



Preliminary report

Effect of 5-aminosalicylate on allergic rhinitis model in mice

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ARTICLE INFO

Article history:

Received 25 December 2009

Received in revised form 27 February 2010

Accepted 12 March 2010

Keywords:

5-aminosalicylate

Peroxisome proliferator-activated receptor γ

Allergic rhinitis

Immunoglobulin E

Interleukin-4

Interleukin-10

ABSTRACT

Previous studies have shown that peroxisome proliferator-activated receptor γ (PPAR γ) is involved in allergic rhinitis. It has been reported that 5-aminosalicylate (5-ASA) has an affinity for PPAR γ , but the effects of 5-ASA on the nasal symptoms of allergic rhinitis are unclear. This study aimed to clarify the effects of 5-ASA on nasal symptoms in an allergic rhinitis model in mice. Female BALB/c mice were sensitized by intraperitoneal injection of ovalbumin (OVA) and aluminium hydroxide hydrate gel (alum) on days 0, 5, 14 and 21. Seven days later, mice were sensitized by the intranasal application of OVA thrice a week. 5-ASA was also administered orally after instillation of the antigen from day 28. The severity of allergic rhinitis was assessed by determining the extent of 2 nasal allergic symptoms—sneezing and nasal rubbing. In addition, serum OVA-specific immunoglobulin E (IgE) antibody, interleukin (IL)-4, and IL-10 levels in nasal lavage fluid and histamine sensitivity were determined. Repeated oral administration of 5-ASA attenuated the progression of nasal symptoms in sensitized mice in a dose-dependent manner. Additionally, 5-ASA prevented an increase in histamine sensitivity. Finally, 5-ASA inhibited both OVA-specific IgE antibody and IL-4 production; however, it had no effect on IL-10 levels. These results indicate that 5-ASA has a prophylactic effect on allergic rhinitis.

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1. Introduction

Peroxisome proliferator-activated receptor γ (PPAR γ) is a subtype of PPAR that serves as a transcriptional regulator of genes involved in adipocyte lipid metabolism [1]. It has been reported that PPAR γ is expressed on monocytes and macrophages; therefore, there has been much interest in the involvement of PPAR γ in inflammatory processes [2]. In addition, PPAR γ is expressed in immune cells and involved in the production of cytokines [3]. Ueki et al. reported that the PPAR γ agonist troglitazone suppresses allergic airway symptoms in mice [4]. It has also been reported that the PPAR γ agonist rosiglitazone suppresses nasal allergy [5–7]. Therefore, the evidence suggests that PPAR γ agonists represent a new target of treatment for allergic rhinitis. However, it is also well known that treatment with thiazolidinediones, namely, pioglitazone, rosiglitazone, and ciglitazone, has been associated with an increase in the risk of heart failure, fluid retention, and peripheral edema [8–11]. Therefore, other types of medication are required for patients suffering from allergic rhinitis.

Recently, Rousseaux et al. [12] and Dubuquoy et al. [13] reported that 5-aminosalicylic acid (5-ASA) has an affinity for PPAR γ . 5-ASA is widely used in the treatment of Crohn's disease and ulcerative colitis, 2 major human forms of chronic inflammatory bowel disease (IBD). It is well known that mast cells play a central role in IBD, and it has been

observed that 5-ASA suppresses antigen-stimulated histamine release from rat peritoneal mast cells [14]. Barrett et al. also reported that sulfasalazine, a precursor of 5-ASA, causes a dose-dependent inhibition of anaphylactic histamine release from rat peritoneal mast cells [15]. Therefore, we hypothesize that 5-ASA suppresses nasal symptoms in a rhinitis model in mice.

In this study, we investigated the effects of repeated oral administration of 5-ASA on nasal symptoms, the progression of sensitivity to histamine, ovalbumin (OVA)-specific immunoglobulin E (IgE) antibody levels, and cytokine (interleukin (IL)-4 and IL-10) levels in sensitized mice to elucidate the mechanism of 5-ASA.

2. Materials and methods

2.1. Animals

Six-week-old female BALB/c mice were obtained from Japan SLC (Shizuoka, Japan).

Animals were maintained in an air-conditioned room with controlled temperature ($24\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$) and humidity ($55\% \pm 15\%$). They were housed in plastic cages lined with sawdust and kept under a light–dark cycle (lights on from 0700 to 1900 h). Food and water were freely available with the exception of test periods. All procedures involving animals were conducted in accordance with the Guidelines for Animal Experiments at Okayama University Advanced Science Research Center, and all procedures were licensed by the Animal Research Control Committee of Okayama University.

