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The effect of minocycline in suppressing TGF-B1 expression to reduce the formation of glial scar in traumatic brain injury model rats

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ABSTRACT

Aims: Traumatic brain injury (TBI) is a long-term disorder that results in lifelong disability, often in the form of cognitive impairment due to glial scar formation. Treatment with minocycline may reduce the transforming growth factor-beta-1 (TGF-β1) pathway. Our assumption was that minocycline also contributed to the inhibition of glial scar via the TGF-β1 pathway in TBI. The study aimed to assess how minocycline affects the TGF-β1 signaling pathway in TBI model rats to inhibit the formation of glial scars.

Methods: Twenty male Sprague Dawley rats were divided in five groups, with four rats per group. Day 0 of TBI using the weight drop model was followed by 14 days of minocycline treatment at doses of 25 mg/kg (MNO1 group), 50 mg/kg (MNO2 group), and 100 mg/kg (MNO3 group). On day 15, the novel object recognition (NOR) test was performed. On day 16, immunofluorescence double staining was carried out.

Results: In rats with TBI models, minocycline inhibited the formation of glial scars. It has been demonstrated that minocycline inhibits the formation of TGF-β1, which in effect inhibits the formation of glial scar in the perilesional area of TBI model rats. In TBI model rats, inhibition of glial scar may ameliorate cognitive function deficit. In TBI model rats, minocycline administration can improve cognitive function.

Conclusion: Through the reduction of TGF-β1 expression, minocycline inhibited glial scar formation in a rat model of TBI.

Keywords: Glial scar, minocycline, rats, TGF-β1, traumatic brain injury

INTRODUCTION

Traumatic brain injury (TBI) is a major cause of mortality and disability, especially in young and productive populations. The majority of TBI are caused by motor vehicle accidents, as suggested by Cortes and Pera.[1] Although there has been a decrease in mortality due to improved trauma management systems and vehicle safety technology, disability rates, particularly neurobehavioral disorders that persist after brain injury, are still high. Currently, there is no effective treatment, creating significant challenges for the state, families, individuals and the patient's environment, as noted by Zhou.[2]

One of the factors causing the disability that persists after a brain injury is the formation of a glial scar, which is believed to inhibit axonal regeneration and counteract the recovery process of brain injuries. This phenomenon occurs because the astrocytes become reactive in response to brain injury, undergo hypertrophy, proliferation, and form a dense tissue called a glial scar around the perilesion area. This glial scar becomes a physical and biochemical barrier that inhibits the healing process in the advanced phase.[3,4] One of the key signaling molecules involved in this process is Transforming Growth Factor-Beta 1 (TGF-β1), which plays a central role in astrocyte activation through the SMAD signaling pathway. [5]

Once TGF-β1 binds to its receptor on the astrocyte surface, it activates intracellular SMAD proteins that translocate into the nucleus and function as transcription factors. These transcription factors bind to specific DNA sequences in the astrocyte nucleus, promoting the expression of genes responsible for cellular hypertrophy, proliferation, and migration. This cascade ultimately contributes to the formation of the glial scar during the chronic phase of TBI. The resulting scar formation interferes with neural regeneration and may cause persistent neurobehavioral deficits, including motor, cognitive, and emotional impairments.[4,6-8]

The research for drugs with neuroprotective properties is still ongoing, and one that is being studied is minocycline, a second-generation derivative of the tetracycline group. Minocycline has attracted attention because it has been shown to have anti-inflammatory, antiapoptotic, and antioxidant effects in previous preclinical studies. Furthermore, minocycline has received approval from the United States Food and Drug

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Administration (FDA) for use in humans, and its availability is affordable.[9] In addition to the benefits already described, studies on conditions such as hydrocephalus, ischemic stroke, spinal cord injury, and neurodegenerative disorders have also shown that minocycline can reduce astrogliosis.[10,11] Studies in different organs and diseases have also found that minocycline has the ability to inhibit TGF-β1.[12,13] Based on these findings, we hypothesized that minocycline may also play a role in inhibiting glial scar formation via the TGF-β1 pathway in TBI.

