Therapeutic potential of bone marrow-derived mesenchymal stem cells in formed aortic aneurysms of a mouse model

Aika Yamawaki-Ogata, Xianming Fu, Ryotaro Hashizume, Kazuro L. Fujimoto, Yoshimori Arakio, Hideki Oshimao, Yuji Narita, Akihiko Usuia

Department of Cardiac Surgery, Nagoya University Graduate School of Medicine, Nagoya, Japan
Department of Pathology and Matrix Biology, Mie University Graduate School of Medicine, Mie, Japan

Abstract

OBJECTIVES: An aortic aneurysm (AA) is caused by atherosclerosis with chronic inflammation. Mesenchymal stem cells (MSCs) have potential anti-inflammatory properties. In this study, we examined whether an already-formed AA can be treated by intravenous injection of bone marrow-derived (BM)-MSCs in a mouse model.

METHODS: AA was induced in apolipoprotein E-deficient mice by angiotensin II-infusion for 28 days through sub-cutaneous osmotic mini-pumps. After that, 1 × 10⁶ BM-MSCs (in 0.2 ml saline) or 0.2 ml saline as a control was injected via the tail vein. Mice were sacrificed at 2 (saline group n = 10, BM-MSC group n = 10), 4 (saline group n = 6, BM-MSC group n = 7) or 8 weeks (saline group n = 5, BM-MSC group n = 6) after injection. The aortic tissues of each group were dissected. Aortic diameter, elastin content, matrix metalloproteinase (MMP)-2 and -9 enzymatic activity and cytokine concentrations were measured, as was macrophage infiltration, which was also evaluated histologically.

RESULTS: The incidence of AA in the BM-MSC group was reduced at 2 weeks (BM-MSC 40% vs saline 100%, P < 0.05), and aortic diameter was reduced at 2 and 4 weeks (2 weeks: 1.40 vs 2.29 mm, P < 0.001; 4 weeks: 1.73 vs 2.32 mm, P < 0.05). The enzymatic activities of MMP-2 and -9 were reduced in the BM-MSC group at 2 weeks (active-MMP-2: 0.28 vs 0.45 unit/ml, P < 0.05; active-MMP-9: 0.16 vs 0.34 unit/ml, P < 0.05). Inflammatory cytokines were down-regulated in the BM-MSC group at 2 weeks (interleukin-6: 2 weeks: 1475.6 vs 3399.5 pg/ml, P < 0.001; 4 weeks: 1745.6 vs 3399.5 pg/ml, P < 0.005) and insulin-like growth factor (IGF)-1 and tissue inhibitor of metalloproteinase (TIMP)-2 were up-regulated in the BM-MSC group at 2 weeks (IGF-1: 4.7 vs 2.0 ng/ml, P < 0.05; TIMP-2: 9.5 vs 4.0 ng/ml, P < 0.001). BM-MSC injection inhibited infiltration of M1 macrophages and preserved the construction of elastin.

CONCLUSIONS: Our results suggest that BM-MSCs might be an effective treatment for AA. Further investigation is necessary to optimize the injected dosage and the frequency of BM-MSCs to prevent a transient effect.

Keywords: Aortic aneurysm • Bone marrow-derived mesenchymal stem cells • Intravenous injection • Elastin • Matrix metalloproteinases • Macrophage

INTRODUCTION

An aortic aneurysm (AA) develops as a result of atherosclerosis and chronic inflammation. Surgical repair of AA is effective and prevents rupture. However, the surgical procedures for thoracic and thoracoabdominal AA are invasive and associated with high mortality and morbidity [1]. Although endovascular aneurysm repair (EVAR) and thoracic EVAR are minimally invasive interventions, they are anatomically and clinically limited, and have drawbacks such as endoleaks and graft migrations [2]. Thus, a new alternative strategy for AA treatment that reduces surgical invasion and the risk of rupture is needed.

The pathology of AA is characterized by destruction of the extracellular matrix (ECM) with chronic inflammation of the aortic wall, where the infiltrating macrophage release matrix metalloproteinases (MMPs), inflammatory cytokines and chemokines, leading to loss of elastin in the aortic wall [3]. MMPs, in particular MMP-2 and -9, cause the degradation of ECM, including collagen and elastin, and are overexpressed in AA [3]. In addition, various inflammatory cytokines and chemokines such as interleukin (IL)-1β, IL-6, tumour necrosis factor (TNF)-α and monocyte
chemotactic protein (MCP)-1 are expressed in the AA wall, and induce inflammatory cell activation [4] and MMP secretion [5] and promote monocyte recruitment [6].

