

Minocycline Inhibits Glial Scar Formation Through CNTF Expression and Ameliorates Cognitive Impairment in Traumatic Brain Injury Rats

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Received: 11 January 2024, Revised: 6 March 2024, Accepted: 13 March 2024, Published: 10 August 2024

Abstract

Traumatic brain injury (TBI) is a chronic condition that causes permanent disability, particularly cognitive impairment resulting from glial scar formation. Minocycline treatment inhibits glial scar formation through the Ciliary Neurotropic Factor (CNTF) pathway in multiple sclerosis. We hypothesized that minocycline could also inhibit CNTF, which would play a role in the inhibition of glial scar formation in TBI. The objective was to evaluate the role of minocycline in inhibiting glial scar formation through the CNTF signaling pathway and ameliorating cognitive impairment in TBI model rats. Male Sprague Dawley rats (n = 16) were divided into 4 groups (n = 4/group). TBI through the weight drop model is performed on day 0, followed by minocycline treatment of 25 mg/kg (MNO1 group), 50 mg/kg (MNO2 group) and 100 mg/kg (MNO3 group) given for 14 days. The NOR test is performed on day 15, followed by immunofluorescence double staining on day 16. Minocycline plays a role in inhibiting glial scar formation in TBI model rats. Minocycline inhibits the formation of CNTF with an effect proportion of 66.3 %, which plays a role in inhibiting glial scar in the perilesional area in TBI model rats. Inhibition of glial scar improves cognitive function impairment in TBI model rats. Administration of minocycline improves cognitive function in TBI model rats with an effect proportion of 46.7 %. It can be concluded that minocycline inhibits glial scar through the inhibition of CNTF expression and ameliorates cognitive impairment in a rat model of TBI.

Keywords: CNTF, Cognitive impairment, Glial scar, Minocycline, Rats, Traumatic brain injury

Introduction

Traumatic brain injury (TBI) causes high mortality and disability rates in young and productive people around the world, including in Indonesia, the majority of which are caused by motor vehicle accidents [1]. Although the mortality rate is starting to be reduced due to the improved trauma management system and safety technology in motor vehicles, the disability rate in the form of persistent neurobehavioral disorders

after TBI is still high and there is no effective treatment, causing problems for the country, family, individual and patient environment [2].

The search for neuroprotective drugs is still under research, one of which is minocycline, a second-generation tetracycline derivative, because it has been shown to have anti-inflammatory, antiapoptotic and antioxidant effects in previous preclinical studies and has been recommended by the United States food and drug regulatory agency (FDA approved) for use in humans and is available at an affordable price [3]. Previous research on the effect of minocycline on TBI with cell targets including neuronal cells, oligodendrocytes and microglia through anti-inflammatory, antiapoptotic and antioxidant mechanisms in TBI has been widely conducted, but no one has observed its effect on astrocyte cells [4]. In studies of hydrocephalus [3], ischemia stroke [5,6], spinal cord injury and neurodegenerative disorders, minocycline was also shown to reduce astrogliosis [7,8].

Astrocytes that are reactive due to TBI, become hypertrophied, proliferative and form a dense network of glial scar in the perilesional area that will cause a physical and biochemical barrier to healing in the later phase [9,10]. Previous research has proven that TBI through the weight drop model can induce glial scar formation that leads to cognitive impairment in rats [11]. Several signaling molecules cause astrocytes to become reactive (astrogliosis) and can progress to glial scar. From a theoretical basis, one of the signaling pathways involved in glial scar formation is CNTF. After trauma, CNTF mRNA and protein increased in the perilesion, coinciding with the accumulation of CNTF bioactivity in the wound cavity. Similarly, after stereotactic biopsy of the striatum, CNTF mRNA accumulated in the tissue, and the timing of its accumulation coincided with the appearance of reactive protoplasmic astrocytes [12].

