

DP2 (CRTh2) Antagonism Reduces Ocular Inflammation Induced by Allergen Challenge and Respiratory Syncytial Virus

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Key Words

DP2 · CRTh2 · Allergic conjunctivitis · Prostaglandin D₂ · Allergy · Eye · Respiratory syncytial virus

Abstract

Background: Allergic conjunctivitis is characterized by itchy, watery and swollen eyes which occur in response to exposure to seasonal or environmental allergens. The early phase reaction of allergic conjunctivitis is primarily mediated by mast cell degranulation while the late phase reaction is driven by Th2 cells and eosinophils. Prostaglandin D₂ (PGD₂), released from mast cells, is present in allergic conjunctival tears and may elicit classical allergic responses via interaction with the high-affinity DP2 receptor (chemoattractant receptor-homologous molecule expressed on Th2 cells, CRTh2). Furthermore, antagonism of this receptor is well known to inhibit eosinophil chemotaxis, basophil activation and Th2 cytokine production. PGD₂, therefore, may be involved in both early and late phase reactions in response to allergen challenge. **Methods:** Thus, we explored whether our novel and selective DP2 antagonist AM156 would be efficacious in animal models of allergic conjunctivitis. Furthermore, as respiratory syncytial virus (RSV) has been implicated in the pathogenesis of allergic conjunctivitis, we examined the effects of DP2 antagonism in a murine model of RSV

ocular infection. **Results:** Utilizing a guinea pig ovalbumin model and a murine ragweed model we demonstrated that AM156 reduces redness, discharge and swelling in response to allergen challenge. These effects were equal to or greater than those of current clinical treatment options for allergic conjunctivitis including topical corticosteroids and a dual-mechanism antihistamine and decongestant. AM156 significantly reduced RSV-induced ocular inflammation and IL-4 production. **Conclusion:** These results suggest that a topical DP2 antagonist such as AM156 may represent a novel therapeutic for allergic conjunctivitis.

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Introduction

Allergic conjunctivitis is a disease of the ocular surface which manifests as itchy, watery and swollen eyes. This occurs in response to intermittent (seasonal) allergens such as pollen and persistent (perennial) allergens such as house dust mite and affects approximately 20% of the global population. The early phase of allergic conjunctivitis is a type I hypersensitivity reaction mediated via IgE-activated mast cells while the late phase is a classical Th2 response.

Respiratory syncytial virus (RSV) is a significant pathogen involved in respiratory tract infections, particularly among newborns and infants. As with allergic conjunctivitis, RSV infection is associated with a Th2 inflammatory profile including RSV-specific IgE [1], Th2 cytokines [2] and CD4+ T cells [3]. A causal role for RSV infection in the pathogenesis of allergic conjunctivitis has, therefore, been suggested [4, 5].

Prostaglandin D₂ (PGD₂) is a member of a family of biologically active prostaglandins derived from arachidonic acid via the action of COX-1, COX-2 and PGD₂ synthases. Upon release from mast cells and, to a lesser extent, antigen-presenting cells and Th2 cells [6–8], PGD₂ binds with high affinity to two G protein-coupled receptors, DP1 and DP2 [also known as chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTh2)]. PGD₂ is rapidly metabolized in vivo and several of its metabolites are potent and selective agonists of DP2. Thus, PGD₂ release may result in sustained activation of DP2. DP2 is expressed predominantly on eosinophils, Th2 lymphocytes and basophils and to a lesser extent on monocytes [9], mast cells [10], fibroblasts [11, 12] and lung epithelial cells [11, 13, 14]. The biological consequence of the PGD₂-DP2 interaction includes eosinophil shape change, basophil degranulation, Th2 cytokine production, upregulation of adhesion molecules and chemotaxis of eosinophils, basophils and Th2 lymphocytes.

In the early phase of allergic conjunctivitis, mast cells synthesize and release PGD₂ in response to allergen exposure. PGD₂ via DP2 is a potent chemoattractant for eosinophils which function as effector cells of the late-phase allergic response. Thus, PGD₂ release during the early phase may facilitate the recruitment of eosinophils and thereby exacerbate the late-phase allergic response in the eye. Indeed, PGD₂ is elevated in tears collected from allergic conjunctivitis eyes following ocular allergen challenge [15]. Furthermore, in severe allergic conjunctivitis, pre-allergen challenge baseline concentrations of PGD₂ and PGJ₂, a metabolite of PGD₂ and a ligand for DP2, are elevated in tears collected [16, 17]. Preclinically, PGD₂ applied topically to guinea pig eyes elicits conjunctival redness, edema and eosinophil infiltration [18]. In a rat model of allergic conjunctivitis, ramatroban (Baynas®), a dual DP2 antagonist/TP antagonist reduced the number of conjunctival eosinophils in antigen-challenged eyes. Ramatroban also reduced conjunctival edema, although this effect may have been mediated by its action on the TP receptor since a role for TXA₂ in edema has been established.

