Effects of Multiple Dexamethasone Treatments on Aggravation of Allergic Conjunctivitis Associated with Mast Cell Hyperplasia

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In a Japanese cedar pollen-induced allergic conjunctivitis model in guinea pigs, symptoms were aggravated by repeated pollen challenges. In addition, the number of mast cells in the conjunctiva was increased by multiple challenges. The amount of a mast cell mediator, histamine in ophthalmic lavage fluid was also increased by multiple challenges. In the present study, we evaluated the effects of multiple dexamethasone treatments to assess the relationship between the aggravation of symptoms and mast cell hyperplasia. Sensitized guinea pigs were challenged by dropping a pollen suspension onto their eye surface once a week until the 15th challenge. Dexamethasone (10 mg/kg, p.o.) was administered once 3 h before the 15th challenge or 3 h before every 1st—15th challenge. Mast cells in the conjunctival tissue were detected by toluidine blue staining. Histamine was fluorometrically assayed by high-performance liquid chromatography. Serum Cry j 1-sipecific IgE titer was measured by an enzyme-linked immunosorbent assay. The results indicated that a single treatment with dexamethasone did not affect the 15th challenge-induced symptoms; however, multiple treatments with the corticosteroid suppressed not only conjunctivitis symptoms after every challenge but also the mast cell hyperplasia and the increase in histamine in the lavage fluid. Conversely, the increase in the IgE titer in the serum was not affected by multiple treatments with dexamethasone. In conclusion, increased numbers of mast cells in the conjunctival tissue may be associated with the aggravation of allergic conjunctivitis symptoms.

Key words allergic conjunctivitis; corticosteroid; mast cell; histamine; pollen

Patients with allergic conjunctivitis are repeatedly exposed to allergen, leading to a chronic stage of the disease. Therefore, to elucidate mechanisms underlying the pathogenesis of this disease, we must use an experimental model in which allergic conjunctivitis develops after multiple challenges with allergen. We have developed a guinea pig model in which sensitized animals are repeatedly challenged by dropping a Japanese cedar pollen suspension onto the eye. Consequenctly, we found that multiple challenges aggravate the disease in proportion to the number of challenges. In addition, multiple antigen challenges increase the number of mast cells in conjunctival tissue. In agreement with our previous findings, it has been well demonstrated that aggravation of allergic conjunctivitis is accompanied by an increased number of mast cells in human conjunctival tissue. Although the types of mast cells that are increased in our guinea pig model have yet to be elucidated, it has been reported that among tryptase-positive, chymase-positive mast cells and tryptase-positive, chymase-negative mast cells, the predominant type increased in the conjunctival tissue of vernal conjunctivitis patients is tryptase-positive, chymase-positive mast cells.

On the other hand, we attempted to evaluate the effect of the anti-histaminic drug mepyramine on conjunctivitis induced after the respective 1st—15th challenges in the sensitized guinea pig. Similar to other models, we observed that mepyramine significantly suppressed responses at the 1st—5th challenges. However, allergic responses at the 7th—15th challenges were not suppressed by the compound. Thus, we regarded conjunctivitis responses at the 1st to approximately the 5th challenges as the acute stage, and responses at the 7th and subsequent challenges as the chronic stage. In addition, we have found that symptoms at the chronic stage were resistant to a single treatment with various pharmacologically active substances such as cycloxyegenase inhibitor.

In the present study, in order to determine how allergic conjunctivitis at the chronic stage can be pharmacologically controlled, we evaluated the effects of single and multiple treatments with dexamethasone on allergic responses at the acute and chronic stages. In addition, because the aggravation of allergic conjunctivitis coincides with the production of serum antigen-specific IgE and the infiltration of mast cells into the conjunctiva, we evaluated the effects of multiple dexamethasone treatments on the levels of IgE and mast cells to assess the relationship between the aggravation of symptoms and the IgE-mast cell response.

MATERIALS AND METHODS

Animals Male, 4-week-old, Hartley guinea pigs (Japan SLC, Hamamatsu, Japan) were used. This animal study was approved by the Experimental Animal Research Committee at Kyoto Pharmaceutical University.

Antigen and Adjuvant Japanese cedar pollen (Cryptomeria japonica) was collected in Gifu and Shiga Prefectures in Japan in 1998. Al(OH)₃ gels were prepared as previously described.

Sensitization and Challenge As previously described, guinea pigs were sensitized by intraperitoneal injections with a pollen extract plus Al(OH)₃ (10 μg pollen protein/20 mg Al(OH)₃/ml/animal) twice within a week. Two weeks after the second sensitization, animals were challenged by dropping a pollen suspension (2 mg pollen/10 μl/eye) without Al(OH)₃ into each of their eyes. This initial challenge was followed by repeated challenges once every week until the 15th challenge. In selected experiments, the 15th challenge was performed at pollen doses of 0.02 and 0.2 mg/10 μl/eye.

