CHAPTER 1

INTRODUCTION

1.1. Background

Atherosclerosis is one of the leading causes of cardiovascular mortality and morbidity in the world, this disease starts early in our life and develops silently and slowly.[1] Atherosclerosis is a chronic inflammation of the blood vessels. Cholesterol is transported in the blood in particles called low density lipoprotein (LDL) that can accumulate in the vessel walls. This triggers the immune system to react against LDL, which then cause inflammation in the vessels, and eventually induce thrombus formation. Atherosclerosis occurs as grow older and leads to damaging effects in the heart, brain, kidneys, gastrointestinal system, testes and ovaries. The walls of the arteries cause abnormal narrowing which leads to clotting and obstruction of the blood vessels. If the thrombus forms in the coronary artery, the patient suffers a myocardial infarction; if it forms in the brain, a stroke can result. When fats build up in the damaged walls of arteries and become oxidized, this causes further damage and eventually causes atherosclerotic plaque with the combination of foam cells, calcification and lipid accumulation. The factors that lead to atherosclerosis include hyperinsulinism, high blood glucose, elevated triglycerides,, low vitamin K, low nitric oxide, low HDL, elevated LDL, low free testosterone, elevated fibrinogen, elevated homocysteine, elevated C reactive protein, hypercholesterolemia, low EPA

and DHA (oxidized omega 3 fatty acids) and oxidized LDL. To get the appropriate treatment, stem cells can be made to inhibit rather than aggravate the inflammation around the LDL particles in the blood vessels.[2]

Mesenchymal stem cells (MSCs) are multipotent stem cells which able to differentiate into a variety of cell types. This phenomenon has been documented in specific cells and tissues in living animals and their counterparts growing in tissue culture. They can include multipotent cells derived from bone marrow, umbilical cord blood, adipose tissue, adult muscle, corneal stroma or the dental pulp of deciduous baby teeth, but do not have the capacity to reconstitute an entire organ.[3]

Stem cell treatments are a type of intervention strategy by introducing stem cells into damaged tissue in order to treat disease or injury.[4] The ability of stem cells to self-renew and give rise to subsequent generations with variable degrees of differentiation capacities, offers significant potential for generation of tissues that can potentially replace diseased and damaged areas in the body, with minimal risk of rejection and side effects. The regenerative potential of stem cells is enforced by the fact that stem cells tend to migrate automatically to sites of damage.[5]

Vasculitis atherosclerosis occurs when the immune system mistakenly sees blood vessel cells as foreign. The immune system then attacks those cells as if they were an invader. Blood vessels affected by vasculitis become inflamed, which can cause the layers of the blood vessel wall to thicken. This narrows the blood vessels, reducing

the amount of blood and therefore oxygen and vital nutrients that reaches the tissues. In some cases, a blood clot may form in an affected blood vessel, obstructing blood flow.[6]

This inflammation appears many cytokines, including (IL-1, IL-6, IL-10 and TNF-α). Macrophages produce IL-1 and TNF-α which increase adhesion of leukocytes. Several chemokines generated by macrophages, including monocyte chemotactic protein-1 (MCP-1), may recruit more leukocytes into the plaque. T lymphocytes (both $CD4^+$ and $CD8^+$) are also recruited to the intimae by chemoattractants.[7,8] IL-10 and TNF-α are cytokines which appear at atherosclerotic plaque in abdominal aorta wall as inflammatory cytokines, which will be as a good marker to know the ratio of inflammation response and the impact of Mesenchymal stem cells in treatment of atherosclerotic lesion.

Mesenchymal stem cell therapy has potential for treatment of patients with atherosclerotic disease. However, many details remain unknown.[9] This study investigate the benefit of mesenchymal stem cells derived from umbilical cord in atherosclerotic vessel abdominal aortic wall in Sprague Dawley rats.

1.2. Research Questions

Does mesenchymal stem cells administration improve atherosclerosis lesion in Sprague Dawley Rats?

1.3. Research Objective:

1.3.1. General Objective

To investigate the effect of mesenchymal stem cells administration on atherosclerotic lesion.

1.3.2. Specific Objective

a. To know the expression of IL-10 of abdominal aorta atherosclerotic Sprague Dawley rats treated with MSCs.
b. To know the expression of TNF-α of abdominal aorta atherosclerotic Sprague Dawley rats treated with MSC.
c. To know atherosclerotic appearance of abdominal aorta atherosclerotic Sprague Dawley rats treated with MSC.

1.4. Research Benefits:

This research gives additional evidence to use mesenchymal stem cells administration to improve atherosclerotic lesion.

1.5. Research Originality

This research is original and different from previous studies regarding the following:

1- This study shows the effect of mesenchymal stem cells derived from umbilical cord to improve atherosclerotic lesion.

2- This study evaluating ratio of interleukin-10 (IL-10) and tumor necrosis factor alpha (TNF-α) after treated by mesenchymal stem cells.

3- This study use different animal type from previous study.

