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Original Research Article

The Role of NMDA, GLUR4, NF-κB, and Nrf2 in Glaucoma Pathophysiology: Evidence from Wistar Rat Model

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Abstract

Background: Glaucoma is a neurodegenerative disorder involving retinal ganglion cell loss. Increased intraocular pressure can overstimulate N-methyl-D-aspartate (NMDA) and Glutamate Receptor 4 (GluR4), triggering downstream inflammatory signaling through nuclear factor-kappa B (NF-κB) and altering the antioxidant response mediated by nuclear factor erythroid 2-related factor 2 (NRF2), which contributes to disease progression and informs therapeutic insights.

Objective: To explore NMDA, GluR4, NF-κB, and NRF2 roles in glaucoma pathophysiology and identify therapeutic targets using Wistar rat models.

Methods: This experiment employed a post-test-only control group design. Fourteen male Wistar rats were randomly allocated into two groups (n = 7 each): a glaucoma group, in which elevated IOP was induced by episcleral vein cauterization (EVC), and an untreated control group. Four weeks post-EVC, NMDA, GluR4, NF-κB, and NRF2 expression in retinal tissue was measured by reverse transcription–polymerase chain reaction (RT-PCR). Data were analyzed using the independent t-test or Mann–Whitney U test, with statistical significance defined as $p < 0.05$.

Results: NMDA expression in the glaucoma group was nearly double that of controls (14.50 ± 4.23 vs. 7.74 ± 3.24; $p = 0.006$). A comparable increase was observed for GluR4 (glaucoma group: 10.77 ± 2.40; control group: 3.03 ± 4.05; $p = 0.012$) and NF-κB (glaucoma group: 14.96 ± 4.87; control group: 6.72 ± 1.84; $p = 0.003$). NRF2, however, showed the opposite trend, with expression in the glaucoma group dropping to roughly half that of controls (3.98 ± 1.10 vs. 7.18 ± 1.61; $p < 0.001$).

Conclusion: All four parameters showed statistically significant differences between groups, with NMDA, GluR4, and NF-κB expression elevated and NRF2 expression reduced in the glaucoma group.

Keywords: *Glaucoma; Glutamate Receptor; NMDA Receptor; Nuclear Factor Erythroid 2-Related Factor 2; Nuclear Factor-Kappa B.*
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INTRODUCTION

Glaucoma is a progressive neurodegenerative condition in which the loss of retinal ganglion cells (RGCs) leads to optic nerve deterioration and visual field defects.[1] Globally, the prevalence of glaucoma reached 76 million cases in 2020, with approximately 1.6 million people losing sight and 4.14 million were visually impaired due to the condition.[2] In Indonesia,

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glaucoma affects 0.46% of the population, with 1.8 million individuals experiencing blindness. [3,4]

The pathogenesis of glaucoma involves various mechanisms, including oxidative stress, mitochondrial dysfunction, excitotoxicity, ischemia, and hypoxia.[5] Among the known risk factors, elevated intraocular pressure (IOP) is the primary modifiable one, driving RGC degeneration via mechanical compression, ischemic injury, and glutamate-mediated toxicity, leading to visual field loss.[6,7]

Glutamate excitotoxicity, mediated by receptors such as GluR4 and NMDA, is a key contributor to RGC damage, leading to calcium overload and activation of destructive pathways.[8] Excessive NMDA receptor stimulation further promotes reactive oxygen species (ROS) generation, which in turn drives apoptosis and oxidative stress.[9]

Elevated IOP induces stress and inflammation, activating NF-κB.[10,11] This upregulates inflammatory cytokines, such as IL-1β, TNF-α, and nitric oxide synthase, thereby worsening RGC damage via inflammatory and oxidative stress.[10,11] Additionally, IOP-induced ROS generation activates the NRF2 pathway, thereby enhancing the transcription of of genes involved in antioxidant defense and providing a protective response against oxidative injury.[12,13]

Elevated IOP is a well-known glaucoma risk factor, but its contribution to RGC degeneration involves complex mechanisms. These include interactions with glutamate signaling through GluR4 and NMDA receptors, generation of oxidative stress, and engagement of inflammatory cascades mediated by NF-κB. A deeper understanding of how these pathways interact is needed to guide the development of targeted therapies, including strategies that harness Nrf2-driven antioxidant protection. The present study therefore examined the effects of elevated IOP on retinal expression of NMDA, GluR4, NF-κB, and Nrf2 receptors in a glaucoma Wistar rat model and by comparing these effects with those in a normal Wistar rat.

