

Cyclosporine A Eye Drops Inhibit the Early-Phase Reaction in a Type-I Allergic Conjunctivitis Model in Mice

Daisuke Shii, Tomoko Oda, Katsuhiko Shinomiya, Osamu Katsuta, and Masatsugu Nakamura

Abstract

Purpose: The effects of cyclosporine A eye drops on the early-phase reaction were investigated in a type-I allergic conjunctivitis model.

Methods: Mice were actively sensitized with ragweed (RW) absorbed on aluminium hydroxide gel and challenged with RW for 10 days (single challenge model) or 10–14 days (repetitive challenge model) after the first sensitization. For the evaluation of itching, ovalbumin was used as an antigen instead of RW. The effects of cyclosporine A eye drops on increased vascular permeability, mast cell degranulation, and itching were evaluated and compared with those of other anti-allergic eye drops.

Results: In the single challenge model, cyclosporine A eye drops significantly inhibited the increase in vascular permeability and histological evaluations showed suppressed degranulation of mast cells. Disodium cromoglycate (DSCG) eye drops showed only a slight tendency to inhibit the increase in both pathophysiological parameters. Ketotifen or betamethasone eye drops significantly inhibited the increase in vascular permeability. The order of potency in the single challenge model was ketotifen > cyclosporine A > betamethasone. In the repetitive challenge model, cyclosporine A eye drops significantly inhibited the increase in vascular permeability and DSCG eye drops showed only slight inhibition. Ketotifen or betamethasone significantly inhibited the increase in vascular permeability. The order of potency in the repetitive challenge model was cyclosporine A > betamethasone > ketotifen. The effect of cyclosporine A eye drops on the itch–scratch response was studied. Cyclosporine A and DSCG significantly reduced the itch–scratch response in the single and repetitive challenge models; the effect of cyclosporine A in the repetitive challenge model was more potent than in the single challenge model.

Conclusions: Those results suggest that administration of cyclosporine A eye drops inhibit the early-phase reaction in type-I allergic conjunctivitis, which may be mediated by the suppression of mast cell degranulation. This action of cyclosporine A eye drops may be involved in the therapeutic effect of cyclosporine A on allergic conjunctivitis.

Introduction

CYCLOSPORINE A IS A cyclic polypeptide calcineurin inhibitor consisting of 11 amino acids. It was discovered and purified from a culture of the fungus *Tolypocladium inflatum* Gams by Novartis Pharma in the 1970s. Cyclosporine A has been used to suppress not only graft rejection after transplantation, but also immunoreaction disorders often seen in autoimmune diseases. That is, cyclosporine A is a well-known immunosuppressant. The therapeutic effect of eye drops of cyclosporine A on vernal keratoconjunctivitis (VKC), one of the most severe refractory allergic conjunctival diseases, was also reported in several articles.^{1–3}

Cyclosporine A displays an immunosuppressive effect by inhibiting the production of cytokines from T cells,^{4,5} but the mechanism of cyclosporine A in allergic disease is poorly understood.

The early-phase reaction (eg, histamine release from mast cells) is the main reaction of type-I allergy that causes allergic conjunctivitis (including VKC). Histamine induces acute itching, vascular permeability, and edema formation.⁶ Histamine H1 receptor antagonists and mast cell stabilizers were used for the treatment of allergic conjunctiva (eg, seasonal allergic conjunctivitis and perennial allergic conjunctivitis). The early-phase reaction (including histamine

release from mast cells) was known to be a critical factor in allergic disease. Several articles reported that cyclosporine A inhibited histamine release from mast cells *in vitro*.^{7,8} These reports suggested that cyclosporine A eye drops may suppress the early-phase reaction by the inhibition of histamine release from mast cells in allergic disease, but the effects of cyclosporine A eye drops on the early-phase reaction in a model of allergic disease have not been studied.

To study the effect of cyclosporine A on the early-phase reaction in type-I allergy, we examined the effects of cyclosporine A eye drops on the degranulation of mast cells and symptoms such as vascular permeability and acute itching in an allergic conjunctivitis model in mice. We have demonstrated that the effects of a histamine H1 receptor antagonist and corticosteroid eye drops were different in the early-phase reaction between single and repetitive challenges with short ragweed pollen (RW) using an allergic conjunctivitis model in mice. Therefore, the effects of cyclosporine A eye drops on vascular permeability in the single and repetitive challenge models were compared with an anti-histamine, a mast cell stabilizer, and corticosteroid eye drops.

Methods

Animals

Experiments were carried out in accordance with the ARVO statement for the Use of Animals in Ophthalmic and Vision Research, and were approved by Santen Institutional Animal Care and Use Committee (Osaka, Japan).

Male BALB/c mice were purchased from Japan Charles River (Kanagawa, Japan) and used at 5–6 weeks. They were housed under a 12-h light–dark cycle (light on from 07:00 to 19:00), with room temperature maintained at $23 \pm 3^\circ\text{C}$ and humidity at $50 \pm 20\%$. Food and water were given *ad libitum*.

