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PROVISION OF LOW-DOSE PROPRANOLOL IN PATIENTS WITH ACUTE ISCHEMIC STROKE AGAINST THE LEVELS OF TNF-A, IL-10, AND THE RATIO OF TNF-A / IL-10 AT "ABDUL MOELOEK" GENERAL HOSPITAL, BANDAR LAMPUNG, INDONESIA

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ABSTRACT

Brain and the immune system are linked through sympathetic, parasympathetic nervous system and HPA axis. Relationship between brain and immune system is thought to be through molecules secreted by the necrotic brain tissues. Immune system alterations in ischemic stroke can be seen with the presence of lymphopenia, immediate increment and followed by decrement of proinflammatory cytokines (TNF-α) and elevated anti-inflammatory cytokines (IL-10). According to animal study, this condition can be controlled by giving propranolol. The aim of this study was to demonstrate the effect of 2x10 mg propranolol on TNF-α, IL-10, and TNF-α/IL-10 ratio in acute ischemic stroke patients with lymphopenia. This is an experimental research with pretest-postest match control group study design. Samples used in this study were 40 ischemic stroke patients with lymphopenia. The study was conducted in "Abdul Moeloek" General Hospital, Bandar Lampung, Indonesia from July, 2016 to February, 2017. Patients were divided into 2 groups; ischemic stroke group without propranolol, and ischemic stroke group with propranolol. TNF-α and IL-10 levels were examined by ELISA. The results showed that reduction of in TNF-α levels in the group treated with propranolol was lower (p=0.221). IL-10 levels increased in the group without propranolol (p=0.547). TNF-α/IL-10 ratio in the group with propranolol was higher (p=0.053). Ischemic stroke affects the patient's immune system that show in alteration of TNF-α,IL-10 levels and ratio of TNFα/IL-10. Provision of propranolol 2x10 mg increase TNF-α level, decrease IL-10 level and inrease ratio TNFα/IL-10, but not significant statistically.

KEYWORDS: Ischemic Stroke, Propranolol, IL-10,TNF-α, TNF-α/IL-10 Ratio.

INTRODUCTION

Stroke has become a global public health problem, killing millions people and causing major disabilities worldwide.[1] Until now ischemic stroke therapy has not been satisfactory due to various factors and the complexity of pathophysiology. Food and Drug Administration (FDA) approved therapy is rTPA (recombinant Tissue Plasminogen Activator). However, this therapy is limited due to the short time for intervention, only up to 4.5 hours post-stroke onset. In addition, the strict criteria of inclusion and exclusion results in the few patients who are able to receive rTPA treatment even in the United States are less than 5% of patients meet the criteria and are able to get the treatment.[2] Treatment with rTPA is quite effective in reopening the clogged blood vessels, but increasing the risk of cerebral hemorrhage and reperfusion itself induces other pathological processes, because the entry of circulating immune cells into brain tissue exacerbates inflammation and damage.[3]

In the early stroke phase there was an increase in Tumor Necrosis Factor-α (TNF-α) due to excessive monocyte activation followed by a decrease due to monocyte fatigue.[4] TNF-α is a potent proinflammatory cytokine. Produced mainly by active macrophages. Other cells can also produce these cytokines such as CD4 + lymphocytes, NK cells, neutrophils, mast cells, eosinophils and neurons.[5] TNF-α plays an important role during brain ischemia, disrupts endothelial function, induces thrombogenesis through elevated levels of Plasminogen Activator Inhibitor (PAI-1) and Platelet

Activating Factor (PAF) and inhibits endogenous TPA activity. Some apoptotic pathways and neuronal damage are also caused by elevated levels of NF-α [6]

Interleukin 10 (IL-10) is an anti-inflammatory cytokine. This substance decreases expression of Th1 cytokines, macrophages, increases B cell survival, proliferation, and antibody production. [42] In mice with B cell deficiency, the infarct volume increasingly spread, this suggests the role of IL-10 in the regulation of the post-stroke immune system. IFN-ɣ production by Th1, T cell proliferation and cytokine response, TNF-α production by monocytes and macrophages, are all inhibited by IL-10.[7] The TNF-α/IL-10 ratio reflects a balance between Th1 and Th2 proinflammation and cellular immunity versus anti-inflammatory and humoral immunity. In patients with ischemic stroke, there was a decrease in the ratio of TNF-α/IL-10.[8,9]

