ABSTRACT

The efficacy of stem cell therapy using of Adipocyte- Mesenchymal Stem Cell (h-AMSC) in post-acute myocardial infarction has been limited because of AMSC is not compatible to the new microenvironment that may leads to cell death. Hypoxic preconditioning of sub lethal low oxygen (O_2 1%) during culture has been reported as an effective strategy to overwhelmed this condition. This study was conducted to evaluate the expression of CD 44, VEGF, SCF, OCT-4, BCL2, HSP27, and apoptosis on culture under hypoxic preconditioning. The main objective of this study is to explain the mechanism of survival enhancement of h-AMSC during culture with hypoxia. The other objective of this study is also looking for the role of hypoxic preconditioning on specific marker expression such as CD44, VEGF, SCF, OCT-4, BCL2, HSP27, and inhibition of apoptosis. This trial also aimed to study the optimal time duration of hypoxic preconditioning exposure to improve survival of h-AMSC so that could be used as a reference for h-AMSCs culture strategy before transplantation.

This research is an experimental laboratory explorative study (in vitro study). Samples were derived from abdominal human adipocyte with minor surgery and afterward the tissue divided into 24 culture units, randomly allocated into two groups; 12 unit as treatment group and 12 unit as control group. Hypoxic group were cultured under hypoxic condition (O_2: 1 %) and control group were cultured under normoxia condition (O_2 : 21%) for 24, 48, 72 hours. The two groups above were observed for the expression of CD44, VEGF, SCF, OCT4, BCL2, HSP27, and apoptotic inhibition. The marker of CD44 was evaluated with flowcytometry method, the expression of VEGF, SCF, OCT-4, BCL2, HSP27 were evaluated with immunocytochemistry method, and the apoptosis was counted by Tunnel Assay method. Data were analyzed using Multivariate Analysis of Variance (MANOVA), and path of mechanism analysis with Multiple Linear Regression.

This study results showed that the expression of CD44 was not significantly different between hypoxia versus normoxia (p=0.066). Hypoxic preconditioning significantly increased the expression of VEGF (p=0.000; b=0.774) as compare to normoxia, but time difference of hypoxia exposure resulted significant VEGF expression where the highest expression VEGF has been observed in 24 hours (p=0.000). All other markers such as SCF, OCT-4, BCL2 and HSP27 revealed significant increased of expression under hypoxia versus normoxia, but the time difference of hypoxic preconditioning exposure to SCF, OCT-4, BCL2, and HSP 27 showed that exposure at 24-hour culture under hypoxic preconditioning is the most optimal time for culture to enhance all markers expression as well as the occurrence of apoptosis. On multiple linier analysis, the expression of VEGF positively affected SCF expression (b=0.889), SCF expression positively affected OCT4 expression (b=0.985). OCT-4 expression positively affected BCL2 expression (b=0.878). Hypoxic preconditioning also significantly increased HSP27 expression versus normoxia (p=0.000). BCL2 expression inhibited the number of apoptosis (b=-

0.442), and HSP27 expression also inhibited the number of cell apoptosis (b=-0.487).

Conclusion: Hypoxic preconditioning of h-AMSC culture has proven increased the expression of VEGF, SCF, OCT-4, and BCL2 and HSP27, apoptosis inhibition as well, but not the expression of CD44. This study demonstrated and explained the existence of a new path mechanism of improvement h-AMSC survival in culture under hypoxia (O_2 : 1%) via VEGF, SCF, OCT-4, BCL2, and HSP 27, and inhibition of apoptosis, but CD 44 did not play a role in this path mechanism

Keywords: h-AMSC, Survival, Hypoxia, Time