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THE ROLE OF RECOMBINANT IL-10 ON THE SERUM LEVEL OF TNF-α, ONE HOUR POST TRAUMATIC BRAIN INJURY OF THE WISTAR RAT

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Background: Brain injury often occurs not only primary brain injury, but often also occur secondary brain injury. Inflammation is a process that occurs immediately after trauma characterized by activation of the mediator substance. TNF-α is a major cytokinee involved in the inflammatory processes that have adverse effects if the serum level are excessive. There needs to be a balance of the inflammatory process in the brain injury so things that harm does not occur. As anti-inflammatory IL-10 plays an important role in maintaining the balance. The objective of this study is to determine the effect of IL-10 intervention as an anti-inflammatory will decrease the serum level of TNF-α in traumatic brain injury. **Material And Method:** Experimental Study in the Rattus Wistar rats, post test control group design, male, aged 3-4 months, with body weight (BW) 300-400g, were obtained from the Laboratory Animal Faculty of Medicine, University of Hasanuddin as much as 24 tails, which is the result of breeding. Subjects were divided into four groups, each group of six rats, treated with controlled cortical impact model (Feeney's weight-drop) of traumatic brain injury. Blood taken with capillary tube in retro-orbita plexus or sinus.This study has approved by ethical clearance for research. **Results:** Levels of TNF-α group of rats 1 hour post-trauma without administration of recombinant IL-10 (28.58 ± 7.28) pg / mL; was significantly higher ($p < 0.05$) than the levels of TNF-α rats without kraniektomi group (22.06 ± 3.34) pg / mL and group kraniektomi rats without brain injury (23.07 ± 2.51) pg / mL. Levels of TNF-α group of rats 1 hour post-trauma by administration of recombinant IL-10 (23.39 ± 6.30) pg / mL; significantly lower ($p < 0.05$) than the group of rats 1 hour post-trauma without administration of recombinant IL-10; and did not different significantly ($p > 0.05$) in the group without craniectomy or craniectomy group without head injury. **Conclusions:** Intervention of recombinant IL-10 decreases levels of TNF-α serum soon after traumatic brain injury in rats.

Keywords: TNF-α; IL-10; traumatic; brain; injury.

INTRODUCTION

Tumor Necrosis Factor (TNF-α) is another cytokinee that has a role in TBI. TNF-α mRNA and protein increased in the initial period after TBI trial and before the infiltration of leukocytes showed that the initial source of production of TNF-α is a collection of the Cell. Increased levels of TNF-α was also observed clinically in patients with TBI. TNF-α is a proinflammatory that are similar to IL-1 and exacerbate inflammation and secondary brain damage after TBI. Early up regulation by TNF-α neurons after TBI was found to contribute to the subsequent neurological dysfunction.[1]

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In the inflammatory response, endothelial cell adhesion molecule triggers for leukocytes, including E-selectin, intracellular adhesion molecule1 (ICAM-1) and vascular cell adhesion molecule1 (VCAM-1). The combination of the release of chemokines (including IL-8, MCP-1 and IP-10), this response facilitates the binding of different populations of leukocytes free on antigen recognition. In addition, many special features of inflammation that can be produced through local effects of TNF on endothelial cells. TNF triggers the expression of cyclooxygenase-2 in order to trigger the production of PGI2 vasodilatori which produces vasodilation, causing rubor and heat through increased blood flow around. This is a new concept in which two double role of TNF is a proinflammatory mediators as well as dangerous cytokine an in vitro data base neutoxik. And in vivo data showed neuro protective by pharmacological inhibition models neuro inflamasi and neuro degeneration.[2] Cytokine detected in

primary brain produced by lymphocytes and monocytes infiltrating active nerve system. In the media injuries, particularly TNFα increased at 1hour, 4 hours reaching a maximum level at 1.4 times that of the control. In ischemic culture media, TNF-α did not change between1 to 2 hours. then increased at 4 h, the maximuml evel of 2. 2 times the control value with value of 8 hours.[2] Cytokinees are protein hormones that mediate a wide class of inflammatory and immune responses are complex, sensitive way relation ship. Major cytokinees that play a role in response to trauma, including tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), IL-2, IL-6, IL-8, IL-4 and IL-18 course. In other words, the cytokinee IL-10 counter the effects of pro-inflammatory cytokinees IL-1, IL-6 and TNF-α in diverse contexts.[3] Such as IL-1β, TNF-α is considered as pure pro inflammatory cytokinees in the short termin TBI research. TNF-α release time out side consistent in experimental paradigm focal TBI in rats (closed cortex damage, fluid percussion, or stab wounds), by detecting the level of 1 hour post-damage, the maximum concentration of 3-8 hours, and a decrease in there lease of 24 hours in the brain. Similarly, TNF-α, IL-6 shows the role of the neuro-inflammation was detected in 1 hour post-injury in animals, followed by the peak concentrations between 2 to 8 hours. Intra thecal production facts on anti-inflammatory cytokinee IL-10 increased in acute within 24 hours of damage, associated with a decrease in TNF-α. In addition, Transforming growth factor-β1 (TGF-β1) increased in CSF serum on 1 day and 3 weeks post-damage. Interestingly, serum levels of IL-10 is increased in severe brain injury, such as polytrauma patients, potentially bringing this cytokineeas a marker non specific TBI as prevalent mechanism in response to damage to TBI.[4]

