

Invited Review

Mesenchymal Stem Cells: A Potential Promise in Treating Atherosclerosis?

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Abstract

Atherosclerosis is a chronic inflammatory disease which results in thickening of the vessel wall and narrowing of the lumen. It is a leading cause of death worldwide. Preventive treatment is taken into prioritized consideration since currently no effective approaches to cure atherosclerosis are available. These treatments mainly focus on lowering blood cholesterol levels, especially LDL-C, by statins. Even so, lowering lipid levels is not sufficient to reduce the risk of cardiovascular events in all patients. Recently, atherosclerosis has increasingly been recognized as a chronic inflammatory disease involving the immune system, initiating new therapeutic approaches which could alleviate or prevent atherosclerosis by modulating inflammation. Mesenchymal stem cells (MSCs) have emerged as a promising option to relieve inflammation and balance immune responses in inflammatory diseases. Several studies including our group also reported that MSCs may be a new therapeutic option for atherosclerosis. This review summarizes the updated state of our knowledge in the administration of MSCs to alleviate atherosclerosis and discusses some of the key unresolved challenges that need to be solved in future studies.

Keywords: atherosclerosis, mesenchymal stem cells, inflammation.

Introduction

Atherosclerosis is characterized by endothelial dysfunction, lipid deposition, and inflammatory cell accumulation within the vascular wall[1,2]. This pathophysiological process may end in ischemic heart disease, myocardial infarction or stroke if abnormal luminal remodeling, plaque erosion or thrombus formation occurs. Atherosclerosis is initiated by endothelial injury, followed with inflammatory responses of vascular endothelial cells, adhesion and infiltration of immune cells, as well as subendothelial lipoprotein retention. Members of the innate immune system such as monocytes, macrophages, and NK cells, in conjunction with T cells and B cells of the adaptive immune system, are important players in the pathogenesis and development of atherosclerosis. Additionally, dyslipidemia, obesity, diabetes, hypertension, smoking and disruptions in circadian rhythm are also risk factors involved in atherosclerosis[1].

Despite significant improvement in diagnosis and treatment strategies for atherosclerosis in recent years, atherosclerosis and consequential diseases remain major contributors to mortality and morbidity worldwide. Combatting the pathological characteristics of atherosclerosis, including lowering plasma cholesterol levels, remain the current major treatment for atherosclerosis. However, recent studies targeting vascular inflammation and imbalanced immune systems attempt to uncover new therapeutic approaches. One encouraging finding from a recent large clinical trial was that antibody-mediated inhibition of the proinflammatory cytokine IL-1 β can decrease cardiovascular events in high-risk populations, providing evidence that anti-inflammation measures in atherosclerosis may work[3,4]. Cell therapy achieved by injection of regulatory T cells (Tregs), stem cells and other functional cells has also been shown to be effective in various autoimmune and inflammatory diseases[5-8].

Mesenchymal stem cells, also referred to as multipotent stromal cells, are a cluster of well-established cells characterized by non-hematopoietic abilities of self-renewal and multipotent differentiation. Due to their multiple differentiation capabilities, inflammatory and immune modulation function, MSCs have been explored as an attractive therapeutic agent in various diseases including allergic conjunctivitis, acute lung injury, myocardial infarction, allergic rhinitis, Alzheimer's disease, and type 1 diabetes[9-15]. In light of the critical role inflammatory processes have in the initiation and progression of atherosclerosis, our group and others have transferred MSCs to atherosclerotic animal models revealing MSCs were able to alleviate atherosclerosis[16-18]. These results are important for further investigation into its potential clinical application.