Ciliary Neurotropic Factor (CNTF) through the Janus Kinase (JAK-2) receptor will activate Signal Transducer and Activator of Transcription (STAT-3). STAT-3 will dimerize and move to the nucleus where it will be recognized by STAT-3 Responsive Elements (SRE) in the promoter area of target genes, which will induce transcription. Then the activated transcription factors will bind to DNA in the nucleus of astrocyte cells and will cause protein synthesis which will give orders for astrocyte cells to hypertrophy, proliferate and migrate into glial scar in the chronic phase. So that this situation causes disruption of nerve regeneration which causes persistent sequelae (disability) in the form of neurobehavioral disorders, namely cognitive, affective and motor [9,13-15]. In other studies with different target organs and diseases, it was found that minocycline inhibits CNTF [16]. Therefore, this study aimed to investigate the role of minocycline in glial scar formation through the CNTF pathway and ameliorate cognitive impairment in TBI model rats.

Materials and methods

Animals

Sixteen male Sprague Dawley rats (3 months old, 250 - 350 g) were purchased from Universitas Gadjah Mada, Yogyakarta, Indonesia. Rats were acclimatized for 7 days before experimental methods. Rats were housed in controlled conditions with a 12-hour light/dark cycle, constant temperature (23 ± 2 °C), humidity (55 ± 5 %) and had ad libitum access to standardized food and water. Each rat was used at a time to reduce anxiety. Rats suffering from postoperative wound infections, death or diseases unrelated to the experiment were excluded from the study. Ethical clearance for this study was approved by the Research Ethics Commission of the Faculty of Medicine, Universitas Brawijaya, Indonesia (Approval No. 246/EC/KEPK-S3/11/2022).

Experimental procedure

Rats were grouped into: (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). TBI was induced using the weight drop model on day 0, followed by minocycline treatment from day 1 to 14. On day 15, the Novel Object Recognition (NOR) Test was conducted on all rats. On day 16, rats were decapitated for immunofluorescence histopathological examination. Every group experienced the same consistent standardization of procedures. The outcomes were assessed by experts blinded to the treatment group of the animals. The experimental procedure is presented in **Figure 1**.

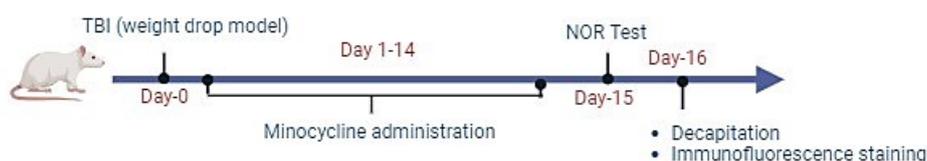


Figure 1 The experimental procedure of the study.

TBI (Weight drop model)

TBI in rats was carried out through the weight drop model method by Wardhana [11]. Rats were randomly selected based on randomization results using a random generator application. Rats were given prophylactic antibiotic Cefazolin sodium 33 mg/kg and anesthesia ketamine xylazine 0.05 cc i.p. Rats were fixed on a stereotactic device then the scalp was shaved and cleaned with 10 % povidone-iodine solution. Next, a craniectomy with a diameter of 10 mm was performed on the right side of the brain, 2.5 mm posterior to the bregma and 3 mm lateral right of the midline. A 10-gram weight with a tip diameter of approximately 2.5 mm was dropped onto the brain surface from a height of 10 cm, and the piston was allowed to compress the tissue a maximum of 2.5 mm. This procedure resulted in moderate brain injury with small focal lesions, clearly visible swelling and good recovery in the animals. The skin was sutured closed with interrupted 6 - 0 silk sutures. Subsequently, rats were put into cages of 1 rat per cage and maintained at room temperature (23 ± 1 °C). Rats were randomly assigned to the MNO1, MNO2, MNO3 and WD groups.

