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MESENCHYMAL STROMAL CELLS

Bone marrow–derived mesenchymal stromal cells regress aortic aneurysm via the NF-kB, Smad3 and Akt signaling pathways

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Abstract

Background aims. We have confirmed that aortic aneurysm (AA) can be regressed by the administration of bone marrow–derived mesenchymal stromal cells (BM-MSCs). We investigated the kinetics of signaling pathways in AA following treatment with BM-MSCs. *Methods.* Angiotensin II-infused apolipoprotein E–deficient mice were treated by intravenous injection of 1×10^6 BM-MSCs in 0.2 mL saline (BM-MSCs group, $n = 5$) or 0.2 mL saline (saline group, $n = 5$). Mice were sacrificed 2 weeks after injection and subjected to measurements of the incidence of AA and levels of phosphorylated proteins. Levels of proteins in conditioned media of BM-MSCs were also measured. *Results.* The incidence of AA in the BM-MSCs group was reduced (BM-MSC 40% versus saline 100%, $P < 0.05$). Levels of pNF-kB and pSTAT1 were reduced (pNF-kB: 0.28 versus 0.45 unit/mL, $P < 0.05$, pSTAT1: 0.16 versus 0.34, $P < 0.05$), whereas levels of pAkt and pSmad3 were elevated (pAkt: 0.13 versus 0.07, $P < 0.01$, pSmad3: 1.07 versus 0.47, $P < 0.05$) in the BM-MSCs group. The levels of pNF-kB, pAkt, and pSmad3 were correlated with aortic diameters. Trophic factors including IGFPB-3, NRF, Activin A and PDGF-AA were secreted from BM-MSCs (IGFBP-3: 35.2 pg/mL, NRF: 3.1 pg/mL, Activin A: 3.1 pg/mL, PDGF-AA: 0.45 pg/mL). *Conclusions.* Our findings suggested that the therapeutic mechanism of BM-MSC–mediated AA regression could contribute to regulation of the NF-kB, Smad3 and Akt signaling pathways. In addition, paracrine actions by factors including NRF, IGFBP-3, Activin A and PDGF-AA might have affected these signaling pathways.

Key Words: *Akt, aortic aneurysm, bone marrow–derived mesenchymal stromal cells, cell therapy, NF-kB, signaling pathways, Smad, trophic factors*

Introduction

Aortic aneurysm (AA) is life-threatening disease associated with risk of aortic rupture. AA is caused by atherosclerosis and chronic inflammation, which lead to vulnerability of the aortic wall and contribute to expansion of the aorta. The incidence of AA is elevated in elderly men, and the prevalence of abdominal aortic aneurysm (AAA) ranges up to 12.5% for patients from 75 to 84 years of age [1]. Surgical replacement with prosthetic graft to prevent rupture is the standard therapy for AA due to the feasibility and effectiveness of this procedure; however, the surgery poses risks of fatal and severe complications such as paraplegia, stroke and renal failure [2]. To address this problem, endovascular repair, a less invasive technique than open surgery, has recently been developed. However, despite being a less invasive intervention, endovascular treatment has some crucial limitations, such as restrictive indication criteria and endovascular graft-related complications (e.g., endoleaks and migration) [2].

Inflammatory sites associated with AA exhibit overexpression of matrix metalloproteinases (MMPs), inflammatory cytokines, and chemokines [3]. In particular, MMP-2 and MMP-9 secreted from macrophages and vascular smooth muscle cells, respectively, degrade extracellular matrix (ECM) including collagen and elastin [4]. Various inflammatory cytokines and chemokines, such as interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α and monocyte chemotactic protein (MCP)-1, are secreted from inflammatory cells, including monocytes, macrophages and helper T cells. These cytokines and chemokines

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induce activation of inflammatory cells [5], MMPs secretion [6], monocyte recruitment [7] and their own overexpression in AA [4]. To ameliorate the chronic inflammation and loss of ECM observed in AA, several studies have investigated treatment of AA using the alternative therapeutic strategies such as pharmacological therapy [8,9] and cell therapy [10–12] to reduce the risk of rupture.