Characteristics of mesenchymal stem cells

Mesenchymal stem cells can be derived from a variety of different sources including bone marrow, adipose tissue, skin, umbilical cord, placenta, and human gingiva[2]. When cultivated under adapted conditions, MSCs can differentiate into osteoblasts, adipocytes, chondrocytes, vascular smooth muscle cells, endothelial cells and other cell types. MSCs do not have a defined profile of surface antigen expression. A consensual definition of MSCs suggested by the International Society for Cellular Therapy is as followed: MSCs must be plastic-adherent, express CD75, CD90, and CD105 but not CD45, CD34, CD14 or CD11b, CD79 α or CD19 and HLA-DR surface molecules, and are able to differentiate into chondrocytes, osteoblasts, and adipocytes[19]. In recent years, MSCs have been increasingly appreciated for their immunosuppressive and anti-inflammatory function. Several studies have suggested that MSCs can alter both innate and adaptive immune responses, modulating the conversion of inflammatory macrophages to anti-inflammatory, suppressing the proliferation of the activated CD4+ T helper cells and CD8+ cytotoxic T cells, decreasing the activation and proliferation of Th1 and Th17, and enhancing the expression of regulatory T cells[20-22]. The underlying mechanisms mediating these modulation functions involve CD39/CD73/, IDO, HO-1, iNOS, TGF- β , IL-10, TSG6, NOD2 or PGE2 signaling[23-26].

With regards to the function of MSCs *in vivo*, the bio-distribution of MSCs is important. The method and location of MSC infusion usually affects the distribution of MSCs *in vivo*. Systemic administration can be achieved by intravenous injection, intraperitoneal injection, intra-arterial injection, or intracardiac injection[27]. Intravenous delivery is the least invasive, while intra-arterial or intracardiac delivery may lead to higher engraftment rates than intravenous delivery. Intraperitoneal delivery is rarely used[28]. A number of studies have explored the therapeutic effect of MSCs by intravenous administration in animals and humans. These studies used various techniques including radioactive labeling, fluorescent vital dyes, contrast agents, reporter gene transduction, and donor cell-specific DNA markers to label MSCs cultured and expanded *in vitro*, tracking their distribution over time[27-32]. The tissue source of MSCs was in most cases not influential. The primary common results of MSC bio-distribution after intravenous injection were that MSCs distribute to a variety of tissues after intravenous injection; the injected cells were found early at the highest frequencies in the lungs, followed by liver and spleen[33]. Obstructive events during lung passage are expected and often observed after intravenous administration of MSCs partly due to their relatively large

average cell size, estimated to be around 30 μm in suspension[11,34]. Studies comparing intra-arterial and intravenous application of MSCs found that the intra-arterial route of administration is more effective to avoid pulmonary entrapment and increase accumulation of MSCs in therapeutic target tissues [35-37]. However, it should be noted that intra-arterial administration may lead to an increased probability of microvascular occlusions[35].

MSCs in atherosclerosis

Bone marrow-derived MSCs in atherosclerosis

To the best of our knowledge, MSCs from at least 8 different sources, including human or murine bone marrow, murine skin, human cardiac adipose tissue, rat umbilical cord blood, human gingiva, human induced pluripotent stem cells and vessel walls, have been studied in atherosclerosis.

Bone marrow-derived mesenchymal stem cells (BM-MSCs) are the most frequently investigated cell type in atherosclerosis and related diseases. Dr. Fang et al. found that allogeneic BM-MSCs transplantation can stabilize and repair ruptured plaques, which may help to reduce acute coronary syndrome (ACS) and stroke[38]. They used male New Zealand rabbits to establish atherosclerotic disrupted plaque models by liquid nitrogen frostbite and eight weeks of high-fat diet. After successful establishment of atherosclerotic ruptured plaque models, 1×10^7 BrdU labeled BM-MSCs were transplanted *via* the right common carotid artery immediately after triggering of atherosclerotic plaque rupture. Four weeks after BM-MSCs transplantation, they found the transplanted BM-MSCs can migrate to plaque areas and reduce the atherosclerotic plaque rupture related hs-CRP, MMP-9, and PAI-1 levels markedly, implying that transplanted BM-MSCs were able to “hone in” on the ruptured plaque regions and differentiate into endothelial cells and collagen fibers. They also observed tightly arranged endothelial cells and abundant collagen fibers at plaque areas, which they considered were possibly differentiated from BM-MSCs. However, they did not explore the expression of PAI-1, MMP-9 and hs-CRP in plaque areas with or without MSC transplantation and the underlying mechanisms of how BM-MSCs migrate to plaque site are not clear either. Additionally, whether those newly formed endothelial cells and collagen fibers are really differentiated from BM-MSCs or just promoted by BM-MSCs transplantation need to be further clarified. Wang et al. further confirmed that BM-MSCs stabilize atherosclerotic vulnerable plaque in New Zealand rabbits by anti-inflammatory properties[16].

