ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 10, Issue 2, 2017

Online - 2455-3891
Print - 0974-2441

Research Article

RESPONSES OF BRAIN TISSUES AGAINST HYPOXIC CONDITION IN HEMORRHAGIC STROKE PATIENTS: NEUROGLOBIN EXPRESSION IN BRAIN TISSUE AND PLASMA

NINIK MUDJIHARTINI[1,2]*, LASMA NURHAYATI[3], MOHAMAD SAEKHU[4], SRI WIDIA A JUSMAN[1,2], JAN PURBA[5], MOHAMAD SADIKIN[1,2]

[1]Department of Biochemistry and Molecular Biology, Fakultas Kedokteran Universitas Indonesia, Jakarta 10430, Indonesia. [2]Department of Biochemistry and Molecular Biology, Centre of Hypoxia and Oxidative Stress Studies, Fakultas Kedokteran Universitas Indonesia, Jakarta 10430, Indonesia. [3]Department of Biomedical Sciences, FKUI, Jakarta 10430, Indonesia. [4]Department of Neurosurgery, Fakultas Kedokteran Universitas Indonesia, Jakarta 10430, Indonesia. [5]Department of Neurology, Fakultas Kedokteran Universitas Indonesia, Jakarta 10430, Indonesia. Email: ninikbiokim@gmail.com

Received: 01 November 2016, Revised and Accepted: 15 November 2016

ABSTRACT

Objective: Strokes remain a significant health concern and are the highest cause of mortality and physical or mental disability in productive and the elderly hospitalized patients in Indonesia. Neuroglobin (Ngb) mostly located in the central and peripheral nervous system, predicted enhanced neuronal survival under hypoxic condition, such as in a stroke. The aim of this study is to observe the response of the brain tissue of hemorrhagic stroke patients against hypoxic/ischemic conditions. The objectives are to recognize the pattern of Ngb expression in the brain tissue and plasma of hemorrhagic stroke patients, and furthermore, to compare the level of Ngb in the brain tissue and plasma of hemorrhagic stroke patients.

Methods: This is an observational study with consecutive sampling methods using cerebral cortex and the blood of hemorrhagic stroke patients, who underwent craniotomies to evacuate hematomas at Cipto Mangunkusumo Hospital (RSCM) and other hospitals in Jakarta. Ngb expression was measured in brain tissue and blood using real time reverse transcription polymerase chain reaction, while the ELISA method was adopted to measure Ngb protein in plasma.

Results: Hypoxia/ischemia in the brain tissue of hemorrhagic stroke patients increased the expression of Ngb in brain tissue compared to the blood. The level of Ngb protein in plasma of hemorrhagic stroke patients increased significantly compared to normal subjects; however, there is no significant difference between the plasma and brain tissue of hemorrhagic stroke patients.

Conclusion: Hypoxia/ischemia in hemorrhagic stroke patients increases the expression of Ngb mRNA and protein level.

Keywords: Neuroglobin, Hypoxia, Hemorrhagic stroke.

 DOI: http://dx.doi.org/10.22159/ajpcr.2017.v10i2.15971

INTRODUCTION

Stroke is a common neurological disease and a leading cause of severe disability and death in developed countries [1]. Until now, strokes remain a serious health problem in Indonesia. According to the Indonesian Ministry of Health in 2007, strokes were the primary cause of mortality in hospitalized patients. Strokes are also a cause of physical or mental disability in the productive age and the elderly.

A hemorrhagic stroke occurs when the blood vessel in the brain is ruptured, and the blood disseminates into the subarachnoid space and brain parenchyma [2]. Furthermore, bleeding vessels cause hypoxia downstream of the ruptured vessels. Neuron cells are very active and require a considerable amount of energy, which means that the neuron is extremely aerobic from a metabolic point of view. Naturally, living things attempt to survive in conditions that threaten life. Neuroglobin (Ngb), the third globin protein family, predominantly located in the central and peripheral nervous system is supposed to enhance neuronal survival under hypoxic conditions, such as in a stroke [3,4]. As Ngb is a member of the globin family, it is suggested that the protein could bind with oxygen.

Burmester *et al*. [5] stated that Ngb acted as an oxydase that regenerates NAD^+ under anaerobic conditions, thereby sustaining adenosine triphosphate production. Ngb may also be involved in the detoxification of harmful reactive oxygen species, which are known to be generated under hypoxic conditions [6-8]. In addition, Ngb could also detoxify other noxious reactive molecules, NO. Finally, Ngb might act as a sensor to detect cellular oxygen concentration [7-9].

