Free Radical Biology and Medicine 71 (2014) 176–185

Contents lists available at ScienceDirect

Free Radical Biology and Medicine

journal homepage: www.elsevier.com/locate/freeradbiomed

Original Contribution

Angiotensin II type 1 receptor blockade suppresses light-induced neural damage in the mouse retina

Toshio Narimatsu [a,b], Yoko Ozawa [a,b,*], Seiji Miyake [a], Norihiro Nagai [a,b], Kazuo Tsubota [b]

[a] Laboratory of Retinal Cell Biology, Keio University School of Medicine, Shinjuku-ku, Tokyo 160-8582, Japan
[b] Department of Ophthalmology, Keio University School of Medicine, Shinjuku-ku, Tokyo 160-8582, Japan

ARTICLE INFO

Article history:
Received 4 December 2013
Received in revised form
6 March 2014
Accepted 16 March 2014
Available online 22 March 2014

Keywords:
Light
Retina
Apoptosis
Angiotensin II type 1 receptor
Oxidative stress

ABSTRACT

Exposure to light contributes to the development and progression of retinal degenerative diseases. However, the mechanisms underlying light-induced tissue damage are not fully understood. Here, we examined the role of angiotensin II type 1 receptor (AT1R) signaling, which is part of the renin–angiotensin system, in light-induced retinal damage. Light-exposed Balb/c mice that were treated with the AT1R blockers (angiotensin II receptor blockers; ARBs) valsartan, losartan, and candesartan before and after the light exposure exhibited attenuated visual function impairment, compared to vehicle-treated mice. This effect was dose-dependent and observed across the ARB class of inhibitors. Further evaluation of valsartan showed that it suppressed a number of light-induced retinal effects, including thinning of the photoreceptor cell layer caused by apoptosis, shortening of the photoreceptor cell outer segment, and increased levels of reactive oxygen species (ROS). The role of ROS in retinal pathogenesis was investigated further using the antioxidant *N*-acetyl-L-cysteine (NAC). Treatment of light-exposed mice with NAC before the light exposure suppressed the visual function impairment and photoreceptor cell histological changes due to apoptosis. Moreover, treatment with valsartan or NAC suppressed the induction of *c-fos* (a component of the AP-1 transcription factor) and the upregulation of *fasl* (a proapoptotic molecule whose transcript is regulated downstream of AP-1). Our results suggest that AT1R signaling mediates light-induced apoptosis, by increasing the levels of ROS and proapoptotic molecules in the retina. Thus, AT1R blockade may represent a new therapeutic approach for preventing light-induced retinal neural tissue damage.

Light is indispensable for vision; however, it may also accelerate age-related diseases resulting in visual impairment. In fact, light exposure is a reported risk factor associated with the progression of age-related macular degeneration [1] and retinitis pigmentosa [2], major causes of blindness. Recent reports have demonstrated some of the pathological effects of light exposure on the development of these diseases [3,4]. However, many of the underlying molecular mechanisms of light-induced tissue damage remain elusive and are not fully understood.

The pathogenic mechanisms associated with acute exposure to excessive light that result in photoreceptor cell apoptosis and retinal damage have been extensively studied [2,5–7]. The process is initiated by the enhanced metabolism of rhodopsin, a visual pigment, in response to excessive light exposure [8]; the resulting excessive activation of the visual cycle, in which the light-transformed rhodopsin chromophore is enzymatically converted to 11-*cis*-retinal, induces photoreceptor cell apoptosis [8]. Genetically induced rhodopsin deficiency [9] or inhibition of the visual cycle by in vivo treatment with 13-*cis*-retinoic acid can block the apoptosis and retinal damage [10]. It was also shown that c-Fos, a component of the transcription factor activator protein-1 (AP-1)[1], is required for the photoreceptor cell loss, as shown by the lack of retinal photodamage in *c-fos*-knockout mice [11,12]. Light-induced retinal damage also involves DNA fragmentation [13–15], calcium influx [16], and mitochondrial dysfunction [12,17]. These events can all be inhibited by decreasing the light that reaches the retina with light-blocking glasses [18] or intraocular lenses [19,20]. We are interested in identifying complementary protective approaches that can be used in combination with light-blocking materials.

Here, we focused on the renin–angiotensin system (RAS), which is activated in response to environmental stimuli and is characterized as a stress response system [21]. Angiotensin II type 1 receptor (AT1R) signaling is well known for causing tissue damage [22], as shown in the cardiovascular system [23,24] and kidneys [25,26], as well as in the retina under inflammatory conditions [27–32]. AT1R is a

Abbreviations: AP-1, activator protein-1; RAS, renin–angiotensin system; AT1R, angiotensin II type 1 receptor; ARB, angiotensin II receptor blocker; ROS, reactive oxygen species; NAC, *N*-acetyl-l-cysteine

* Corresponding author at: Keio University School of Medicine, Department of Ophthalmology, Laboratory of Retinal Cell Biology, 35 Shinanomachi, Shinjuku-ku, Tokyo, 160-8582 Japan. Fax: +81 3 3359 8302.
E-mail address: ozawa@a5.keio.jp (Y. Ozawa).

http://dx.doi.org/10.1016/j.freeradbiomed.2014.03.020

seven-transmembrane, G-protein-coupled receptor that induces various intracellular signaling cascades [33]. We hypothesized that AT1R signaling may be involved in mediating light-induced tissue damage.

AT1R blockers (angiotensin II receptor blockers; ARBs) such as valsartan, losartan, and candesartan, all of which bind to the AT1R to suppress its signal, are standard treatments for hypertension. Recent reports show that ARBs have tissue-protective effects in addition to reducing blood pressure [30,32,34–36]. ARB-targeted diseases are induced by oxidative stress [37–40], similar to light-induced retinal degeneration [15,41].