Mesenchymal stromal cells (MSCs; also known as mesenchymal stem cells) are widely used as source material for cell therapy of various diseases, including graft-versus-host disease [13], stroke [14], heart failure [15] and renal failure [16]. MSCs exert immunosuppressive and anti-inflammatory effects and accumulate at the inflammatory site and also secrete cytokines, chemokines, protease inhibitors and growth factors. We previously reported that AAs that have already formed in angiotensin II (AT-II)-infusion apolipoprotein E-deficient (apoE$^{-/-}$) AA model mice can be regressed by intravenous injection of bone marrow (BM)-derived MSCs (BM-MSCs). This treatment induces the following effects: inhibition of MMP-2 and MMP-9 enzymatic activity, reduction in the number of infiltrated inflammation macrophages and prevention of elastin degradation; these outcomes are associated with down-regulation of IL-6, MCP-1 and MMP-2 and up-regulation of IGF-1 and tissue inhibitor of metalloproteinase (TIMP)-2 [17]. However, the mechanisms underlying the effects of MSCs on AA regression, reported in our own work and other studies of cell-based therapy [10–12], remain unknown.

Several signaling pathways, including the c-Jun N-terminal kinase (JNK) [18], nuclear factor-kappa B (NF-kB) [19], signal transducer and activator of transcription (STAT) [20,21], extracellular signal-regulated kinase (ERK) [22] and Sma- and Mad-related proteins (Smad) [23–25] pathways, are activated, whereas protein kinase B (Akt) [26] signaling is reduced, in AA tissues of human patients or experimental animals. To obtain insight into the roles of these signaling pathways in AA, we investigated several signaling pathways in mouse AA tissues following treatment with BM-MSCs.

Methods

Animals

All animals were cared for in accordance with the *Guide for the Care and Use of Laboratory Animals* published by the U.S. National Institutes of Health (Publication No. 85-23). All experiments and procedures were approved by the Animal Experiment Advisory Committee of the Nagoya University School of Medicine (Protocol No. 28343). For this study, male apolipoprotein E deficient (apoE$^{-/-}$) mice were purchased from the Jackson Laboratory.

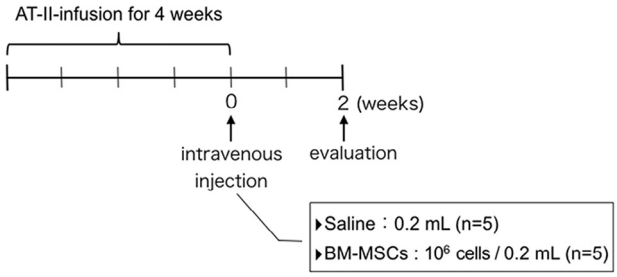

Figure 1. Diagram of *in vivo* protocol. AA was induced by AT-II infusion for 4 weeks. On day 28, 1×10^6 BM-MSCs (in 0.2 mL saline, n = 5) or 0.2 mL saline (n = 5) was injected via the tail vein. Mice were sacrificed and evaluated at 2 weeks after injection.

Cell culture and characterization of BM-MSCs

BM-MSCs used for intravenous injection were obtained from bone marrow cells collected from femurs and tibiae of mice using established techniques, as in our previous study [27]. Cells were identified as positive for Sca-1, CD44 and CD106 and negative for CD11b, CD31, CD34, CD45, CD86 and CD117 by flow cytometry (FACS Canto II), and tripotential differentiation capacity was assessed using differentiation medium, as previously described [28].

Aortic aneurysm mice model and treatment

apoE$^{-/-}$ mice 24–32 weeks of age were induced to form AAs by infusion of angiotensin II (AT-II, 1000 ng/kg/min, Calbiochem) for 28 days through subcutaneous osmotic mini-pumps (model 2004; DURECT) implanted under anesthesia with isoflurane, as previously described [29]. After infusion, 1×10^6 BM-MSCs (in 0.2 mL saline) or 0.2 mL saline was injected via the tail vein. Mice were divided randomly into the saline group ($n = 5$) and BM-MSCs group ($n = 5$). The protocol is shown in Figure 1. After euthanasia by an overdose of isoflurane, the length of the aorta from the thoracic to the abdominal was carefully exposed and photographed alongside a calibrated ruler using a DP70 digital camera (Olympus) attached to a stereomicroscope using the DP controller software (Olympus). The maximum aortic diameter at the infra-diaphragm was measured using digital image analysis software (ImageJ v.1.41, National Institutes of Health) by calibration with ruler correction. Ten-millimeter lengths of aorta, from the infra-diaphragm to the suprarenal, were harvested for Western blotting.

Co-culture of macrophages or SMCs with BM-MSCs

To investigate the effects of BM-MSCs on macrophages or SMCs, a co-culture model was adopted,