Table 1. Previous studies

N0	Title publication and authors	Method	Results
1.	Effects of MSCs on the progression of atherosclerosis plaque in ApoE-knock out mice.[10] Wang ZX, Mao S, Li Y, Zhan ZQ, He CR, Wang CQ.	ApoE -- mice mesenchymal stem cells (MSCs)were isolated and identified. Thirty ApoE -/ - mice were divided into negative control group (Neg, n = 10), positive control group (Pos, n = 10) and MSCs group (n = 10).MSCs were injected through caudal vein into the body ofPos and MSCs 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 cyto-kines in the supernatant were determined by ELISA.	Compared with controls, MSCs 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 MSCswas significantly suppressed. The supernatant levels ofTGF-f3 and IL-10 in MSCs group were increased while IFN-y decreased significantly.

2.	Transfusion of allogeneic mesenchymal stem cells promotes progression of atherosclerotic plaque in rabbits.[11] Liu PX, Zhang L, Liao WB, DU WT, Gu DS, Liu M, Lu SH, Han ZC.	Allogeneic MSCs 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 5 x 10(7) MSCs (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.	The results showed that the aortic sinus lesion size significantly increased in rabbits infused with MSCs 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.
3.	Allogeneic bone marrow mesenchymal stem cells transplantation for stabilizing and repairing of atherosclerotic ruptured plaque.[12] Fang SM1, Du DY, Li YT, Ge XL, Qin PT, Zhang QH, Liu Y.	28 male New Zealand rabbits were randomly divided into 2 groups after establishment of atherosclerotic disrupted plaque model by liquid nitrogen frostbite: MSCs transplantation group and control group. MSCs were isolated, cultured in vitro, and labeled with BrdU. BrdU-incorporated MSCs (MSCs transplantation group) or an equal amount of IMDM medium without MSCs (control group) were transplanted into vessels with ruptured plaque. PAI-1, MMP-9 and hs-CRP were determined by ELISA of blood 3 days and 4 weeks after	Four weeks after MSCs transplantation, PAI-1, MMP-9 and hs-CRP were reduced significantly in all experimental animals ($p < 0.001$). The reduction was more evident in the transplantation group than in the control group ($p < 0.01$). In addition, the transplantation group showed dramatically higher numbers of newly formed endothelial cells, collagen fibers, and proliferative BrdU-positive

		transplantation. Rabbits were sacrificed 4 weeks after transplantation and plaque repair was assessed by HE and Masson's trichrome staining. Transplanted BrdU-positive cells were identified by immunohistochemistry.	cells at plaque areas.
4.	Rapid Endothelial Turnover in Atherosclerosis-Prone Areas Coincides With Stem Cell Repair in Apolipoprotein E–Deficient Mice.[13]GeorgiosFoteinos, PhD; Yanhua Hu, MD; Qingzhong Xiao, PhD; Bernhard Metzler, MD; QingboXu, MD, PhD.	animal is ApoE mice were crossed with TIE2-LacZ mice in laboratory. Tissue Harvesting and Preparation: Blood was obtained from the inferior vena cava for lipid analysis. The procedure using immunofluorescent staining.	Observed occasionally in wild-type mice and frequently at sites prone to lesion develop in apoE?/? mice (0.18?0.1% versus 1.12?0.2%; P?0.001). Endothelial integrity tests demonstrated that the areas with high rate of cell turnover displayed Evans blue leakage, low levels of VE-cadherin expression, and increased cell attachment, as evidenced by Evans blue dye injection, immunostaining, and scanning electron microscopy.

5.	Protective paracrine effect of mesenchymal stem cells on cardiomyocytes.[14] Mei-xiang XIANG, Ai-na HE, Jian-an WANG, Chun GUI.	MSCs from Sprague-Dawley (SD) rats were separated and cultured. MSC medium was collected from MSCs cultured in serum-free Dulbecco's modified eagle medium (DMEM) under hypoxia. The apoptotic cardiomyocytes were stained with Annexin-V-fluorescein isothiocyanate (FITC), Hoechst 33342 and terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL).	Data demonstrated that MSC medium reduced H/R-induced cardiomyocyte apoptosis, increased the Bcl-2/Bax ratio, and reduced the release of cytochrome C and AIF from mitochondria into the cytosol. Conclusion: MSCs protected the cardiomyocytes from H/R-induced apoptosis through a mitochondrial pathway in a paracrine manner.
6.	Intravenous Mesenchymal Stem Cells Improve Survival and Motor Function in Experimental Amyotrophic Lateral Sclerosis.[15]AntonioUccelli, Marco Milanese, Maria Cristina Principato, Sara Morando, TizianaBonifacino, Laura Vergani, Adriana Voci, Enrico Carminati, Francesco Giribaldi, Claudia Caponnetto, and GiambattistaBonanno	MSCs were intravenously injected in mice expressing human superoxide dismutase 1 (SOD1) carrying the G93A mutation (SOD1/G93A) presenting with experimental ALS. Survival, motor abilities, histology, oxidative stress.	observed a reduced accumulation of ubiquitin agglomerates and of activated astrocytes and microglia in the spinal cord of MSC-treated mice, with no changes in the number of choline acetyl transferase and glutamate and observed that MSCs reverted both spontaneous and stimulus-evoked neuronal release of [3H]D-aspartate, a marker of endogenous glutamate, which is upregulated in mice.