• Basic Research •

Effect of the Notch signaling pathway on retinal ganglion cells and its neuroprotection in rats with acute ocular hypertension

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Abstract

• AIM: To explore the effect of the Notch signaling pathway on retinal ganglion cells (RGCs) and optic nerve in rats with acute ocular hypertension (OH).

• METHODS: Totally 48 Sprague-Dawley (SD) rats were included, among which 36 rats were selected to establish acute OH models. OH rats received a single intravitreal injection of 2 μL phosphate buffered solution (PBS) and another group of OH rats received a single intravitreal injection of 10 μmol/L γ-secretase inhibitor (DAPT). Quantitative real-time polymerase chain reaction (qPCR) and Western blot assay were adopted to determine the mRNA level of Notch and the protein levels of Notch, Bcl-2, Bax, caspase-3, and growth-associated protein 43 (GAP-43). The RGC apoptosis conditions were assessed by TUNEL staining.

• RESULTS: The OH rats and PBS-injected rats had increased expression levels of Notch1, Bax, caspase-3, and GAP-43, decreased expression levels of Bcl-2, and increased RGC apoptosis, with severer macular edema and RGCs more loosely aligned, when compared with the normal rats. The DAPT-treated rats displayed increased expression levels of Notch1, Bax, caspase-3, and GAP-43, decreased expression levels of Bcl-2, and increased RGC apoptosis, in comparison with the OH rats and PBS-injected rats. RGCs were hardly observed and macular edema became severe in the DAPT-treated rat.

• CONCLUSION: The Notch signaling pathway may suppress the apoptosis of retinal ganglion cells and enhances the regeneration of the damaged optic nerves in rats with acute OH.

• KEYWORDS: Notch signaling pathway; ocular hypertension; retinal ganglion cells; anti-apoptotic; neuroprotection; growth-associated protein

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INTRODUCTION

The definition of ocular hypertension (OH) is accepted as intraocular pressure (IOP) increased more than 21 mm Hg, and it occurs without obvious injury to the papilla or visual field[1]. OH makes more than 3 000 000 individuals suffer in the United States, and 4%-7% of which are 40y or older[2]. OH has been well recognized as the commonest risk factor for the occurrence of primary open angle glaucoma (POAG)[3]. According to the reports of Ocular Hypertension Treatment Study (OHTS), the risk contributing to POAG reduced from 9.5% to 4.4%, when the patients with OH underwent a reduction of more than 20% IOP[4]. The reduction of IOP to a favorable level is beneficial in stopping or slowing down the onset and progression of POAG and it can protect the optic nerve head from the direct mechanic or vascular effects caused by IOP elevation[5]. The elevated IOP has been commonly highlighted to be linked with death of retinal ganglion cells (RGCs)[6]. However, OH therapy for glaucoma is not always sufficient in preventing the progressive loss of ganglion cells and a subset of treated patients continues to lose vision[7-8].

Multiple studies pointed out that the retina acts as a model system, and they focused on the mechanisms of cell diversity generation during the development of vertebrate nervous system[9-11]. The critical role of one cell-cell signaling system has been indicated in the development of retina, which is controlled by Notch, which is a cell surface receptor. In the retinal cells, Notch1 and its Notch ligand, Delta-1, are expressed during cellular development[12-13]. Furthermore, the

Notch signaling pathway is conserved and exerts its effect on a broad range of cell types and at different stages during normal development[14]. The Notch signaling pathway is clearly implicated in regulating the RGC development. Previous studies supported the view that the inhibition of the Notch signaling pathway intensifies the generation of RGC during the peak period for RGC genesis[15-17]. On the other hand, the Notch activity is repressed just before the differentiation of the RGC differentiation[18]. In addition, the neuroprotection is conferred through the activation of the Notch signaling pathway to accelerate the survival of the neuronal cells[19]. The cell-cell interactions are associated to some extent by the Notch signaling pathway, which in the early developing retina affects RGC production. Currently, the association has been documented between RGC apoptosis and OH, and studies have been performed for investigating the effect of molecular factors on RGCs in OH[20-22]. This study was conducted with the establishment of rat models of OH, in which the alternations of the Notch signaling pathway were identified in order to explore the effect of the Notch signaling pathway on RGCs and the involvement in rats with OH.

MATERIALS AND METHODS

Experimental Animals A total of 48 healthy Sprague-Dawlay (SD) rats (SCXK 2011-0011, Beijing Vital River Laboratory Animal Technology Co., Ltd., Beijing, China) weighing between 160 g and 80 g were bred in quiet, well-ventilated and clean cages. The environment of the cages was controlled with a temperature of 21℃-22℃, with intermittent illumination of 12h light and dark periods as well as free access to water and food intake. After one week of adaptation feeding, the rats were prepared for the following experiment. This study was performed in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Institutional Animal Care and Use Committee of the First Affiliated Hospital of Hainan Medical University.

Animal Grouping and Treatment Totally, 48 SD rats were evenly classified into four groups (n=12 for each group): normal group, model group (rat models with acute OH), phosphate buffered solution (PBS) group (rats with acute OH were subjected to a single intravitreal injection of 2 μL PBS), and γ-secretase inhibitor (DAPT) group [rats with acute OH were subjected to a single intravitreal injection of 10 μmol/L γ-secretase blocker DAPT, an inhibitor of the Notch signaling pathway (Sigma Aldrich Chemical Co., Saint Louis, MI, USA)].