Another group showed that BM-MSCs play an atheroprotective role by enhancing the number and function of Tregs and inhibiting the formation of macrophage foam cells[32]. In this experiment, they fed male C57BL/6 ApoE-KO mice a western-type high fat diet (WTD) for 12 weeks to induce the formation of atherosclerotic lesions. Mice were administrated BM-MSCs (1×10^7) through the tail vein. The injections were administered three times at intervals of 3 weeks. Their results revealed that infusion of BM-MSCs reduced the size of plaque, as well as increased the expansion and function of Treg cells. Moreover, they also observed that the anti-inflammatory cytokines TGF- β and IL-10 were significantly upregulated while the pro-inflammatory cytokines IFN- γ ,

MMP-1 and hs-CRP were downregulated after BM-MSCs treatment. Since TGF- β is essential for Treg cell induction[39,40], upregulated expression of TGF- β may partly account for an increase of Treg cells after BM-MSCs administration. They also observed that BM-MSCs treatment inhibited macrophage foam cell formation *in vitro*. In this experiment, they did not make it clear that whether BM-MSCs were injected before or after the formation of atherosclerotic lesions. The mechanisms of how BM-MSCs enhance the expression and function of Treg cells as well as inhibit foam cell formation *in vitro* and *in vivo* also need to be further studied.

Lin et al. reported that human BM-MSCs restored endothelial function *via* activating the Akt/eNOS pathway in endothelium and eventually alleviated atherosclerosis[41]. In this study, the researchers fed ApoE-KO mice on the 58Y1 diet (60% fat and 0.03% cholesterol) for 5 weeks to induce atherosclerotic lesions before administering a single dose of BM-MSCs (2×10^5 cells) by intravenous tail vein injection. Mice were sacrificed after 5-week treatment period with high-fat diet and 1-week normal chow. They found that infusion of BM-MSCs restored Akt/eNOS activation, stabilized eNOS in endothelial cells, and inhibited plaque formation while having no effect on the plasma lipid concentrations. They also ruled out that IL-8 or MIP-2, which both play an essential role in the chemotaxis and activation of leukocytes at the early stage of atherogenesis, are required for MSC-mediated restoration of Akt/eNOS activation and endothelial function. They also demonstrated that a paracrine effect, rather than differentiation of BM-MSCs, contributes to the therapeutic role in atherosclerosis by detecting CFSE-labeled BM-MSCs at areas close to, but not actually in the endothelium. Song et al. also found that small molecules with a 1H-pyrrole-2,5-dione moiety are important for initiating the endothelial cell differentiation of BM-MSCs[42], indicating a great potential to improve the efficacy of MSCs-based cell therapy for vascular diseases.

In line with previous results, Vanessa Frodermann et al. also displayed that BM-MSCs were able to reduce atherosclerosis *via* modulating inflammatory responses and reducing dyslipidaemia in mice[43]. In this study, they fed LDLr KO mice a WTD for 8 weeks to build an atherosclerotic model. Prior to the induction of atherosclerosis, LDLr KO mice were treated with three intravenous injections of MSCs every other day. They observed that MSC administration was able to modulate immune response, decrease circulating CD4⁺ T cells and increase circulating Tregs in the early days after MSCs injection, while total CD8⁺ T cell numbers were not affected throughout the entire experiment. MSC treatment also reduced inflammatory response, IFN- γ , IL-6, and TNF- α expression but increased IL-10 levels. They also observed a significant reduction in serum cholesterol levels in MSC-treated mice, which is contradicted with what Lin et al. reported[41]. Yamawaki-Ogata et al. observed that BM-MSCs were effective to improve aortic aneurysm, which develops as a result of atherosclerosis and chronic inflammation, *via* their anti-inflammatory modulation function[44].

