

Image analyses of conjunctival hyperemia in guinea pig allergic conjunctivitis

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Dear Editor,

We recently reported that the histamine challenge-induced conjunctival hyperemia of guinea pigs can be quantified by image analysis [1]. This model reflects the release of histamine in the ocular allergy cascade, but a more realistic model would provoke histamine release by adding an antigen (Ag) into the tear film [2]. Therefore, we asked whether the conjunctival hyperemia induced in Ag-sensitized guinea pigs by Ag challenge can also be quantified.

Six male Hartley guinea pigs (bred in an animal facility of Cine-Science Lab. Co., Ltd., Tokyo, Japan) were sensitized by intravenous injection with 1 ml pooled ovalbumin (OVA)-specific hyperimmune serum confirmed by passive cutaneous anaphylaxis method. All research adhered to the guidelines of the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research. Forty-eight hours later, the conjunctival sac was challenged with OVA solution (25 mg/ml, 20 μ l). As previously reported [1], conjunctival hyperemia images taken by a digital camera every 10 seconds for 20 minutes after OVA challenge were binarized by the software program ImageJ, and a region of interest (ROI) unobscured by corneal and scleral vessels was selected for evaluation. Absolute pixel values (Fig. 1a),

fold increases of pixels relative to the pixel value before OVA challenge (Fig. 1b), and fractal dimensions (Fig. 2) were determined. The Mann–Whitney *U*-test was used for statistical analysis, and $P < 0.05$ was considered significant. Both pixel ratios and fractal dimensions rose for 5 minutes before stabilizing: statistical significance was first observed 4 minutes ($P = 0.004$) and 3 minutes after challenge ($P = 0.025$) respectively (Figs. 1b and 2). Similar kinetic changes were observed when the absolute pixel values were estimated, except significant upregulation was first noted 5 minutes after OVA challenge ($P = 0.006$, Fig. 2). Thus, our analytical method can be used to measure hyperemia in both the Ag-mediated ocular allergy model and histamine-induced conjunctivitis.

In histamine-induced conjunctivitis, the absolute pixel values, pixel ratios and fractal dimensions all rose significantly 1 minute after challenge [1], whereas in Ag-induced conjunctival hyperemia, these parameters only achieved statistical significance after 3 to 5 minutes. This may reflect the fact that it takes IgE- and Ag-binding mast cells/basophils several minutes to become activated and undergo degranulation of proinflammatory mediators [3]. Thus, our analytical method appears to reliably detect conjunctival hyperemia in a real-time fashion. That the fractal dimensions rose significantly as early as 3 minutes after OVA challenge suggests that this parameter is sensitive enough for detecting small conjunctival hyperemia changes.

These observations together indicate our analytical method is suitable for clinical use. We are currently developing a device that automatically binarizes conjunctival hyperemia images and calculates fractal dimensions. Although several critical issues remain, including the determination of ROI, this device will be helpful for clinicians who manage red-eyed patients.

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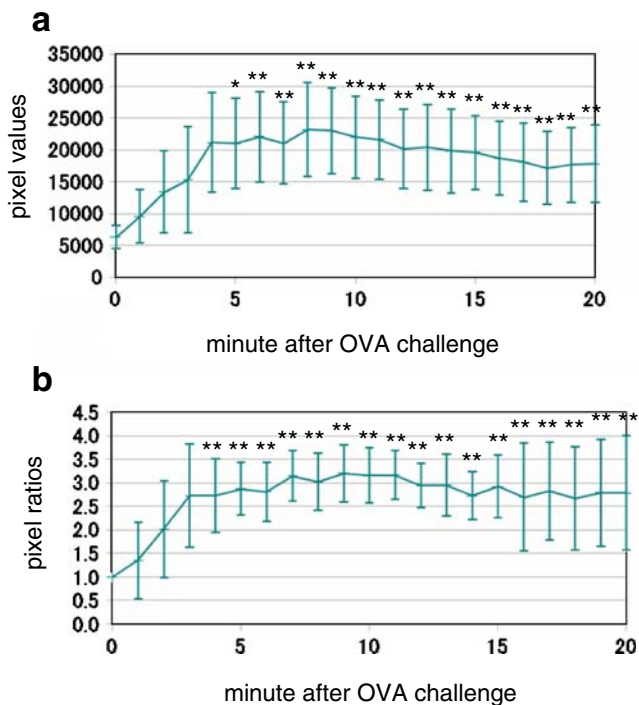


Fig. 1 Quantitation of kinetic changes in the bulbar hyperemia induced by type I allergic reaction. Guinea pigs sensitized with OVA-specific hyperimmune serum were challenged with OVA solution in eye drops. Conjunctival hyperemia images taken by a digital camera for 20 minutes after OVA challenge were converted into binary images by using the software program ImageJ. Data are expressed as absolute pixel values (**a**) and fold increase of pixels compared to before OVA challenge (0 min) (**b**). Data are shown as median $\pm$ SEM. * P < 0.05 and ** P < 0.01 compared to 0 min

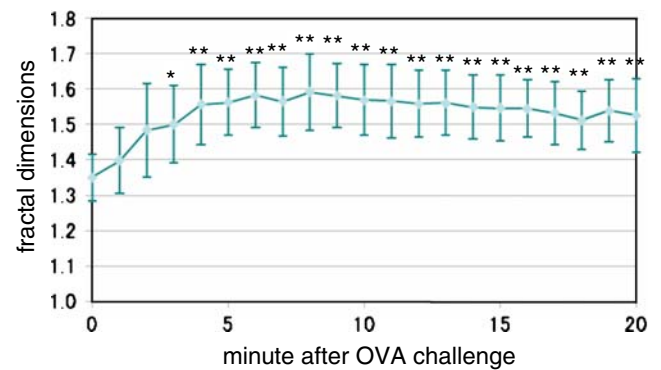


Fig. 2 Fractal analysis of kinetic changes in the bulbar hyperemia induced by type I allergic reaction. The box size and number of boxes needed to cover the boundary were plotted on a log-log plot. Fractal dimension was determined from the slope. Data are shown as median $\pm$ SEM. * P < 0.05 and ** P < 0.01 compared to 0 min

References

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