Reagents

Cyclosporine A was obtained from Novartis Ringaskiddy Limited (Cork, Ireland). Cyclosporine A eye drops (Papilock mini ophthalmic solution 0.1%) and vehicle were prepared at Santen Pharmaceutical Company Limited (Osaka, Japan). Cyclosporine A eye drops (0.01%) were diluted 10-fold with vehicle. Disodium cromoglycate eye drops (DSCG; Intal ophthalmic solution, 2%) were purchased from Astellas Pharma Incorporated (formerly Fujisawa Pharmaceutical Company Limited, Osaka, Japan). Betamethasone sodium phosphate eye drops (betamethasone, Rinderon solution, 0.1%) was purchased from Shionogi & Company Limited (Osaka, Japan). Ketotifen fumarate eye drops (ketotifen, Zaditen ophthalmic solution, 0.05%) was purchased from Novartis Pharma K. K. (Tokyo, Japan). RW (Polysciences, Warrington, PA, USA), ovalbumin (OVA; Sigma, St. Louis, MO, USA), Evans blue dye (Sigma) were also used in the experiments. Aluminium hydroxide gel (ALUM; 50 mg/mL) was prepared as previously described.⁹

Experimental allergic conjunctivitis induced by active sensitization with RW

Mice were actively sensitized by intraperitoneal injection of 0.5 mL of RW absorbed on ALUM (containing 0.2 mg

RW and 2.5 mg ALUM) on days 0 and 5. They were challenged by topical instillation with 2 μL per right eye of 20% RW in phosphate-buffered saline (PBS) on day 10 (single challenge) or days 10–14 (once a day, repetitive challenge). Conjunctival tissues were removed 30 min after the last challenge (day 10 or 14) for vascular permeability and histological observation.

Vascular permeability

A dye solution of 0.1% Evans blue (15 mL/kg) was administered intravenously followed by the last challenge (day 10 or 14) and the dye-leakage area of right eyes removed 30 min after the challenge. Extravasated Evans blue dye could then be extracted from tissues using a solvent including sodium sulfate and acetone. Evans blue dye was extracted for >48 h and absorbance of the extracts at 620 nm was determined with a spectrophotometer (DU-640; Beckman Coulter, Fullerton, CA, USA). In single challenge, cyclosporine A or vehicle was instilled for 1 or 3 h, ketotifen was instilled for 0.5 h, or DSCG and betamethasone were instilled 3 h before the RW challenge. In repetitive challenges, for daily treatment, cyclosporine A, vehicle, DSCG, or betamethasone was instilled 3 times; 3 h before, and 1 and 5 h after each RW challenge, or ketotifen was instilled 3 times; 0.5 h before, and 1 and 5 h after each RW challenge. Eye drops (2 μL) were instilled into the right eye. Because ketotifen showed strong inhibition in early-phase reaction 30 min before single antigen challenge, ketotifen was instilled 30 min before antigen challenge in these studies.

Histological analysis

Conjunctival tissues were removed 30 min after single RW challenge and fixed in 10% neutral buffered formalin solution and embedded in paraffin. Vertical sections (thickness, 2 μm) were cut. Samples were stained with Toluidine blue (pH 4.1). The area for cell counting was the lamina propria of the tarsal and bulbar conjunctivas throughout each section, in accordance with the method of Fukushima and colleagues.¹⁰ The sections counted were those of the internal canthus from the central portion of the eye (about 200 μm), which included the pupil and optic nerve head. The phenotype of mast cells was classified into degranulated (>20% of the cytoplasmic granules exhibiting fusion or discharge, staining alterations, and extraction from the cell) or normal. The ratio of degranulated cells was calculated.¹¹ Each drug (2 μL per right eye) was instilled 3 h before RW challenge.

Measurement of scratching behavior

Mice were actively sensitized by intraperitoneal injection with 0.5 mL of OVA absorbed on ALUM (containing 10 μg OVA and 1 mg ALUM) on days 0 and 5. They were challenged by a topical instillation with 2 μL per right eye of 15% OVA in 10% glycerol on days 10–14. On day 10 (single OVA challenge) or day 14 (repetitive OVA challenges), mice were put into an acrylic cage in the observation room for about 1 h for acclimatization before recording behavior. Immediately after OVA challenge, they were returned to the same cage and scratching behavior was videotaped. Itch–scratch response was defined as an interrupted cluster of rapid movements

of the hind-paws that was precisely directed toward the eye, and behaviors were counted at intervals of 30 min. Each drug (2 μ L per right eye) was instilled 3 h before and 1 and 5 h after each OVA challenge.

Statistical analysis

Data from 2 groups were statistically evaluated using Student *t*-test or the Aspin-Welch *t*-test based on the variance of data examined with the *F*-test. Data from multiple groups were evaluated using Dunnett's multiple comparison test. $P < 0.05$ was considered statistically significant. The means of data are presented with SEM.

Results

Effect of cyclosporine A eye drops on vascular permeability 30 min after single RW challenge in actively sensitized mice

To examine the effect of cyclosporine A on the early-phase reaction in type-I allergic conjunctivitis, cyclosporine A eye drops were instilled 1 or 3 h before RW challenge, and vascular permeability enhanced by single antigen challenge in actively sensitized mice was evaluated. Instillation of cyclosporine A 1 h before RW challenge did not inhibit the increase in vascular permeability 30 min after antigen challenge compared with that of the control group (Fig. 1A). Instillation of 0.01% and 0.1% cyclosporine A 3 h before RW challenge inhibited the increase in vascular permeability in a dose-dependent manner compared with that of the control group, and the inhibitory effect of 0.1% cyclosporine A was significant (Fig. 1B). Instillation of DSCG 3 h before RW challenge suppressed the increase in vascular permeability slightly compared with that of the control group. These results suggest that cyclosporine A eye drops can inhibit the early-phase reaction in type-I allergic conjunctivitis.

