Asian Jr. of Microbiol. Biotech. Env. Sc. Vol. 20 (December Suppl.) : 2018 : S10-S13

ISSN-0972-3005

EFFECT OF ALPHA TOCOPHEROL SUPPLEMENTATION ON EXPRESSION OF PLATELET DERIVED GROWTH FACTORIN HUMAN BONE MARROW MESENCHYMAL STEM CELLS (IN VITRO STUDY)

ISNOE SURYANDANU[1], ANDRA RIZQIAWAN[2*], EDUWARD[1] AND PURWATI[3]

[1] Residency Program, Department of Oral and Maxillofacial Surgery, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia
[2] Department of Oral and Maxillofacial Surgery, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia
[3] Stem Cell Research and Development Center, Universitas Airlangga, Surabaya, Indonesia

(Received 25 September, 2018; accepted 15 November, 2018)

Key words *: Platelet derived growth factors, Alpha tocopherol, Human bone marrow mesenchymal stem cells.*

Abstract– Alpha-tocopherol is a natural macromolecule that acts as a biological antioxidant in the cell membranes. Antioxidant has been shown to be beneficial in suppressing the damaging effects of oxygen free radicals in cells during bone healing, however, the mechanism is not clearly understood. The cascade of bone healing involves the recruitment of platelets and white blood cells and the subsequent release of essential growth factors such as platelet derived growth factor (PDGF) which induce mesenchymal cell migration, activation and proliferation, and angiogenesis. In vitro studies have demonstrated PDGF to be mitogenic for osteoblasts. This in vitro study evaluated the effect of alpha-tocopherol supplementation on PDGF expression in bone marrow mesenchymal stem cell culture. Human bone marrow mesenchymal stem cells (hBM-MSC) culture was divided into experimental and control group. In experimental group hBM-MSC was cultured in 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 and subsequently immune-stained to observe the expression of PDGF using fluorescent microscope. Data was statistically analysed with ANOVA with p value < 0.05. The expression of PDGF was significantly higher in experimental group compared to that in control group in all observation periods. Alpha tocopherol supplementation in human bone marrow mesenchymal stem cells increases platelet derived growth factor expression.

INTRODUCTION

Facial defects in oral and maxillofacial surgery can occur due to the resection of mandible involved in the jaw tumor. Reconstruction of the defects with bone grafts expected to bind and become new bones with the native bone (Elsanlanty *et al*., 2009).

The ideal bone graft is an autograft, because it has 3 biological properties that are needed in bone healing: osteogenesis, osteoinductive, osteoconductive. Osteogenesis is a bone graft containing osteoblast cells to produce bone matrix. Osteoinductive is a bone graft that contains various cytokines such as transforming growth factor (TGF-β), platelet derived growth factor (PDGF), insulin like growth factor (IGF), fibroblast growth factor (FGF), and bone morphogenetic protein (BMP) which functions interestingly, stimulates osteoprogenitor cells to proliferate and differentiate into osteoblasts which will then produce new bone. Osteoconductive is a bone graft that has a matrix that functions as a scaffold where new bone deposition (Ferdiansyah *et al*., 2011).

In the process of bone healing, osteogenesis occurs which is an active process initiated by Bone Marrow Mesenchymal Stem Cell (BMSc) in bone fractures which proliferate and differentiate into osteoblasts due to induction of growth factor, co-

***Corresponding author's email**: andra-r@fkg.unair.ac.id

factor, hormone, transcription factor which will subsequently produce new bone tissue (Pramono, 2011; Zhang *et al*., 2008; Mehta *et al*., 2012).