MATERIALS AND METHODS

Ethical Statement

Ethical clearance for all animal procedures was granted by the Faculty of Medicine Research Ethics Commission, Universitas Diponegoro, Semarang (approval No. 27/EC-H/KEPK/FK-UNDIP/III/2023). Animal housing, experimental interventions, and termination were carried out at the Integrated Development and Research Institute, Unit 4, Universitas Gadjah Mada, Yogyakarta. RT-PCR analysis of NMDA, GluR4, NF-κB, and NRF2 expression was performed at the Pathology Anatomy Laboratory, Universitas Gadjah Mada.

Animal Model and Experimental Design

The sample size was determined in accordance with World Health Organization (WHO) guidelines, which recommend a minimum of five rats per group. A total of 14 male Wistar rats (age: 6–8 weeks; body weight: 150–200 g) were equally distributed into a glaucoma group and a control group (n = 7 each). The glaucoma model was made by EVC.[14] Inclusion criteria for the glaucoma group were IOP ≥30 mmHg. Rats with anatomical eye abnormalities, infectious diseases, aggressive behavior, or illness, as well as those that died during the adaptation phase, were excluded. Prior to experimentation, all rats were housed under standard laboratory conditions for two weeks and provided food and water without restriction.

Retinal tissue preparations and quantitative RT-PCR

Four weeks after EVC, all animals were euthanized by decapitation and both eyes were immediately enucleated. Retinal tissue preparation followed a protocol that included fixation in 10% neutral buffered formalin, then processed through standard dehydration, clearing, and impregnation steps before being embedded in paraffin. The paraffin blocks were sectioned at 5 µm with a microtome, mounted on poly-L-lysine-coated slides, and the RGC layer was then scraped off and transferred to microtubes for further analysis.

RNA was isolated using the Quick-RNA FFPE Kit (Zymo Research, USA) as directed by the manufacturer. The primers used in this study are listed in Table 1. The isolated RNA was then subjected to RT-qPCR using the Bioneer AccuPower GreenStar RT-qPCR Master Mix on a DTlite Real-Time PCR System (DNA-Technology, Russia). Thermal cycling parameters were as follows: reverse transcription at 50–70°C for 15 min, initial denaturation at 95°C for 5 min, and 40 amplification cycles (95°C for 30 s; 55–60°C for 30 s), with a final melting curve analysis step.[15] MDA, GluR4, NF-κB, and Nrf2 transcript levels were quantified using gene-specific primers under the same RT-qPCR conditions. All expression data were normalized against GAPDH using the 2^-ΔCt method, in which ΔCt denotes the difference between target and GAPDH threshold cycle values within each sample. Final values are expressed as fold changes calculated from the mean of the normalized data.

Table 1. Primer of Sequences

Receptor	Forward	Reverse
NMDA	GCTGTACCTGCTGGACCGCT	GCAGTGTAGGAAGCCACTATGQATC
GluR4	ATTGCCTATGGAACACTTGATTCG	TCTTGTCCTTACTTCCGGAGTCCTT
NFκB	GAAATTCCTGATCCAGACAAAAAC	ATCACTTCAATGGCCTCTGTGTAG
Nrf2	CCA GCT ACT CCC AGG TTG C	CCA AAC TTG CTC CAT GTC CT

NMDA = N-methyl-D-aspartate, GluR4 = Glutamate Receptor 4, NFκB = Nuclear Factor-Kappa B, Nrf2 = Nuclear Factor Erythroid 2-Related Factor 2

RESULTS

The expression profiles of NMDA, GluR4, NF-κB, and NRF2 were analyzed to assess molecular alterations associated with elevated intraocular pressure. The data for NMDA, NF-κB, and NRF2 were normally distributed and homogeneous, whereas GluR4 expression was not normally distributed. As illustrated in Figure 1, the glaucoma group showed increased expression of NMDA, GluR4, and NF-κB, while NRF2 expression was reduced. NMDA expression showed a higher median and a broader distribution in the glaucoma group, indicating increased receptor activity. Similarly, GluR4 and NF-κB expressions were elevated in the glaucoma group, suggesting enhanced glutamatergic signaling and inflammatory activity. In contrast, NRF2 expression was reduced, indicating a potential decrease in antioxidant defense mechanisms.

