Effect of Phosphorylated-Extracellular Regulated Kinase 1/2 Inhibitor on Retina from Light-induced Photoreceptor Degeneration

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Abstract

Background: The demonstrated role of mitogen-activated protein kinase (MAPK) in both cell apoptosis and the inflammation pathway makes it an attractive target for photoreceptor protection. The aim of this study was to investigate the protective effects of MAPK antagonists against photoreceptor degeneration and retinal inflammation in a rat model of light-induced retinal degeneration.

Methods: Sprague Dawley rats were treated with intravitreal injections of MAPK antagonists, inhibitors of p-P38, phosphorylated-extracellular regulated kinase (p-ERK) 1/2, and p-c-Jun N-terminal kinase (JNK) just before they were assigned to dark adaptation. After dark adaptation for 24 h, rats were exposed to blue light (2500 lux) in a light box for 24 h, and then returned to the normal 12-h light/12-h dark cycle. Samples were collected at different time points. MAPK expression during light exposure was examined with immunofluorescence. Photoreceptor death was detected with histopathology and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining. The expression of retinal p-ERK1/2, caspase 3, activated caspase 3, tumor necrosis factor (TNF)-α, and interleukin (IL)-1β was examined by Western blotting. Differences between groups were evaluated using unpaired one-way analysis of variance and least significant difference *post hoc* tests.

Results: MAPKs (P38, ERK1/2, and p-JNK) were phosphorylated and activated in the light injury groups, compared with normal group, and their expressions were mainly elevated in the outer nuclear layer (ONL). Among the selected MAPK antagonists, only the p-ERK1/2 inhibitor attenuated the loss of photoreceptors and the thinning of ONL in light injury groups. Besides, p-ERK1/2 inhibitor refrained light-induced photoreceptor apoptosis, which was presented by TUNEL positive cells. Light injury significantly increased the expression of p-ERK1/2 (1.12 ± 0.06 vs. 0.57 ± 0.08, $t = 9.99$, $P < 0.05$; 1.23 ± 0.03 vs. 0.57 ± 0.08, $t = 11.90$, $P < 0.05$; and 1.12 ± 0.12 vs. 0.57 ± 0.08, $t = 9.86$, $P < 0.05$; $F = 49.55$, $P < 0.001$), and induced caspase 3 activating (0.63 ± 0.06 vs. 0.14 ± 0.05, $t = 13.67$, $P < 0.05$; 0.74 ± 0.05 vs. 0.14 ± 0.05, $t = 16.87$, $P < 0.05$; and 0.80 ± 0.05 vs. 0.14 ± 0.05, $t = 18.57$, $P < 0.05$; $F = 100.15$, $P < 0.001$), compared with normal group. The p-ERK1/2 inhibitor significantly reduced p-ERK1/2 overexpression (0.61 ± 0.06 vs. 1.12 ± 0.06, $t = -9.26$, $P < 0.05$; 0.77 ± 0.06 vs. 1.23 ± 0.03, $t = -8.29$, $P < 0.05$; and 0.68 ± 0.03 vs. 1.12 ± 0.12, $t = -7.83$, $P < 0.05$; $F = 49.55$, $P < 0.001$) and downregulated caspase 3 activating (0.23 ± 0.04 vs. 0.63 ± 0.06, $t = -11.24$, $P < 0.05$; 0.43 ± 0.03 vs. 0.74 ± 0.05, $t = -8.86$, $P < 0.05$; and 0.58 ± 0.03 vs. 0.80 ± 0.05, $t = -6.17$, $P < 0.05$; $F = 100.15$, $P < 0.001$), compared with light injury group. No significant change in the total level of caspase 3 was seen in different groups ($F = 0.56$, $P = 0.75$). As for inflammation, light injury significantly increased the expression of TNF-α (0.42 ± 0.04 vs. 0.25 ± 0.05, $t = 5.99$, $P < 0.05$; 0.65 ± 0.03 vs. 0.25 ± 0.05, $t = 14.87$, $P < 0.05$; and 0.86 ± 0.04 vs. 0.25 ± 0.05, $t = 22.58$, $P < 0.05$; $F = 160.27$, $P < 0.001$) and IL-1β (0.24 ± 0.01 vs. 0.19 ± 0.02, $t = 2.33$, $P < 0.05$; 0.35 ± 0.02 vs. 0.19 ± 0.02, $t = 7.97$, $P < 0.05$; and 0.48 ± 0.04 vs. 0.19 ± 0.02, $t = 14.69$, $P < 0.05$; $F = 77.29$, $P < 0.001$), compared with normal group. P-ERK1/2 inhibitor significantly decreased the overexpression of TNF-α (0.22 ± 0.02 vs. 0.42 ± 0.04, $t = -7.40$, $P < 0.05$; 0.27 ± 0.02 vs. 0.65 ± 0.03, $t = -14.27$, $P < 0.05$; and 0.33 ± 0.03 vs. 0.86 ± 0.04, $t = -19.58$, $P < 0.05$; $F = 160.27$, $P < 0.001$) and IL-1β (0.13 ± 0.03 vs. 0.24 ± 0.01, $t = -5.77$, $P < 0.05$; 0.17 ± 0.01 vs. 0.22 ± 0.02, $t = -9.18$, $P < 0.05$; and 0.76 ± 0.05 vs. 0.48 ± 0.04, $t = -13.12$, $P < 0.05$; $F = 77.29$, $P < 0.001$), compared with light injury group.

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DOI: 10.4103/0366-6999.246064

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Received: 09-10-2018 **Edited by:** Peng Lyu
How to cite this article: Ding XY, Gu RP, Tang WY, Shu QM, Xu GZ, Zhang M. Effect of Phosphorylated-Extracellular Regulated Kinase 1/2 Inhibitor on Retina from Light-induced Photoreceptor Degeneration. Chin Med J 2018;131:2836-43.

Conclusion: The p-ERK1/2 inhibitor might protect the retina from light-induced photoreceptor degeneration and retinal inflammation.

