Article

IL-32γ Plays a Neuroprotective Role after Acute Stroke in Middle Cerebral Artery Occlusion Mouse Model

Jaewoo Hong [1,†], Suyoung Bae [2,†], Yasmin Hisham [3], Taewon Han [4], and Hyunjung Jhun [4,*]

[1] Department of Physiology, School of Medicine, Daegu Catholic University, Daegu 42472, Republic of Korea
[2] Cardiovascular & Neurology Products Division, National Institute of Food and Drug Safety Evaluation, Ministry of Food and Drug Safety, Cheongju 28159, Republic of Korea
[3] Laboratory of Cytokine Immunology, Department of Biomedical Science and Technology, Konkuk University, Seoul 05029, Republic of Korea
[4] Infrastructure Support Team, Korea Food Research Institute, Wanju-gun 55365, Republic of Korea
* Correspondence: vetian@kfri.re.kr; Tel.: +82-63-219-9092; Fax: +82-63-219-9876
† These authors contributed equally to this work.
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Abstract: Stroke is the second leading cause of death and a major cause of long-term disability worldwide. Within minutes of onset, cerebral ischemia triggers a cascade of pathophysiological events that ultimately result in irreversible tissue damage. Post-ischemic inflammation plays a critical role in cerebral ischemia-reperfusion injury, characterized by the elevated release of cytokines and chemokines. Interleukin (IL)-32 is known to induce several cytokines, particularly pro-inflammatory ones such as IL-6, tumor necrosis factor (TNF)-α, and IL-1β. Targeting inflammatory pathways are of great interest in stroke research. In this study, we investigated the role of IL-32γ in a middle cerebral artery occlusion (MCAO) model using transgenic (TG) mice expressing human IL-32γ. Compared to wild-type (WT) mice, IL-32γ TG mice exhibited a significantly reduced infarct volume after MCAO. Accompanying the decreased brain tissue damage, the levels of pro-inflammatory cytokines IL-6, TNF-α, and IL-1β were markedly lower in IL-32γ TG mice than in WT controls. These findings suggest that IL-32γ attenuates the inflammatory response in ischemic brain injury. Specifically, IL-32γ reduced the expression of pro-inflammatory cytokines and the number of apoptotic cells following ischemic insult. In conclusion, our results demonstrate that IL-32γ protects the neuroinflammatory response in brain injury and may serve as a potential neuroprotective therapeutic target.

Keywords: stroke; IL-32γ; proinflammatory cytokine; IL-32γ TG; middle cerebral artery occlusion

1. Introduction

Stroke is a leading cause of long-term disability and the second leading cause of death worldwide. It is primarily caused by a blockage in the brain's blood supply, resulting in the malfunction of affected brain regions [1,2]. Subsequent events, such as oxygen deprivation in brain tissue, formation of reactive oxygen species (ROS), and activation of inflammatory responses, are major contributors to secondary brain injury [3,4]. Inflammatory signaling begins immediately after the onset of stroke. While inflammation plays a crucial role in brain recovery, it often exacerbates secondary brain damage. As part of the neuroprotective response, proinflammatory cytokines including interleukin (IL)-6, tumor necrosis factor (TNF)-α, and IL-1β are rapidly induced, accompanied by activation of microglia and astrocytes.

Additionally, peripheral immune cells such as macrophages and neutrophils infiltrate the brain and release further proinflammatory cytokines [4–9]. The subsequent inflammatory responses, including cytokines, chemokines, adhesion molecules, leukocytes, and infiltrating immune cells critically influence the patient's outcome. Accumulating evidence supports that suppressing detrimental inflammatory processes offers a promising therapeutic strategy for improving outcomes in stroke patient [10,11].

IL-32 is a cytokine that induces the expression of various proinflammatory cytokines such as IL-6, TNF-α, and IL-1β. It has been implicated in numerous pathological conditions, including infectious diseases, autoimmune disorders, and cancers [12–17]. IL-32 has also been reported in neuroinflammatory diseases such as cerebral ischemia, multiple sclerosis, and Alzheimer's disease [18–21]. However, its precise role in the pathophysiology

of many conditions remains unclear. Notably, IL-32 exists in several isoforms, including IL-32α, IL-32β, IL-32γ, and others, each exhibiting distinct biological activities and inflammatory profiles. Among these isoforms, IL-32γ has shown significant potential as a modulator of inflammatory responses in various pathological conditions [22]. Similar to interferon-γ, whose inflammatory role depends on regulation by IL-18 and IL-18 binding protein [23], the pro- or anti-inflammatory effects of IL-32 may also vary depending on isoform and context. Therefore, understanding isoform-specific regulation of IL-32 is essential for elucidating its molecular mechanisms and identifying novel therapeutic targets for stroke. In this study, we investigated the role of the IL-32γ isoform using IL-32γ transgenic (TG) mice expressing human IL-32γ in a surgically induced middle cerebral artery occlusion (MCAO) model.

2. Materials and Methods

2.1. Animals

Human IL-32γ TG mice and wild-type (WT) mice of the C57BL/6J strain were prepared as previously described [12]. The human IL-32γ TG mice exhibited normal body size and showed no apparent physical or behavioral abnormalities, as reported earlier. Both human IL-32γ TG and WT mice were male, 10 to 13 weeks old, and weighed between 23 and 28 g. All mice were housed in a specific pathogen-free barrier facility at the Laboratory Animal Research Center of Konkuk University (Seoul, Republic of Korea). The animal facility was maintained under 12 h light/dark cycle, with 10 air changes per hour, a temperature of 23 ± 2 °C, and relative humidity of 50% ± 10%. Mice were housed in individually ventilated cages (MVCS, Three Shine, Daejeon, Republic of Korea) and provided ad libitum access to a sterilized standard laboratory diet (5L79, PMI Nutrition International, St. Louis, MO, USA) and water. All animal care and experimental protocols were approved by the Institutional Animal Care and Use Committee (IACUC, Approval No: KU12056) of Konkuk University.