AM156 is a selective antagonist of DP2 which has been described previously [19, 20]. In our previously published

experiments, we demonstrated that AM156 is effective in murine models of allergic disease. Specifically, AM156 prevented ovalbumin (OVA)-induced sneezes and nasal rubs and reduced plasma IgE in a murine model of allergic rhinitis [19]. Furthermore, in a model of house dust mite-induced pulmonary inflammation, AM156 reduced eosinophil and lymphocyte influx into the bronchoalveolar lavage fluid and reduced secreted and stored mucin in the lung [19]. In the experiments described herein, we utilized guinea pig and murine models to probe the efficacy of topically applied AM156 against experimental allergic conjunctivitis. In addition, we examined topical AM156 in a murine model of RSV-induced ocular inflammation in which the Th2 cytokine IL-4 is known to play a role [21].

Material and Methods

Guinea Pig Model of Allergic Conjunctivitis

Male Hartley guinea pigs (300–350 g, Charles River Laboratories, Wilmington, Mass., USA) were allowed to acclimate for 1 week prior to sensitization. On day 0, guinea pigs were sensitized to OVA via injection of OVA adsorbed to Imject Alum [50 µg intraperitoneal (IP) and 50 µg subcutaneous near cervical lymph nodes] (fig. 1). On days 21 and 23, guinea pigs were topically challenged in each eye with 10 µl of either saline or 2.5% OVA. On days 21, 22 and 23, guinea pigs were treated with 10 µl of PBS or AM156 solution (300 ng in 10 µl) delivered to the medial cornea followed by 3–5 forced blinks. Systemic mepyramine (5 mg/kg IP) and topical Opcon-A (10 µl/eye) were used as positive controls. Drug treatment occurred 30 min prior to antigen challenge. Following drug treatment on day 23, animals were transferred to a new cage and allowed to acclimate for 30 min and then received their final OVA challenge. The itch and scratch response was monitored for up to 30 min following antigen challenge (methods adapted from Sanchis-Merino et al. [22]). When more than 11 clusters of scratching had occurred, a maximum score was achieved and scratch monitoring stopped. Ninety minutes after OVA challenge (day 23), clinical features were assessed macroscopically and scored for redness, lid edema and discharge using the methods described by Sanchis-Merino et al. After recording clinical scores, guinea pigs were euthanized by CO₂ inhalation. Eyes were dissected and frozen at –80°C for biochemical analysis.

Evans Blue Dye Extravasation

On day 23, a subset of guinea pigs was anesthetized via intramuscular injection of ketamine (70 mg/kg) and xylazine (10 mg/kg) and injected intravenously with Evans blue dye (12.5 mg/kg). Immediately following dye injection, eyes were topically challenged with saline or OVA. Ninety minutes later, animals were euthanized by CO₂ inhalation. Eyes and eyelids were dissected and extracted overnight in 2 ml formamide. Extracted samples were centrifuged (2,000 g, 5 min). 200-µl aliquots were then added to a 96-well plate and read on a spectrophotometer at 610 nm. The data were baseline corrected and normalized as a percentage of PBS/OVA values.

Biochemical Analysis of Eyes

Eyes were thawed on ice and 1.0 ml of homogenization buffer (0.5% Triton-X 100, 15 mM Tris-HCl, pH 7.4 [21]) was added to each tube containing both the left and the right eye from each animal. Tissue was minced and homogenized using a Polytron (Kinematica, Bohemia, N.Y., USA). Samples were centrifuged (10,000 g, 10 min, 4°C), supernatants were aliquoted and frozen at -80°C until biochemical analysis.

Eosinophil peroxidase (EPO) was analyzed using modifications of published methods [23–25]. Briefly, a standard curve of human EPO (EMD Biosciences, San Diego, Calif., USA) was constructed in PBS. 50 µl of homogenate or EPO standard was added to a 96-well plate along with 100 µl of substrate buffer [1 mM *o*-phenylenediamine HCl, 0.05 M Tris-HCl buffer (pH 8), 0.1% Triton X-100, 1 mM H₂O₂]. The reaction was incubated in the dark at room temperature. After 1 h, the reaction was stopped by the addition of 50 µl 4 M H₂SO₄. Since addition of the stop solution caused precipitate to occur, the plate was centrifuged (1,000 g, 10 min), and supernatants transferred to a new microtiter plate, and A₄₉₂ of these clarified supernatants was determined using a spectrophotometer (Molecular Devices, Sunnyvale, Calif., USA).

For determination of IL-4 in the homogenates, samples were concentrated 5-fold using Amicon 30,000 MWCO filters (Millipore, Billerica, Mass., USA). Guinea pig IL-4 was analyzed by ELISA according to the manufacturer's instructions (CosmoBio, Carlsbad, Calif., USA).

Murine Model of Allergic Conjunctivitis

Female BALB/c mice (Harlan, San Diego, Calif., USA, 6–8 weeks upon arrival) were allowed to acclimate for at least 1 week prior to sensitization with ragweed using the methods adapted from Miyazaki et al. [26]. On day 0, a 2 mg/ml suspension of short ragweed (Greer, Lenoir, N.C., USA) was prepared in Alhydrogel (Sigma-Aldrich, St. Louis, Mo., USA) and 25 µl was injected into each rear footpad (50 µg/foot; fig. 2).

