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Correlation Between Hypoxia Inducible Factor-1α and Vesicular Endothelial Growth Factor in Male Wistar Rat Brain Tissue After Anaerobic Exercise

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ABSTRACT

During physical activity, oxygen reduction leading to hypoxia affects brain cell metabolisms, induces stabilization of Hypoxia Inducible Factor-1α (HIF-1α) and up-regulate Vesicular Endothelial Growth Factor (VEGF). This study aimed to investigate the correlation of HIF-1α and VEGF of brain tissue in male wistar rat after anaerobic exercise. Twenty four rats were divided into four groups: control, 1x, 3x and 7x in a week of anaerobic exercise. A rat treadmill was used at speed 35 m min^{-1}, 20 min for every anaerobic exercise. The HIF-1α and VEGF level were measured by ELISA. The correlation between HIF-1α and VEGF was analyzed using Pearson test. The HIF-1α and VEGF level increased in 1x and 3x a week of anaerobic exercise. The highest HIF-1α and VEGF level were observed in 1x a week of anaerobic exercise. Pearson test between HIF-1α and VEGF showed $r = 0.709$ and $p = 0.000$. These findings showed that HIF-1α and VEGF are correlated and anaerobic exercise affects HIF-1α and VEGF level.

Key words: HIF-1α, VEGF, brain tissue

INTRODUCTION

Brain has an important role during physical exercise. It needs the most glucose and oxygen of the body. The brain of adult human consists of 2% of the total body weight, consumes 20% oxygen and 25% glucose of total body consumption (Clarke and Sokoloff, 1999; Magistretti *et al*., 1993). The brain basal metabolic activity is supported by glucose oxidation (Dienel, 2012). Brain tissue is very vulnerable to hypoxic condition for mitochondrial oxidative phosphorylation in brain cell requires oxygen to generate energy (Nioka *et al*., 1990). Hypoxic condition in brain tissue can occur when oxygen demand during physical exercise is not supplied with sufficient oxygen (Folkman and Shing, 1992).

Hypoxia induces adaptive mechanisms in systemic and molecular level. In molecular level, hypoxic condition induces stabilization and expression of HIF-1α (Zagorska and Dulak, 2004). Under hypoxic condition, HIF-1α will be stabilized and translocated into the nucleus. In the nucleus, HIF-1α will be dimerized with HIF-1β to form HIF-1 complex (Wang *et al*., 1995). The HIF-1 is a transcription factor, a heterodimer of two basic helix-loop-helix PAS proteins (HIF-1α and HIF-1β) (Wang *et al*., 1995). Through the activity of HIF-1, the expression of some genes are activated particularly genes for maintaining energy metabolisms and oxygen balance including erythropoeisis (*EPO* gene), vasodilatation, glucose metabolisms (*SLC*2A1 gene) and angiogenesis (*VEGF* gene) (Greer *et al*., 2012).

Angiogenesis is a process of blood veins development that regulated by VEGF. The VEGF induces recruitment of endothelial cells to hypoxia and avascular area and stimulates endothelial cell proliferation, survival and cell migration (Ahluwalia and Tarnawski, 2012). Under hypoxic condition, the VEGF expression is regulated by the HIF-1 as a master regulator of oxygen regulated gene expression. The angiogenesis increases the vascular density and decreases the distance of oxygen diffusion which made cells around the blood vessel formation get sufficient oxygen and nutrition (Lee *et al*., 2004; Ahluwalia and Tarnawski, 2012).