2.2. Middle Cerebral Artery Occlusion Induced Ischemia Reperfusion Model

Focal cerebral ischemia/reperfusion (I/R) injury was induced by transient occlusion of the right MCAO, as previously described [24] with minimal modifications. Briefly, mice in both groups were anesthetized using 5.0% isoflurane for induction in a closed chamber, followed by 1.5% isoflurane for maintenance in a gas mixture of 70% N_2O and 30% O_2 via a face mask. Rectal temperature was continuously monitored and maintained at 37 ± 0.5 °C using a water-heated surface throughout the surgical procedure.

Laser-Doppler flowmetry (LDF) was used for measuring the cerebral cortical perfusion, to confirm appropriate probe placement (PeriFlux system 5000, Stockholm, Sweden) in all mice. Anesthetized mice were placed in the left lateral position under an operating microscope and the right temporoparietal region of the head between the orbit and ear was shaved. A small plastic tube (internal diameter, 1 mm) was affixed with glue to the right temporal skull (ipsilateral hemisphere) that had been exposed through a transverse incision [25]. Mice were placed in the supine position, and the LDF probe was secured perpendicular to the right temporal skull surface by inserting into the affixed tube [24]. Cortical perfusion values after occlusion are expressed as a percentage relative to baseline. LDF recordings were made 10 min prior to MCAO.

A midline skin incision was made in the neck, and the right common carotid artery (CCA), external carotid artery (ECA), and internal carotid artery (ICA) were carefully isolated from the vagus nerve. The superior thyroid, lingual, and maxillary arteries were gently cauterized and cut. Two closely spaced silk ligatures were placed on the distal ECA. The ECA was then cut between the knots, leaving a stump attached to the proximal ligature. Microvascular aneurysm clips were applied to the right CCA and ICA. A silicone rubber-coated 7–0 monofilament (7023PK5Re, Doccol Corp., Sharon, MA, USA) was inserted through an arteriotomy in the ECA stump and advanced 11 mm into the ICA from the carotid bifurcation. Proper placement of the filament was confirmed by a sudden drop in local cortical blood flow to less than 20% of baseline, as measured by LDF. In addition, LDF values were recorded 5 min after MCAO to verify the success of the occlusion. The resulting occlusion was visualized as an 80–90% abrupt reduction in cerebral blow flow by the LDF. After 60 min of ischemia, the filament and CCA clamp were gently removed to initiate reperfusion. The collar suture at the base of the ECA was carefully tightened, and the skin was sutured. Anesthesia was discontinued, and the mice were allowed to recover in pre-warmed cages.

2.3. 2,3,5-Triphenyltetrazolium Chloride (TTC) Staining for Brain Infarction Volume Measurement

Infarct size determination was performed as previously described [26]. Briefly, 24 h after I/R, all mice were euthanized and perfused with ice-cold phosphate-buffered saline (PBS) via the ascending aorta. Brains were removed and coronally sectioned into 2 mm-thick slices (as illustrated in Figure 1B). The brain slices were then

fixed in 10% neutral-buffered formalin (pH 7.4), followed by staining with 2% 2,3,5-triphenyltetrazolium chloride (TTC; Sigma-Aldrich, Munich, Germany) at 37 °C for 15 min. Infarct volumes were assessed by photographing stained slices using a digital camera (DSC-HX1, Sony Corp., Tokyo, Japan). Images were analyzed with Image J software (NIH, Bethesda, MD, USA). Unstained pale areas were considered as ischemic lesions. For each brain slice, the infarct area and the total area of both hemispheres were measured. The edema index was calculated by dividing the volume of the left hemisphere by that of the right hemisphere. The actual infarct volume was corrected for edema by dividing the measured infarct volume by the edema index. Infarct volumes are presented as a percentage of total brain volume ± standard error of the mean (SEM).