The statistical analysis presented in Table 2 supports these findings. NMDA expression in the glaucoma group (14.50 ± 4.23) was significantly greater than in the control group (7.74 ± 3.24; p = 0.006). A similar pattern was observed for GluR4, with higher expression in the glaucoma group (10.77 ± 2.40) than in controls (3.03 ± 4.05; p = 0.012). Because the data of GluR4 were not normally distributed, the Mann–Whitney U test was used for analysis. NF-κB expression also showed a significant increase in the glaucoma group (14.96 ± 4.87) relative to the control group (6.72 ± 1.84; p = 0.003). NRF2 expression was significantly reduced in the glaucoma group (3.98 ± 1.10) compared with the control group (7.18 ± 1.61; p < 0.001.

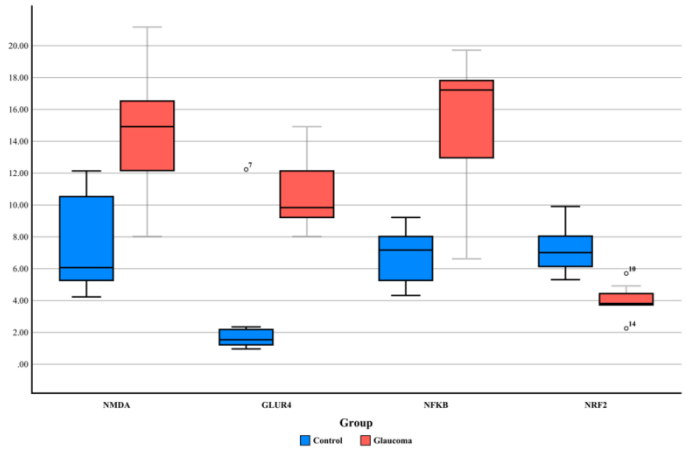

Figure 1. Mean NMDA, GluR4, NFκB, NRF2 receptor level between the control and glaucoma groups

Table 2. Variables Observed between Control and Glaucoma Group

Variables	Control			Glaucoma			p-value
	Min	Mean ± S.D	Max	Min	Mean ± S.D	Max	
NMDA	4.29	7.74 ± 3.24	12.13	8.00	14.50 ± 4.23	21.11	P = 0.006 [a]
GLUR4	0.93	3.03 ± 4.05	2.29	8.00	10.77 ± 2.40	14.93	P = 0.012 [b]
NFkB	4.29	6.72 ± 1.84	9.19	6.50	14.96 ± 4.87	19.70	P = 0.003 [a]
NRF2	5.65	7.18 ± 1.61	9.84	2.14	3.98 ± 1.10	5.66	p < 0.001 [a]

NMDA = N-methyl-D-aspartate, GluR4 = Glutamate Receptor 4, NFkB = Nuclear Factor-Kappa B, Nrf2 = Nuclear Factor Erythroid 2-Related Factor 2
[a] = t-test
[b] = Mann-Whitney U tes

DISCUSSION

Glaucoma is a major cause of blindness worldwide.[16] Therefore, developing an animal model that is reliable, cost-effective, and highly reproducible is urgently needed to investigate its underlying mechanisms and potential treatment approaches.[16] Over the past decade, various rat models with elevated intraocular pressure have been widely utilized to explore the mechanisms underlying glaucoma and to develop new therapeutic approaches for the disease.[17] EVC was used to induce a glaucoma model characterized by increased IOP.[16,17]

In this study, a distinct pattern of molecular changes was observed in the glaucoma group, characterized by increased expression of NMDA, GluR4, and NF-κB, alongside decreased NRF2 expression. Therefore, the current findings suggest that glaucoma progression involves a complex interaction between excitotoxicity, inflammation, and oxidative stress. The modulation of NMDA, NFκB, and NRF2 pathways may therefore offer potential therapeutic targets for neuroprotection in glaucoma.