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Treatment with Dexamethasone  Dexamethasone was orally administered once 3 h before the 1st or 15th pollen challenge at a dose of 10 mg/kg. In a multiple dosing study, dexamethasone (10 mg/kg, p.o.) was given 3 h before the respective 1st—15th challenges. As it is well known that the guinea pig is relatively resistant to glucocorticoids in comparison with other species such as rat and mouse, high doses (3—10 mg/kg) of dexamethasone have generally been used in guinea pig models of allergic inflammation.

Evaluation of Conjunctivitis The magnitude of conjunctival edema and redness was macroscopically judged and expressed as a conjunctivitis intensity score (CIS) according to an arbitrary 5-point graded scale in which a score of 0 indicates no symptoms; 1, light symptoms; 2, mild symptoms; 3, moderate symptoms; and 4, severe symptoms. Additionally, symptoms with scores falling between 0 and 1 were given a score of 0.5.

Scratching frequencies at 0—0.5, 0.5—1, and/or 1—2 h after the respective pollen challenges were counted. The scratch response was defined as an uninterrupted cluster of rapid hind limb movements that were precisely directed to the ocular surface.

Measurement of the Number of Mast Cells Procedures to estimate the number of mast cells in the isolated conjunctival tissue were conducted as previously described. Animals were sacrificed by bleeding under pentobarbital (40 mg/kg, i.p.) anesthesia on day 7 after the 15th challenge. The center part of the conjunctival tissue at the eyelid was isolated and fixed in 10% phosphate buffered formalin. Tissues were embedded in paraffin, sliced into 4-μm-thick sections, and stained with toluidine blue (pH 4.1, Wako Pure Chem., Osaka, Japan). Mast cells in 4 to 12 high power fields were counted under a light microscope with a magnification of ×400.

Measurement of Histamine in Ophthalmic Lavage Fluid (OLF) Ophthalmic lavage was performed before and 5 min after the 1st—15th challenges. Physiologic saline (10 μl) was applied to the eye using a micropipette without touching the eye. After 2 or 3 forced blinks, the OLF was collected. The lavage was repeated 5 times in each eye, and the OLF obtained from both eyes was combined. Following centrifugation, histamine in the supernatant was assayed fluorometrically by high performance liquid chromatography over a cation exchange column (TSK gel SP-2SW, 4.6φ×50 mm, Tosoh, Tokyo, Japan).

Measurement of Cry j 1-Specific IgE Antibody in Sera Cry j 1 and Cry j 2 are major allergenic proteins contained in Japanese cedar pollen. The amount of Cry j 1 in the pollen was approximately 10 times greater than the amount of Cry j 2. Thus, we determined the level of Cry j 1-specific IgE antibody in sera, which was obtained 7 d after the 15th challenge, by a previously described method using an enzyme-linked immunosorbent assay (ELISA) kit (Guinea pig IgE ELISA Marupi, Dainippon Pharmaceutical Co., Osaka, Japan). Because this kit has been developed to specifically measure guinea pig total IgE, we changed a detection antibody provided by the manufacturer to 100 ng/ml of biotinylated Cry j 1 (Hayashibara Biochem. Lab., Inc., Okayama, Japan), followed by incubation with avidin–horse-radish peroxidase conjugate (BD Pharmingen, San Diego, CA, U.S.A.).

Values for Cry j 1-specific IgE levels in tested sera were expressed in arbitrary units relative to the value of a pooled standard serum from the sensitized-challenged guinea pigs. The standard serum was prepared by i.p. injection of the pollen extract adsorbed onto Al(OH)3 once every week for a total of 9 times in naïve guinea pigs. The sera were collected 2 weeks after the last sensitization, followed by a subsequent pooling of all sera obtained. The Cry j 1-specific IgE titer of the pooled serum was regarded as 1000 u/ml.

Statistical Analysis If statistical significance was detected by one-way analysis of variance, individual group differences were evaluated by Bonferroni’s multiple comparison test. Probability values (p values) less than 0.05 were considered statistically significant.

RESULTS

Effects of a Single Treatment with Dexamethasone Effects of a single dexamethasone treatment on allergic conjunctivitis symptoms at the acute and chronic stages were evaluated at the 1st and 15th pollen challenges, respectively. When dexamethasone was given 3 h before the 1st challenge, the increase in CIS after the challenge was significantly reduced by approximately 50% (Fig. 1A). However, the scratching response was hardly affected by the treatment (Fig. 1B). In contrast, a single treatment with dexamethasone 3 h before the 15th challenge did not reduce CIS and scratching frequency (Figs. 1C, D).