METHODS

Ethics

This study was approved by the Universitas Brawijaya Ethics Committee (Approval Date: 03.11.2022, Decision No: 246/EC/KEPK-S3/11/2022). All procedures were carried out in accordance with the ethical rules and the principles, and adhered to animal welfare regulations.

Animals

Twenty male Sprague Dawley rats weighing between 250 and 350 grams at three months old were acquired from Universitas Brawijaya. Before using them in the experiment, the rats were acclimated for seven days. The rats were kept in controlled environments with a 12-hour cycle of light and dark, a constant temperature of 23±2°C, a constant humidity of 55±5%, and free access to standardized food and water. Anxiety was decreased by using a single rat at a time. Rats with postoperative wound infections, fatalities, or illnesses unrelated to the trial were not allowed to participate in the research.

Experimental Procedure

Five groups of rats were created: (1) WD: brain injury (n=4); (2) MNO1: brain injury+minocycline 25 mg/kg (n=4); (3) MNO2: brain injury+minocycline 50 mg/kg (n=4); (4) MNO3: brain injury+minocycline 100 mg/kg (n=4); and (5) Sham: healthy rats (n=4). By using the weight drop model, brain injury was created on day 0, and was given minocycline treatment from day 1 to day 14. On day 15, the novel object recognition (NOR) test was administered to every rat. On day 16, rats were euthanized to be examined histologically using immunofluorescence. Every group experienced the same consistent standardization of procedures. The outcomes were assessed by specialists who were unaware of the treatment group for the animals. The experiment's procedure is shown in **Figure 1A**.

TBI (Weight Drop Model)

The weight drop model method was used by Wardhana[14] to induce TBI in rats. Using a computer program, rats were chosen at random from the randomization results. Prophylactic antibiotics Cefazolin sodium 33 mg/kg and ketamine xylazine 0.05 cc i.p. were administered to rats as an anesthetic. Rats were fixed using a stereotactic device, and their scalps were subsequently shaved and washed using a 10% povidone-iodine solution. After that, a 10 mm-diameter craniectomy was performed on the right side of the brain, 3 mm lateral right of the midline, and 2.5 mm posterior to the bregma. The brain surface was injured by a 10-gram weight with a tip diameter of roughly 2.5 mm, which was dropped from a height of 10 cm. The piston was only permitted to compress the tissue to the extent of 2.5 mm. The rats had an adequate recovery after this operation, with minor localized lesions, obvious edema, and a moderate brain injury. A series of interrupted 6-0 silk sutures were used to seal the skin. The rats were then housed at room temperature (23±1°C) in cages with one rat per cage. The rats were divided into WD, MNO1, MNO2, and MNO3 group (n=4 for each group).

Minocycline Administration

For 14 days, minocycline was taken orally once a day, beginning the day after the weight drop treatment. The doses were as follows: 25 mg/kg (MNO1 group), 50 mg/kg (MNO2 group), and 100 mg/kg (MNO3 group). The drug was delivered via oral gavage using a flexible feeding tube to ensure accurate and consistent dosing. Drug administration was performed by laboratory staff who were blinded to the experimental group.

Novel Object Recognition Test

The test was carried out in an arena (40 cm x40 cm x40 cm), with 20 lux lighting, regulated humidity and temperature, and an overhead camera for the best viewing. To minimize anxiety, the testing environment was maintained at a constant volume for every group. Blinded observers conducted observations to the groups. The test consists of three stages: habituation, familiarization, and testing. In the habitation phase, rats are placed in an empty arena for 5 minutes. After 24 hours, the rats enter the training phase in which the rats are put back into an arena containing two identical objects placed on opposite quadrants in the arena. The size of both objects corresponds to the size of a mouse or slightly larger to encourage exploration. The rats were allowed to explore objects for 10 minutes. One hour after the training, the test was carried out by replacing one of the objects with a new one and placed in the same position as in the training phase. After testing, the animals were returned to the cage, while the arena and objects were cleaned using 70% ethanol (**Figure 1B**). The definition of exploration was described as climbing on an object or sniffing or contacting it with the nose and/or front paws within a distance of two to three centimeters. Using Ethovision software, the Discrimination Index [(time spent near the new object-old object)/(time spent near the new+old object)] was used to assess cognitive impairment.