Key words: Apoptosis; Inflammation; Retinal Degeneration

Introduction

Photoreceptor degeneration is an important pathological process in degenerative retinal diseases such as age-related macular degeneration and retinitis pigmentosa.[1,2] Intense ultraviolet and blue light initiate photoreceptor damage and death.[3-5] In animal models of light-induced retinal degeneration, the death of the photoreceptor cells is predominantly caused by apoptosis.[6-8] Inflammatory chemokines are also increased and microglia are activated in the light-stressed retina,[9,10] and anti-inflammatory measures have been shown to reduce photoreceptor degeneration in the retina.[11,12]

The mitogen-activated protein kinases (MAPKs) are a group of serine-threonine protein kinases that can be activated by different extracellular stimuli including cytokines, neurotransmitters, hormones, and cell stress. Extracellular regulated kinase (ERK) 1/2 (MAPK1/3), P38 (MAPK14), c-Jun N-terminal kinase (JNK) (MAPK8), and ERK5 (MAPK7) are four components of MAPK pathways.[13] On activation, MAPKs phosphorylate and activate an array of transcription factors that regulate many important physiological and pathological processes in the cell including growth and differentiation, stress, adaptation to the environment, and the inflammatory response.[14-17]

Several studies have demonstrated the involvement of ERK1/2, P38, and JNK in inflammation and cell apoptosis. Phosphorylated-ERK1/2 (p-ERK1/2) promotes cell survival, and anti-inflammatory agents can protect cells from damage by activating the ERK1/2 pathway.[18-21] However, under certain conditions, p-ERK1/2 can exert proapoptotic effects, for example, activation of ERK1/2 in response to some DNA damage stimuli promotes cell apoptosis by enhancing activity of some pro-apoptotic signaling molecules.[20,22] Previous studies have shown that P38 MAPK is neuroprotective[23] and plays an important role in the induction of inflammatory cytokines and chemokines, such as interleukin 1 (IL-1), tumor necrosis factor (TNF), and IL-6, in different diseases, both *in vitro* and *in vivo*.[24-27] The activation of p-JNK might be involved in aggravated nerve cell apoptosis and brain damage and may induce retinal degeneration during light-induced retinal damage.[28,29]

The demonstrated roles of MAPKs in cellular apoptosis and the inflammatory pathway make them attractive therapeutic targets for photoreceptor protection. However, the roles of MAPKs in photoreceptor degeneration are not yet fully understood. Therefore, in this study, we tested the effects of activating ERK1/2, P38, and JNK in a model of light-induced retinal degeneration. We also used specific MAPK antagonists, inhibitors of p-P38 (SB203580), p-ERK1/2 (U0126), and p-JNK (SP600125), to investigate the effects of MAPKs on photoreceptor degeneration and inflammation during light exposure.

Methods

Animals

All procedures were approved by the Animal Ethics Committee of the Eye and ENT Hospital of Fudan University (Shanghai, China) and were conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Adult male Sprague Dawley (SD) rats (approximately 250 g, animal production license No. SCXK[Shanghai] 2012-0002) were maintained under a 12-h light/12-h dark cycle with free access to food and water. The rats were treated humanely and finally killed by cervical dislocation under anesthesia induced with ketamine (80 mg/kg) and xylazine (10 mg/kg).

Light exposure of Sprague Dawley rats

The SD rats assigned to the light exposure groups were placed individually in cages and their pupils dilated. After dark adaptation for 24 h, they were exposed to blue light (intensity of 2500 lux) in a light box for 24 h, and then returned to the normal 12-h light/12-h dark cycle. After that, rats were executed, and samples were collected at 1, 3, 5, and 7 days, respectively.

Drug injection

The rats were randomly divided into four groups: (1) p-p38 inhibitor (SB203580, Sigma-Aldrich, Billerica, MA, USA) injected light exposure group; (2) p-ERK1/2 inhibitor (U0126, Sigma-Aldrich, Billerica, MA, USA) injected light exposure group; (3) p-JNK inhibitor (SP600125, Sigma-Aldrich, Billerica, MA, USA) injected light exposure group; (4) light exposure group, and (5) nomal group. Each experimental group (including different time points) included six animals, and all experiments were performed at least three times to ensure reproducibility. The MAPK inhibitors were first dissolved in dimethyl sulfoxide (DMSO, Sigma-Aldrich, Billerica, MA, USA) and then diluted with phosphate-buffered saline (PBS, Beyotime, Shanghai, China). The final concentration of inhibitors was 0.1 mmol/L with a concentration of 5% DMSO. Two microliters of the inhibitor solution was injected into the vitreous with a 32G syringe just before the rats were assigned to dark adaptation.

Histopathology

The anesthetized rats were transcardially perfused with 0.9% saline. Their eyes were enucleated, fixed in 4% paraformaldehyde (PFA, Beyotime, Shanghai, China) for 24 h, and then dehydrated and embedded in paraffin.

The paraffin cubes were sectioned to 5 μm thickness, and the sections through the optic nerve were stained with hematoxylin and eosin (H & E, Beyotime, Shanghai, China). One slice was selected from each eye and recorded with a digital slice scanning system (Panoramic Scan; 3DHistech, Budapest, Hungary). Three defined regions in each slice were selected for measurement: two were located at points 450 μm away from the ora serrata and the optic nerve head, and the third was chosen at the midpoint between them. The thicknesses of the total retina, outer nuclear layer (ONL), and inner nuclear layer were determined with the Panoramic Viewer software (3DHistech Ltd., Budapest, Hungary).

Immunofluorescence

The anesthetized rats were transcardially perfused with 0.9% saline. The eyes were excavated and the anterior segments were removed. The eyecups were fixed in 4% PFA for 1 h and dehydrated in graded sucrose solutions (20–30%) overnight at 4°C. The eyecups were then embedded in optimal cutting temperature compound (Tissue-Tek; Ted Pella, Inc., Redding, CA, USA) and snap-frozen at −80°C until they were sectioned (10 μm). The frozen sections were stored in a −20°C freezer. The slices were fixed in 4% PFA for 30 min and washed three times with PBS. They were then treated with 0.5% Triton X-100 (Beyotime, Shanghai, China) for 15 min. The slices were blocked with 10% goat serum in PBS for 1 h at room temperature and then incubated with a primary antibody overnight at 4°C. Antibodies directed against the following proteins were used: p-P38 (1:100, Cat #4511, CST, Boston, MA, USA), p-JNK (1:100, Cat #4668, CST, Boston, MA, USA), or p-ERK1/2 (1:100, Cat #4370, CST, Boston, MA, USA). The slices were then washed three times with PBS, incubated with the corresponding secondary antibody (1:1000, Cat #A21428, Invitrogen, Carlsbad, CA, USA) for 1 h, and counterstained with 4′,6-diamidino-2-phenylindole (diluted 1:1000; Sigma-Aldrich, St. Louis, MO, USA). Finally, the slices were scanned with a laser confocal microscope (Leica Microsystems, Bensheim, Germany).

