

# Expression of Nogo-A on the retina in rat model with chronic ocular hypertension

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## Abstract

• **AIM:** To study the expressive variation of Nogo-A on rat retina in the process of chronic ocular hypertension.

• **METHODS:** Thirty-six healthy adult male Wistars were randomly divided into control group (6 rats) and chronic hypertension group (30 rats). Chronic hypertension was created by cauterizing the superficial scleral veins. Immunohistochemistry technique was used to evaluate the expressive varieties of Nogo-A at different time points during the course of chronic ocular hypertension.

• **RESULTS:** The success of the model was indicated by over 40% of increase in the IOP as compared with normal rats. Compared with control group, as time passed chronic hypertension group gradually had detectable morphology changes in the retina. At the 21st day of chronic ocular hypertension, retinas became thinner and the quantity of retinal ganglion cells (RGC) decreased ( $P < 0.05$ ). Associated with the morphological changes, the expression of Nogo-A was strongly increased ( $P < 0.05$ ).

• **CONCLUSION:** Myelin associated protein Nogo-A plays a part in the process of chronic ocular hypertension.

• **KEYWORDS:** retina; chronic hypertension; Nogo-A; retinal ganglion cell

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## INTRODUCTION

The pathological mechanism of glaucomatous optic neuropathy is progressive death of retina ganglion cells,

which leads to irreversible damage. Regeneration of damaged central nervous system, including optic nerve, is difficult to achieve because of a number of reasons, such as inhibitors of axonal regeneration are present in myelin, lack of neurotrophic factors, and formation of the glia scar. It has been postulated that the regeneration of central nervous system is affected by myelin associated protein Nogo-A. Nogo-A, which is predominantly present in oligodendrocytes and myelin in the adult central nervous system (CNS), not only restricts neurite growth, plasticity, and axonal regeneration, but also limits the invasion and migration of cells and tumors in the CNS<sup>[1]</sup>. Therefore, our finding on the expressive variation of Nogo-A in rat retina indicates an important role of Nogo-A in the optic nerve injury in the process of chronic ocular hypertension and suggests a new approach to rehabilitate glaucomatous optic nerve.

## MATERIALS AND METHODS

**Materials** Thirty-six male Wistar rats, weighing between 250-300g were supplied by experiment animal department of China Medical University. The animals and experimental conditions were performed in accordance with laboratory animal regulations of State Science and Technology Commission. Animals were randomly divided into 2 groups, which are 6 in control group (12 eyes), and 30 in chronic hypertension group (60 eyes). Rabbit anti-rat Nogo-A (Wuhan Boster Biotechnology Co. Ltd), and SP kit (Fuzhou Maixin Biotechnology) were used.

**Methods** Rat model of chronic IOP elevation. Rats were anesthetized by intraperitoneal injection of 3.5mL/kg of chloral hydrate (100g/L). Bulbar conjunctiva was cut and two superficial venous tributaries were burnt. Signs of successful burn were shown as disappeared episclera venous blood flow on the distal end of the burnt point, and distension and darkness of the vessels near comeoscleral limbus. Bulbar conjunctiva was then reset with Tobradex drops and pasted with eyedrop. IOP was measured with Tonopen XL before the operation, half an hour after the operation, and at the 3<sup>rd</sup> day, the 7<sup>th</sup> day, the 14<sup>th</sup> day, the 21<sup>st</sup> day and the 28<sup>th</sup> day after operation. IOP that is 40% beyond preoperative value (9-16mmHg) indicates a success of the model.

