stage of the multiple antigen challenges would be required for significant inhibition of nasal blockage.

Late phase, but not early phase, nasal blockage was found to be significantly attenuated by pranlukast, a cysteinyl LT₁ (CysLT₁) receptor antagonist (Mizutani *et al.*, 2001), and N^ω-nitro-L-arginine methyl ester (L-NAME), which has been known to non-selectively inhibit nitric oxide (NO) synthases, has been found to inhibit early and late nasal blockage (Imai *et al.*, 2001). Furthermore, LTD₄-induced nasal blockage (nasal hyperresponsiveness) was found to be strongly inhibited by L-NAME (Mizutani *et al.*, 2001), and LTD₄ instillation was found to cause a significant increase in NO₂[−] and NO₃[−] in NCLF compared with that before LTD₄ instillation in sensitized–challenged guinea pigs (Mizutani *et al.*, 2001), indicating that late phase nasal blockage is mostly due to NO production via CysLT₁ receptor activation. Therefore, we examined whether long-term treatment with GL inhibited the increase of NO₂[−] and NO₃[−] in NCLF induced by LTD₄ instillation. Levels of NO₂[−] and NO₃[−] in the sensitized–challenged-GL (1000 mg/kg)-treated group showed only a slight tendency to increase in response to LTD₄, although LTD₄ application caused a significant increase in levels of NO₂[−] and NO₃[−] in the sensitized–challenged group. These data suggest that the inhibitory effect of GL on late phase nasal blockage may be associated with the suppression of NO production via CysLT₁ receptor activation. On the other hand, NO can be involved in not only late but also early phase nasal blockage (Imai *et al.*, 2001), although histamine (Yamasaki *et al.*, 2001a), CysLTs (Mizutani *et al.*, 2001), kinins (Sugahara *et al.*, 2003) and tachykinins (Nabe *et al.*, 2008) do not take part in the early phase response, suggesting that the inhibition of early phase nasal blockage by GL may also be associated with the reduction of NO production.

It has been reported that GL significantly showed a dose-dependent inactivation of the production of reactive oxygen species (ROS), such as superoxide anions and hydroxyl radicals (Lee *et al.*, 2001). Furthermore, ROS can be involved in early and late phase nasal blockage because ebselen (Mizutani *et al.*, 2008), which has been known to scavenge ROS, inhibited early and late nasal blockage, suggesting that the development of nasal blockage was associated with ROS production. From the findings in this literature and our finding that ROS can be involved in nasal blockage, it was suggested that the inhibition of antigen-induced nasal blockage with treatment with GL could be due to suppressed production of ROS. We have to further analyse the effects of GL on the production and elimination of ROS in future studies.

Regarding the development of nasal hyperresponsiveness to LTD₄, we have reported that both kinins (Sugahara *et al.*, 2003) and tachykinins (Nabe *et al.*, 2008) mediate the development via the stimulation of bradykinin B₁ and B₂ receptors, and tachykinin NK₁ and NK₂ receptors. We have suggested the possibility that kinins immediately formed could stimulate release

of tachykinins in the nasal tissues, leading to the development of nasal hyperresponsiveness. The initiator of the hyperresponsiveness, kinins, have been known to be produced through the kininogenase activity of trypsin, which is released from the nasal mucosal mast cells by antigen-antibody reaction (Proud *et al.*, 1988; Walls *et al.*, 1992; Imamura *et al.*, 1996). In the present study, the development of nasal hyperresponsiveness was suppressed by GL, suggesting that the inhibitory mechanism might be due to the diminishment of kinins by the inhibition of trypsin produced by IgE-mediated mast cell activation.

On the other hand, it has been suggested that GL influenced various cytokine production in both *in vitro* and *in vivo* studies, and thereby GL may modulate immune functions (Lin, 2005). However, there has been no literature suggesting a relationship between the immune systems and GL with regard to allergic symptoms. Thus, detailed mechanisms underlying the inhibition of GL should be analysed in future from an immunological perspective.

