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The Effect of Intervention Recombinant IL-10 on Level of TNF-α and GFAP Serum in the Wistar Rats with Traumatic Brain Injury Treatment

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Abstract Traumatic brain injury (TBI) can trigger an inflammation and activation the mediator substances. TNF-α is a major cytokine that has adverse effects in high level. Glial Fibrillary Acidic Protein (GFAP) in brain cells increases in TBI thus can be an accurate biomarker for brain damage. The purposes of this study are to know the intervention effect of IL-10 as an anti-inflammatory to inflammatory processes (pro anti-inflammatory balance) in TBI. This is an experimental laboratory study with a post-test control group design consisting of four treatment groups and one control group. Measurement of Tumor Necrosis Factor α (TNF-α) and GFAP using Enzyme-Linked Immunoabsorbent Assay (ELISA) methods. The result of this study shows Groups with provoked brain injury had significantly higher levels of serum TNF-α ($p < 0.05$) than the control group. Group provoked a brain injury and given recombinant IL-10 did not have increased levels of TNF-α in serum after 1 hour, differ significantly with no intervention of IL-10. The levels of GFAP have no significant difference 1 hour after TBI, and these levels increase at 24 hours. The conclusion is Intervention by IL-10 could decrease the levels of TNF-α serum immediately after TBI in the Wistar rats and the levels of GFAP 24 hours after TBI are increasing persistently although given by intervention of IL-10 or not.

Keywords: *Glial Fibrillary Acidic Protein, Interleukin-10, traumatic brain injury*

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1. Introduction

Brain injury is a major concern for health as it can lead to disability and death. Patients with brain injuries, particularly severe traumatic brain injury, appears to undergo two major issues namely damage to his brain and systemic disorders that are not directly [1]. Hypermetabolism which was related to the severity of the brain injury. The increase in intracranial pressure has a relationship with the increase in metabolism. Similarly, the increased levels of cytokines (Tumor Necrosis Factor α (TNF-α), IL-10) will increase metabolism and has an effect on secondary brain injury [2].

Inflammation, as well as other organs, is the most important of the pathophysiology of traumatic brain injury. Cytokines are released soon after brain injury will cause a negative nitrogen balance, and hyperglycemia occurs [3]. A brain injury needs to be a balance between the cytokines that cause an inflammatory reaction, with the aim of limiting and restoring the damage, as well as cytokines anti-inflammatory (IL-10) that restrict the functionality of the inflammatory cytokine is not an exaggeration, which when there will be effects adverse [4].

Detection of cytokines in the brain that is produced primarily by lymphocytes and monocytes infiltrating functioning nerve system. In the media wounds, particularly TNF-α increased at 1 hour, 4 hours reaching a maximum level at 1.4 times that of the control. In ischemic culture media, TNF-α did not change between 1 to 2 hours. Then increased at 4 hours, the maximum level of 2.2 times the control value with the value of 8 hours [5].

Glial Fibrillary Acidic Protein (GFAP) obtained only in glial cells and astrocytes, increases in brain injury and neurodegenerative related to the severity of the incident (damage to brain cells). So that serum GFAP is a biomarker specific to damage brain cells [6].

Mosman and scientists first described the cytokine produced by T helper two (Th2) cells and Th2 cells that inhibit the formation of interferon (IFN). Cytokines Synthesis Inhibiting Factor (CSIF) is known as Interleukin (IL-10) [7].

Based on the facts above, this research attempts to reveal the extent to which the inflammatory process (TNF-α) and the anti-inflammatory (IL-10), beneficial or detrimental in patients with severe traumatic brain injury, and the extent to which the role of intervention IL-10 affects the inflammatory process. [8].

2. Materials and Methods

2.1. Design and Research Variables

The study was conducted at the Animal Laboratory, Faculty of Medicine, Hasanuddin University in July 2016. This research is an experimental research laboratory on mice with the approach of post-test control group design consisting of four treatment groups and one control group. The study variables consist of a dependent variable (brain injury, TNF-α, IL-10, and GFAP) and the independent variables (Recombinant IL-10).