The lack of inhibition by dexamethasone on the 15th challenge-induced increase in CIS might be due to the magnitude of the allergic conjunctivitis symptoms at the chronic stage, at which a marked increase in CIS was induced compared with the 1st challenge, as shown in Figs. 1A and C. Thus, in the next experiment, the effect of dexamethasone on symptoms at the chronic stage was re-evaluated using a weakened response. Sensitized guinea pigs had been challenged with 2 mg/eye of the pollen at the 1st—14th challenges; subsequently, the 15th challenge was performed with lower doses of the pollen. In a preliminary experiment, we found that CIS was dose-dependently increased at 0.02, 0.2, and 2 mg/eye (data not shown), and that the magnitude of increase in CIS at 0.2 mg/eye at the 15th challenge was almost equal to that at 2 mg/eye at the 1st challenge (Figs. 1A, 2A). Therefore, the effect of a single dexamethasone treatment on the 15th challenge-induced increase in CIS was re-assessed at the pollen dose of 0.2 mg/eye. We found that even the weakened conjunctivitis was not affected by the single treatment with dexamethasone (Fig. 2).

Effects of Multiple Treatments with Dexamethasone To determine how the induction of allergic conjunctivitis symptoms can be controlled at the chronic stage, we evaluated the effects of multiple dexamethasone treatments. When dexamethasone was given 3 h before the 1st—15th challenges, the increase in CIS at respective peaks (30 min) was significantly suppressed (Fig. 3A). Scratching frequency within 30 min after respective challenges was also suppressed (with or without statistical significance) by multiple treatments (Fig. 3B).

Figure 4 shows the effect of multiple dexamethasone treatments on the number of mast cells in the conjunctival tissue 7 d after the 15th challenge. Although not statistically signifi-
Sensitized guinea pigs were repeatedly challenged by dropping a pollen suspension (2 mg/10 μl/eye) into each of their eyes at the 1st—15th challenges. Dexamethasone (10 mg/kg) was orally administered 3 h before the 1st or 15th challenge. Each point and column represents the mean±S.E. of 6—26 animals.

Fig. 2. Effects of Dexamethasone on Conjunctivitis Intensity Score (CIS; A) and Scratching Frequency (B) at the 15th Challenge in Sensitized Guinea Pigs Exposed to a Low Dose of Pollen

Sensitized guinea pigs were repeatedly challenged by dropping a pollen suspension (2 mg/10 μl/eye) into each of their eyes at the 1st—14th challenges. At the 15th challenge, the guinea pigs were challenged with a low pollen dose (0.2 mg/10 μl/eye). Dexamethasone (10 mg/kg) was orally administered 3 h before the challenge. Each point and column represents the mean±S.E. of 6 animals.

Fig. 3. Effects of Multiple Administrations of Dexamethasone on Conjunctivitis Intensity Score (CIS; A) and Scratching Frequency (B) during the 1st to 15th Pollen Challenges in Sensitized Guinea Pigs

Dexamethasone (10 mg/kg) was orally administered 3 h before every challenge. Each point and column represents the mean±S.E. of 12—14 animals. *p<0.05, **p<0.01 vs. control.
cant, mast cell hyperplasia was suppressed by approximately 60% after multiple dexamethasone treatments (Fig. 4A). In addition, consistent with our previous study, the amount of histamine in OLF at 5 min was dramatically increased by repeated challenges with the pollen (Fig. 4B). When dexamethasone was administered 3 h before every challenge, the increase in the histamine level was almost completely suppressed (Fig. 4B). In contrast, the dexamethasone treatments hardly affected the increase in the Cry j 1-specific IgE antibody level in the serum of the sensitized-challenged guinea pigs (Fig. 4C).

**DISCUSSION**

In association with the aggravation of allergic conjunctivitis symptoms, not only the antigen-specific IgE level in sera but also the number of mast cells in the conjunctiva was increased by multiple pollen challenges in our guinea pig model of allergic conjunctivitis. In the present study, to assess the relationship between the aggravation of symptoms and increases in the levels of IgE and mast cells, we evaluated the effects of multiple dexamethasone treatments on these parameters.

First, we evaluated the effects of a single dexamethasone treatment on the 1st or 15th challenge-induced allergic conjunctivitis. Although the 1st challenge-induced increase in CIS was significantly suppressed by the treatment, the 15th challenge-induced response was hardly affected. We suspected that the ineffectiveness of dexamethasone at the 15th challenge might be due to the fact that the 15th challenge-induced symptoms were considerably stronger than the 1st challenge-induced response. Thus, we evaluated the single dexamethasone treatment using a weakened challenge, in which one-tenth of the pollen dose was used at the 15th challenge. However, the single dose of dexamethasone showed no effect even on the weakened response. These results strongly suggest that mechanisms underlining the allergic response at the chronic stage are substantially different from mechanisms at the acute stage. Moreover, as some steroid-resistant patients with ocular allergy have been reported, the response at the chronic stage should be useful for analyzing the pathogenesis of such severe or chronic allergic conjunctivitis resistant to glucocorticoids in clinical settings.