Cytokine has become the focus of scientific interest for more than a decade now. The scientific analyzes the expression better understanding of the pathogenesis of various diseases. In addition, they are now beyond the level when their interest to study the pathophysiology; some cytokinee therapy is ready to use from the clinical practice, ranging from initial exploration well established test the therapists who are ready to be proven.[5]

Mosman and scientists first described that cytokinees produced by T helper cell2 (Th2) and Th2 cells that inhibit the formation of interferon (IFN). Cytokinee synthesis inhibiting factor (CSIF) is known as interleukin (IL-10).[5]

MATERIALS AND METHODS

Rattus Wistar, male, aged 3-4 months, with body weight (BW) 300-400g were obtained from the Laboratory Animal Faculty of Medicine, University of Hasanuddin as much as 24 tails, which is the result of breeding. Subjects were divided into four groups, each group of six rats, randomly selected, that is without CT =without traumatic brain injury and craniectomy / normal rats (TNF-α check) CT without TBI = normal rats performed a craniectomy, without traumatic brain injury treated (TNF-α check), CT+TBI (1 Hour) = Group performed a craniectomy and given a traumatic brain injury (TNF-α check1 hour post-treatment) CT+TBI+Rec IL-10 (1 Hour) = Group performed a craniectomy was given a traumatic brain injury and immediately give the intervention of recombinant IL-10 (check TNF-α 1 hour post-treatment).

Rats then adapted to the new environment for 1 week before the study is done. Animals kept in a cage made of a material that is water proof, robustand easy to clean, with an area of 80-100 cm 2 and a height of 14-15 cm. Acclimatization animal made 7 days prior to the study.

After a midline skin incision, a 5-7 mm craniectomy was made from 3 mm right posterior of the bregma and 2 mm right lateral to the midline. The animals were then subjected to TBI using a small iron bar, receiving a contact velocity of 571.17 Newton/mm^2. Cylindrical tube 25 cm long, 5 mm in diameter, directing the iron ball weighing 2.5 g to obtain 571.17 newtons / mm2 with the calculation of the I = 2 / 5mr^2 (I = force, m = mass, r = distance) dropped to the top areas have been already craniectomy. Cylindrical tubes are kept to a 90-degree angle using iron ball and spaced one inch from the surface of the rat brain to maintain the compressed air. Recombinant IL-10 treated mice at a dose of 20 mg / kg.[6]

Data were analyzed by SPSS. Applied data normality test TNF-α serum Shapiro-Wilk test. Data showed TNF in each group of data are normally distributed. To assess the levels of TNF-α comparison between groups use One Way Anova followed by LSD (least significant defference). Limit the use of significance α = 5%.

RESULTS

The comparison serum levels of TNF-α By Group

Table1
The comparison serum levels of TNF-α By Group

Variable	Group	Test Statistic		
		N	Mean (SD)	*p*
Serum Level TNF-α (pg/mL)	Without CT	6	22.06 (3.34)[b]	0.032
	CT without TBI	6	23.07 (2.51)[b]	
	CT+TBI (1 Hour)	6	28.58 (7.28)[c]	
	CT+TBI+ Rec IL-10 (1 Hour)	6	23.39 (6.30)[b]	

CT= craniectomy; TBI= Traumatik Brain Injury; Rec=rekombinan. The same super scriptin the same column indicatep > 0.05(not significant), and if different shows p<0.05 (significant). This data analized with One Way Anova + LSD;

Table 1 shows that the group of rats with brain injury treatment had higher levels of serum TNF-α were significantly higher (p<0.05) than the control (group without CT and CT groups); 1 hour after treatment of brain injury. Group who suffered a brain injury and was given recombinant IL-10 did not have elevated levels of TNF-α in serum after 1 hour after brain injury; significant difference (p<0.05) with a group of rats with brain injury without being given recombinant IL-10; and did not differ significantly (p>0.05) with the two control groups.