In this study, NMDA receptor expression was markedly increased in the glaucoma group. This finding is supported by previous experimental studies demonstrating that elevated intraocular pressure is associated with increased NMDA receptor expression, including in DBA/2J mice.[18] Elevated IOP has been suggested to increase the expression of NR2B subunits of the NMDA receptor.[18] Under conditions of elevated intraocular pressure, glutamate excitotoxicity may occur, resulting in increased NMDA receptors. An increase in NMDA receptors can lead to NMDA-induced retinal injury, oxidative stress, and subsequent apoptosis of RGCs.[9] Previous studies have demonstrated that NMDA receptor expression is altered in glaucomatous conditions induced by elevated IOP.[19] In particular, the NR2B subunit of the NMDA receptor was found to be upregulated in glaucomatous rats following episcleral vein cauterization, indicating enhanced glutamate-mediated excitotoxicity in retinal ganglion cells.[19] These findings indicate that NMDA receptor activation plays an important role in the neurodegenerative process observed in glaucoma.[19]

Glutamate excitotoxicity under conditions of elevated IOP is a key hypothesis in glaucoma research.[8] Studies highlight the susceptibility of RGCs to excessive glutamate levels, where overstimulation of glutamate receptors leads to intracellular calcium accumulation, activating destructive pathways that damage cells.[8] This process involves mechanisms such as calcium-dependent proteases, nitric oxide synthase, and mitochondrial dysfunction, contributing to neuronal death.[8] The role of glutamate receptors, particularly GLUR4, has been implicated in this excitotoxic process.[8] It is consistent with our finding that GluR4 expression is significantly increased in the glaucoma group compared with the control group.

The NF-κB signaling pathway plays an important role in inflammation by regulating the expression of pro-inflammatory cytokines and mediators.[10,11] In glaucoma, activation of this pathway is closely related to cellular stress and inflammatory responses.[10,11] This study found that NF-κB expression is significantly higher in the glaucoma group than in the control group, which is consistent with previous studies. NF-κB has been reported to be activated in glaucomatous conditions in response to elevated intraocular pressure and oxidative stress, leading to increased production of pro-inflammatory cytokines such as IL-1β and IL-6 and contributing to retinal ganglion cell degeneration.[20] Research has shown that mechanical stress and oxidative stress associated with elevated IOP can activate the NF-κB signaling pathway, resulting in upregulation of inflammatory cytokines such as IL-1β, TNF-α, and IL-6.[21] Similarly, the overlap in NFκB levels between control and glaucoma groups may indicate that not all glaucomatous eyes exhibit the same degree of inflammatory activation. NFκB is a redox-sensitive transcription factor, and its expression can vary depending on oxidative stress intensity and local cytokine signaling.[19]

While elevated IOP triggers the production of reactive oxygen species (ROS) in the retina and trabecular meshwork, it has been hypothesized that early activation of the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway following IOP elevation, where Nrf2 phosphorylation enhances the transcription of antioxidant genes.[12,13] Nuclear factor erythroid 2-related factor 2 (NRF2) plays an essential role in protecting cells against oxidative stress by regulating the expression of enzymes involved in redox metabolism.[22] Naguib et al. found that Nrf2 phosphorylation is increased in C57B1/6 J mice using microbead occlusion to elevate IOP in mice.[12] In contrast, NRF2 expression was significantly reduced in the glaucoma group compared with the control group. Similar findings have been reported in previous studies, including those by Arana et al.[21]

The limitation of this study is that the study did not include morphological or histological assessment of RGCs, which limits the ability to directly correlate molecular changes with structural neuronal damage. While the expressions of NMDA, GluR4, NF-κB, and Nrf2 provide important insights into the molecular mechanisms underlying glaucoma, they do not confirm whether these biochemical alterations translate into actual RGC loss or degeneration. Therefore, without morphological validation such as retinal histology, immunohistochemistry, or cell counting, the findings remain at the molecular level and cannot fully describe the extent of neurodegeneration.

CONCLUSION

This study demonstrates that experimental glaucoma induced by episcleral vein cauterization (EVC) alters key molecular pathways related to excitotoxicity, inflammation, and oxidative stress. The glaucoma group was characterized by increased NMDA, GluR4, and NF-κB expression and decreased NRF2 levels when compared with the control group. Having a better understanding of these mechanisms can help in developing effective therapeutic strategies against glaucoma. Further studies should implement using histopathological assessment which was needed to fully describe the extent of neurodegeneration.

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