In this study, we analyzed whether the light-induced ROS accumulation in the retina and resulting visual function impairment are mediated by AT1R signaling, focusing on the molecular mechanisms underlying light-induced photoreceptor cell apoptosis.

Materials and methods

Animals

Six-week-old Balb/c male mice were purchased (CLEA Japan, Tokyo, Japan) and housed in an air-conditioned room maintained at 22 ± 2 °C under a 12-h dark/light cycle (light on from 0800 to 2000 hours), with free access to a standard diet (CLEA Japan) and tap water. All animal experiments were conducted in accordance with the Association for Research in Vision and Ophthalmology *Statement for the Use of Animals in Ophthalmic and Vision Research* and the guidelines of the Animal Care Committee of Keio University (Approval No. 08002).

Light exposure

The light-exposure experiments were performed as described previously [15,42]. Briefly, before light exposure, the 6- to 7-week-old mice were allowed to rest for several days and were then dark-adapted by maintaining them in complete darkness for 12 h. Their pupils were dilated with a mixture of 0.5% tropicamide and 0.5% phenylephrine (Mydrin-P; Santen Pharmaceutical, Osaka, Japan) just before exposure to light. The mice were then exposed to 5000 lux from a white fluorescence lamp (FHD100ECW, Panasonic, Osaka, Japan) for 1 h (starting at 0900 hours), in a dedicated exposure box maintained at 22 ± 2 °C, containing stainless steel mirrors on each wall and on the floor (Tinker N, Kyoto, Japan). After light exposure, the mice were returned to their cages and maintained under dim cyclic light (5 lux, 12 h on/off) until they were euthanized at the time of sampling at various time points, according to each experimental protocol. Control mice were also maintained under dim cyclic light and euthanized at the time of sampling.

Administration of ARBs or N-acetyl-L-cysteine (NAC)

Valsartan (LKT Laboratories, St. Paul, MN, USA) and candesartan (Sigma–Aldrich, St. Louis, MO, USA) were solubilized in dimethyl sulfoxide (Sigma–Aldrich), followed by dilution with phosphate-buffered saline (PBS). Losartan (Sigma–Aldrich) was solubilized in distilled water, followed by dilution with PBS. Mice were separated into treatment groups and intraperitoneally injected with either ARB (valsartan, 5, 20, 200 mg/kg; candesartan, 0.5, 2, 20 mg/kg; losartan, 3.125, 12.5, 125 mg/kg) or vehicle, twice, just before and after light exposure. The doses were adjusted to deliver equally potent doses of the ARBs [43].

For experiments with the antioxidant, NAC (Nakalai Tesque, Kyoto, Japan), mice were separated into treatment groups and intraperitoneally injected with either NAC diluted with PBS (250 or 500 mg/kg) or vehicle, twice, before dark adaptation and before light exposure.

Electroretinogram (ERG)

The ERG was performed as previously described [44–46]. Briefly, mice were dark-adapted for at least 12 h, maintained under dim red illumination, and then anesthetized with 70 mg/kg pentobarbital sodium (Dainippon Sumitomo Pharmaceutical, Osaka, Japan). The mice were placed on a heating pad throughout the experiment. The pupils were dilated with one drop of a mixture of 0.5% tropicamide and 0.5% phenylephrine (Santen Pharmaceutical). The ground electrode was placed on the tail, and the reference electrode was placed in the mouth. Active electrodes were gold wires placed on the cornea. Electrical responses were recorded (PowerLab System 2/25; AD Instruments, NSW, Australia) and differentially amplified and filtered through a digital bandpass filter ranging from 0.313 to 1000 Hz to yield a- and b-waves. Light pulses of 3.20 cd-s/m^2 and 4-ms duration were delivered through a commercial stimulator (Ganzfeld System SG-2002; LKC Technologies, Gaithersburg, MD, USA). Electrode impedance was checked before and after each measurement in all animals using a built-in feature of the instrument. Implicit times of the a- and b-waves were measured from the onset of the stimulus to the peak of each wave. The amplitude of the a-wave was measured from the baseline to the trough of the a-wave, and the amplitude of the b-wave was from the trough of the a-wave to the peak of the b-wave. Each treatment group consisted of six animals, and all groups were analyzed 6 days after light exposure, the time point at which clear changes in ERG responses were previously found to occur [15,42].

Measurement of outer nuclear layer (ONL) thickness and outer segment (OS) length of the retina

The eyes were enucleated and immediately frozen in Tissue-Tek OCT compound (Sakura Finetek Japan, Tokyo, Japan). Unfixed cryosections (6 μm thick) that included the optic nerve head were fixed in 4% paraformaldehyde (PFA) and stained with hematoxylin and eosin (H-E). The ONL thickness was measured at each point from the optic nerve head to the most peripheral region of the retina using the ImageJ program (developed by Wayne Rasband, National Institutes of Health, Bethesda, MD, USA; available at http://rsb.info.nih.gov/ij/index.html), and the average values were calculated. The OS length was measured 750 μm from the optic nerve in the posterior upper retina using ImageJ. All of the sections were examined under a microscope equipped with a digital camera (Biorevo BZ-9000, Keyence, Osaka, Japan). Each group consisted of six animals, and all groups were analyzed 6 days after light exposure, the time point previously shown to exhibit a clear change in retinal morphology after light exposure [15,42].