2.2. Population and Sample

The study population was a rat model of traumatic brain injury and mice without brain injury as a control. The research sample was a rat model of traumatic brain injury and mice without brain injury, with the inclusion criteria: Rat (Rattus norvegicus) Wistar males, aged about 3-4 months, weight about 300-400 grams, the mice were healthy. In this study, 30 research subjects met the inclusion criteria.

2.3. Method of Collecting Data

This study uses experimental laboratory method in mice by using an entirely randomized design. The division of the mice to the treatment group and the control is carried out through random permutation block. Efforts to study the effects of time administration of IL-10 as an anti-inflammatory, performed through immunohistochemical examination to see changes "Damage to brain tissue of mice with levels of TNF-α, IL-10And GFAP post-traumatic brain injury". Immunohistochemical analysis time of TNF-α, IL-10 and GFAP made after 1 hour and 3 hours post-treatment. Some mice were given the intervention of IL-10 immediately after the rat made a head injury.

2.3.1. ELISA Methods

This study uses quantitative ELISA method with direct type.

2.4. Data Analysis

Statistical analysis using SPSS 22. Data were tested by One Way ANOVA test and Post Hoc Test Kruskal-Wallis method. 95% confidence level and is considered significant if $p < 0.05$.

2.5. Ethical Clearance

This study had passed ethical approval from Ethic Committee of Health Research of Medical Faculty of Hasanuddin University. Reg no UH16060500.

3. Result

In a comparison, study results mean levels of TNF-α in each group was significantly different ($p = 0.029$). From Table 1 shows that the mean serum levels of TNF-α highest in the group 1 hour post – Traumatic Brain Injury (TBI) without recombinant IL-10 and decreased at 24 hours post-TBI.

Table 2 shows that the average levels of TNF-α in serum in group one hour after the TBI without the administration of recombinant IL-10 was significantly higher ($p = 0.027$) than the control group, as well as the group 24 hours after the TBI without recombinant IL-10, but no significant difference ($p = 0.824$).

Table 1. Data normality test levels of TNF-α

Groups	N	Mean	Median (min-max)	*P
Control	6	22.06 (3.34)	23.31 (19.03-27.59)	0.029
Craniectomy + TBI (1 hour)	6	28.58 (7.28)	29.32 (20.56-38.10)	
Craniectomy + TBI + recombinant IL-10 (1 hour)	6	23.39 (2.43)	23.47 (19.95-27.00)	
Craniectomy + TBI (24 hours)	6	22.68 (6.19)	25.73 (17.45-34.02)	
Craniectomy + TBI + IL-10 recombinant (24 hours)	6	18.86 (2.57)	20.03 (16.32-23.75)	

*One Way ANOVA p = 0.029.

Table 2. Kruskal-Wallis Post Hoc Test with TNF-α levels

Treatment group		Sign
Control	Craniectomy + TBI (1 hour)	.027
	Craniectomy + TBI IL-10 Rec (1 hour)	.633
	Craniectomy + TBI (24 hours)	.824
	Craniectomy + TBI + IL-10 Rec (24 hours)	.260
Craniectomy + TBI (1 hour)	Craniectomy + TBI + IL-10 Rec (1 hour)	.073
	Craniectomy + TBI (24 hours)	.043
	Craniectomy + TBI + IL-10 Rec (24 hours)	.002
Craniectomy + TBI (24 hours)	Craniectomy + TBI (24 hours)	.798
	Craniectomy + TBI + IL-10 (24 hours)	.115
Craniectomy + TBI (24 hours)	Craniectomy + TBI + IL-10 Rec (24 hours)	.181

Table 3. Normal distribution of data normality test levels of GFAP

Groups	N	Mean	Median (min-max)	P*
Control	6	322.78 (26.29)	312.10 (277,37-346,84)	.058
Craniectomy + TBI (1 hour)	6	365.71 (54.55)	375.23 (307,13-443,34)	
Craniectomy + TBI + recombinant IL-10(1 hour)	6	315.14 (35.36)	314.31 (270,43-358,20)	
Creniectomy + TBI (24 hours)	6	367.91 (32.11)	379.92 (332,10-427,74)	
Craniectomy + TBI + IL-10 recombinant (24 hours)	6	345.60 (41.54)	356.88 (270,43-443,34)	

* One Way ANOVA p = 0.058.