Effect of cyclosporine A eye drops on the degranulation of mast cells after single RW challenge in actively sensitized mice

To elucidate the effect of cyclosporine A eye drops on mast cells, the degranulation of mast cells was evaluated in the conjunctival tissue treated with Toluidine blue staining after single RW challenge in actively sensitized mice. Figure 2A and B shows a normal mast cell and a degranulated mast cell, respectively. Normal mast cells and degranulated mast cells could be distinguished because the cytoplasmic granules released from mast cells were seen 30 min after RW challenge. The ratio of degranulated mast cells to total mast cells in the conjunctiva is shown in Figure 3. The ratio of degranulated mast cells in RW-challenged conjunctiva was significantly increased 30 min after RW challenge compared with that of the normal group. Instillation of cyclosporine A 3 h before RW challenge significantly suppressed the ratio of degranulated mast cells to total mast cells in the conjunctiva, and DSCG instillation suppressed the ratio slightly compared with that of the control group. Cyclosporine A eye drops therefore had a stronger inhibitory effect on mast cell degranulation than that of DSCG eye drops.

Effects of eye drops of ketotifen, betamethasone, and cyclosporine A on vascular permeability 30 min after single or repetitive RW challenges in actively sensitized mice

We demonstrated a difference in efficacy between ketotifen and betamethasone in single and repetitive RW challenges (data not shown). We therefore examined the effect of cyclosporine A eye drops on the increase in vascular permeability in single and repetitive RW challenges in actively sensitized mice. The dose-response relationship of cyclosporine A eye drops and the effect of DSCG eye drops were also examined in a repetitive RW challenge model (Fig. 4). The treatment timing of cyclosporine A eye drops was identical

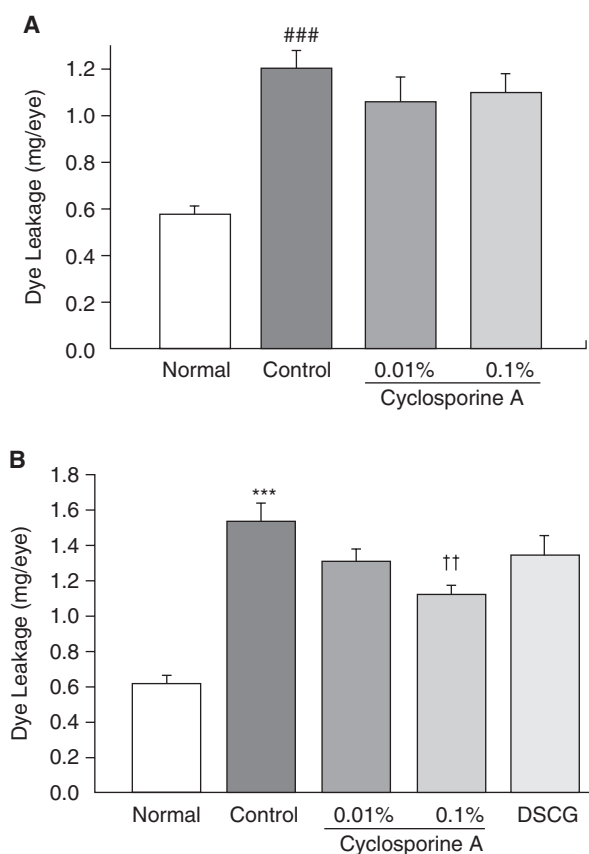


FIG. 1. Effects of cyclosporine A and disodium cromoglycate (DSCG) eye drops on increase in vascular permeability in actively sensitized mice. Allergic conjunctivitis was induced by ragweed (RW) challenge in actively sensitized mice. Each drug (2 μ L/eye) was instilled 1 h (A) or 3 h (B) before RW challenge. Instillation of cyclosporine A at 3 h, but not at 1 h, before RW challenge significantly inhibited the increase in vascular permeability and DSCG at 3 h before RW challenge (B) slightly inhibited the increase in vascular permeability compared with the control group. Normal, sensitized, and unchallenged group; control, sensitized, and challenged group. Each column shows the mean and SEM of 10 mice. ### $P < 0.001$, compared with the normal group by Student *t*-test. *** $P < 0.001$, compared with the normal group by the Aspin-Welch *t*-test. †† $P < 0.01$, compared with the control group by Dunnett's multiple comparison test.