Flora *et al*. (2012) study mentioned that strong correlation ($r = 0.69$; $p = 0.00$) between HIF-1α and VEGF in rat myocardium after anaerobic exercise. Ahluwalia *et al*. (2010) showed a correlation between HIF-1α and VEGF in the impaired angiogenesis of aging myocardial microvascular endothelial cells. Lack of importin α, a protein that carries HIF-1α into the nucleus could impair transport of HIF-1α into the nucleus, reduces VEGF expression and thereby significantly decrease angiogenic capability (Ahluwalia *et al*., 2010). In early stage of brain neurons development, hypoxic condition induces expression of HIF-1α (Rosenstein *et al*., 2010). In brain tissue, the correlation between HIF-1α and VEGF is not studied yet. Therefore, this study aims to investigate the correlation between HIF-1α and VEGF in rat brain tissues after treated with various anaerobic exercise which could contribute to develop a physical therapy for neurodegenerative patient, for VEGF has ability to enhance nerve regenerative, glial cell growth, blood vessel formation and neuroprotective (Rosenstein *et al*., 2010).

MATERIALS AND METHODS

Animals: Twenty four healthy and adult male wistar rats (*Rattus norvegicus*), 6-8 weeks old, 150-220 g of body weight and healthy were used. Those criteria were used to get rat that easily run in the treadmill during the treatment. The amount of sample was determined using Federer's formula (Federer, 1991):

$$(n-1)\ (t-1) \geq 15$$

The 4 groups of treatment were conducted ($t = 4$) including negative control, 1x, 3x and 7x in a week anaerobic exercise and each group was consist of $n = 6$ healthy and adult male rats.

Anaerobic exercise: This study used cross-sectional experimental design. A rat treadmill for anaerobic treatment was used at speed 35 m min^{-1} during 20 min and 90 sec with interval every 5 min (Soya *et al*., 2007; Flora *et al*., 2012). Before the measurement of HIF-1α and VEGF, rats were decapitated and the brain tissue was stored at -70°C. The research has been approved by the Research Ethics Committee, Faculty of Medicine, Sriwijaya University.

Hypoxia inducible factor-1α measurement: A 100 μL homogenates of rat brain tissue were used for HIF-1α assay. The HIF-1α level was measured by sandwich ELISA using Surveyor™ IC Human/Mouse total HIF-1α Immunoassay (SUV 1935, R and D System).

Vesicular endothelial growth factor measurement: A 100 μL homogenates of rat brain tissue were used for VEGF assay. The VEGF level was measured by sandwich ELISA using Quentikine Mouse VEGF (MMVOO, R and D System).

Statistical analysis: The data was analyzed using IBM SPSS 17.0 software and presented as Mean±Standard Deviation (SD). The Pearson correlation test was conducted to know the correlation between HIF-1α and VEGF. Significant differences of HIF-1α and VEGF level in each treatment analyzed using one way ANOVA, α = 0.05 was considered as the significance level and followed by post hoc tests multiple comparison (Tukey HSD).

RESULTS

The HIF-1α level was higher than VEGF level (Table 1). The HIF-1α and VEGF level were lower following an increase of anaerobic exercise frequency (Fig. 1). Seven times anaerobic exercise showed the lowest level of HIF-1α and VEGF. Once anaerobic exercise showed the highest level of HIF-1α and VEGF (Table 1 and 2). The Pearson-correlation test and a scatter plot showed a significant positive and strong correlation ($p<0.05$, $r = 0.709$) between HIF-1α and VEGF (Table 3, Fig. 2).

Table 1: Effects of various anaerobic exercise treatments toward HIF-1α and VEGF

Treatment groups	HIF-1α (pg mL^{-1})	VEGF (pg mL^{-1})
Negative control	162.29 ± 29.44^{b}	65.48 ± 13.82^{a}
1x	242.53 ± 27.72^{c}	113.09 ± 27.89^{b}
3x	206.45 ± 37.55^{bc}	88.64 ± 16.79^{ab}
7x	104.34 ± 20.15^{a}	83.21 ± 10.75^{a}