Mesenchymal stem cells (MSCs) have the potential to accumulate into damaged tissue [7]. They also have immunosuppressive and anti-inflammatory potential, and secrete various factors, including tissue inhibitor of metalloproteinases (TIMPs), transforming growth factor (TGF)-β1 [8] and insulin-like growth factor (IGF)-1, which have been reported to promote elastin synthesis [9]. Focusing on these properties, we have previously reported that MSC may be an effective AA cell therapy in an angiotensin II (ATII)-infusion mice AA model either by surgically implanting a bone marrow-derived mesenchymal stem cell (BM-MSC) sheet onto the aortic adventitial surface, or by expanded and single-suspended cells, which was then directly injected intravenously at the initiation of ATII-infusion [10, 11]. These experiments tested the ability of BM-MSC to prevent AA development, rather than their ability to treat already-formed AA.

In this study, we examined whether BM-MSCs are effective for the treatment of already-formed AA using ATII-infusion apolipoprotein E-deficient (apoE−/−) mouse model.

**MATERIALS AND METHODS**

**Animals**

All animals were cared for in accordance with the ‘Guide for the Care and Use of Laboratory Animals’ published by the US National Institutes of Health (Publication 85-23, National Academy Press, Washington, DC, USA, revised in 1996). All experiments and procedures were approved by the Animal Experiment Advisory Committee of the Nagoya University School of Medicine (Protocol No. 24061). For this study, male apoE−/− mice were purchased from the Jackson Laboratory (Sacramento, CA, USA).

**Cell culture and characterization of bone marrow-derived mesenchymal stem cells**

Passage 6–8 murine BM-MSCs used for intravenous injection (IV) were obtained from bone marrow cells collected from femurs and tibiae using established techniques as in our previous study [10]. Cells were identified positive for sca-1, CD44 and CD106, and negative for CD11b, CD31, CD34, CD45, CD86 and CD117 by flow cytometry (FACS CantoII, Becton Dickinson, NJ, USA) at Passage 6. The tripotential differentiation capacity of cells was assessed using a differentiation medium as previously described [12]. Briefly, osteogenic differentiation was induced for 4 weeks, and cells were stained with alkaline phosphatase (ALP, Sigma). Adipogenic differentiation was induced for 2 weeks, and the cells were stained with Oil Red-O (Wako, Osaka, Japan). Chondrogenic differentiation was induced for 4 weeks, and the cells were stained with Alcian blue (Muto Pure Chemicals, Tokyo, Japan) (data not shown).

**Animal model**

AA was induced in 24–32-week (6–8 months) old apoE−/− mice on normal chow diets by infusion of ATII (1000 ng/kg/min,
Calbiochem, Darmstadt, Germany) for 28 days through subcutaneous osmotic mini-pumps (model 2004; DURECT, Cupertino, CA, USA) implanted under anaesthesia with isoflurane, as previously described [13]. After ATII infusion for 28 days, $1 \times 10^6$ BM-MSCs (in 0.2 ml saline) or 0.2 ml saline was injected via the tail vein. Mice were divided randomly into six groups: (i) ATII infusion with BM-MSC treatment for 2 weeks ($n = 10$), (ii) ATII infusion with saline injection for 2 weeks ($n = 10$), (iii) ATII infusion with BM-MSC treatment for 4 weeks ($n = 6$), (iv) ATII infusion with saline injection for 4 weeks ($n = 7$), (v) ATII infusion with BM-MSC treatment for 8 weeks ($n = 5$) and (vi) ATII infusion with saline injection for 8 weeks ($n = 6$). The injection protocol is shown in Fig. 1. BM-MSCs were labelled with PKH26 red fluorescent cell linker (SIGMA-Aldrich, St Louis, MO, USA) for detection according to the manufacturer’s protocol, before injection. Following euthanasia by an overdose of isoflurane, the entire length of the aorta was carefully exposed and photographed alongside a calibrated ruler, with a DP70 digital camera (Olympus, Tokyo, Japan) attached to a stereomicroscope using the DP controller software (Olympus). The maximum aortic diameter at the infradiaphragm was measured using the digital image analysis software (Image J v.1.41, National Institutes of Health, Bethesda, MD, USA) by

