RESEARCH ARTICLE

Vascular Endothelial Growth Factor and Brain-Derived Neurotrophic Factor Levels in Ischemic Stroke Subject

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Abstract

BACKGROUND: Vascular endothelial growth factor (VEGF) and brain-derived neurotrophic factor (BDNF) present during early neuronal development and play important roles in the process of neurorepairing includes angiogenesis, neurogenesis and neuronal plasticity after ischemic stroke. In this study, we observed VEGF and BDNF levels of subjects with ischemic stroke in different onset time.

METHODS: A cross sectional study was designed. Study subjects were 51 ischemic stroke subjects, aged 30-80 years old, recruited from Gatot Subroto Army Central Hospital, Jakarta, Indonesia. Ischemic stroke was diagnosed by neurologist, based on clinical examination and magnetic resonance imaging (MRI) result. Subjects were divided into 3 groups based on onset time of stroke: <7 days (group A), 7-30 days (group B) and >30 days (Group C). VEGF and BDNF levels from serum were measured using luminex Magpix. The data was analyzed for comparison and correlation.

RESULTS: VEGF and BDNF levels of group B and C were significantly different with p=0.034 and p=0.007, respectively. Group B had the highest VEGF levels, whereas Group C had the highest BDNF level. VEGF and BDNF levels in each group were not significantly correlated.

CONCLUSION: Each stage of time after ischemic stroke has different recovery activities like angiogenesis, neurogenesis and plasticity. Angiogenesis process was optimum in 7-30 days after onset. In more than 30 days onset, Low VEGF with high BDNF have important role in a long period of time after the onset of stroke in the regeneration and repair, such as maintaining neuronal survival and plasticity.

KEYWORDS: ischemic stroke, VEGF, BDNF

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Introduction

Some growth factors are increased after ischemic stroke, include basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), brain-derived neurotrophic factor (BDNF) and others.(1-3) All neurotrophins can enhance recovery of ischemic stroke patients through the process of angiogenesis, neurogenesis and plasticity of neurons.(1) Angiogenic genes are upregulated within minutes upon the onset of cerebral ischemia and angiogenic proteins remain increased in the ischemic area for days to weeks.(1) The primary neurovascular responses during stroke recovery are thought to involve angiogenesis and neurogenesis.(4,5)

VEGF is a hypoxia-inducible protein that promotes angiogenesis through receptor tyrosine kinases on endothelial cells.(6) In patients with ischemic stroke, VEGF increases process and stimulates angiogenesis in penumbra.(7) The number of new blood vessel in penumbra is correlated with survival period.(8) VEGF also acts as an angiogenic protein with neurotrophic and neuroprotective effects (9), including

increase neurogenesis (6) and can stimulates axon growth from dorsal root ganglia (DRG), superior cervical ganglion (SCG) and primary cortical neurons (10).

VEGF and BDNF are both present during early neuronal development.(11) Among other mechanisms, it has been reported that VEGF stimulates brain endothelial cells (BEC) to secrets BDNF (12), which promotes proliferation and differentiation of progenitor cells (13) and in higher level, it can improves functional recovery (14). Since BDNF can be produced by endothelial cells, the BDNF can later increase neuronal migration to ischemic area.(1) BDNF also have neuroprotective effects.(15) In the other hand, BDNF also regulates VEGF secretion and angiogenesis through tryptomyosin receptor kinase B (TrkB), phospholipase C (PLC)-γ, protein kinase C (PKC)-α and Hypoxia-inducible factor (HIF)-1α signaling pathways.(16)

In Framingham study, lower BDNF and higher VEGF were associated with the increase of incident stroke risk.(10) In ischemic stroke condition, increasing VEGF and BDNF will promote neurogenesis and neuroplasticity in the ischemic boundary zone (17), and also create vascular niche for neurogenesis (18) and neuroblast migration to penumbra (1).

Many studies for stroke therapy are directed to improve the process of endogenous repair with marker VEGF or BDNF, such as through the provision of phosphodiesterase-5 inhibitors (19), erythropoietin (20), statins (13). In this study, we observed the VEGF and BDNF levels of subjects with ischemic stroke in different onset time, which has not been conducted in Indonesian patients. This study can add information regarding VEGF and BDNF levels in different time stage of ischemic stroke patients.