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2.2. Materials

The following drugs were used in this study: 5-ASA (Sigma, St. Louis, MO, USA), histamine dihydrochloride (Sigma), OVA (grade VII; crystallized and lyophilized, essentially salt-free, Sigma), and aluminium hydroxide hydrate gel (alum, LSL, Tokyo, Japan). 5-ASA was suspended in 0.5% carboxymethylcellulose (CMC) solution. After antigen instillation, 5-ASA was administered orally thrice a week from day 28 until day 63.

2.3. Sensitization

Female BALB/c mice were sensitized by administering an intraperitoneal injection of OVA (1 µg) in 200 µl of saline containing alum (100 µg) as an adjuvant on days 0, 5, 14, and 21. Local sensitization was then performed thrice a week, starting from day 28, by instilling OVA in physiological saline (100 mg/ml, 2 µl) into the bilateral nasal cavities.

2.4. Evaluation of nasal symptoms in mice

Before the experiment, the animals were placed in an observation cage (31 × 25 × 18 cm) for approximately 10 min for acclimatization. After nasal instillation of the antigen solution (OVA 100 mg/ml, 2 µl) into the bilateral nasal cavities using a micropipette, the animals were placed back into the observation cage (one animal/cage), and the frequency of sneezing and nasal rubbing was counted for 30 min. Nasal symptoms were observed weekly.

2.5. Measurement of nasal responsiveness to histamine

To estimate the nasal responsiveness to histamine, nasal symptoms were measured after intranasal instillation of a histamine solution. On day 63, 2 µl of increasing doses of histamine in saline solution (0.1, 1, 10, 100, and 1000 nmol/mouse) were consecutively instilled into each nasal cavity at 60-min intervals. Sneezing and nasal rubbing were counted for 10 min after the instillation of each histamine dose.

2.6. Measurement of serum OVA-specific IgE antibody

Blood specimens were collected from the ophthalmic veins 24 h after the last intranasal provocation with the antigen. Serum was obtained by centrifugation (700 × g for 10 min at 4 °C) and stored at −20 °C until measurement. OVA-specific IgE antibody in the serum was measured by enzyme-linked immunosorbent assay (ELISA), using a DS mouse IgE ELISA (OVA) kit (Dainippon Sumitomo Pharma, Osaka, Japan).

2.7. Collection of nasal lavage fluid

The nasal lavage fluid was collected 24 h after the last intranasal provocation with the antigen. After euthanasia, the trachea was ligated at the upper level and a catheter was guided into the nasopharynx. The nasal passages were gently perfused with 1 ml cold phosphate-buffered saline and collected in a tube. Corrected nasal lavage fluid was centrifuged at 700 × g for 10 min at 4 °C to separate cells and supernatants.

2.8. Measurement of amounts of IL-4 and IL-10 in the nasal lavage fluid

The amounts of IL-4 and IL-10 in the nasal lavage fluid were measured with ELISA, using a mouse IL-4 and IL-10 ELISA quantitation kit (R&D Systems, Inc., Minneapolis, MN, USA).

2.9. Statistical analysis

All experimental data are shown as the mean ± S.E.M. Statistical analysis of the data was performed with one-way analysis of variance (ANOVA) followed by a Dunnett's test for multiple comparisons. A probability value less than 0.05 was considered statistically significant.

3. Results

3.1. Effect of 5-ASA on nasal symptoms in sensitized mice

As shown in Fig. 1, repeated oral administration of 5-ASA caused a dose-dependent inhibition of sneezing and nasal rubbing in mice. Significant effects were observed from day 35 at a dose of 10 mg/kg and from day 49 at a dose of 1 mg/kg throughout the experimental period.

3.2. Effects of 5-ASA on acquisition of histamine sensitivity

Fig. 2 shows the frequency of sneezing and nasal rubbing induced by histamine in non-sensitized, sensitized, and 5-ASA (10 mg/kg)-treated sensitized mice. Intranasal application of the histamine solution resulted in an increase in nasal symptoms in non-sensitized mice. In the case of sensitized mice, sensitivity to histamine was increased. In contrast, repeated 5-ASA treatments inhibited the acquisition of histamine sensitivity.