**Figure 2:** AA induced by ATII infusion. (A) Microscopic images show aorta; arrows indicate AA at the infradiaphragmatic aorta. Aortic diameters (B) and EVG staining (C) of infradiaphragmatic aorta were performed on the pre-ATII and post-ATII groups. Scale bars = 200 μm. ***$P < 0.001$ vs pre-ATII, assessed by unpaired t-test.
calibration with a ruler correction. Whole aortas, from the ascending to the iliac bifurcation, were harvested, and 2-mm lengths of aorta were cut at the infradiaphragm for immunofluorescence staining and Elastica van Gieson (EVG) staining, and cut longitudinally for \( \alpha \)-elastin quantification, MMP enzymatic activity and protein expression.

Gelatin zymography

MMP-2 and MMP-9 enzymatic activities in aortic tissues were detected by gelatin zymography as previously described [10]. Briefly, aortic tissues were homogenized with a protein extraction buffer containing 20 mM Tris-HCl (pH 7.5) and 0.01% Brij-35 (MP Biomedicals). The protein concentration in each lysate was measured using a BCA assay kit (PIERCE, Rockford, IL, USA) according to the manufacturer’s protocol. An equal concentration of protein was loaded into gels for electrophoresis and detected using a gelatin zymography electrophoresis kit (Life Kenkyusho, Yamagata, Japan) according to the manufacturer’s protocol. Slides were photographed with an FSX-100 microscope (Olympus). Images were quantified using the Image J software. The number of unbroken elastic lamellae throughout the cross-sectional aortic wall, the percent area of elastin and the medial components between the elastic lamellae (gap area) as compared with the total medial tissue area were quantified using the images [14].

Elastica van Gieson staining

The aorta at the infradiaphragmic level was embedded in OCT compound (Leica microsystems, Buffalo Grove, IL, USA), and cut using a microtome cryostat (Leica microsystems). The frozen cross-sections (5 \( \mu \)m) were stained for elastic lamellae specific stain using Weigert’s resorcin-fuchsin (Muto Pure Chemicals, Tokyo, Japan) according to the manufacturer’s protocol. Slides were photographed with an FSX-100 microscope (Olympus). Images were quantified using the Image J software. The number of unbroken elastic lamellae throughout the cross-sectional aortic wall, the percent area of elastin and the medial components between the elastic lamellae (gap area) as compared with the total medial tissue area were quantified using the images [14].

Measurement of \( \alpha \)-elastin content in aortic tissue

Soluble \( \alpha \)-elastin in the freeze-dried longitudinal aortas was measured using a Fastin elastin assay kit (Biocolor, County Antrim, UK) according to the manufacturer’s protocol. Aortas were cut into pieces with scissors and heated at 100°C for 60 min with 0.25 M
oxalic acid. Samples were then centrifuged at 3000 × g for 10 min, and the supernatant was measured. Soluble α-elastin values were standardized by the corresponding dry weight.

Immunofluorescence staining of macrophages

To identify M1 inflammatory and M2 anti-inflammatory macrophages in AA, immunofluorescence staining was performed. Frozen cross-sections were fixed with 4% paraformaldehyde for 30 min, and blocking was performed with 10% bovine serum albumin. Primary antibodies used were F4/80 antibody (1:100, AbD Serotec, Oxford, UK) for M1 macrophage and CD206 antibody (1:1000, Abcam, Cambridge, MA, USA) for M2 macrophage. Secondary antibodies used were antirat IgG Alexa fluor 555 conjugated antibody (1:1000) binding to F4/80 antibody and antirabbit IgG Alexa fluor 488 conjugated antibody (1:1000) binding to CD206 antibody. Cell nuclei were stained with DAPI (Vectashield, Vector Laboratories, Burlingame, CA, USA). Slides were photographed with an FSX-100 microscope, and images were quantified using the Image J software.