Modeling of Acute Ocular Hypertension Rats The rat models of OH was established[23]. After an aesthesia with 3% pentobarbital sodium (30 mg/kg, Sigma, Saint Louis, MI, USA) through intraperitoneal injection, the rats were fixed in the prone position. Their eyes were rinsed by using chloramphenicol eye drops, and then with topical anesthesia with 0.4% benoxil for three times with one drop every 5min. A steel sleeve of 30 degrees was connected with a container containing isotonic saline, which was stuck into the anterior chamber near corneal limbus. The container was lifted to increase the IOP to 120 mm Hg, and the changes of anterior segment were observed. The Tono-Pen tonometer was adopted to record IOP before the operation and 1, 2, 3, 4, 5d after the operation by touching the corneal with the stylus. The IOP of the moments of initial touching and initial leaving of the stylus was abandoned for unreliability. Three values of IOP were recorded each time with the mean value calculated. After the operation, the eyes were supplemented with chloramphenicol ophthalmic solution to prevent infection.

Separation of Retina and Optic Nerve Tissues of Rats Five days following the grouping, the rats were anaesthetized by intraperitoneal injection with 3% pentobarbital sodium (30 mg/kg, Sigma, Saint Louis, MI, USA) and then fixed in the supine position on a perfusion table. A scissor was used to open the abdominal skin and abdominal wall into the abdominal cavity, through the septum into the chest cavity, and the ribs were cut to expose the heart. A needle was inserted into the aorta *via* the left atrium, and the blood bled with the incision on right auricle. The heart of rats was perfused with 0.9% physiological saline rapidly and then into the liver. When the effluent water was clear, the rats were then perfused with cold fixative containing 4% paraformaldehyde to stiffen the limbs and tail. After removal of the rat eyeball, 8 mm of the retrobulbar optic nerve was kept. And the eyeball was fixed in 4% paraformaldehyde/PBS for 24h, then eye cup was made after detachment of the eye lens and removal of anterior segment. The eyeball was dehydrated at room temperature and then gradient dehydrated 1h using different concentrations of ethanol. Then the eye cup was soaked in mixed wax I for 2h, and followed by mixed wax II for 1h, soft wax for 1.5h, and hard wax for 1.5h, finally embedded in paraffin and cut into 3 μm thickness slices for reservation.

Hematoxylin-eosin Staining The tissue sections were baked for 2h at 62℃, which were subsequently soaked in xylene I for 10min, xylene II for 10min, absolute alcohol for 5min, 95% alcohol for 5min, and 80% alcohol for 5min, finally followed by rinsing with distilled water. There was no need of repair of high temperature and high pressure antigen. Following the nuclear staining with hematoxylin for 5min, and then color development was conducted by using alcohol solutions of hydrochloric acid for 1min, distilled water for 1min, 70% ethanol for 2min, and 80% ethanol for 2min. Then the sections were dyed with fine wine for 1 to 2min, and 95% ethanol for 2min, and absolute ethanol for 3min. The sections were cleared in xylene and finally mounted with neutral balsam. Under the light microscope, the staining results were observed.

RNA Isolation and Quantitation A single-step Trizol method, Invitrogen (Waltham, MA, USA) was employed to extract the total RNA, based on the instructions. The purity and concentration of RNA were evaluated by ultra-violet (UV) analysis and formaldehyde gel electrophoresis. Reverse transcription was carried out to transform 1 μg RNA into cDNA through the avian myeloblastosis virus (AMV) reverse transcriptase. The primers for quantitative real-time polymerase chain reaction (qPCR) were designed and synthesized by Invitrogen (Waltham, MA, USA). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the internal reference (Table 1). The PCR amplification conditions were listed as follows: pre-denaturation at 94℃ for 5min, denaturation at 94℃ for 40s, annealing at 60℃ for 40s, extension at 72℃ for 1min, which run for 40 cycles and a final extension at 72℃ for 10min. The samples were treated in agarose gel electrophoresis, and the PCR results were evaluated by using Opticon Monitor 3 software (Bio-Rad Laboratories, Hercules, CA, USA). The cycle threshold (Ct) of each reaction tube was the inflection point on the logarithmic amplification power curves, and the gene expression was calculated with the $2^{-\Delta\Delta Ct}$ method: $\Delta\Delta Ct=(Ct_{\text{target gene}}-Ct_{\text{reference gene}})_{\text{experimental group}}-(Ct_{\text{target gene}}-Ct_{\text{reference gene}})_{\text{control group}}$. The experiment was repeated in triplicates and the mean values were calculated.

Western Blot Assay Analysis The bicinchoninic acid (BCA) Protein Assay Kit (Wuhan Boster Biological Technology, Ltd., Wuhan, Hubei Province, China) was used to determine the concentration of proteins extracted from the rat retina and optic nerve, according to the manufacturer's instructions. Then the extracted proteins were supplemented with the loading buffer, boiled at 95℃ for 10min. Subsequently, 30 μg protein was supplemented to each well. The 10% polyacrylamide gel electrophoresis (Wuhan Boster Biological Technology, Ltd., Wuhan, Hubei Province, China) was adopted to separate proteins, with a voltage changed from 80 V to 120 V. Then the protein was transferred onto the polyvinylidene fluoride (PVDF) membranes with a voltage of 100 mV for 45-70min. The membrane was blocked with 5% bovine serum albumin (BSA) for 1h and then cultured with the following primary antibodies (Notch1, Bcl-2, Bax, caspase-3, GAP-43, 1:1000 dilution, Cell Signaling Technologies, Beverly, MA, USA) at 4℃ for overnight. The samples were rinsed by using tris-buffered saline with tween-20 (TBST) solution for 3 times (5min each time), and cultured with the corresponding second antibodies (Miao Tong Biological Technology Co., Ltd., Shanghai, China) for 1h at room temperature. After the membrane was rinsed for 3 times (5min each time) and coloration, β-actin was set as an internal reference. The samples were then developed using a Bio-Rad Gel Doc EZ Imager (Bio-Rad, Hercules, CA, USA). The gray values of the target bands were analyzed by the Image J software. The experiment was repeated in triplicates and the mean values were calculated.