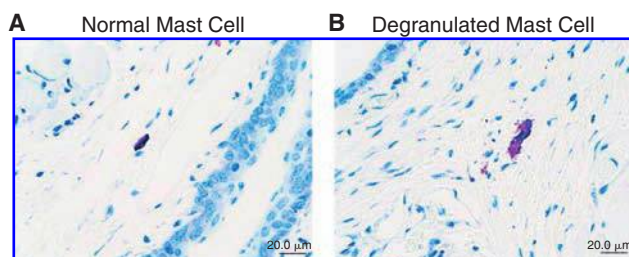


FIG. 2. Histological features of conjunctival mast cells after RW challenge in Toluidine blue staining. **(A)** Normal mast cell and **(B)** degranulated mast cell. Allergic conjunctivitis was induced by ragweed (RW) challenge in actively sensitized mice, or normal mice were instilled with phosphate-buffered saline (PBS) instead of RW into the conjunctiva. Vehicle (2 μ L/eye) was instilled 3 h before RW challenge or PBS instillation. Conjunctival tissues were removed 30 min after RW challenge or PBS instillation. Mast cells were degranulated in the lesion site by RW challenge and showed isolated and scattered secretory granules around the cells.

to that shown in Figure 1B because administration of cyclosporine A eye drops 3 h before RW challenge significantly decreased the increase in vascular permeability. Instillation of 0.01% and 0.1% cyclosporine A significantly inhibited the increase in vascular permeability in a dose-dependent manner compared with that of the control group. DSCG showed slightly inhibited the increase in vascular permeability compared with the control group in a repetitive RW challenge model as well as in a single challenge model. Next, we examined the effects of eye drops of ketotifen, betamethasone, and cyclosporine A on vascular permeability 30 min after single or repetitive RW challenges in actively sensitized mice. In single RW challenge, eye drops of ketotifen, betamethasone, and cyclosporine A significantly inhibited the increase in vascular permeability compared with that of the control group (Fig. 5A). Percentage inhibition due to ketotifen, betamethasone, and cyclosporine A was 65%, 44%, and 60%, respectively. The efficacy of betamethasone eye drops on vascular permeability was significantly less than that of ketotifen eye drops in the single RW challenge. In the repetitive RW challenges, ketotifen, betamethasone, and cyclosporine A eye drops also significantly inhibited the increase in vascular permeability compared with that of the control group (Fig. 5B). Percentage inhibition due to ketotifen, betamethasone, and cyclosporine A eye drops was 35%, 49%, and 66%, respectively. Cyclosporine A eye drops showed the strongest inhibitory effect on vascular permeability and the efficacy of cyclosporine A eye drops was significantly stronger than that of ketotifen eye drops in the repetitive RW challenges. Cyclosporine A eye drops therefore strongly inhibited the early-phase reaction in the single and repetitive RW challenge models. The order of potency was ketotifen > cyclosporine A > betamethasone in the single challenge model, and cyclosporine A > betamethasone > ketotifen in the repetitive challenge model.

Effect of cyclosporine A eye drops on the itch-scratch response for 30 min after the first or fifth OVA challenge in actively sensitized mice

To elucidate the effect of cyclosporine A eye drops on the itch-scratch response induced in type-I allergy, the number

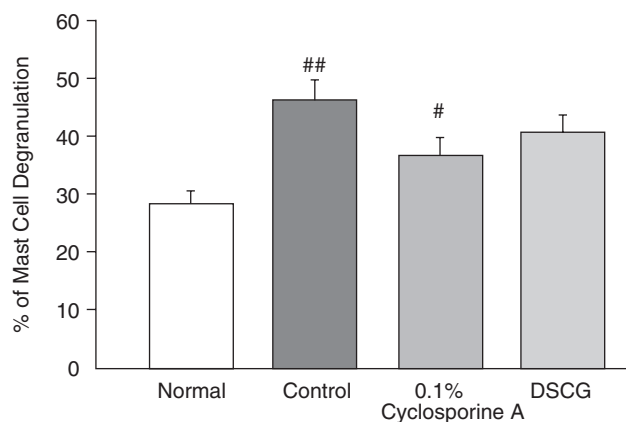


FIG. 3. Effects of cyclosporine A and disodium cromoglycate (DSCG) eye drops on the ratio of degranulated mast cells in actively sensitized mice. Allergic conjunctivitis was induced by ragweed (RW) challenge in actively sensitized mice. Each drug (2 μ L/eye) was instilled 3 h before RW challenge. The number of mast cells in the lamina propria mucosae of the upper tarsal and bulbar conjunctiva was counted, and the mast cells in which >20% of the cytoplasmic granules exhibited fusion or discharge, staining alterations and extractions from the cells were classified as “degranulated.” Cyclosporine A significantly suppressed the ratio of degranulated mast cells to total mast cells in the conjunctiva, and DSCG suppressed the ratio slightly compared with the control group. Normal, sensitized, and unchallenged group; control, sensitized, and challenged group. Each column shows the mean and SEM of 10 mice. ^{##} $P < 0.01$, compared with the normal group by Student t -test. [#] $P < 0.05$, compared with the control group by Student t -test.

of scratching episodes for 30 min after the first and fifth OVA challenge in actively sensitized mice was counted. Application of OVA induced a significant increase in itch-scratch response compared with the normal group in the first and fifth challenges. Instillation of cyclosporine A significantly inhibited the itch-scratch response for 30 min after the first and fifth OVA challenges compared with those of the control group (Fig. 6A and B). Instillation of DSCG also significantly inhibited the itch-scratch response for 30 min after the first and fifth OVA challenges compared with those of the control group. Percentage inhibition due to cyclosporine A eye drops was 48% and 76% in the first and fifth OVA challenges, respectively. Compared with the first OVA challenge, cyclosporine A showed a stronger effect on the itch-scratch response for 30 min after the fifth OVA challenge. Percentage inhibition due to DSCG eye drops was 41% and 48% in the first and fifth OVA challenges, respectively.