Minocycline administration

Minocycline (p.o) once per day starting 24 h after weight drop treatment for 14 days. The doses were 25 mg/kg (MNO1 group), 50 mg/kg (MNO2 group) and 100 mg/kg (MNO3 group). First, the average body weight of rats in each group was calculated, followed by determining the drug requirement for a single dose administration ($1 \times$ sonde) in each rat group. Minocycline, available in capsule form, was dissolved to achieve a 100 mg/mL concentration. Subsequently, multilevel dilution was carried out for groups MNO1, MNO2 and MNO3. The minocycline solution was then prepared in small tubes for single-dose administration. Minocycline was orally administered using a specialized pipette, ensuring thorough ingestion by the rats. WD group that was not treated with minocycline received a placebo. Drug administration was performed by laboratory personnel who were blinded to the experimental group.

NOR test

The test was conducted in a 40×40×40 cm³ arena with 20 lux lighting, controlled temperature and humidity, featured an overhead camera for optimal viewing. During testing, the room was kept quiet to reduce anxiety and was ensured to be consistent across all groups. Blinded observers conducted observations to the groups. The first phase was habituation by placing the rats in the center of an empty arena for 5 min. After 24 h, the training phase was conducted, where the rats were randomly selected and put back into the arena where 2 identical objects had been placed. The size of these 2 objects matched the size of the rat or was slightly larger to encourage exploration. The objects are placed in the opposite quadrant of the arena, at a diagonal distance of 7 cm from the rat, and then the rat is allowed to explore the objects for 10 min. At 1 h after training, testing was conducted by replacing one of the objects with a new object and placed in the same position as in the training phase. Rats were randomly selected and placed back in the arena to explore the object for 10 min (**Figure 2**). As soon as possible after testing, the animals were returned to the cage, while the arena and objects were cleaned using 70 % ethanol. Exploration was defined as sniffing or touching with the nose and/or front paws within a distance of 2 - 3 cm around the object or climbing on the object. Video recordings during the testing phase were analyzed using Ethovision software (Noldus, version 17.0.1630) to ascertain the duration of rats' exploration of both familiar and novel objects. Consistent with prior studies [17-27], cognitive impairment was assessed through the discrimination index formula: $\frac{\text{time spent near the new object} - \text{old object}}{\text{time spent near the new} + \text{old object}}$. Healthy rats typically exhibit a positive discrimination index as they tend to explore new objects more extensively. Conversely, rats with cognitive impairments display diminished interest in exploring new objects, resulting in a negative discrimination index.

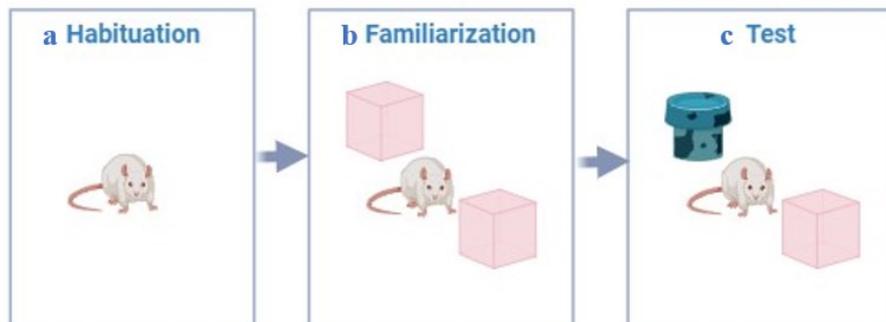


Figure 2 Schematic of the NOR test. (a) Rats are habituated to an empty arena; (b) Rats are familiarized to an arena that has been given 2 identical objects; (c) Rats are tested in an arena with one of the objects replaced with a novel object.

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 h, coronal brain sections (5 µm) were obtained from the perilateral region. 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.