Table 1 The primers sequence of Notch1 and GAPDH used for qPCR

Gene	Sequence
Notch1	F: 5'-GCCGCCTTTGTGCTTCTGTTC-3'
	R: 5'-CCGGTGGTCTGTCTGGTCGTC-3'
GAPDH	F: 5'-CAAGTTCAACGGCACAGTCA -3'
	R: 5'-CCCCATTTGATGTTAGCGGG-3'

GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; qPCR: Quantitative real-time polymerase chain reaction.

TUNEL Staining The sections were dehydrated with concentration of gradient ethanol, and rinsed by using PBS 3 times for 3min. Then the sections were soaked in 200 mL of 0.1 mol/L sodium citrate buffer with microwave heating at 350 W for 5min. After that, the samples were removed and rinsed with 80 mL distilled water, and followed by washing with PBS 3 times for 3min after cooling. The samples were supplemented with TUNEL reaction mixture, and then blocked in a damp dark box at 37℃ for 1h and rinsed by using PBS 3 times for 3min. The samples were then supplemented with converter-POD and blocked in a damp dark box at 37℃ for 1h and rinsed by using PBS 3 times for 3min again. And the samples were cultured with direct aluminum bonded (DAB) substrates for 15min at room temperature, finally rinsed by using PBS 3 times for 3min. The samples were re-stained with hematoxylin and dehydrated with gradient concentration of ethanol, and then were cleared in xylene and finally mounted with neutral gum. Cells stained with brownish-yellow granules in the nucleus or cytoplasms were considered as positive cells. The number of apoptotic neurons was counted by NIK0N80i software to calculate the apoptotic index.

Statistical Analysis The SPSS software 21.0 (SPSS Inc., Chicago, IL, USA) was employed for the statistical analysis in the present study. The measurement data were presented using the mean±standard deviation (SD). Comparisons between two groups for normal distributed measurement data were performed by the *t*-tests. The comparisons between two groups were analyzed with the least significant difference (LSD) method, and comparisons among multiple groups were conducted by one-factor analysis of variance (ANOVA). The level of significance was $P<0.05$.

RESULTS

Successfully Established Rat Models of Acute Ocular Hypertension During establishment of acute OH, no rats died during the course of rat models, and the anterior capsule of the lens and the lens were non-injury in the process. Tono-Pen tonometer was used to measure IOP of rats in the normal group and the model group every day. IOP in rat models of OH in all the groups stabilized

approximately at 120 mm Hg (the normal IOP: 10-21 mm Hg), which was higher than that in the normal group. Based on the Figure 1, the IOP of the DAPT, PBS and model groups were all significantly higher than the normal group, and it was stabilized. No difference was identified in IOP among the three groups, which suggested the successful establishment of rat models with acute OH.

DAPT Suppresses the Activation of the Notch Signaling Pathway The results of PCR detection and Western blot assay for Notch1 mRNA and protein expression in rat retinal and optic nerve tissues of each group exhibited that Notch1 mRNA and protein expression in rat models of OH were significantly increased when compared with the normal group (P<0.05), which demonstrated that the Notch signaling pathway was activated by the occurrence of OH. In comparison with the model group and the PBS group, the Notch1 mRNA and protein expression in rats in the DAPT group were decreased (P<0.05). Compared with the normal group, the DAPT group showed higher exression of Notch1 without significant difference (P>0.05). DAPT successfully repressed the activation of the Notch signaling pathway in retinal and optic nerve tissues (Figure 2). No difference was identified in Notch1 expression between the model group and the PBS group.

HE Staining Identified the Morphological Changes of Rat Retinal Tissues In the normal group, every layer of retina was clear, with the RGCs densely arranged. The nucleus boundaries were clear. Retinal pigment epithelial cells were uniformly aligned in a single layer. While in the model group and the PBS group, all the retinal layers, inner and outer nuclear layers, and nerve fiber layer were observed with severe edema. The inner plexiform layer and outer plexiform layer got thinner with vauge boundaries. The inner and outer nuclear layers showed reduced and loosely arranged cells and enlarged nucleus. Part of the nucleus underwent changes of getting dissolved and broken. The cells of inner and outer nuclear layers in the DAPT group were irregularly aligned and reduced with almost invisible RGCs. The severe edema was observed in all the retinal layers, and the edema was more serious when compared with the model group and the PBS group, which indicated that the inhibition of the Notch signaling pathway exacerbated the damage of retina and optic nerve in rat models of OH (Figure 3A). The model and PBS group had thicker full-thickness layers, inner and outer nuclear layers, and retinal nerve fiber layers than the normal group (P<0.05); the DAPT group showed significantly far thicker full-thickness layers, inner and outer nuclear layers, and retinal nerve fiber layers than the model and PBS groups (Figure 3B-3E).

The Inhibition of the Notch Signaling Pathway Enhances RGC Apoptosis TUNEL staining was performed to observe the apoptosis of RGCs in rat retinal tissues (Figure 4). The apoptotic RGCs in the model, PBS and DAPT groups were

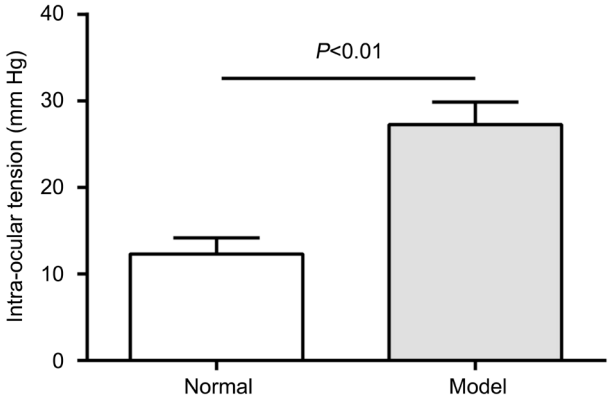

Figure 1 Changes of IOP in rats between the normal group and the model group.