In conclusion, these studies have supported that BM-MSCs were able to alleviate atherosclerosis, mainly by restoring endothelial function, stabilizing and repairing ruptured plaques, and modulating inflammatory and immune responses. BM-MSCs are the most commonly used type of stem cells in preclinical studies on cell-based therapies for atherosclerosis so far. However, the relative rarity of these cells and the

invasive procedures required for their harvesting have limited their clinical application [45]. Moreover, the ability of expansion and differentiation of BM-MSC is donor-dependent and their ability of differentiation and tissue regeneration decreases with age and disease, also limiting their use [46].

Skin-derived mesenchymal stem cells in atherosclerosis

Compared to bone marrow, skin is a more easily accessible and a readily available source of stem cells, requiring less invasive procedures without risk of oncogenesis after transplantation[47]. Considering these characteristics, skin-derived mesenchymal stem cells(S-MSCs) possess advantages for clinical use since they can be obtained from a patient's own skin biopsy, avoiding the problem of immune rejection after transplantation. Qun Li et al. found that S-MSC transplantation was able to inhibit atherosclerosis *via* modulating inflammatory responses [48]. In the experiment, they established atherosclerotic plaque models by feeding 8 week old male apoE^{-/-} mice on a high-fat diet for 24 weeks, and 5X10⁵ S-MSCs were administered for the last 5 weeks. Their results suggested S-MSCs were capable of modulating the pro-inflammatory cytokine TNF- α and anti-inflammatory cytokine IL-10 expression in plaque sites as well as inhibiting the progression of atherosclerosis, which is dependent on the activation of NF- κ B. They also revealed that the injected S-MSCs migrated toward inflamed tissues and settled nearby CD11b⁺ cells, implicating a possibility that S-MSCs interact with activated macrophages within atherosclerotic plaque to alleviate atherosclerosis.

Human gingival-derived MSCs in atherosclerosis

Human gingival derived MSCs (GMSCs) are another member of MSCs and have been considered as a promising source of MSCs for their ease and safe isolation and fast proliferation properties [49]. In line with other studies, our group recently observed that infusion of GMSCs can also alleviate atherosclerosis in ApoE^{-/-} Mice[17]. In this study, atherosclerotic models were built by feeding 8 week old male ApoE^{-/-} mice a high fat diet for 12 weeks, and GMSCs (2X10⁶) were administered every three weeks from the first day of high fat diet for a total of three times. We observed that GMSCs were able to modulate the differentiation and activation of macrophages, converting inflammatory macrophages into anti-inflammatory ones. Additionally, GMSCs directly inhibited macrophage foam cell development, which indicates that GMSCs may hinder the initiation of atherosclerosis. The Ly-6C^{hi} monocytes, which are a major source of lesion local macrophages [50,51], were also markedly decreased after GMSC treatment, and helped to shrink reservoirs, lowering the migration of infiltrated inflammatory macrophages. These effects resulted in the improvement of plaque lesions in atherosclerosis. We further observed that the modulatory function of GMSCs on macrophage may partly be mediated by IDO and CD73 signals, which is in line with our previous study of the mechanism of GMSCs in their immunoregulatory function on T cells[22]. IFN- γ and IL-4, which may be involved in inflammatory responses in atherosclerosis, were also decreased significantly after GMSCs administration.