5 µl of AM156 (15 µg in PBS), prednisolone acetate (1%, Falcon Pharmaceuticals, Fort Worth, Tex., USA) or PBS vehicle was administered to the central cornea of each eye 3 times daily beginning on day 9 and continuing throughout the experiment.

Allergen challenge occurred 30 min after drug treatment on day 13 (1,000 µg/eye in 5 µl) and then again on days 14 and 15 (750 µg/eye in 5 µl). Control animals received PBS challenge. Microscopic ophthalmic scoring was performed 15 min after the final antigen challenge (day 15) and consisted of hyperemia, lid swelling, chemosis and discharge as described previously [26, 27].

Murine Model of RSV-Induced Ocular Inflammation

Female BALB/c mice (6–8 weeks old) were purchased from Charles River Laboratories (Wilmington, Mass., USA). Eyes were inoculated with RSV as previously described [21]. Briefly, on day 0 mice were anesthetized by sodium pentobarbital (50 mg/kg, IP) and 2 µl virus (10⁶ PFU) or sucrose/PBS was pipetted onto the corneal surface of one eye and massaged in with closed eyes (fig. 3). AM156 (60 ng/2 µl) or saline was topically applied to the cornea of the RSV-treated eye 40 min after RSV inoculation on day 0 and then once daily for 14 days.

Eyes were examined and ocular pathology scores were evaluated daily using a slit lamp biomicroscope as described previously [21]. Ocular pathology was scored by an independent blinded examiner on a scale of 0–4: 0 = clear eye; 1 = slight redness in the

corners; 2 = moderate redness and injection; 3 = conjunctival and corneal injection with ciliary flush; 4 = extensive injection, generally associated with some mucus, and 5 = most extensive injection, associated with excess mucus. Three mice from each treatment group were sacrificed and the eyes enucleated on the following days after RSV infection: day 2, 4, 6, 8, 10 and 14.

To assess whether DP2 antagonism had an effect on RSV replication in the eye, ocular tissue was analyzed for RSV using both Western blot and viable virus assay as described previously [21]. Ocular tissue IL-4 was quantified using RT-PCR.

Results

Guinea Pig Model of Allergic Conjunctivitis

Following OVA challenge, guinea pig eyes and eyelids were red and swollen. Scratching of the ocular region was also observed. AM156 inhibited redness, edema and discharge (fig. 4). Inhibition by AM156 was comparable to that achieved with systemic antihistamine or topical Opcon-A. The cumulative clinical score was slightly improved by all treatments (fig. 4d). None of the compounds tested significantly reduced the scratch response, although all showed a trend toward a reduction (data not shown). Neither AM156 nor mepyramine reduced the Evans blue dye extravasation. Opcon-A reduced Evans blue dye leak by approximately 50% (table 1).

The results of the biochemical analysis of EPO and IL-4 in guinea pig eye homogenates were inconclusive with very little increase observed. Allergen challenge led to a small, nonsignificant increase in EPO measured in eye homogenates (table 1). Even though the increase was small, AM156 treatment significantly decreased EPO relative to vehicle-treated OVA-challenged eyes. The values were lower than PBS-challenged eyes suggesting a modest effect of PBS on this endpoint. Mepyramine had no effect on EPO.

As with EPO, allergen challenge caused a small nonsignificant increase in IL-4 (table 1). Both AM156 and mepyramine reduced this response to a similar extent although neither was statistically significant.

Murine Model of Allergic Conjunctivitis

Ocular ragweed challenge resulted in robust eye pathology which could be observed with a dissecting microscope. AM156 significantly reduced ocular discharge and lid swelling to a degree that was comparable to that of prednisolone (fig. 5). AM156 also reduced chemosis and this effect was greater than that of prednisolone. Hyperemia was increased slightly but not significantly and was not affected by AM156. Prednisolone appeared to de-

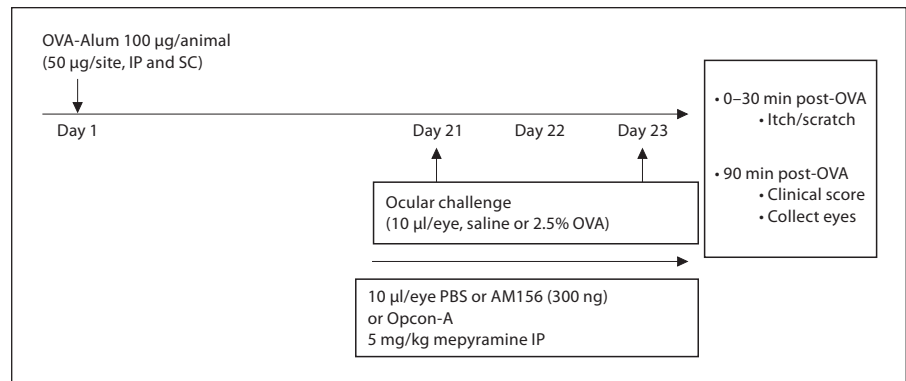


Fig. 1. Study design for a guinea pig model of OVA-induced allergic conjunctivitis. Male Hartley guinea pigs were sensitized to OVA on day 0. OVA was prepared in Imject Alum and 50 µg was injected IP and 50 µg was injected subcutaneously (SC) near the cervical lymph nodes. On days 21–23, PBS vehicle or AM156 or Opcon-A was topically administered bilaterally in 10 µl. Mepyra-

mine (5 mg/kg) was administered IP. Ocular challenge (10 µl of 2.5% OVA) occurred 30 min after drug treatment on days 21 and 23. On day 23, scratching was monitored for the first 30 min after challenge. 90 min after the day 23 challenge, clinical scores were evaluated. Guinea pigs were euthanized with CO₂ inhalation and eyes were collected for biochemical analyses.

