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THE EFFECT OF ALPHA TOCOPHEROL SUPPLEMENTATION ON THE EXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTOR ON HUMANBONE MARROW MESENCHYMAL STEM CELL (*IN VITRO* STUDY)

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Key word : *Autogenous bone grafts, Bone Marrow Stem Cell Mesenchymal (hBM-MSC), Alpha tocopherol, Vascular endothelial growth factor (VEGF).*

Abstract–Surgical reconstruction is a major challenge in oral and maxillofacial surgery, autogenous bone graft considered to be the gold standard reconstruction for Bone Augmentation. Supplementation of Alpha tocopherol enhance healing that can help process bone regeneration. Improvement for vascular endothelial growth factor expression in the culture medium of bone marrow mesenchymal stem cells. The Purpose of this study is to determine the effect of alpha tocopherol supplementation of the expression of VEGF on hBM-MSC. In this study we divided into 2 main groups: Alpha Tocopherol group and control group. Each group consist of 5 samples and was observed on the 1st, 3rd, and 7th days. Human bone marrow mesenchymal stem cell multiplicated towed to $5x10^4$ in 96 well. The cells the supplemented with VEGF and then observed under microscope fluorescent. The results of statistical analysis based on the findings of this study were conducted using the Mann-Whitney test There was no significant difference between alpha tocopherol and control group on observation day 1 and day 7 (p=0,549 and p=0,419) but there was significant differences in the 3rd day (p=0.042). There was no increase in VEGF expression in hBM-MSC after supplemented with Alpha tocopherol on observation day 1 and 7, but there was anincreasedin day 3.

INTRODUCTION

Bones or large bone defects resulting from trauma or surgical removal of lesions in the hard tissue of the jawbone and face area always causes complications or disability and becomes a problem in its reconstruction (Laurencin *et al*., 2006). Autologous bone graft or Autogenous bone graft Bone graft has the nature of biological mechanisms namely osteogenic, osteoconduction and osteoinduction (Samartzis *et al*., 2005).

Healing process of bone defect is a process of bone regeneration that occurs due to proliferation and differentiation of bone marrow mesenchymal stem cells which promoted by vascular endothelial growth factor (VEGF) (Madeddu, 2005). Alpha tocopherol is known to improve wound healing and that is marked at the molecular level in the form of increased VEGF expression which needed to increase the formation of new blood vessels that will increase proliferation and differentiation of bone marrow mesenchymal stem cells to become osteoblasts in the process of bone regeneration (Pradel *et al*., 2009). Based on the background above, this study tries to prove whether alpha tocopherol supplementation affects VEGF expression from bone marrow mesenchymal stem cell culture.

MATERIALS AND METHODS

This Research was true experimental laboratory study, with post-test control group design to

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evaluate alpha tocopherol supplementation on the expression of VEGF in bone marrow mesenchymal stem cell culture. The study sample group used was comprised of two groups of hBM-MSCs (Human bone marrow Mesenchymal Stem Cell). The culture preparations of bone marrow mesenchymal stem cell were available at thestem cell research and Development Center, Universitas Airlangga, Surabaya. First group are hBM-MSCs culture supplemented with 25ìM Alpha tocopherol, and the control groups is not treated with alpha tocopherol. The effect was observed after 1,3,7 days and prepared for immunocytochemistry staining with monoclonal Antibody Anti VEGF. Observations then conducted under a Fluorescent microscope.

The data is processed in scores 0-4 to categorize the percentage of cells presenting luminescence to represent the amount VEGF 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 VEGF 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 VEGF expression after 25 ìM alpha tocopherol supplementation in human bone marrow mesenchymal stem cell culture which were evaluated inday 1, 3 and 7 observationwith immunocytochemistry method under fluorescent microscope are presented in Figure 1.

The results of this study showed no significant differences between the alpha tocopherol group and control group on days 1 and 7 (pe"0.05), whereas on day 3 there were significant differences (p <0.05). In