Discussion

It is well known that mast cell degranulation induces the early-phase reaction in type-I allergy. Although cyclosporine A is reported to inhibit degranulation of mast cells *in vitro*,^{12,13} the effect of cyclosporine A on the early-phase reaction is not well studied *in vivo*. We therefore examined the effect of cyclosporine A eye drops in the early-phase reaction such as plasma exudation and mast cell degranulation in the conjunctiva 30 min after RW challenge in actively sensitized

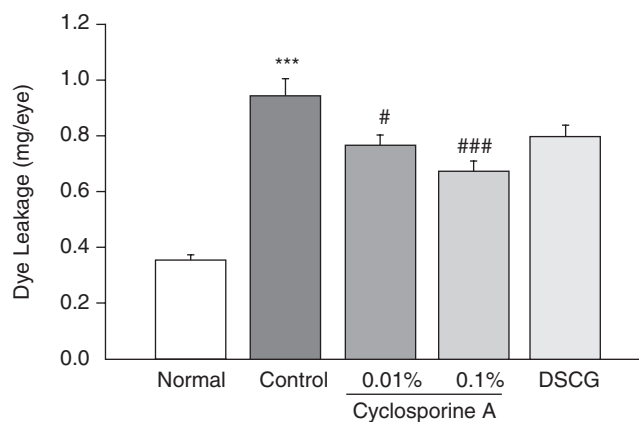


FIG. 4. Effects of cyclosporine A and disodium cromoglycate (DSCG) eye drops on increase in vascular permeability after repetitive ragweed (RW) challenge in actively sensitized mice. Allergic conjunctivitis was induced by RW challenge in actively sensitized mice. Each drug (2 μ L/eye) was instilled 3 h before, and 1 and 5 h after each RW challenge. Cyclosporine A significantly inhibited the increase in vascular permeability in a dose-dependent manner, and DSCG inhibited it slightly compared with the control group. Normal, sensitized, and unchallenged group; control, sensitized, and challenged group. Each column shows the mean and SEM of 10 mice. *** P < 0.001, compared with the normal group by the Aspin-Welch t -test. # P < 0.05, #### P < 0.001, compared with the control group by Dunnett's multiple comparison test.

mice. We demonstrated that cyclosporine A eye drops significantly inhibited the increase in conjunctival vascular permeability and suppressed degranulation of mast cells in the conjunctiva as shown by histological evaluation. DSCG eye drops tended to inhibit the increase in vascular permeability and degranulation of mast cells. These results clearly indicate that cyclosporine A eye drops inhibit the early-phase reaction in type-I allergy, and that the effects of cyclosporine A may be mediated by the inhibition of mast cell degranulation in the conjunctiva. It was also suggested that the effect of cyclosporine A eye drops on mast cell degranulation was more potent than that of DSCG eye drops (DSCG is a topical mast cell stabilizing agent). Regarding the inhibitory effect of cyclosporine A on degranulation of mast cells *in vitro*, it has been reported that antigen-induced histamine release and beta-hexosaminidase secretion in the mucosal-type mast cell line RBL-2H3 were inhibited by cyclosporine A,⁷ and that the IC₅₀ for both events was ~20 nM. In human skin mast cells, cyclosporine A (2.4–800 nM) was reported to inhibit histamine release induced by anti-IgE.⁸ It was reported that cyclosporine H, which exhibited potent binding to calmodulin with no affinity for cyclophilin, had no effect on histamine release,⁸ and the authors concluded that the inhibition of mast cell degranulation by cyclosporine A is due to cyclophilin binding. Thus, cyclosporine A was reported to inhibit histamine release from mast cells *in vitro*. In this study, we clearly demonstrated that cyclosporine A eye drops can inhibit mast cell degranulation in the conjunctiva in *in vivo* allergy models.

Instillation of cyclosporine A at 3 h, but not at 1 h, before antigen challenge showed a significant inhibitory effect on the increase in vascular permeability, although cyclosporine