Terminal deoxynucleotidyl transferase dUTP nick end labeling

Frozen section of the retina were prepared as described previously. The terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL)-based *in situ* Cell Death Detection Kit, Fluorescein (Roche, Nutley, NJ, USA) was used to detect the apoptosis of the photoreceptor cells in the sections. Nine fields were chosen randomly from every retina with microscopy (Leica Microsystems). The number of TUNEL-positive cells was counted to determine the ratio of TUNEL-positive cells to the total number of cells.

Western blotting analysis

The rats were killed, and their retinas were homogenized with ultrasound. The protein concentrations were measured with the BCA Protein Assay Kit (Beyotime, Shanghai, China). Equal amounts of protein were then fractionated with sodium dodecyl sulfate- polyacrylamide gel electrophoresis (SDS-PAGE, Beyotime, Shanghai, China) and transferred to polyvinylidene difluoride membranes (Millipore, Billerica, MA, USA). The membranes were blocked with 5% nonfat milk in Tris-buffered saline containing Tween-20 for 1 h and then incubated with antibodies directed against the following proteins: p-ERK1/2 (1:100, Cat #4370, CST, Boston, MA, USA), TNF-α (1:1000, Cat #ab6671, Abcam, Cambridge, MA, USA), IL-1β (1:1000, Cat #ab9722, Abcam, Cambridge, MA, USA), caspase 3 (1:1000, Cat #ab13847, Abcam, Cambridge,

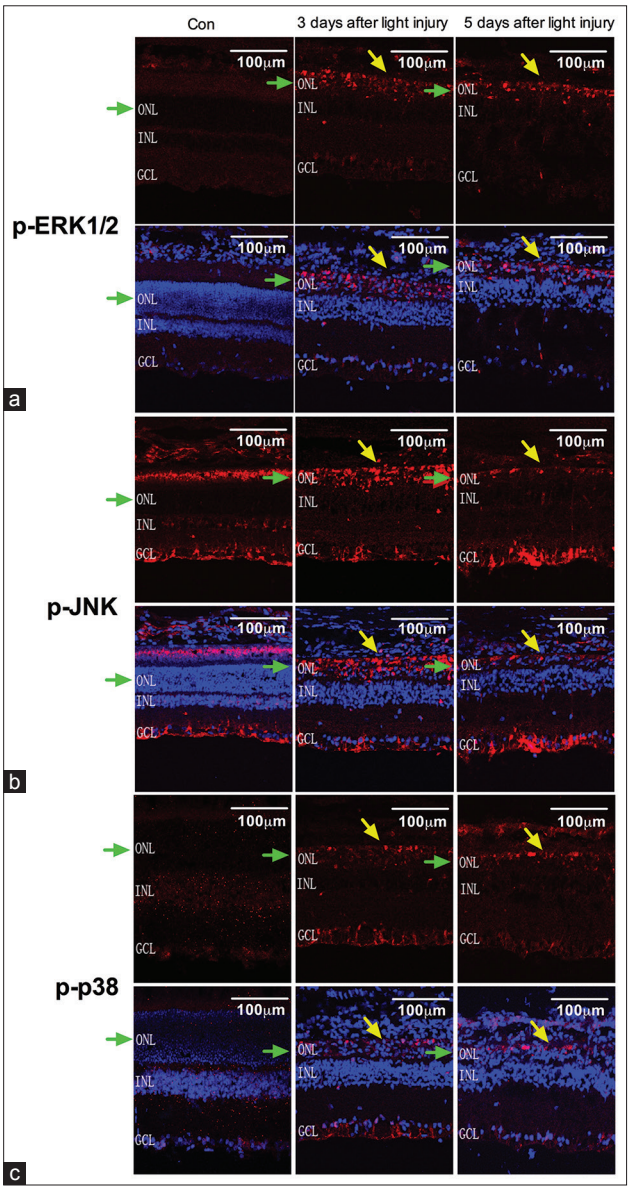

Figure 1: Light exposure increased the expression of p-MAPKs in the retina. MAPKs activation in retina, represented by the levels of p-ERK1/2, p-JNK and p-P38, were detected with immunofluorescence. The green arrows points to the ONL, and the yellow arrows points to the MAPKs enhancement area after light exposure. p-ERK1/2 (a), p-JNK (b) and p-P38 (c) were elevated in the rat model of light-induced retinal degeneration, mainly in the ONL, on days 3 and 5 when compared with their levels in the normal retina. MAPK: Mitogen-activated protein kinase; p-ERK1/2: Phosphorylated-extracellular regulated kinase 1/2; JNK: c-Jun N-terminal kinase; ONL: Outer nuclear layer; INL: Inner nuclear layer; GCL: Ganglion cell layer.

MA, USA), and activated caspase 3 (1:1000, Cat #ab2302, Abcam, Cambridge, MA, USA). After the membranes were washed three times, specific secondary antibodies (1:1000, Cat #A21428, Invitrogen, Carlsbad, CA, USA) were added to them. The labeled proteins were then visualized with enhanced chemiluminescence reagent (ECL, Amersham Pharmacia GE, Pittsburgh, PA, USA). Chemiluminescent images were captured, and the band intensities were analyzed with the Bio-Rad Quantity One software (Bio-Rad, Hercules, CA, USA). The relative levels of the target proteins were normalized to that of β-actin.

Statistical analysis

All statistical analyses were performed using GraphPad Prism (version 6.0, GraphPad, Inc., La Jolla, CA, USA). Measurement data were presented as mean ± standard deviation (SD). Differences between groups were evaluated using unpaired one-way analysis of variance (ANOVA) and least significant difference *post hoc* tests. Each experiment was repeated three times; A value of $P < 0.05$ was considered statistically significant.