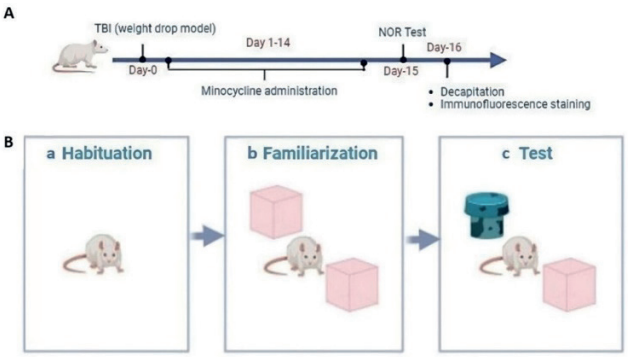

Figure 1. (A) Experimental procedure of the study; (B) Schematic of the novel object recognition test. (a) Rats are habituated to an empty arena; (b) Rats are familiarized to an arena that has been given two identical objects; (c) Rats are tested in an arena with one of the objects replaced with a novel object

Tissue Preparation

On day 16 post-injury, rats were anesthetized with ketamine and xylazine i.p, secured on a table, underwent a small skull incision, and the brain was carefully removed. The brain samples were placed in identical containers that had

been filled with 10% neutral buffered formalin. All parts of the brain were ensured to be completely submerged in formalin. After 24 hours, coronal brain sections (5 µm) were obtained from the perilateral region (Figure 2). Dehydration, paraffin infiltration, and freezing followed. Paraffin blocks were then thinly sectioned, and mounted on slides for immunofluorescence staining. Slide preparation was performed by an anatomical pathologist who was blinded to the experimental group.

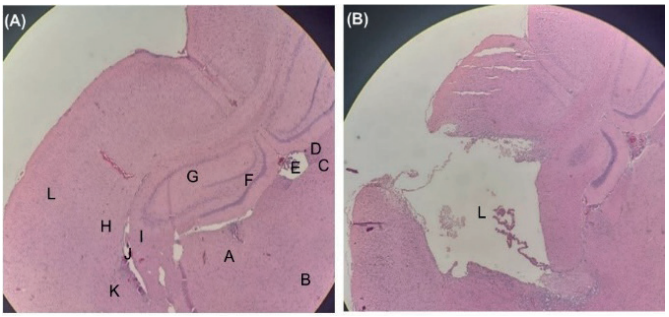

Figure 2. Hematoxylin and eosin staining of coronal sections of the right frontal region of the rat brain. (A) Sham group (healthy rats) with the following parts: A: Thalamus, B: Hypothalamus, C: Habenular nuclei, D: Stria medullaris thalami, E: 3rd ventricle, F: Dentate gyrus, G: Hippocampus, H: Corpus callosum, I: Fimbria, J: Lateral ventricle, K: Caudate putamen (Striatum), L: Neocortex. (B) WD group (rats with injury), L: Neocortex, injury area due to weight drop model. Observations were made using light binocular Olympus BX51 microscope at 40x magnification