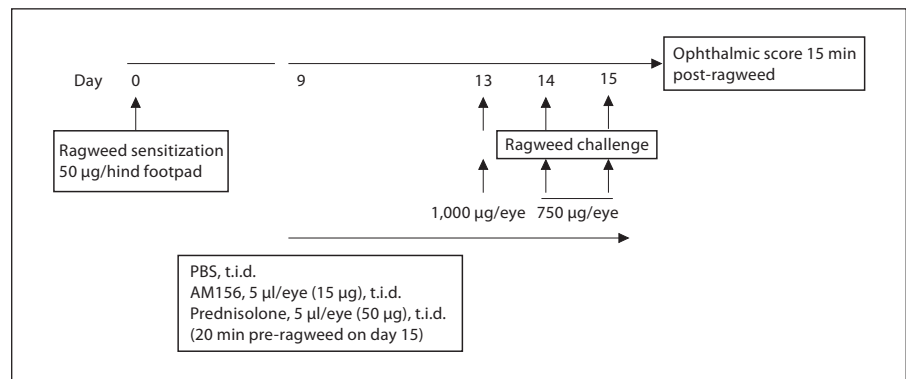


Fig. 2. Study design for a murine model of ragweed-induced allergic conjunctivitis. Female BALB/c mice were sensitized to ragweed on day 0. Ragweed was prepared in Alhydrogel and 50 µg was injected into each hind footpad. Beginning on day 9, PBS vehicle, AM156 or prednisolone was topically administered bilaterally in 5 µl thrice daily. On day 13, eyes were challenged with

1,000 µg ragweed prepared as a suspension in PBS. On days 14 and 15, eyes were challenged with 750 µg ragweed. Control eyes were challenged with PBS. On day 15, drug was administered 20 min prior to ragweed challenge and ophthalmic scoring was performed 15 min after the time of challenge.

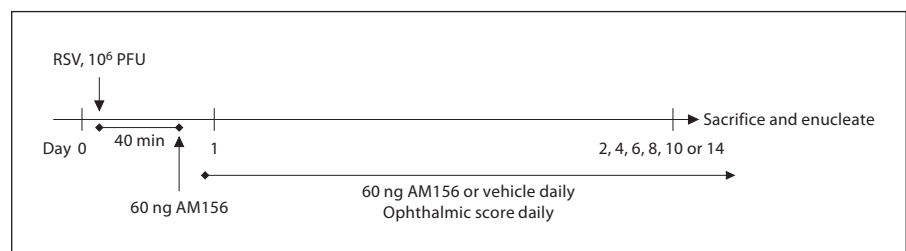


Fig. 3. Study design for a murine model of RSV-induced ocular inflammation. On day 0, 10⁶ PFU of RSV was dosed to one eye of female BALB/c mice, and 40 min later, PBS vehicle or AM156 (60 ng) was topically applied in 2 µl to that eye. Treatment con-

tinued daily for 14 days. Ophthalmic scoring was performed daily. A subset (n = 3) was euthanized every 2 days (days 0, 2, 4, 6, 8, 10 and 14), and the eyes removed and homogenized for further analyses.

Fig. 4. Effect of AM156 on redness (**a**), edema (**b**), ocular discharge (**c**) and cumulative clinical score (**d**) in a guinea pig model of OVA-induced allergic conjunctivitis (PBS, n = 8 eyes; OVA, n = 30 eyes; AM156, n = 24 eyes; mepyramine (MEP IP), n = 8 eyes; Opcon-A, n = 6 eyes). Data are means $\pm$ SEM. ### p < 0.001 vs. PBS/Sal; * p < 0.05, ** p < 0.01 vs. PBS/OVA by ANOVA; + p < 0.05 vs. PBS/OVA by t test.

