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RESEARCH ARTICLE

Interleukin-1 beta (IL-1β) and Interleukin-6 (IL-6) as Pro-Inflammatory Cytokines in Traumatic Brain Injury (TBI) of Rat (*Sprague-Dawley*): A Study of Propofol Administration

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Abstract

The increase in the prevalence of traumatic brain injury (TBI) followed by increased morbidity and mortality challenges anesthetists to perform the treatment patients with TBI. Menwhile, interleukin-1 beta (IL-1ß) and interleukin-6 (IL-6) are the first pro-inflammatory markers expressed after secondary injury occurs. In addition, propofol (2,6-disopropylphenol) is a short-acting intravenous agent that reported to play role in inflammatory process. This study aims to determine the effects of propofol administration on the neuroinflammatory pathway. Thirty adult female Sprague Dawley rats were randomly assigned as TBI rats (T), rats that received propofol infusion (P), or TBI rats that received propofol infusion (TP). The rat model of TBI was developed using the Marmarou Weight Drop method. Clinical assessment was performed using the Neurobehavioral Severity Score-Revised (NSS-R). Brain tissues were taken 24 h after TBI and the levels of interleukin-1 (IL-1) and interleukin-6 (IL-6) mRNA expression levels were examined using qRT-PCR, while the concentration were examined using ELISA and immunohistochemistry. On clinical examination, a decrease in the median value of NSS-R in the TP group was found from 30th minute to 120th minute. 24 hours after the occurrence of TBI, there was a decrease in both the TP and T groups. However, the NSS-R values in the TP group were lower than those in the T group. The expression of IL-1ß was found in the nuclei and cytosol, while IL-6 was found in the axons and cytosol. The expression and concentration of IL-1ß and IL-6 were the highest in the TP group, followed by the P and then the T group. Propofol can improve neurological function in rats with TBI, probably through the mechanism involves neuroinflammatory pathways, particularly IL-1ß and IL-6.

Keywords: *Traumatic brain injury, Pro-inflammatory cytokines, IL-1β, IL-6, Propofol.*

Introduction

In the United States, TBI accounts for approximately 30% of all deaths due to injury and causes a substantial health burden [1]. In 2013, approximately 2.8 million people in the United States suffered from TBI which resulted in approximately 282.000 hospitalizations and 56.000 deaths. TBI is most common in young children (ages 0-4 years), adolescents and young adults (ages 15-24 years), and the elderly (ages ≥ 75 years). The increase in the prevalence of TBI followed by increased morbidity and mortality challenges anesthetists to carry out the treatment, stabilization, and prevention

of worsening in patients with TBI [2].TBI causes primary and secondary injuries. Primary injury occurs shortly after impact and is exacerbated by acute systemic damage such as hypoxia, bleeding, and activation of the neurotoxic pathway [3]. Hypoxia then activates the secondary injury cascade pathway [4].

Secondary injury can occur within seconds, hours, months and years and cause biochemical, metabolic, and cellular changes. All of the secondary injury cascade pathways result in neuronal cell death [4].The secondary injury cascade pathway consists of blood-brain barrier disorders, excitotoxicity, apoptosis, mitochondrial damage, and neuroinflammation.

In the neuroinflammatory cascade, the involvement of immune cells, microglia, cytokines, chemokines, and other inflammatory mediators causes damage to the brain's endogenous ability to repair its own damage. This cascade takes longer to cause cell death than the other cascade pathways. This circumstances provides ample opportunities for preventive interventions in the neuroinflammatory cascade pathway [5].

Interleukin-1 beta (IL-1β) and interleukin-6 (IL-6) are the first pro-inflammatory markers expressed after secondary injury occurs. It expressions increase within the first one to three hours and lasts up to seven days [6].This disorder provides a therapeutic window for modulation therapy in secondary brain injury, thereby inhibiting neuronal cell death.

Propofol (2, 6-disopropylphenol) is a short-acting intravenous agent that has been used as an anesthetic and sedative. Previous studies have shown that propofol inhibits glutamate secretion, prevents the active expression of cathepsin B which functions to activate caspase, [7] mitochondrial translocation and cytochrome-C secretion in the apoptotic pathway, [8] reduces excess intracellular calcium, prevents swelling of the mitochondria, inhibits the formation of lipid peroxides [9], reduces reactive oxygen species (ROS) formation by interfering with malondialdehyde (MDA) and nitric oxide (NO) signaling, and inhibiting the formation of inflammatory factors [10].