Immunofluorescence

Immunofluorescence double staining Glial Fibrillary Acidic Protein (GFAP) and CNTF was performed in all groups to evaluate the intensity of GFAP and CNTF. Paraffin-embedded tissue slides were incubated at 40 °C overnight and then immersed in xylol solution 1 and xylol solution 2 (10 min each); ethanol absolute 1, ethanol absolute 2, ethanol 90 %, ethanol 70 %, PBS + Tween 1, PBS + Tween 2 and PBS + Tween 3 (5 min 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 min), and then allowed to stand at room temperature (10 min). The slides were washed with PBS + Tween (3×5 min), dried, blocked with 2 % BSA solution in PBS + Tween and incubated at room temperature (1 h). The slides were washed with PBS + Tween (3×8 min), dried and then stained with primary antibody solutions including antiastrocyte-GFAP antibody (1022 Mouse monoclonal IgG2b, Santa Cruz Biotechnology) and anti-CNTF (Rabbit, Thermo Fisher Scientific) in 2 % BSA, incubated overnight at 4 °C, washed with PBS + Tween 3×8 min, and dried. Slides were stained with secondary antibody solution containing fluorescent dye in 2 % BSA, incubated at room temperature (1 h), washed with PBS + Tween 3×8 min, dried, stained with 25 µL of 10 % glycerol solution, and covered with cover glass. Slides were observed with 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 analyses

The analysis was performed using SPSS ver. 26 (IBM Corp) and visualized using GraphPad Prism ver. 10.0.0.153 (GraphPad Software LLC). The analyzed data was documented as mean ± SD of 4 replicates in each group. One Way ANOVA and Post Hoc Bonferroni were used for normal data, while Kruskal-Wallis and Mann-Whitney tests were applied for nonnormal data. The correlation between variables was assessed through the Pearson Correlation test, while the influence of the independent variable on the dependent variable through linear regression. A *p*-value < 0.05 was considered statistically significant.

Results and discussion

During the study, there were no rats that became ill or died, so all rats were included in the analysis without any dropouts.

Minocycline inhibits CNTF intensity

Comparison of GFAP intensity of WD and Sham groups was performed through unpaired T test. The results of the comparison of the 2 groups are shown in the significance value column. There was a significant difference in GFAP intensity between WD and Sham with a significance value of 0.000 (*p*-value < 0.05).

Table 1 Comparison of GFAP intensity of WD and Sham groups.

Group	Mean ± SD	Significance value	Mean difference
WD	2,433,038 ± 543,386	0.000*	2,092,539
Sham	278,316 ± 21,190		(1,419,766 - 2,765,311)

Table 2 Comparison of CNTF intensity of WD and Sham groups.

Group	Mean \pm SD	Significance value	Mean difference
WD	1,695,04 \pm 552,205	0.003*	1,400,150
Sham	294,895 \pm 151,158		(699,696 - 2,100,601)

Comparison of CNTF intensity of WD and Sham groups was performed through unpaired T-test. The results of the comparison of the 2 groups are shown in the significance value column. There was a significant difference in CNTF intensity between WD and Sham with a significance value of 0.003 (p -value < 0.05).

Based on the T-test results, there was a significant difference in GFAP intensity in the WD and Sham groups ($p < 0.05$) indicating that TBI treatment through the weight drop model had an impact on glial scar formation (**Table 1**). In **Table 2**, there was also a significant difference in CNTF intensity at the glial scar location between the WD and sham groups. Through fluorescence observation, there was an increase in CNTF intensity after TBI. In the MNO group, it appears that the intensity of CNTF decreases according to the increase in drug dose (**Figure 3**). Through Post Hoc Bonferoni test, there was a significant difference in CNTF intensity in the WD group with MNO1 ($p = 0.008$), WD with MNO2 ($p = 0.004$) and WD with MNO3 ($p = 0.002$) (**Figure 4**). The regression test proved the effect of minocycline in inhibiting CNTF with an effect proportion of 66.3 % (**Table 3**).