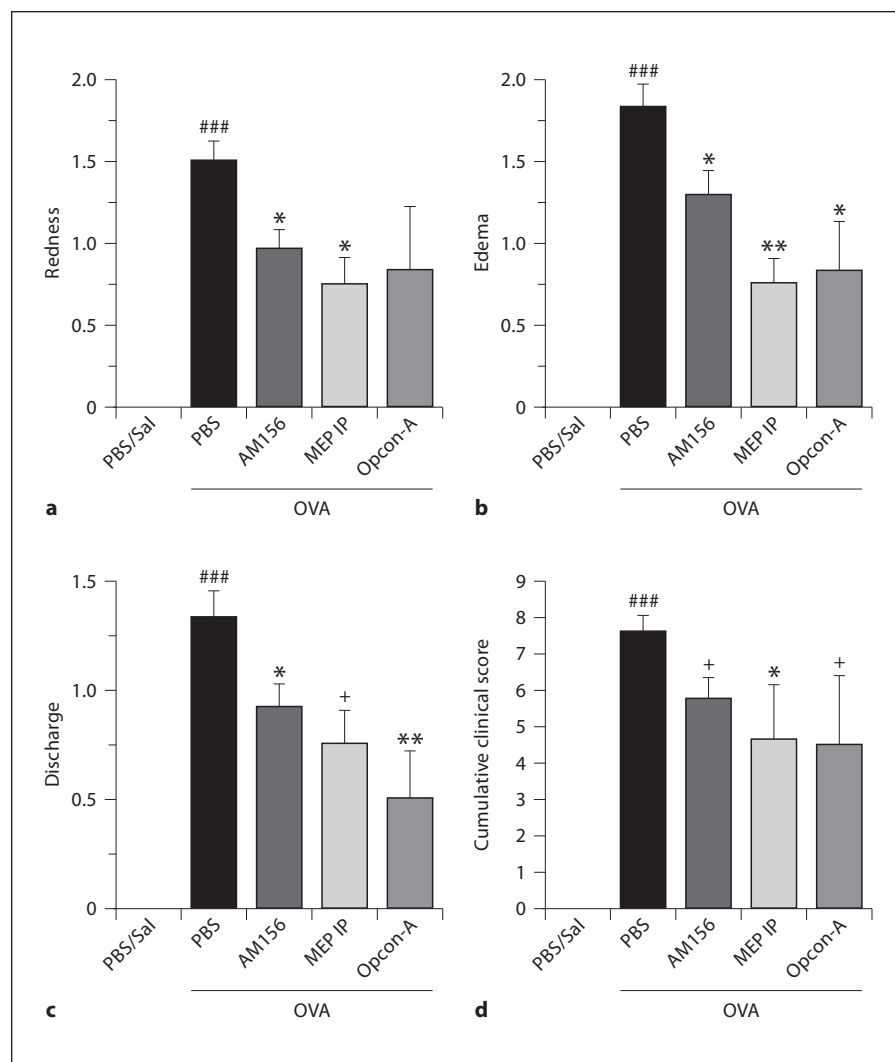


Table 1. Effect of AM156 on biomarkers of inflammation in guinea pig eye homogenates obtained 90 min after ocular OVA challenge

	Treatment: Challenge:	PBS Saline	PBS OVA	AM156 OVA	Mepyramine OVA	Opcon-A OVA
IL-4, pg/ml		1.7 $\pm$ 0.7	3.9 $\pm$ 1.4	1.9 $\pm$ 0.4***	1.9 $\pm$ 0.6	n.d.
EPO, pg/ml		156 $\pm$ 13.6	163 $\pm$ 25	137 $\pm$ 7.9*	172 $\pm$ 27	n.d.
Evans blue		n/a	100 $\pm$ 5.7	106 $\pm$ 20	107 $\pm$ 9	54 $\pm$ 9**

All data are means $\pm$ SEM. Evans blue dye extravasation is expressed as percent of PBS/OVA averages. * p < 0.05 vs. PBS/OVA, Student's t test; ** p < 0.01 vs. PBS/OVA, ANOVA; *** p = 0.057. n.d. = Not determined; n/a = not applicable.

