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Article *in* Journal of Postgraduate Medical Institute · January 2018

THE EFFECT OF MODERATE EXERCISE ON VASCULAR ENDOTHELIAL GROWTH FACTOR EXPRESSION DURING TOOTH SOCKET WOUND HEALING AFTER TOOTH EXTRACTION

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Date Received:
June 19, 2017
Date Revised:
January 12, 2018
Date Accepted:
January 20, 2018

ABSTRACT

Objective: To determine vascular endothelial growth factor (VEGF) expression during the tooth socket wound healing process post-extraction after moderate exercise.

Methodology: Wistar Rats (Rattus norvegicus) were divided into control group (group I) and treatment group (group II). The group II was given moderate exercise with 50% maximal work capacity of time, every day, for 2 weeks. The VEGF expression was observed in macrophages 3 days after tooth extraction and analyzed using immunohistochemistry. Statistical analysis was conducted using an independent t-test.

Results: The treatment group had a higher expression of mean VEGF as compared to the control group (194.43 +17.213 vs. 131.29 +21.085, respectively). The difference in test results of VEGF expressions was significant, with a p value of =0.000.

Conclusion: Moderate exercise increased the expression of VEGF during the tooth socket wound healing process after tooth extraction.

Key Words: Moderate exercise, Vascular endothelial growth factor, Wound healing, Wistar rats

This article may be cited as: **Irmawati A, Giffari FZ, Oki AS. The effect of moderate exercise on vascular endothelial growth factor expression during tooth socket wound healing after tooth extraction. J Postgrad Med Inst 2018; 32(1): 19-23.**

INTRODUCTION

The most common dental treatments or procedures performed by the dentists in the Indonesia are tooth extractions that can lead to alveolar bone defect[1]. According to health-related statistics 2016, the percentage of dental treatments culminating in tooth extraction reached as high as 80.6%[2]. Previous research highlighted the prevalence of tooth extraction-related complications such as fractures 31.82%, bleeding 4.54% and swelling 2.27%[3].

However, some will heal through a primary healing process without complications. The complex and dynamic process of replacing devitalized missing cellular structures and tissue layers is known as wound healing. It is achieved through hemostasis, inflammation, proliferation and remodeling. Injury caused by tooth extraction is categorized as acute inflammation that can occur about 1-3 days and chronic inflammation can occur if the inflammation duration prolonged about 4-5 days. There are many factors that can affect wound healing and may cause improper or impaired tissue repair. The process of wound healing can sometimes be delayed, especially in patients who have to consume certain drugs (anticoagulants) or in patients who have a systemic disease (diabetes) or other external and internal factors[4,5].The growth factor which stimulates angiogenesis and vasculogenesis is called vascular endothelial growth factor (VEGF). VEGF has critical role in the new bone formation, hematopoiesis and wound healing. VEGF can be produced by neutrophils, macrophages and platelets. Exercise may stimulate the secretion of VEGF[6].

Exercise can be defined as an activity that can improve or maintain health. Regular exercise can provide lots of benefits including strengthening of skeletal muscles, improving cardiovascular functions, losing weight and maintaining body health. In addition, physical exercise can also prevent cancer by inhibiting the formation of transform cells. Exercise generally can be divided into 2 types, aerobic and anaerobic. Aerobic exercise such as swimming will enhance the overall oxygen consumption of the body[7,8]. The several pathways that are induced by exercise are known to be important for vasculogenesis and angiogenesis. Moderate exercise has been shown to increase VEGF expression. VEGF has critical role in wound healing and vasculogenesis. In addition to mitogen-activated protein kinase (MAPK), the several other signaling pathways that may be the link between metabolism and vasculogenesis are activated by moderate exercise. The activation of several downstream signaling proteins including calmodulin-dependent kinase (CaMK) and CaN by the increasing Ca^{++} level, possibly lead to increased VEGF mRNA expression[9]. Previous studies showed that physical exercise can increase the expression of VEGF. A study conducted by Erekat et al[10] reported that the expression of cardiac VEGF is significantly increased ($p<0.01$) in the the sedentary diabetic group as compared to the exercised diabetic group.

However, no research has been done to study if moderate exercise can increase the expression of VEGF during inflammation and thus accelerate the wound healing process post tooth extraction. The aim of this study was to analyze the effect of moderate exercise on VEGF expression during the tooth socket wound healing process post-extraction.