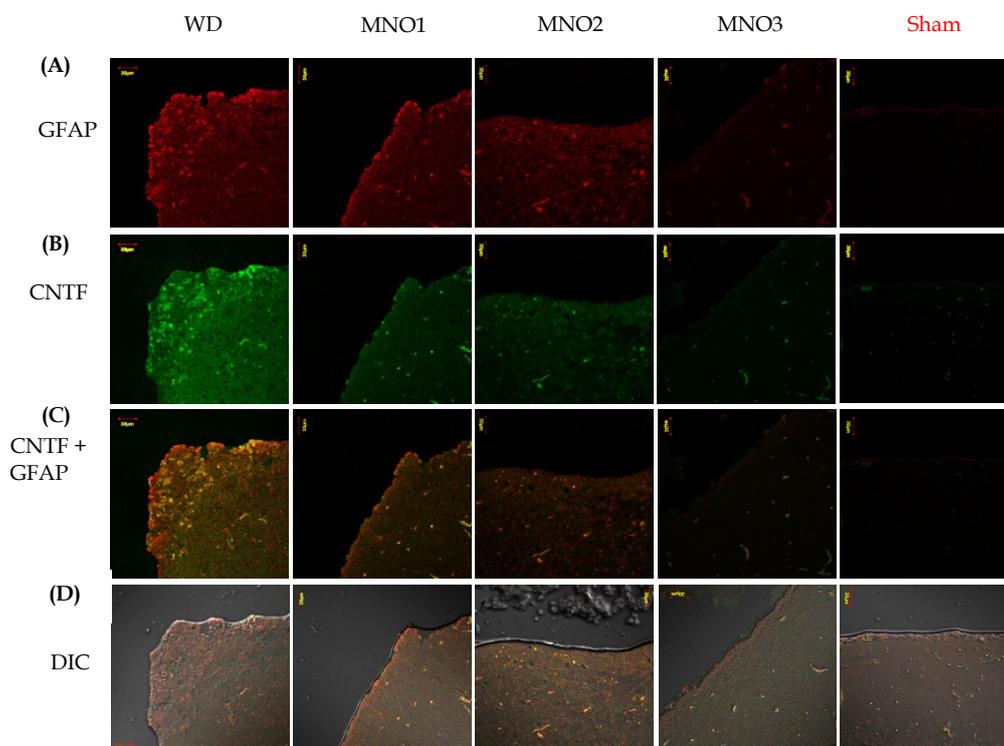


Figure 3 Immunofluorescence double staining analysis of GFAP and CNTF on the brain in the perilesion area. (A) The trend of GFAP intensity in the 5 treatment groups shown through red color luminescence (rhodamine). GFAP indicates the location of glial scar in the brain in the perilesion area. (B) The trend of CNTF intensity in the 5 treatment groups is shown through the green luminescence (FITC) of the antibody

attached to the CNTF antigen in the brain. (C) The trend of GFAP + CNTF intensity through yellow color double staining to assess CNTF at the glial scar location. (D) The trend of DIC images with high resolution and contrast facilitates the determination of data collection locations. The order of images from left to right are WD (injured rats), MNO1, MNO2 and MNO3 (injured rats + minocycline treatment). Staining was performed by immunofluorescence double staining method using antiastrocyte-GFAP antibody (mouse) and anti-CNTF antibody (rabbit). Observations were made using confocal laser scanning microscopy (CLSM, Olympus, Japan) at 400× magnification. Image scale: 30 μm.

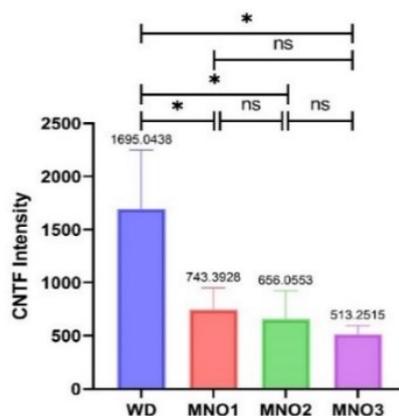


Figure 4 Minocycline inhibits CNTF intensity in TBI model rats. There was a significant difference in CNTF intensity between WD with MNO1, WD with MNO2 and WD with MNO3. WD: Rats were given injury; MNO: Rats were given injury and minocycline treatment. The intensity of CNTF with arbitrary units (au) was assessed through anti-CNTF antibody (rabbit) with anti-astrocyte GFAP (mouse) by immunofluorescence double staining. Differences in intensity between groups were performed through Post Hoc Bonferroni test. The sign (*) indicates a significant difference in the 2 treatment groups with $p < 0.05$. Data are presented as mean \pm standard deviation. CNTF: Ciliary Neurotropic Factor.