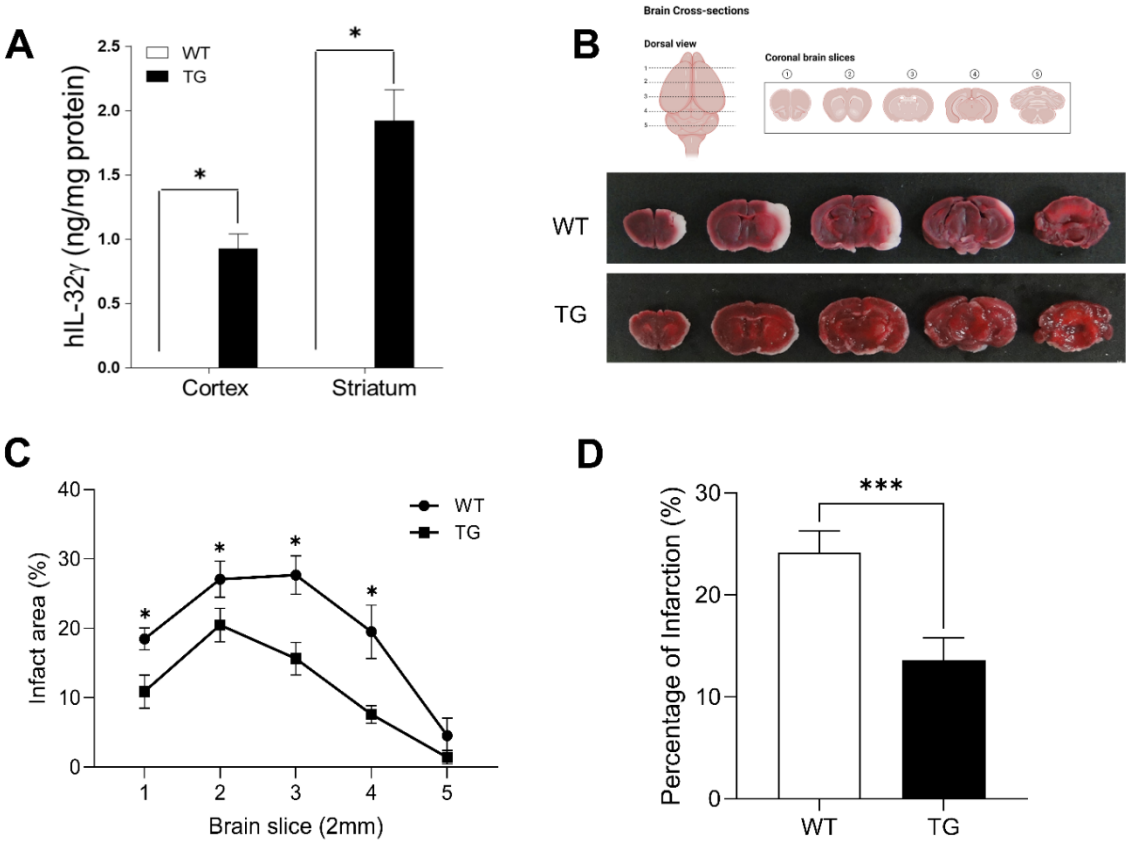

Figure 1. Comparison of human IL-32γ expression in WT and transgenic mice and evaluation of infarct volume following MCAO. (**A**) Quantification of human IL-32γ levels in the cortex and striatum of WT and IL-32γ TG mice. Data are presented as mean ± SEM (n = 6 per group). * $p < 0.05$ compared to WT. (**B**) Representative TTC-stained coronal brain sections 24 h after MCAO. Upper panel: illustration of coronal brain slice levels; lower panel: TTC-stained sections showing infarct areas (white) in WT and IL-32γ TG mice. (**C**) Quantification of infarct areas (%) based on TTC staining revealed a significant reduction in infarct size in IL-32γ TG mice compared to WT mice (* $p < 0.05$). (**D**) Infarct volume expressed as a percentage of total cerebral volume. WT mice showed significantly larger infarct volumes (24.19 ± 0.95%, n = 5; mean ± SEM.) than IL-32γ TG infarct size (13.59 ± 0.99%, n = 5; means ± SEM; *** $p < 0.001$).

2.4. Behavioral Testing

To evaluate neurological deficits, mice were assessed for behavioral changes using the scoring system described by Hatcher et al. [27]. The scores were defined as follows: 0, no observable deficit; 1, flexion of the contralateral torso and forelimb when the mouse is lifted by the tail; 2, circling to the contralateral side when held by the tail on a flat surface; 3, spontaneous circling to the contralateral side; and 4, no spontaneous motor activity. The neurological assessment was performed at least three times to ensure consistency and reliability, and the final score was calculated as the average of the repeated measurements.

2.5. TUNEL Assay

Terminal deoxynucleotidyl transferase biotin-dUTP nick-end labeling (TUNEL) assay was performed using the Click-iT™ TUNEL Alexa Fluor™ 488 Imaging Assay (Invitrogen, Eugene, OR, USA), following the

manufacturer's instructions. Briefly, cryo-frozen brain tissue sections were fixed with 4% paraformaldehyde (Sigma-Aldrich) at 4 °C for 24 h. The tissue was then permeabilized using 0.25% Triton X-100 in PBS. The TUNEL reaction mixture was applied, and the samples were incubated at 37 °C for 1 h, followed by the addition of the Click-iT reaction cocktail according to the protocol. Negative controls were prepared by omitting terminal deoxynucleotidyl transferase from the reaction. All sections were examined using a fluorescence microscope (Olympus, Hamburg, Germany), and green fluorescent signals were considered indicative of apoptotic cells. Six sections from the same coronal level (one per animal) were analyzed for each experimental group. The number of apoptotic cells in MCAO groups was quantified using Image J software and expressed as a percentage.

2.6. Tissue ELISA and Western Blotting

First, homogenization of tissues was performed using the lysis buffer (20 mM HEPES (pH 7.4), 150 mM NaCl, 12.5 mM β-glycerophosphate, 1.5 mM $MgCl_2$, 2 mM EGTA, 10 mM NaF, 2 mM DTT, 1 mM Na_3VO_4, 1 mM PMSF, 100 U/mL aprotinin, 0.5% Triton X-100) using a tissue tearer (BioSpec Products, Bartlesville, OK, USA). Next, homogenized tissues were applied for centrifugation at 10,000 g at 4 °C for 15 min. Cytokine levels of tissues were measured using the supernatant by sandwich enzyme-linked immunosorbent assay (ELISA)-cytokine-specific (R&D Systems, Minneapolis, MN, USA), following the manufacturer's instructions. Human IL-32γ concentrations in both sera and tissues of TG mice were determined using a human IL-32γ-specific sandwich ELISA (YbdY, Seoul, Republic of Korea). Protein concentrations of the homogenate were quantified using bicinchoninic acid assay (Thermo Fisher Scientific, Waltham, MA, USA) following the manufacturer's instructions.

For western blotting analyses, tissue lysates were exposed to SDS-PAGE and transferred to nitrocellulose paper (Millipore, Billerica, MA, USA). The membrane was immunoblotted with respective specific antibodies: mouse anti-phospho-nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) p65 (Ser 536) (7F1) mAb and monoclonal mouse polyclonal rabbit anti-GAPDH (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Bound antibodies were visualized with HRP-conjugated secondary antibodies against primary antibodies using the Super Signal West Substrate (Thermo Fisher Scientific).

2.7. Statistical Analysis

Data is represented as means ± SEM. Statistical significance of differences was assessed using an unpaired two-tailed Student's *t*-test. A p value of < 0.05 was considered statistically significant.

3. Results

3.1. Infarct Volume Reduced in the IL-32γ TG Mice

To investigate the role of IL-32γ, we generated human IL-32γ transgenic mouse [12]. Human IL-32γ was detected in the brain in IL-32γ TG mice but absent in WT mice (Figure 1A). LDF was used to monitor and confirm comparable levels of ischemia in both groups. The average regional cerebral blood flow (rCBF) was 10.91 ± 2.25% in WT mice and 10.03 ± 1.76% in IL-32γ TG mice, indicating that all animals were exposed to a similar ischemic insult. After reperfusion, rCBF increased in both WT and IL-32γ TG mice to 84.84 ± 1.29% and 83.45 ± 1.84%, respectively, with no statistically significant difference between the two groups. These results confirm that the surgical procedure was consistently and successfully performed in both groups.

MCAO was performed in both IL-32γ TG and WT mice, followed by 23 h of reperfusion. Cerebral infarction was assessed using TTC staining. As shown in Figure 1B,C, the infarct areas of brain slices #1, #2, #3, and #4 were significantly smaller in IL-32γ TG mice compared to WT mice (* $p < 0.05$). Consistent with these results, the total infarct volume (Figure 1D) was also significantly reduced in IL-32γ TG mice (WT, 24.19 ± 0.95%; IL-32γ TG, 13.59 ± 0.99%, $p < 0.001$). The total infarct volume observed in WT mice was more than two-fold greater compared to those in IL-32γ TG mice, suggesting that IL-32γ overexpression may confer neuroprotection against ischemic brain injury, particularly in the cortical region.