TdT-mediated dUTP nick-end labeling

Mice were anesthetized with pentobarbital sodium (70 mg/kg). Subsequently, the eyes were enucleated and fixed in 4% PFA overnight at 4 °C. After fixation, the tissues were processed and embedded in Tissue-Tek OCT compound (Sakura Finetek Japan). Cryosections (6 μm thick) that included the optic nerve head were prepared, and the TdT-mediated dUTP nick-end labeling (TUNEL) assay was performed using the ApopTag red apoptosis detection kit (Millipore, Bedford, MA, USA) according to the manufacturer's protocol. Nuclei were stained with 10 μg/ml Hoechst bisbenzimide 33258 (Sigma–Aldrich). Fluorescence images were obtained using confocal fluorescence microscopy (FV 1000, Olympus, Tokyo, Japan), and TUNEL-positive cells in the ONL were counted. Each treatment group consisted of six animals and all groups were analyzed

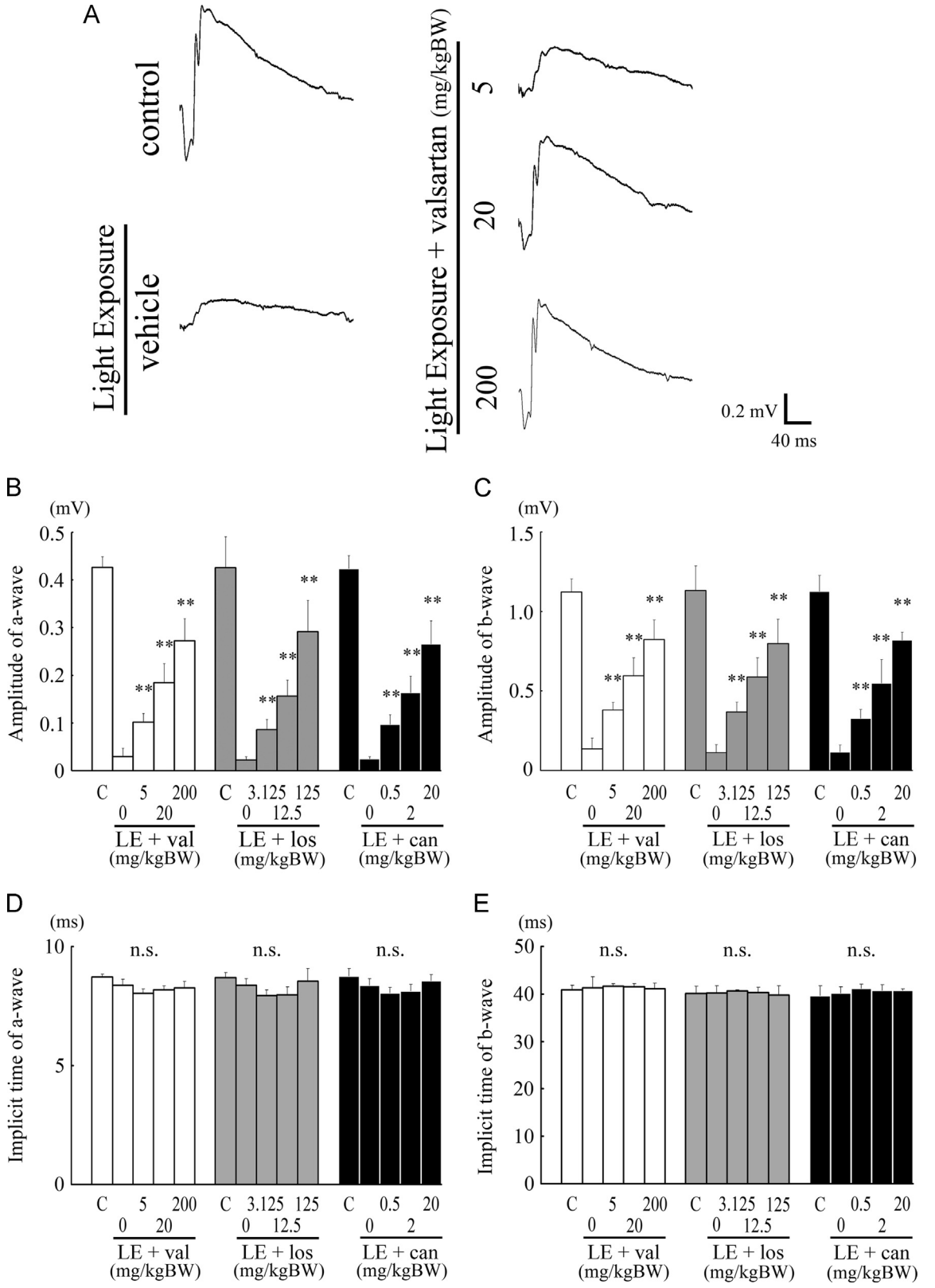

Fig. 1. Suppression of light-induced visual function impairment by ARBs. Analysis of full-field ERG after light exposure. (A) Representative wave forms of the ERG from an individual mouse treated with one of the ARBs, valsartan, in each dosage group in response to one flash. (B, C) Amplitudes of the a-wave and b-wave were decreased 6 days after light exposure, and these changes were suppressed by treatment with ARBs in a dose-dependent manner. (D, E) No differences were observed in the a-wave or b-wave implicit times. ERG, electroretinogram; ARBs, angiotensin II type 1 receptor blockers; C, control; LE, light exposure; val, valsartan; los, losartan; can, candesartan. $n = 6$ in each group. Statistical analysis was performed using one-way ANOVA with Tukey's post hoc test. $^{**}P < 0.01$, $^{*}P < 0.05$.

2 days after light exposure, the time point at which a clear change in photoreceptor cell number was previously detected [15,42].

Measurement of ROS

ROS measurement was performed as described previously [15,46]. Briefly, the eyes were enucleated and immediately frozen in Tissue-Tek OCT compound (Sakura Finetek Japan). Unfixed cryosections (6 μm thick) were incubated with 5 mM dihydroethidium (DHE; Life Technologies, Carlsbad, CA, USA) for 10 min at room temperature. DHE reacts with intracellular superoxide anion and is converted to the red fluorescent compound ethidium. Fluorescence images were obtained using confocal fluorescence microscopy (FV 1000, Olympus) using the equal exposure conditions among the groups; the staining intensity in the ONL was measured at two points in the posterior retina, which were vertically 250 μm away from the optic disc, using the ImageJ program, and averaged. Each treatment group consisted of six animals and all groups were analyzed 1 h after light exposure, the time point at which a clear increase in retinal ROS was previously observed [15].