Table 3 Effect of minocycline in inhibiting glial scar formation through CNTF inhibition.

Model	Coefficient of regression	Significance value of regression	Significance value of ANOVA	Coefficient of determination
Constant	1,695.044	0.000		
MNO1	-951.651	0.001		
MNO2	-1,038.988	0.001	0.001*	0.663
MNO3	-1,181.792	0.000		

The effect of minocycline in inhibiting CNTF intensity was performed through dummy regression. The coefficient of regression indicates the amount of change in the independent variable in influencing the change in the dependent variable; significance value of regression indicates the significance of the effect of the independent variable partially on the dependent variable; significance value of ANOVA indicates the feasibility of the model in estimating the effect of the independent variable on the dependent variable; coefficient of determination indicates the proportion of the influence of all independent variables on the dependent variable. Minocycline had an effect on the inhibition of CNTF intensity with a significance value of 0.001 (p value < 0.005) and an effect proportion of 66.3 %.

Inhibition of CNTF intensity inhibits glial scar formation

The correlation analysis showed a strong significant relationship between CNTF intensity and GFAP intensity of 87.8 %. This relationship is positive, meaning that inhibition of CNTF intensity can inhibit GFAP formation (**Table 4**). Furthermore, regression analysis was carried out which showed that inhibition of CNTF intensity had an effect in inhibiting the formation of glial scar by 75.9 % (**Table 5**).

Table 4 Relationship between CNTF intensity and GFAP (glial scar) intensity in TBI model rats.

Result	Correlation coefficient		Significance value
	GFAP	CNTF	
GFAP	1	0.878	0.000*
CNTF	0.878	1	

The relationship between GFAP and CNTF intensity was conducted through the Pearson Correlation test. The results of the Pearson Correlation test are shown in the significance value column. There was a significant relationship between GFAP and CNTF intensity with a significance value of 0.000 (p -value < 0.05). The relationship between these 2 variables was 87.8 %, including a strong relationship.

Table 5 Effect of CNTF intensity on GFAP (glial scar) intensity in TBI model rats.

Model	Coefficient of regression	Significance value of regression	Significance value of ANOVA	Coefficient of determination
Constant	317,250	0.053	0.000*	0.759
CNTF	1,251	0.000		

The effect of CNTF intensity on GFAP (glial scar) intensity was performed through linear regression. The coefficient of regression indicates the amount of change in the independent variable in influencing the change in the dependent variable; significance value of regression indicates the significance of the effect of the independent variable partially on the dependent variable; significance value of ANOVA indicates the feasibility of the model in estimating the effect of the independent variable on the dependent variable; coefficient of determination indicates the proportion of the influence of all independent variables on the dependent variable. CNTF affects GFAP intensity with a significance value of 0.000 (p value < 0.005) and the proportion of influence is 75.9 %.

Inhibition of glial scar formation improves cognitive function impairment

There is a strong significant relationship between GFAP intensity and cognitive function of rats by 72.8 %. The correlation coefficient is negative, indicating that a decrease in GFAP intensity will increase the cognitive function of rats and vice versa (**Table 6**). Furthermore, regression analysis was carried out which showed that GFAP had a significant effect on the cognitive function of rats with an influence proportion of 50.4 % (**Table 7**).

Table 6 Relationship between GFAP (glial scar) intensity and cognitive function improvement in TBI model rats.

Result	Correlation coefficient		Significance value
	GFAP	Discrimination index	
GFAP	1	-0.728	0.000*
Discrimination index	-0.728	1	

The relationship between GFAP intensity and discrimination index was conducted through the Pearson Correlation test. The results of the Pearson Correlation test are shown in the significance value column. There was a significant relationship between GFAP intensity and discrimination index with a significance value of 0.000 (p value < 0.05). The relationship between these 2 variables was 72.8 %, including a strong relationship.

