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A NOVEL THERAPEUTIC APPROACH FOR ALLERGIC CONJUNCTIVITS BY MOISTENING THE OCULAR SURFACE

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Abstract

Allergic conjunctivitis (AC) and dry eye disease (DED) are inflammatory diseases of the ocular surface. Antigen (Ag) and immunoglobulin E-mediated activation of mast cells in the conjunctiva have a central role in the development of AC. On the other hand, tear film instability and non-specific conjunctival inflammation are the key factors of DED. The site of both diseases is the conjunctiva, but the relationship between AC and DED has not been investigated extensively. To examine whether DED affects the development of AC, DED was induced in BALB/c mice. Then AC was induced in these mice by ovalbumin immunization and challenge in eye drops. Evan's blue (EB) was injected intravenously and EB leakage into the conjunctiva was evaluated as the severity of AC. Regarding the AC-induced mouse groups, EB leakage was significantly higher in DED-induced mice. Next, we investigated whether treatment of DED can suppress AC development. Treatment with diquafosol eye drops significantly decreased the leakage of EB into the conjunctiva. These data support that AC and DED are not only comorbidities but also have a solid relationship. Furthermore, the ocular surface barrier function appears to be a key factor to consider therapeutic approaches for AC.

Introduction

Allergic conjunctivitis (AC) and dry eye disease (DED) are representative inflammatory diseases occurring in the ocular surface [1]. In both diseases, the primary site of inflammation is the conjunctiva. Binding of antigen and immunoglobulin E on the surface of mast cells leads to mast cell activation and release of various kinds of chemical mediators into the conjunctiva. Then, eyes become itchy and the conjunctiva turns red [2]. In contrast, DED is a multifactorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance, and tear film instability and can be potentially damage the ocular surface [3]. For example, DED is known to be accompanied by increased osmolarity of the tear film and inflammation of the ocular surface [4]. Patients with DED also report symptoms of itchness and redness of the eye [5]. Thus, it appears that the signs and symptoms of AC and DED overlap.

In the mid 1990s, Tsubota's group suggested that there is a solid interaction between AC and DED [6, 7]. Especially, in severe cases of AC such as vernal keratoconjunctivitis (VKC) and atopic keratoconjunctivitis(AKC), disturbance of tear function has been demonstrated [8-10].Thus, it appears to be possible that severe ocular surface inflammation induced by VKC or AKC affects the stability of the ocular surface and, therefore, can lead to DED.

The frequency of DED inmild cases of AC has also been investigated. Recently, Hom et al. reported that concomitant features, such as itchness, dry feeling and redness, have been noted in symptomatic patients with mild AC and DED [11]. Kim et al. reported that DED tends to be more severe in pediatric patients with AC [12]. Similarly, Akil et al. reported that the severity of DED markers, such as breakup time (BUT),were significantly higher in pediatric patients with AC [13]. Chen et al. demonstrated that the incidence of DED was higher in young

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children with AC [14]. More recently, Ayaki et al.found that short BUT-type DED cases increased in spring when air pollen counts are highest in Japan [15]. Taken altogether, AC appears to be an important risk factor for development of DED.

As depicted above, AC plays a role, at least in part, in the development of DED. On the other hand, roles of DED in the development of AC have not been investigated. Recently, Gomes et al. reported that signs and symptoms AC were worsened if DED was induced in the AC patients before antigen challenge into the conjunctiva [16]. The data suggested that DED damages the condition of ocular surface and upregulates the penetrating efficacy of antigen in the conjunctiva. However, no reports have been available whether DED affects the development of AC in animal models.

Materials and methods

Mice

BALB/c mice were purchased from Charles River Laboratories, Yokohama, Japan. These mice were maintained under pathogen-free conditions at the animal facility of Santen Nara Research and Development Center. Male mice were used for experiments at 6- to 8-week-old. All research adhered to the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research. The number of mice in each group is 8.

Reagents

Ovalbumin (OVA) was purchased from Worthington Biochemical Corp (Lakewood, NJ, USA). Aluminum hydroxide (alum) was prepared with 42 mg/mLNaOH and 659 mg/mL $Al_2(SO_4)_3$. Evan's blue (EB) was purchased from Sigma (St. Louis, MO).