Methods

A cross sectional study was designed. Study subjects were 51 ischemic stroke subjects, aged 30-80 years old, recruited from Gatot Subroto Army Central Hospital, Jakarta, Indonesia. Ischemic stroke was diagnosed by neurologist, based on clinical examination and magnetic resonance imaging (MRI) result. Study protocol was approved by the Ethical Commission of Faculty of Medicine, Hassanudin University (No. 0024/H04.8.4.5.31/PP36-KOMETIK/2015). Subjects were divided into 3 groups based on onset time of stroke: <7 days (group A), 7-30 days (group B) and >30 days (Group C). Subjects with medical history of carcinoma, hematoma subdural, global ischemia, blood clotting disorders, seizures, or those who could not conduct MRI were excluded. Most subjects got medication of amlodipine, simvastatin, ACE inhibitor and aspirin.

Specimen Collection

Blood were collected intravenously, serum were separated by centrifugation and aliquots were stored frozen at -21°C for subsequent batched analysis for all parameter. VEGF and BDNF levels were measured using multiplex method (catalog number: LXSAHM-03, R&D System, Minneapolis, USA), with luminex Magpix instrument. Multiplex method provided multiple parameters by immunoassay principle at the same time with a high speed. We used BDNF reagents exclude pro-BDNF and the test was conducted at Prodia research laboratory, Jakarta.

Statistical Analysis

The normal distribution was analyzed by Shapiro-Wilk, and the difference significances between groups were analyzed by t-test analysis or Mann-Whitney, depends on distribution results. Meanwhile correlation significances between log VEGF and BDNF were analyzed by Pearson analysis.

Results

We conducted a study to identify the difference between VEGF and BDNF levels in each group. Total ischemic stroke subjects were 51, which were divided into: 8 subjects for group A, 18 subjects for group B and 25 subjects for group C.

General characteristic of 51 subjects for age, body mass index (BMI), systole blood pressure (SBP), diastole blood pressure (DBP) and the results of normality test with Shapiro-Wilk analysis data was reported in Table 1. Age, BMI and BDNF was distributed normally, whereas VEGF was not distributed normally.

As described in Table 2, there was no significant difference between the group onset for age, DBP, SBP and

Table 1. General characteristics of subjects and normality test (n=51).

Variable	Min	Max	Mean±SD	Median	*p**
Age (Year)	38	76	57.18±9.87	55	0.343*
BMI (Kg/m2)	18.25	34.05	24.04±11.63	23.88	0.086*
SBP (mmHg)	57	159	87.9412±17.2446	89	0
DBP (mmHg)	100	220	146.176±695.548	140	0.004
VEGF (pg/mL)	13.99	459.02	83.8761±72.2411	67.78	0
BDNF (pg/mL)	6105	4,000.00	18860±6844.67	18,343.00	0.536*

*Distribution assessment with Shapiro-Wilk test, SD: standard deviation.

Table 2. Comparison of biomarkers among groups. Results were shown as mean±SD.

Clinical Characteristics	Group			*p*		
	(A)	(B)	(C)	A vs. B	A vs. C	B vs. C
BMI	27.43±4.18	24.86±2.85	24.48±3.36	0.121	0.08	0.685
Age	61.75±11.68	57.72±10.70	55.32±8.45	0.442	0.207	0.518
SBP	74.83±29.95	88.17±16.57	83.96±9.97	0.592*	0.234*	0.261*
DBP	142.38±34.43	145.17±30.50	148.12±20.86	0.538*	0.355*	0.394*
VEGF	69.23±41.58	110.45±98.30	69.43±52.01	0.273	0.584	0.034**
BDNF	18291±6437.62	15919±5599.68	21160±7155.45	0.455	0.174	0.007**

*Mann Whitney, ** Independent t-test

BMI. The levels of VEGF and BDNF were significantly different between group B and C, while the other group did not significantly different. VEGF level of group B was the highest and the one of group C was the lowest. Meanwhile, BDNF levels of group B was the lowest and the one of group C was the highest (Figure 1). Using Pearson correlation test, it was found that there was no significant correlation between VEGF and BDNF in each group (Table 3).

