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EFFECT OF DIFFERENT CONCENTRATION OF ALPHA TOCOPHEROL SUPPLEMENTATION ON HUMAN BONE MARROW MESENCHYMAL STEM CELL VIABILITY (*IN VITRO* STUDY)

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Abstract– Bone defect can be reconstructed with autogenous bone graft and considered as an ideal for bone graft material. The process of bone regeneration that occurs because of the proliferation and differentiation of human bone mesenchymal stem cell marrow. Alpha tocopherol is a co-factor that can help osteogenesis as micronutrient supplementation. Biocompability of this supplement still needs to be proven. This study is to determine the effect of alpha tocopherol supplementation on human bone marrow mesenchymal stem cell viablity. Experimental studies performed in vitro on human bone marrow mesenchymal stem cell. This study was divided into 2 main groups: alpha tocopherol concentrations group (25 μM/mL, 50 μM/mL, 75 μM/mL) and control group. Each group is analyzed with MTT assay observed living cells. Alpha tocopherol with 25 μM/mL concentration obtained high results (107,86%) than other samples (p>0,05 in all subjects observed). Optimum concentration of Alpha tocopherol is 25 μM/ml.

INTRODUCTION

Bone defects could be treated by reconstruction procedure using one's own body termed as autogenous bone graft (Randall M, 2004). Autogenous bone graft is considered as an ideal bone graft because of its osteogenic characteristic because it contains many living cells, osteoconductive meaning that it has a woven structure that functions as a scaffold for a growth media or host tissue vascular regeneration, and also has osteoinductive property which is the ability to secrete growth factors that could induce proliferation and differentiation processes of the bone marrow mesenchymal stem cell to turn into osteoprogenitor cells and has the ability to stimulate osteoprogenitor cells to differentiate into osteoblast and could start the process of new bone formation (Axelrad *et al*., 2009; Choi, 2008).

The failure of bone graft could happen because of periosteum damage thus reconstruction with non-vascularized autogenous bone graft often fails marked by graft resorption or incorporation between grafts and recipient bone do not occur (Pramono, 2011). The proliferation stage is the early stage to examine cell viability. Because of this condition the role of growth factor and co factor with its osteoinductive property holds an important role to increase proliferation process and bone marrow mesenchymal stem cell tissue differentiation. Alpha tocopherol is a cofactor that could help supplement micronutrients in the bone marrow mesenchymal stem cell culture media in the osteogenesis process (Granjeiro *et al.,* 2005).

Alpha tocopherol could increase bone marrow mesenchymal stem cell proliferation, inducting osteoblast differentiation, and increase collagen fiber formation (Pradel *et al.,* 2008).Biocompability of this supplement still needs to be proven. This study is an in vitro experimental study with alpha tocopherol concentrations starting 25μM/mL, 50 μM/mL, 75 μM/mL with the aim to determine the effect of alpha tocopherol supplementation on human bone marrow mesenchymal stem cell

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viability.

MATERIALS AND METHODS

This study was a true experimental laboratory study divided into 2 groups, the treatment group, examining the impact of alpha tocopherol supplementation with concentrations of 25μM/mL, 50μM/mL, and 75μM/mL, and the control group, without alpha tocopherol towards human bone marrow mesenchymal stem cell (hBMSC) viability *in vitro*. The sample was taken from human bone marrow mesenchymal stem cell stock with 3 times passages at Stem Cell Research and Development Center in the Institute of Tropical Disease Center, Airlangga University. Every treatment group consisted of 7 samples.