In group 1 hour after the TBI by administration of recombinant IL-10, the mean serum levels of TNF-α is slightly higher than the control group but did not differ significantly (p = 0.633). At 24 hours post-TBI group by administration of recombinant IL-10, the mean serum levels of TNF-α was significantly lower (p = 0.260) than the control group. This suggests that the effect of recombinant IL-10 in reducing serum levels of TNF-α in the first 1 hour after the TBI is not too significant, but only significant at 24 hours post-administration of recombinant IL-10.

In Table 3 for the comparison of the mean levels of IL-10 in each of the groups was not significant (p = 0.058). From Table 3 also shows that the mean serum GFAP levels rose at 1-hour post-TBI and again increased at 24 hours post-TBI. Provision of recombinant IL-10 to reduce levels of serum GFAP 1 hour post-TBI, but the levels increase after 24 hours post-TBI.

4. Discussion

In the serum levels of TNF-α post-TBI, the results show that 1 hour post-TBI in mice models with 571.17 style Newton / mm2 in area craniectomy one-time, equivalent to the power of inflicting mild traumatic brain injury (Feeney's weight drop models / control cortical impact), showed increased serum levels of TNF-α, a significant difference (p <0.05) with serum levels of TNF-α in control. It indicates that soon after the TBI mild in mice models increased production of TNF-α. In theory, it is easy to understand because as soon as the TBI, the accumulation of leukocytes in the injured area of the brain. Leukocytes migrate out of the blood vessels into the brain parenchyma injury through increased endothelial P and E selectins and intercellular adhesion molecules (ICAMs) [9].

Anthony et al. study showed that injection of TNF-α in brain parenchyma with ELISA proved that serum TNF-α decreased 50% after 4 hours and 24 hours to disappear. The accumulation of leukocytes in the TBI cause leukocytes migrate out of the blood vessels into the brain parenchyma and surrounding injuries through increased endothelial P and E-selectin and intercellular adhesion molecules (ICAMs). Leukocyte cells will produce lymphocytes T and B. T helper one (Th-1) cells secrete TNF-α, IL-2, and IFN-γ. Furthermore, IFN-γ activates macrophages and induces MHC-II. Activation of macrophages produce IL-12 stimulates the production of TNF-α, IL-2, and IFN-γ. Leukocytes accumulate in brain parenchymal injury in addition to the release of proinflammatory cytokines (including TNF-α) also release proteases, prostaglandins, complement factor, free oxygen and nitrogen that can damage neurons, microvascular, and the blood-brain barrier and cause vasogenic edema [10].

High levels of serum TNF-α production of TNF-α showed a high, associated with high leukocyte infiltration in brain tissue equivalent to the extent of brain injury due to trauma. According to Allan and Rothwell, elevated levels of TNF-α aggravate inflammation and secondary brain damage after the TBI. TNF-α levels were high after TBI plays a role of neurological dysfunction will occur [11].

24 hours post-TBI, serum levels of TNF-α was significantly lower (p <0.05) on serum levels of TNF-α 1 hour after the TBI, and did not differ significantly (p> 0.05) with the controls. This suggests that TNF-α production decreased at 24 hours post-TBI. This may be associated with an area of just a notch TBI light so that the accumulation of TBI limited and ultimately the release of TNF-α was also slightly [12].

The results of the study Thomas Woodcock and Maria Morganti-Kossmann also showed elevated levels of TNF-α in the serum of mice one hour after the TBI, but remained high and peaked between 4-8 hours post-TBI. Differences in levels of TNF-α serum is probably due to differences in the degree of TBI her (TBI weight), as has been proposed by Thomas (2013), that difference increased levels of TNF-α serum in each study may be due to disagreements and the type of injury, delays cytokine movement of pure Kim into a vein or cerebrospinal fluid [13].

GFAP was first isolated in 1971 and is found only in glial cells of the CNS. GFAP is a protein that forms the cytoskeleton of astrocytes. GFAP is a monomeric protein filament that is specific to the cells of astrocytes. This substance is associated with a wide variety of cellular processes nervous and a little more responsible for the neurological function in the blood-brain barrier. Several clinical studies showed increased GFAP levels in serum in patients with ischemic stroke, with peak levels occur at 2-4 days after the onset of stroke [14].

