Tetramethylpyrazine Nitrone Protects Retinal Ganglion Cells against Acute High Intraocular Pressure Injury

ABSTRACT

Objective To explore whether (2-[[1,1-dimethylethyl(oxidoimino)-methyl]-3,5,6-trimethylpyrazine] (TBN) can protect retinal ganglion cells against acute high intraocular pressure injury.

Methods We used anterior chamber puncture to rapidly increase intraocular pressure to 70 mmHg in rats and continue to infuse for 60 min, resulting in retinal ischemia and death of retinal ganglion cells. After acute high intraocular pressure injury in rats, TBN (70 mg/kg body weight) or the equal volume of NS was administered by intraperitoneal injection for four consecutive days.

Results The results showed that the density of retinal ganglion cells was significantly decreased after acute high intraocular pressure injury, and TBN treatment significantly increased the survival rate of retinal ganglion cells.

Conclusion TBN may serve as a potential candidate to treat glaucoma.

KEYWORDS acute high intraocular pressure, TBN, retinal ganglion cells

INTRODUCTION

Glaucoma is the second leading cause of blindness in developed countries, accompanied by the characteristic defects of the optic nerve fibers of the retina and the optic disc, causing the retinal ganglion cells to gradually lose their functional defects, leading to irreversible blindness. It affects millions of people in the world but its mechanism remains unclear. Therefore, it is particularly important to actively study the mechanism of glaucoma injury and find effective protective treatment programs. (2-[[1,1-dimethylethyl(oxidoimino)-methyl]-3,5,6-trimethylpyrazine] (TBN) is the main active component of Ligusticum wallichii Franchat that has been used to treat ischemic stroke for centuries in China. TBN exhibits stronger anti-oxidative activity and shows significant therapeutic effects in a rat stroke model. It can easily cross the blood-brain barrier, and can strongly block Ca²⁺ overload and eliminate free radicals in rat ischemic stroke model. Furthermore, recent research has shown that TBN protects and rescues dopaminergic neurons by reducing ROS and increasing cellular anti-oxidative defense capabilities in models of Parkinson’s disease. Our recent work also discovered that TBN protects retinal ganglion cells against N-methyl-D-aspartate (NMDA) induced injury. In this study, we used acute high intraocular pressure injury model to test the protective effect of TBN on retinal ganglion cells.

EXPERIMENTS

The animals (Female, SD rats, 200–250 g) were anaesthetized with chloral hydrate (0.38 mg/g body weight, i.p.). Corneal analgesia was achieved using topical drops of oxibuprocaine 0.4%. Pupillary dilation was maintained using 0.5% tropicamide. The anterior chamber of the left eye was cannulated with a 33-gauge needle connected to a 500-ml plastic container of sterile saline, the IOP was raised to 70 mmHg for 60 min by...
elevating the saline reservoir. Retinal ischemia was confirmed by observing whitening of the iris and loss of the red reflex of the retina. Sham procedure was performed without the elevation of the bottle in control rats. TBN was dissolved in 0.9% normal saline (NS). TBN or the equal volume of NS were administered by intraperitoneal (i.p.) injection to injured rats with a concentration of 70 mg/kg body weight, starting on the day of injury and lasted for 4 days. In other batch of experiments, we only performed an acute high intraocular pressure injury, and then harvested retinas between 4 and 7 days after surgery to count the survival rate of ganglion cells.

RESULTS AND DISCUSSION

After the successful establishment of the acute high intraocular pressure model, we chose to label retinal ganglion cells with FG at 4 and 7 days after surgery to count the survival rate of RGCs. As shown in Fig. 1, we found that the density of retinal ganglion cells in the sham-operated group was 2217 ± 77/mm², and the density of cells in the 4 day ganglion after acute ocular hypertension injury was significantly reduced to 382 ± 42/mm² ($P < 0.0001$ compared with the sham group). This achieved 17.2% of the sham group, indicating that the survival rate of retinal ganglion cells after acute high intraocular pressure injury was significantly decreased. Similarly, the cell density 7 days after injury was significantly reduced to 339 ± 19/mm² ($P < 0.0001$ compared to sham group). However, there was no significant difference in the retinal ganglion cell density between 4 and 7 days after acute high intraocular pressure injury ($P > 0.05$). Thus, we chose 4 days after injury as the time point to test the protective effect of TBN. On the fourth day after acute high intraocular pressure injury, we calculated the density of retinal ganglion cells after injury in each group of animals. Compared with the control group in Fig. 2, the retinal ganglion cell density after treatment with 70 mg/kg TBN was 978 ± 127/mm² ($P < 0.01$ compared with the NS group), thus TBN significantly increased the survival rate of retinal ganglion cells.

**Fig. 1** Retinal ganglion cell survival is significantly reduced after acute high intraocular pressure injury. (A) Fluoro-Gold (FG) staining of rat retinal whole-mount in the sham group, 4 and 7 days after acute high pressure injury. (B) Cell density graphs for three groups of rats. ***$P < 0.0001$, comparison between the three sets of data using one-way ANOVA test. Sham: $n = 5$, D4; $n = 3$, D7; $n = 3$; $n$: number of animals.

**Fig. 2** TBN increases the survival rate of retinal ganglion cells after acute high intraocular pressure injury. (A) Fluoro-Gold (FG) staining of retina whole-mount of sham-operated rats and intraperitoneal injection of NS or TBN in acute ocular hypertension rat retina. (B) Statistical plot of cell density of retinal whole-mount of three groups of rats, TBN dose 70 mg/kg. ***$P < 0.0001$, *$P < 0.01$. B was tested using one-way ANOVA. Sham: $n = 5$, NS; $n = 7$, TBN; $n = 5$; $n$: number of animals.
The protective effect of TBN on retinal ganglion cells was consistent with our previous study\(^6\), where NMDA was intravitreously injected to injury ganglion cells. TBN treatment attenuated activation of the apoptotic process following NMDA stimulation, as indicated by the elevated ratios of cleaved caspase-3/caspase-3 and of Bax/Bcl-2. And the other possible protective mechanism of TBN may include its ability to eliminate free radicals and decrease the level of the calcium overload. With its protective effect on retinal ganglion cells against acute high intraocular pressure injury or NMDA injury, TBN shows a strong neuro-protective effect. The protection mechanism of TBN may be related to the nitrone, which makes it show a strong ability to scavenge oxygen radicals. In addition, TBN has the effect of inhibiting apoptosis and calcium overload, so TBN may serve as a potential chemical to treat acute intraocular pressure injury as well as excitotoxicity diseases in retina.

REFERENCES