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Short communication

Establishment of a drug evaluation model against light-induced retinal degeneration using adult pigmented zebrafish

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ABSTRACT

Age-related macular degeneration (AMD) is a major cause of irreversible loss of central vision in the elderly. Zebrafish is an attractive animal model in some respects, lower cost, smaller housing facilities and easier genetic manipulation compared to rodents. The present study aimed to establish a drug evaluation method against light irradiation, as a dry AMD disease model, using adult pigmented zebrafish. Intravitreal administration of an antioxidant, *N*-acetylcysteine, protected against light-induced retinal degeneration in a concentration-dependent manner. We established a new drug evaluation model against light-induced retinal degeneration that can provide new knowledge about dry AMD pathology and therapy.

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Age-related macular degeneration (AMD) is one of the most common causes of severe loss of vision in the elderly. Clinically, AMD is classified into two major phenotypes, “wet” and “dry”. Worldwide prevalence rates of wet and dry AMD are 15% and 85%, respectively (1). Unfortunately, in spite of this situation, at present, effective therapeutics for dry AMD are not established. Risk factors of dry AMD include aging, oxidative stress, and light irradiation (2). Employing these characteristics of dry AMD, light-induced photoreceptor degeneration animal models have been used for studying the mechanisms of dry AMD.

Light-induced retinal degeneration is composed of thinning of the outer nuclear layer (ONL) and the loss of photoreceptor cells. These changes are consistent with the pathology of dry AMD (3,4). Most of light irradiation models for dry AMD disease have been used albino rodents, because the retinae in pigmented wild-type rodents were not damaged by light irradiation. Actually, in our previous study, we could not induce retinal degeneration in wild-type pigmented mice (5). The influence of non-pigmentated retinal pigment epithelium on retinal degeneration is largely unknown.

Zebrafish (*Danio rerio*) have been widely used as disease models because of their fecundity, and their morphological and physiological similarity to mammals. For study mechanisms of retinal regeneration, light induced retinal degeneration model in adult zebrafish has been widely used (6,7). However, the light irradiation model of adult zebrafish has never been used for drug evaluation (8). Moreover, recently some reports of studying regeneration mechanisms showed retinae of adult pigmented wild-type zebrafish were damaged by light irradiation (9). Zebrafish is an attractive model animal because it needs lower costs and smaller facilities for housing. Therefore, in this study, to take these advantages we established a new drug evaluation model for dry AMD using light irradiation in adult pigmented zebrafish.

Adult pigmented wild-type zebrafish (*D. rerio*) were purchased from Meito Systems (Aichi, Japan). Zebrafish were maintained in cycles of 14 h of light: 10 h of dark cycles at 28.5 °C (10). Adult fish (3–10 months of age) were used for all experiments. All experiments were performed in accordance with the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research.

Light irradiation was performed according to the methods of other laboratories (9,11). Briefly, following 14 days dark adaptation,

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zebrafish were transferred to a clear tank (3 L) that was separated at the center by a clear plastic board. Fish were then exposed to constant white light using two halogen lamps as the light source. Light intensity was 16,000–20,000 lux (measured at the outside surface of the tank). The distance from the tank to each light source was 30 cm. An air bubbler and thermometer were present introduced in the tank. In all experiments, the tank contained 20–30 free-swimming zebrafish. To prevent elevating the water temperature, a fan was placed behind the tank to keep the water temperature at 30–33 °C. Fish were euthanized by over-anesthesia in a 0.1% phenoxyethanol solution for about 5 min. Euthanasia was confirmed by observing cardioplegic arrest using a stereomicroscope (Olympus SZX7, Olympus, Tokyo, Japan).

Following euthanasia, eyes were enucleated and immediately transferred to a 4% paraformaldehyde solution. After fixation, eyes were embedded in optimum cutting temperature (O.C.T.) compound (Sakura Fine Technical, Tokyo, Japan). These tissues were then immediately frozen with liquid nitrogen and stored at −80 °C. Serial transverse sections were cut on a cryostat to a thickness of 12 μm and placed on slides (MAS coat; Matsunami Glass Ind. Ltd., Osaka, Japan) for immunohistochemistry.