Quantitative real-time RT-PCR

The eyes were enucleated and the retinas were placed in 100 μl of TRIzol reagent (Life Technologies) to extract the total RNA. Complementary DNA was generated by adding 1 μg of the total RNA to SuperScript VILO master mix (Life Technologies) and performing reverse transcription according to the manufacturer's instructions. PCR was performed using the StepOnePlus real-time PCR system (Life Technologies), and the $\Delta\Delta C_T$ method was used to quantify gene expression. The following gene-specific primers designed by TaqMan Gene Expression Assays (Life Technologies) were used: Mm00487425_m1 (*fos*), Mm00438864_m1 (*fasl*), and Cat. No. 4352339E (*gapdh*). The mRNA levels were normalized to that of *gapdh* mRNA. The data from 6 to 12 animals in each group, analyzed 1 (*fos*) or 6 h (*fasl*) after light exposure, are shown. The time points were selected based on results of preliminary experiments, in which the peak expression was observed at 1 (*fos*) and 6 h (*fasl*) after light exposure.

Statistical analysis

All results were expressed as means ± SD. The values were processed for statistical analyses (one-way ANOVA with Tukey's post hoc test) using SPSS Statistics 21 (IBM, Armonk, NY, USA) software, and differences were considered statistically significant at $P < 0.05$.

Results

Suppressive effect of ARBs on light-induced visual function impairment

To evaluate the protective effect of ARBs against the visual function impairment induced by light exposure, we examined the ERG recordings of mice treated with ARBs or vehicle just before and after exposure to light (Fig. 1A–E). In vehicle-treated mice the amplitudes of both the a-wave (Fig. 1B), which reflects photoreceptor function, and the b-wave (Fig. 1C), which represents subsequent neuronal signaling, were decreased 6 days after light exposure.

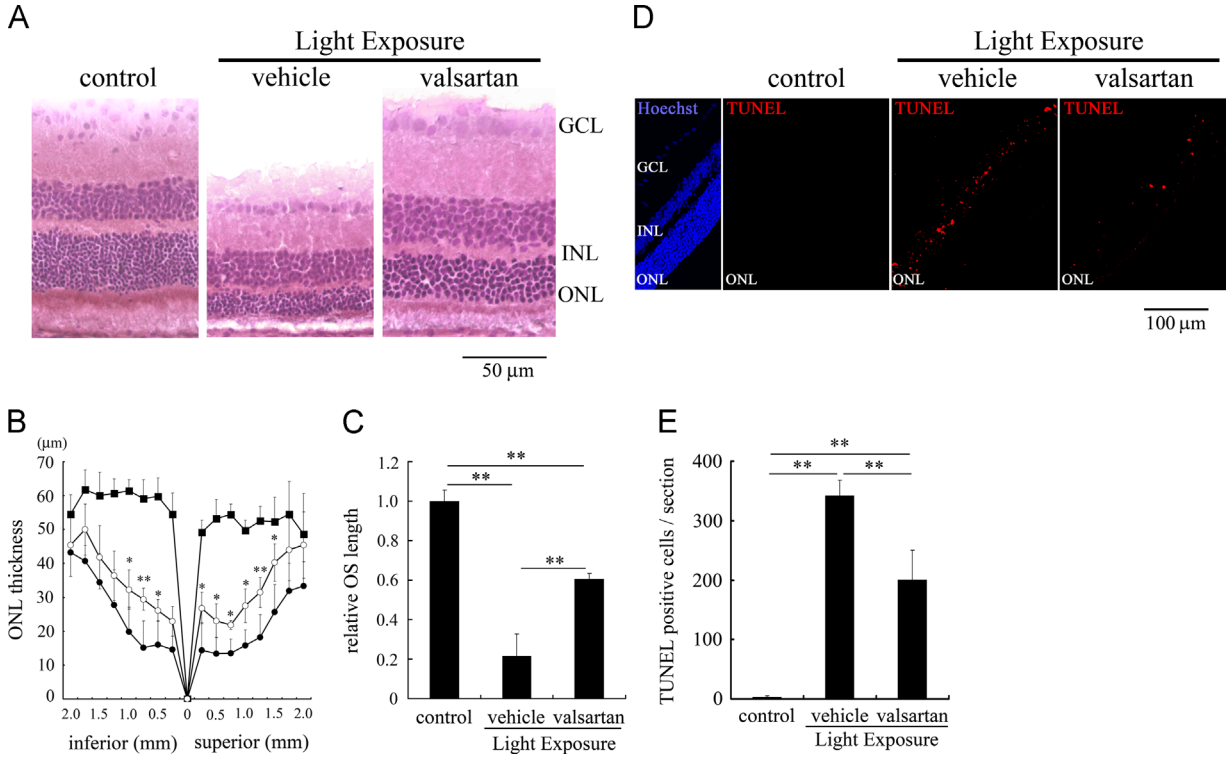

Fig. 2. Suppression of light-induced histological changes in the retina by valsartan. (A) H-E staining of retinal sections 6 days after light exposure. (B) The ONL thickness and (C) the OS length in the retina of light-exposed mice were reduced compared with those of untreated control mice. This reduction was significantly attenuated by valsartan administration (5 mg/kg). (D, E) TUNEL assay performed 2 days after light exposure. TUNEL-positive cells (red) appeared in the ONL after light exposure. These apoptotic cells were significantly reduced by valsartan administration (5 mg/kg). Hoechst staining of the control was shown as a guide for the retinal layers. GCL, ganglion cell layer; INL, inner nuclear layer; ONL, outer nuclear layer; OS, outer segment; ■, control mice with no light exposure; •, light-exposed mice treated with vehicle; ○, light-exposed mice treated with valsartan. $n = 6$ in each group. Statistical analysis was performed using one-way ANOVA with Tukey's post hoc test. $^{**}P < 0.01$, $^{*}P < 0.05$.

