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EFFECT OF ALPHA TOCOPHEROL SUPPLEMENTATION ON FGF EXPRESSION IN HUMAN BONE MARROW MESENCHYMAL STEM CELLS (*IN VITRO* STUDY)

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Abstract– In tissue engineering, the administration of alpha tocopherol is reported to increase wound healing, given the possibility of a lack of microcirculation in the graft area. Alpha tocopherol is also known to increase the proliferation of mesenchymal stem cells, induce osteoblast differentiation, and increase the formation of collagen fibers. This study aims to determine the effect of additionAlpha tocopherol on FGF expression on bone marrow mesenchymal stem cell cultures. Culture samples taken from Bone marrow aspiration from adult human femur bones. To get the homogeneity of the research data, the researcher uses as many as 5 samples for each treatment. The expressions of FGF was then evaluated using immunocytochemistry on the 1st, 3rd and 7th day. The collected data was then analyzed using comparative analysis test. The results showed that there was no significant difference in FGF expression in hBM-MSC by addition of alpha tocopherol supplementation compared with control. Addition of Alpha tocopherol supplementation does not influence of FGF expression on bone marrow masenchymal stem cell.

INTRODUCTION

Post mandibular resection defect needs rehabilitation, one of the options are using autogenous bone graft. Autogenous bone graft is considered as an ideal bone graft because it has three biological properties, first, because it is osteogenic which contains a lot of living cells, second it is osteoconductive, it has a woven structure that functions as a scaffold to place vascular tissue in host tissue, third is osteoinductive the ability to secreted growth factors that can induce the process of tissue proliferation and differentiation mesenchymal stem cell into osteoprogenitor cells and has the ability to stimulate osteoprogenitor cells to differentiate into osteoblasts and can then continue to initiate new bone formation (Movahed *et al*., 2013).

Failure in bone harvesting can occur due to damage to the periosteal tissue, then the reconstruction procedure with non-vascularized autogenous bone graft method often fails, which is characterized by the occurrence of bone resorption or no incorporation between the graft and recipient bone (Pramono, 2011). In such conditions above the role of growth factors and osteoinductive co-factors can play an important role to enhance the process of proliferation and differentiation of bone marrow mesenchymal stem cell tissue. Alpha tocopherol is a co factor that can help as a suplement in bone marrow mesenchymal stem cell culture media in the process of osteogenesis, tocopherol as a micronutrient which hopes to stimulate fibroblast growth factors (FGF), which are involved in the process of angiogenesis (Azzi *et al.*, 2003).

Human bone healing process need many growth factors.increase of this growth factor is related to the process of new blood vessel growth. VEGF and FGF expression occurs due to autocrine or paracrine regulation (Phelps, 2010). This study aims to

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determine FGF expression in hBM-MSC between giving 25 µM alpha tocopherol supplementation.

MATERIALS AND METHODS

This research is a true experimental labolatory study by observe the effect of alpha tocopherol supplementation with a dose of 25 µM to FGF expression in human bone marrow mesenchymal stem cell (hBM-MSC) cultures *in vitro*. Samples was taken from culture stock at Laboratory of Stem Cell, Universitas Airlangga isolated and processed from adult human femoral bone aspiration. The replication used in this study is five samples per study group.

This research was conducted by addition alpha tocopherol with a dose of 25 µM (treatment group) and without alpha tocopherol (control group) in hBM-MSC culture using Immunocytochemistry method. Cell samples from each group were fixated after 1, 3, and 7 days of culture. After incubation, the fixation, washing and blocking processes were carried out then the addition of FGF antibodies labeled FIT-C was added, incubated at 37^{0}C, for 45 minutes and immediately observed with a fluorescent microscope at 40x magnification. Positive expression is determined by green fluorescence in the observation field.

The data is processed in scores 0-4 to categorize the percentage of cells presenting luminescence to represent the amount FGF expression. The scores "0" = if there is no luminescence observed, score "1" = if less than 5% of the cells glow positively, score "2" = if 5-50% of cells glow positive, score "3" = if more than 50% of cells glow with weak intensity, and score "4" = if more than 50% of cells glow with strong intensity are obtained.

Statistical analysis in this study compared FGF expression between treatment and control group using comparative analysis of Mann-Whitney test (SPSS Ver. 15.0 for Windows (SPSS Inc.USA).

RESULTS

This study was analysing FGF expression after 25 ìM alpha tocopherol supplementation in human bone marrow mesenchymal stem cell culture which were evaluated on day 1, day 3 and day 7 and observed under microscope fluorescent with immunocytochemistry method (Figure 1).