Periosteum plays an important role in healing and remodeling of bone fractures because it contains mesenchymal stem cells that can differentiate into bone and cartilage (Zhang *et al*., 2008). Loss of the periosteum tissue as a source of MSc with progenitor cells of bone graft can cause loss of molecular signals or molecular markers for initiation of a process of angiogenesis and osteogenesis (Pramono, 2011). Osteoinductive from growth factors and co-factors may play an important role in improving the proliferation and differentiation processes of BMSc networks. (Pramono, 2011). Alpha tocopherol is a co factor that can help as a micronutrient supplement in BMSc culture media in the process of osteogenesis (Azzi *et al*., 2003; Nuttelmen, 2005). Based on the background, this in vitro study evaluated the effect of alpha-tocopherol supplementation on PDGF expression in bone marrow mesenchymal stem cell culture

MATERIALS AND METHODS

This is true experimental laboratorystudy by observing at the effect of alpha tocopherol supplementation at a dose of 25 µM on PDGF expression in human bone marrow mesenchymal stem cell culture. Samples of bone marrow mesenchymal stem cell is 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.

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.

PDGF expression was analysed using immunocytochemistry method using anti PDGF monoclonal antibodies, as follows: Cells that have been monolayer into one cell underwent a trypsinization process. Performed a 1600rpm centrifuge for 5 minutes. Inserting 1mL 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 ^{o}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 labelled FIT-C, incubate at 37 ^{o}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 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 PDGF 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 PDGF 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 PDGF 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).

The results of the study was presented in Figure 2 below. Comparative analysis of PDGF expression between alpha tocopherol (treatment) and control groups used Mann-Whitney test. The study showed that there were no significant differences in luminescence between treatment group and controls on days 1 and 7 ($p \geq 0.05$), whereas on day 3 there was a significant difference in expression ($p < 0.05$). 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

During the process of bone healing, a number of

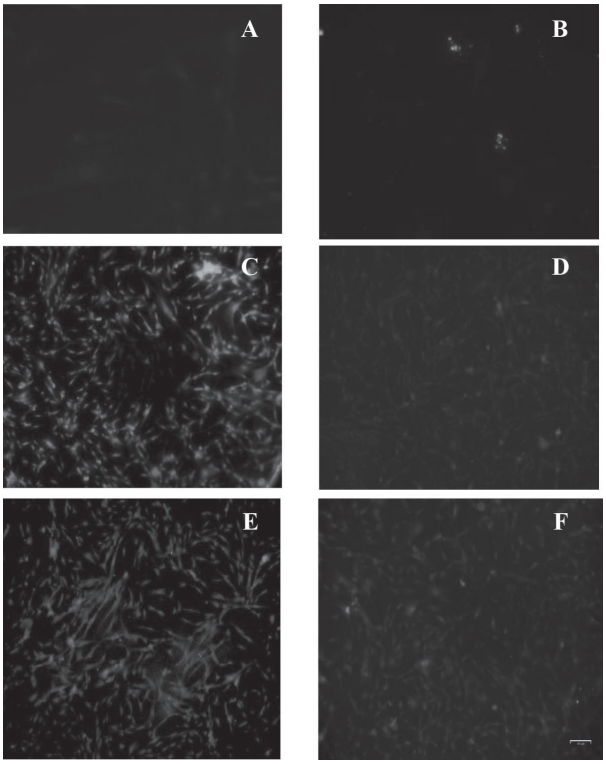

Fig. 1. Microscopic view through immunocytochemistry staining of PDGF expression in hBM-MSC cell culture in (A, C, E) treatment group and (B, D, F) control group with observations on day 1, 3, and 7, respectively

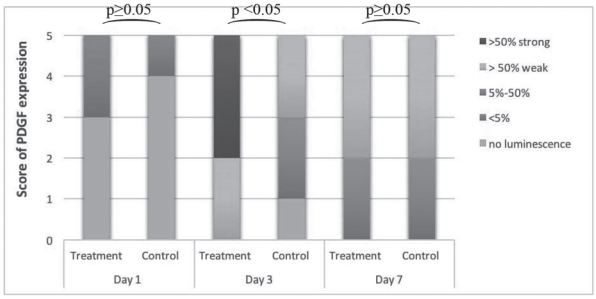

Fig. 2. PDGF expression score on hBMMSC between treatment (apha tocopherol) and control groups after day 1, 3, and 7 observation. The vertical axis showed total amount of sample which were 5 slides in each group and the colour indicated the category of luminescence from immunostaining (0= no luminescence 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).

growth factors, cytokines, and receptors increase inside and around the fracture site, secreted by inflammatory cells at the site of injury. This inductive protein plays an active role in the process of bone healing. During the healing process, releasing growth factors are beginning with PDGF and TGF-β by platelets (Barnes *et al*., 1998).