Data are presented as Mean±SD, n = 6. 1x, 2x and 3x are anaerobic exercise frequency in a week. Superscripts (a, b, c, d) in HIF-1α column: a = p<0.05 vs. negative control, 1x and 3x, b = p<0.05 vs. 1x and 7x, bc = p<0.05 vs. 7x, c = p<0.05 vs. Negative control and 7x, Superscripts (a,b) in VEGF column: a = p<0.05 vs. 1x, b = p<0.05 vs. Negative control and 7x, HIF-1α: Hypoxia inducible factor-1α, VEGF: Vesicular endothelial growth factor, significant differences value based on Tukey HSD post-hoc comparison (p<0.05)

Table 2: Effects of various anaerobic exercise treatments toward percentage of HIF-1α and VEGF by comparing with negative control

Treatment groups	HIF-1α (%)	VEGF (%)
1x	49.44±17.08	72.71±42.6
3x	27.21±23.14	35.37±25.65
7x	-35.71±12.42	27.07±16.41

Data are presented as Mean±SD, n = 6. 1x, 2x and 3x are anaerobic exercise frequency in a week, HIF-1α: Hypoxia inducible factor-1α, VEGF: Vesicular endothelial growth factor

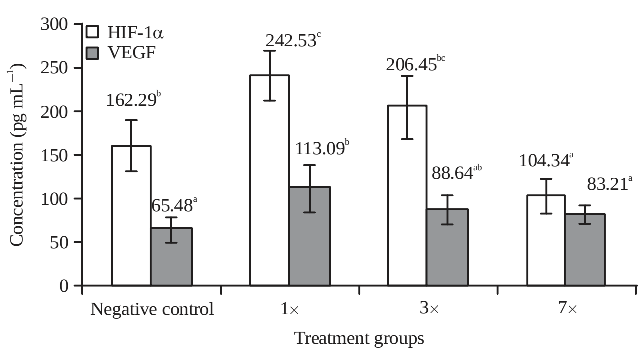

Fig. 1: Column chart of HIF-1α and VEGF level in various anaerobic exercise, superscripts (a, b, c, d) in HIF-1α bar: a = p<0.05 vs. negative control, 1x and 3x, b = p<0.05 vs. 1x and 7x, bc = p<0.05 vs. 7x, c = p<0.05 vs. negative control and 7x, Superscripts (a,b) in VEGF bar: a = p<0.05 vs. 1x, b = p<0.05 vs. negative control and 7x, HIF-1α: Hypoxia inducible factor-1α, VEGF: Vesicular endothelial growth factor, significant differences value based on Tukey HSD post-hoc comparison (p<0.05)

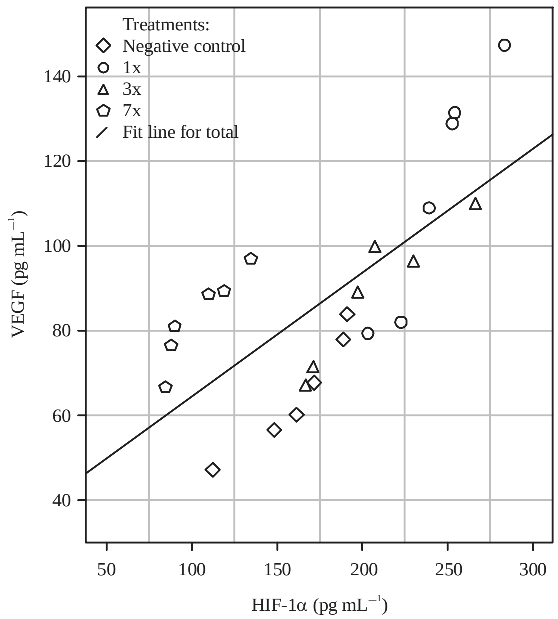

Fig. 2: Scatter plot of HIF-1α and VEGF of all the treatments, HIF-1α: Hypoxia inducible factor-1α, VEGF: Vesicular endothelial growth factor

Table 3: Correlation of HIF-1α and VEGF brain tissue on various anaerobic exercise treatments