METHODOLGY

This was a prospective cohort study. The inclusion criteria were male Wistar Rat (Rattus Novergicus), aged 2-3 months and having body weight 100-200g. Exclusion criteria were rats of other strains, females and being sick or non-healthy. The 14 male Wistar Rats were equally divided into control group (group I) and treatment group (group II). This research had been approved with ethical clearance from Committee of Ethical Clearance of Health Research, Faculty of Dentistry/ Dental Medicine, Universitas Airlangga (No: 99/KKEPK.FKG/VII/2016).

In the control group, rats were only immersed in a tub of clean water, within 50% of maximum work capacity, daily, for 2 weeks. In the treatment group, the rat had moderate exercise (swimming) within 50% of maximum work capacity, at intervals (3x swimming and 2x break), daily, for 2 weeks. After 15 days, 2 of mandibular incisors were removed. After 18 days, rats were sacrifice using ether. Afterwards the jaw resection was fixed in 10% formaldehyde buffer solution for 24 hours. Decalcification was conducted for 1 month using formic acid mixed with formaldehyde at a ratio of 160 ml:100 ml. The measured variable in this research was VEGF. The immunohistochemistry (IHC) staining was performed using monoclonal antibody anti-VEGF (RM0002-7A23, Abcam). Total macrophage VEGF expression was calculated in both the control and treatment group, after that the mean expression of VEGF in both groups were calculated. VEGF expression was read using an e-microscope with 400x magnification, in 5 different fields and was examined by two experts. Data obtained from the research was then reviewed with statistical tests. Prior to the statistical test, a normality test was done in advance using the Shapiro-Wilk test. Results obtained from the Shapiro-Wilk test showed that $p > 0.05$ for both groups, therefore it can be concluded that the two groups were normally distributed. After the normality test, a homogeneity test was then performed with the Levene test to determine whether the two groups were homogeneous or not. The results of the Levene test showed that $p > 0.05$ for both groups, therefore it can be concluded that the two groups were homogeneous.

After finding out that the two groups were normally distributed and homogeneous, an independent t-test was conducted The data obtained were analyzed using SPSS version 21.0 (SPSS Inc., Illinois, Chicago). The independent t-test was conducted to analyze the difference between 02 groups (group I and group II). A p value of <0.05 was considered significant.

RESULTS

According to our results, the treatment group had a higher expression of VEGF as compared to the control group. The treatment group showed a mean of its VEGF expression as 194.43 and the control group showed a mean of its VEGF expression as 131.29. Table 1 shows the difference in test results of VEGF expressions between the two groups, with a p value of =0.000.

On the immunohistochemical preparations of tooth extraction sockets in Wistar rats' mandible, the observed cells were macrophages that secreted VEGF. The control group showed that its macrophage VEGF expression was lower than that of the treatment group (Figure 1 and Figure 2).

DISCUSSION

Wound healing begins when the tissue is injured. In this study, the wound is a tooth-shaped socket formed from the extraction of Wistar rats' mandibular incisors. Healing time depends on several factors in wound healing process. One of the most important factors is angiogenesis. In hemostasis and inflammation phases, some cells will produce VEGF which may accelerate wound healing process[5,6].

The results of this study were in accordance with the previous study by Vital et al[11]. There was an increased VEGF expression in the 60-year-old patient after physical exercise (in 4 samples), while the other 6 samples did not affect VEGF expression. This study is also in accordance with the study done by Jensen et al[12] on skeletal muscle in humans and showed that physical exercise for 4 weeks can induce VEGF mRNA. Physical exercise has a lot of benefits such as increased heart and lung capacity, joint and muscle strength, decreased levels of body fat and blood glucose levels, reduced risk of cor-

Figure 1: Macrophage VEGF expression (white arrows in the control group with immunohistochemical staining (400x magnification).

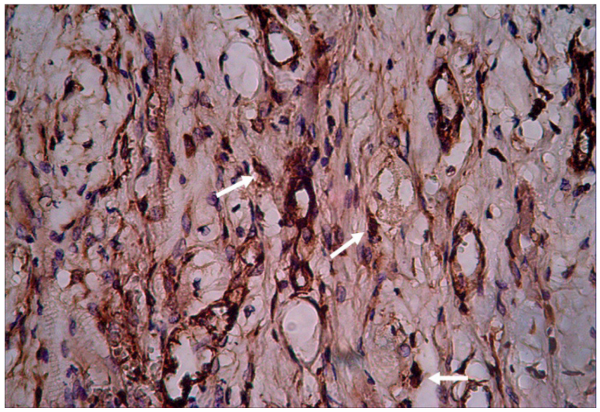

Figure 2: Macrophage VEGF expression (white arrows) in treatment group with immunohistochemical staining(400x magnification). Arrows show the expression of VEGF on macrophages