Immunofluorescence

Immunofluorescence double staining Glial Fibrillary Acidic Protein (GFAP) and TGF-β1 was performed in all groups to evaluate the intensity of GFAP and TGF-β1. Paraffin-embedded tissue slides were incubated at 40°C overnight and then immersed in xylol solution 1 and xylol solution 2 (10 minutes each); ethanol absolute 1, ethanol absolute 2, ethanol 90%, ethanol 70%, phosphate-buffered saline (PBS) + Tween 1, PBS + Tween 2, and PBS + Tween 3 (5 minutes each). The slides were placed in a heat-resistant container filled with 10 mM pH 6 citrate buffer solution, incubated in a 120°C oven (15 minutes), and then allowed to stand at room temperature (10 minutes). The slides were washed with PBS+Tween (3x5 minutes), dried, blocked with 2% bovine serum albumin (BSA) solution in PBS+Tween, and incubated at room temperature (1 hour). The slides were washed with PBS+Tween (3x8 min), dried, and then stained with primary antibody solutions including antiastrocyte-GFAP antibody (1022 Mouse monoclonal IgG2b, Santa Cruz Biotechnology) and anti-TGF-β1 (Rabbit, Thermo Fisher Scientific) in 2% BSA, incubated overnight at 4°C, washed with PBS+Tween 3x8 min, and dried. Slides were stained with secondary antibody solution containing fluorescent dye in 2% BSA, incubated at room temperature (1 hour), washed with PBS + Tween 3x8 minutes, dried, stained with 25 µl of 10% glycerol solution, and covered with cover glass. Slides were observed with confocal laser scanning microscope (CLSM) (Olympus, Japan) and analyzed using OLYMPUS FLUOVIEW FV1000 ver.4.2a software. Observations were made by analysts who were blinded to the treatment groups.

Statistical Analysis

SPSS version 26 (IBM Corp.) was used for the analysis, and GraphPad Prism version 10.0.0.153 (GraphPad Software LLC) was used for the visualization. The mean±SD of four replicates in each group was used to show the studied results. For normal data, One-Way Analysis of Variance (ANOVA) and Post Hoc Bonferroni were utilized; for nonnormal data, Kruskal-Wallis and Mann-Whitney tests were applied. The Pearson Correlation test was used to measure the correlation between the variables, and linear regression was used to measure the impact of the independent variable on the dependent variable. p-values less than 0.05 were regarded as statistically significant.

RESULTS

In this study all rats were included without any drop out.

Minocycline Reduces Glial Scar Formation

Through an unpaired T test, there was a significant difference in GFAP intensity in the WD vs sham groups ($p<0.05$, n=4 for each group) (Table 1). This result confirmed the formation of glial scar after TBI. Furthermore, through immunofluorescent observation, there was a decrease in GFAP intensity in the brain, especially in the perilesion area in the group of rats treated with minocycline as the drug dose increased (Figure 3A). Through post-hoc Bonferoni tests, the intensity of GFAP in the MNO2 (50 mg/kg) and MNO3 (100 mg/kg) groups differs significantly from the WD group (n=4 for each group). Thus, the dosage that has the effect of inhibiting the formation of glial scar is in the range of 50-100mg/kg (Figure 4A). Based on the results of the regression test, minocycline affects the intensity of GFAP with a ratio of 72.2% (Table 2).

Table 1. Comparison of GFAP, TGF-β1, and DI in WD and Sham groups

	Mean±SD		
Variable	WD (n=4)	Sham (n=4)	p-value
GFAP	2433.038±543.386	278.316±21.190	0.000*
TGF-β1	1179.794±266.931	296.779±131.293	0.003*
DI	-0.490±0.056	0.648±0.244	0.002*

GFAP: Glial fibrillary acidic protein, TGF-β1: Transforming growth factor-beta, DI: Discrimination Index, WD: Weight drop, SD: Standard deviation, *p<0.05

Minocycline Reduces TGF-β1 Intensity

The unpaired T test showed a significant difference in TGF-β1 intensity in the WD vs Sham group ($p<0.05$, n=4 for each group) which proved an increase in TGF-β1 intensity after TBI (Table 1). The WD group had the highest TGF-β1 intensity and decreased intensity in the MNO group. The higher the