	Correlation value		
Treatment group	p	r	Correlation level
Negative control	0.003	0.955	Significant, very strong
1x	0.002	0.965	Significant, very strong
3x	0.006	0.934	Significant, very strong
7x	0.007	0.929	Significant, very strong
Total	0.000	0.709	Significant, strong

1x, 2x and 3x are anaerobic exercise frequency in a week. r show correlation value and p show level of significant value from Pearson- correlation 2-tailed test

DISCUSSION

The HIF-1α and VEGF is correlated for hypoxic condition increases expression and stabilization of HIF-1α. Furthermore, HIF-1α and HIF-1β are dimerized to form HIF-1 which activating the VEGF gene transcription (Richardson *et al*., 1999). Anaerobic exercise was able to induce hypoxic condition, this result was verified with previous study that anaerobic exercise treatment (animal treadmill at speed 35 m min^{-1} for 20 min) decrease partial oxygen pressure and oxygen saturation in blood (Flora *et al*., 2012). During intensive physical activity such as anaerobic exercise, skeletal muscle needs much oxygen supply from blood, which made low oxygen supply to the brain that leads to hypoxic condition in the brain. Hypoxic condition increase HIF-1α and VEGF level. This result was consistent with previous study that extensive exercise increase HIF-1α level in rat brain (Correia *et al*., 2013). Rat treated with single bout treadmill exercise showed an increase of HIF-1α proteins in hypothalamus (Berggren, 2013). Hypoxic condition in rat treated with short term one legged exercise increased vascularisation in skeletal muscles through induction of VEGF gene expression by HIF-1α (Gustafsson *et al*., 1999). Once a week of anaerobic exercise showed the highest level of HIF-1α and VEGF, this result accordance to previous result in myocardium cell that once a week physical exercise treatment could increase HIF-1α and VEGF level (Flora *et al*., 2012). Hypoxic condition affects some gene expression that involved in oxygen homeostasis, through HIF-1 (HIF-1α and HIF-1β coupling complex), the expression of some genes such as VEGF increases for inducing angiogenesis to increase oxygen supply to hypoxic tissue (Lee *et al*., 2004; Ahluwalia and Tarnawski, 2012; Zagorska and Dulak, 2004). *In vivo* and *in vitro* study showed that VEGF involved in angiogenesis (Otani *et al*., 1998; Zheng *et al*., 2001). An increase of HIF-1α mRNA related with the increase of VEGF mRNA in rat muscles which four times more abundant by hypoxic condition (Richardson *et al*., 1999; Gustafsson *et al*., 1999).

The HIF-1α and VEGF are important not only in hypoxic condition but also in normoxic condition (Stroka *et al*., 2001). Negative control, untreated rat or normoxic condition had HIF-1α and VEGF level for HIF-1α was necessary for brain cell homeostasis (Stroka *et al*., 2001; Flora *et al*., 2012). In normoxic condition, HIF-1α regulates genes expression specially genes that involved in cellular energy metabolism and VEGF promotes neurogenesis, neural patterning and neuroprotection (Cramer *et al*., 2003; Rosenstein *et al*., 2010).

The HIF-1α and VEGF level decreased in increasing anaerobic exercise frequency. The increasing of the anaerobic exercise frequency reduced the recovery phase. The highest exercise frequency resulted the body had shortest time for recovering and responding hypoxic condition. In seven times anaerobic exercise, resulted no recovery time and interfered hypoxic responses. Previous study showed a reduction of HIF-1α and VEGF level in the increase of physical exercises in myocardial cell (Flora *et al*., 2012). This result was also verified with previous result that intense anaerobic exercise leads to acute hypoxic condition that decreased HIF-1α and VEGF level (Forsythe *et al*., 1996).

CONCLUSION

This study showed a positive and strong correlation between HIF-1α and VEGF level in brain tissue of rat with anaerobic exercise. Low frequency of anaerobic exercise preserves HIF-1α and VEGF level. As suggestion, further animal study aim to observe neurodegenerative healing effect of programmed anaerobic exercise in neurodegenerative rat model.

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