Results

Light exposure increased the expression of p-mitogen-activated protein kinases in the retina

MAPK activation, represented by the levels of p-ERK1/2, p-JNK and p-P38, was detected with immunofluorescence. p-ERK1/2, p-JNK and p-P38 were elevated in the light injury

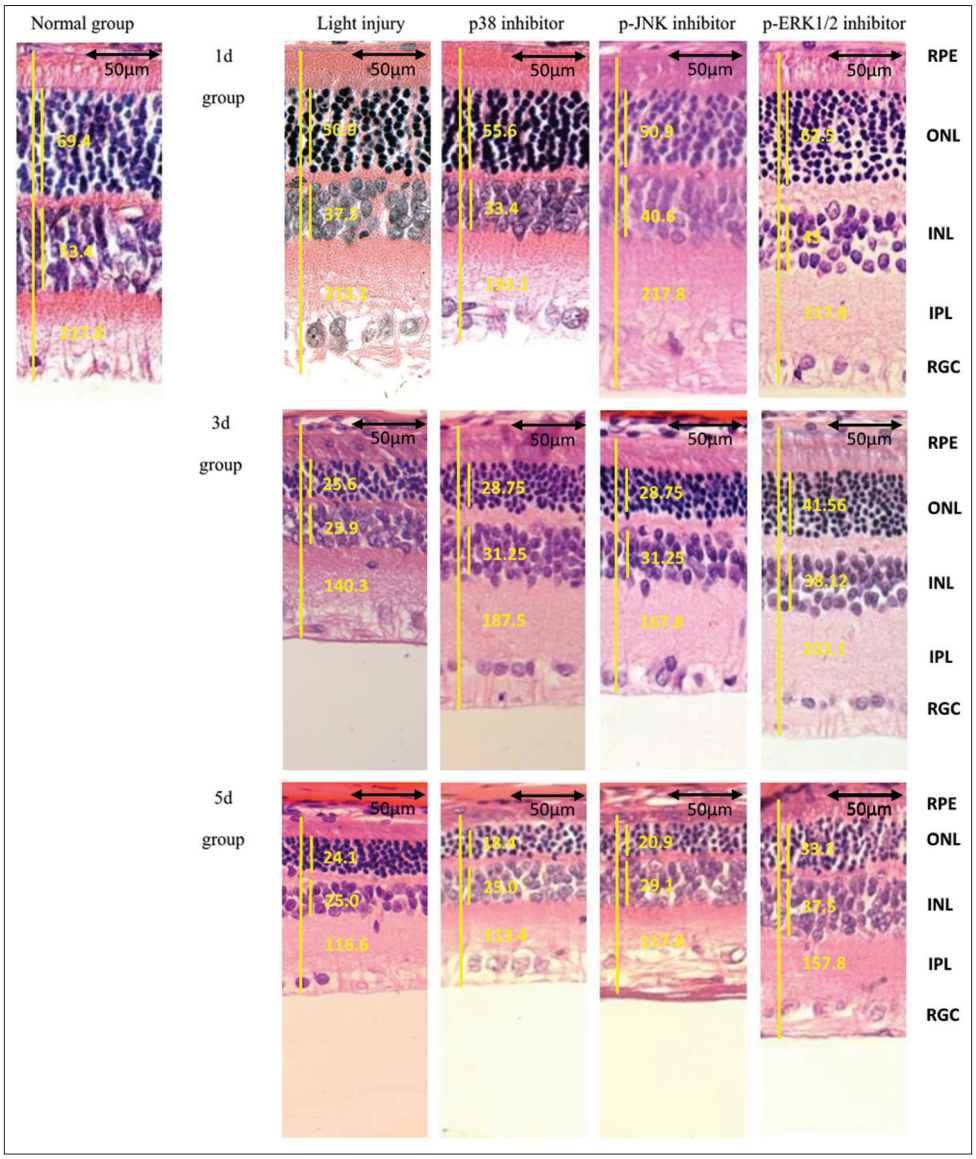

Figure 2: p-ERK1/2 inhibitor attenuated photoreceptor loss and the thinning of the ONL. Photoreceptor loss was detected by histopathology and H & E staining. After light exposure for 24 h, SD rats showed progressive photoreceptor loss and reduced the thicknesses of the ONL and the total retina. Among the selected MAPKs antagonists, only the inhibitor of p-ERK1/2 reduced photoreceptor loss and thinning of ONL compared to light exposure group. MAPK: Mitogen-activated protein kinase; p-ERK1/2: Phosphorylated-extracellular regulated kinase 1/2; SD: Sprague-Dawly; H & E: Hematoxylin and eosin; RPE: Retinal pigment epithelium; ONL: Outer nuclear layer; INL: Inner nuclear layer; GCL: Ganglion cell layer; IPL: Inner plexiform layer.

groups, mainly in the ONL, on day 3 and day 5 compared with their levels in normal group [Figure 1a-1c].

Phosphorylated-extracellular regulated kinase 1/2 inhibitor attenuated photoreceptor loss and the thinning of the outer nuclear layer

After light exposure for 24 h, the SD rats showed progressive photoreceptor loss and reduced thicknesses of the ONL and the total retina, compared with normal group, and detected with H and E staining [Figure 2].

When we used MAPK antagonists, inhibitors of p-P38 (SB203580), p-ERK1/2 (U0126), and p-JNK (SP600125), to treat the SD rats exposed to light, as shown in Figure 2, only the inhibitor of p-ERK1/2 reduced the photoreceptor loss and thinning of the ONL in light injury groups, compared with light injury groups at the same time.

Phosphorylated-extracellular regulated kinase 1/2 inhibitor refrained light-induced photoreceptor apoptosis

To confirm the protective effects of the p-ERK1/2 inhibitor against light-induced photoreceptor degeneration, we used a TUNEL assay and caspase 3 levels to evaluate photoreceptor apoptosis and the activation of the apoptotic signaling pathway. As shown in Figure 3, the green signal presents TUNEL-positive cells. Compared to normal group, TUNEL-positive cells were mainly observed in the ONL and peaked at 1 day, and then, the number of TUNEL-positive cells gradually decreased due to photoreceptor loss. After treatment with the p-ERK1/2 inhibitor, there was a significant reduction in TUNEL-positive cells on day 1, day 3, and day 5, compared with light injury groups at the same time. At 7 days after light exposure, nearly no TUNEL-positive cell can be observed while in the p-ERK1/2 inhibitor injected group, TUNEL-positive cells are still observable.