Table 2. Effects of minocycline in inhibiting GFAP intensity, TGF-β1 intensity, and improving cognitive function

	Mean±SD					
Variable	WD (n=4)	MNO1 (n=4)	MNO2 (n=4)	MNO3 (n=4)	p-value	Coefficient of determination
GFAP	2433.038±543.386	1673.138±339.169	1194.055±325.319	888.237±128.993	0.000*	0.722
TGF-β1	1179.794±266.931	898.893±379.590	619.930±270.418	411.674±158.022	0.012*	0.485
DI	-0.490±0.056	-0.094±0.578	0.247±0.254	0.464±0.339	0.014*	0.467

GFAP: Glial fibrillary acidic protein, TGF-β1: Transforming growth factor-beta, SD: Standard deviation, WD: Weight drop, MNO: Minocycline, DI: Discrimination Index, *p<0.05

MNO dose, the lower the TGF-β1 intensity (Figure 3B). Statistically, the intensity of TGF-β1 in the WD group was significantly different from MNO3 (p-value 0.013) (Figure 4B) (n=4 for each group). The regression test proved the effect of minocycline on TGF-β1 intensity with an effect proportion of 48.5% (Table 2).

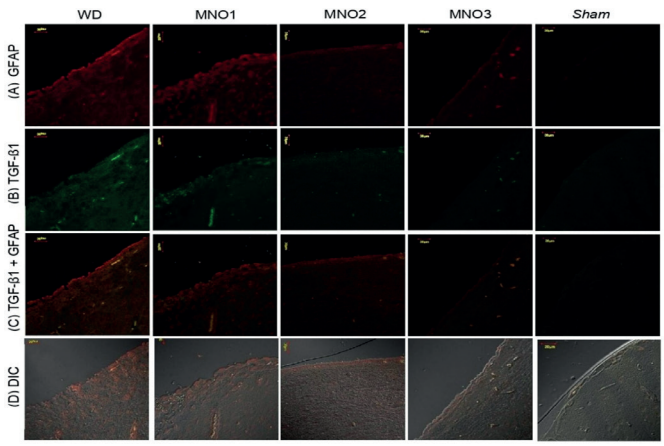

Figure 3. The representation of the results of immunofluorescence double staining analysis of GFAP and TGF-β1 in the brain in the perilesional area. (A) The trend of GFAP intensity shown through red color (rhodamine). GFAP indicates the location of glial scar in the brain in the perilesion area. (B) The trend of TGF-β1 intensity shown through the green (FITC) of the antibody attached to the TGF-β1 antigen in the brain. (C) The trend of GFAP+TGF-β1 intensity through yellow color double staining to assess TGF-β1 at the glial scar location. (D) The trend of DIC image with high resolution and contrast to facilitate the determination of data collection locations. Staining was performed by immunofluorescence double staining method using antiastrocyte-GFAP antibody (mouse) and anti-TGF-β1 antibody (rabbit). Observations were made using confocal laser scanning microscopy (CLSM, Olympuse, Japan) at 400x magnification. Image scale: 30 µm

GFAP: Glial fibrillary acidic protein, TGF-β1: Transforming growth factor-beta, FITC: Fluorescein isothiocyanate, DIC: Differential interference contrast, WD: Weight drop, MNO: Minocycline