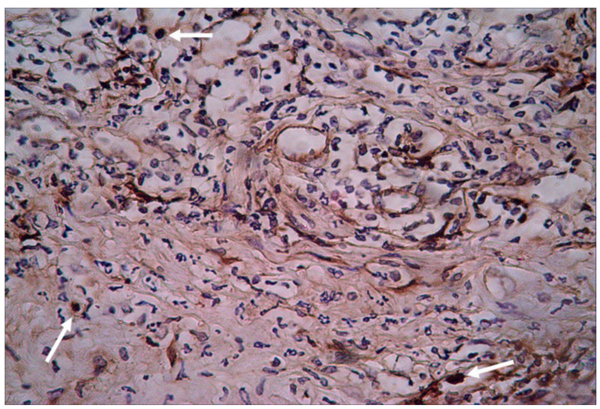

Table 1: Independent t-test between the 2 groups

Group	n	Mean	Standard Deviation	Independent t-test
Control	7	131.29	21.085	0.000*
Treatment	7	194.43	17.213	

Note: significant if $p<0.05$

onary heart disease, as well as immunity modulation. It can thus help in optimization of physiological performance[13]. Regular physical exercise can reduce inflammatory markers in wound healing process. Decreased inflammation will help faster wound healing process[14].

The physical exercise has anti-inflammatory effect that can be obtained via decrease in visceral fat mass, decrease in the Toll-like receptors (TLRs) expression on macrophages and monocytes and release of anti-inflammatory cytokines. Several studies have revealed that anti-inflammatory effects of physical exercise can inhibit infiltration of macrophages and monocytes to the tissues. Previous studies also showed that physical exercise may decrease the number of monocytes and decrease the number of macrophages in the circulation[15,16].

Macrophages are functionally and phenotypically different in response to various stimuli. Macrophages can be classified phenotypically by the expression of its cell surface molecules, cytokine expression and effector function. Macrophages are divided into the M1 (pro-inflammatory macrophages) and M2 (reparative macrophages). Interleukin-1β (IL-1β), IL-6, IL-12, tumor necrosis factor α (TNF-α) and other pro-inflammatory cytokines are triggered by M1. On the other hand, the anti- inflammatory cytokines e.g. tumor growth factor β (TGF-β) and IL-10 are produced by M2. M2 macrophages can also secrete VEGF that has important role in the process of neovascularization and wound healing[12]. Previous studies have shown that there was a relationship between physical exercise and increased expression of VEGF in certain age groups, but the ideal type of physical exercise is debatable[17,18].

M2 macrophages within the tissues will be more efficient in wound healing process post- tooth extraction because M2 macrophages may produce angiogenic factors such as VEGF. Regular exercise can increase adrenaline which helps open the Ca++ channels in the cell membranes of macrophages which ultimately lead to increased concentration of Ca++ in the cytoplasm. This will activate MAPK through Src and Ras-GAP signal transduction pathway. Consequently, the activated MAPK will activate the mRNA transcription factor for VEGF. Increased synthesis of VEGF will induce translation in ribosomes that will generate new VEGF[11,13,19].

Physical activity can also stimulate macrophages on existing wound tissue to produce nitric oxide (NO)[20]. NO is important for physiological and pathological conditions[21]. It can increase VEGF through hypoxia-inducible factor 1α (HIF-1α). HIF-1α can be regarded as one of the transcription factors involved in the wound healing process[22]. During the wound healing process, activated macrophages need high oxygen consumption therefore it will decrease the level of oxygen in the surrounding tissue. This condition will lead to hypoxia. Hypoxia stimulates the formation of new blood vessels and increased HIF-1α level in the injured area. The dimerization of HIF-1α and HIF-1β and binding to cis-acting hypoxia response elements (HREs) HREs can increase the expression of VEGF. HIF-1α expression has an important role in cell regulatoion during hypoxia. Moreover, it can increase the expression of several genes involved in wound healing and angiogenesis[23,24]. The function of a system of gene regulation of HIF-1/HRE, which is active in the state of hypoxia, is a proposed mechanism for expression of a specific gene target[25,26].

CONCLUSION

Moderate exercise increased the expression of VEGF during the tooth socket wound healing process after tooth extraction.

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CONTRIBUTORS

AI conceived the idea, planned the study and drafted the manuscript. FZG and ASO helped acquisition of data, did statistical analysis, editing and final approval of manuscript. All authors contributed significantly to the submitted manuscript.