Enzyme-linked immunosorbent assay

Lysate protein concentration was measured using a BCA assay kit (Pierce). An equal concentration of total protein was applied to each assay kit and detected according to the manufacturer’s protocol for each enzyme-linked immunosorbent assay (ELISA) kit (IGF-1: Mediagnost, Reutlingen, Germany; TGF-β1, TIMP-1: R&D Systems, Minneapolis, MN, USA; TIMP-2: RayBiotech, Norcross, GA, USA; IL-1β, IL-6, TNF-α and MCP-1: Bender MedSystems, Vienna, Austria).

Statistical analysis

Statistical significance between groups was calculated by χ² test or unpaired t-test, as appropriate, using GraphPad Prism for Mac.
RESULTS

Incidence of aortic aneurysms, aortic diameter and distribution of injected cells

No deaths, and no abnormal behaviour indicating pain or distress, or paraplegia due to ischaemia were observed in any of the mice in this study. We determined whether AA was formed by ATII infusion for up to 28 days. Figure 2A shows AA formation at the infra-diaphragmatic aorta in the pre- and post-ATII groups. Aortic diameters of the post-ATII group were significantly expanded compared with the pre-ATII group (2.3-fold, 1.89 ± 0.17 vs 0.82 ± 0.01 mm, \( P < 0.001 \); Fig. 2B). EVG staining of aortic cross-sections in the post-ATII group revealed fragmented elastic lamellae (Fig. 2C). After receiving treatment with BM-MSC or saline, both groups of mice were observed to have AA through 8 weeks. Morphological changes were ameliorated by BM-MSC treatment at 2 weeks (Fig. 3A). Aortic diameter was significantly smaller in the BM-MSC group than in the saline group at 2 and 4 weeks (2 weeks: 1.40 ± 0.18 vs 2.29 ± 0.20 mm, \( P < 0.001 \); 4 weeks:

Figure 5: Comparative histology of elastic lamellae and \( \alpha \)-elastin content. (A) EGV staining indicates prevention of elastin lamellae destruction and degradation in the BM-MSC group compared with the saline group. Scale bars = 200 \( \mu m \). Quantitative analysis of medial elastin area and medial gap area (B), and \( \alpha \)-elastin content (C). Data are means ± SEM. * \( P < 0.05 \), ** \( P < 0.01 \) vs saline assessed by unpaired t-test.
1.73 ± 0.22 vs 2.32 ± 0.14 mm, P < 0.05, respectively; Fig. 3B). However, the diameter in the BM-MSC group had expanded similarly in the saline group at 8 weeks. These results suggest that the tested treatment protocol might have a transient effect. The incidence of AA was significantly decreased in the BM-MSC group compared with the saline group at 2 weeks (40 vs 100%, P < 0.05; Fig. 3C), and there was no significant difference at 4 and 8 weeks. The injected BM-MSCs were detected in the infradiaphragmatic aorta at 2 weeks (Fig. 3D).

### Matrix metalloproteinases enzymatic activity

Zymographic analysis of MMP activity showed detectable signals of pro- and active-MMP-2 and MMP-9 (Fig. 4A), and they were significantly decreased in the BM-MSC group compared with the saline group at 2 weeks (pro-MMP-2: 0.26 ± 0.06 vs 0.46 ± 0.07 unit/ml, P < 0.05; active-MMP-2: 0.28 ± 0.03 vs 0.45 ± 0.07 unit/ml, P < 0.05; pro-MMP-9: 0.32 ± 0.04 vs 0.72 ± 0.17 unit/ml, P < 0.05; active-MMP-9: 0.16 ± 0.03 vs 0.34 ± 0.06 unit/ml, P < 0.05, respectively; Fig. 4B). However, there were no significant differences at 4 and 8 weeks.

### Elastic lamellae

EVG staining showed considerable destruction of the elastic lamellae in the saline group, whereas less loss of construction of the elastic lamellae was observed in the BM-MSC group through 8 weeks (Fig. 5A). The medial elastin area was preserved from degradation (2 weeks: 59.5 ± 1.8 vs 48.2 ± 3.4%, P < 0.05; 4 weeks: 51.5 ± 3.3 vs 33.4 ± 3.9%, P < 0.01; 8 weeks: 45.9 ± 2.7 vs 32.7 ± 4.8%, P < 0.05, respectively; Fig. 5B). The medial gap area was significantly decreased in the BM-MSC group compared with the saline group (2 weeks: 40.5 ± 1.8 vs 51.8 ± 3.4%, P < 0.05; 4 weeks: 48.5 ± 3.3 vs 66.6 ± 3.9%, P < 0.01; 8 weeks: 54.1 ± 2.7 vs 67.3 ± 4.8%, P < 0.05, respectively; Fig. 5B). The BM-MSC group had significantly more α-elastin in aortic tissue compared with the
saline group at 2 weeks (30.2 ± 1.8 vs 21.4 ± 2.1%, P < 0.01; Fig. 5C).

