主論文の要旨

Intravenous administration of mesenchymal stem cells prevents angiotensin II-induced aortic aneurysm formation in apolipoprotein E-deficient mouse

（間葉系幹細胞の静注療法はアポＥノックアウト+アンギオテンシンII負荷大動脈瘤モデルマウスの大動脈瘤形成を予防する）

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符　显明

Introduction

Aortic aneurysm (AA) is a common life-threatening disease and its prevalence is increasing with the ageing society. The pathogenesis of AA is characterized by chronic inflammation in the aortic wall with accumulation of macrophages and degradation of extracellular matrix with increased matrix metalloproteinases (MMPs). Accumulating evidence indicates that anti-inflammation is a promising therapeutic strategy for AA. Mesenchymal stem cells (MSCs) are known to be capable of suppressing inflammatory responses. Intra-abdominal implantation of bone marrow-derived MSCs (BM-MSCs) sheet by laparotomy has been reported to attenuate angiotensin II (AngII)-induced AA growth in apolipoprotein E-deficient ($apoE^{-/-}$) mice through anti-inflammation effects. However, cell delivery by laparotomy is invasive; we here demonstrated the effects of multiple intravenous administrations of BM-MSCs on AngII-induced AA formation.

Object and Methods

BM-MSCs were isolated from femurs and tibiae of male $apoE^{-/-}$ mice (6 to 8 weeks old). Experimental AA was induced by AngII infusion (1000 ng/kg/min) for 28 days in male $apoE^{-/-}$ mice (24 to 28 weeks old). Mice received weekly intravenous administration of BM-MSCs (n=12, group MSC4, 1×10^6 cells in 0.2 ml saline) or saline (n=10, group CONT, 0.2 ml saline) (Figure 1A). After 4 weeks, the outer aortic diameter was measured at ascending, phrenic, and infrarenal levels. AA was defined as an increase in aortic diameter of ≈ 50%. AA incidence was evaluated. Elastin content in aortic tissue was quantified using A Fastin elastin assay Kit (Biocolor, County Antrim, UK). Histology and immunofluorescence staining were performed to evaluate aortic wall morphology and macrophage infiltration. Gelatin zymography was performed to evaluate MMP-2 and MMP-9 enzymatic activities in aortic tissues. In addition, enzyme-linked immunosorbent assay (ELISA) was performed to quantify protein expression including MMP inhibitors (TIMP-1 and -2), chemokine (MCP-1), cytokines (IL-1ß, IL-6, and TNF-α, and growth factors (IGF-1 and TGF-ß1).

Results

1. BM-MSCs inhibited development of AA in Ang II-infused $apoE^{-/-}$ mice

The AA incidence in group CONT was 100%, which was significantly decreased to 50% in group MSC4 (Figure 1C). At the ascending, phrenic, and infrarenal levels, group CONT mice showed significantly larger aortic diameters compared with group Sham. Treatment with BM-MSCs resulted in significantly decreased aortic diameters compared with group CONT at ascending and infrarenal levels, but not at phrenic level (Figure 1D-F).

2. BM-MSCs suppressed aortic elastin degradation in AngII-infused $apoE^{-/-}$ mice.

EVG-stained sections from suprarenal aortas of group Sham showed normal wavy elastic lamina structure in the aortas, but sections from group CONT showed disruption of elastic fibers and aneurysm formation. Intravenous administration of BM-MSCs partially maintained the wavy structure of the elastic lamellae (Figure 2A-C). In addition, the elastin content of the aorta in group MSC4 was

significantly increased compared with group CONT, and showed no significant difference compared with group Sham (group MSC4 vs. group CONT, 39.69 ± 7.65 vs 24.80 ± 2.78, μg/mg, $P < 0.01$; group MSC4 vs. group Sham, 39.69 ± 2.21 vs. 48.35 ± 3.28, μg/mg, $P > 0.05$; Figure 2D).

3. BM-MSCs decreased inflammatory response in AngII-infused apoE$^{-/-}$ mice

Immunofluorescence staining demonstrated that F4/80-positive macrophages were detected abundantly in the adventitia and media of the suprarenal aortic walls in group CONT. The macrophages infiltration was ameliorated by BM-MSCs administration (Figure 3 A-E). Quantitation of the area of F4/80 staining as a ratio of the DAPI staining area was shown in Figure 3 J.