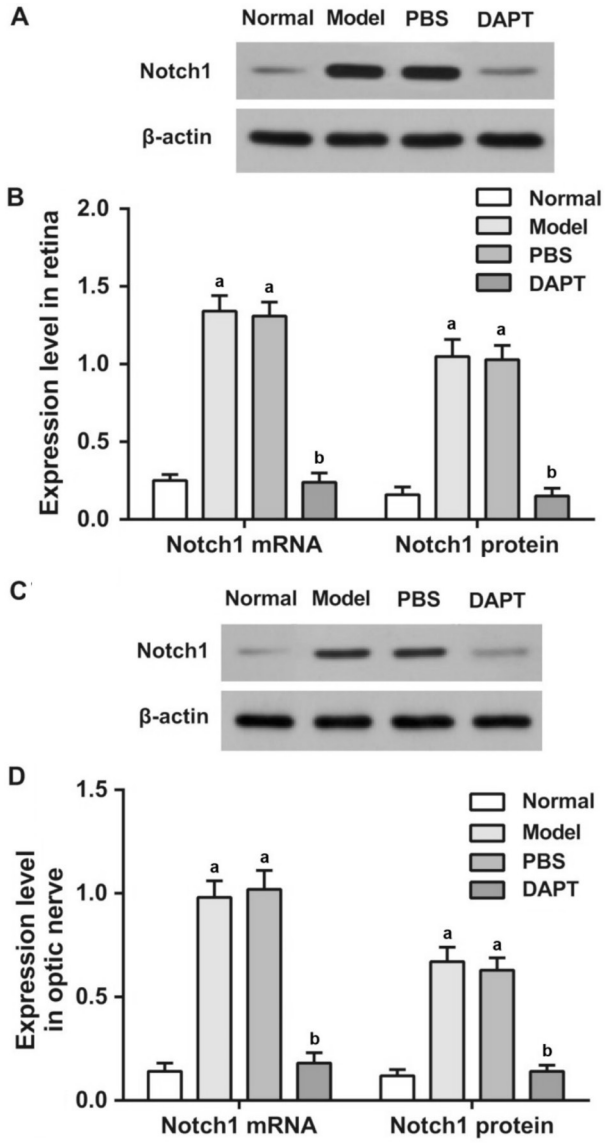

Figure 2 Expression of Notch1 in rat retina and optic nerve tissues among normal, model, PBS, and DAPT groups A: Grey value of the Notch1 protein bands in retinal tissues; B: Expression levels of Notch1 mRNA and protein in retinal tissues, measured using qPCR and Western blot assay; C: Grey value of the Notch1 protein bands in optic nerve tissues; D: Expression levels of Notch1 mRNA and protein in optic nerve tissues. [a]P<0.05 in comparison with the normal group; [b]P<0.05 in comparison with the model group. PBS: Phosphate buffered solution; DAPT: γ-secretase inhibitor; qPCR: Quantitative real-time polymerase chain reaction.

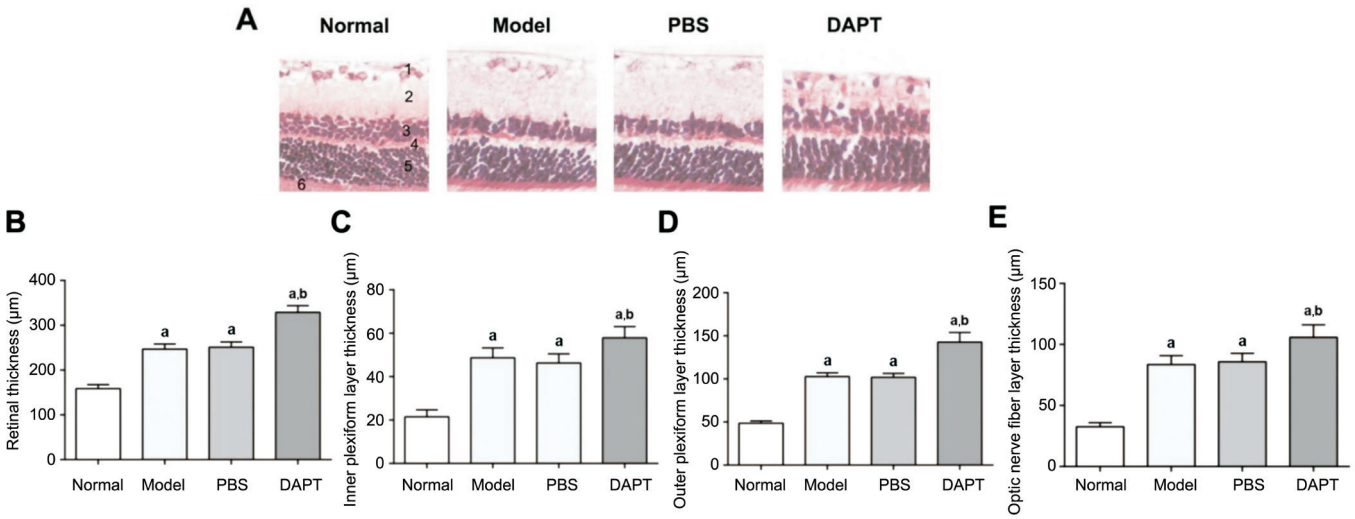

Figure 3 RGCs of DAPT-treated OH rats were almost invisible and the retinal pigment epithelial cells irregularly-arranged and significantly decreased. DAPT-treated OH rats had severe edema in retinal layers (×400) A: Morphological changes of rat retinal tissues of all the layers; B: The thickness of full-thickness layer; C: The thickness of inner plexiform layer; D: The thickness of outer plexiform layer; E: The thickness of retinal nerve fiber layer. 1: Ganglion cell layer; 2: Inner plexiform layer; 3: Inner nuclear layer; 4: Outer plexiform layer; 5: Outer nuclear layer; 6: Layer of rods and cones. [a]P<0.05 compared with the normal group; [b]P<0.05 compared with the model group. PBS: Phosphate buffered solution; OH: Ocular hypertension.