3.2. Mortality and Neurological Deficits Following MCAO

Twenty-four hours after reperfusion, mortality was 25% in WT mice and 15% in IL-32γ TG mice. In addition, the degree of neurological deficits following stroke was significantly greater in WT mice compared to IL-32γ TG mice (2.85 ± 0.21 to 2.2 ± 0.22, * $p < 0.05$; Figure 2). These results indicate that neurological motor function was

significantly improved in IL-32γ TG mice. Therefore, IL-32γ overexpression appears to mitigate stroke-induced neurological impairments, demonstrating a statistically significant difference between the two groups.

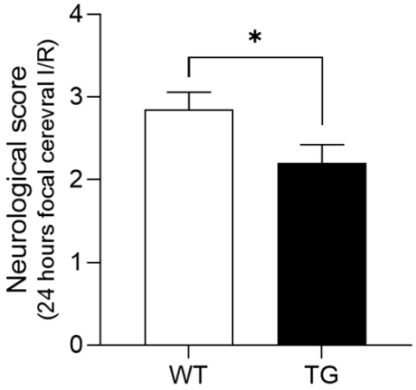

Figure 2. Neurological function assessment in WT and IL-32γ TG mice after cerebral I/R. Neurological function was assessed using the Hatcher scoring system, based on the average of three independent evaluations. Scoring criteria were as follows: 0 = no deficit; 1 = contralateral torso and forelimb flexion when lifted by the tail; 2 = circling to the contralateral side while suspended; 3 = spontaneous contralateral circling; 4 = no spontaneous motor activity. WT mice exhibited significantly higher neurological deficits than IL-32γ TG mice (* $p < 0.05$; mean ± SEM).

3.3. Brain Cytokine Levels Showed Lower Pro-Inflammatory Cytokines in the IL-32γ TG Mice

To evaluate inflammatory activation in the MCAO groups, we measured the expression levels of pro-inflammatory cytokines (TNF-α, IL-6, and IL-1β) and the anti-inflammatory cytokine (IL-10) in brain tissue lysates 24 h after MCAO using ELISA (Figure 3). The levels of pro-inflammatory cytokines—TNF-α, IL-6, and IL-1β—were significantly elevated in the brain tissues of WT mice, and to a lesser extent in IL-32γ TG mice (* $p < 0.05$ and ** $p < 0.01$; Figure 3A–C). These findings indicate that the post-ischemic expression of TNF-α, IL-6, and IL-1β was significantly reduced in IL-32γ TG mice. Conversely, the level of the anti-inflammatory cytokine IL-10 was similarly elevated in both groups, with no statistically significant difference between IL-32γ TG and WT mice (Figure 3D). Collectively, these results suggest that IL-32γ overexpression under ischemic conditions suppresses the production of pro-inflammatory cytokines in vivo.

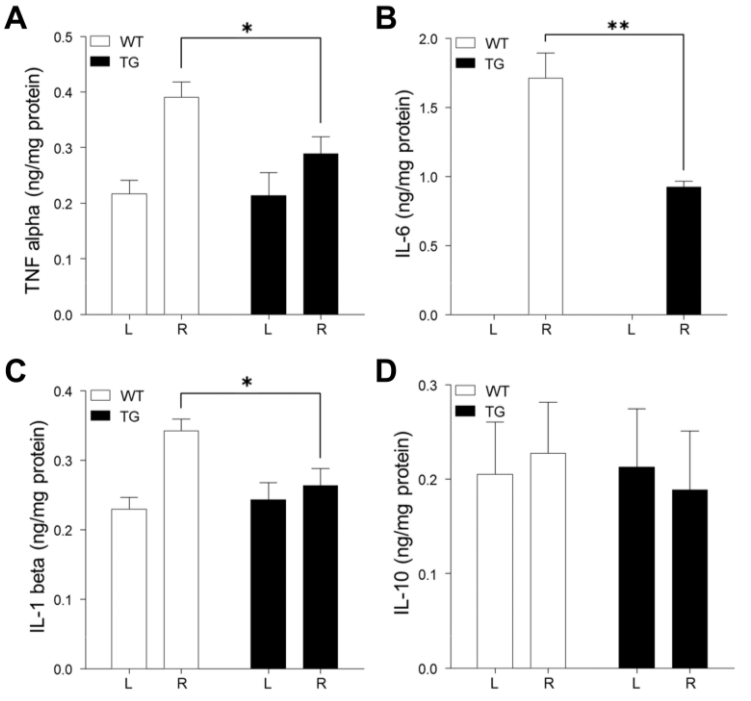

Figure 3. Cytokine levels in brain tissue 24 h after cerebral I/R in WT and hIL-32γ TG mice. (A–C) Levels of pro-inflammatory cytokines TNF-α, IL-6, and IL-1β were significantly reduced in IL-32γ TG mice compared to WT

mice. (**D**) The level of the anti-inflammatory cytokine IL-10 showed no significant difference between the two groups. Cytokine levels were measured by ELISA using brain tissue lysates collected 24 h after I/R injury (n = 5 mice per group). Data are presented as mean ± SEM. * $p < 0.05$, ** $p < 0.01$ indicate statistically significant differences.

3.4. TUNEL Staining Revealed Apoptosis Inhibition in IL-32γ TG Mice

Apoptosis in MCAO groups was quantified using TUNEL staining. As shown in Figure 4A,B, the number of TUNEL-positive apoptotic cells was more than 20% lower in IL-32γ TG mice compared to WT mice at 24 h after ischemia-reperfusion injury. These findings suggest that IL-32γ overexpression inhibits apoptosis in the brain following cerebral ischemia-reperfusion.