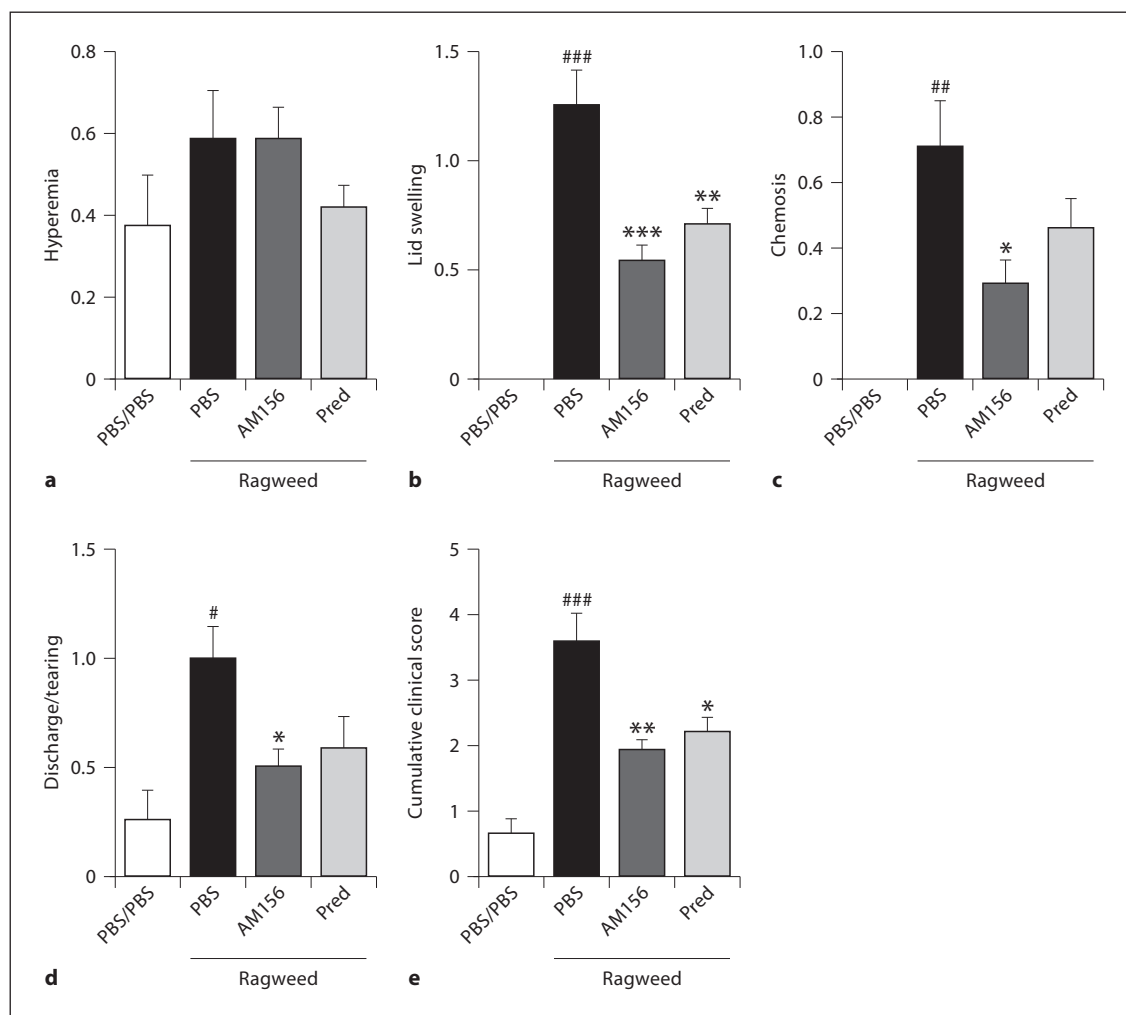


Fig. 5. Effect of AM156 on hyperemia (a), lid swelling (b), chemosis (c), ocular discharge (d) and cumulative clinical score (e) in a murine model of ragweed-induced allergic conjunctivitis. Data are means $\pm$ SEM (n = 12 eyes/group). * p < 0.05, ** p < 0.01, *** p < 0.001 vs. PBS/ragweed by ANOVA; # p < 0.05, ## p < 0.01, ### p < 0.001 vs. PBS/PBS. Pred = Prednisolone.

crease this response but again was not statistically significant. Overall, both AM156 and prednisolone improved the cumulative clinical score to a similar degree (~50%).

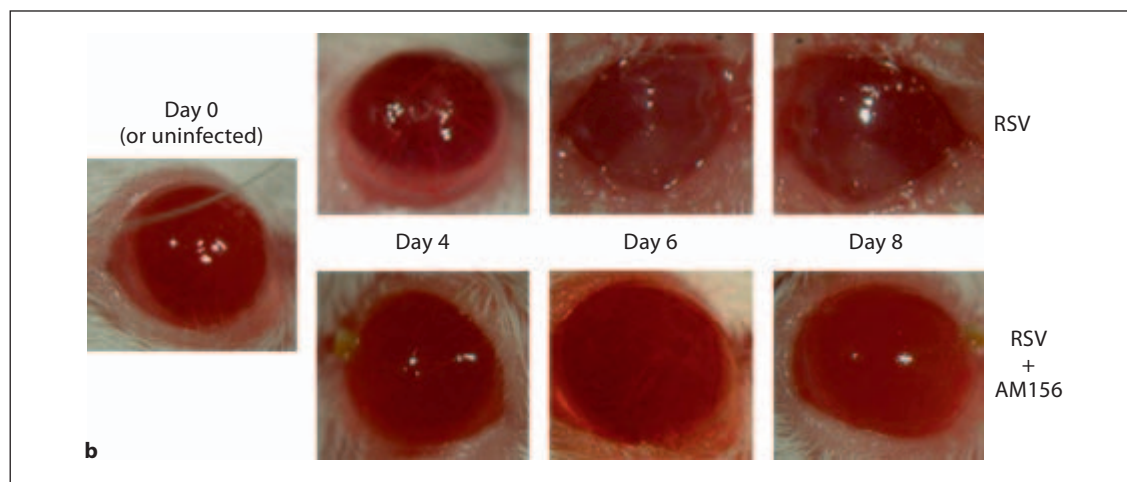
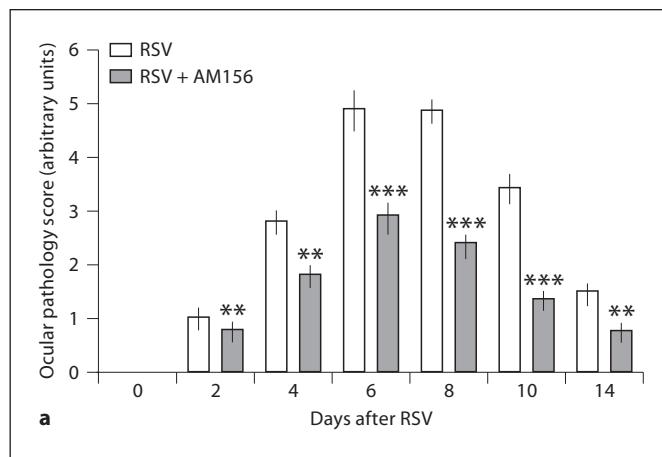
Murine Model of RSV-Induced Ocular Inflammation

