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EFFECT OF ALPHA TOCOPHEROL SUPPLEMENTATION ON TUMOR NECROSIS FACTOR-ALPHAEXPRESSION IN HUMAN BONE MARROW MESENCHYMAL STEM CELL (*IN VITRO* STUDY)

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Abstract– Inflammatory processes started first before osteogenic differentiation begin. Tumor necrosis factor alpha (TNF-α) is a major pro-inflammatory cytokine, the over expression of which may prolong inflammation which may inhibit initiation of bone healing. It is beneficial in suppressing damaging effects of free radicals oxygen in cells during bone healing. Alpha-tocopherol (Vitamin E) is a natural macromolecule that acts as a biological antioxidant. For optimal bone healing, the healing process need supplementation like alpha-tocopherol for increase the formation of new bone. Results of another study showed that it is clear that alpha-tocopherolplays a role in normal bone mineralization, either by its antioxidant effects or by increasing calcium availability for bone deposition. The aim of this study is to evaluate the effect of alpha tocopherol supplementation on TNFα expression in human bone marrow mesenchymal stem cell (hBM-MSC). This study was post test only experimental study in hBM-MSC culture, divided into two groups, control group were4 x 10^5hBM-MSC and experimental group were 4 x 10^5hBM-MSC supplemented with alpha tocopherol 25 μM. Observation was carried for 1, 3, and 7 days. Each group was evaluated with immunocytochemistry staning with monoclonal antibody anti TNFα with fluorescein (FITC). Data were analyzed statistically one way ANOVA, with p value < 0.05 considered statistically significant. There were significantly lower TNF-α expression in experimental group compared to control group on 7th day of observation (p =0,014), but there was no significant in the other day experiment. Alpha tocopherol supplementation not significantly decreases TNF-α expression in human bone marrow mesenchymal stem cell culture.

INTRODUCTION

Process of bone healing is divided into four phases: inflammation, angiogenesis, resorption of graft and remodeling (Xinping *et al.*, 2008). During the inflammatory phase of thebone healing process, four types of cytokines play in thatrole, there were Platelet Derived Growth Factor (PDGF), Tumor Necrosis Factor-Alpha (TNF-α), Transforming Growth Factor - Beta (TGF-beta) and Interleukin (IL-1, IL-6, IL-10 and IL-12). TNF-α was pleiotropic inflammatory cytokine that serves various in functions. The effect of TNF-α on inflammation was the main mediator of inflammation. Controlled inflammatory process was very necessary in the bone healing process, but an increase or elongation of the inflammatory phase conditions will result the inhibition of the bone healing process (Openheimer *et al.*, 2008). For optimal bone healing, the healing process need supplementation like alpha-tocopherolfor increase the formation of new bone.

The role of alpha-tocopherol in cell homeostasis is seen through modulation of specific signaling pathways and genes involved in proliferation, metabolic, inflammatory, and antioxidant functions (Galli, 2010). Alpha tocopherol was known to increase the proliferation of bone marrow mesenchymal stem cells, and increase the formation

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of collagen fibers. In the study, it was proved that lack of alpha tocopherol intake caused structural changes in osteoblasts formed from bone marrow mesenchymal stem cell (Miyazawa, *et al.*, 2008).

In this study, human mesenchymal stem cell bone marrow was used as the sample. According to the source, stem cells can be obtained from the bone marrow or commonly referred to as bone marrow mesenchymal stem cell (BMC). The main function was to help the tissues formation and help the hematopoietic cell system (Saputra, 2006).

The aims of the study was to determine the effect of alpha-tocopherolsupplementation on TNF-á expression in bone marrow mesenchymal stem cell cultures with fluorophore conjugated immunocytochemistry (ICC) staining.

MATERIAL AND METHODS

This wasa true experimental laboratory study to evaluatethe effect of 25 µMalpha tocopherol supplementation doses on the expression of TNF-á in bone marrow mesenchymal stem cells culture in vitro. The sample of this study used a culture preparations of human bone marrow mesenchymal stem cells which were available at Stem Cell Research and Development Center, Universitas Airlangga, Surabaya, Indonesia.

Human bone marrow mesenchymal stem cells (hBM-MSC) culture was divided into experimental and control group. In experimental group hBM-MSC was cultured in 25 µM alpha-tocopherol-supplemented medium, while in control group the cells was cultured in basic medium. Cell samples from each group were fixated after 1, 3, and 7 days of culture.

Characterization of mesenchymal stem cell bone marrow used anti TNFα antibodies using immunocytochemistry techniques, as follows: Cells that have been monolayer into one cell underwent a trypsinization process. Performed a 1600rpm centrifuge for 5 minutes. Inserting 1 mL of media into cell pellets, resuspending and producing 20 µL of special glass. Place a glass object in a box that is already in wet paper, then incubated at 37 °C, for an hour. Fix it with 3% Formaldehyde for 15 minutes at room temperature. Wash with PBS 4 times, then dry. Blocking with PBS containing 1% serum, for 15 minutes, at room temperature. Wash with PBS 4 times, then dried. Install the PDGF antibody labeled FIT-C, incubate at 37 °C, for 45 minutes. Wash with PBS 4 times, then air dried around the glass object with tissue paper. Drop Glicerin 50% above the glass object, then strain it seen with a fluorescent microscope at 40x magnification. Positive expression is determined by number of cells that glow in the observation field.