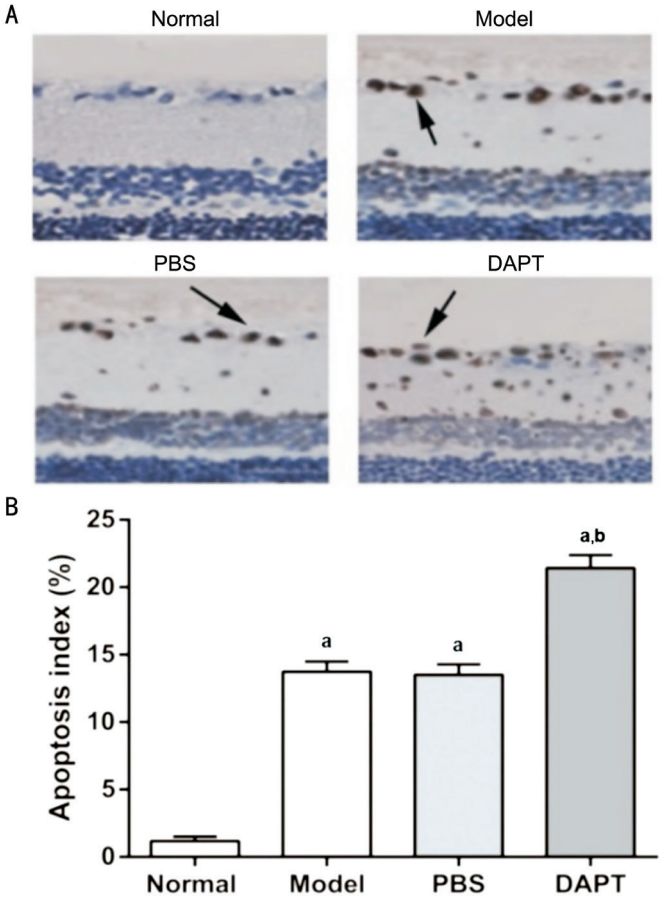

Figure 4 DAPT-treated OH rats showed increased RGC apoptosis in retinal tissues A: TUNEL-stained RGCs (×400). The arrows point to brown-stained cells that are defined as apoptotic cells. The DAPT group showed the most brown-yellow granules, and the model and PBS groups took the second place; B: The apoptosis index of RGCs in rat retinal tissues. [a]P<0.05 compared with the normal group. [b]P<0.05 compared with the model group. PBS: Phosphate buffered solution; OH: Ocular hypertension; RGC: Retinal ganglion cell.

increased, with significant increase of apoptotic index, compared with the normal group (all P<0.05). As compared with the model group and the PBS group, the apoptotic RGCs and apoptotic index increased more significantly further in the DAPT group (all P<0.05).

The Notch Signaling Pathway Controls the Expression of Apoptosis-related Factors Western blot assay was performed to determine the expression levels of Bcl-2, Bax, and caspase-3 in rat retinal tissues so as to further find out the effect of the Notch signaling pathway on RGC apoptosis. As Figure 5 shown, when compared with the normal group, the expressions of pro-apoptotic proteins, Bax and caspase-3, were up-regulated in model, PBS and DAPT groups, while the expression of anti-apoptotic protein, Bcl-2, was down-regulated (all P<0.05). However, the protein expressions of Bax and caspase-3 in the DAPT group were increased more significantly, and the protein expression of Bcl-2 was reduced more significantly as compared with the model and PBS groups (all P<0.05). These results concluded that the Notch signaling pathway repressed the apoptosis of RGCs in retina tissues of OH rats.

The Notch Signaling Pathway Accelerates Optic Nerve Regeneration In order to find out the effect of the Notch signaling pathway on optic nerve regeneration, Western blot assay was used to measure the alternation of GAP-43 expression that was a specific protein during axonal regeneration of optic nerve in rat optic nerve tissues in each group. And the results indicated that expression of GAP-43 was enhanced in the model group when compared with the normal group, which indicated that GAP-43 was activated after the optic nerve in rats with OH was damaged. When the Notch signaling pathway was repressed by DAPT, the expression of GAP-43

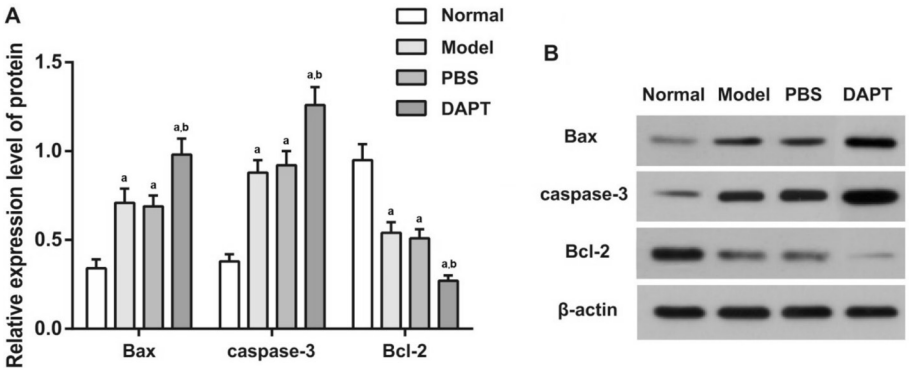

Figure 5 Protein expressions of apoptosis-related factors in rat retinal tissues among normal, model, PBS, and DAPT groups A: Protein levels of Bax, caspase-3, and Bcl-2 in rat retinal tissues, using Western blot assay; B: Grey value of Bax, caspase-3, and Bcl-2 protein bands, using Western blot assay. [a]P<0.05 in comparison with the normal group; [b]P<0.05 in comparison with the model group. PBS: Phosphate buffered solution; DAPT: γ-secretase inhibitor.