Virus was detectable in the eyes on days 2 and 4 and titers were similar between vehicle- and AM156-treated eyes (data not shown). RSV induced an ocular pathology beginning on day 2, which was significantly reduced by AM156 (fig. 6a, b). RSV inoculation increased expression of IL-4 in the eye in a pattern which followed the ocular pathology. IL-4 expression was increased with RSV infection and this was reduced by AM156 (fig. 7).

Discussion

The role for PGD₂ and DP2 in allergic airway and allergic cutaneous disease has been extensively explored [19, 28–32]. However, to our knowledge, these data are the first to report efficacy of a selective DP2 antagonist in in vivo ocular models of allergic conjunctivitis and RSV inflammation. Ramatroban (Baynas), a dual-function DP2 antagonist/TXA₂ antagonist, has been reported to inhibit scratching in a rat model of allergic conjunctivitis, conjunctival edema and eosinophil infiltration after an oral dose of 10 mg/kg [33, 34]. However, at the time of these publications, the ramatroban activity was thought to be

Fig. 6. Effect of AM156 on RSV-induced ocular pathology. Data are means $\pm$ SEM. ** $p < 0.01$, *** $p < 0.001$ vs. RSV-treated eye by ANOVA.



mediated through TXA_2 antagonism rather than with DP2 antagonism. However, given the results that we have presented here, it is likely that at least some of the activity observed by Minami et al. [33, 34] was due to DP2 antagonism rather than TXA_2 antagonism alone.

Allergic conjunctivitis affects approximately 20% of the global population and is often comorbid with allergic rhinitis. As is the case with other allergic diseases, mast cell degranulation plays a central role in the allergic cascade, both in the immediate phase and in perpetuating the allergic response. Therefore, therapeutics which target the mast cell and its degranulation products may provide clinical benefit for allergic conjunctivitis. At present, current treatment options for allergic conjunctivitis are primarily dual-action agents which combine antihistamines and mast cell stabilizers. Oral antihistamines are often the first line of therapy for allergic conjunctivitis, particularly for rhinoconjunctivitis. However,

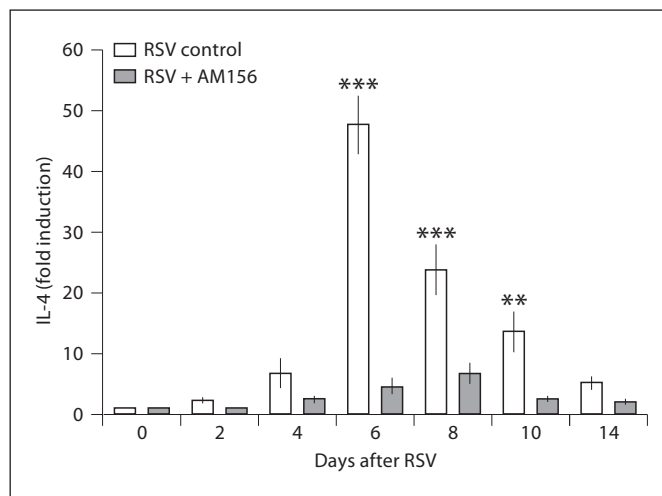


Fig. 7. Inhibition of RSV-induced ocular IL-4 mRNA by AM156. Data are means $\pm$ SEM. ** $p < 0.01$, *** $p < 0.001$ vs. AM156-treated eyes by ANOVA.

er, reports are somewhat conflicting as to whether or not these provide significant clinical benefit for the ocular symptoms [35]. Topical antihistamines provide more rapid relief and seem most effective at relieving the itching [36]. Over-the-counter dual-function solutions combining antihistamines and decongestants provide short-term relief; however, long-term use has been shown to cause rebound hyperemia [37]. Topical corticosteroids provide benefit but due to their potential for side effects, namely glaucoma and cataracts, only short-term usage is recommended. Thus, there exists an unmet clinical need for a safe and effective treatment for allergic conjunctivitis. DP2 antagonism, via inhibition of Th2 cells and eosinophil chemotaxis, could represent a novel mechanism for treating the late phase of allergic conjunctivitis. Furthermore, DP2 antagonism could ameliorate the PGD₂-mediated component of the early phase response by preventing interaction of PGD₂ with the DP2 receptor.