Phosphorylated-extracellular regulated kinase 1/2 inhibitor downregulated light-induced phosphorylated-extracellular regulated kinase 1/2 overexpression and caspase 3 activating

The level of p-ERK1/2 and caspase 3 expression in the retina was detected with Western blotting as shown in Figure 4a-4c. Light injury significantly increased the expression of p-ERK1/2 [1.12 ± 0.06 vs. 0.57 ± 0.08, $t = 9.99$, $P < 0.05$; 1.23 ± 0.03 vs. 0.57 ± 0.08, $t = 11.90$,

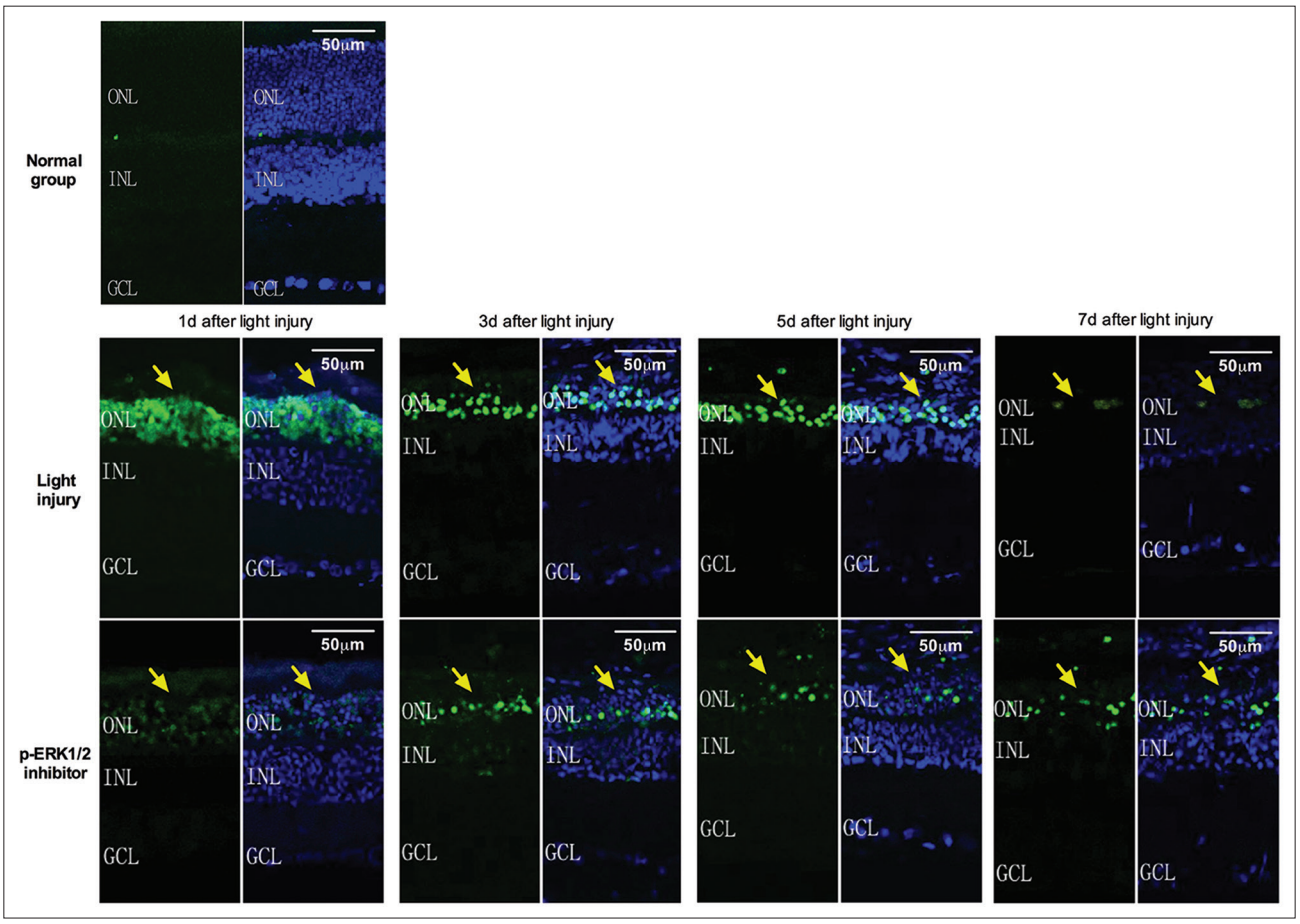

Figure 3: p-ERK1/2 inhibitor restricted light-induced photoreceptor apoptosis. We used a TUNEL assay to evaluate photoreceptor apoptosis. The green signal represents TUNEL-positive cells, which is pointed by the yellow arrows. TUNEL-positive cells were mainly observed in the ONL and peaked after 24 h. After treatment with the p-ERK1/2 inhibitor, TUNEL-positive cells were significantly reduced on days 1, 3, and 5. At 7 days after light exposure, almost no TUNEL-positive cell can be observed while in the p-ERK1/2 inhibitor injected group, TUNEL-positive cells are still observable. MAPK: Mitogen-activated protein kinase; p-ERK1/2: Phosphorylated-extracellular regulated kinase 1/2; ONL: Outer nuclear layer; TUNEL: Terminal deoxynucleotidyl transferase dUTP nick end labeling; INL: Inner nuclear layer; GCL: Ganglion cell layer.