Yamasaki *et al.* (2001a) previously reported that sneezing induced within 1 h after challenge was almost completely suppressed by treatment with antihistaminics. This indicates that histamine released from mast cells immediately after pollen inhalation plays a major role in the occurrence of sneezing. In this study,

long-term treatment with GL showed a tendency to inhibit the induction of sneezing. Furthermore, Kohda *et al.* (1985) reported that GL extract inhibited the release of histamine from mast cells induced by compound 48/80 *in vitro*. Together, these results show that GL may exert inhibitory effects on the release of histamine induced by IgE-mediated mast cell activation in the nasal mucosa.

In conclusion, we have demonstrated that long-term treatment with GL inhibited pollen-induced biphasic nasal blockage in this guinea pig model of allergic rhinitis. These results suggest that GL may be a useful therapeutic drug for treating patients with allergic rhinitis.

Acknowledgements

This work was supported in part by the High-Tech Research Center Project for Private Universities: matching fund subsidy from MEXT (Ministry of Education, Culture, Sports, Science and Technology), 2004–2008.

Conflict of interest

The authors have declared that there is no conflict of interest.

REFERENCES

- Andoh T, Zhang Q, Yamamoto T, *et al.* 2010. Inhibitory effect of the methanol extract of *Ganoderma lucidum* on mosquito allergy-induced itch-associated response in mice. *J Pharmacol Sci* **114**: 292–297.
- Iijima H, Ishii M, Yamauchi K, *et al.* 1987. Bronchoalveolar lavage and histological characterization of late asthmatic response in guinea pigs. *Am Rev Respir Dis* **136**: 922–929.
- Iliopoulos O, Proud D, Adkinson Jr NF, *et al.* 1990. Relationship between the early, late, and rechallenge reaction to nasal challenge with antigen: observations on the role of inflammatory mediators and cells. *J Allergy Clin Immunol* **86**: 851–861.
- Imai A, Nabe T, Mizutani N, Sakurai H, Takenaka H, Kohno S. 2001. Involvement of nitric oxide in pollen-induced biphasic nasal blockage in sensitised guinea pigs. *Eur J Pharmacol* **423**: 63–70.
- Imamura T, Dubin A, Moore W, Tanaka R, Travis J. 1996. Induction of vascular permeability enhancement by human trypsin: dependence on activation of prekallikrein and direct release of bradykinin from kininogens. *Lab Invest* **74**: 861–870.
- Kanthawattana S, Maturim W, Fooanant S, Manorot M, Trakultivakorn M. 1997. Evaluation of threshold criteria for the nasal histamine challenge test in perennial allergic rhinitis. *Asian Pac J Allergy Immunol* **15**: 65–69.
- Kimura Y, Taniguchi M, Baba K. 2002. Antitumor and antimetastatic effects on liver triterpenoid fractions of *Ganoderma lucidum*: mechanism of action and isolation of active substance. *Anticancer Res* **22**: 3309–3318.
- Kohda H, Tokumoto W, Sakamoto K, *et al.* 1985. The biologically active constituents of *Ganoderma lucidum* (Fr.) Karst. Histamine release-inhibitory triterpenes. *Chem Pharm Bull* **33**: 1367–1374.
- Lakshmi B, Ajith TA, Sheena N, Gunapalan N, Janardhanan KK. 2003. Antiperoxidative, anti-inflammatory, and antimutagenic activities of ethanol extract of mycelium of *Ganoderma lucidum* occurring in South India. *Terato Carcinog Mutagen* **1**: 85–97.
- Lee JM, Kwon H, Jeong H, Lee JW, Lee SY, Baek SJ, Surh YJ. 2001. Inhibition of lipid peroxidation and oxidative DNA damage by *Ganoderma lucidum*. *Phytother Res* **15**: 245–249.
- Lin ZB. 2005. Cellular and molecular mechanisms of immunomodulation by *Ganoderma lucidum*. *J Pharmacol Sci* **99**: 144–153.
- Maruyama H, Yamazaki K, Murofushi S, Konda C, Ikekawa T. 1989. Antitumor activity of *Sarcodon aspratus* (Berk.) S. Ito and *Ganoderma lucidum* (Fr.) Karst. *J Pharmacobiodyn* **12**: 118–123.
- Min BS, Gao JJ, Nakamura N, Hattori H. 2000. Triterpenes from the spores of *Ganoderma lucidum* and their cytotoxicity against Meth-A and LLC tumor cells. *Chem Pharm Bull* **48**: 1026–1033.
- Mizutani N, Nabe T, Sasaki K, Takenaka H, Kohno S. 1999. Nasal hyperresponsiveness to histamine induced by repetitive exposure to cedar pollen in guinea-pigs. *Eur Respir J* **14**: 1368–1375.
- Mizutani N, Nabe T, Imai A, Sakurai H, Takenaka H, Kohno S. 2001. Markedly increased nasal blockage by intranasal leukotriene D₄ in an experimental allergic rhinitis model: contribution of dilated mucosal blood vessels. *Jpn J Pharmacol* **86**: 170–182.
- Mizutani N, Nabe T, Fujii M, Yoshino S, Kohno S. 2008. Involvement of peroxynitrite in pollen-induced nasal blockage in guinea pigs. *Eur J Pharmacol* **582**: 139–144.
- Nabe T, Shinoda N, Yamashita K, Yamada M, Yamamura H, Kohno S. 1997a. Comparative studies on nebulizers for antigen inhalation in experimental asthma. *Allergol Int* **46**: 261–267.
- Nabe T, Shimizu K, Mizutani N, *et al.* 1997b. A new model of experimental allergic rhinitis using Japanese cedar pollen in guinea pigs. *Jpn J Pharmacol* **75**: 243–251.
- Nabe T, Mizutani N, Shimizu K, Takenaka H, Kohno S. 1998. Development of pollen-induced allergic rhinitis with early and late phase nasal blockage in guinea pigs. *Inflamm Res* **47**: 369–374.
- Nabe T, Kubota K, Terada T, Takenaka H, Kohno S. 2005. Effect of oral immunotherapy on nasal blockage in experimental allergic rhinitis. *J Pharmacol Sci* **98**: 380–387.
- Nabe T, Tsuzuki N, Ohtani Y, *et al.* 2008. Important roles of tachykinins in the development of allergic nasal hyperresponsiveness in guinea-pigs. *Clin Exp Allergy* **39**: 138–146.
- Naclerio RM. 1991. Allergic rhinitis. *N Eng J Med* **325**: 860–869.
- Nagata T, Nabe T, Fujii M, Mizutani N, Kohno S. 2008. Effects of multiple dexamethasone treatments on aggravation of allergic conjunctivitis associated with mast cell hyperplasia. *Biol Pharm Bull* **31**: 464–468.
- Pelikan Z. 1978. Late and delayed responses of the nasal mucosa to allergen challenge. *Ann Allergy* **41**: 37–47.