However, these decreases were attenuated by the administration of ARBs (valsartan, losartan, or candesartan), in a dose-dependent manner, indicating that this class of drugs was effective in suppressing the light-induced visual function impairment. Because 5 mg/kg valsartan was sufficient to produce a protective effect, the following experiments were performed using this dose of valsartan.

Suppressive effect of valsartan on light-induced photoreceptor changes

To elucidate the effect of ARBs on retinal histology, light-exposed mice were treated with either vehicle or valsartan just before and after light exposure, and retinal sections were prepared and analyzed by H-E staining (Fig. 2A). Retinal sections prepared from vehicle-treated mice 6 days after light exposure indicated that the thickness of the ONL that is composed of photoreceptor cells (Fig. 2B) and the length of the OS (Fig. 2C) were all decreased. However, these changes were significantly suppressed by valsartan treatment. There were no obvious changes in the other retinal layers.

Next, we performed TUNEL assays to determine whether the retinal thinning was mediated by apoptosis. TUNEL-positive apoptotic cells increased in the retina 2 days after light exposure, and there were significantly fewer TUNEL-positive cells in the retinas of mice in the valsartan-treated group compared with the vehicle group (Fig. 2D and E). This result was consistent with valsartan's suppression of light-induced ONL thinning shown above.

Inhibitory effect of valsartan on light-induced ROS accumulation

We next analyzed the effects of valsartan on light-induced ROS levels in the ONL by measuring the fluorescence intensity of DHE, which increases in the presence of ROS, mainly superoxide anions. Consistent with our previous reports [15,46], ROS levels in the ONL of the retina from control mice increased 1 h after light exposure. However, the ROS levels in the ONL from mice treated with valsartan just before and after light exposure were substantially suppressed (Fig. 3A and B).

Inhibitory effect of NAC on light-induced retinal damage

We next investigated whether oxidative stress was involved in promoting the light-induced retinal damage described above, using the antioxidant NAC. Interestingly, light-induced changes in ERG responses (Fig. 4A–E), histological characteristics (Fig. 4F–H), and photoreceptor cell apoptosis (Fig. 4I and J) were all suppressed by pretreatment with NAC. Thus, we concluded that oxidative stress also plays a role in the light-induced retinal damage mediated by AT1R.

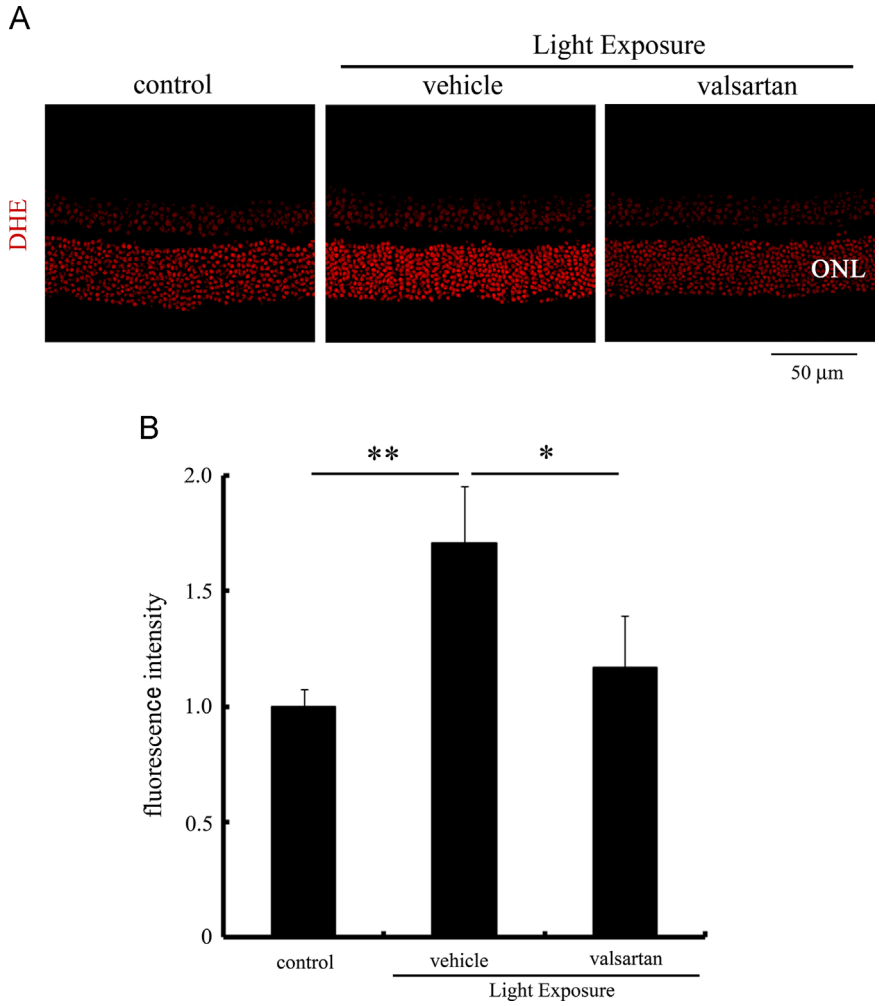

Fig. 3. Inhibition of light-induced ROS accumulation by treatment with valsartan. Detection of ROS by DHE staining 1 h after light exposure. (A, B) The fluorescence intensity of DHE in the ONL measured by ImageJ was increased after light exposure. The light-induced increase in ROS levels was prevented by treatment with valsartan (5 mg/kg). ONL, outer nuclear layer. $n = 6$ in each group. Statistical analysis was performed using one-way ANOVA with Tukey's post hoc test. $^{**}P < 0.01$, $^{*}P < 0.05$.