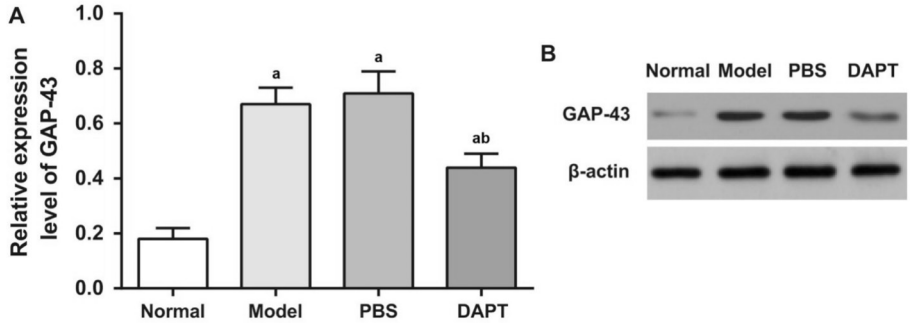

Figure 6 The protein expression of GAP-43 in rat retinal tissues among normal, model, PBS, and DAPT groups A: Protein levels of GAP-43 in rat retinal tissues, using Western blot assay; B: Grey value of GAP-43 protein bands, using Western blot assay. [a]P<0.05 in comparison with the normal group; [b]P<0.05 in comparison with the model group. PBS: Phosphate buffered solution; DAPT: γ-secretase inhibitor.

was significantly declined, showing that the Notch signaling pathway accelerated the regeneration of optic nerve in rats with OH (Figure 6).

DISCUSSION

The conflicting effects of the Notch signaling pathway have been reported in various brain injury models. Arumugam *et al*[24] reported that the blockade of Notch signaling pathway improved functional outcome in stroke. However, Yang *et al*[25] found that the activation of Notch signaling pathway confers neuroprotection against transient cerebral ischemia by increasing neuronal survival in mice. In this study, we explored the neuroprotective role of Notch signaling pathway in rat models of acute OH. We found that the activation of Notch signaling pathway may prevent OH progression in rats with OH.

Initially, we measured the expression levels of Notch1 in retinal and optic nerve tissues in rats with acute OH. The Notch signaling pathway was activated once acute OH occurred at early. Actually, OH may cause retinal ischemia-reperfusion (I/R) injury[26]. Li *et al*[27] presented the results that the Notch1 signaling pathway was activated at the early stage. More consistent with our study, Guan *et al*[28] obtained the results in a rat model of middle cerebral artery occlusion/reperfusion (MCAO/R), in which MCAO/R resulted in cerebral infarction, hippocampus neuronal injury and apoptosis, and also activated the Notch1 signaling pathway. However, the study also found that osthole treatment further activated Notch1 signaling pathway to reduce the cerebral infarction as well as the hippocampus neuronal injury and apoptosis. In addition, we found the inhibition of Notch1 signaling pathway facilitated RGC apoptosis. Notch signaling pathway exists in all known animal cells, and it is a classic signaling pathway implicated in modulating neural stem cell (NSC) proliferation and differentiation, and dendrite morphology[29]. Notch1 activation promotes the proliferation and differentiation of neural progenitor cells after ischemic stroke[30]. Hill *et al*[31] indicated that NSCs protected rat RGCs and promoted their regeneration. Furthermore, Notch1 activates microglia cells following focal cerebral ischemia, and Notch1 shows the ability to regulate the inflammation, clear the damaged neurons, and repair the injury after ischemia[32]. Chiu *et al*[33] reported through promoting the growth of microglia cell, whereby RGCs were rescued from OH. In our study, we also determined the expression levels of anti-apoptotic factor, Bcl-2, pro-apoptotic factors Bax, and caspase-3, in retinal tissues of rats with the blockade of Notch signaling pathway, further indicating the anti-apoptotic effect of this signal on RGCs. Oishi *et al*[34] found Notch increased

Bcl-2 expression and promoted the survival of neural precursor cells, and Yu *et al*[35] found that the drug treatment increased Notch1 intracellular domain, Bcl-2 expression and p-Akt/Akt ratio, and down-regulates Bax and caspase-3 expression, thus offering cardioprotective effect against myocardial I/R injury. In the study reported by Sánchez-Migallón *et al*[36], they found that down-regulation of caspase-3 might delay RGC loss, thus suggesting Notch1 down-regulates caspase-3 to prevent RGC loss.

Another important finding in our study indicated that the activation of Notch signaling pathway confers neuroprotection in rats with acute OH. Retinal I/R injury is a common pathological process in many eye disorders. Oxidative stress and inflammation play a role in retinal I/R injury[37]. In an OH-induced retinal I/R injury model, and Chen *et al*[38] showed that oxidative stress reduction was achieved to alleviate retinal ischemia-reperfusion injury. Zhou *et al*[39] supported the protective effects of Notch1 on myocardial I/R injury and found it inhibited cardiomyocyte apoptosis and reducing reactive oxygen species formation, which is also consistent with the study reported by Pei *et al*[40] suggesting the Notch signaling pathway offers protection in myocardial I/R injury by reduction of oxidative/nitrative stress. Meanwhile Shi *et al*[41] considered that activating Notch1 signaling pathway *in vivo* and *in vitro* had neuroprotective effects against cerebral I/R injury. Besides, we found the Notch signaling pathway facilitated the expression of GAP-43. GAP-43 plays an important role in the development and regeneration of central nervous system and the drug treatment causes an up-regulation of GAP-43, exhibiting a neuroprotection in glaucoma[42]. Since phospho-GAP-43 is active in both the early and late stages of optic nerve regeneration[43], the down-regulation of GAP-43 in retinal tissues of rats with the blockade of Notch signaling pathway further indicated the protective effect of Notch signaling pathway against optic nerve injury. However, the underlying mechanism involving Notch signaling pathway and GAP-43 during optic nerve regeneration is beyond the scope of this article.