Inoculation 200 cfu *S. pnemoniae* to MCAO mice can cause severe pneumonia, whereas in control mice 200,000 cfu are required to obtain the same severity of infection.[10] It has been shown that there was a post-stroke immunosuppression in animals and cerebral arterial media occlusion cause spontaneous infection in mice.[11] Similar infections may also be observed in stroke patients.[12,13] The suppression of the immune system by the nervous system actually protects the brain from further inflammatory damage, but at the same time increases susceptibility to infection.[14] Infection is a complication of 30% of patients with acute stroke.[15] Post-stroke infection is directly correlated with the severity of stroke (high NIHSS value), and extent of infarction.[16] Post-stroke infections prolong the duration of treatment and 80% are due to pneumonia and urinary tract infections.[17] Pneumonia in stroke patients will increase 3 times mortality within 30 days and worse clinical outcomes.[18]

Propranolol is a beta blocker, used to treat hypertension, irregular heart rhythm, thyrotoxicosis, anxiety and tremor. These drugs include a class of non-cardioselective sympatholytic beta blockers that can penetrate the blood-brain barrier. As non-selective beta blockers, drugs work to inhibit epinephrine and noerpinephrine at β1- and β2- receptors.[19] Propranolol 2x10 mg can overcome the symptoms of tachycardia, hyperhidrosis, hypertension and hyperthermia, caused by sympathetic hyperactivity in post-stroke patients.[20]

The production of TNF-α is regulated by the α2- and β-adrenoreceptors present in the terminal adrenergic nerves and macrophages. Using α2- and β-adrenergic drugs (clonidine, CH-38083, isoproterenol, propranolol), it was found that α2- and β- adrenoreceptors were involved in the production of TNF-α induced by lipopolysaccharide (LPS).[21] In addition to inducing an anti-apoptotic pathway, propranolol therapy significantly increases the activity of TNF-α and interferon γ (IFNγ).[22] In macrophages, endogenous norepinephrine inhibits the ability of phagocytosis to limit its immune system activity. In addition, norepinephrine decreases the cytotoxicity activity of natural killer (NK) cells, expansion of CD8 + T cells, and increases T helper 2 response.[23]

MATERIAL AND METHODS

This study was an experimental study with pretest-postes control group, conducted from July, 2016 to February, 2017. Subjects consisted of 40 ischemic stroke patients with chronic hypertension and lymphopenia who fulfilled the inclusion and eclusion criteria at "Abdul Moeloek" General Hospital, Bandar Lampung, Indonesia. Subjects are divided into two groups with the same characteristics (match control group). The first group consisted of 20 ischemic stroke patients not given propranolol. The second group consisted of 20 ischemic stroke patients given propranolol 2x10 mg. Diagnosis of ischemic stroke is confirmed through CT-Scan head examination performed maximum until the third day. The clinical weight of stroke was measured using NIHSS. Examination of TNF-α and IL-10 levels was performed on the fourth day using ELISA method using Quantikine® HS Human Immunoassay, R & D Systems, specifically for research by Prodia Laboratory, Jakarta, Indonesia.

This study has passed the ethical test from the ethics committee of The Faculty of Medicine at University of Andalas, Padang, Indonesia. Participants who participated in the study have signed an informed consent form. Propranolol 2x10 mg was inspired by a case report by [20] given after the first blood sample was taken. Seven days later re-sampled blood. The TNF-α/IL-10 ratio was obtained by comparing TNF-α and IL-10 levels. Statistical tests were performed by comparing the TNF-α, IL-10 deltas and the pretest-postest TNF-α/IL-10 delta ratios between groups using independent T tests. TNF-α and IL-10 were tested using the Mann-Whitney test. Differences in levels of TNF-α, IL-10, and TNF-α/IL-10 ratios were found to be significant when $p < 0.05$.

RESULTS AND DISCUSSION

Provision of Low Dose Propranolol In Acute Ischemic Stroke Patients Against TNF-α level, IL-10 level, and TNF-α/IL-10 Ratio are presented in Table 1, Table 2, and Table 3.

Table 1: TNF-α level.

	TNF-α without Propranolol (*Mean* ± *SD*)	**TNF-α with Propranolol (*Mean* ± *SD*)**	***P***
Pretest	8,300 ± 6,070	6,769 ± 5,920	
Post Test	5,380 ± 3,740	5,953 ± 3,900	
ΔTNF-α	2,920± 4,870	0,816 ± 7,370	0,221

Table 1 shows that a decrease in TNF-α levels of more groups not given propranolol 2x10 mg but, the difference in TNF-α levels decreased in both groups was not statistically significant (p> 0.05).

Table 2: IL-10 level.

	IL-10 without Propranolol (*Mean* ± *SD*)	**IL-10 with Propranolol (*Mean* ± *SD*)**	***P***
Pretest	0,702 ± 0,60	0,761 ± 0,39	
Post Test	0,884 ± 1,22	0,663 ± 0,62	
ΔIL-10	0,182 ± 1,25	0,098 ± 0,64	0,547

Based on Table 2., in groups not given propranolol, an increase in IL-10 levels was found. The group given proranolol 2x10 mg was decreased. However, the difference in IL-10 between groups was not statistically significant (p> 0.05).