4. BM-MSCs suppressed activities of MMP-2 and MMP-9 in AngII-infused apoE$^{-/-}$ mice

Gelatin zymography was conducted to evaluate MMP-2 and -9 enzymatic activities in the aortic tissues. A representative zymogram was shown in Figure 4A. Quantitation of the band intensity shows that (pro- and active-) MMP-2 and (pro- and active-) MMP-9 activities were significantly decreased in group MSC4 compared with group CONT (Figure 4B-E).

5. BM-MSCs regulated aortic cytokines, chemokine, and growth factors expression

Protein expression in aortic tissue was evaluated by ELISA. There were no statistical differences in TNF-α expression between group CONT and group MSC4, while expressions of IL-1β, IL-6, and MCP-1 that promote inflammatory reaction were significantly decreased in group MSC4 compared with group CONT (Figure 5A-D). At the same time, the expression of IGF-1 and TIMP-2 which promote elastin synthesis were significantly increased in group MSC4 compared with group CONT, though expression of TGF-β1 and TIMP-1 showed no significant difference (Figure 5E-H).

6. Cell tracking of transplanted BM-MSCs

We investigated whether intravenous injected BM-MSCs mobilized into the aortic walls. BM-MSCs were labeled with fluorescent PKH26 and then intravenously injected into the apoE$^{-/-}$ mice via tail vein. PKH26 labeled BM-MSCs were detected in the media and adventitia of aortas four weeks after operation (Figure 6).

Discussion

MSCs are known to be capable of suppressing inflammatory responses. Intra-abdominal implantation of BM-MSCs sheets by laparotomy has been reported to inhibit the development of Ang II-induced aortic aneurysm in apoE$^{-/-}$ mice by anti-inflammation and elastin preservation. However, BM-MSCs implantation by laparotomy is an invasive approach; an alternative delivery approach is needed. Studies have shown that intravenous delivery of MSCs is an effective approach for treatment of a variety of diseases such as myocardial infarction, stroke, lung injury, and diabetes mellitus. Moreover, it is considered a suitable route for translation into clinical application, due to its low invasiveness. In the present study, we demonstrated that intravenously administered BM-MSCs were capable of engrafting into the aortic wall in Ang II-infused apoE$^{-/-}$ mice, and that multiple intravenous administrations of BM-MSCs could effectively prevent AA formation.

In the present study, we found that multiple intravenous administrated BM-MSCs suppressed infiltration of macrophages and activities of MMPs in the aortic walls. BM-MSCs also markedly decreased proinflammatory cytokines secretions including IL-1β, IL-6, and MCP-1, while increasing growth factor expressions like IGF-1 and TIMP-2, which promote elastin synthesis. These results suggested that the effects of multiple intravenous administrated BM-MSCs on Ang II-induced AA in apoE$^{-/-}$ mice may be associated with anti-inflammatory actions.

It should be noted that in this study multiple intravenous administrations of BM-MSCs effectively inhibited the development of AngII-induced AA in apoE$^{-/-}$ mice, while single intravenous administration of BM-MSCs showed modest effects (in the preliminary experiment). We presumed that the timing and cell dose of BM-MSCs might account for the different results. BM-MSCs were shown to be able to migrate into sites of inflammation or injury when transplanted locally or systemically, which was believed to be mediated by chemotactic proteins such as MCP-1, and IL-8 secreted from injured or inflammatory tissues. The timing of single BM-MSCs injection in this study was just after pump implantation, and at that time aortic inflammation did not occur. On the other hand, we intravenously administrated 4×10^6 BM-MSCs for each mouse in multiple injection group while 1×10^6 in single injection group. The different cell dose may affect the strength of anti-inflammatory effects of BM-MSCs. The appropriate timing and cell dose for intravenous administrations of BM-MSCs treatment of AA remain unclear. Further studies are needed.

Conclusions

Multiple intravenous administrations of BM-MSCs were effective to suppress inflammatory reactions in Ang II-infused apoE$^{-/-}$ mice, and inhibit the development of AAs. It may therefore serve as a new therapeutic strategy for patients with AA.