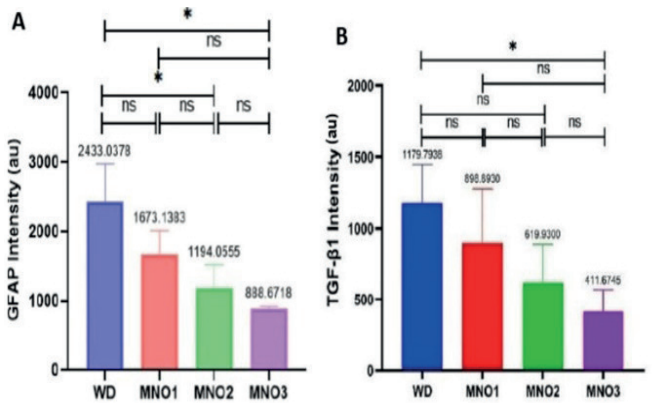

Figure 4. Comparison of GFAP and TGF-β1 intensity in WD vs MNO groups in TBI model rats. (A) There is a significant difference in GFAP intensity between WD vs MNO2 (p-value 0.002) and WD vs MNO3 (p-value 0.000). The intensity of GFAP was assessed through the color luminescence of antiastrocyte-GFAP antibody (mouse) by immunofluorescence double staining. (B) There was a significant difference in TGF-β1 intensity between WD vs MNO3 (p-value: 0.013). The intensity of TGF-β1 was assessed through the color luminescence of anti-TGF-β1 antibody (rabbit) by immunofluorescence double staining. Data are presented as mean±standard deviation. Differences in intensity between groups were performed through Post Hoc Bonferroni test (n=4 for each group). *p<0.05 vs. WD group.

ns: not significant, indicates there is no difference between the two groups, WD: Weight drop, rats were given injury, MNO: Minocycline, rats were given injury and minocycline treatment; au: Arbitrary unit. units of fluorescence-labeled antibody color luminescence intensity

Inhibition of TGF-β1 Intensity Reduces Glial Scar Formation

The correlation analysis showed a strong significant relationship between TGF-β1 intensity and GFAP intensity of 66.3%. This relationship is positive, meaning that inhibition of TGF-β1 intensity can inhibit GFAP formation. Furthermore, regression analysis was carried out which showed that inhibition of TGF-β1 intensity had an effect in inhibiting the formation of glial scar by 40.9% (Table 3).

Table 3. Effect of TGF-β1 intensity on GFAP (glial scar) intensity

Variable	Correlation of TGF-β1 and GFAP		Effect of TGF-β1 on GFAP	
	p-value	Coefficient	p-value	Coefficient
TGF-β1	0.001*	0.663	0.001*	0.409
GFAP				

TGF-β1: Transforming growth factor-beta, GFAP: Glial fibrillary acidic protein, *p<0.05

Inhibition of Glial Scar Formation Improves Cognitive Function Impairment

Through correlation analysis, there was a strong significant relationship between GFAP intensity and cognitive function of rats by 72.8%. A decrease in GFAP intensity will improve the cognitive function of rats. Through regression analysis, it was found that GFAP had a significant effect on the cognitive function of rats with an effect proportion of 50.4% (Table 4).

Table 4. Effect of GFAP (glial scar) intensity on cognitive impairment

Variable	Correlation of GFAP and DI		Effect of GFAP on DI	
	p-value	Coefficient	p-value	Coefficient
GFAP	0.000*	-0.728	0.000*	0.504
DI				

GFAP: Glial Fibrillary Acidic Protein; DI: Discrimination Index, *p<0.05

Minocycline Improves Cognitive Function

We conducted a Mann-Whitney test which showed a significant difference in the cognitive condition of rats in the WD vs Sham group, confirming the presence of cognitive impairment after injury (p<0.05, n=4 for each group) (Table 1). Through different tests of WD vs MNO, there was a significant difference in cognitive function in the WD vs MNO2 (p-value 0.029), and WD vs MNO3 (p-value 0.029); however, there were no significant differences between MNO2 vs MNO3 (p-value 0.2) (n=4 for each group) which indicated that a dose of 50-100 mg/kg had an improvement effect on cognitive function in rats (Figure 5). The results of regression analysis showed that minocycline have an influence on cognitive function with a ratio of influence of 46.7% (Table 2).