Comparative analysis between alpha tocopherol and control groups were done using the Mann-Whitney test. The results of the analysis presented in Figure 2 below showed that there was no significant difference in luminescence between the alpha tocopherol group and the control group on

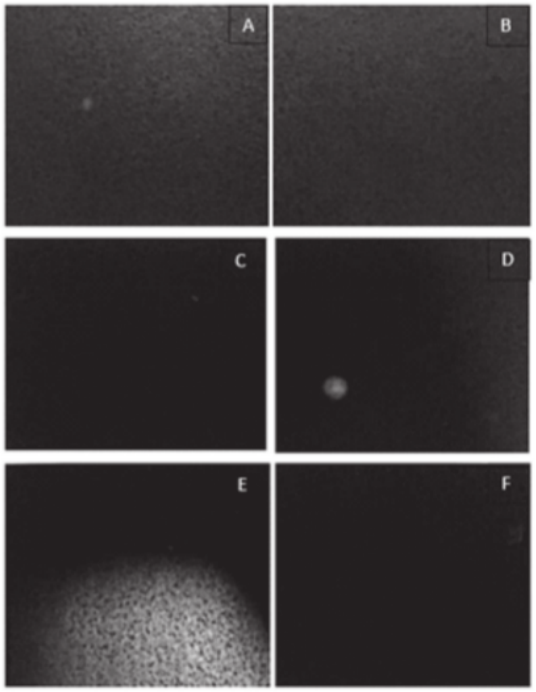

Fig. 1. Microscopic view through immunocytochemistry staining of FGF expression in hBM-MSC cell treatment group (A, C, E) culture in (B, D, F) control group with observations on day 1, 3, and 7, respectively

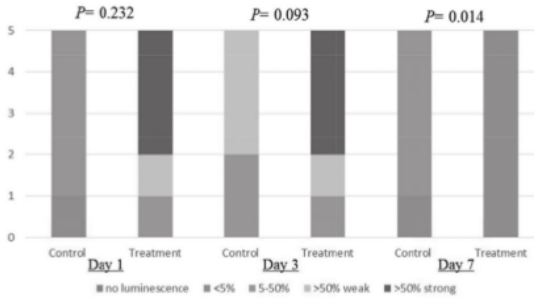

Fig. 2. FGF expression score on Human Bone Marrow Mesenchymal Stem Cells between treatment (apha tocopherol) and control groups after day 1, 3, and 7 observation (horizontal axis) using immunocytochemistry method. The vertical axis showed total amount of sample which were 5 slides in each group and the color indicated the category of luminescence from immunostaining.

days 1 and 3 ($P > 0.05$), whereas on day 7, there was a significant difference ($P<0.05$) in FGF expression. In an analysis of the comparison of values between days in each group, obtained the results of the significance of the different test $p < 0.05$, it explained that there were significant differences in expression in the comparison between time groups in the alpha group and overall control.

DISCUSSION

This study attempt to analyze the effect of alpha tocopherol on the expression of FGF in MSC culture in vitro, because FGF has been widely known to be a potent stimulator for angiogenesis which is one of the most important steps in bone defect healing (Bahramsoltani *et al*., 2010).

Angiogenic effect of FGF is mediated by interaction of FGF-tirosin kinase receptors which has high affinity (FGFRs) and integrin receptors and proteoglikan heparan sulfat which has low affinity. The two types of receptor lead to activation of various transduction signaling pathways which consist of various pathways such as phospholipase C-γ, phosphatidylinositol 3-kinase (PI3K) and mitogen-activated protein kinase (Andrés *et al*., 2009).

The role of FGF in inducing angiogenesis is via indirect pathway that is through *chemokine-dependent* pathway. Stimulated FGF produces chemotactic factors which are able to attract mononuclear phagocyts involved in amplification of angiogenic responseby releasing *monocyte-derived pro-angiogenic cytokines* (Andrés *et al*., 2009).

The results of this study found no significant differences between the alpha tocopherol group and the control group on observations on the 1st and 3rd days. This is probably due to the fact that in the control group, in a physiologically normal state the initial process of inflammation will occur in the wound healing stage, triggered by the surgical procedure itself, wherein the normal process of angiogenesis is controlled by several proteins and matrix-bound growth factors such as VEGF and FGF-2 (Robbins, 2009).

The provision of alpha tocopherol supplementation can also help to prevent the stages of wound healing that are too long, because the wound healing stage is a series of events related to one stage and the next stage is expected that by providing micro nutrient supplementation can optimize each stage in a short period of time.

CONCLUSION

There was no difference in FGF expression in hBM-MSC between giving 25 μM alpha tocopherol supplementation compared with no alpha tocopherol supplementation.

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