In conclusion, we established rat models of acute OH and found that the Notch signaling pathway may modulate RGC survival and had neuroprotective effects on rats with acute OH. These findings may provide some research basis for minimizing the conversion of OH to glaucoma. However, we fail to observe the persistence of Notch1 activation in rats with acute OH, which may be investigated in further study. Meanwhile, further studies are warranted to report the more specific mechanism responsible for the Notch signaling pathway in acute OH.

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Conflicts of Interest: Li L, None; **Chen LP,** None; **Liu QH,** None.

REFERENCES

1 Toyokawa N, Kimura H, Matsumura M, Kuroda S. Incidence of late-onset ocular hypertension following uncomplicated pars plana vitrectomy in pseudophakic eyes. *Am J Ophthalmol* 2015;159(4):727-732.

2 Lee AJ, Goldberg I. Emerging drugs for ocular hypertension. *Expert Opin Emerg Drugs* 2011;16(1):137-161.

3 Cheng YH, Tsai TH, Jhan YY, Chiu AW, Tsai KL, Chien CS, Chiou SH, Liu CJ. Thermosensitive chitosan-based hydrogel as a topical ocular drug delivery system of latanoprost for glaucoma treatment. *Carbohydr Polym* 2016;144:390-399.

4 Varma R, Ying-Lai M, Francis BA, Nguyen BB, Deneen J, Wilson MR, Azen SP; Los Angeles Latino Eye Study Group. Prevalence of open-angle glaucoma and ocular hypertension in Latinos: the Los Angeles Latino Eye Study. *Ophthalmology* 2004;111(8): 1439-1448.

5 Rao S, Narayanan PV. A randomised open label comparative clinical trial on the efficacy of latanoprost and timolol in primary open angle glaucoma. *J Clin Diagn Res* 2016;10(1):FC13-15.

6 Qu J, Matsouaka R, Betensky RA, Hyman BT, Grosskreutz CL. Calcineurin activation causes retinal ganglion cell degeneration. *Mol Vis* 2012;18:2828-2838.

7 Sullivan TA, Geisert EE, Hines-Beard J, Rex TS. Systemic adeno-associated virus-mediated gene therapy preserves retinal ganglion cells and visual function in DBA/2J glaucomatous mice. *Hum Gene Ther* 2011;22(10):1191-1200.

8 Fu J, Sun F, Liu W, Liu Y, Gedam M, Hu Q, Fridley C, Quigley HA, Hanes J, Pitha I. Subconjunctival delivery of dorzolamide-loaded poly(ether-anhydride) microparticles produces sustained lowering of intraocular pressure in rabbits. *Mol Pharm* 2016;13(9):2987-2995.

9 CalazaKda C, Gardino PF. Neurochemical phenotype and birthdating of specific cell populations in the chick retina. *An Acad Bras Cienc* 2010; 82(3):595-608.

10 Wallace VA. Concise review: making a retina--from the building blocks to clinical applications. *Stem Cells* 2011;29(3):412-417.

11 Demb JB, Singer JH. Functional circuitry of the retina. *Annu Rev Vis Sci* 2015;1:263-289.

12 Varnum-Finney B, Wu L, Yu M, Brashem-Stein C, Staats S, Flowers D, Griffin JD, Bernstein ID. *J Cell Sci* 2000;113 Pt 23:4313-4318.

13 Yaron O, Farhy C, Marquardt T, Applebury M, Ashery-Padan R. Notch1 functions to suppress cone-photoreceptor fate specification in the developing mouse retina. *Development* 2006;133(7):1367-1378.

14 Andersson ER, Sandberg R, Lendahl U. Notch signaling: simplicity in design, versatility in function. *Development* 2011;138(17):3593-3612.

15 Austin CP, Feldman DE, Ida JA Jr, Cepko CL. Vertebrate retinal ganglion cells are selected from competent progenitors by the action of Notch. *Development* 1995;121(11):3637-3650.

16 Ahmad I, Dooley CM, Polk DL. Delta-1 is a regulator of neurogenesis in the vertebrate retina. *Dev Biol* 1997;185(1):92-103.

17 Waid DK, McLoon SC. Ganglion cells influence the fate of dividing retinal cells in culture. *Development* 1998;125(6):1059-1066.

18 Nelson BR, Gumuscu B, Hartman BH, Reh TA. Notch activity is downregulated just prior to retinal ganglion cell differentiation. *Dev Neurosci* 2006;28(1-2):128-141.

19 Yoon JH, Ann EJ, Kim MY, Ahn JS, Jo EH, Lee HJ, Lee HW, Lee YC, Kim JS, Park HS. Parkin mediates neuroprotection through activation of Notch1 signaling. *Neuroreport* 2017.

20 Wu J, Zhang S, Sun X. Neuroprotective effect of upregulated sonic Hedgehog in retinal ganglion cells following chronic ocular hypertension. *Invest Ophthalmol Vis Sci* 2010;51(6):2986-2992.

21 Tao YJ, Gao DW, Yu M. TGF-β(1) in retinal ganglion cells in rats with chronic ocular hypertension: its expression and anti-apoptotic effect. *Int J Ophthalmol* 2011;4(4):396-401.

22 Wu Z, Wang L, Hou S. Alpha B-crystallin improved survival of retinal ganglion cells in a rat model of acute ocular hypertension. *Neural Regen Res* 2012;7(19):1493-1497.

23 Oka T, Tamada Y, Nakajima E, Shearer TR, Azuma M. Presence of calpain-induced proteolysis in retinal degeneration and dysfunction in a rat model of acute ocular hypertension. *J Neurosci Res* 2006;83(7):1342-1351.

24 Arumugam TV, Chan SL, Jo DG, Yilmaz G, Tang SC, Cheng A, Gleichmann M, Okun E, Dixit VD, Chigurupati S, Mughal MR, Ouyang X, Miele L, Magnus T, Poosala S, Granger DN, Mattson MP. Gamma secretase-mediated Notch signaling worsens brain damage and functional outcome in ischemic stroke. *Nat Med* 2006;12(6):621-623.