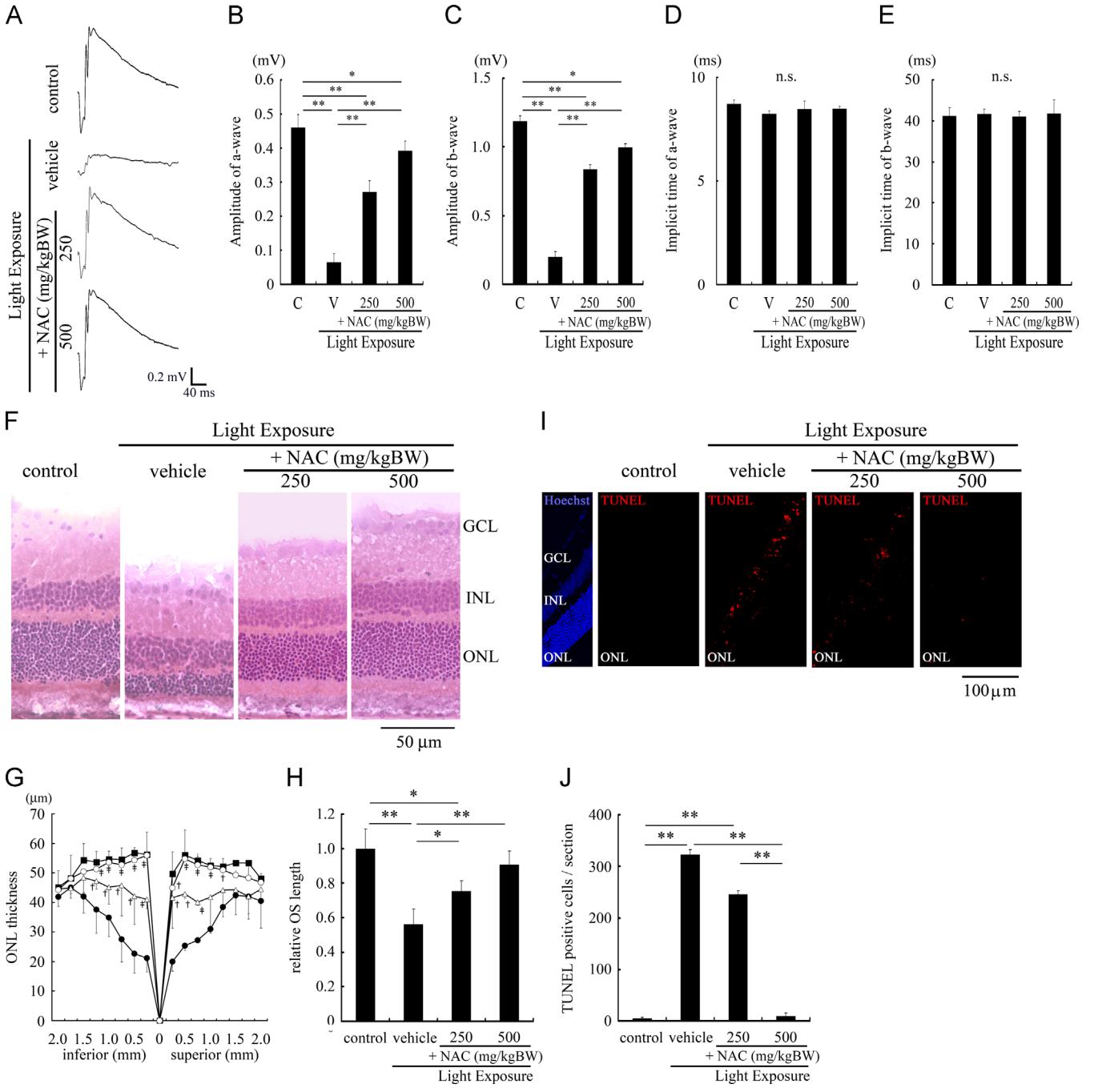

Fig. 4. Suppression of light-induced retinal damage by treatment with NAC. (A–E) Analysis of full-field ERG 6 days after light exposure. (A) Representative wave forms of the ERG from an individual mouse in each group in response to one flash. (B, C) Amplitudes of the a-wave and b-wave were decreased after light exposure, and these changes were suppressed by treatment with NAC in a dose-dependent manner. (D, E) No differences were observed in the a-wave or b-wave implicit times. (F) H-E staining of the retina 6 days after light exposure. The reductions in (G) ONL thickness and (H) OS length in the retina of light-exposed mice were significantly attenuated by NAC administration, in a dose-dependent manner. (I, J) TUNEL assay performed 2 days after light exposure. Light-induced TUNEL-positive cells (red) were significantly reduced by NAC administration. Hoechst staining of the control is shown as a guide for the retinal layers. GCL, ganglion cell layer; INL, inner nuclear layer; ONL, outer nuclear layer; OS, outer segment; C, control; V, vehicle; ■, control mice with no light exposure; ●, light-exposed mice treated with vehicle; Δ, light-exposed mice treated with NAC at 250 mg/kg; ○, light-exposed mice treated with NAC at 500 mg/kg. $n = 6$ in each group. Statistical analysis was performed using one-way ANOVA with Tukey's post hoc test. $^{**}P < 0.01$, $^{*}P < 0.05$, all compared with the data from light-exposed mice treated with vehicle.