Under hypoxic conditions, Sun *et al*. [4] reported a ~2.5-fold up-regulation of Ngb mRNA and protein levels after 24 hrs anoxia-reperfusion in relation to cerebral tissue culture [3,4].

Wang *et al*. [10] concluded that Ngb could reduce tissue infarction in transient focal cerebral ischemic transgenic mice. Tissue with overexpression of Ngb may be sustained for up to 2 weeks after ischemia.

Research conducted with stroke ischemic patients' revealed upregulation of Ngb in peri-infarct (ischemic penumbra) compared to normal brain and ischemic core [11]. Research on acute cerebral ischemic (ACI) patients demonstrated an increase in Ngb level in serum, 1-6 hrs after the onset of ACI patients. The levels peaked at 24 hrs, and gradually descended to normal level at 72 hrs [12].

This research is intended to observe the pattern of Ngb mRNA expression in the brain tissue and plasma of hemorrhagic stroke patients, in addition to comparing the level of Ngb between the brain tissue and plasma of hemorrhagic stroke patients.

METHODS

Expression of Ngb mRNA

Expression of Ngb mRNA was measured using real time reverse transcription polymerase chain reaction according to the CFX program (MiniOpticon Bio Rad). The Ngb mRNA primer was (F:5'-TGGAAGACCTGTCCTCACTG-3', R:5'-GAGCAGAGACTCACCCACTG-3') [13]; 18 sRNA primer was (F:5'-AAACGGCTACCACATCCAAG-3',R:5'-ACTTCTCCTCGGTGACGTTC-3')[14].

Measurement of Ngb protein
Ngb protein was measured by means of the ELISA method.

Total RNA isolation
Total RNA from the blood was isolated using a total mini kit (Blood/Cultured Cell) Geneaid® (Taiwan), while the total RNA isolation from the brain tissue used a total mini kit (Tissue) Geneaid® (Taiwan).

Brain tissue homogenates
Brain tissue was homogenized using 0.1 M phosphate buffer solution pH 7.4 (1:10 dilution), centrifuged for 10 minutes at 4°C and the supernatant was collected (stored at −80°C if the measurement is not conducted on the same day) for determination of Ngb levels.

Total protein concentration of tissues
The total protein concentration of tissues was measured by spectrophotometric technique at a wavelength of 280 nm. A standard curve was performed using bovine serum albumin.

Ngb protein level
The Ngb protein measurement level was determined by utilizing a human Ngb ELISA kit USCN® (Wuhan, PRC).

Statistical analysis
All data were analyzed with SPSS 16.0 software. A significance difference was defined at a level of $p<0.05$.

RESULTS AND DISCUSSION

General data of patients
Samples were obtained from the cerebral cortex and venous blood of hemorrhagic stroke patients who underwent craniotomy <24 hrs previously. From August 2012 to March 2013, we collected 11 bloods, 19 plasmas, and 12 cerebral cortex samples. As a control, we used venous blood from 10 normal healthy subjects.

The pattern of Ngb mRNA expression
The relative expression of Ngb mRNA in brain tissue was 0.025 times higher than in the blood of hemorrhagic stroke patients (Fig. 1).

The level of Ngb protein in the brain tissue and plasma of hemorrhagic stroke patients
The level of Ngb protein in the plasma of hemorrhagic stroke patients was significantly higher compared to normal plasma (Mann–Whitney, $p<0.05$). Meanwhile, the level of Ngb protein between the plasma and brain tissue of hemorrhagic stroke patients were not significantly different (Mann–Whitney, $p>0.05$) (Fig. 2).

The level of Ngb protein in normal plasma, brain tissue, and plasma patients
The level of Ngb protein in the plasma was higher than in the brain tissue; however, it revealed no significant difference (Mann–Whitney, $p>0.05$) to the negative correlation (Spearman's Rho, $R=-0.370$, $p>0.05$) (Fig. 3).

The relative expression of Ngb mRNA in brain tissue was 0.025 times higher than in the blood of hemorrhagic stroke patients. This result was in accordance with the previous studies, confirming that the expression of Ngb increased in hypoxia/ischemia conditions [4,11,15].

Brain tissue in a hypoxic condition experienced an ischemic area at central and surrounded by the penumbra. Brain cells are extremely sensitive to oxygen lacking and can begin to die within 5 minutes after an oxygen supply has been cut-off [16]. In the ischemic area cell death occurred, whereas within the penumbra selective genes were expressed, [17] including Ngb in an attempt to improve the oxygen supply. This result is also in accordance with a previous study, which noted that hypoxia increased Ngb expression within the penumbra [11].