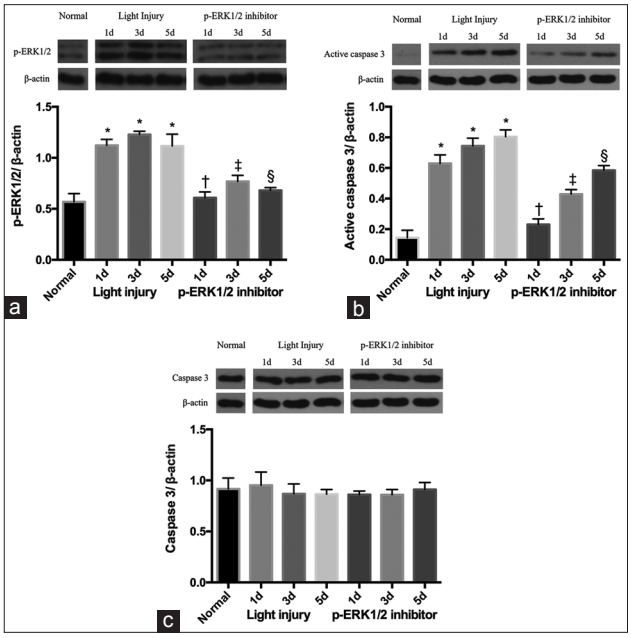

Figure 4: p-ERK1/2 inhibitor downregulated light-induced p-ERK1/2 overexpression and caspase 3 activating. The level of p-ERK1/2 and caspase 3 expression was detected with Western blotting. (a) Light injury significantly increased the expression of p-ERK1/2 compared with normal group. The p-ERK1/2 inhibitor significantly reduced p-ERK1/2 overexpression compared with light injury group. (b) Light injury significantly induced caspase 3 activating compared with normal group. The p-ERK1/2 inhibitor significantly downregulated caspase 3 activating compared with light injury group. (c) No significant change in the total level of caspase 3 was seen in different groups ($F = 0.56$, $P = 0.75$). *$P < 0.05$ versus normal group; †$P < 0.05$ versus light injury group in 1 day; ‡$P < 0.05$ versus light injury group in 3 days; §$P < 0.05$ versus light injury group in 5 days. p-ERK1/2: Phosphorylated-extracellular regulated kinase 1/2.

$P < 0.05$; and 1.12 ± 0.12 vs. 0.57 ± 0.08, $t = 9.86$, $P < 0.05$; $F = 49.55$, $P < 0.001$, Figure 4a] and induced caspase 3 activating [0.63 ± 0.06 vs. 0.14 ± 0.05, $t = 13.67$, $P < 0.05$; 0.74 ± 0.05 vs. 0.14 ± 0.05, $t = 16.87$, $P < 0.05$; and 0.80 ± 0.05 vs. 0.14 ± 0.05, $t = 18.57$, $P < 0.05$; $F = 100.15$, $P < 0.001$, Figure 4b] compared with normal group. The p-ERK1/2 inhibitor significantly reduced p-ERK1/2 overexpression in [0.61 ± 0.06 vs. 1.12 ± 0.06, $t = -9.26$, $P < 0.05$; 0.77 ± 0.06 vs. 1.23 ± 0.03, $t = -8.29$, $P < 0.05$; and 0.68 ± 0.03 vs. 1.12 ± 0.12, $t = -7.83$, $P < 0.05$; $F = 49.55$, $P < 0.001$, Figure 4a], and downregulated caspase 3 activating [0.23 ± 0.04 vs. 0.63 ± 0.06, $t = -11.24$, $P < 0.05$; 0.43 ± 0.03 vs. 0.74 ± 0.05, $t = -8.86$, $P < 0.05$; and 0.58 ± 0.03 vs. 0.80 ± 0.05, $t = -6.17$, $P < 0.05$; $F = 100.15$, $P < 0.001$, Figure 4b] compared with light injury group. No significant change in the total level of caspase 3 was seen in different groups [$F = 0.56$, $P = 0.75$; Figure 4c].

Phosphorylated-extracellular regulated kinase 1/2 inhibitor protects the retina from light-induced inflammation

The release of proinflammatory cytokine in the retina was detected with Western blotting for TNF-α and IL-1β. Light injury significantly increased the expression of TNF-α [0.42 ± 0.04 vs. 0.25 ± 0.05, $t = 5.99$, $P < 0.05$; 0.65 ± 0.03 vs. 0.25 ± 0.05, $t = 14.87$, $P < 0.05$; and 0.86 ± 0.04 vs. 0.25 ± 0.05, $t = 22.58$, $P < 0.05$; $F = 160.27$, $P < 0.001$, Figure 5a] and IL-1β [0.24 ± 0.01 vs. 0.19 ± 0.02, $t = 2.33$, $P < 0.05$; 0.35 ± 0.02 vs. 0.19 ± 0.02, $t = 7.97$, $P < 0.05$; and 0.48 ± 0.04 vs. 0.19 ± 0.02, $t = 14.69$, $P < 0.05$; $F = 77.29$, $P < 0.001$, Figure 5b] compared with normal group. P-ERK1/2 inhibitor significantly decreased the overexpression of TNF-α [0.22 ± 0.02 vs. 0.42 ± 0.04, $t = -7.40$, $P < 0.05$; 0.27 ± 0.02 vs. 0.65 ± 0.03, $t = -14.27$, $P < 0.05$; and 0.33 ± 0.03 vs. 0.86 ± 0.04, $t = -19.58$, $P < 0.05$; $F = 160.27$, $P < 0.001$, Figure 5a] and IL-1β [0.13 ± 0.03 vs. 0.24 ± 0.01, $t = -5.77$, $P < 0.05$; 0.17 ± 0.01 vs. 0.22 ± 0.02, $t = -9.18$, $P < 0.05$; and 0.76 ± 0.05 vs. 0.48 ± 0.04, $t = -13.12$, $P < 0.05$; $F = 77.29$, $P < 0.001$, Figure 5b] compared with light injury group.

Discussion

The exposure of the retina to excessive light causes photoreceptor cell death, retinal inflammation, and other degenerative changes.[5,30,31] The mechanisms of these phenomena and their potential treatment require further study. Due to MAPKs play important roles in cell apoptosis and tissue inflammation, we investigated whether MAPKs are involved in light-induced retinal damage and have potential utility in the treatment of this damage.