Suppressive effects of valsartan and NAC on molecular changes in the retina of light-exposed mice

We further analyzed the light-induced molecular changes in the retina. In these analyses also, valsartan was administered just before and after light exposure, and NAC was before light exposure.

c-Fos, a component of the AP-1 transcription factor, is a major pathogenic factor involved in light-induced photoreceptor damage [11], and its expression in the retina increases after light exposure [18]. We therefore measured the *c-fos* mRNA level in the retinas of mice treated with vehicle, valsartan, or NAC 1 h after light exposure, when the ROS levels were significantly increased (Fig. 5A and B). Treatment with either valsartan or NAC suppressed the level of light-induced *c-fos* mRNA.

Then, we also examined the expression of *fasl*, an apoptotic factor and one of the genes regulated by AP-1 (Fig. 5C and D). The

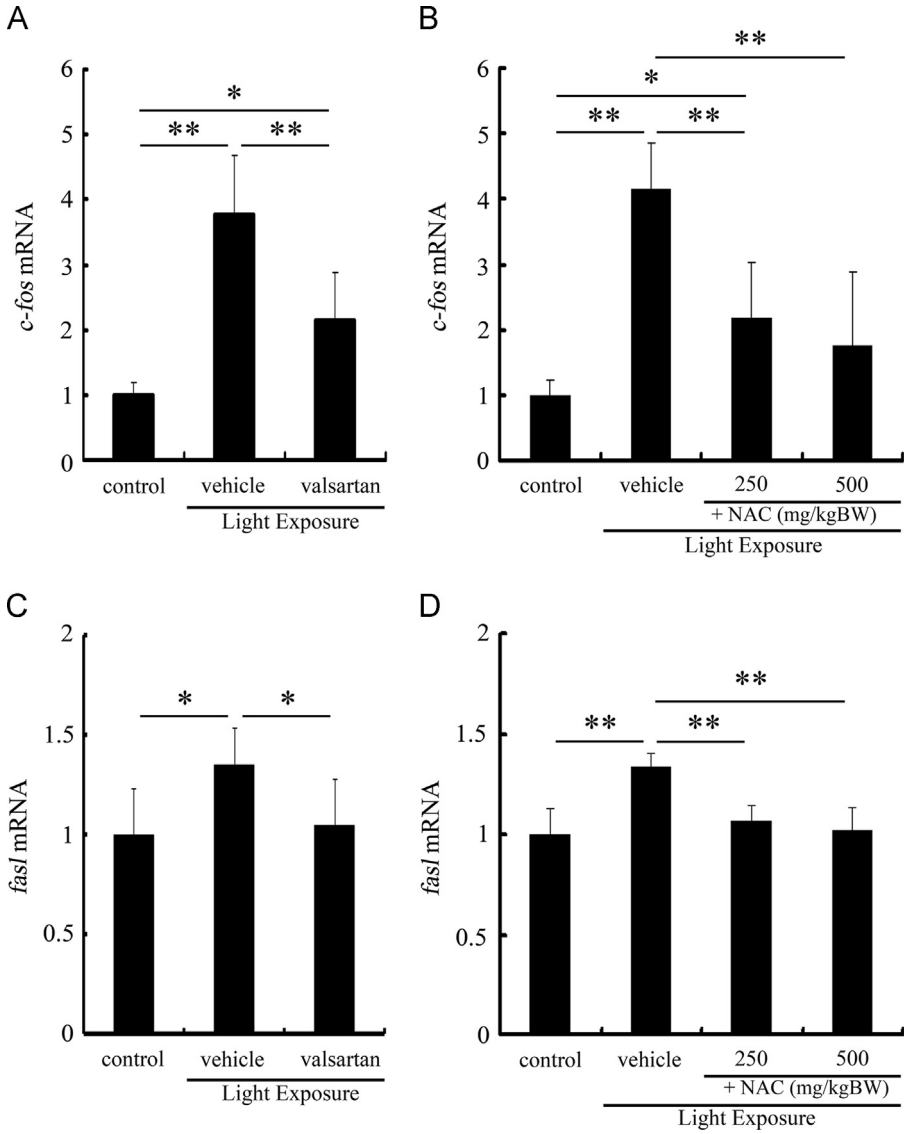

Fig. 5. Attenuation of light-induced molecular changes by treatment with either valsartan or NAC. (A, B) The mRNA level of *c-fos* measured by quantitative real-time RT-PCR, 1 h after light exposure, was significantly attenuated in the retinas of light-exposed mice treated with either (A) valsartan or (B) NAC, compared with vehicle-treated mice. (C, D) The increase in the *fasl* mRNA level 6 h after light exposure was significantly attenuated in the retinas of light-exposed mice treated with either (C) valsartan or (D) NAC, compared with vehicle-treated mice. Valsartan was administered at 5 mg/kg and NAC was at 250 or 500 mg/kg. The NAC-induced suppression was dose-dependent (B, D). NAC, *N*-acetyl-L-cysteine. $n = 6$. Statistical analysis was performed using one-way ANOVA with Tukey's post hoc test. $^{**}P < 0.01$, $^{*}P < 0.05$.

fasl mRNA level was elevated 6 h after light exposure in vehicle-treated mice, and this increase was attenuated by treatment with either valsartan or NAC. Taken together, these findings indicated that apoptosis-associated molecules were indeed induced in the retina after light exposure in vivo and that these inductions were mediated by AT1R and oxidative stress signaling.

Discussion

In this study we demonstrated that light-induced visual impairment was suppressed by the ARB class of drugs administered just before and after light exposure, in a dose-dependent manner (Fig. 1). Treatment of mice with one of the ARBs, valsartan, resulted in attenuated photoreceptor cell apoptosis and degeneration, leading to reduced thinning of the photoreceptor cell layer (Fig. 2). Valsartan treatment also prevented the light-induced accumulation of ROS in the retina (Fig. 3). The suppression of ROS induction by NAC treatment before light exposure was effective at preventing the light-induced visual function impairment and the associated histological changes in photoreceptor cells, in a dose-dependent manner (Fig. 4). Light-induced increases in the expression of *c-fos,* a known pathogenic molecule involved in light-induced retinal damage, and *fasl,* a proapoptotic molecule, were also attenuated by treatment with either valsartan or NAC.