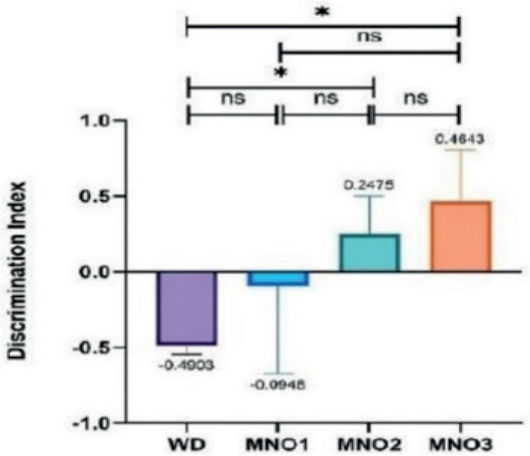

Figure 5. Comparison of Discrimination Index in WD vs MNO groups in TBI model rats. There was a significant difference in the discrimination index between WD vs MNO2 (p-value 0.029), and WD vs MNO3 (p-value 0.029). Data are presented as mean±standard deviation. Differences in discrimination index were performed through the Mann-Whitney test (n=4 for each group). *p<0.05 vs. WD group.

ns: not significant, indicating there is no difference between the two groups. WD: Weight drop, rats were given injury; MNO: Minocycline, rats were given injury and minocycline treatment

DISCUSSION

Research involving the role of minocycline as a neuroprotectant to improve cognitive impairment in brain-injured rat models is growing.[15-19] The results of this study showed cognitive improvement through the NOR test in the group of brain-injured rats that received minocycline therapy, especially significant at the second dose (50 mg/kg) and the third dose (100 mg/kg) (**Figure 5**). This finding is in line with previous studies, such as those conducted by Kovesdi,[15] Kumar,[16] Lam,[17] Sangobowale,[18] and Simon[19] which also support the role of minocycline in improving cognitive function with dose variations between 22.5 to 90 mg/kg. The neuroprotective benefits of the drug might be observed at lower dosages in the prior investigation since the intraperitoneal route was utilized to provide the lowest dose of 22.5 mg/kg. Minocycline was given in this study at doses ranging from 25 to 100 mg/kg. However, in accordance with the results of Kumar's study,[16] a dose of 50 mg/kg administered orally demonstrated to considerably give neuroprotective effects. Given that the majority of neuroprotective medications taken by humans are often administered orally over an extended period of time, the oral route was selected to enable further application in humans. These findings support the clinical relevance of oral minocycline delivery, although further translational studies are needed to confirm its efficacy and safety in humans.

There have been many previous investigations on the effects of minocycline on the central nervous system, with cell targets including microglia, oligodendrocytes, and neuronal cells through antioxidant, anti-inflammatory, and antiapoptotic pathways in brain injury.[15-21] However minocycline's effect on astrocyte cells has not been investigated.[22] Mincycline's specific effects on astrocyte cells-which are known to be involved in the formation of glial scars-were the focus of this study. Regeneration of the central nervous system is hypothesized to be inhibited by these glial scars. This study used the weight drop model, as described in Wardhana,[14] to induce glial scar formation by increasing GFAP intensity in rats that had brain injuries. Once minocycline was administered to the rat, the effect of this medication on the suppression of GFAP intensity was observed. A considerable reduction in GFAP intensity in the perilesion area was observed in the 50 mg/kg and 100 mg/kg dosing groups after two weeks of minocycline administration (**Figure 4A**). When rats with brain injury were given minocycline, the intensity of their GFAP was reduced by 72.2% (**Table 2**). These results highlight the potential of minocycline in modulating astrocyte response, although the underlying mechanisms and long-term effects require further elucidation.

The researchers intend to determine how the role of minocycline in the inhibition of glial scar formation. **Figure 3B** shows that the administration of minocycline significantly reduced the intensity of TGF-β1 in rats with brain injury at a dose of 100 mg/kg. The administration of minocycline contributed to the decrease in TGF-β1 intensity by 48.5% (**Table 2**). Inhibition of TGF-β1 through minocycline treatment has also been proven in previous research by Ataie-Kachoie[12] on ovarian cancer. In that study, minocycline administration by targeting NF-kB through suppression of TGF-β1-TAK1-IkB signaling can downregulate TGF-β1 expression.