Therefore, this study aims to determine the effects of propofol administration on the neuroinflammatory pathway, particularly IL-1β and IL-6, in TBI; therefore it is expected to initiate the innovative interventions in order to improve the quality of life of TBI patients.

Method

This research is an experimental study using rats and has been approved by the ethics committee of the Faculty of Medicine, Universitas Indonesia, with number of letter: KET- 1054/UN2.F1/ETIK/PPM.00.02/2019 and number of protocol: 19-08-1008.

Animals and Groupings

Thirty adult female Sprague-Dawley rats were randomly assigned to a TBI (T) group, a propofol infusion (P) group, and a TBI plus propofol infusion (TP) group. The TP rats received propofol (50 mg/kg) for one hour intravenously through the lateral tail vein 40 minutes after TBI. All rats were raised under controlled temperature and humidity conditions with 12 hours of light and dark and ad libitum access to food and water.

Rat Model of Traumatic Brain Injury

Rats were put into an induction chamber (Kent Scientific®) and anesthetized with isoflurane (3–5%) in oxygen flow at 800–1500 mL/minute. After induced, the rats were removed from the induction chamber and placed on the operating table with all four legs fixed.

A nose cone provided continuous isoflurane (2–2.5%) in fresh gas flow (oxygen only) of 400–800 mL/minute to keep the rat anesthetized. A 0.5 cm incision was made on the scalp of the rats. A plate 10 mm in diameter and 3 mm thick was glued with dental acrylic in the Lambda area and Bregma fissures of the rat. The modified Marmarou Weight Drop model [14] was adopted to inflict the moderate TBI in this model.

Assessment of Neurological Functions

The neurological functions of the rats were assessed at the 30th and 120th minutes, and then 24 hours after TBI using the modified Neurological Severity Score Revised (NSS-R) [17]. A score of 20 indicates a maximum reduction of neurological function, 11-14 indicates a moderate injury, less than 10

indicates a mild injury, and a score of 0 indicates the absence of a neurological problem.

Table 1: The modified Neurological Severity Score Revised (NSS-R)

No.	NSS-R	0	1	2
1	General Balance	Balance and walk	Balance and no walk	No balance/ fall
2	Landing Test	Normal landing	Partial compromised	No reflex/ falls flat
3	Tail Raise Test	Normal reflex	Partial/ weak reflex	No reflex/ limp
4	Drag Test	Extension and resistance	Partial response	No response
5	Righting Reflex	Immediate and complete	Partial response	No response
6	Ear Reflex	Full response	Partial response	No response
7	Eye Reflex	eyeblink	Partial response	No response
8	Sound Reflex	Start then freeze	Slow or no freeze	No response
9	Tail Reflex	Immediate squeak	Delayed squeak	No response
10	Paw Flexion Reflex	Normal withdrawal reflex	Partial withdrawal reflex	No response

Brain Tissue Preparation

At 24 h after TBI, rats were anesthetized intraperitoneally with ketamine-xylazine and the brains were quickly removed. Some brain tissue was stored at -80 ℃ for qRT-PCR, ELISA analysis and some tissues were processed for immunohistochemistry and histological analysis of the brain tissue damage.

QRT-PCR Analysis

The qRT-PCR analysis was performed as described by Yisarakun et al [18]. RNA was extracted from cortical tissue using Trizol reagent according to the manufacturer's instructions. The extracted RNA was added to SensiFAST™ SYBR No-ROX PCR reagent One-Step Mix, containing the primers (forward and reverse), RNAse inhibitor, reverse transcriptase, RNA template and nuclease-free water in a total volume of 20 µL in a well plate.

The plate was sealed with optical adhesive film and subjected to 40 cycles of qRT-PCR amplification. The qRT-PCR was performed using the Real Time 7300 Applied PCR system with reverse transcription at 45 ℃ (10 min), pre-denaturation at 95 ℃ (2 min), denaturation at 95 ℃ (5 seconds), annealing at 60 ℃ (10 min), and extension at 72 ℃ (5 seconds).

Elisa

In ELISA examination, the brain tissues of each group were crushed and the lysis buffers along with protease inhibitor were added. Quantification of IL-1b and IL-6 by using an enzyme-linked immunosorbent assay (ELISA) kit sandwich (Rat IL-1 beta from ELISA Kit MBS825017 and IL-6 from ELISA Kit MBS726707, My Bio Source, San Diego, California, USA. Various concentrations of standard solutions were prepared first. Then, the wells were covered with specific antibody to IL-1 and IL-6, which was continued by the addition of 100 µL standard solution and 100 µL supernatant from tissue homogenate (100 mg tissue sample in 1 mL PBS buffer). Then, the solution was put in into wells which then kept at 37°C for 2 hours. After removing the solution, 100 µL biotin antibody was placed in the wells and incubated at 37°C for 1 hour. After that, the plate was rinsed with buffer four times and held for 2 minutes.