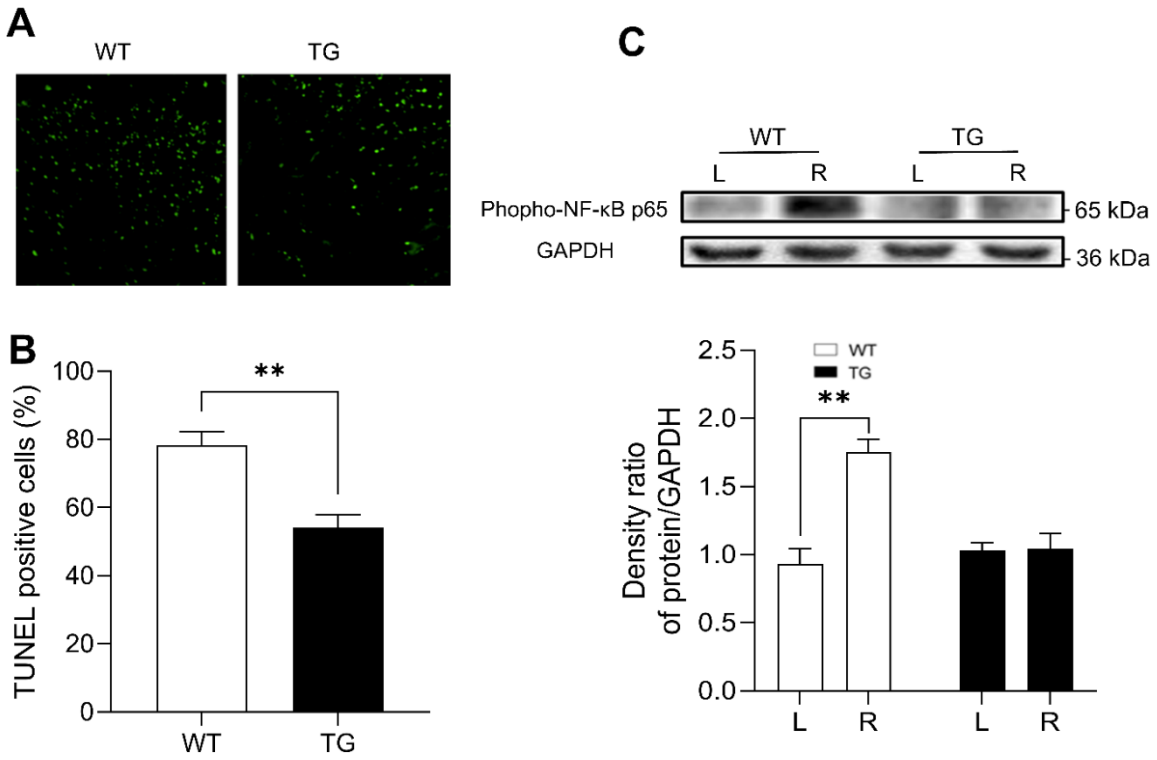

Figure 4. TUNEL staining and phospho-NF-κB expression in MCAO groups 24 h after cerebral I/R in WT and hIL-32γ TG mice. (**A**) TUNEL staining demonstrated a marked reduction in apoptotic cells in the IL-32γ TG group compared to WT mice. (**B**) Quantification of apoptotic cells was performed using Image J. IL-32γ TG mice showed a significantly lower percentage of TUNEL-positive cells (54.0%) compared to WT mice (78.3%). (**C**) Expression levels of pNF-κB in brain tissues of WT and IL-32γ TG mice were analyzed by Western blotting 24 h after MCAO. GAPDH was used as a loading control. Densitometric analysis shows significantly increased pNF-κB p65 expression in the ipsilateral hemisphere of WT mice compared to TG mice. L: contralateral hemisphere; R: ipsilateral hemisphere. Data are presented as mean ± SEM (n = 3). * $p < 0.05$, ** $p < 0.01$ indicate statistically significant differences.

3.5. NF-κB Inflammatory Signaling Is Reduced in IL-32γ TG Mice

Nuclear translocation of NF-κB has been observed in stroke patients, suggesting its involvement in cerebral I/R injury [28]. As the NF-κB pathway plays a critical role in amplifying post-stroke inflammatory responses, it has emerged as a promising therapeutic target [29]. To explore the mechanism by which IL-32γ overexpression confers protection against cerebral injury, we analyzed NF-κB p65 activation in IL-32γ TG and WT mice. Twenty-four hours post-ischemia, p-p65 levels (normalized to GAPDH) were significantly higher in WT mice than in IL-32γ TG mice, as shown in Figure 4C. These findings suggest that IL-32γ overexpression may attenuate NF-κB activation following cerebral I/R injury.

4. Discussion

Inflammation, particularly neuroinflammation, is a major contributor to tissue damage and secondary injury following ischemic stroke. Therefore, substantial attention has been focused on understanding and solving this devastating problem. In this current investigation, we aimed to explore the role of human IL-32γ in a cerebral I/R mouse model. The transient occlusion of the MCAO model closely resembles human stroke [30]. Interestingly, our data strongly suggest that IL-32γ overexpression showed a protective effect on the mouse brain from I/R-induced injury.

In this study, we determined the role of IL-32γ in an MCAO model using IL-32γ TG mice expressing human IL-32γ to study its role in acute ischemic stroke. Compared with WT mice, IL-32γ TG mice demonstrated a significant reduction of the infarct area following MCAO surgery. In line with the diminished brain tissue damage, IL-6, TNF-α, and IL-1β cytokine levels showed a significant reduction in the IL-32γ TG mice compared with WT mice. Moreover, the expression levels of phosphorylated NF-κB were significantly lower in IL-32γ TG mice compared to the WT mice. These data suggest that IL-32γ regulates regenerative inflammatory brain diseases and reduces ischemic damage through the inhibition of NF-κB activation.

IL-32 was originally characterized as a cytokine in 2005, and to date, more than nine isoforms have been identified, all of which are known to have distinct biological functions [31]. IL-32 is well known for its pro-and anti-inflammatory cytokine manipulation under health and diseases [16,32]. Furthermore, the association of IL-32 with the NF-κB signaling pathway has been reported in several inflammatory conditions [33] and cancer studies [34–38]. Limited studies have addressed the development of stroke with the expression of IL-32. However, lately, the role of IL-32 through stroke or brain injury is of interest to be investigated.