In glial scar, inhibition of TGF-β1 will reduce the TGF-β/SMAD signaling pathway, reducing glial scar formation and facilitating intrinsic axonal growth and regeneration.[5] Given the critical role of TGF-β signaling in glial scar formation, blocking TGF-β is an effective antifibrotic therapeutic strategy in treating central nervous system (CNS) injury. This is supported by the results of the regression analysis in this study which showed a significant positive effect of TGF-β1 on GFAP by 40.9% (**Table 3**). Thus, inhibition of TGF-β1 will inhibit the formation of glial scar in rats modeling brain injury. Nevertheless, the statistical analysis should be interpreted with caution due to the small sample size, which may limit the robustness and generalizability of these findings.

The development of glial scar, which prevents axon healing and neuronal function recovery in the central nervous system and triggers aberrant molecular mechanisms in the late stages of injury, maybe the cause of persistent impairment of cognitive function. Therefore, it is believed that inhibiting glial scar is a useful tactic in the recovery of cognitive function. This study revealed a substantial inverse connection between GFAP intensity and cognitive function, suggesting that glial scar formation inhibition or GFAP intensity reduction may be important in restoring CNS function that has been compromised by injury. Glial scar inhibition was found to have a 50.4% improvement in cognitive function. While glial scar has a significant inhibitory effect, other factors also seem to play a role. These include intrinsic factors like phosphatase and tensin homolog (PTEN) and suppressor of cytokine signaling 3 (SOCS3) expression in nerve cells that are regenerating axons as well as extrinsic factors like meningeal cells, oligodendrocytes, and extracellular matrix (ECM). This relationship between glial scar suppression and cognitive recovery may hold translational relevance and deserves more attention in future CNS injury studies.

Treatment with minocycline for brain injury results in an improvement in cognitive function, but the condition is still not completely cured. Regression analysis results may provide information about the impact of minocycline on cognitive function, which is shown to be 46.7%. Other factors not included in the regression model account for 53.3% of the effect (**Table 2**). Given the small sample size and exploratory nature of the analysis, the regression findings should be interpreted as preliminary and hypothesis-generating rather than conclusive.

Limitations

There are a number of limitations to this study that should be considered for future research. Rats are nocturnal animals, meaning they are active at night, but as there are no facilities for night observations, the cognitive test observations in this study were done during the day. Furthermore, the absence of a standardized room posed a risk to the rats' behavior throughout maintenance and cognitive test observations by causing them to become stressed. There's no dedicated silent observation area, especially when observing experimental animals who are sensitive to noise. Aside from that, there was no observation area with adjustable lighting because nocturnal animal observation and video use are light-sensitive. Every group of rats was monitored simultaneously to minimize bias. Moreover, the small sample size (n=4 per group) and the absence of a priori power analysis may limit the statistical strength and generalizability of the findings.

CONCLUSION

Minocycline has been shown to inhibit the expression of TGF-β1, thereby reducing glial scar formation in the

perilesional area of rats with TBI. This reduction in glial scarring is associated with improvements in cognitive function. These findings may inform future translational research aimed at developing glial scar-targeted therapies for human TBI.

ETHICAL DECLARATIONS

Ethics Committee Approval

This study was approved by the Universitas Brawijaya Ethics Committee (Approval Date: 03.11.2022, Decision No: 246/EC/KEPK-S3/11/2022).

Informed Consent

Because the study has no study with human and human participants, no written informed consent form was obtained.

Peer Review Process

This manuscript was subject to external peer review.

Conflict of Interest

The authors declare no conflicts of interest related to this study.

Financial Disclosure

The authors received no financial support for the conduct or publication of this research.

Author Contributions

All authors contributed significantly to the study's conception, design, data acquisition, analysis, and interpretation. All authors reviewed and approved the final version of the manuscript.

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