3.3. Effects of 5-ASA on serum OVA-specific IgE antibody and nasal IL-4 and IL-10 levels

Repeated oral administration of 5-ASA at a dose of 10 mg/kg resulted in a significant decrease in serum OVA-specific IgE antibody

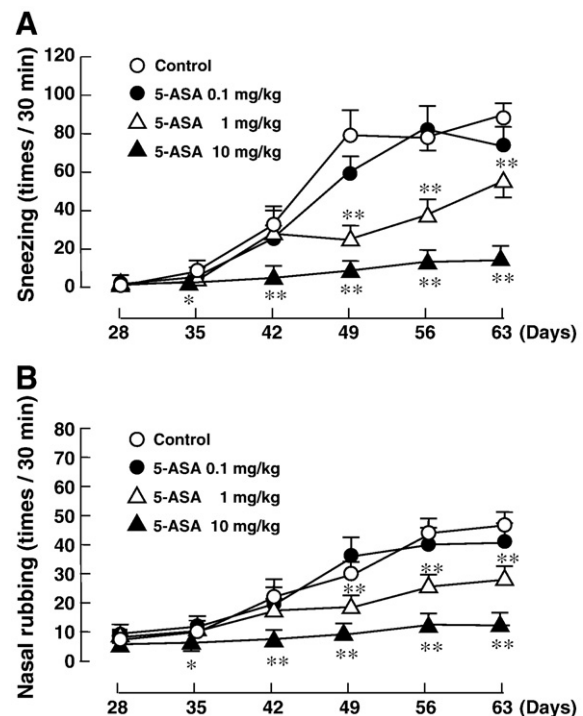


Fig. 1. Preventive effect of 5-ASA on sneezing (A) and nasal rubbing (B) induced by antigen in sensitized mice. 5-ASA was administered orally after instilling the antigen three times a week from day 28. The number of sneezing and nasal rubbing were counted for 30 min immediately after the nasal challenge. Open circle: control group. Closed circle: 5-ASA (0.1 mg/kg) treated group. Open triangle: 5-ASA (1 mg/kg) treated group. Closed triangle: 5-ASA (10 mg/kg) treated group. Each point and vertical bar shows the mean ± S.E.M. of 10 mice. *, **: Significantly different from control group at $P < 0.05$ and $P < 0.01$, respectively.

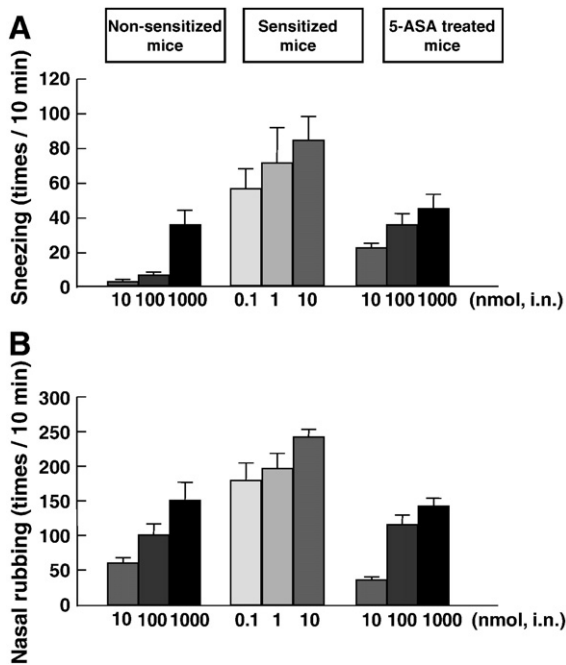


Fig. 2. Effect of repeated treatment of 5-ASA on sensitivity for histamine. Sneezing (A) and nasal rubbing (B) induced by histamine solution were counted for 10 min, using non-sensitized mice, sensitized mice (control mice at day 63) and 5-ASA (10 mg/kg) treated mice at day 63. Each column and vertical bar shows the mean $\pm$ S.E.M. of 10 mice.

levels (Fig. 3A). In addition, 5-ASA dose-dependently inhibited the production of IL-4 in the nasal lavage fluid, with a significant effect observed at a dose of 10 mg/kg (Fig. 3B). In contrast, 5-ASA had no significant effect on the production of IL-10 in the nasal lavage fluid (Fig. 3C).