Table 7 Effect of GFAP (glial scar) intensity on cognitive function improvement in TBI model rats.

Model	Coefficient of regression	Significance value of regression	Significance value of ANOVA	Coefficient of determination
Constant	0.771	0.000	0.000*	0.504
GFAP	0.000	0.000		

The effect of GFAP intensity on cognitive function (discrimination index) was performed through linear regression. The coefficient of regression indicates the amount of change in the independent variable in influencing the change in the dependent variable; significance value of regression indicates the significance of the effect of the independent variable partially on the dependent variable; significance value of ANOVA indicates the feasibility of the model in estimating the effect of the independent variable on the dependent variable; coefficient of determination indicates the proportion of the influence of all independent variables on the dependent variable. GFAP affects the discrimination index with a significance value of 0.000 (p value < 0.005) and the proportion of influence is 50.4 %.

Minocycline improves cognitive function

Comparison of the discrimination index of the WD and Sham groups was performed through the Mann-Whitney test. The results of the comparison of the 2 groups are shown in the significance value column. There was a significant difference in discrimination index between WD and Sham with a significance value of 0.002 (p value < 0.05).

Table 8 Comparison of discrimination index of WD and Sham groups.

Group	Mean \pm SD	Significance value	Mean Difference
WD	-0.490 \pm 0.056	0.002*	-1,138
Sham	0.648 \pm 0.244		-1,516 - (-0,759))

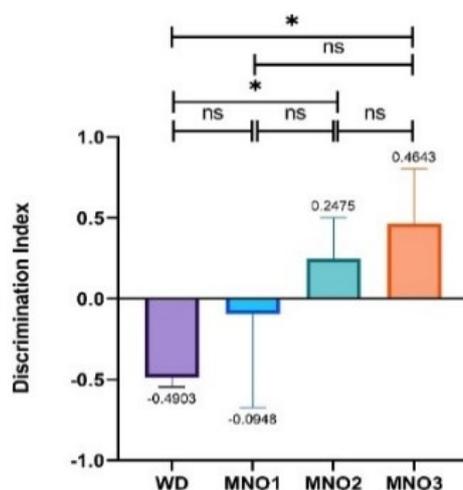


Figure 5 Minocycline improves cognitive function of TBI model rats. WD: Rats given injury; MNO: Rats with injury and minocycline treatment. Differences in discrimination index between groups were performed through Mann-Whitney test. The sign (*) indicates a significant difference in the 2 treatment groups with p value < 0.05 . There was a significant difference in discrimination index between WD with MNO2 and WD with MNO3. Data are presented as mean \pm standard deviation.

Table 9 Effect of minocycline in improving cognitive function of TBI model rats.

Model	Coefficient of regression	Significance value of regression	Significance value of ANOVA	Coefficient of determination
Constant	-0.490	0.018		
MNO1	0.395	0.146		
MNO2	0.738	0.013	0.014*	0.467
MNO3	0.954	0.003		

The effect of minocycline in improving cognitive function was performed through dummy regression. The coefficient of regression indicates the amount of change in the independent variable in influencing the change in the dependent variable; significance value of regression indicates the significance of the effect of the independent variable partially on the dependent variable; significance value of ANOVA indicates the feasibility of the model in estimating the effect of the independent variable on the dependent variable; coefficient of determination indicates the proportion of the influence of all independent variables on the dependent variable. Minocycline had an effect on cognitive function with a significance value of 0.014 (p value < 0.005) and an effect proportion of 46.7 %.

Table 8 shows a significant difference in the cognitive function of rats in the WD and Sham groups ($p < 0.05$). Furthermore, when compared with the dose group, there was a significant difference in the cognitive function of rats in the WD group with MNO2 ($p = 0.029$) and WD with MNO3 ($p = 0.029$). However, there is no significant difference between MNO2 and MNO3 ($p = 0.2$), indicating that doses of 50 - 100 mg/kg provide an improved effect on the cognitive function of rats (**Figure 5**). The results of regression analysis showed that minocycline had an effect on cognitive function with an effect proportion of 46.7 % (**Table 9**).