Streptavidin-Horseradish Peroxidase was added to the wells and incubated at 37°C for 1 hour. Then, tetramethyl-benzidine (TMB substrate) was added to the wells and incubated at 37°C for 30 minutes. The reaction was stopped by 50 µL Stop Solution and the absorbance measured in an ELISA reader at a wavelength of 450 nm. IL-1 and IL-6 quantification was then defined by comparing the optical density (OD) value of the sample with the standard curve.

Immunohistochemistry

In immunohistochemistry examination, paraffin preparations that have been cut with microtomes will be deparaffinized and rehydrated. Then, the preparations were immersed in citrate buffer which acted as retrieval antigen at 95℃ for 20 minutes, followed by cooling process. After the preparations were rinsed with PBS (3 times, each for 5 minutes), blocking was done using SNIPER® solution for 30 minutes. 10% fetal bovine serum (FBS) was added to the preparations for 30 minutes, then continued by adding and incubating preparations with three different antibodies, namely anti-HIF-

1α, IL-1β, and IL-6 antibodies at 4℃ over night.

Trekkie Universal® was added as a secondary antibody reaction (2nd antibody) for 30 minutes. The preparations were rinsed with PBS (3 times, each for 5 minutes). The Avidin-HRP® solution was added for 20 minutes. Antibody visualization was performed by adding 3, 3'-diaminobenzidine (DAB) reaction. Counterstain was performed with hematoxylin. The preparation was rinsed under running water and distilled water. The final step included dehydration, clearing, and mounting with a cover glass.

Results

This study used thirty Sprague-Dawley rats aged 25-26 weeks with an average body weight of 233.2 (± 23.9) grams. On clinical examination, a decrease in the median value of NSS-R in the TP group was found from 30th minute to 120th minute. 24 hours after the occurrence of TBI, there was a decrease in both the TP and T groups. However, the NSS-R values in the TP group were lower than those in the T group. Meanwhile, there was no change in the value of neurological deficits in the P group (Figure 1).

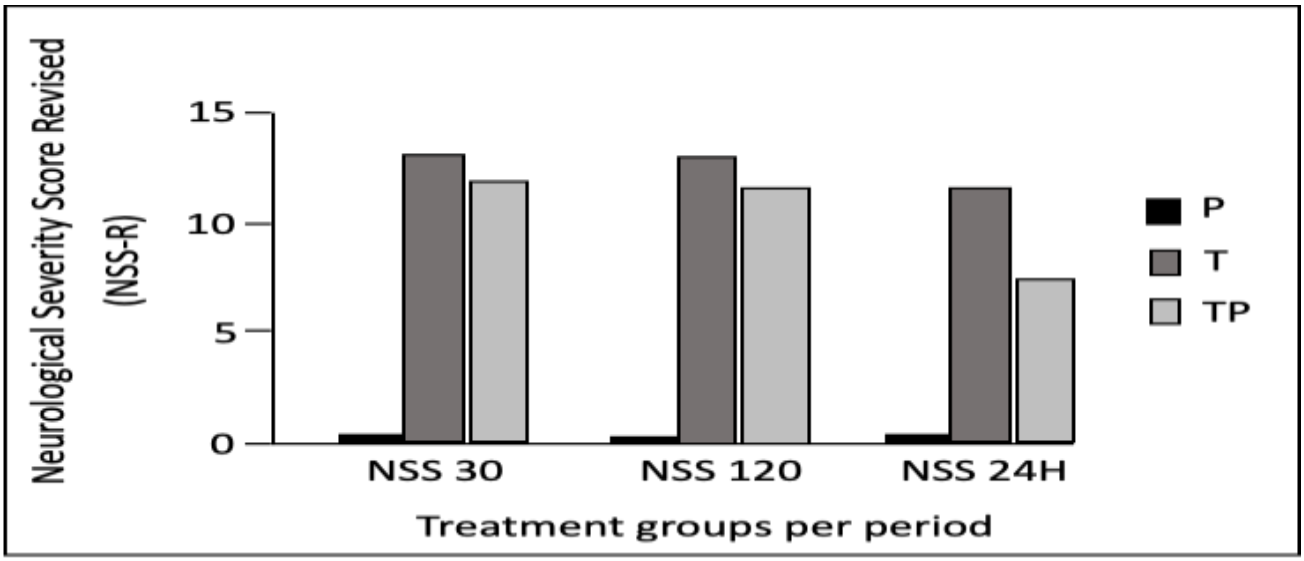

Figure 1: Neurological Severity Score Revised (NSS-R) on the 30th minute (NSS-R 30), 120th minute (NSS-R 120), and 24 hours (NSS-R 24H) after the occurrence of TBI in groups