In a guinea pig model of allergic conjunctivitis we demonstrated that DP2 antagonism with AM156 was effective on the overall clinical score comprised of redness, lid edema and ocular discharge. Furthermore, efficacy was comparable to that achieved with systemic antihistamine and topical antihistamine plus decongestant (Opcon-A). Therefore, DP2 antagonism may provide similar clinical benefit and may have a better safety profile by avoiding the rebound hyperemia which can occur with long-term topical decongestant usage. Longer-term experiments will need to be performed to see if this is indeed the case. We failed to demonstrate an effect on itching and scratching with any of the pharmacological mechanisms tested. The dose of AM156 used in our guinea pig experiments was very low (300 ng per eye or 10 µl of a 0.003% solution dose once daily). Therefore, it is likely that had we explored higher and/or more frequent doses; we may have observed effects on scratching with AM156 as well as greater effects on the other clinical readouts. In a cedar pollen model, mepyramine (10 mg/kg) was effective against the first antigen challenge but ineffective against multiple antigen challenges administered over several weeks [38]. In our guinea pig model we did not look for pathology following the first antigen challenge but recorded clinical events following only the second challenge. It is possible that had we monitored clinical scores following the initial antigen challenge; we may have observed an effect with mepyramine or AM156 on the itch/scratch response. Evaluating AM156 in a more chronic setting may be an important area of future research.

Using a mouse model of ragweed-induced allergic conjunctivitis, we demonstrated that DP2 antagonism with AM156 inhibits lid swelling, chemosis and ocular discharge. These effects were similar or even slightly superior to those of topical corticosteroid. DP2 antagonism had no effect on hyperemia while prednisolone reduced hyperemia to baseline levels. These data are consistent with our guinea pig results and together suggest that topical application of a DP2 antagonist such as AM156 may provide clinical benefit for allergic conjunctivitis. Importantly, DP2 antagonism may provide an improved safety profile over topical corticosteroids.

OVA challenge caused only modest increases in IL-4 and EPO in whole eye homogenates, and these effects did not reach statistical significance. The reason for this may be due to the fact that we had few subjects and pooled eyes ($n = 3$ sets of pooled eyes for PBS/Sal and $n = 6$ sets of pooled eyes for PBS/OVA). Statistical significance may have been reached had we homogenized individual eyes from each animal rather than pooled both eyes from each animal. Alternatively, ocular lavage may have yielded samples with more concentrated IL-4 and EPO than did the whole eye homogenates. Since allergic conjunctivitis is a disease of the surface of the eye, we likely diluted IL-4 and EPO significantly by examining whole eye homogenates. We may have collected the samples too early following a challenge to see a peak response. In an earlier time course study, for example, EPO in conjunctiva homogenates was not elevated 1 h after antigen challenge, but was increased 6 h after challenge and reached a peak 24 h after challenge [23]. We collected the eyes 90 min after challenge and so may have been too early to see a robust increase. That being said, our model utilized 2 challenges, administered 48 h apart with tissue collection occurring 90 min after the second challenge. We expected that this regimen might allow us to see a late-phase component. However, the time between the two challenges may have been too great to see such a late-phase response elicited by the initial challenge. In fact, in most animals, macroscopically the eyes appeared normal prior to the second challenge suggesting that the late-phase response had already resolved. Regardless, the trend toward an increase in IL-4 was reduced by DP2 antagonism with AM156. The effect of AM156 is consistent with previous reports of DP2 antagonism reducing Th2 cytokines [19, 32, 39] but to our knowledge these data are the first to examine this effect in an ocular setting. Despite the very modest increase in EPO detected in OVA-challenged eyes, AM156 treatment significantly reduced EPO values suggesting that AM156 has the potential to modulate a

late-phase allergic response in the eye. These data are consistent with previous reports that DP2 antagonism blocks eosinophil degranulation [40].

RSV is implicated in asthma and allergy, although reports are conflicting with some suggesting that prior RSV infection protects against development of atopy as determined by skin prick test [41] and others suggesting that RSV infection predisposes to the development of allergic conditions such as asthma [42, 43]. Serum collected from RSV-infected patients contains elevated Th2 cytokines and IgE [44, 45], suggesting that viral infection induces an allergy-like condition. In mice, virus infection resulted in enhanced allergic airway responses in OVA-sensitized animals including airway hyperresponsiveness, IgE, IL-4 and IL-13 [46]. In children, ocular RSV inoculation results in efficient spread of virus to the lower respiratory tract, and viral infection has been shown to exacerbate allergic airway responses [47–49]. A role for RSV in the pathogenesis of allergic conjunctivitis has also been suggested [5]. Using a polymerase chain reaction method, Fujishima et al. [5] demonstrated that a higher percentage of RSV-positive conjunctival epithelial cells were present in samples obtained from allergic conjunctivitis patients than from normal control subjects (23 vs.

5%). In the current experiments, AM156 reduced IL-4 mRNA in RSV-infected eyes. The reduction in IL-4 mRNA correlated with an improvement in pathological score, suggesting a role for this Th2 cytokine in RSV-induced ocular inflammation. This is a novel finding as neither PGD₂ nor DP2 activation has been previously linked to RSV eye inflammation.

To our knowledge, these data are the first report of efficacy of selective DP2 antagonism in in vivo models of allergic conjunctivitis and RSV eye infection. Our results suggest that topical delivery of DP2 antagonists such as AM156 may represent a novel therapy for ocular conditions such as allergic conjunctivitis. Furthermore, when RSV infection of the eye is suspected, treatment by topical application of a DP2 antagonist may limit the extent of the outward signs of the infection, which would be of great benefit to the patient.

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