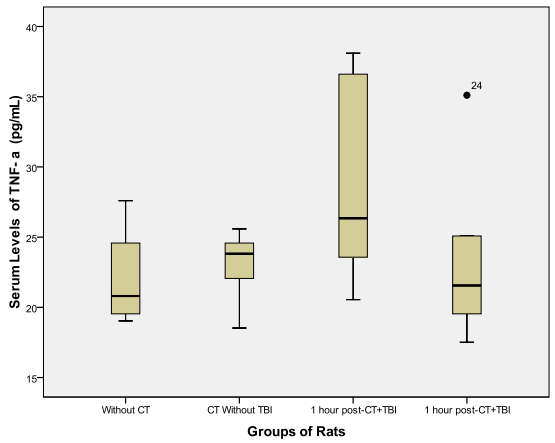

Figure 1
Graph error bars serum levels of TNF-α by groups of rats

From Figure 1 it can be seen that the serum levels of TNF-α was higher in the group of rats treated brain injury an hour earlier. Rats fed a brain injury and was given the commission of recombinant IL-10; one hour after brain injury have serum levels of TNF-α in common with the two control groups. Craniectomy can increase the levels of TNF-α. Groups of rats with brain injury treatment had higher levels of serum TNF-α were significantly higher (p<0.05) than the control (group without craniectomy and craniectomy group). Group who suffered a brain injury and was given recombinant IL-10 did not have elevated levels of TNF-α in serum after 1 hour after brain injury, a different meaning to the group who suffered a brain injury without being given recombinant IL-10.

From the results of the above table shows that craniectomy actions can increase the levels of TNF-α significantly, any intervention that causes injury to body tissues can increase the levels of TNF-α, a proinflammatory cytokinee that is dominant and not specific to brain tissue damage alone. Increased levels of TNF-α was highest in the early trauma or the first hour. Shows the pro-inflammatory response occurs shortly after a traumatic brain injury, administration of recombinant IL-10 can reduce levels of TNF-α significantly in the first hour.

The accumulation of leukocytes in traumatic brain injury causes leukocytes migrate out of the blood vessels into the brain parenchyma injury by binding to endothelia lP-selectin and intercellular adhesion molecule E and (ICAMs). Leukocyte cell will produce T lymphocytes and B lymphocytes, T lymphocytes, consisting of cell Th-1 and Th-2 cells. Th-1 cells secrete IFN-Y, TNF-α, IL-2. IFN-Y is what works activates macrophages and induces MHC-II. Macrophages will also produce IL-12 which serves to stimulate the production of IFN-Y, TNF-α, and IL-2. TNF-α will increase inflammation in endothelial cells. [7,8]

Provision of recombinant IL-10 as an anti-inflammatory role big enough in lowering serum levels of TNFα and work quickly immediately after the first hour of administration. Provision of recombinant IL-10 can increase production of IL-10 itself by TH2.[7,8]

At the same time when there is inflammation, Th-2 cells will produce IL-10, IL-5, and IL-4. IL-10 is an inhibitor of macrophages and dendritic cells, which play a role in the control of non-specific immune response and cellular immune. In dendritic cells, lL-10 will prevent the production of IL-12 and expressing kostimulator and MHC-II. By administering recombinant IL-10, it will inhibit the activation of macrophages and inhibit the activation of Th1 by blocking IL-12 in producing TNFα.[7,8]

CONCLUSIONS

Based on the research results it can be concluded that administration of IL-10, reduce levels of TNFα serum immediately after traumatic brain injury in rats (p<0.05).

REFERENCE

1. Knoblach S.M., Faden A.I., Interleukin-10 Improves Outcome and Alter proinflamatory Cytokinee Expression After Experimental Traumatic Brain Injury, Exp Neurol, 1998, Sep;153(1):143-51.
2. Schmidt O.L., Heyde C.E., Ertel W., Stahel P.F., Closed Head Injury-an inflammatory disease? in Brain Research Reviews, Germany, PubMed 2005 April,48(2):388-399 URL: http://www.ncbi.nlm.nih.gov/pubmed/15850678
3. Lok Ting L., Albert C.H.Y., Journal Neurotrauma : Astrocyt Produce And Release Interleukin-1, interleukin-6, tumor Necroting Factor Alpha and Interferon-Gamma Following Traumatic and Metabolic Injury, Vol. 18;Issue.3: Pages. 351-359

4. Namas R., Ghuma A., Hermus L., et al., The acute inflammatory response in trauma / hemorrhage and traumatic brain injury : current state and emerging prospects, Pittsburgh URL:http://indexmedicus.afro.who.int/iah/fulltext/LJM/vol%204%20n3/The%20Acute%20Inflammatory.pdf
5. Asadullah K., Sterry W., Vilk H.D., Interleukin 10 Therapy – Review of a New Approach, Berlin, Germany, 2003 Jun;55(2):241-269 URL:http://www.ncbi.nlm.nih.gov/pubmed/12773629
6. Dixon E.C., Clifton L. Guy, Lightall W. James, Yaghmay A. Amy, Hayes L. Ronald, A Controlled Cortical Impact model of Traumatic Brain Injury in the Rat, Journal of Neuroscience Methods, October 1991, vol.39(3):253-262
7. Peiying Li, Yu Gan, Leilei Mao, Rehana Leak, Jun Chen, Xiaoming Hu, Chapter 2; The Critical Role of Immune Cell in Acute Brain Injury-Immunological Mechanisms and Therapies in Brain Injuries and Stroke,. USA : Springer ; 2014. Page 9-20.
8. Karnen Garna B., Iris R., Imunologi Dasar, Edisi ke -11, Badan Penerbit Fakultas Kedokteran Universitas Indonesia, Jakarta, 2014.