Meanwhile, on qRT-PCR examination, it was found that the mRNA expression of both IL-1β and IL-6 was the highest in the TP group, followed by the P group then the T group (Figure 2).

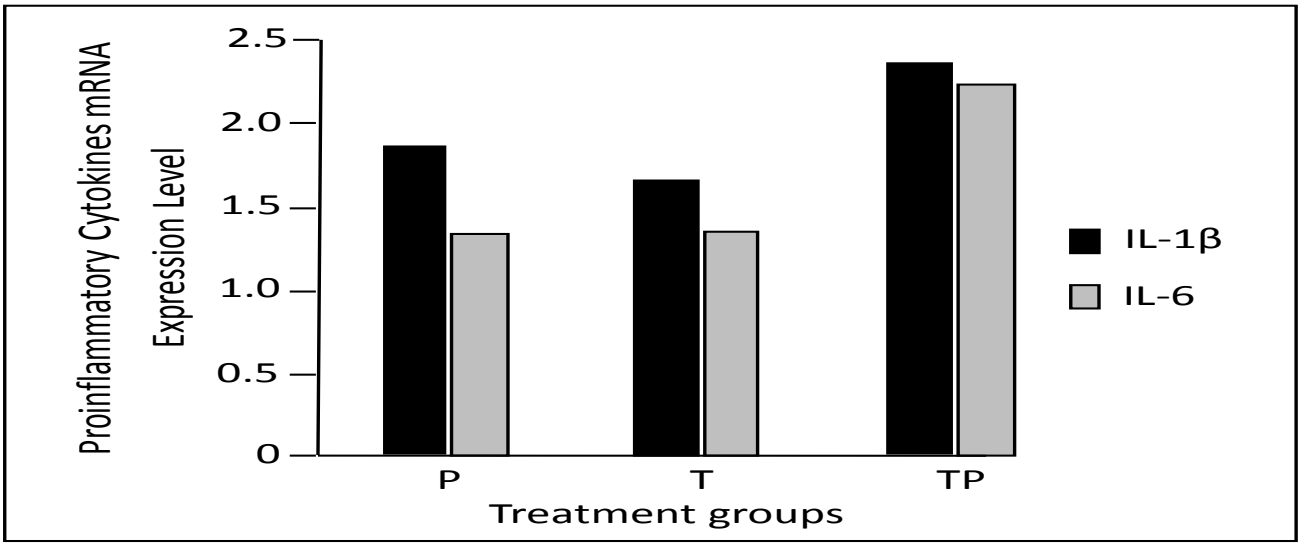

Figure 2: Expression of proinflammatory cytokines (IL-1β and IL-6) in groups

In addition, the highest IL-1β concentration was obtained in the TP group on ELISA examination. Likewise, with IL-6 which was found the most in the TP group, followed by the P group and finally the T group (Figure 3).