The level of Ngb protein in the plasma of hemorrhagic stroke patients was higher than normal. This result was in agreement with the previous studies, which established that the level of Ngb increased in plasma

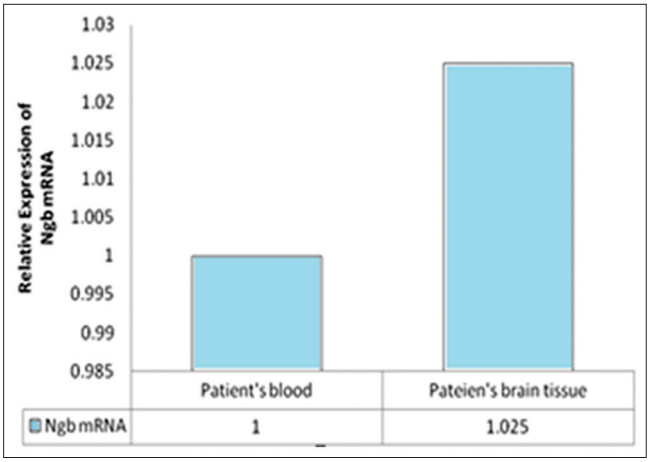

Fig. 1: The relative expression of neuroglobin mRNA in the blood and brain tissue of hemorrhagic stroke patients (n=10)

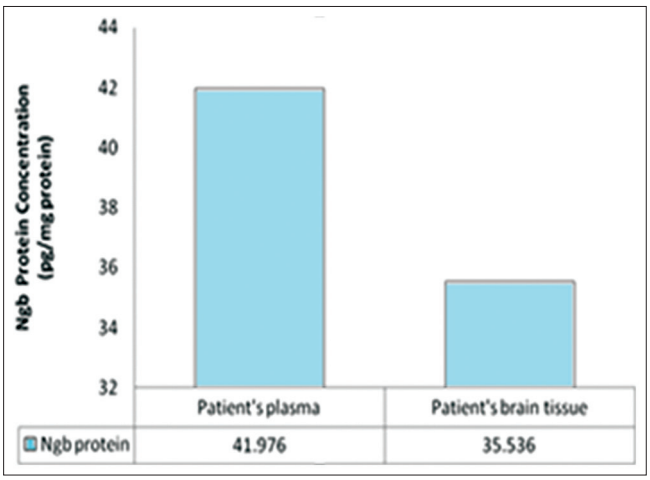

Fig. 2: The level of neuroglobin protein in normal plasma, plasma, and brain tissue of hemorrhagic stroke patients (n=10 for normal subjects, n=19 for patient's plasma, and n=12 for patient's brain tissue)

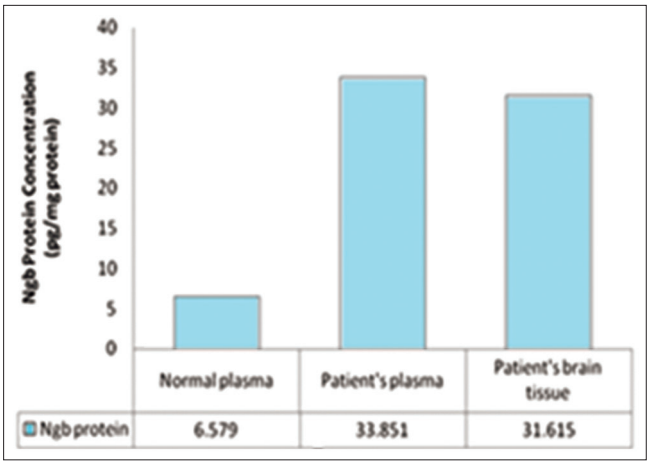

Fig. 3: The level of neuroglobin protein in the plasma and brain tissue of hemorrhagic stroke patients (n=10), in pairs

1-6 hrs after onset, peaked at 24 hrs, subsequently decreased over time in acute atherosclerotic cerebral infarction (ACI) [12].

The increase in Ngb level in plasma remains ambiguous, although Casado *et al*. discovered Ngb protein in cerebrospinal fluid [18]. In addition,

hypoxia caused cell death through two mechanisms, necrosis and apoptosis. Necrosis caused edema cell impairments of the cytoskeleton and ultimately membrane rupture. Meanwhile, apoptosis at the end caused cell lysis. The two mechanisms predicted that Ngb leaked out cerebrospinal fluid, crossed the blood-brain barrier and entered into systemic circulation. Therefore, Ngb is observed in the plasma.