In the comparison analysis in each group, there were significant differences in circulation in the comparison between time groups in the alpha group and overall control ($p<0.05$). This could be explained as follows: Alpha tocopherol is an antioxidant in the lipids of biomembranes, prevents lipid peroxidation and plays an important role in Reactive Scavenger (ROS) and Reactive Nitrogen Species (RNS). According to Castelini *et al*., 2007, vitamin E can prevent the damaging effects of ROS or RNS on enzymes and molecules involved in signal transduction and gene expression, allowing stem cells given vitamin E to release more growth factors than stem cells which not given vitamin E.

In the comparison analysis between each time group seen from the control group, it was found that there was a significant difference in luminescence between groups of day 1 and day 7, while there was no significant difference between day 1 and 3 and day 3 with 7. This is consistent with the research of Heldin *et al*., 2005, that at the site of injury, due to vascular endothelial damage is activation of platelet aggregation, and release of the contents of α granule. This platelets degranulation release growth factors and trigger chemotactic signals. PDGFs are produced by platelets, monocytes, activated macrophages, and endothelial cells in callus fractures. After the inflammatory phase decreases, PDGF expression rises and remains constant throughout repair.

CONCLUSION

Based on the result of this study, it was concluded that Alpha tocopherol supplementation increases expression of platelet derived growth factor in human bone marrow mesenchymal stem cells culture.

REFERENCES

Azzi, A., Ricciarelli, R. and Zingg, J. 2002. Non-antioxidant molecular functions of α-tocopherol (vitamin E). *FEBS Letters.* 519 (1-3) : 8-10.

Barnes, G., Kostenuik, P., Gerstenfeld, L. and Einhorn, T. 1999. Growth Factor Regulation of Fracture Repair. *Journal of Bone and Mineral Research.* 14 (11) : 1805-1815.

Castellini, C., Mourvaki, E., Dal Bosco, A. and Galli, F.

2007. Vitamin E Biochemistry and Function: A Case Study in Male Rabbit. *Reprod Domest Anim*. 42 (3) : 248-256.

Elsalanty, M. and Genecov, D. 2009. Bone Grafts in Craniofacial Surgery. *Cranial Maxillofac Trauma Reconstruction.* 2 (3) : 125-134.

Ferdiansyah, Djoko Rushadi, Fedik Abdul Rantam, Aulani'am Galli, Francesco, Aisa Maria Chritina Anneti Claudie and Floridi Ardesio, 2011. Vitamin e and cell signalling in *The Encyclopedia of Vitamin* E. eds. Preedy, Victor R and Ronald Ross Watson. *CABI*. pp 365-380.

Heldin, C. and Westermark, B. 2005. Platelet-derived growth factor: mechanism of action and possible in vivo function. *Molecular Biology of the Cell*. 1 (8) : 555-566.

Mehta, M., Schmidt-Bleek, K., Duda, G. and Mooney, D. 2012. Biomaterial delivery of morphogens to mimic the natural healing cascade in bone. *Advanced Drug Delivery Reviews*. 64 (12) : 1257-1276.

Nuttelmen, C.R., Tripodi, M.C. and Anseth, KS. 2005. Synthetic hydrogel niches that promote hMSC viability. *Matrix Biol.* 24 : 208-218.

Pramono, C. 2011. Mandibular reconstruction using non-vascularized autogenous bone graft applied in decorticated cortical bone. *Italian Journal of Maxillofacial Surgery.* 22 (1) : 47-56.

Zhang, X., Awad, H., O'Keefe, R., Guldberg, R. and Schwarz, E. 2008. A Perspective: Engineering Periosteum for Structural Bone Graft Healing. *Clin Orthop Relat Res.* 466 (8) : 1777-1787.

Zhou, J. and Dong, J. 2012. Vascularization in the Bone Repair. *Osteogenesis*.