In this study showed that there was no change in serum GFAP levels of up to 1 hour after light TBI. Increased serum GFAP levels were significantly (p <0.05) was found in 24-hour observation post-TBI. This is possibly related to the condition of the blood-brain barrier permeability so that the levels of GFAP in brain parenchyma can not cross the blood-brain barrier into the serum [15].

Although the levels of TNF-α showed elevated levels of 1-hour post-TBI was not immediately followed by an increase in GFAP levels in serum. This means that the TNF-α cytokine that plays a role though as the blood-brain barrier damage, apparently, blood-brain barrier damage is

not imminent. This is supported by research conducted by Feuerstein et al. and Yang et al. showed in his study that TNF-α plays a leading role in the destruction of the blood-brain barrier caused by traumatic brain injury. This is easier for leukocytes infiltrate into the brain tissue. Rosenberg et al. explain that TNF-α increases the permeability of blood vessels and release proteolytic enzymes after 24 hours do an intracerebral injection of TNF-α, they assume that elevated levels of TNF-α to extend the increased permeability of blood vessels [16].

Nicola and Andrew's study showed that the injection of recombinant TNF-α at 1.5 hours there has been no significant aggregation of leukocytes that has not happened damage the blood brain barrier. MRI with contrast to the picture of a 5.5 hours after injection of TNF-α occurs aggregation of leukocytes characterized by a hyperintense illustration showing the occurrence of damage to the blood-brain barrier. On histopathologic examination at 5.5 hours, it has been demonstrated that increased vascular permeability and contained macrophages in the brain parenchyma. In ED-1 (Anti-CD68 antibody) stained macrophages / brown-stained cells (a specific marker for microglia, monocytes, and macrophages) are accumulated around the surface of the macrophage in the brain parenchyma than in the brain tissue of injected TNF-α. [17].

MRI with contrast to the picture of 24 hours after the injection of recombinant TNF-α in brain parenchyma occurs diffuse large area and hyperintensities on brain blood barrier that shows the brain tissue damage was advancing. This is in line with the findings that the damage is massive after 24 hours post-TBI characterized by elevated levels of serum GFAP [18].

Balasingam et al found that astroglial very minimal activity after brain injury in neonates stab wounds, in contrast to a single microinjection post-derived macrophage cytokines (IL-1 or TNF-α), an extensive astroglial reactivity [19].

Astroglial reactivity is a manifestation of brain pathology in some adult TBI. Reactive astrocytes become more widespread, much thicker, longer process, increase serum GFAP levels, increase metabolism of mitochondria, glycogen content, and various enzyme levels [20].

In this study showed that 24 hours after light TBI evidence of increased levels of GFAP were significantly ($p < 0.05$), in contrast to 3 hours post-TBI. This reinforces the notion that high levels of GFAP are a result of reactivity of astrocytes following the inflammatory process that occurs after the TBI. Microglia activity, mobilization of blood-derived macrophage, and astroglial reactivity is a response to the post-TBI. A cytokine produced by macrophages/microglia cause astrocytes become reactive. High levels of serum GFAP in astroglial reactivity require long and extensive inflammation, and levels of pro-inflammatory cytokines (e.g., TNF-α) is elevated. In this study, the distance interval between the occurrence of inflammation (heightened levels of TNF-α) and the elevation of serum GFAP levels long enough. Serum levels of TNF-α increased 1 hour after the first TBI whereas serum GFAP levels increased 24 hours after the TBI. It can be deduced that an increase in TNF-α was not immediately followed by an increase in GFAP. Reactivity astroglial GFAP takes to release into the serum.

5. Conclusions and Recommendations

According to this study, concluded that IL-10 recombinant treatment can suppress brain damages after traumatic brain injury by inflammation process, with the fact that IL-10 treatment can elevate IL-10 level and lowered TNF-α serum in mice after traumatic brain injury.

A further study about dosage, method and the effect of IL-10 recombinant treatment with mice with the different grading of traumatic brain injury and a study about IL-10 recombinant in human with traumatic brain injury.

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