4. Discussion

In the present study, the nasal symptoms of untreated mice increased with systemic sensitization and repeated local sensitization. Yokota et al. reported that nasal allergies in BALB/c mice increase in the same manner [16]. In contrast, 5-ASA administration caused a dose-related suppression of sneezing and nasal rubbing in sensitized mice. In ulcerative colitis, 5-ASA has been found to have an anti-inflammatory effect via the inhibition of inflammatory cell infiltration, suppression of platelet activating factor (PAF) generation, and inhibition of histamine release [17,18]. It is well known that histamine is one of the major chemical mediators of allergic symptoms [19,20]. Therefore, it is suggested that 5-ASA inhibition of histamine release from mast cells contributed to the anti-allergic effect in the present study.

Additionally, sensitization increased the sensitivity to histamine in mice, that is, lower doses of histamine increased sneezing and nasal rubbing in sensitized mice more than in normal mice. For this reason, nasal allergy progressed in connection with sensitization as a result of nerve elongation to the nasal dermis. In human patients with nasal rhinitis, detectable levels of nerve growth factor (NGF) increase in the nasal mucosa, and the sensory nerve is extended to the dermis [21]. However, mice that received repeated doses of 5-ASA did not acquire sensitivity to histamine due to protection from destruction of the barrier function of the nasal mucosa. In addition, it has been reported that PPAR agonists inhibit tumor necrosis factor- α (TNF- α) production in mast cells [22]. TNF- α is said to be an important factor for inflammation and is also associated with NGF production [23]. Furthermore, increased NGF production by keratinocytes results in nerve elongation. Thus, 5-ASA

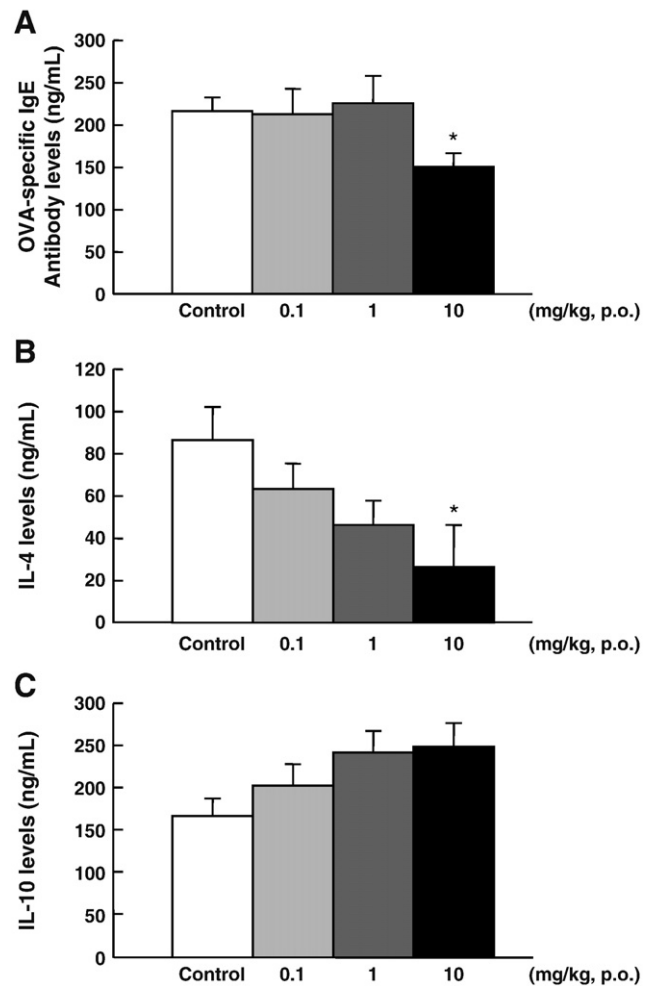


Fig. 3. The effects of 5-ASA on serum OVA-specific IgE antibody, and nasal IL-4 and IL-10 levels. OVA-specific IgE antibody (A) measured from serum of mice at day 64. IL-4 (B) and IL-10 (C) were measured from nasal lavage fluid of mice at day 64. Each column and vertical bar shows the mean $\pm$ S.E.M. of 10 mice. *: Significantly different from control group at $P < 0.05$.

may prevent sensory nerve growth due to the inhibition of TNF- α production that results from stimulation of PPAR γ .