Thawing process was conducted on human bone marrow mesenchymal stem cell until monolayer was formed on a petridish after 7 days. The monolayer was split, then moved into 96 wheel microplate with every wheel given about 5 x 10^4cells with á-MEM 100 mL, incubated until the cell reached80% confluence. After the cell achieved 80% confluence, it was administered with staged Alpha tocopherol (25 mM/mL, 50 mM/mL, 75 mM/mL) and á-MEM 50 ìM/mL then incubated for 20 hours in 37°C. MTT Assay was done after 4 hours of incubation, optical density (OD) was evaluated using Elisa Reader, and viable cells were counted using formula below:

$$\% \text{ living cells} = \frac{\text{OD treatment} + \text{OD media} \times 100\%}{\text{OD cell control} + \text{OD media}}$$

Note: % living cells : The percentage of living cells after the examination.
OD treatment : OD value in every sample after Elisa Reader result examination.
OD media : Optical density stem cell value in control media.
OD cell control : Optical density stem cell value in control cell.

Toxicity was determined when cell viability is <60% (Freshney, 2000). The data was analysed statistically using One-way ANOVA, $p<0.05$ was considered significantly different.

RESULT

The result of this study also showed that all treatment and control groups revealed no living cell percentage above 60% which indicated that none of the groups has cytotoxicity (Figure 1). The decline of living cells percentage were documentedin 50 μM/mL and 75 μM/mL groups,however they were not significantly different ($p=0.682$; $p> 0.05$).

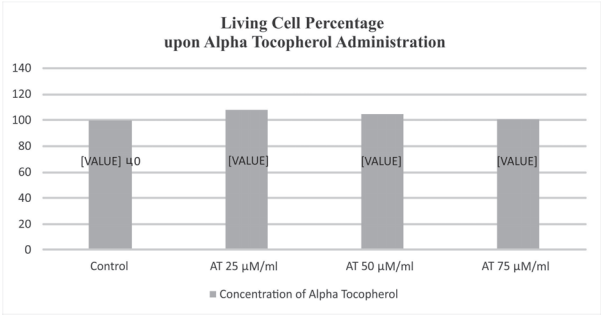

Fig. 1. The amount of living cells percentageshowed bone marrow mesenchymal stem cellviability in each concentration.

DISCUSSION

Mesenchymal stem cells are progenitor cells that have the ability of differentiation. Mesenchymal stem cells could originate from fibroblastic cells, muscle cells, bone cells, tendons, ligaments and fatty tissues (Kaveh, 2011; Banfi, 2009).Alpha tocopherol induced the differentiation of mesenchyme cells including fat cells, osteoblasts, myoblasts and chondrocytes. Alpha tocopherol deficiency can decrease the proliferation of chondrocytes, the number of cells to osteoblasts and disturbance towards the synthesis of matrix(Tecla M et al., 2010). This study was conducted to determine the effect of alpha tocopherol supplementation on human bone marrow mesenchymal stem cell viability. Alpha tocopherol has an important role in collagen synthesis and marks differentiation into osteoblast (Pradel, 2008).

The result of the study proved that various concentrations (25 μM/mL, 50 μM/mL, and 75 μM/ml) of Alpha tocopherol could influence Bone Marrow Mesenchymal Stem Cell viability. Upon the concentration of 25 μM/mL, the percentage of living cells was 107.86%; highest amongst the other concentrations. This is possibly a result of the characteristic of Alpha tocopherol as a lipid soluble antioxidant thus preventing free radicals in the cell membrane. In addition, alpha tocopherol could increase the proliferation of mesenchymal stem cells inducing osteoblast differentiation and increasing collagen fibres production. The administration of Alpha tocopherol 50 μ M/mL caused decline to the percentage of living cells due to the stationary phase or decrease in cell count in compliance to the cell

cycle. Administration of a relatively high dose of alpha tocopherol would influence the presence of fatty acid in the mitochondria membrane. This is in accordance to the study by Lauridsen and Jansen (2011) which stated that administration of alpha tocopherol dose needs to be considered because of the variation in composition of membrane fatty acid and its relation with gene regulation.

CONCLUSION

Various concentrations of alpha tocopherol influenced hBMSC viability. Alpha tocopherol at25 μM/ml concentration showed the highest percentage of living cells.

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