CHAPTER 1

INTRODUCTION

1.1. Background

Atherosclerosis is the leading cause of morbidity and mortality in the world (1). Through its manifestations of cardiovascular disease and stroke, it will become the leading global killer by the year 2020 (1). It is hypothesized as a chronic response to injury which is initiated by endothelial dysfunction (2). Such dysfunction increases lipoprotein entry, produces adhesion molecule, releases inflammatory cytokine, and produces chemokines (1). Those processes will recruit monocytes (3, 4) that differentiate into macrophage and imbibe oxidized low density lipoprotein to form foam cells in vessel wall (5). Furthermore, foam cells release platelet derived growth factor which stimulates migration of smooth muscle cells to subintimal space, release cytokines and growth factors that further stimulate smooth muscle cell proliferation and synthesis of extracellular matrix proteins (1, 5). Cytokines play a role in reinforcing and maintaining chronic inflammation of lesion. They induce smooth muscle cell and leukocyte activation to promote further cytokine release (1). Microenvirontment consists of signaling molecule, intercellular communication and interaction between cell and their neighbouring extracellular matrix (6). Change of microenvironment in atherosclerotic vessel wall from healthy vessel wall make some abnormal

processes inside vessel wall. Cytokines such as IL-1α, IL-6 and TGF-β1 are part of microenvironment which play a povital role in atherosclerosis (7, 8).

There are a lot of studies which demonstrate the role of IL-1α, IL-6 and TGF-β1 cytokines in atherosclerosis. IL-1α was considered to play a role in the propagation of vessel wall inflammation in atherosclerosis. It facilitates early lesion of atherosclerosis by increasing adhesion molecules (9), mediating leukocyte transmigration (10), and maintaining an inflammatory milieu (11). Whereas IL-6 leads to an increase in endothelial cell adhesiveness and release of inflammatory mediators, including MCP-1, IL-8, and IL-6 itself (12). On the other hand, TGF-β1 attenuates foam cell formation, increases cholesterol efflux (13) and inhibits expression of lipoprotein lipase (14). Although IL-1α and IL-6 have a strong role in pro-atherosclerosis process, and TGF-β1 serve as anti-atherosclerosis process, however it remains controversial.

Mesenchymal stem cells (MSC) are progenitors that give rise to multiple mesodermal derivatives. They are widespread in the organism and are implicated in a variety of physiological and pathological processes (15). They can differentiate multilineage into mesoderm cells, non-mesoderm cells, and may have a role in immunoregulatory functions (15-17). They can be isolated from bone marrow, gut, lung, liver, adipose, dental pulp, periodontal ligament, peripheral blood, umbilical cord blood, amniotic fluid, and fetal membranes (17-25). MSC are one of the most common stem cell observed by researchers, because they have favorable biological characteristics. First, they are easy to be

isolated and expanded ex vivo (26). Second, they are hypoimmunogenic, so allogenic MSC transplantation may be feasible (27). Lastly, it is feasible to administer MSC intravenously and home to the damaged tissues (28).

The effect of MSC on progression of atherosclerotic plaques remains controversial. Liu et al. found that the allogeneic MSC transfusion may result in an increase in atherosclerotic lesion size in rabbits (29). On the other hand, Wang et al. found that MSCs resulted in a significant decrease in atherosclerotic plaques size and a significant increase in CD4 CD25 regulatory T cells in spleen of ApoE-knockout mice (30). This study investigated the effect of MSC administration on atherosclerosis in rats.

1.2. Research Question

"Does intravenous allogeneic mesenchymal stem cells administration can reduce atherosclerosis plaque in Spague Dawley rats?"

1.3. Research Objective:

1.3.1. The general Objective:

To investigate the effect of intravenous allogeneic mesenchymal stem cells administration on atherosclerosis vessel wall in abdominal aorta of Sprague Dawley rats.

1.3.2. The Special Objective:

1. To describe atherosclerotic event of Sprague Dawley rats fed on atherogenic diet and atherogenic diet plus mesenchymal stem cell.
2. To describe IL-1α expression in abdominal aorta Sprague Dawley rats fed on atherogenic diet and atherogenic diet plus mesenchymal stem cell.
3. To describe IL-6 expression in abdominal aorta Sprague Dawley rats fed on atherogenic diet and which received atherogenic diet plus mesenchymal stem cell.
4. To describe TGF-β1 expression in abdominal aorta Sprague Dawley rats which received atherogenic diet and which received atherogenic diet plus mesenchymal stem cell.

1.4. Research Benefit:

1. For science:

   Give medical scientific information about the benefits of intravenous allogeneic mesenchymal stem cell administration on atherosclerotic vessel wall.

2. For community:

   Give information about the benefits of intravenous allogeneic mesenchymal stem cell administration based on scientific evidence to improve community life expectancy.

3. For other researcher:

   Give the additional scientific study as a basis of further research about mesenchymal stem cell and atherosclerosis.

1.5. Research Originality

This study was original and different from previous studies regarding the following:

1. This study used different samples called Sprague Dawley rats.
2. This study observed the effect of intravenous allogeneic MSC administration on IL-1α, IL-6, and TGF-β1 of atherosclerotic Sprague Dawley rats.

Table 1.1. Research Originality

No	Publication Title and Author	Method	Result
1	Effect of MSCs on progression of atherosclerosis plaque in ApoE-knockout mice. Wang ZX, Mao S, Li Y, Zhan ZQ, He CR, Wang CQ.	ApoE-mice mesenchymal stem cells were isolated and identified. Thirty ApoE -/ - mice were divided into negative control group (Neg, n = 10), positive control group (Pos, n = 10) and MSC group (n = 10). MSC were injected through caudal vein into the body of Pos and MSC groups. The plaque area of all subjects were compared, the percentage of CD4 CD25' regulatory T cells in different tissues were analyzed by FACS, proliferation response of splenocytes to mesenchymal stem cells and cytokines in the supernatant were determined by ELISA.	Compared with controls, MSC resulted in a significant decrease of the atherosclerotic plaques size (P <0.05), and a significant increase of CD4 CD25 regulatory T cells in spleen (P<0.05). Specific proliferation response of CD4' CD25' regulatory T cells in splenocytes to MSC was significantly suppressed. The supernatant levels of TGF-f3 and IL-10 in MSC group were increased while IFN-γ decreased significantly.

2	Transfusion of allogeneic mesenchymal stem cells promotes progression of atherosclerotic plaque in rabbits. Liu PX, Zhang L, Liao WB, DU WT, Gu DS, Liu M, Lu SH, Han ZC.	Allogeneic MSC were obtained from rabbit bone marrow aspirates and expanded in vitro. New Zealand white rabbits were divided into three groups: 24 rabbits with hypercholesterolemia receiving intravenous injection of either 5x10 (7) MSC (n = 12) or saline (n = 12) after 5 weeks on a high lipid diet and additional rabbits (n = 6) fed with standard rabbit diet were served as controls. Body weight and blood lipids were measured at weeks 0, 5, 9 and 13 during the study. All rabbits were sacrificed at week 13. Atherosclerotic lesion size and vasa vasorum were evaluated by using pathological analysis and immunocytochemical technique.	The results showed that the aortic sinus lesion size significantly increased in rabbits infused with MSC as compared with controls receiving saline (23.35 +/- 3.51% and 11.39 +/- 3.08% respectively). The lesion size in whole aortas of MSC-treated rabbits was 76.64 +/- 12.70% versus 57.61 +/- 9.00% in saline-treated animals ($p < 0.05$). Moreover, vasa vasorum networks in MSC-treated aortas were more numerous and had increased capillary density.