Table 3: TNF-α/IL-10 Ratio.

	TNF-α/IL-10 Rratio Without Propranolol (*Mean* ± *SD*)	**TNF-α/IL-10Ratio with Propranolol (*Mean* ± *SD*)**	***P***
Pretest	14,79 ± 1,24	9,73 ± 6,56	
Posttest	10,81 ± 8,13	19,97 ± 3,06	
ΔTNFα/IL10	-3,98 ± 1,24	+10,24 ± 2,92	0,053

Table 3 presented that The TNF-α / IL-10 ratio in the group without propranolol decreased, whereas propranolol was elevated, indicating the effect of propranolol in improving anti-inflammatory conditions in ischemic stroke patients. However, the difference in TNF-α / IL-10 ratio between groups was not statistically significant (p> 0.05).

Increased proinflammatory cytokines (TNF-α and IL-1β) post-stroke causes sympathetic nerve activation and HPA axis,[24] further increased norepinephrine release from sympathetic nerve endings and glucocorticoids from the adrenal cortex and increased epinephrine and norepinephrine release from medulla adrenal.[12] All mentioned above cause immunosuppresion conditions characterized by decreased levels of TNF-α and elevated levels of IL-10[25,26,12] In addition,[27] have described clearly that T cells in the spleen (T-ChAT cells) expressing β2AR will be activated by norepinephrine released at the splenic nerve to produce acetylcholine. Acetylcholine binds to the α7nAch receptor, consequently the production of proinflammatory cytokines by macrophages is inhibited. In the liver, norepinephrine (NE) released by the sympathetic nerve endings changes the iNCT cell response that initially produces IFN-γ (Th1) changes producing IL-10 (Th2). It is clear that NE can change the production of cytokines from Th1 to Th2.[27] Another mechanism occurs through the bonding of acetylcholine with the α7nAch receptor which inhibits IkB phosphorylation so that NFkB does not undergo translocation to the nucleus, consequently the production of proinflammatory cytokines is inhibited. While other pathways are suspected through JAK2 pathways that activate STAT3 monomer changes to STAT3 dimer and into NFkB binding nuclei so that NFkB is inactivated.[28]

Propranolol is a non-selective adrenergic beta blocker, which can decrease sympathetic nervous activity in both β1 and ad adrenergic receptors[19] The β-adrenergic receptor can regulate the production of TNF-α through catecholamines. Propranolol therapy causes blockade of NE bond with β AR, TNF- α production is not suppressed, and IL-10 is reduced so that systemic immunosuppression that occurs after stroke is reduced.[11,10] The results of the research are not significant, the explanation is as follows. The path is inhibited only the sympathetic nerve pathway, while the HPA axis path is not. Glucocorticoids may inhibit the expression of chemoatractant and pro-inflammatory cytokines such as IL-1β, IL-6 and TNF-α.[29] In this study, the first TNF-α examination was performed on day 4, and the second day-to-11 post stroke examination. In this phase there has been a decrease in TNF-α levels due to post-stroke biphasic response.[30] Within 1 week of therapy, the mean TNF-α level was lowered from 6.769 ± 5.92 (pretest) to 5.953 ± 3.9 (posttest). This shows that the immunosuppression process continues. At a dose of 2x10 mg, propranolol can overcome the symptoms of tachycardia, hyperhidrosis, and hyperthermia, caused by sympathetic hyperactivity in post stroke patients,[20] but can not overcome post stroke immunosuppression.

Overcoming post stroke immunosuppression in experimental animals, determined by the dose of propranolol administered. The higher the dose of propranolol, the stronger inhibitory effect on immunosuppression.[11] At a dose of 80 mg, BEST found poor patient outcomes due to decreased cerebral blood flow. However, the dose was administered from the first day of stroke.[30,31] used a dose of 160 mg but his research was stopped without a clear explanation. Even though there is no definite dose of propranolol to treat

immunosuppression in stroke patients, it appears that a dose of 2 × 10 mg is not sufficiently strong to overcome severe immunosuppression. While high doses increase the risk of death especially when given in the early stroke phase.[30]

The results were not significant in this study may also be due to differences in age range between research subjects too far (44 years-87 years).[32] examined the effect of age with TNF levels. In the study, high levels of TNFα were found in 81-year-old subjects compared to young and healthy controls. However, in the study of[43] stated that in older rats the production of TNFα in macrophages decreased compared with young rats. In another study,[33] suggested that postmenopausal women had higher levels of TNFα than did younger controls.[34] also stated the same thing. TNFα in menopausal women is higher than in women of reproductive age. It is influenced by estrogen hormones that decrease at the age of menopause. The hormone estrogen can suppress the production of inflammatory cytokines such as IL-1β, IL-6 and TNFα. In addition, estrogen affects the Th1/Th2 balance with stimulation dominance on Th2. Meanwhile, according to[35] levels of TNFα also showed an increase in men with decreased testosterone, and decreased testosterone occurred in elderly men. Thus, it can be concluded that TNFα levels are equally high in women and older men.