AT1R signaling is known to play a pathogenic role in hypertension [47,48], atherosclerosis [49], and renal disorders [50,51] and in promoting focal inflammatory diseases of the retina [27–32,52–54]. In addition, the AT1R is well known to promote proinflammatory signaling, which can induce inflammatory cytokine production and/or recruit inflammatory cells [55]. Here we report that light-induced neural tissue damage also involves AT1R signaling, suggesting that photodamage may be due at least in part to AT1R-mediated proinflammatory signaling.

All ARBs act as antagonists of the AT1R, but because they are artificial products, it has been suggested that some of them may bind to other receptors, such as CCR2B [56] and PPARγ [57,58]. In addition, some ARBs are known to function as inverse agonists, meaning they can suppress the constitutive agonist-independent activity of AT1Rs [59]. These are known as molecule-specific effects, whereas the

AT1R-antagonizing effect is known as an ARB class-specific effect. In this study, three different ARBs all exhibited dose-dependent effects in preventing visual function impairment. Therefore, we conclude that these are class-specific effects of the ARBs.

The protective effects of the ARBs against light-induced visual function impairment, such as the suppression of the reduced a-wave amplitudes in the ERG, were consistent with their effects on the histological changes in the photoreceptor cells. Light-induced thinning of the ONL and the photoreceptor cell layer was found to result from photoreceptor cell apoptosis and shortening of the OS, which contains the photosensitive discs, consistent with previous reports [6,18]. These findings indicated that even the surviving photoreceptor cells were affected and showed signs of degeneration. All of these photoreceptor changes were attenuated by ARB treatment, indicating that AT1R blockade promoted photoreceptor survival and maintained cell health after light stimulation. Fewer changes were observed in the inner retina after light exposure, suggesting that the reduction in b-wave amplitude may reflect the reduced electrical activity of the downstream network of photoreceptor cells.

The induction of ROS in photoreceptor cells after light exposure was also consistent with previous reports [15,60]. Because of the extremely high oxygen gradient extending from the choroid to the inner segment of the photoreceptors [11,60], it has been suggested that the oxygen-consuming mitochondria in the inner segments play a primary role in oxidative stress reactions of the outer retina [12,60,61]. There are some reports suggesting that AT1R signaling can stimulate mitochondrial oxidant release leading to energy metabolism depression in renal and cardiovascular diseases [55,58], suggesting that ARBs may function in part by suppressing this pathway. Alternatively, a recent report suggests that nicotinamide adenine dinucleotide phosphate oxidase (Nox) enzymes contribute to ROS generation in photoreceptor cells [60,62]. The AT1R-induced ROS accumulation is reported to occur through the action of Nox in vascular endothelial cells [63] and cardiac fibroblasts [64]. Our findings indicating that the ROS induction in response to light is regulated by AT1R signaling raise the possibility that the Nox pathway is involved as well.

An increase in AP-1's DNA-binding activity has been shown to be a key event in regulating retinal photodamage [65]. The induction of the mRNA for c-Fos, an AP-1 component, after light exposure was also previously reported [18], consistent with results of the current study. Upregulated c-Fos expression may also contribute to dysregulated gene expression through AP-1. In our system the light-induced *c-fos* upregulation was attenuated by treatment with either an ARB or NAC, suggesting that these treatments may have suppressed c-Fos-mediated dysregulated gene expression that contributes to light-induced retinal damage. This idea is supported by the increased mRNA levels of *fasl*, which was also found to be regulated downstream of both signaling pathways.

Photoaging involves the accumulated effects of daily light exposure on tissues and organs over time [15,66,67]. In this study, we showed that AT1R signaling was activated in the retina in response to light stimulation. Because the pathogenesis of a number of age-related diseases, such as hypertension and diabetes, involves the dysregulation of the RAS and its downstream signaling pathways [33], the results of this study may lead to further investigation on the involvement of RAS activation in tissue aging caused by light exposure.

The limitations of this study include the fact that the model was generated by an acute and intense light exposure, which humans would not experience in daily life; therefore, the study may not absolutely mimic the influence of light exposure in humans. Mice have no macula in the retina, where photoreceptor cells are concentrated, in contrast to humans; thus, the phenotype of the light-exposed mice may not completely reflect the damage in humans. In addition, the doses of ARBs and NAC used in this study (the lowest doses per body weight used in this study were approximately four or five times more than ordinary doses of human use for treating hypertension by ARBs and for detoxification by NAC) may not be simply converted to estimate the effects in humans, because there would be differences in the pharmacokinetics between humans and mice, and the effect may not be equal when using the same doses. Studies on therapeutic approaches for light-induced retinal damage have focused largely on treatment with neurotrophic factors, such as erythropoietin [68], FGF [69], CNTF [70], and LIF [71], in addition to light-blocking mechanisms [18–20]. In the current study, we prevented light-induced retinal damage by suppressing dysregulated cell signaling. This approach may show promise as a stand-alone therapy or as an additional therapy for use in combination with trophic factors or with light-blocking glasses and/or intraocular lenses, which are currently used for cataract treatment.

Conclusion

We found that in vivo AT1R blockade in mice was an effective therapeutic approach for protecting photoreceptor neurons from the effects of light-induced cellular damage. ARB treatment suppressed the elevation of ROS levels, resulting in reduced proapoptotic signaling and increased survival of photoreceptor cells.

Acknowledgment

We appreciate Dr. Toshihide Kurihara for technical guidance and Ms. Eriko Toda for technical assistance. This work was supported by a Grant JSPS KAKENHI 2459647.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.freeradbiomed.2014.03.020.

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