Immunohistochemical staining was performed in accordance with the following protocol. After blocking with goat serum, tissue sections were incubated with the zpr1 [1:250 dilution] (Abcam, Cambridge, MA, USA) primary antibody at 4 °C overnight. The sections were then incubated with an Alexa Fluor®546 goat anti-mouse IgG (Thermo Fisher Scientific, Carlsbad, CA, USA) secondary antibody for 1 h and counterstained for 10 min using Hoechst 33342 [1:2000 dilution] (Thermo Fisher Scientific). Finally, tissue sections were mounted in Fluoromount (Diagnostic BioSystems, Pleasanton, CA, USA). Images were acquired using a confocal microscope (FLUOVIEW FV10i; Olympus). All images were captured at the central part of dorsal area, which was the most damaged area. Cell counting conducted in the range of 212 μm square images.

Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) staining was performed in accordance with the following protocol. Briefly, tissue sections were permeabilized with 0.1% Triton X-100 in 0.1% sodium citrate for 10 min. After rinsing with phosphate-buffered saline, the tissues were labeled with the In Situ Cell Death Detection Kit, TMR red (Roche Diagnostics, Rotkreuz, Switzerland). Tissues were incubated for 1 h at 37 °C in a humidified chamber with the staining mixture. They were then counterstained for 10 min using Hoechst 33342 (Thermo Fisher Scientific) and mounted. The ONL thickness was measured in all images and the ONL cell number determined using ImageJ software

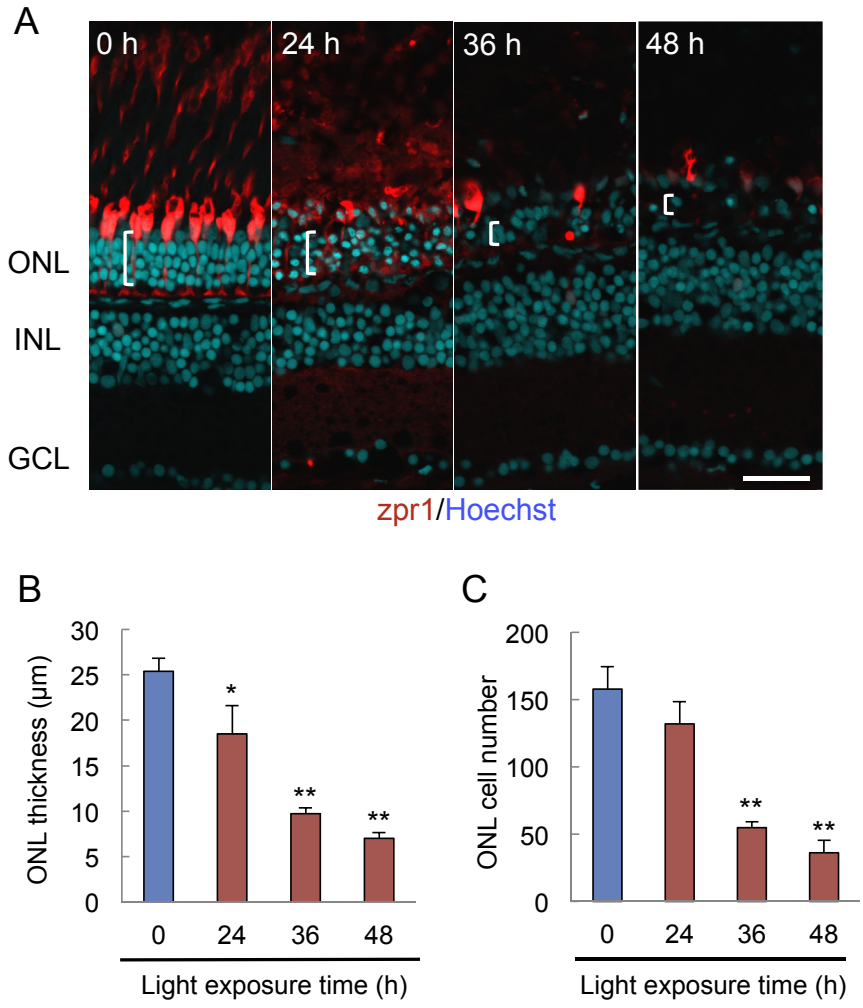

Fig. 1. Retinal degeneration induced by light irradiation shows time dependency. (A) Typical images immunostained for zpr1 (red) and Hoechst 33342 (blue). (B–C) Quantification of ONL thickness and ONL cell number using immunostaining. Data are shown as means ± SEM (n = 5–8). $^{**}p < 0.01$ and $^{*}p < 0.05$ vs. 0 h (Student's *t*-test). Scale bar = 30 μm. GCL, ganglion cell layer; INL, inner nuclear layer; ONL, outer nuclear layer; OS, outer segment.

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