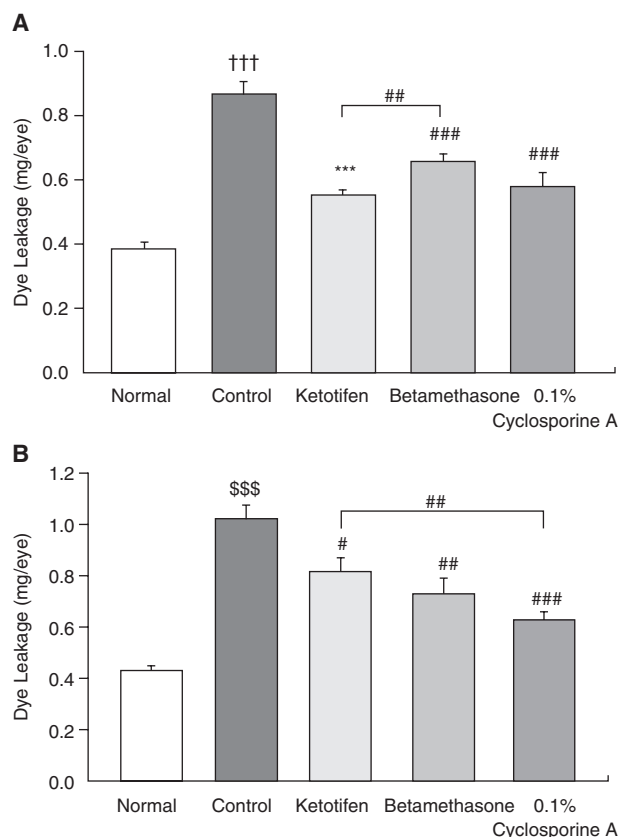


FIG. 5. Effects of cyclosporine A, ketotifen, and betamethasone eye drops on increase in vascular permeability in actively sensitized mice. (A) Single challenge and (B) repetitive challenge. Allergic conjunctivitis was induced by ragweed (RW) challenge in actively sensitized mice. Cyclosporine A, vehicle, or betamethasone was instilled 3 h before, and 1 and 5 h after each RW challenge (only 3 h before in single challenge), or ketotifen was instilled 0.5 h before, and 1 and 5 h after each RW challenge (only 0.5 h before in single challenge). Cyclosporine A showed a significant inhibitory effect in single and repetitive challenges compared with the control group. Normal, sensitized, and unchallenged group; control, sensitized, and challenged group. Each column shows the mean and SEM of 10 mice. ††† P < 0.001, compared with the normal group by Student t -test. \$\$\$ P < 0.001, compared with the normal group by the Aspin-Welch t -test. # P < 0.05, ## P < 0.01, ### P < 0.001, compared with the control group by Student t -test. *** P < 0.001, compared with the control group by the Aspin-Welch t -test.

A added only several min before antigen application was effective in mast cell degranulation induced by antigen *in vitro*. It was therefore speculated that cyclosporine A eye drops may need >1 h to reach a sufficient concentration in the conjunctiva to enable cyclosporine A to exhibit an inhibitory effect on mast cell degranulation.

A comparison of the effects of an anti-histamine, a corticosteroid, and cyclosporine A on the increase in vascular permeability was performed. It was reported that anti-histamine is a potent inhibitor of the early-phase reaction.^{12,14} Ketotifen instillation showed the most potent inhibitory effect on plasma exudation after single RW challenge.

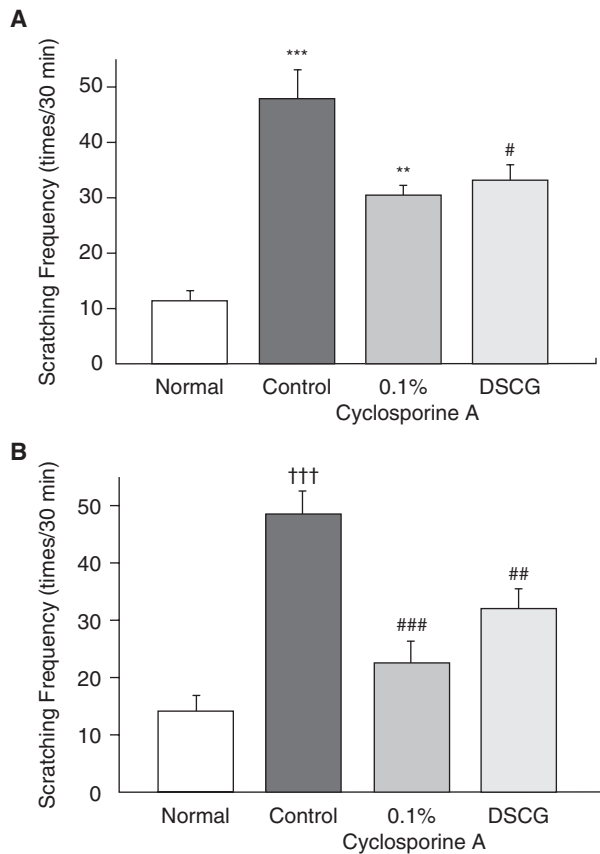


FIG. 6. Effects of cyclosporine A and disodium cromoglycate (DSCG) eye drops on the itch-scratch response in actively sensitized mice. **(A)** Single ovalbumin (OVA) challenge, **(B)** repetitive OVA challenges. Allergic conjunctivitis was induced by OVA challenge in actively sensitized mice. Each drug (2 μ L/eye) was instilled 3 h before, and 1 h and 5 h after each OVA challenge (only 3 h before in single challenge). Scratching behavior was counted at 30-min intervals. Cyclosporine A significantly inhibited the itch-scratch response for 30 min after the first and fifth OVA challenges, and DSCG also significantly inhibited this response, compared with the control group. Normal, sensitized, and unchallenged group; control, sensitized, and challenged group. Each column shows the mean and SEM of 10 mice. *** $P < 0.001$, compared with the normal group by the Aspin-Welch t -test. ††† $P < 0.001$, compared with the normal group by Student t -test. ** $P < 0.01$, compared with the control group by the Aspin-Welch t -test. # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$, compared with the control group by Student t -test.