The mechanisms underlying the differences in the effect of a single treatment of dexamethasone on increases in CIS at the acute and chronic stages remain unclear. Leung and Bloom demonstrated that exposure of peripheral blood mononuclear cells to a combination of IL-2 and IL-4 (or IL-13) reduced dexamethasone affinity for the glucocorticoid receptors and induced resistance to its anti-inflammatory action in vitro. Iruzen et al. also reported that a combination of IL-2 and IL-4 activates p38 mitogen-activated protein kinase (MAPK) phosphorylation of glucocorticoid receptors, reducing affinity and nuclear localization of glucocorticoid receptors in human peripheral blood mononuclear cells, and that these effects were reversed by an inhibitor of p38 MAPK. Therefore, in the present model of allergic conjunctivitis, the inflammatory cytokines produced by repeated pollen challenges may have altered the sensitivity of glucocorticoid receptors to dexamethasone in inflammatory cells, which contribute to the formation of allergic conjunctivitis.

On the other hand, it has been reported that dexamethasone does not inhibit histamine release from human mast cells in the airway, intestine and skin. However, Schleimer et al. reported that 24-h treatment with dexamethasone in vitro inhibited anaphylactic release of histamine from guinea pig lung tissue. In contrast, we have found that antigen-induced histamine release from lung fragments of passively sensitized guinea pigs is never affected by 3-h treatment with dexamethasone in vitro (Kohno et al., unpublished). Thus, we have believed that treatment with glucocorticoids for several hours does not affect antigen-induced immediate release of chemical mediators from guinea pig lung. In agreement with our concept, the single treatment with dexamethasone did not affect the 1st challenge-induced scratching behaviour, which has been reported to be sensitively inhibited by an anti-histaminic drug, although the increase in CIS after the 1st challenge was significantly suppressed by the single treatment. Thus, we can speculate that the inhibition on the increase in CIS at the acute stage by a single treatment is not related to anaphylactic histamine release from the conjunctival mast cells. The inhibition action of dexamethasone on the increase in CIS at the acute stage may be associated with the inhibition of the glucocorticoid on various inflammatory changes resulting in suppression of vascular permeability.

We next evaluated whether multiple treatments with dexam-
amethasone suppressed the aggravation of allergic conjunctivitis. We found that multiple dexamethasone treatments significantly suppressed increases in CIS during the 1st—15th challenges. Additionally, mast cell hyperplasia but not Cry j 1-specific IgE production was suppressed by repeated corticosteroid treatment. In agreement with our previous report, the increase in the number of mast cells coincided with a dramatic increase in levels of a mast cell mediator, histamine in OLF immediately after the 1st—15th challenges. Consistent with the suppression of mast cell hyperplasia, the increase in histamine levels was almost completely inhibited by the treatment. Although we have reported that histamine is not involved in the chronic stage of ocular allergy, the present results suggest that the suppressive effect of dexamethasone on the aggravation of allergic conjunctivitis may be associated with the inhibition of the increase in mast cells. In addition, Kitamura et al. recently reported that repeated exposures to an antigen induced nasal allergy-like symptoms in rats, and that histamine content, histidine decarboxylase (HDC) expression, and HDC activity were increased in the nasal mucosa. Furthermore, they demonstrated that these allergic changes were suppressed by systemic treatment with dexamethasone. Indeed, increased HDC mRNA was observed in patients with allergic rhinitis. Therefore, in our allergic model, increases in HDC expression and activity in mast cells may also be involved in the increase in the histamine level. In future, we must also evaluate this expression and activity in conjunctival tissue.

It is well known that proliferation and recruitment of mast cells are induced by various cytokines such as stem cell factor (c-kit ligand) and interleukin-3. The generation of mast cell proliferation factors may be suppressed by repeated dexamethasone treatments, as previously demonstrated in vitro. However, because dexamethasone suppresses various aspects of inflammatory reactions, we cannot deny the possibility that the inhibition of mast cell hyperplasia by the glucocorticoid is not a direct cause of the suppression of the increase in CIS. More specific research is required in order to demonstrate a direct relationship between increases in CIS and mast cell number.

In conclusion, although a single treatment with dexamethasone did not suppress allergic conjunctivitis at the chronic stage, multiple treatments inhibited the aggravation of the disease. The inhibitory effect may be associated with the suppressive effect on mast cell hyperplasia, but not related to the formation of antigen-specific IgE. Thus, we can speculate that increased levels of mast cells may be involved in the induction of aggravated symptoms.

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