Other tissue-derived MSCs in atherosclerosis

Adipose tissue is now recognized as an accessible, abundant, and reliable source for the isolation of stem cells[52]. Adutler-Lieber et al. showed that human cardiac adipose tissue derived MSCs (AT-MSCs) can polarize human macrophages into anti-inflammatory phenotypes[53]. Due to their proximity to the heart, the potential interaction between AT-MSCs and macrophages could be relevant to the pathogenesis of atherosclerosis. Human induced pluripotent stem cell derived MSCs, which possess the ability to maintain stability during proliferation and do not lose this ability easily as they age, were also able to clearly reduce the size of plaques, reducing the levels of inflammatory cytokines, TNF- α and IL-6, in serum[18]. Rat umbilical cord blood-derived MSCs also showed therapeutic effects on atherosclerosis in albino rats[54]. Vessel wall-resident MSCs were abundant in the adventitia and migrated across the vessel wall, where they subsequently differentiated into SMCs and contributed to neointima formation[55]. However, some beta-gal(+) vessel wall-resident MSCs were reported to migrate into atherosclerotic lesions and enhance development of lesions[56].

Conclusion and perspective

In conclusion, MSCs acquired from different sources may be a promising therapeutic option for atherosclerosis treatment. As atherosclerosis is increasingly observed to be a chronic inflammatory disease, it is not surprising that atherosclerosis can benefit from MSC treatment, since MSCs possess the capability to modulate inflammation. Additionally, administrated MSCs are able to migrate to injury sites, which may help repair damaged endothelium *via* secretion of anti-inflammatory cytokines and nutritional factors, improving the recruitment and production of immune regulatory cells, or differentiating into endothelial cells directly. This endothelial cell repair function may help to inhibit the initiation processes of atherosclerosis. Finally, MSCs not only inhibit the activation and differentiation of inflammatory monocytes/macrophages, but also control the macrophage to foam cell formation *in vitro*, which further helps to suppress the initiation of atherosclerosis. Whether foam cell formation inhibition is gained from reduction of lipids or by direct modulation of macrophage differentiation, needs further investigation in the future.

The sum of present studies regarding MSCs in atherosclerosis reveal source-dependent differences in MSCs, a difference in animals and methods used to establish atherosclerotic models, which have been treated with varied doses and times of MSCs *via* different ways of delivery. These contrasts make it difficult to delineate what source of MSCs is most suitable for atherosclerosis treatment. More studies are needed to further assess the best suitable source of MSCs and mode of infusion for atherosclerosis treatment. Additionally, MSCs are heterogeneous, making some functional and others not[57,58]. For this reason, it would be valuable to identify the biomarkers of functional MSCs, so as to purify them for atherosclerosis treatment. Finally, the migration, differentiation, molecules and tissue targets of MSCs in atherosclerosis also deserve further studies. MSCs from elderly individuals with atherosclerosis secrete high levels of IL-6, IL-8/CXCL8 and MCP-1/CCL2 which mediate their reduced immunopotency[59]. Consequently, strategies aimed at modification of MSCs could enhance their efficacy in cell-based therapies, particularly in the aged population. Age and age-associated diseases, such as type II diabetes, impair

the functions of MSCs[60-62]. These details emphasize the relevance of appropriate donor selection for MSC based therapies and the potential for modulating the MSCs secretome as a way to enhance their therapeutic benefit.

Although MSCs multilineage potential and ability to modulate host tissue biology clearly holds immense therapeutic promise, these characteristics may be a double-edged sword [52]. Adipose tissue derived MSCs are reported to primarily undergo osteogenic and chondrogenic differentiation *in vivo*, which might result in formation of undesirable cell types in target tissues leading to other pathological changes such as cyst formation or tissue calcification. Additionally, modified LDL and free cholesterol are potent inducers of MSC migration and differentiation, which may cause harmful changes such as vascular calcification, inflammation and vascular remodeling[63,64]. Several studies also suggest that adipose tissue derived MSCs and BM-MSCs retain an ability to facilitate tumorigenesis[65-67]. Administration of BM-MSCs in graft-versus-host disease was also implicated with increased risk of relapse[68]. These potential risks of MSC therapy underline the importance of determining their efficacy and safety through clinical trials in multiple patient facilities.

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