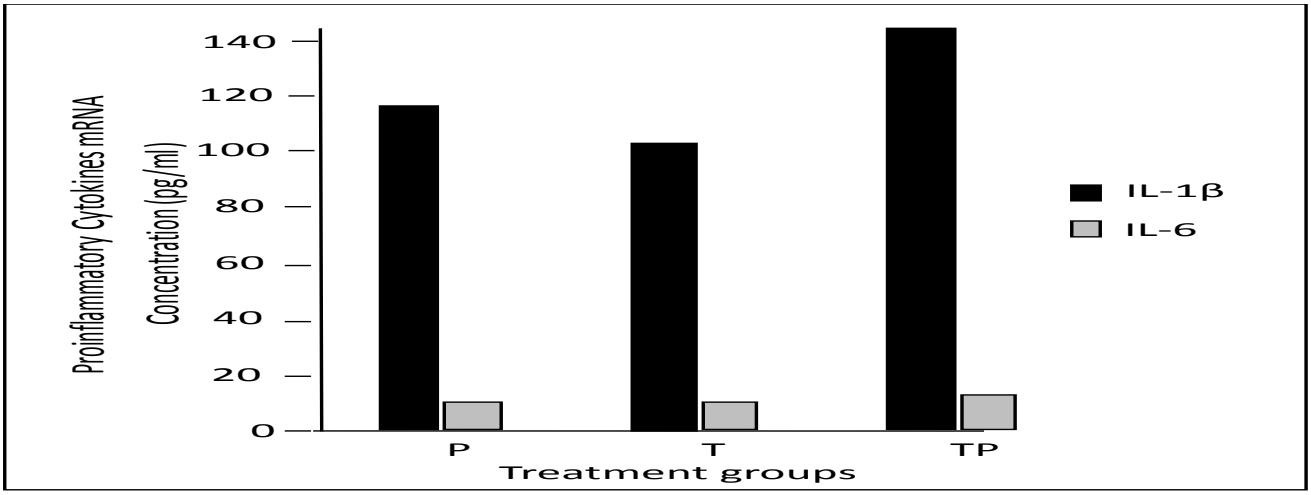

Figure 3: Concentration of Pro-inflammatory Cytokines (IL-1β and IL-6) in groups

Furthermore, on immunohistochemical examination, IL-1 ß expression was found in the nuclei of neuron cell and cytosol. Compared to group C, IL-1ß expression was more seen in CP and P groups where the IL-1ß immunohistochemical was thicker (Figure 4).

On immunohistochemical examination of IL-6, the expression was found in the cytosol and along the axons of the neuron cells. Compared to group C, IL-6 expression was seen higher in CP and P groups where the IL-6 immunohistochemical was thicker (Figure 5).

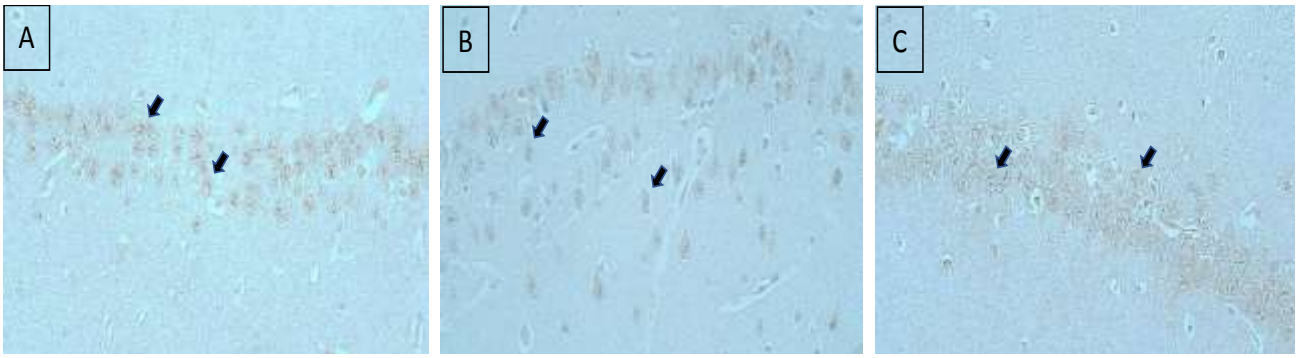

Figure 4: Immunohistochemistry of IL-1β in the (A) P group, (B) T group and (C) TP group with a 40x magnification. Arrow sign point to IL-1β expression in the nuclei and cytosol.

