THE EFFECTS OF ALLOGENEIC MSC ADMINISTRATION ON ATHEROSCLEROTIC VESSEL WALL

(EXPERIMENTAL STUDY OF TGF-β1, IL-1α, AND IL-6 ON ATHEROSCLEROTIC SPRAGUE DAWLEY RATS)

PENGARUH PEMBERIAN MSC ALLOGENIK TERHADAP DINDING PEMBULUH DARAH ATEROSKLEROSIS

(STUDI EKSPERIMENTAL TGF-β1, IL-1α DAN IL-6 PADA TIKUS SPRAGUE DAWLEY ATEROSKLEROSIS)

Thesis

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APPROVAL PAGE

The Effects of Allogeneic MSC Administration on Atherosclerotic Vessel Wall

(Experimental Study of TGF-β1, IL-1α, and IL-6 on Atherosclerotic Sprague Dawley Rats)

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DECLARATION

I hereby declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of reference is given.

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LIST OF ABBREVIATION

IL-1α	: Interleukin 1 alpha
IL-6	: Interleukin 6
TGF-β1	: Transforming growth factor betha 1
MSC	: Mesenchymal stem cells
CD4	: Cluster of differentiation 4
CD25	: Cluster of differentiation 25
ApoE	: Apolipoprotein E
FACS	: Fluoresence absorbance cell sorting
TGF-f3	: Transforming growth factor f3
IFN-γ	: Interferon gamma
IL-1	: Interleukin 1
TNFα	: Tumor necrosis factor alpha
NO	: Nitrit Oxide
LDL	: Low density lipoprotein
mLDL	: Modified low density lipoprotein
LAM	: Leukosite adhesion molecule
PDGF	: Platelet-derived growth factor
MMP	: Matrix metaloproteinase
TF	: Tissue factor
SMC	: Smooth muscle cell
BAEC	: Bovine aortic endothelial cell

HUVEC	: Human umbilical vein endothelial cell
eNOS	: e nitrit oxide synthase
mRNA	: Mesenger ribonucleat acid
iNOS	: Inducible nitric oxide synthase
LAP	: Latencyassociated protein
LTBP	: Latent TGF-β1 binding protein-1
MMP-2	: Matrix metalloproteinase-2
MMP-9	: Matrix metalloproteinase-9
IL-1Ra	: IL-1 receptor antagonist
IL-1β	: Interleukine-1 betha
ICE	: intracellular IL-1β-converting enzyme
IL-1RI	: IL-1 receptor type I
IL-1RIAcP	: IL-1R accessory protein
MAPK	: mitogen-activated protein kinase
NF-κB	: Nuclear factor kappa B
AP-1	: Activating protein-1
IL-4	: Interleukine-4
IL-1RII	: Interleukine-1 receptor type II
Th1	: T helper 1
sIL-6R	: Soluble IL-6Rα subunit
gp130	: g protein 130

ICAM-1	: Intracellular adhesion molecule
VCAM-1	: Vascular cell adhesion molecule
LPS	: Lipopolysaccharide
DNA	: Deoxyribonucleat acid
APT	: Asexually Produced Totipotent
ESC	: Embryonic stem cells
ASC	: Adult stem cell
MHC class 1	: Major histocompatibility complex class I
MHC class II	: Major histocompatibility complex class II
HLA-DR	: Human leukocyte antibody DR
MT1-MMP	: Membrane type 1 matrix metalloprotease
HGF	: Hepatocyte growth factor
TLR	: Toll like receptor
HSP60	: Heat shock protein 60
ECM	: Extracellular matrix
ALP	: Alkali phosphatase
NK cell	: Natural killer cells
DC	: Dendritic cells
Treg	: Regulatory T cells
AHSCT	: Allogeneic hematopoietic stem cell transplantation
HSC	: Hematopoietic stem cells
GvHD	: Graft versus host disease