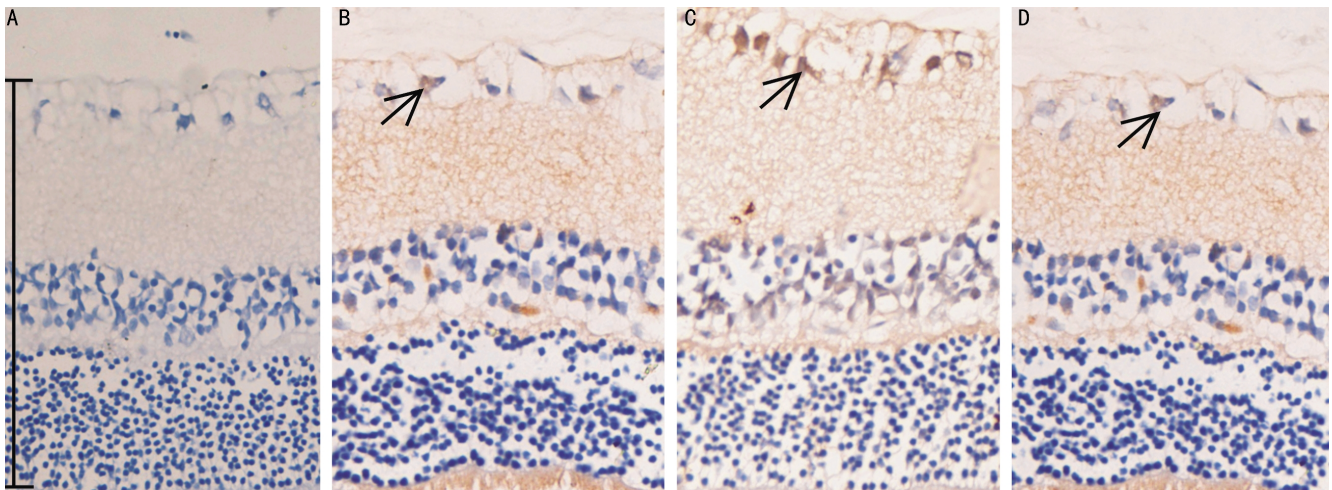


Figure 1 Expression of Nogo-A in retinas of rats with chronic ocular hypertension A: Control; B: 3 days; C: 7 days; D: 28 days

After sampling fixation, dehydration and paraffin imbedding were performed according to the instruction of the kit. Positive cells were those with yellow or brownish-yellow granules deposited in cytoplasm or nuclei. We selected 5 discontinued high power fields from each section to assess the expression intensity with metaMorph/BX51 microgram analytical system to determine the integrated A of positive cells.

**Statistical Analysis** Quantitative data were expressed as the mean $\pm$ SEM, and were analyzed with one-way analysis of variance (ANOVA) followed by Bonferroni test for multiple comparisons among experimental groups and control groups. Statistical analysis was performed using the SPSS 17.0 statistical software. The results were considered statistically different at  $P<0.05$ .

## RESULTS

After 21 days of chronic ocular hypertension the retina became thinner ( $28.3\pm 8.0\mu\text{m}$ ,  $17.0\pm 6.5\mu\text{m}$ ,  $P<0.05$ ) and the number of RGC decreased as compared with control group. In the control group only trace amount of Nogo-A was detected in the layer of ganglion cells in the retina. In the retina from rat model with chronic ocular hypertension, the level of Nogo-A protein (IOD) were found to increase at 7 days ( $64.17\pm 2.68$ ,  $24.93\pm 1.31$ ,  $P<0.01$ ) after establishment of the model, and the increase remained significantly at 28 days ( $37.69\pm 3.15$ ,  $24.93\pm 1.31$ ,  $P<0.05$ ) after the model establishment compared with control group (Figure 1).

## DISCUSSION

Optic nerve damage of glaucoma is a chronic course. However, most animal models for glaucoma research are ischemia-reperfusion models and have disadvantage for observation of retina protection. Reports about morphological changes under chronic ocular hypertension are rare. Regenerative nerve fiber growth and structural plasticity are limited in the CNS of adult mammalian,

including optic nerve, in part because of the presence of neurite growth inhibitory constituents<sup>[2]</sup>. An important step in elucidating the mechanisms of this inhibition was the discovery of Nogo-A, which is an oligodendrocyte-associated neurite growth inhibitor<sup>[3-5]</sup>. The nogo gene encodes three major protein products, Nogo-A, -B, and -C, by alternative splicing and alternative promoter usage<sup>[6,7]</sup>. Nogo-A was shown to be inhibitory for fibroblast spreading and neurite outgrowth and to induce growth cone collapse in rat dorsal root ganglion (DRG) and chick retinal ganglion cell (RGC) neurons. Our results suggest the change of expression of Nogo-A protein in the retina was associated with the elevated ocular pressure. The dramatically increased Nogo-A indicated that Nogo-A may play an important role in obstructing regeneration of optic nerve. Suppression of the Nogo-A might be a new treatment for glaucoma.

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