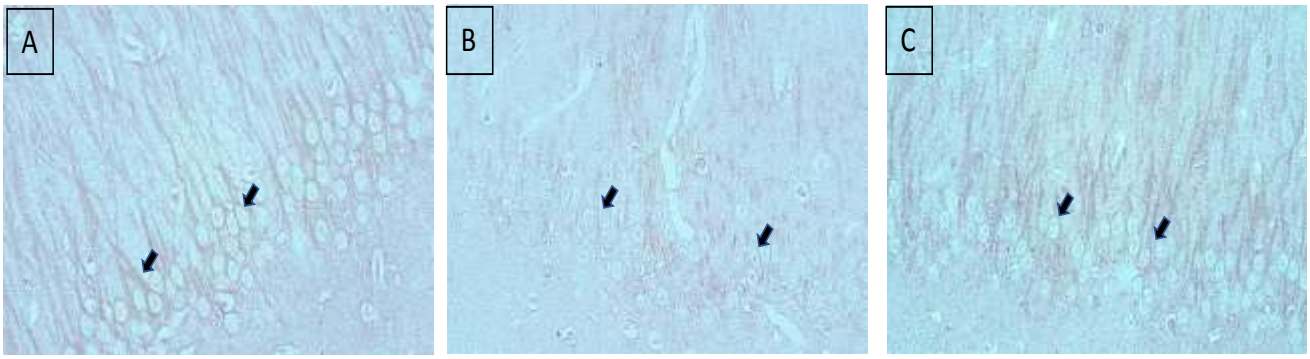

Figure 5: Immunohistochemistry of IL-6 in the (A) P group, (B) T group and (C) TP group with a 40x magnification. Arrow sign point to IL-6 expression in the axons and cytosol

Discussion

This study shows that propofol can improve neurological function in rats with TBI which mechanism involves a neuroinflammatory pathway. Neuroinflammation is an important part of the pathophysiology following TBI, which is associated with secondary brain injury and deficits in neurological function [14-16]. The acute increase in pro-inflammatory cytokines after TBI in the first 48 hours had been investigated in detail in a study by Harting et al which showed that IL-1ß increased 60-80-fold from baseline in 6 hours, 18-22-fold in 12 hours, and remained elevated 5-fold in 24 hours after the occurrence of TBI. On the other hand, IL-6 will increase 30-36-fold from baseline in 6

hours, 23-30 fold in 12 hours, 17-21 fold in 24 hours, and continue to increase 4-fold in 48 hours after the occurrence of TBI [17].The neuroprotective effects of propofol have long been recognized in animal studies such as in ischemia models, most of which focus on the propofol complex mechanism to relieve brain edema, neuronal apoptosis, inflammatory response, and so on [18, 19].

Through a model of ischemia in rats, a study by Shi showed that propofol reduces inflammatory reactions and brain damage in focal cerebral ischemia in rats [20]. However, only a few studies have investigated the role of propofol in TBI. In an in vitro study, Luo et al demonstrated that propofol pre-treatment can suppress IL-1ß production in microglial cells [21]. Furthermore, Ding et al found that IL-1ß decreased significantly in rats with TBI after being given propofol 10 minutes at 12 hours post-TBI [22].

In summary, previous studies have shown that the neuroprotective effect of propofol on TBI-induced brain damage is associated with anti-inflammatory properties. In this study, it was found that propofol administration in the TP group showed an increase in IL-1ß and IL-6. This result is inversely proportional to other studies regarding the effects of propofol that have been conducted, in which propofol can suppress proinflammatory cytokines.

The researchers' opinion, which may differ from the results of other studies, is that propofol alone can induces an increase in proinflammatory cytokines in acute phase as evidenced by an increase in IL-1ß and IL-6 in the P group compared to the T group. In a study that evaluated the concentration of proinflammatory cytokines in the hippocampus of rats with a global model of transient ischemia, it was found that in a state of oxygen deficiency, hypoxia induced factor 1 alpha (HIF-1α) induced the release of proinflammatory cytokines such as IL-1ß and IL-6, which when resuscitated and reperfused, the increased HIF-1a would increase IL-1ß and IL-6 even more [23].

In addition, a research regarding the effect of Dimethyloxaloylglycine (DMOG) on TBI conducted by Sen et al also showed an increase in cytokines, which is thought to promote neovascularization via vascular endothelial growth factor (VEGF) induction which may repair damaged cells [24].

Furthermore, it was found that there was an increase in NSS-R 24 hours after the occurrence of TBI in the TP group that was given 1 hour of propofol intervention. These results are supported by research by Liu et al which reported that NSS would gradually decline starting from day 1 after TBI, but that propofol administration significantly improved neurological function [25].

This study showed improvements in neurological function in experimental animals with TBI after propofol administration, however, it still has several limitation. The sham group is needed to see whether the effects of stress are also involved when administering propofol to experimental animals so as to increase proinflammatory cytokines in the rats brain tissues.

Moreover, it is also necessary to carry out anti-inflammatory examination such as on IL-10 to determine its possible role so that there would be an improvement in the TP group. In addition, this study focused only on the effects of propofol in the acute phase. More research is needed to see the effects of propofol for a longer duration.

Conclusion

Propofol can improve neurological functions in rats after the occurrence of TBI which mechanism involves neuroinflammatory pathways, particularly IL-1ß and IL-6. However, further research is needed to determine the effect of propofol on the neuroinflammatory pathway for a longer duration.

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