DISERTASI

PREKONDISI HIPOKSIA UNTUK MENCAPAI *LONG TERM MAINTENANCE* (LTM) DARI *QUIESCENCE BONE MARROW MESENCHYMAL STEM CELLs* (BMSCs)

ERMA SAFITRI

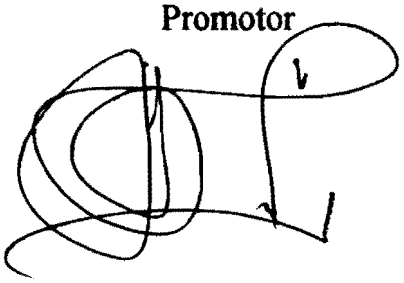
PROGRAM STUDI ILMU KEDOKTERAN JENJANG DOKTOR
FAKULTAS KEDOKTERAN
UNIVERSITAS AIRLANGGA
SURABAYA
2014

LEMBAR PERSETUJUAN

Disertasi ini telah disetujui
Pada tanggal, 19 Juni 2014

OLEH

Promotor



Prof. H. Mas'ud Hariadi, PhD., MPhil., Drh
NIP. 195105021976031003

Ko Promotor

Prof. Dr. Fedik Abdul Rantam, Drh.
NIP. 195910031987011001

ABSTRACT

HYPOXIA PRECONDITION TO ACHIEVE LONG TERM MAINTENANCE (LTM) FROM QUIESCENCE BONE MARROW MESENCHYMAL STEM CELLs (BMSCs)

Erma Safitri

In vitro conventional culture from BMSCs with high oxygen tension (O_2>20%) reported by other researchers caused senescence cells formation, apoptotic, and gene mutation. It caused the reduction of stem cells viability before transplantation. The stem cels experienced to death between 93-99% in 1^{st}– 7^{th} days after transplantation.

Low O_2 tension (hypoxia) is needed as condusive microenvironment supporting for in vitro culture in order to maintain viable and adaptive condition at the time of transplantation. This condition is called long term maitenance (LTM). LTM occur if the stem cells reside at silent condition (G0), is not experienced cycling state (G1/S/G2/M), but still in proliferation and undifferentiated. This condition in vivo is called as quiescence cells.

This research was done in five phases: **First phase** was isolation and culture of MSCs from bone marrow of healthy male rabbit (New Zealand strain); **Second phase** was BMSCs characterisation by identification of p63 protein as quiescence cells marker, identification of CD44, CD90, CD73, CD105 and CD45 before hypoxia precondition;**Third phase** was hypoxia precondition treatment on the stem cells culture of several O_2 concentration (21, 1 and 3%) and several cultivation time (1, 2, 4, 8 days); **Fourth phase** analysed four main keys of quiescence LTM such as slow proliferation, optimal concentration of ROS intracellular, pluripotency and undifferentiated cells; **Fifth phase** was Quiescence Cells BMSCs characterisation by identification of p63 protein expresion after hypoxia precondition

The overall results were: 1. Slow proliferation based on least like CFU-Fs at O_2 1%; 2. Optimal concentration of ROS intracellullar by measurement of MDA concentration at O_2 1% for 2 days; 3. Optimal Pluripotency based on phenotype and genotype expressions of genes OCT4 and SOX2 at O_2 1% for 2 days; and 4.Undifferentiated cells after hypoxia precondition based on genotype and phenotype identification of CD44, CD90, CD73, CD105 and CD45.

The new discovery from this research was LTM quiescence BMSCs can be made by hypoxia precondition based on four of main keys such slow proliferation, optimal concentration of ROS intracellular, expresion of pluripotency factor OCT4 and SOX2, and undifferentiated from BMSCs by genotype of CD44 or phenotype of CD44, CD90, CD73, CD105 and CD45.

In conclusion the results of the research was LTM *quiescence* BMSCs can be obtained by given of hypoxia precondition with optimal consentration of O_2 1% for 2 days.

Key words: Hypoxia Precondition, Long Term Maintenance, Quiescence BMSCs