Data from the present study show that repeated treatment with 5-ASA induces a decrease in OVA-specific IgE antibody production in sensitized mice. In addition, IL-4 levels in nasal lavage fluid are affected by 5-ASA treatment. IL-4 is one of the T-helper 2 (Th2) cytokines, which exacerbate nasal allergy. IL-10 seems likely to have a suppression effect on inflammation; it is said to be a suppressive cytokine [24]. It has been reported that 5-ASA has an affinity for PPAR γ , one of the family of nuclear receptors that is known to be involved in lipid metabolism and glucose metabolism [12]. Recently, PPAR γ expression has been found in eosinophils and mast cells, which are types of immune cells [4,25]. Additionally, Kang et al. reported that the expression of PPAR γ mRNA and protein in the nasal mucosa increased in patients with perennial allergic rhinitis [26]. Thus, it is suggested that the activation of PPAR γ negatively controls the activation of mast cells. It has also been reported that rosiglitazone suppresses nasal allergy [5–7]. Sugiyama et al. reported that ciglitazone regulates the cytokine production of mast cells [27]. Furthermore, it has been reported that rosiglitazone modulates the production of Th2 cytokines *in vivo* [6]. Because PPAR γ activation plays an important role in the production of cytokines, it is thought that 5-ASA also regulates cytokine production via PPAR γ activation.

Tachibana et al. reported that PPAR γ is involved in histamine synthesis [25]. In addition, Maeyama et al. reported that the histamine content in mast cells decreases by incubation with the endogenous PPAR γ ligand 15-deoxy- Δ (12, 14)-prostaglandin J₂, because expression of histidine decarboxylase, the sole histamine synthesis enzyme, is suppressed [28]. Moreover, Maeyama et al. reported that the long-term challenge of PPAR γ to mast cells induced inhibition of mast cell maturation [28]. Thus, 5-ASA may suppress maturation of mast cells via PPAR γ . These findings suggest that the inhibitory effects of both histamine and cytokine production may contribute to the anti-allergic activity of 5-ASA.

In clinical practice, the controlled-release 5-ASA preparation that is prescribed for IBD seems to exert its effect directly and locally on the large intestine. Therefore, it is thought that controlled-release 5-ASA is largely unabsorbed by the small intestine. For this reason, 5-ASA is thought to lack efficacy in treating allergic rhinitis in clinical practice. We propose that 5-ASA can affect allergic symptoms in clinical practice if the blood concentration of 5-ASA rises sufficiently by the administration of 5-ASA itself.

In conclusion, 5-ASA suppresses nasal allergy through inhibitory effects on serum IgE and nasal IL-4 levels. Moreover, we suggest that PPAR γ activation is involved in the anti-allergic effect induced by 5-ASA. Therefore, 5-ASA is promising as an innovative agent that targets PPAR γ for the suppression of the underlying processes of allergic rhinitis.