The level of Ngb protein in paired samples indicated that the level of plasma Ngb protein was higher than in brain tissue, although it was not observed to be significantly different. Correlation of Spearman's Rho demonstrated a negative correlation ($R=-0.370$ and $p>0.05$).

CONCLUSION

Hypoxia/ischemia in the brain tissue of hemorrhagic stroke patients increased the expression of Ngb in brain tissue compared to the blood. The level of Ngb protein in plasma of hemorrhagic stroke patients increased significantly compared to normal subjects; however, there is no significant difference between the plasma and brain tissue of hemorrhagic stroke patients. Hypoxia/ischemia in hemorrhagic stroke patients increases the expression of Ngb mRNA and protein level.

ACKNOWLEDGMENTS

Authors wish to express our gratitude to Directorate of Research and Public Service University of Indonesia for the Research Grant.

REFERENCES

1. Patil ML, Patel SJ. Pharmacogenomic analysis of single-nucleotide polymorphisms of angiotensin-I converting enzyme gene in stroke. Asian J Pharm Clin Res 2015;8(2):308-10.
2. Prince SA, Wilson LM. Patofisiologi: Konsepklinis Proses-Proses Penyakit. 6th ed. Jakarta: EGC; 2006. p. 1119-20.
3. Burmester T, Hankeln T. Neuroglobin: A respiratory protein of the nervous system. News Physiol Sci 2004;19:110-3.
4. Sun Y, Jin K, Mao XO, Zhu Y, Greenberg DA. Neuroglobin is up-regulated by and protects neurons from hypoxic-ischemic injury. Proc Natl Acad Sci U S A 2001;98(26):15306-11.
5. Burmester T, Weich B, Reinhardt S, Hankeln T. A vertebrate globin expressed in the brain. Nature 2000;407(6803):520-3.
6. Garry DJ, Mammen PP. Neuroprotection and the role of neuroglobin. Lancet 2003;362(9381):342-3.
7. Brunori M, Giuffrè A, Nienhaus K, Nienhaus GU, Scandurra FM, Vallone B. Neuroglobin, nitric oxide, and oxygen: Functional pathways and conformational changes. Proc Natl Acad Sci U S A 2005;102(24):8483-8.
8. Herold S, Fago A, Weber RE, Dewilde S, Moens L. Reactivity studies of the Fe(III) and Fe(II)NO forms of human neuroglobin reveal a potential role against oxidative stress. J Biol Chem 2004;279(22):22841-7.
9. Bunn HF, Poyton RO. Oxygen sensing and molecular adaptation to hypoxia. Physiol Rev 1996;76(3):839-85.
10. Wang X, Liu J, Zhu H, Tejima E, Tsuji K, Murata Y, *et al.* Effects of neuroglobin overexpression on acute brain injury and long-term outcomes after focal cerebral ischemia. Stroke 2008;39(6):1869-74.
11. Jin K, Mao Y, Mao X, Xie L, Greenberg DA. Neuroglobin expression in ischemic stroke. Stroke 2010;41(3):557-9.
12. Zhao S, Chu Z, Ma L, Chen Y, Wang L, Wang B, *et al.* Significance of neuroglobin in serum of acute atherosclerotic cerebral infarction patients. Neural Regen Res 2011;6(27):2140-5.
13. Zhang W, Tian Z, Sha S, Cheng LY, Philipsen S, Tan-Un KC. Functional and sequence analysis of human neuroglobin gene promoter region. Biochim Biophys Acta 2011;1809(4-6):236-44.
14. Hardiany NS, Wanandi SI. Correlation between oxidative stress and tumor grade in glioma cells from patients in Jakarta. Med J Indones 2012;21(3):122-7.
15. Shang A, Zhou D, Wang L, Gao Y, Fan M, Wang X, *et al.* Increased neuroglobin levels in the cerebral cortex and serum after ischemia-reperfusion insults. Brain Res 2006;1078(1):219-26.
16. Paulbabu K, Singh KD, Prashanti P, Padmaja M. Neuroprotective potential and efficacy of neurodegenerative disorders of fruit extract of *Aegle marmelos*. Int J Pharm Pharm Sci 2014;7(1):155-9.
17. Dirnagl U, Iadecola C, Moskowitz MA. Pathobiology of ischaemic stroke: An integrated view. Trends Neurosci 1999;22(9):391-7.
18. Casado B, Pannell LK, Whalen G, Clauw DJ, Baraniuk JN. Human neuroglobin protein in cerebrospinal fluid. Proteome Sci 2005;3(1):2.