Based on the above discussion, the results of this study are supported by many existing literatures, in which TNF-α changes are not significant although in the propranolol therapy group. Other risk factors that contribute to changes in the immune system are not examined further. A considerable age range among study subjects may also be influential. In the group not given propranolol there was an increase in IL-10 levels. In the group given propranolol there was a decrease in IL-10 levels. Differences in IL-10 levels in both groups were not statistically significant (P> 0.05).

Patients at risk for stroke-related infection were patients with high IL-10 levels (≥ 14.5 pg / mL) with different KIT ELISA, low TNF-α levels and low TNF-α / IL-10 ratios.[9] Because propranolol can inhibit the NE bond with β AR, propranolol therapy may reduce IL-10 levels as an anti-inflammatory cytokine.[25,12] In this study, one patient died of severe pneumonia, where the level of IL-10 in second examination increased more than 17x compared to the first examination (data not shown). It can not be concluded whether infection is caused by high levels of IL-10 or an infection that causes elevated levels of IL-10.

Many studies support the theory above. Some of them are as follows. Oxprenolol therapy (β receptor antagonists) invivo in LPS-induced rats, significantly reducing serum IL-10 levels.[36] Propranolol may decrease elevated levels of IL-10 due to norepinephrine stimulation in THP-1 cells.[37] High levels of IL-10 were found in stroke-related infections.[12,9] The results of this study indicate that in the group whom not given propranolol still increased in levels of IL-10 means still decrease immune function. While in the second group, the level decreased in the second examination within 1 week interval. Although not significant, the increase of IL-10 in the first group was consistent with existing theories, that during the immunosuppression period there was an increase in IL-10 levels,[25,26,12] and decreased levels of IL-10 after provision of propranolol according to the theory of,[28,27] blockade of norepinephrine bonds with β-adrenergic receptors by propranolol will also decrease the immunosuppression response through decreased levels of IL-10 due to suppression of Treg cells.[37]

Research about the association levels of IL-10 and age is still inconclusive. In the study of,[43] IL-10 production in rat macrophages was not affected by age. These results are in contrast to the study of[44] which suggests that in women of menopausal age the levels of IL-10 increase.[38] also stated the same thing. Levels of IL-10 in menopausal women increase, but in men of the same age did not increase.

It is recalled that the HPA pathway is not inhibited where glucocorticoids may increase the expression of anti-inflammatory cytokines such as IL-10 and TGF-β.[29] Other risk factors that contribute to changes in the immune system are not examined further. A considerable age range among study subjects may also be influential. The TNF-α / IL-10 ratio in the first group decreased while the second group increased. The ratio difference was not statistically significant (p> 0.05).

Under normal circumstances, there is a balance between pro- and anti-inflammatory cytokines. This balance will be impaired when there is a pathological process in the brain, and potentially determine the ability of subsequent immunological responses.[9,26] This TNF-α/IL-10 ratio, being the balance reference between Th1 and Th2 (proinflammatory and cellular immunity vs. antiinflammatory and humoral immunity). In stroke patients this ratio changed, even this decrease in ratio was already seen 1 day after onset of ischemic stroke.[39]

In this study, it was found that TNF-α / IL-10 in the group without propranolol decreased, whereas propranolol was increased. Although not significant in both groups, the TNF-α / IL-10 ratio decrease in the first group occurred due to immunosuppression due to sympathetic system activation.[25,40,12] The increase in TNF-α / IL-10 ratio in the second group (propranolol therapy) was due to propranolol therapy inhibiting norepinephrine bond with βAR.[37,28,27]

The study (retrospectively) by[45] showed that beta-blockers in the acute phase of ischemic stroke showed a decrease in the incidence of infection and a 3-month mortality rate compared with ischemic stroke patients

who did not receive beta-blockers.[45] investigated the administration of propranolol in the infarct stroke model and found that propranolol may protect experimental animals from immunosuppression and life-threatening infections, despite an increase in specific autoreactivity of brain antigens, but did not exacerbate functional outcomes.[41]

CONCLUSION

Low dose propranolol may improve the clinical symptom of sympathetic nerves hyperactivity but have not significantly improved immune system disorders in patients with ischemic stroke. Ischemic stroke affects the patient's immune system that show in alteration of TNF-α,IL-10 levels and ratio of TNFα/IL-10. Provision of propranolol 2x10 mg increase TNF-α level, decrease IL-10 level and inrease ratio TNFα/IL-10, but not significant statistically.

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