Instillation of the strong corticosteroid betamethasone was significantly less effective than that of ketotifen in single challenge. In repetitive antigen challenges, betamethasone inhibited plasma exudation significantly and the inhibitory effect was stronger than that of ketotifen. Thus, the effects of anti-allergic eye drops on plasma exudation are different between the single and repetitive antigen challenge models. These results may suggest that histamine is dominant in the early-phase reaction induced by single antigen challenge; however, after repetitive antigen challenges, not only histamine but also multiple inflammatory factors are involved

in the symptoms of the early-phase reaction. Fukushima and colleagues reported that involvements of histamine in inductions of conjunctivitis symptoms are decreased by the multiple antigen challenge.¹⁵ Our results are consistent with their reports. We also demonstrated that instillation of cyclosporine A inhibited the increase in vascular permeability in the single and repetitive antigen challenge models. The potency of cyclosporine A was slightly weaker than that of ketotifen in single antigen challenge, but significantly stronger than those of ketotifen in repetitive antigen challenges. Cyclosporine A was known to inhibit not only histamine release from mast cells but also other mediator,⁸ which may be involved in the strong effects of cyclosporine A in repetitive antigen challenge model. Repetitive exposure of antigens in mice closely mimics human allergic conjunctivitis because patients are repeatedly exposed to antigens in clinical environments. Anti-histamines were more effective than corticosteroids in the antigen-provocation test in human,¹⁶ but not in an environmental study.¹⁷ Therefore, cyclosporine A eye drops may show a strong therapeutic effect in allergic conjunctivitis in clinical environments. The allergic conjunctivitis model induced by repetitive antigen challenge may be useful to test the effects of anti-allergic eye drops in clinical environments.

Itching is the predominant symptom of allergic conjunctivitis, and many mediators released from mast cells (eg, histamine, prostaglandins, and platelet-activating factor) are thought to induce itching.^{13,18,19} It is well known that mast cell degranulation induces the symptoms of the early-phase reaction such as itching in type-I allergy; however, the effect of cyclosporine A eye drops on the itch-scratch response induced by antigen challenge has not been elucidated. In this experiment, the itch-scratch response was induced by OVA solution as the antigen. RW is a very common antigen for ocular allergy, but is insoluble in water; therefore, the challenge of RW suspension might cause a foreign body sensation that may affect sensitivity to itching. In fact, ragweed did not induce enough scratching to study the effect of cyclosporine A (data not shown). OVA was therefore used as the antigen instead of RW. Nakahara and colleagues²⁰ reported that increase of vessel permeability induced by OVA markedly decreased in actively sensitized histamine H1-deficient mice compared with wild-type mice and they concluded that the change in vascular permeability in allergic conjunctivitis were completely regulated by histamine released from activated mast cells. We also showed that increase of vessel permeability induced by ragweed in actively sensitized mice was markedly inhibited by H1 blocker and mast cells were degranulated in our model. Those results demonstrated that early-phase reaction induced by both of OVA and ragweed was mediated by mast cell degranulation. Although Fukushima and colleagues¹⁵ reported that multiple cedar pollen challenge increased the itch-scratch reaction and conjunctivitis intensity score in guinea pig, the number of scratching in both of single challenge and repetitive challenge was almost the same in our study. Before this experiment, we had measured scratching frequency and ocular surface symptom in guinea pigs in daily repetitive antigen challenge, but increase of frequency was not detected (data not shown). Because Fukushima and colleagues¹⁵ challenged cedar pollen once every week until the 15th challenge, time schedule of antigen challenge may affect the difference of results in both of experiments. Instillation of cyclosporine A

eye drops significantly inhibited the itch-scratch response after the first and fifth OVA challenges. Instillation of DSCG eye drops caused significant inhibition in the first and fifth challenges. The result was consistent with a report stating that DSCG suppresses itching in patients with allergic conjunctivitis.^{21,22} In our study, the effect of cyclosporine A was stronger than that of DSCG in both conditions. Cyclosporine A eye drops may be highly effective in relieving pruritus in patients with allergic conjunctivitis. Similar to DSCG, the mechanisms of cyclosporine A in itching may be related to suppression of mast cell degranulation. We demonstrated that the effect of cyclosporine A on the itch-scratch response in the fifth OVA challenge was more effective than the first challenge, unlike DSCG. Another action of cyclosporine A might be involved in the anti-itching effects in the fifth OVA challenges.

We demonstrated that cyclosporine A eye drops strongly suppressed the symptoms of the early-phase reaction in type-I allergic conjunctivitis by inhibiting mast cell degranulation. This action of cyclosporine A eye drops may contribute to the treatment of allergic conjunctivitis, including VKC.

Acknowledgments

We thank Satsuki Yamaguchi and Harue Miyata for technical assistance.