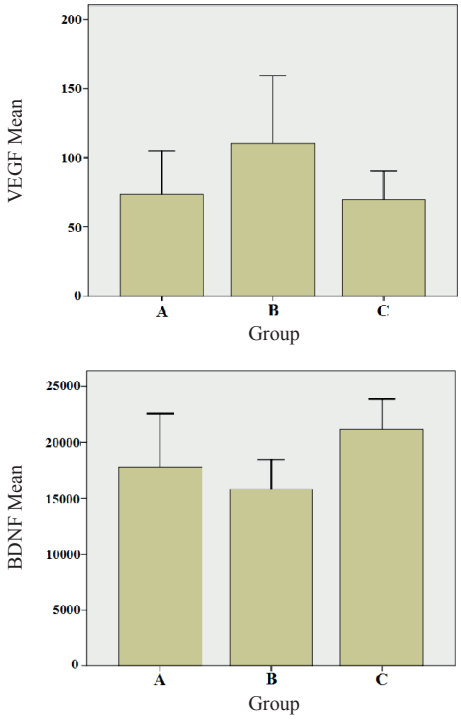

Figure 1. Comparison between VEGF and BDNF mean among groups.

Table 3. Correlation analysis between VEGF and BDNF.

Group	P	R
A (n=8)	0.399	0.321
B (n=18)	0.083	0.42
C (n=25)	0.564	-0.116

Discussion

VEGF levels were significantly different between group B and C, whereas group B had the highest VEGF level, while group C had the lowest VEGF level. These results are in line with the process of angiogenesis that occurs due to ischemic stroke with peak between day 7th to 14th after onset and then decreased.(21) The profile is also similar to the research results of Hayashi, *et al*., where VEGF levels increased immediately after the stroke and decreased until day 21st of observation. It is known that angiogenic genes increase 1 hour after onset and some genes remain elevated until day 21st, as well as the total number of vessel in the cortex. This suggests that angiogenesis is still running.(22) The peak of endogenous neurorepair processes including angiogenesis occurs at day 6th after onset and decrease by 1 month. It opens up opportunities for restoration therapy post stroke with a longer time.(23)

BDNF levels were significantly different between group B and C. BDNF level of group B was lower than group A, and group C had the highest BDNF level. This suggests that BDNF level was increased 2 weeks after VEGF level increased. This phenomenon was also observed in study of Louissaint, *et al.*, although triggered by different factors. It shows that BDNF level is changes slightly in the first week, but then increased steadily. The delayed rise of BDNF level was contrasted with VEGF level, which was increased rapidly until 1-2 weeks but then decreased.(12) Others study showed that VEGF could stimulate BECs in order to secrete BDNF.(18) The resulted BDNF could support neuron differentiation and migration.(12) In current study, the highest level of BDNF was observed after 30 days onset, suggesting that an important role of BDNF in a long period of time after the onset for regeneration and repair, such as maintaining neuronal survival and plasticity.

VEGF and BDNF are involved in process of neurogenesis and plasticity of synaptic.(24) Lin, *et al*.,

showed that BDNF also increased VEGF through TrkB and HIF-1 signal pathway.(16,25) Low BDNF and high VEGF levels were reported in patients with vascular risk, like atherosclerosis and cerebrovascular disease.(11) This profile similar to our result in group B but not in group C. This suggests that correlation between VEGF and BDNF can not be calculated, but the profile of the results can be assessed.

Conclusion

VEGF and BDNF in ischemic stroke have positive effect on long-term potentiation, neural remodeling, and functional motor recovery.(11) Each stage of time after ischemic stroke has different levels of VEGF and BDNF, this is related to the different recovery activities like angiogenesis, neurogenesis and plasticity and angiogenesis process was optimum in 7-30 days after onset. In onset more than 30 days, low VEGF with high BDNF probably needed for next regeneration process such as maintaining neuronal survival and plasticity. Assessing the levels of VEGF and BDNF in ischemic stroke could possibly be used to determine the recovery process. Yet, it needs more study to determine the recovery process.

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