In 2015, a study aimed to evaluate the neuroinflammation changes using the MCAO mouse model showed that IL-32α-overexpressing TG mice demonstrated a reduction in cell death of the ischemic neurons. In addition, the anti-neuroinflammatory factor (IL-10) was improved as well as neuroinflammatory cytokines (IL-6, IL-1β, TNF-α), while the activation of astrocytes was reduced. Thus, indicating crosstalk between IL-32, specifically IL-32α and IL-6, IL-1β, and IL-10 in brain tissue under ischemia. In addition, the generation of ROS, lipid peroxidation, inducible nitric oxide, and cyclooxygenase-2 expression were also reduced, and they observed a lower level of NF-κB (blocking) but higher levels of active signal transducer and activator of transcription (STAT)3 (enhancement) in IL-32α-overexpressing mice brains compared to those of WT mice brain [39]. On the other hand, IL-32β worsens the condition through the same pathways, NF-κB and STAT3, and as a result, memory impairment increased, glia activated, amyloid-genesis and neuroinflammation increased [40]. Together, it suggested that IL-32α can prevent cerebral ischemia damage through anti-neuroinflammatory cytokine upregulation and activation of STAT3, but NF-κB inhibition, while IL-32β showed the opposite role. Here, our data suggests that IL-32γ plays a similar role to IL-32α, and both isoforms were shown to improve cerebral ischemia through inhibition of NF-κB pathway activation. Our investigation suggests that IL-32γ overexpression inhibits the activation of NF-κB. However, further exploration studies are needed to discover in depth the underlying IL-32γ signaling pathways in cerebral ischemia-reperfusion injury.

Recently, extremely limited studies have been published regarding IL-32 and its effect in response to hypoxia [41,42]. Out of two recent studies using a hypoxia/reoxygenation model, oxygen-glucose deprivation and reoxygenation (OGD/R) PC12 cells revealed that significant upregulated IL-32 expression, induced by OGD/R, corresponds to the up-regulated phosphorylation level of p65 and inhibitor of kappa Bα. In addition, they showed that IL-32 knockdown resulted in less production of intracellular ROS, higher superoxide dismutase activity, and cell viability improvement [41]. The other study similarly found that inhibiting IL-32 expression ameliorates cerebral ischemia-reperfusion injury via the nucleotide-binding oligomerization domain/mitogen-activated protein kinase/NF-κB signaling pathway [42]. Although, these data confirm that IL-32 redirects the response to hypoxia via mediated cytokine release and NF-κB signaling pathway. Yet, these two studies were subjected to a major limitation, which is ignoring the IL-32 dominant isoform. It is universally acknowledged that different IL-32 isoforms undoubtedly have distinct roles. Therefore, investigating pathophysiological mechanisms for each isoform of IL-32 will provide either targeted or therapeutic potential neuroprotective options after ischemic stroke.

In conclusion, the current in vivo experimental investigation provides insight into the effect of IL-32γ on the ischemic damage caused by acute stroke. IL-32γ showed a powerful effect as a neuroprotective role post-ischemic in which it provides a way for inhibiting acute neuronal damage. Further studies in this context are necessary to ascertain the efficacy of IL-32γ, as well as its other isoform in models of cerebral injury, to be screened for clinical use against ischemic neuronal damage.

Author Contributions: J.H., S.B., and H.J. performed the experiments, and wrote the first draft of the manuscript. J.H., S.B., Y.H and H.J analyzed the data. Y.H., T.H. and H.J. edited the manuscript. H.J. designed the study, supervised the project, and wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The animal experiments and procedures involved in this study were approved by the Institutional Animal Care and Use Committee of Konkuk University (Approval No. KU12056).

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The author declares no potential conflict of interest.

Abbreviations

CCA, Common carotid artery; ECA, External carotid artery; ELISA, Enzyme-linked immunosorbent assay; GAPDH, Glyceraldehyde-3-phosphate dehydrogenase; ICA, Internal carotid artery; IL, Interleukin; I/R, ischemia/reperfusion; LDF, Laser-Doppler flowmetry; MCAO, Middle cerebral artery occlusion; NF-κB, Phospho-nuclear factor kappa-light-chain-enhancer of activated B cells; OGD/R, Oxygen-glucose deprivation and reoxygenation; PBS, Phosphate-buffered saline; pNF-κB, Phosphorylated NF-κB; rCBF, Regional cerebral blood flow; ROS, Reactive oxygen species; STAT, Signal transducer and activator of transcripsion; TG, Transgenic; TNF, Tumor necrosis factor; TTC, Triphenyl tetrazolium chloride; TUNEL, Terminal deoxynucleotidyl transferase biotin-dUPT nick-end labeling; WT, wild-type.