However, there were no significant differences at 4 and 8 weeks.

Immunofluorescence of M1 and M2 macrophage

F4/80-positive M1 macrophages were detected in the thrombus and the medial layers, and CD206-positive M2 macrophages were detected in the adventitia (Fig. 6A). At 2 weeks, the percentage of M1 macrophages decreased (12.5 ± 1.2 vs 23.3 ± 3.9%, P < 0.05; Fig. 6B) and the percentage of M2 macrophages increased in the BM-MSC group compared with the saline group (21.7 ± 2.8 vs 14.6 ± 1.7%, P < 0.05; Fig. 6C).

Tracking of PKH26-labelled BM-MSCs

The injected BM-MSCs were distributed to other tissues in addition to aortic tissue at 2 weeks. Many BM-MSCs were observed in the spleen, liver, kidney and lung, but not in the heart (Fig. 7).

Table 1: Protein expression in aortic tissues, measured by ELISA

<table>
<thead>
<tr>
<th>Protein</th>
<th>Two weeks</th>
<th>Four weeks</th>
<th>Eight weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline (n = 10)</td>
<td>BM-MSC (n = 10)</td>
<td>Saline (n = 6)</td>
</tr>
<tr>
<td>IL-1β (pg/ml)</td>
<td>478.9 ± 123.0</td>
<td>437.4 ± 65.6</td>
<td>489.8 ± 59.4</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>3399.5 ± 644.5</td>
<td>1475.6 ± 329.6</td>
<td>3712.8 ± 575.3</td>
</tr>
<tr>
<td>MCP-1 (pg/ml)</td>
<td>352.7 ± 61.0</td>
<td>208.0 ± 22.6</td>
<td>380.3 ± 61.6</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>56.1 ± 3.6</td>
<td>57.6 ± 4.2</td>
<td>68.9 ± 7.3</td>
</tr>
<tr>
<td>IGF-1 (ng/ml)</td>
<td>2.0 ± 0.6</td>
<td>4.7 ± 1.0</td>
<td>1.8 ± 0.2</td>
</tr>
<tr>
<td>TGF-β1 (pg/ml)</td>
<td>355.4 ± 24.4</td>
<td>387.7 ± 25.4</td>
<td>294.1 ± 16.1</td>
</tr>
<tr>
<td>TIMP-1 (pg/ml)</td>
<td>1462.9 ± 289.0</td>
<td>1998.1 ± 580.6</td>
<td>1156.0 ± 191.4</td>
</tr>
<tr>
<td>TIMP-2 (ng/ml)</td>
<td>4.0 ± 0.6</td>
<td>9.5 ± 1.0***</td>
<td>4.5 ± 0.9</td>
</tr>
</tbody>
</table>

Data for the variables are mean ± SEM.
*P < 0.05.
**P < 0.001 vs saline group.

Protein expression in aortic tissue

There was no statistical difference in IL-1β, TNF-α, TGF-β1 and TIMP-1 between the two groups. However, IL-6 and MCP-1 in the BM-MSC group were significantly decreased compared with the saline group at 2 and 4 weeks (IL-6: 2 weeks: 1475.6 ± 329.6 vs 3399.5 ± 644.5 pg/ml, P < 0.05; 4 weeks: 2184.7 ± 297.3 vs 3712.8 ± 575.3 pg/ml, P < 0.05 and MCP-1: 2 weeks: 208.0 ± 22.6 vs 352.7 ± 61.0 pg/ml, P < 0.05, respectively). IGF-1 (4.7 ± 1.0 vs 2.0 ± 0.6 ng/ml, P < 0.05) and TIMP-2 (9.5 ± 1.0 vs 4.0 ± 0.6 ng/ml, P < 0.001) were significantly increased at 2 weeks in the BM-MSC group compared with the saline group (Table 1).