References

- Avunduk, A.M., Avunduk, M.C., Erdol, H., et al. Cyclosporine effects on clinical findings and impression cytology specimens in severe vernal keratoconjunctivitis. *Ophthalmologica*. 215:290–293, 2001.
- Pucci, N., Novembre, E., Cianferoni, A., et al. Efficacy and safety of cyclosporine eyedrops in vernal keratoconjunctivitis. *Ann. Allergy Asthma Immunol.* 89:298–303, 2002.
- Spadavecchia, L., Fanelli, P., Tesse, R., et al. Efficacy of 1.25% and 1% topical cyclosporine in the treatment of severe vernal keratoconjunctivitis in childhood. *Pediatr. Allergy Immunol.* 17:527–532, 2006.
- Pahl, A., Zhang, M., Kuss, H., et al. Regulation of IL-13 synthesis in human lymphocytes: implications for asthma therapy. *Br. J. Pharmacol.* 135:1915–1926, 2002.
- Schmidt, J., Fleissner, S., Heimann-Weitschat, I., et al. Effect of corticosteroids, cyclosporin A, and methotrexate on cytokine release from monocytes and T-cell subsets. *Immunopharmacology*. 27:173–179, 1994.
- Jutel, M., Blaser, K., and Akdis, C.A. Histamine in allergic inflammation and immune modulation. *Int. Arch. Allergy Immunol.* 137:82–92, 2005.
- Narenjkar, J., Assem, E.L.-S.K., Wan, B.Y., et al. Effect of cyclosporin and tacrolimus (FK506) on the antigen-induced mediator release, membrane potential and $86\text{Rb}^+/\text{K}^+$ and Ca^{2+} fluxes in the RBL-2H3 cell line. *Int. Immunopharmacol.* 6:742–749, 2006.
- Stellato, C., de Paulis, A., Ciccarelli, A., et al. Anti-inflammatory effect of cyclosporin A on human skin mast cells. *J. Invest. Dermatol.* 98:800–804, 1992.
- Kato, M., Imoto, K., Miyake, H., et al. Apafant, a potent platelet-activating factor antagonist, blocks eosinophil activation and is effective in the chronic phase of experimental allergic conjunctivitis in guinea pigs. *J. Pharmacol. Sci.* 95:435–442, 2004.
- Fukushima, A., Yamaguchi, T., Ishida, W., et al. Cyclosporin A inhibits eosinophilic infiltration into the conjunctiva mediated by type IV allergic reactions. *Clin. Exp. Ophthalmol.* 34:347–353, 2006.
- Yamashita, H., Makino, T., Mizukami, H., et al. Pharmacological characterization of a chronic pruritus model induced by multiple application of 2,4,6-trinitrochlorobenzene in NC mice. *Eur. J. Pharmacol.* 563:233–239, 2007.
- Schoch, C. Effect of ketotifen fumarate, olopatadine, and levocabastine on ocular active anaphylaxis in the guinea pig and ocular immediate hypersensitivity in the albino rat. *Ocul. Immunol. Inflamm.* 13:39–44, 2005.
- Nakano, T., Andoh, T., Tayama, M., et al. Effects of topical application of tacrolimus on acute itch-associated responses in mice. *Biol. Pharm. Bull.* 31:752–754, 2008.
- Fukushima, A., Shii, D., Sumi, T., et al. Cryptomeria japonica-induced allergic conjunctivitis in mice. *Biol. Pharm. Bull.* 30:1745–1747, 2007.
- Fukushima, Y., Nabe, T., Mizutani, N., et al. Multiple cedar pollen challenge diminished involvement of histamine in allergic conjunctivitis of guinea pigs. *Biol. Pharm. Bull.* 26:1696–1700, 2003.
- Berdy, G.J., Stoppel, J.O., and Epstein, A.B. Comparison of the clinical efficacy and tolerability of olopatadine hydrochloride 0.1% ophthalmic solution and loteprednol etabonate 0.2% ophthalmic suspension in the conjunctival allergen challenge model. *Clin. Ther.* 24:918–929, 2002.
- Koo, E.H., Samudre, S.S., Williams, P.B., et al. Loteprednol etabonate ophthalmic suspension 0.2% (Alrex) and olopatadine hydrochloride ophthalmic solution 0.1% (Patanol) in the treatment of allergic conjunctivitis. *ARVO. abstracts #422*, 2008.
- Kato, M., Kurose, T., Oda, T., et al. The role of platelet activating factor and the efficacy of apafant ophthalmic solution in experimental allergic conjunctivitis. *J. Ocul. Pharmacol. Ther.* 19:315–324, 2003.
- Nakajima, M., Goh, Y., Azuma, I., et al. Effects of prostaglandin D_2 and its analogue, BW245C, on intraocular pressure in humans. *Graefes Arch. Clin. Exp. Ophthalmol.* 229:411–413, 1991.
- Nakahara, H., Izumi, K., Sugimoto, Y., et al. Vascular permeability in allergic conjunctivitis in mice lacking histamine H1 receptor. *Eur. J. Pharmacol.* 409:313–317, 2000.
- Leino, M., Ennevaara, K., Latvala, A.L., et al. Double-blind group comparative study of 2% nedocromil sodium eye drops with 2% sodium cromoglycate and placebo eye drops in the treatment of seasonal allergic conjunctivitis. *Clin. Exp. Allergy*. 22:929–932, 1992.
- Montan, P., Zetterstrom, O., Eliasson, E., et al. Topical sodium cromoglycate (Opticrom®) relieves ongoing symptoms of allergic conjunctivitis within 2 minutes. *Allergy*. 49:637–640, 1994.

Received: January 21, 2009

Accepted: May 18, 2009

Address correspondence to:

Dr. Daisuke Shii

Research and Development Center
Santen Pharmaceutical Co., Ltd.

8916-16 Takayama-cho

Ikoma-shi, Nara 630-0101

Japan

E-mail: daisuke.shii@santen.co.jp