References

1. Virani, S.S.; Alonso, A.; Aparicio, H.J.; Benjamin, E.J.; Bittencourt, M.S.; Callaway, C.W.; Carson, A.P.; Chamberlain, A.M.; Cheng, S.; Delling, F.N.; et al. Heart Disease and Stroke Statistics-2021 Update: A Report From the American Heart Association. *Circulation* **2021**, *143*, e254–e743. https://doi.org/10.1161/CIR.0000000000000950.
2. Katan, M.; Luft, A. Global Burden of Stroke. *Semin. Neurol.* **2018**, *38*, 208–211. https://doi.org/10.1055/s-0038-1649503.
3. Sveinsson, O.A.; Kjartansson, O.; Valdimarsson, E.M. Cerebral ischemia/infarction—Epidemiology, causes and symptoms. *Laeknabladid* **2014**, *100*, 271–279. https://doi.org/10.17992/lbl.2014.05.543.
4. Orellana-Urzua, S.; Rojas, I.; Libano, L.; Rodrigo, R. Pathophysiology of Ischemic Stroke: Role of Oxidative Stress. *Curr. Pharm. Des.* **2020**, *26*, 4246–4260. https://doi.org/10.2174/1381612826666200708133912.
5. Huang, J.; Upadhyay, U.M.; Tamargo, R.J. Inflammation in stroke and focal cerebral ischemia. *Surg. Neurol.* **2006**, *66*, 232–245. https://doi.org/10.1016/j.surneu.2005.12.028.
6. Shi, K.; Tian, D.C.; Li, Z.G.; Ducruet, A.F.; Lawton, M.T.; Shi, F.D. Global brain inflammation in stroke. *Lancet Neurol.* **2019**, *18*, 1058–1066. https://doi.org/10.1016/S1474-4422(19)30078-X.
7. Jin, R.; Liu, L.; Zhang, S.; Nanda, A.; Li, G. Role of inflammation and its mediators in acute ischemic stroke. *J. Cardiovasc. Transl. Res.* **2013**, *6*, 834–851. https://doi.org/10.1007/s12265-013-9508-6.
8. Kim, E.; Cho, S. Microglia and Monocyte-Derived Macrophages in Stroke. *Neurotherapeutics* **2016**, *13*, 702–718. https://doi.org/10.1007/s13311-016-0463-1.
9. Drieu, A.; Buendia, I.; Levard, D.; Helie, P.; Brodin, C.; Vivien, D.; Rubio, M. Immune Responses and Anti-inflammatory Strategies in a Clinically Relevant Model of Thromboembolic Ischemic Stroke with Reperfusion. *Transl. Stroke Res.* **2020**, *11*, 481–495. https://doi.org/10.1007/s12975-019-00733-8.
10. Drieu, A.; Levard, D.; Vivien, D.; Rubio, M. Anti-inflammatory treatments for stroke: From bench to bedside. *Ther. Adv. Neurol. Disord.* **2018**, *11*. https://doi.org/10.1177/1756286418789854.
11. Heinz, R.; Brandenburg, S.; Nieminen-Kelha, M.; Kremenetskaia, I.; Boehm-Sturm, P.; Vajkoczy, P.; Schneider, U.C. Microglia as target for anti-inflammatory approaches to prevent secondary brain injury after subarachnoid hemorrhage (SAH). *J. Neuroinflamm.* **2021**, *18*, 36. https://doi.org/10.1186/s12974-021-02085-3.
12. Choi, J.; Bae, S.; Hong, J.; Ryoo, S.; Jhun, H.; Hong, K.; Yoon, D.; Lee, S.; Her, E.; Choi, W.; et al. Paradoxical effects of constitutive human IL-32{gamma} in transgenic mice during experimental colitis. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 21082–21086. https://doi.org/10.1073/pnas.1015418107.
13. Joosten, L.A.; Netea, M.G.; Kim, S.H.; Yoon, D.Y.; Oppers-Walgreen, B.; Radstake, T.R.; Barrera, P.; van de Loo, F.A.; Dinarello, C.A.; van den Berg, W.B. IL-32, a proinflammatory cytokine in rheumatoid arthritis. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 3298–3303.
14. Shioya, M.; Nishida, A.; Yagi, Y.; Ogawa, A.; Tsujikawa, T.; Kim-Mitsuyama, S.; Takayanagi, A.; Shimizu, N.; Fujiyama, Y.; Andoh, A. Epithelial overexpression of interleukin-32alpha in inflammatory bowel disease. *Clin. Exp. Immunol.* **2007**, *149*, 480–486. https://doi.org/10.1111/j.1365-2249.2007.03439.x.