MAPKs are a group of serine-threonine protein kinases that can be activated by different extracellular stimuli and contribute to many important physiological and pathological processes in the cell.[14-17] ERK1/2, P38, JNK, and ERK5 are four members of the MAPK pathways. In previous studies, the expression of MAPK family members P38, JNK, and ERK1/2 was shown to be upregulated by exposure to light.[32,33] However, how they function in light-induced retinal damage is unclear. We first used immunofluorescence to examine the expression and location of MAPKs. We found that P38, ERK1/2, and JNK were phosphorylated and activated in a rat model of light-induced retinal degeneration and were mainly elevated in the ONL. Therefore, we assumed that the MAPK pathways participate in the processes underlying light-induced photoreceptor degeneration. Then, we used specific MAPK antagonists, i.e., inhibitors of p-P38 (SB203580), p-ERK1/2 (U0126), and p-JNK (SP600125), to investigate the effects of these MAPKs on photoreceptor degeneration and inflammation during light exposure.

In our previous study, we successfully established a model of photoreceptor apoptosis induced by light.[11,12] In this study, the results indicating that light injury induces progressive photoreceptor loss and reduced thicknesses of the ONL, are consistent with previous research findings [Figure 2]. Among the MAPK antagonists, only the p-ERK1/2 inhibitor attenuated the progressive photoreceptor loss and thinning of the ONL. Thus, we will use the p-ERK1/2 inhibitor in our

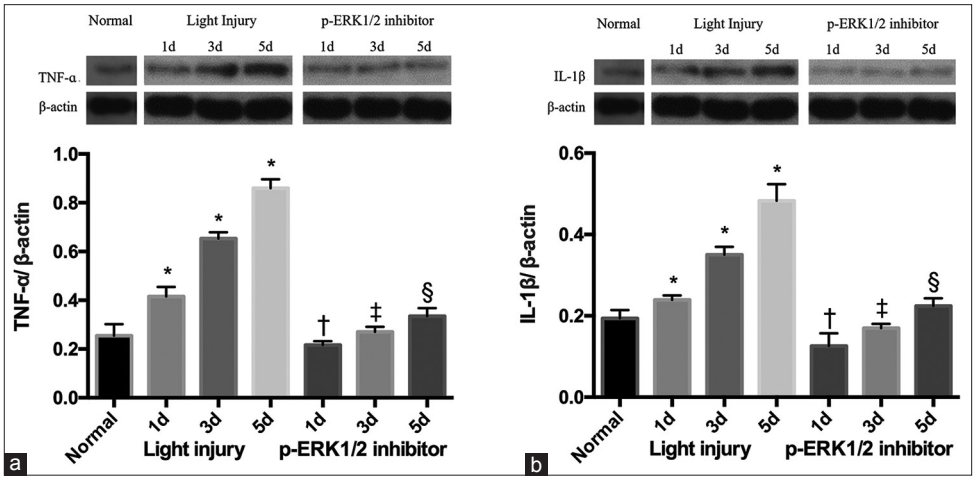

Figure 5: p-ERK1/2 inhibitor protects the retina from light-induced inflammation. The release of proinflammatory cytokine in the retina was detected with Western blotting for TNF-α and IL-1β. (a) Light injury significantly increased the expression of TNF-α compared with normal group. p-ERK1/2 inhibitor significantly decreased the overexpression of TNF-α compared with light injury group. (b) Light injury significantly increased the expression of IL-1β compared with normal group. p-ERK1/2 inhibitor significantly decreased the overexpression of IL-1β compared with light injury group. *$P < 0.05$ versus normal group, †$P < 0.05$ versus light injury group in 1 day; ‡$P < 0.05$ versus light injury group in 3 days; §$P < 0.05$ versus light injury group in 5 days. p-ERK1/2: Phosphorylated-extracellular regulated kinase 1/2; TNF-α: Tumor necrosis factor-α; IL-1β: Interleukin-1β.

following study to test its effect on photoreceptor apoptosis and retina inflammation.

As shown in Figure 3, photoreceptor apoptosis, marked by TUNEL-positive cells in ONL, is significantly reduced by the p-ERK1/2 inhibitor in 1, 3, and 5 days, compared with light injury groups. At 7 days after light exposure, the photoreceptors in the posterior retina were almost completely lost, thus, few TUNEL-positive cells can be observed. However, in the p-ERK1/2 inhibitor injected group, TUNEL-positive cells are still observable, mainly because the p-ERK1/2 inhibitor apparently decreases the speed of photoreceptor loss in ONL. In Figure 4, we saw that the p-ERK1/2 inhibitor downregulated light-induced p-ERK1/2 overexpression and caspase 3 activating in 1, 3, and 5 days. These results suggested that the excessive activation of p-ERK1/2 induced by light injury might be related to photoreceptor apoptosis, the p-ERK1/2 inhibitor protects light injured retina from photoreceptor apoptosis, and inhibition of caspase 3 activation might play a key role in this process.

Inflammation is another important pathological process in light-induced retina damage. In Figure 5, we found that the rising trend of inflammatory factors and the trend of over expression of p-ERK1/2 were basically the same; the p-ERK1/2 inhibitor has anti-inflammation effects on light injured retina by attenuating the expression of TNF-α and IL-1β.

ERK1/2 signal transduction pathway is a very complex system. After activation of ERK1/2, the main role of ERK1/2 on cells is still positive such as promoting cell division and proliferation, and anti-inflammation.[18-21] In the study, we demonstrate the overactivation of p-ERK1/2 in the process of light-induced photoreceptor apoptosis, and the protective effect of p-ERK1/2 inhibitor against photoreceptor apoptosis and retinal inflammation, which opposes the main role of p-ERK1/2. According to previous studies, there are also reports that excessive activation of ERK1/2 under certain condition can lead to cell injury, death, and inflammation, for example, when stimulated by some DNA damage stimuli.[20,22,34] Research confirms that light triggers a sequence of events that damage photoreceptor cells including DNA damage, and activation of ERK1/2 in response to DNA-damaging stimuli promotes cell apoptosis by enhancing activity of some pro-apoptotic signaling molecules, for example, bax, caspase 3, and cytochrome c. This might account for the negative role played by p-ERK1/2 in the study. This hypothesis might need further experimental confirmation.

The main limitation of the study is that the mechanism by which the p-ERK1/2 inhibitor protects retina from light-induced photoreceptor degeneration, and retinal inflammation has not been intensively studied. In addition, since, according to our current and previous studies, photoreceptor cell apoptosis induced by light injury peaked in 1 day, it is better to set more time points before the conclusion of this 24h period to observe the progressive process of p-ERK elevation and cell apoptosis. Finally, this study only involved animal experiment. Therefore, to fully understand the mechanism by which p-ERK1/2 participates in light-injured photoreceptor apoptosis, we need to advance future experimental design.