- Pennock BE, Cox CP, Rogers RM, Cain WA, Wells JH. 1979. A noninvasive technique for measurement of changes in specific airway resistance. *J Appl Physiol* **46**: 399–406.
- Proud D, Siekierski ES, Bailey GS. 1988. Identification of human lung mast cell kininogenase as tryptase and relevance of tryptase kininogenase activity. *Biochem Pharmacol* **37**: 1473–1480.
- Sugahara S, Nabe T, Mizutani N, Takenaka H, Kohno S. 2003. Kinins are involved in the development of allergic nasal hyperresponsiveness in guinea pigs. *Eur J Pharmacol* **476**: 229–237.
- Walls AF, Bennett AR, Suieras-Diaz J, Olsson H. 1992. The kininogenase activity of human mast cell tryptase. *Biochem Soc Trans* **20**: 260S.
- Yamasaki M, Mizutani N, Sasaki K, *et al.* 2001a. Involvement of thromboxane A2 and peptide leukotrienes in early and late phase nasal blockage in a guinea pig model of allergic rhinitis. *Inflamm Res* **50**: 466–473.
- Yamasaki M, Sasaki K, Mizutani N, *et al.* 2001b. Pharmacological characterization of the leukocyte kinetics after intranasal antigen challenge in a guinea pig model of allergic rhinitis. *Inflamm Res* **50**: 474–482.