Induction of DED

On day 0, under anesthesia, bilateral extra orbitallacrimal glands were excised from mice. As a control, sham surgery was performed [17].

Induction of AC

OVA (100 μg) was suspended in phosphate buffered saline (PBS) and adsorbed on alum (2.5 mg) and injected intraperitoneally twice, at day 22 and 27. On day 32, the right eyes of the mice were challenged with OVA in PBS (5%, w/v). The control groups were the mice that were immunized but not challenged (Figure 1).

Treatment with diquafosol eye drops

One group of mice was treated with diquafosol eye drops daily for 6 times from day 16 to day 31. The other groups of mice did not receive eye drop treatment (Figure 1).

Evaluation of the early phase reaction using extravasation of EB

EB (15 μg/g body weight) was injected intravenously into all the mice, 30 minutes prior to challenge with eye drops containing OVA in PBS. After 30 min, mice were sacrificed and their conjunctivas were harvested (Figure 1). EB was extracted for 48 h ina 2 ml of a 0.5 % Na_2SO_4 and acetone mixture (3:7) for 48 hours. After centrifugation, the absorbance (620 nm) of the supernatant was determined using a spectrophotometer. Data are presented as EB content/weight of conjunctiva. Analyses were performed using the Tukey-Kramer test. Probability values (p) < 0.05 were considered significant.

Results and discussion

To investigate the roles of DED in AC, DED was induced by excision of extra orbital lacrimal glands and AC was induced by immunization of OVA emulsified in alum(Figure 1).DED was induced 3 weeks before the initial sensitization of OVA. Ten days after the initial sensitization, the mice were challenged with OVA (5% w/v) in eye

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drops. Control mice received sensitization but were not challenged with OVA in eye drops. To evaluate the severity of AC, early phase reaction in the conjunctiva was evaluated by leakage of EB into the conjunctiva. As shown in Figure 2, compared with the control mice (group 1), leakage was increased by induction of AC, regardless of induction of DED (group 3 and 4). Importantly, in the AC-induced mouse groups, EB leakage was significantly higher in DED-induced mice than in control mice without DED (group 4). Thus, our data confirmed that DED appears to exacerbate the severity of AC.

To further clarify the role of DED in the development of experimental AC, we treated DED-induced AC developing mice with diquafosol eye drops, which are commercially available for DED, 30 minutes prior to OVA challenge for 2 weeks (group 5). As shown in Figure 2, treatment with diquafosol significantly decreased the leakage of EB into the conjunctiva compared to the DED-induced AC developing mice without diquafosol eye drop treatment (group 4). Thus, treatment of DED with diquafosol appeared to suppress AC in this experiment. Because diquafosol upregulates mucin and tear secretion in the ocular surface, moistening the ocular surface may be a therapeutic approach to treat AC. These data could also be interpreted that keeping ocular surface barrier function is beneficial to suppress AC.

Increased impairment of the skin barrier has a crucial role in the development of atopic dermatitis [18]. Filaggrin has been found to be a key molecule in the skin barrier [19]. Loss of filaggrin functionality has been found to be associated with skin barrier dysfunction, and concomitant upregulation of IL-4 and thymic stromal lymphopoietin in the skin [20]. Thus, an intact skin barrier blocks penetration of antigens inhibits inflammation. Our results support that this concept is also applicable to the conjunctiva.

Conclusion

Our data suggest that research into methods to keep the conjunctival barrier intact may lead to a new treatment approaches for AC, which may cure various kinds of ocular surface inflammatory diseases.