Discussion

The role of minocycline as a neuroprotectant that can improve cognitive impairment in TBI model rats has begun to be widely practiced [28-32]. In this study, cognitive improvement using the NOR test was obtained in the group of brain-injured rats that received minocycline therapy, with significant improvements seen in the second dose (50 mg/kg) and third dose (100 mg/kg) groups (**Figure 5**). This is in line with previous studies [28-32] which support the proof of the role of minocycline in improving cognitive function with a dose variation of 22.5 - 90 mg/kg. In the previous study [32], the lowest dose used was 22.5 mg/kg with an intraperitoneal route of administration which allows smaller doses to begin to show neuroprotectant effects. In this study, minocycline was administered starting from doses of 25, 50 to 100 mg/kg. However, the dose that significantly provided neuroprotective effects was a dose of 50 mg/kg (p.o) following the research [31]. The oral route is used to facilitate further application in humans because currently neuroprotectant drugs consumed by humans are dominated by the oral route in the long term.

In this study, it was proven that the administration of minocycline significantly reduced the intensity of CNTF in rats with TBI. Minocycline reduced CNTF intensity starting at doses of 25, 50 and 100 mg/kg (**Figure 4**) with an effect of 66.3 % (**Table 3**), which means that the higher the coefficient of determination, the greater the contribution of MNO's effect on CNTF. Similar findings have also been proven in study on multiple sclerosis disease in the brain which showed that minocycline treatment for 2 weeks in the remyelination phase resulted in a significant decrease in CNTF mRNA levels compared to placebo-treated rats [16]. CNTF inhibition on the mechanism of glial scar inhibition leads to a decrease in the canonical JAK-STAT3 pathway, where STAT3 is involved in a complex signaling cascade in the cell that will suppress GFAP expression and astrocyte hypertrophy thereby inhibiting glial scar formation. This is in line with the results of the regression analysis which obtained a significant positive effect of CNTF on GFAP by 75.9 % (**Table 5**).

In this study, there was a significant negative relationship between GFAP intensity and cognitive function, indicating that decreasing GFAP intensity or inhibiting glial scar formation has a role in the repair of the central nervous system (CNS) function damaged by injury (**Table 6**). The effect of glial scar inhibition on cognitive function improvement was found to be 50.4 % (**Table 7**). Although the effect of glial scar inhibition is quite large, it appears that there are other factors that contribute to this process, namely intrinsic factors of nerve cells in regenerating axons such as phosphatase and tensin homolog (PTEN) and suppressor of cytokine signaling 3 (SOCS3) expression and extrinsic factors such as oligodendrocytes, meningeal cells and extracellular matrix (ECM) [33].

This study has several limitations that need to be evaluated for further research development. First, rats are nocturnal animals (active at night), but the cognitive test observations in this study were conducted during the day because there are no facilities for night observations. To reduce bias, all groups of rats were observed at the same time. Secondly, there was no standard room to ensure that the rats did not experience stress during maintenance and cognitive test observations because it could affect the nature and behavior of the rats. Third, there is no special quiet observation room, especially when observing the behavior of sound-sensitive experimental animals. Fourth, there was no room with adjustable lighting for observation, as the use of video and observation of nocturnal animals is sensitive to light.

Conclusions

Minocycline inhibits glial scar through inhibition of CNTF intensity. Through regression analysis, it was revealed that CNTF has a key role in inhibiting glial scar in the perilesional area in TBI model rats. Inhibition of glial scarring can improve impaired cognitive function in this model through NOR test.

Acknowledgements

Authors acknowledge the Professor Grant program of the Faculty of Medicine, Universitas Brawijaya, Indonesia which has funded the research and publication of this article with the basis of payment DPA-FK-271101/2023-0 Fiscal Year 2023.

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