DISCUSSION

In this study, we used an ATII infusion mice AA model to determine whether already-formed AA can be treated by IV of BM-MSCs. Injection of BM-MSCs resulted in transient regression...
of formed AA, transiently inducing the following effects: (i) inhibition of MMP-2 and MMP-9 enzymatic activity, (ii) increased protein expression of TIMP-2, (iii) decreased protein expression of inflammatory cytokine and chemokine involving IL-6 and MCP-1, (iv) increased protein expression of IGF-1, which helps promote of elastin synthesis and (v) prevention of elastin degradation while promoting elastin synthesis.

There have been several reports of AA treatment using various cells. Sharma et al. [15] reported that attenuation of IL-17 and abdominal AA (AAA) formation were inhibited by IV of human placental-derived MSCs in an elastase-perfusion mice model. However, the primary purpose of AA therapy in the clinical setting is to prevent the rupture of formed AA. An advantage of the present study is that we evaluated the impact of MSC cell therapy on aortic remodelling in a clinically relevant setting, an already-formed AA model. Durand et al. [16] reported that an already-formed carotid arterial aneurysm was regressed by catheter delivery of gingival fibroblast using an elastase-perfusion mice model. Fibroblasts also have potential anti-inflammatory and immunosuppressive properties similar to MSC, but were not observed to migrate into the injury site following IV [17]. Thus, IV of fibroblasts may not adequately decrease the size of aneurysms.

Schneider et al. [18] reported that BM-MSCs stabilize already-formed tentative rat AAA model using xenograft obtained from guinea pig. BM-MSCs were injected into the lumen of clamped xenograft aorta using a catheter and allowed to attach for 8 min. The results showed that a decrease of inflammation and MMP-9 proteolysis, and stabilization of AAA diameter at 4 weeks after injection. However, this delivery method requires a surgical procedure or an advanced catheter intervention because the cells must be injected into the lumen of the clamped aorta. In contrast, our method of IV is simple, easy and minimally invasive. In addition, in a clear difference between our study and ours, the pathophysiology of an elastase-infused aneurysm model and a xenograft model is different from that of aneurysms based on atherosclerosis [19]. Our study is the first to report on the efficacy of MSC treatment for atherosclerotic-formed AA using apoE−/− mice with ATII.

The localization of M1 and M2 macrophages has not been examined in the aneurysmal site in other studies to date. M1 proinflammatory macrophages were decreased and M2 anti-inflammatory macrophages were increased in the BM-MSC group. These phenomena are supported by a recent study. Adutler-Lieber et al. [20] reported that human cardiac adipose tissue-derived MSCs can polarize macrophages into M2 macrophages through IL-10 secretion in coculture. Moreover, the down-regulation of MCP-1, which promotes macrophage infiltration into the AA wall, might decrease the number of M1 macrophages [21].

MMP-2 and -9 are abundantly expressed in human AA and contribute to the elastin degradation in aortic expansion [6]. The catalytic activities of MMPs are controlled by forming a complex with TIMPs [3]. Our results suggested that an increase of TIMP-2 in tissues led to decreased MMP-2 activity, and a decrease of M1 inflammatory macrophage infiltration led to decreased MMPs activity in the BM-MSC group.

Elastin provides the aortic strength and flexibility, and the gene expression of elastin is modulated by IGF-1 [9]. Our data indicated that the level of IGF-1 increased in the BM-MSC group. Thus, BM-MSCs might contribute to elastin synthesis via IGF-1 up-regulation in aortic tissue, and elastin synthesis might lead to AA regression.

Our study has some limitations. First, the number of MSCs injected in our study was relatively lower than that in previous studies where 1–5 × 10^6 cells/kg were administered in an attempt to treat human graft and host disease [22], or 1–4 × 10^7 cells/kg were administered for a mouse or rat myocardial infarction [23, 24]. On the other hand, a study of cell therapy for AA was performed using the same dosage as in our study [15]. Our results were transient effects, with the greatest difference between BM-MSCs and saline groups at 2 weeks; however, our effects may have been because of the low dose of injected cells. Thus, we must attempt to optimize the number of injected cells in the future. Secondly, the measurements of AA diameter are endpoints of each mouse, and mice were not assessed continuously throughout the 8 weeks following treatment. Also, the effects of AA treatment with BM-MSCs are not long term in duration; therefore, injection of BM-MSCs might be required intermittently to obtain a sustained clinical effect. Thirdly, we must verify the efficiency of BM-MSCs using another animal model such as an elastase-perfusion or CaCl2-treatment mouse model. Finally, we have not investigated the impact of cell therapies using different cell sources or allogenic MSCs. A study using other cell types would better define which cell source is optimal. As a further step towards the clinical application, we will attempt to investigate the optimal cell number, along with better timing of administration of BM-MSC and the identification of a cell source, in a study using a larger animal.