Legends for Figures

Figure 1.Experimental Protocol

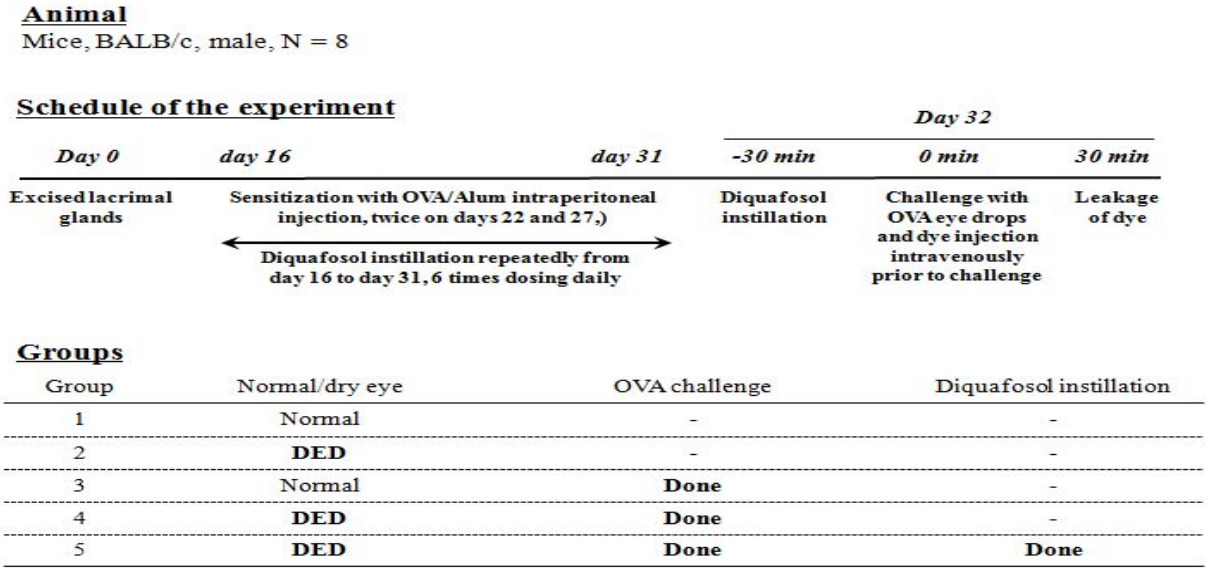

Figure 1 Study methods

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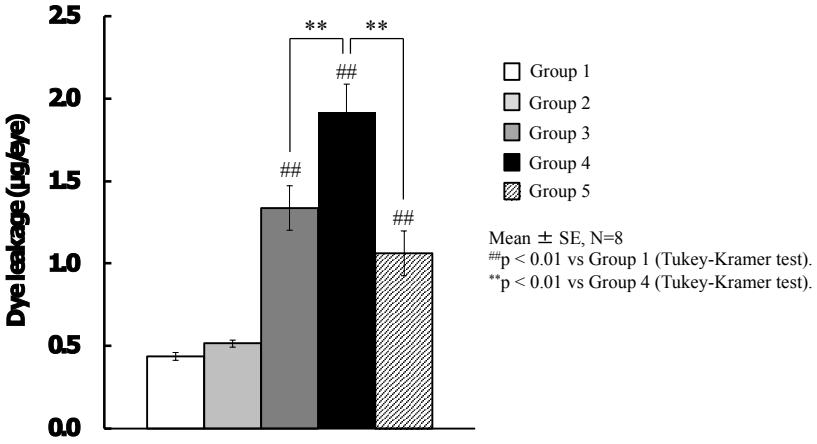
This experiment used 6- to 8-week-old male BALB/c mice. To induce DED, the extraorbital lacrimal glands were excised. As a control, sham treatment was performed. After 22 and 27days, the mice were intraperitoneally injected with OVA in alum. After 32 days later, the mice were intravenously injected with Evans blue dye. Concomitantly, the mice were challenged with OVA solution eye drops. The mice were sacrificed 30 minutes after the challenge, and the conjunctivas were harvested for evaluation of Evans blue leakage into the conjunctiva, as detailed in the Figure 2 legend. In one DED-induced group, the mice were treated with diquafosol eye drops 6 times per day, from day 16 to day 31. On day 32 (the day of the OVA challenge), the mice were finally treated with diquafosol eye drops. Each group had 8 mice.



Group	Normal/dry eye	OVA challenge	Diquafosol instillation
1	Normal	-	-
2	**DED**	-	-
3	Normal	**Done**	-
4	**DED**	**Done**	-
5	**DED**	**Done**	**Done**

Figure 2 Study result

Figure 2. EB was extracted by using a 2 mL of mixture of 0.5 % Na_2SO_4 solution and acetone (3:7) for 48 hours. After centrifugation, the absorbance of the supernatant at 620 nm was determined by using a spectrophotometer. Data are presented as EB content/weight of conjunctiva.

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