Data collected in this study was number of cells which showed positive expression of TNF-α under fluorescence microscope observation using anti-TNFα monoclonal antibodieswith immunocytochemistry technique and observed under fluorescence microscope with 400x magnification.

The TNF-α expressions were observed after 1, 3, and 7 days culture and prepared for immunostaining using anti-TNFα monoclonal antibodieswith immunocytochemistry technique andobserved under fluorescence microscope with 400x magnification. The difference in TNF-α expression between treatment (25 µM alpha tocopherol supplementation) and control group

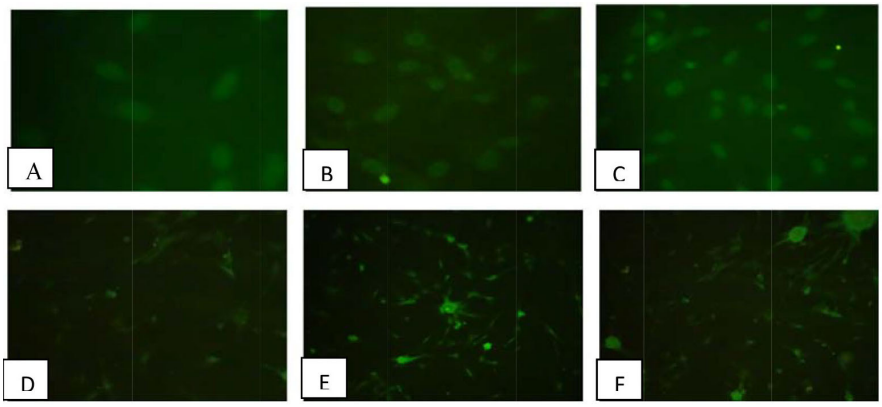

Fig. 1. Expression of TNF- á in treatment and control group observed underfluorescence microscopy with 400x magnification,A, B, C are control group and C, D, E are treatment group with observations on day 1, 3, and 7respectively.

(without supplementation) were analyzed witht-test using SPSS®version 23.

RESULTS

Expression of TNF-α in hBM-MSC culture in both treatment and control groups were observed underfluorescence microscope with immunocytochemistry staining as seen on Figure 1. TNF-α expression in both groups were then evaluated on day 1, 3 and 7.

The result of this study showed TNF-α expression in treatment groups were lower than control groups in all observation days. Statistical analysis showed that there was significant differences in TNF-α expression between control and treatment groupsin day 7, while there was no significant differencesbetween the two groups in day 1 and 3 (Fig. 2). It was noted from Figure 2 that TNF-α expression was consistently increased along observation period in control group; whereas it was relatively stable in day 1 and day 3; followed by declined in day 7 in Alpha Tocopherol group.

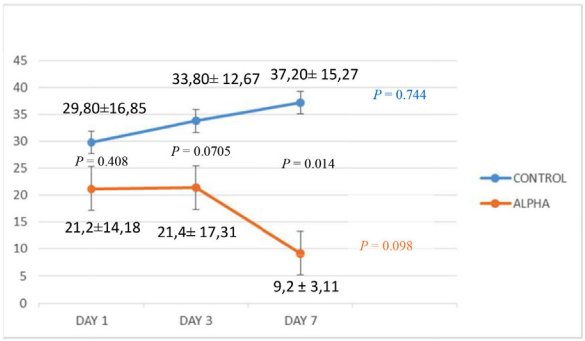

Fig. 2. Expression of TNF-α between control and alpha tocopherol groups after day 1, 3, and 7 observation. Significant different was found on 7 day observation.

DISCUSSION

Process of wound or bone healing, the inflammatory phase is an important step. Inflammatory cells (macrophages, monocytes, lymphocytes and PMN) as well as fibroblasts infiltrate the bone that is fractured by mediating prostaglandin. The result of this infiltration is the formation of granulation tissue, the growth of new vascular tissue and migration of mesenchymal cells. This study used TNF-α as a pro-inflammatory cytokine that can be expressed in culture cells both before and after treatment by giving alpha tocopherol. The alpha tocopherol dose used in this study is 25 μM which was obtained from preliminary study (unpublished data).

The result of this study showed that administration of Alpha Tocopherol to medium of hBM-MSC culture decreased TNF-α expression along the observation periods. This indicates that Alpha Tocopherol has, to a certain extent, anti-inflammatory effect on cell culture in vitro.

The decrease in TNF-α expression in this study is consistent with previous studies which stated that giving alpha tocopherol supplementation significantly decreased monocyte pro-atherogenic activity (Devaraj and Jialal, 2005). Monocyte pro-atherogenic activity is releasing monocyte superoxide anion, lipid oxidase, release of cytokines such as interleukin 1β, tumor necrosis factor α, and interleukin 6(Sack, 2002). At the time of alpha tocopherol supplementation, cyclooxygenase pathway was inhibited by inhibiting 5-Lipooxygenase and decreasing NF-κB activity resulting in a decrease in the synthesis and secretion of TNF-α (Devaraj and Jialal, 2005). This led to alpha tocopherol supplementation resulting in decrease in TNF-α expression.

CONCLUSION

Alpha tocopherol supplementationto human bone marrow mesenchymal stem cell culturemedium decrease TNF-α expression *in vitro*.

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