In conclusion, our results suggest that IV of BM-MSCs might be an effective treatment for already-formed AA. However, further investigations are necessary to identify the optimal injecting cell number, along with the frequency and optimal timing of BM-MSCs administration to achieve a sustained effect.

ACKNOWLEDGEMENTS

The authors acknowledge the Division for Medical Research Engineering, Nagoya University Graduate School of Medicine, for the usage of FACS Canto II and Crioimctome.

Funding

This work was supported by the Japan Society for the Promotion of Science (JSPS) KAKENHI Grant Number 23791566 and 25462161.

Conflict of interest: none declared.

REFERENCES

APPENDIX. CONFERENCE DISCUSSION

Dr I. Dimarakis (Manchester, United Kingdom): Recent research utilizing the same small animal model knockout has implicated an array of molecular pathways leading to attenuation of aneurysm formation, as you mentioned, including beta-arrin and cathepsin-S. Similarly, suppression of angiotensin II-induced aortic aneurysm formation has been described with pharmacologic therapy alone.

As with stem cell research for myocardial regeneration, a huge amount of discrepancy appears regarding cell type, dosage, frequency, as well as route of administration. As far as reported outcomes are concerned, once again a great deal of inconsistency exists amongst groups, mainly reporting selected parameters of successful end effects. In this body of literature, reporting of observed outcomes most commonly does not lead to identification of specific molecular and cellular pathways involved. Furthermore, the inherent heterogeneity of the mesenchymal stem cell populations may account for a myriad of interactions, especially as passaging of cells may result in cell populations different to their parent ones. Taking this further, mesenchymal stem cells are currently investigated for their favourable or even unfavourable role in tumour biology.

Although many queries from the clinical translation standpoint arise, I believe it is premature to go down that path today. With this in mind, I’d like to ask two questions. First, have you identified, or do you have preliminary data on any specific pathways mesenchymal stem cell delivery resulted in the observed outcomes? Did your most recent work with repeated infusions and overall higher doses provide any further insight? If so, do you believe that this may be interrupted by targeted pharmacological strategies?

Dr Dimarakis: Have you identified any specific molecular pathways, like kinase pathways for example, responsible for this? And do you think you can use drugs? Because if you need continuous repeated infusions of stem cells and you can achieve the same effect with medication, the latter would be preferable. Do you have any insight, any data on that?

Dr Narita: Rho-kinase?

Dr Dimarakis: That’s an example. Do you have any data about any specific pathways?

Dr Narita: We did not have data for other pathways, so I can’t answer your question correctly.

Dr C. Hagi (Munch, Germany): The question is: What is the mechanism?

Dr Narita: We have previously investigated cell-to-cell interaction in vitro by indirect co-cultured system. MSC was co-cultured with smooth muscle cells or macrophages, which, as you know, are donor cells for secretion of cytokine or metalloproteinase. These results were an expression of inflammatory cytokines, and matrix metalloproteinase were significantly downregulated co-cultured with MSC. So we think the mechanism of this effect was induced by the paracrine effects of MSC.

Dr Dimarakis: And secondly, the heterogeneity of mesenchymal stem cells has suggested the potential for stem cells to contribute to both reparative as well as destructive aortic remodelling processes. Have you identified any stem cell subpopulations for the reparative effects seen?

Dr Narita: You mean differentiation of mesenchymal stem cells?

Dr Dimarakis: Stem cell markers of any subpopulations responsible for the effect seen?

Dr Narita: We didn’t check this differentiation of the injected MSC. But we have checked differentiation of intraperitoneal implantation of MSC in a previous study. They did not differentiate into osteoblasts or chondrocytes or adipocytes.