In conclusion, in this study, we used a light-induced rat model of retinal degeneration to analyze the roles of MAPK antagonists (inhibitors of p-P38, p-ERK1/2, and p-JNK) in photoreceptor degeneration and inflammation. We proved that the p-ERK1/2 inhibitor attenuated the apoptosis of photoreceptor cells, the changes in retinal thickness (ONL), and retinal inflammation associated with light damage. The results indicated that the p-ERK1/2 pathway might be

involved in light-induced retinal damage and suggested that the p-ERK1/2 inhibitor protects retina from light-induced photoreceptor degeneration and retinal inflammation.

Financial support and sponsorship

This work was supported by grants from the National Natural Science Foundation for Young Scholars of China (No. 81400410) and National Natural Science Foundation of China (No. 81570854).

Conflicts of interest

There are no conflicts of interest.

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P- ERK1/2抑制剂对光损伤致视网膜感光细胞退化的保护作用

摘要

背景：丝裂原活化蛋白激酶（MAPK）在细胞凋亡和炎症途径中的作用使其成为光感受器保护的可能重要靶点。本研究旨在探讨MAPK拮抗剂对光诱导大鼠视网膜变性模型中光感受器变性和视网膜炎症的保护作用。

方法：SD大鼠在暗适应前给与玻璃体腔内注射MAPK拮抗剂，包括p-P38抑制剂、p-细胞外调节激酶（ERK）1/2抑制剂和p-c-Jun N末端激酶（JNK）抑制剂。暗适应24小时后，大鼠在照光箱蓝光中暴露（2500勒克斯）24小时，然后恢复到正常的12小时光/12小时暗循环，随后在不同时间点采集样品。本研究用免疫荧光法检测光暴露后细胞MAPK的表达，用组织病理学和末端脱氧核苷酸转移酶dUTP缺口末端标记（TUNEL）染色检测光感受器凋亡，并用免疫印迹法检测视网膜p-ERK1/2、caspase 3、活化caspase 3、肿瘤坏死因子（TNF-α）、白细胞介素（IL-β）的蛋白表达。各组之间的差异采用非配对单因素方差分析和LSD事后分析测算。

结果：MAPK在光诱导视网膜变性模型中被磷酸化和激活，其表达上调主要表现在视网膜外核层（ONL），提示MAPK途径参与了光诱导的视网膜变性。在我们所选择的MAPK拮抗剂中，只有p-ERK1/2抑制剂显著减轻了光损伤带来的感光细胞丢失和视网膜外核层（ONL）的厚度变薄。此外，P-ERK1/2抑制剂减少了光损伤诱导的视网膜外核层（ONL）细胞TUNEL染色阳性率。与对照组相比，光损伤1天、3天、5天组p-ERK1/2（1.12 ± 0.06 vs. 0.57 ± 0.08, t=9.99, P <0.05; 1.23 ± 0.03 vs. 0.57 ± 0.08, t=11.90, P <0.05; and 1.12 ± 0.12 vs. 0.57 ± 0.08, t=9.86, P <0.05；F=49.55, P <0.001）和活化caspase 3（0.63 ± 0.06 vs. 0.14 ± 0.05, t=13.67, P <0.05; 0.74 ± 0.05 vs. 0.14 ± 0.05, t=16.87, P <0.05; and 0.80 ± 0.05 vs. 0.14 ± 0.05, t=18.57, P <0.05；F=100.15, P <0.001）的表达显著增加。p-ERK1/2 抑制剂显著降低光损伤组相应时间点p-ERK1/2 的表达（0.61 ± 0.06 vs. 1.12 ± 0.06, t=-9.26, P <0.05; 0.77 ± 0.06 vs. 1.23 ± 0.03, t=-8.29, P <0.05; and 0.68 ± 0.03 vs. 1.12 ± 0.12, t=-7.83, P <0.05; F=49.55, P <0.001）和活化caspase 3的水平（0.23 ± 0.04 vs. 0.63 ± 0.06, t=-11.24, P <0.05; 0.43 ± 0.03 vs. 0.74 ± 0.05, t=-8.86, P <0.05; and 0.58 ± 0.03 vs. 0.80 ± 0.05, t=-6.17, P <0.05; F=100.15, P <0.001）. 所有组间总caspase 3的表达无显著变化（F=0.56, P =0.75）。视网膜炎症指标的检测显示，与对照组相比，光损伤1天、3天、5天组TNF-α（0.42 ± 0.04 vs. 0.25 ± 0.05, t=5.99, P <0.05; 0.65 ± 0.03 vs. 0.25 ± 0.05, t=14.87, P <0.05; and 0.86 ± 0.04 vs. 0.25 ± 0.05, t=22.58, P <0.05; F=160.27, P <0.001）和 IL-1β（0.24 ± 0.01 vs. 0.19 ± 0.02, t=2.33, P <0.05; 0.35 ± 0.02 vs. 0.19 ± 0.02, t=7.97, P <0.05; and 0.48 ± 0.04 vs. 0.19 ± 0.02, t=14.69, P <0.05; F=77.29, P <0.001）的表达显著增加。P-ERK1/2抑制剂显著降低光损伤组相应时间点TNF-α（0.22 ± 0.02 vs. 0.42 ± 0.04, t=-7.40, P <0.05; 0.27 ± 0.02 vs. 0.65 ± 0.03, t=-14.27, P <0.05; and 0.33 ± 0.03 vs. 0.86 ± 0.04, t=-19.58, P <0.05; F=160.27, P <0.001）和 IL-1β（0.13 ± 0.03 vs. 0.24 ± 0.01, t=-5.77, P <0.05; 0.17 ± 0.01 vs. 0.22 ± 0.02, t=-9.18, P <0.05; and 0.76 ± 0.05 vs. 0.48 ± 0.04, t=-13.12, P <0.05; F=77.29, P <0.001）的表达。

结论：p-ERK抑制剂可能对光诱导的感光细胞变性和视网膜炎症反应有保护作用。