15. Calabrese, F.; Baraldo, S.; Bazzan, E.; Lunardi, F.; Rea, F.; Maestrelli, P.; Turato, G.; Lokar-Oliani, K.; Papi, A.; Zuin, R.; et al. IL-32, a novel proinflammatory cytokine in chronic obstructive pulmonary disease. *Am. J. Respir. Crit. Care Med.* **2008**, *178*, 894–901. https://doi.org/10.1164/rccm.200804-646OC.
16. Dinarello, C.A.; Kim, S.H. IL-32, a novel cytokine with a possible role in disease. *Ann. Rheum. Dis.* **2006**, *65* (Suppl. 3), 61–64.
17. Hong, J.T.; Son, D.J.; Lee, C.K.; Yoon, D.Y.; Lee, D.H.; Park, M.H. Interleukin 32, inflammation and cancer. *Pharmacol. Ther.* **2017**, *174*, 127–137. https://doi.org/10.1016/j.pharmthera.2017.02.025.
18. Gong, L.; Dong, C.; Cai, Q.; Ouyang, W. Interleukin 32: A novel player in perioperative neurocognitive disorders. *Med. Hypotheses.* **2020**, *144*, 110158. https://doi.org/10.1016/j.mehy.2020.110158.
19. Safari-Alighiarloo, N.; Taghizadeh, M.; Mohammad Tabatabaei, S.; Namaki, S.; Rezaei-Tavirani, M. Identification of common key genes and pathways between type 1 diabetes and multiple sclerosis using transcriptome and interactome analysis. *Endocrine* **2020**, *68*, 81–92. https://doi.org/10.1007/s12020-019-02181-8.
20. Hamzaoui, K.; Borhani-Haghighi, A.; Dhifallah, I.B.; Hamzaoui, A. Elevated levels of IL-32 in cerebrospinal fluid of neuro-Behcet disease: Correlation with NLRP3 inflammasome. *J. Neuroimmunol.* **2022**, *365*, 577820. https://doi.org/10.1016/j.jneuroim.2022.577820.
21. Cho, K.S.; Park, S.H.; Joo, S.H.; Kim, S.H.; Shin, C.Y. The effects of IL-32 on the inflammatory activation of cultured rat primary astrocytes. *Biochem. Biophys. Res. Commun.* **2010**, *402*, 48–53. https://doi.org/10.1016/j.bbrc.2010.09.099.
22. Sohn, D.H.; Nguyen, T.T.; Kim, S.; Shim, S.; Lee, S.; Lee, Y.; Jhun, H.; Azam, T.; Kim, J.; Kim, S. Structural Characteristics of Seven IL-32 Variants. *Immune Netw.* **2019**, *19*, e8.
23. Kim, S.; Yu, H.; Azam, T.; Dinarello, C.A. Interleukin-18 Binding Protein (IL-18BP): A Long Journey From Discovery to Clinical Application. *Immune Netw.* **2024**, *24*, e1.
24. Hua, F.; Ma, J.; Ha, T.; Kelley, J.; Williams, D.L.; Kao, R.L.; Kalbfleisch, J.H.; Browder, I.W.; Li, C. Preconditioning with a TLR2 specific ligand increases resistance to cerebral ischemia/reperfusion injury. *J. Neuroimmunol.* **2008**, *199*, 75–82. https://doi.org/10.1016/j.jneuroim.2008.05.009.
25. Chen, Y.; Ito, A.; Takai, K.; Saito, N. Blocking pterygopalatine arterial blood flow decreases infarct volume variability in a mouse model of intraluminal suture middle cerebral artery occlusion. *J. Neurosci. Methods* **2008**, *174*, 18–24. https://doi.org/10.1016/j.jneumeth.2008.06.021.
26. Reglodi, D.; Tamas, A.; Somogyvari-Vigh, A.; Szanto, Z.; Kertes, E.; Lenard, L.; Arimura, A.; Lengvari, I. Effects of pretreatment with PACAP on the infarct size and functional outcome in rat permanent focal cerebral ischemia. *Peptides* **2002**, *23*, 2227–2234.
27. Hatcher, J.P.; Virley, D.; Hadingham, S.J.; Roberts, J.; Hunter, A.J.; Parsons, A.A. The behavioural effect of middle cerebral artery occlusion on apolipoprotein-E deficient mice. *Behav. Brain Res.* **2002**, *131*, 139–149.
28. Nurmi, A.; Lindsberg, P.J.; Koistinaho, M.; Zhang, W.; Juettler, E.; Karjalainen-Lindsberg, M.L.; Weih, F.; Frank, N.; Schwaninger, M.; Koistinaho, J. Nuclear factor-kappaB contributes to infarction after permanent focal ischemia. *Stroke* **2004**, *35*, 987–991. https://doi.org/10.1161/01.STR.0000120732.45951.26.
29. Howell, J.A.; Bidwell, G.L., 3rd. Targeting the NF-kappaB pathway for therapy of ischemic stroke. *Ther. Deliv.* **2020**, *11*, 113–123. https://doi.org/10.4155/tde-2019-0075.
30. Ginsberg, M.D.; Busto, R. Rodent models of cerebral ischemia. *Stroke* **1989**, *20*, 1627–1642. https://doi.org/10.1161/01.str.20.12.1627.
31. Kim, S.H.; Han, S.Y.; Azam, T.; Yoon, D.Y.; Dinarello, C.A. Interleukin-32: A cytokine and inducer of TNFalpha. *Immunity* **2005**, *22*, 131–142.
32. Aass, K.R.; Kastnes, M.H.; Standal, T. Molecular interactions and functions of IL-32. *J. Leukoc. Biol.* **2021**, *109*, 143–159. https://doi.org/10.1002/JLB.3MR0620-550R.
33. Park, H.M.; Park, J.Y.; Kim, N.Y.; Kim, H.; Kim, H.G.; Son, D.J.; Hong, J.T.; Yoon, D.Y. Recombinant Human IL-32theta Induces Polarization Into M1-like Macrophage in Human Monocytic Cells. *Immune Netw.* **2024**, *24*, e27. https://doi.org/10.4110/in.2024.24.e27.
34. Lee, Y.S.; Han, S.B.; Ham, H.J.; Park, J.H.; Lee, J.S.; Hwang, D.Y.; Jung, Y.S.; Yoon, D.Y.; Hong, J.T. IL-32gamma suppressed atopic dermatitis through inhibition of miR-205 expression via inactivation of nuclear factor-kappa B. *J. Allergy Clin. Immunol.* **2020**, *146*, 156–168. https://doi.org/10.1016/j.jaci.2019.12.905.
35. Nam, S.Y.; Kim, H.M.; Jeong, H.J. Attenuation of IL-32-induced caspase-1 and nuclear factor-kappaB activations by acteoside. *Int. Immunopharmacol.* **2015**, *29*, 574–582. https://doi.org/10.1016/j.intimp.2015.09.026.
36. Al-Shobaili, H.A.; Farhan, J.; Zafar, U.; Rasheed, Z. Functional role of human interleukin-32 and nuclear transcription factor-kB in patients with psoriasis and psoriatic arthritis. *Int. J. Health Sci.* **2018**, *12*, 29–34.
37. Lin, J.; Xu, R.; Hu, L.; You, J.; Jiang, N.; Li, C.; Che, C.; Wang, Q.; Xu, Q.; Li, J.; et al. Interleukin-32 induced thymic stromal lymphopoietin plays a critical role in the inflammatory response in human corneal epithelium. *Cell. Signal.* **2018**, *49*, 39–45. https://doi.org/10.1016/j.cellsig.2018.05.007.

38. Cao, H.; Pan, X.F.; Zhang, K.; Shu, X.; Li, G. Interleukin-32 expression is induced by hepatitis B virus. *Zhonghua Gan Zang Bing Za Zhi* **2013**, *21*, 442–445. https://doi.org/10.3760/cma.j.issn.1007-3418.2013.06.014.
39. Hwang, C.J.; Yun, H.M.; Jung, Y.Y.; Lee, D.H.; Yoon, N.Y.; Seo, H.O.; Han, J.Y.; Oh, K.W.; Choi, D.Y.; Han, S.B.; et al. Reducing effect of IL-32alpha in the development of stroke through blocking of NF-kappaB, but enhancement of STAT3 pathways. *Mol. Neurobiol.* **2015**, *51*, 648–660. https://doi.org/10.1007/s12035-014-8739-0.
40. Yun, H.M.; Kim, J.A.; Hwang, C.J.; Jin, P.; Baek, M.K.; Lee, J.M.; Hong, J.E.; Lee, S.M.; Han, S.B.; Oh, K.W.; et al. Neuroinflammatory and Amyloidogenic Activities of IL-32beta in Alzheimer's Disease. *Mol. Neurobiol.* **2015**, *52*, 341–352. https://doi.org/10.1007/s12035-014-8860-0.
41. Yin, H.; Wu, M.; Jia, Y. Knockdown of IL-32 protects PC12 cells against oxygen-glucose deprivation/reoxygenation-induced injury via activation of Nrf2/NF-kappaB pathway. *Metab. Brain Dis.* **2020**, *35*, 363–371. https://doi.org/10.1007/s11011-019-00530-0.
42. Liu, C.; Xu, X.; Huang, C.; Shang, D.; Zhang, L.; Wang, Y. Inhibition of IL-32 Expression Ameliorates Cerebral Ischemia-Reperfusion Injury via the NOD/MAPK/NF-kappaB Signaling Pathway. *J. Mol. Neurosci.* **2020**, *70*, 1713–1727. https://doi.org/10.1007/s12031-020-01557-0.