References

- [1] Kliewer SA, Forman BM, Blumberg B, Ong ES, Borgmeyer U, Mangelsdorf DJ, et al. Differential expression and activation of a family of murine peroxisome proliferator-activated receptors. *Proc Natl Acad Sci U S A* 1994;91:7355–9.
- [2] Moraes LA, Piqueras L, Bishop-Bailey D. Peroxisome proliferator-activated receptors and inflammation. *Pharmacol Ther* 2006;110:371–85.
- [3] Spears M, McSharryw C, Thomson NC. Peroxisome proliferator-activated receptor- γ agonists as potential anti-inflammatory agents in asthma and chronic obstructive pulmonary disease. *Clin Exp Allergy* 2006;36:1494–504.
- [4] Ueki S, Matsuwaki Y, Kayaba H, Oyama H, Kanda A, Usami A, et al. Peroxisome proliferator-activated receptor γ regulates eosinophil functions: a new therapeutic target for allergic airway inflammation. *Int Arch Allergy Immunol* 2004;134 (Suppl 1):30–6.
- [5] Jeon EJ, Lee SK, Park YS, Kim DH, Yum JH, Park CS. The effects of peroxisome proliferator-activated receptor- γ agonist on a murine model of experimental allergic rhinitis. *Otolaryngol Head Neck Surg* 2008;139:124–30.
- [6] Han JL, Ding RY, Zhao L, Ren Z, Jiang XJ. Rosiglitazone attenuates allergic inflammation and inhibits expression of galectin-3 in a mouse model of allergic rhinitis. *J Int Med Res* 2008;36:830–6.
- [7] Fukui N, Honda K, Ito E, Ishikawa K. Peroxisome proliferator-activated receptor γ negatively regulates allergic rhinitis in mice. *Allergol Int* 2009;58:247–53.
- [8] Rubenstrunk A, Hanf R, Hum DW, Fruchart JC, Staels B. Safety issues and prospects for future generations of PPAR modulators. *Biochim Biophys Acta* 2007;1771:1065–81.
- [9] Tang WH, Maroo A. PPAR γ agonists: safety issues in heart failure. *Diabetes Obes Metab* 2007;9:447–54.
- [10] Yang T, Soodvilai S. Renal and vascular mechanisms of thiazolidinedione-induced fluid retention. *PPAR Res* 2008;2008:943614.
- [11] Krentz A. Thiazolidinediones: effects on the development and progression of type 2 diabetes and associated vascular complications. *Diabetes Metab Res Rev* 2009;25:112–26.
- [12] Rousseaux C, Lefebvre B, Dubuquoy L, Lefebvre P, Romano O, Auwerx J, et al. Intestinal antiinflammatory effect of 5-aminosalicylic acid is dependent on peroxisome proliferator-activated receptor- γ . *J Exp Med* 2005;201:1205–15.
- [13] Dubuquoy L, Rousseaux C, Thuru X, Peyrin-Biroulet L, Romano O, Chavatte P, et al. PPAR γ as a new therapeutic target in inflammatory bowel diseases. *Gut* 2006;55:1341–9.
- [14] Bissonnette EY, Enciso JA, Befus AD. Inhibitory effects of sulfasalazine and its metabolites on histamine release and TNF- α production by mast cells. *J Immunol* 1996;156:218–23.
- [15] Barrett KE, Tashof TL, Metcalfe DD. Inhibition of IgE-mediated mast cell degranulation by sulphasalazine. *Eur J Pharmacol* 1985;107:279–81.
- [16] Yokota E, Kuyama S, Sugimoto Y, Ogawa M, Kamei C. Participation of histamine H₃ receptors in experimental allergic rhinitis of mice. *J Pharmacol Sci* 2008;108:206–11.
- [17] Capasso F, Tavares IA, Bennett A. Release of platelet-activating factor (PAF) from human colon mucosa and its inhibition by 5-aminosalicylic acid. *Drugs Exp Clin Res* 1991;17:351–3.
- [18] Fox CC, Moore WC, Lichtenstein LM. Modulation of mediator release from human intestinal mast cells by sulfasalazine and 5-aminosalicylic acid. *Dig Dis Sci* 1991;36:179–84.
- [19] Wang DY, Clement P. Pathogenic mechanisms underlying the clinical symptoms of allergic rhinitis. *Am J Rhinol* 2000;14:325–33.
- [20] Van Hoecke H, Vandenbulcke L, Van Cauwenberge P. Histamine and leukotriene receptor antagonism in the treatment of allergic rhinitis: an update. *Drugs* 2007;67:2717–26.
- [21] Bresciani M, Lilibertè F, Gramiccioni C, Bonini S. Nerve growth factor localization in the nasal mucosa of patients with persistent allergic rhinitis. *Allergy* 2009;64:112–7.
- [22] Iwasaki M, Saito K, Takemura M, Sekikawa K, Fujii H, Yamada Y, et al. TNF- α contributes to the development of allergic rhinitis in mice. *J Allergy Clin Immunol* 2003;112:134–40.
- [23] Takei Y, Laskey R. Interpreting crosstalk between TNF- α and NGF: potential implications for disease. *Trends Mol Med* 2008;14:381–8.
- [24] Ogawa Y, Duru EA, Ameredes BT. Role of IL-10 in the resolution of airway inflammation. *Curr Mol Med* 2008;8:437–45.
- [25] Tachibana M, Wada K, Katayama K, Kamisaki Y, Maeyama K, Kadowaki T, et al. Activation of peroxisome proliferator-activated receptor gamma suppresses mast cell maturation involved in allergic diseases. *Allergy* 2008;63:1136–47.
- [26] Kang HJ, Cinn YG, Hwang SJ, S, Woo JS et al. Up-regulation of peroxisome proliferator-activated receptor γ in perennial allergic rhinitis. *Arch Otolaryngol Head Neck Surg* 2006;132:1196–200.
- [27] Sugiyama H, Nonaka T, Kishimoto T, Komoriya K, Tsuji K, Nakahata T, et al. Peroxisome proliferator-activated receptors are expressed in human cultured mast cells: a possible role of these receptors in negative regulation of mast cell activation. *Eur J Immunol* 2000;30:3363–70.
- [28] Maeyama K, Emi M, Tachibana M. Nuclear receptors as targets for drug development: peroxisome proliferator-activated receptor gamma in mast cells: its roles in proliferation and differentiation. *J Pharmacol Sci* 2005;97:190–4.