PGE2	: Prostaglandin E2
IDO	: Indoleamine 2, 3-dioxgenase
HLA-G	: Humam leukocyte antigen G
COX	: Cyclooxygenase enzymes
VEGF-A	: Vascular endothelial growth factor A
PBMC	: Peripheral blood mononuclear cell
PAR1	: Protease-activated receptors 1
TGFβR	: Transforming growth factor β receptor
ALK	: Alkali phosphatase
JNK	: Jun N kinase
JAK	: Janus kinase

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ABSTRACT

Background: Atherosclerosis is the leading cause of morbidity and mortality in the world. Mesenchymal stem cells (MSCs) are implicated in a variety of physiological and pathological processes. They may able to ameliorate atherosclerosis by interfering IL-1α, IL-6 and TGF-β1 expression. However, the effect of MSCs to them in atheroscherotic vessel wall has never been reported.

Objective: To investigate the effect of intravenous administration of allogeneic MSCs on atherosclerotic vessel wall.

Method: Sprague Dawley rats were divided into negative control group (standard diet, n=6), positive control group (atherosclerotic diet, n=6), and MSC treatment group (atherosclerotic diet and treated by single dose of $5x10^6$ MSCs, n=8). MSCs were isolated by plastic adherent method from umbilical SD rats. Atherosclerotic event in abdominal aorta was stained by hematoxilin and eosin, while IL-1α, IL-6 and TGF-β1 expression were observed by immunohitochemistry.

Results: Atherosclerotic plaque in the MSC treatment group was lower than in the positive control group (p=0.006). IL-1α and IL-6 expression in endothel, smooth muscle and macrophage of three groups were not different. TGF-β1 expression by macrophage inside vessel wall in MSC tretment group was lower than in positive control group (p=0.046), but there was no different of TGF-β1 expression in both endothel and smooth muscle.

Conclusion: MSCs may have role in ameliorating atherosclerotic plaque in SD rats mediated in part by reducing of TGF-β1 expression in macrophage, but not by IL-1α and IL-6.

Keywords: Mesenchymal stem cells, atherosclerosis, IL-1α, IL-6, TGF-β1

ABSTRAK

Latar Belakang: Aterosklerosis adalah penyebab utama morbiditas dan mortalitas di dunia. Sel punca mesenkimal (MSC) terlibat dalam berbagai proses fisiologi dan patologi. Mereka dapat memperbaiki aterosklerosis dengan mempengaruhi ekspresi IL-1α, IL-6 dan TGF-β1. Bagaimanapun juga, efek MSC terhadap mereka dalam dinding pembuluh darah aterosklerosis belum dilaporkan.
Tujuan: Untuk menginvestigasi efek pemberian MSC allogenik secara intravena terhadap dinding pembuluh darah aterosklerosis.
Metode: Tikus Sprague Dawley dibagi menjadi kelompok kontrol negatif (diet standar, n=6), kelompok kontrol positif (diet aterosklerosis, n=6), dan kelompok pengobatan MSC (diet aterosklerosis dan diobati dengan dosis tunggal MSC $5x10^6$, n=8). MSC diisolasi dengan metode *plastic adherent* dari umbilikal tikus SD. Kejadian aterosklerosis di aorta abdominal diobservasi dengan pewarnaan hematoksilin dan eosin, sedangkan ekspresi IL-1α, IL-6 dan TGF-β1 diobservasi dengan immunositokimia.
Hasil: Kejadian plak sterosklerosis pada kelompok pengobatan MSC lebih rendah dibandingkan kelompok kontrol positif (p=0,006). Ekspresi IL-1α dan IL-6 di endotel, otot polos dan makrofag pada ketiga kelompok tidak berbeda. Ekspresi TGF-β1 oleh makrofag di dinding pembuluh darah kelompok pengobatan MSC lebih rendah dibanding kelompok kontrol positif (p=0,046), tetapi tidak ada perbedaan ekspresi TGF-β1 di endotel dan otot polos.
Kesimpulan: MSC mungkin mempunyai peran memperbaiki plak aterosklerosis pada tikus SD dengan menginterfensi penurunan ekspresi TGF-β1 di makrofag, tidak melalui interfensi ekspresi IL-1α dan IL-6.

Kata kunci: Sel punca mesenkimal, aterosklerosis, IL-1α, IL-6, TGF-β1