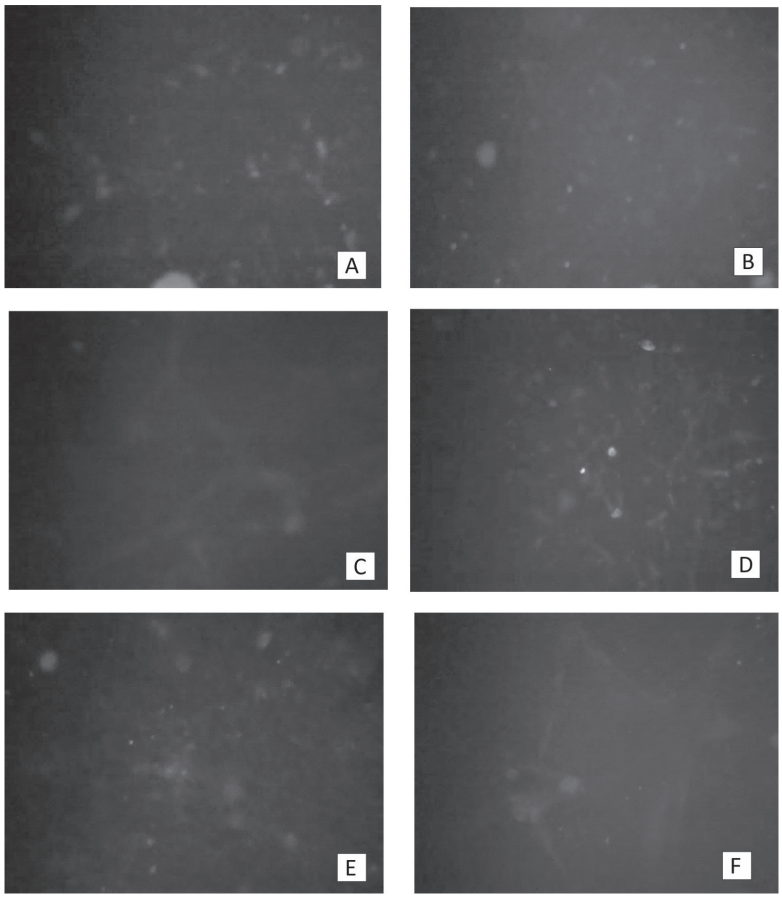

Fig. 1. hBM-MSC cell culture in (A, C, E) control group (without alpha tocopherol supplementation) and (B, D, F) treatment group (alpha tocopherol supplementation) under fluorescent microscope with 100x magnification in day 1, 3, and 7 respectively.

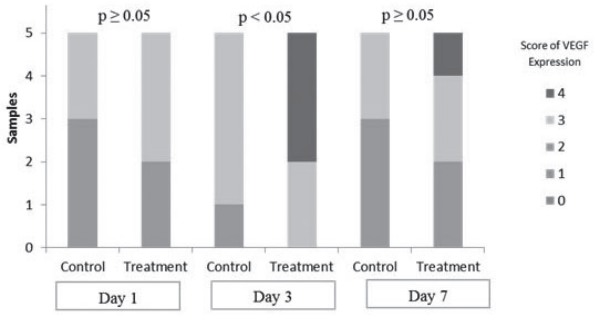

Fig. 2. VEGF expression score on hBM-MSC between treatment (Alpha tocopherol) and control groups after day 1, 3, and 7 observation. The vertical axis showed total amount of sample which were 5 slides in each group and the colour indicated the category of luminescence from immunostaining (0= no luminescence at all, 1= less than 5% of the cells glow positively, 2= between 5-50% of cells that glow positively, 3= if more than 50% of cells glow with weak intensity, and 4= if more than 50% of cells glow with strong intensity).

an analysis of the comparison of values between days in each group showed there was a higher increase on day 3 and began to decline on day 7.

DISCUSSION

Stem cell therapy has become an important topic and has been widely researched in the field of regenerative tissue engineering, especially multipotentmesenchymal stem cells which have the ability to proliferate into various cell types and have high bioavailability.

One of the angiogenic factors that has endothelial cell targets to stimulate the mitotic process isVEGF which signalling will attached to the vascular endothelial growth factor receptor (VEGF-R), after which there will be dimerization events or conformational changes in VEGF-R. Next, autophosphorylase or transfhosphorylase occurs. The new blood vessels will grow quickly, increasingly release VEGF, and in turn further trigger the growth of new blood vessels. These viable cells can also be supplemented with micronutrients in this case giving alpha tocopherol which hopes to stimulate VEGF, which are involved in the process of angiogenesis.Based on the results of research from Pradel *et al*., (2009), á-tocopherol increases VEGF in phosphatidylinositol-3-kinase gamma (PI3Kã) which will increase vascular permeability and proliferation of new blood vessels (angiogenesis). Increased PI3Kc activity mediated by alpha tocopherol is needed to increase the formation of new blood vessels which will increase proliferation and differentiation of bone marrow mesenchymal stem cells to become osteoblasts in the process of bone regeneration.

Alpha tocopherol is known to increase the proliferation of mesenchymal stem cells, induce osteoblast differentiation, and increase the formation of collagen fibres which differentiated into osteoblasts. Physiologically, the spread of VEGF expression on bone marrow mesenchymal stem cells increased on day 5 to day 7 (Frais *et al*., 2009), but in the findings of this study, giving alpha tocopherol causedincrease in VEGF expression on day 3. This could be explained that the result of *invitro* study may be influenced by several factors, such as the concentration of supplemented agent, the cell density of mesenchymal stem cell culture, the number of cell passages at which the treatment is performed, the culture medium used an many more. The significant increase of VEGF expression found in alpha tocopherol group might indicate its influence on VEGF expression at tissue level, however, further study is required to confirm this assumption.

CONCLUSION

The supplementation of alpha tocopherol does not increase VEGF expression in human bone marrow mesenchymal stem cells culture on observation day 1 and 7, but increase in VEGTis exhibited on day 3.

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