25 Yang Q, Yan W, Li X, Hou L, Dong H, Wang Q, Dong H, Wang S, Zhang X, Xiong L. Activation of canonical notch signaling pathway is involved in the ischemic tolerance induced by sevoflurane preconditioning in mice. *Anesthesiology* 2012;117(5):996-1005.

26 Nucci C, Gasperi V, Tartaglione R, Cerulli A, Terrinoni A, Bari M, De Simone C, Agrò AF, Morrone LA, Corasaniti MT, Bagetta G, Maccarrone M. Involvement of the endocannabinoid system in retinal damage after high intraocular pressure-induced ischemia in rats. *Invest Ophthalmol Vis Sci* 2007;48(7):2997-3004.

27 Li S, Zyang X, Wang Y, Ji H, Du Y, Liu H. DAPT protects brain against cerebral ischemia by down-regulating the expression of Notch 1 and nuclear factor κB in rats. *Neurol Sci* 2012;33(6):1257-1264.

28 Guan J, Wei X, Qu S, Lv T, Fu Q, Yuan Y. Osthole prevents cerebral ischemia-reperfusion injury *via* the Notch signaling pathway. *Biochem Cell Biol* 2017;95(4):459-467.

29 Ding XF, Gao X, Ding XC, Fan M, Chen J. Postnatal dysregulation of Notch signal disrupts dendrite development of adult-born neurons in the hippocampus and contributes to memory impairment. *Sci Rep* 2016;6:25780.

30 Wang L, Chopp M, Zhang RL, Zhang L, Letourneau Y, Feng YF, Jiang A, Morris DC, Zhang ZG. The Notch pathway mediates expansion of a progenitor pool and neuronal differentiation in adult neural progenitor cells after stroke. *Neuroscience* 2009;158(4):1356-1363.

31 Hill AJ, Zwart I, Samaranayake AN, Al-Allaf F, Girdlestone J, Mehmet H, Navarrete R, Navarrete C, Jen LS. Rat neurosphere cells protect axotomized rat retinal ganglion cells and facilitate their regeneration. *J Neurotrauma* 2009;26(7):1147-1156.

32 Wei Z, Chigurupati S, Arumugam TV, Jo DG, Li H, Chan SL. Notch activation enhances the microglia-mediated inflammatory response associated with focal cerebral ischemia. *Stroke* 2011;42(9):2589-2594.

33 Chiu K, Chan HC, Yeung SC, Yuen WH, Zee SY, Chang RC, So KF. Erratum: modulation of microglia by Wolfberry on the survival of retinal ganglion cells in a rat ocular hypertension model. *J Ocul Biol Dis Infor* 2009;2(3):127-136.

34 Oishi K, Kamakura S, Isazawa Y, Yoshimatsu T, Kuida K, Nakafuku M, Masuyama N, Gotoh Y. Notch promotes survival of neural precursor cells via mechanisms distinct from those regulating neurogenesis. *Dev Biol* 2004;276(1):172-184.

35 Yu L, Li F, Zhao G, Yang Y, Jin Z, Zhai M, Yu W, Zhao L, Chen W, Duan W, Yu S. Protective effect of berberine against myocardial ischemia reperfusion injury: role of Notch1/Hes1-PTEN/Akt signaling. *Apoptosis* 2015;20(6):796-810.

36 Sánchez-Migallón MC, Valiente-Soriano FJ, Nadal-Nicolás FM, Vidal-Sanz M, Agudo-Barriuso M. Apoptotic retinal ganglion cell death after optic nerve transection or crush in mice: delayed RGC loss with BDNF or a caspase 3 inhibitor. *Invest Ophthalmol Vis Sci* 2016;57(1):81-93.

37 Ren L, Tao J, Chen H, Bian Y, Yang X, Chen G, Zhang X, Liang G, Wu W, Song Z, Wang Y. Myeloid differentiation protein 2-dependent mechanisms in retinal ischemia-reperfusion injury. *Toxicol Appl Pharmacol* 2017;317:1-11.

38 Chen YI, Lee YJ, Wilkie DA, Lin CT. Evaluation of potential topical and systemic neuroprotective agents for ocular hypertension-induced retinal ischemia-reperfusion injury. *Vet Ophthalmol* 2014;17(6):432-442.

39 Zhou XL, Fang YH, Zhao Y, Zou B, Xu QR, Xu H, Rao CX, Liu JC. The effect of Notch1 on myocardial ischemia reperfusion injury. *Zhonghua Yi Xue Za Zhi* 2016;96(20):1591-1596.

40 Pei H, Yu Q, Xue Q, Guo Y, Sun L, Hong Z, Han H, Gao E, Qu Y, Tao L. Notch1 cardioprotection in myocardial ischemia/reperfusion involves reduction of oxidative/nitrative stress. *Basic Res Cardiol* 2013;108(5):373.

41 Shi F, Dong Z, Li H, Liu X, Liu H, Dong R. MicroRNA-137 protects neurons against ischemia/reperfusion injury through regulation of the Notch signaling pathway. *Exp Cell Res* 2017;352(1):1-8.

42 Stankowska DL, Minton AZ, Rutledge MA, Mueller BH 2nd, Phatak NR, He S, Ma HY, Forster MJ, Yorio T, Krishnamoorthy RR. Neuroprotective effects of transcription factor Brn3b in an ocular hypertension rat model of glaucoma. *Invest Ophthalmol Vis Sci* 2015;56(2):893-907.

43 Kaneda M, Nagashima M, Nunome T, Muramatsu T, Yamada Y, Kubo M, Muramoto K, Matsukawa T, Koriyama Y, Sugitani K, Vachkov IH, Mawatari K, Kato S. Changes of phospho-growth-associated protein 43 (phospho-GAP43) in the zebrafish retina after optic nerve injury: a long-term observation. *Neurosci Res* 2008;61(3):281-288.