Bone Marrow Mesenchymal Stem Cells Stabilize Already-formed Aortic Aneurysms More Efficiently than Vascular Smooth Muscle Cells in a Rat Model

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WHAT THIS PAPER ADDS

Stabilization of aneurysmal growth using mesenchymal stem cells in an already-formed abdominal aortic aneurysm model has not been published. Direct cellular seeding using catheter-based procedures could be an alternative to treat aortic expansion associated with type II endoleak after endovascular aortic aneurysm repair.

Purpose: Abdominal aortic aneurysms (AAAs) expand because of aortic wall destruction. Enrichment in Vascular Smooth Muscle Cells (VSMCs) stabilizes expanding AAAs in rats. Mesenchymal Stem Cells (MSCs) can differentiate into VSMCs. We have tested the hypothesis that bone marrow-derived MSCs (BM-MSCs) stabilizes AAAs in a rat model.

Material and methods: Rat Fischer 344 BM-MSCs were isolated by plastic adhesion and seeded endovascularly in experimental AAAs using xenograft obtained from guinea pig. Culture medium without cells was used as control group. The main criteria was the variation of the aortic diameter at one week and four weeks. We evaluated the impact of cells seeding on inflammatory response by immunohistochemistry combined with RT-PCR on MMP9 and TIMP1 at one week. We evaluated the healing process by immunohistochemistry at 4 weeks.

Results: The endovascular seeding of BM-MSCs decreased AAA diameter expansion more powerfully than VSMCs or culture medium infusion (6.5% $\pm$ 9.7, 25.5% $\pm$ 17.2 and 53.4% $\pm$ 14.4; $p = .007$, respectively). This result was sustained at 4 weeks. BM-MSCs decreased expression of MMP-9 and infiltration by macrophages (4.7 $\pm$ 2.3 vs. 14.6 $\pm$ 6.4 mm^2 respectively; $p = .015$), increased Tissue Inhibitor Metallo Proteinase-1 (TIMP-1), compared to culture medium infusion. BM-MSCs induced formation of a neo-aortic tissue rich in SM-alpha active positive cells (22.2 $\pm$ 2.7 vs. 115.6 $\pm$ 30.4 cells/surface units, $p = .007$) surrounded by a dense collagen and elastin network covered by luminal endothelial cells.

Conclusions: We have shown in this rat model of AAA that BM-MSCs exert a specialized function in arterial regeneration that transcends that of mature mesenchymal cells. Our observation identifies a population of cells easy to isolate and to expand for therapeutic interventions based on catheter-driven cell therapy.

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INTRODUCTION

Abdominal aortic aneurysms (AAAs) expand because of wall atrophy, a consequence of proteolytic injury of extracellular matrix (ECM), disappearance of vascular smooth muscle cells (VSMCs), and absence of compensatory aortic reconstruction.[1,2] In expanding experimental AAAs, correction of depletion in VSMCs triggers wall reconstruction and stabilizes the diameter of the diseased aorta.[3–5] Accordingly, increasing VSMC content by endovascular cell seeding could be a therapeutic strategy. It requires the identification of cells easy to isolate and to expand.

A subset of bone marrow (BM) cells, referred to as Mesenchymal Stem Cells (MSCs) can be isolated by adherence to plastic wells and can easily be expanded.[6] As MSCs are multipotent and can home at sites of injury, it has been

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proposed that BM-MSCs contribute to post-natal tissue repair.[7] *In vitro*, MSCs have been shown to differentiate into VSMC-like cells upon PDGF-BB stimulation.[8] *In vivo*, MSCs have been shown to contribute to healing of mechanically injured arteries.[9] This repair process results in intimal hyperplasia, a VSMC-rich tissue hypertrophy that contrasts with the VSMC-deprived atrophy of the aneurysmal wall. Previous reports have suggested that MSCs may be of interest in AAAs.[10] However, there is no demonstration that MSCs stabilize expanding AAAs. This demonstration requires a model of AAA with statistically significant diameter expansion after cell seeding, with thrombus at wall/blood interface.[3,4] The xenograft model, in which aneurysmal degeneration is driven by inflammation, matrix metalloprotease activity regulated by the plasmin pathway,[11,12] fulfills these requirements.

In this study, we have tested the hypothesis that local endovascular seeding of BM-MSCs induces aortic tissue growth and stabilizes the diameter of expanding experimental AAAs, a strategy compatible with future catheter-based cell therapy approaches in aortic aneurysm disease.

MATERIAL AND METHODS

BM-MSC isolation

Cells were isolated from 12 week old male Fischer 344 rats (Charles River, St Quentin Fallavier, France). Aortic VSMCs were isolated[5] and grown in RPMI 1640 and Medium 199 (1:1), with L-glutamine and 10% fetal calf serum (FCS) (Invitrogen, Corporation, Paisley, UK). BM-MSCs were isolated from femurs and tibias by centrifugation and selected by plastic adhesion after overnight incubation in α-MEM with 20% FCS. After this step, medium was removed after 72 h. Antibiotics and antifungic were added in each culture medium. Adherent cells were passaged when reaching 80% confluence. BM-MSCs phenotypes were shown to express MSC markers (CD44, CD73, CD90, CD105), but not leukocyte markers (CD45, CD11b).

Surgery

Animals were housed and taken care of according to the European Union Standards. They received analgesia and were anesthetized with .1 ml/100 g body weight pentobarbital i.p. AAAs were generated in 250 g-12 week old, male Fischer 344 rats by implanting a segment of Hartley guinea pig aorta (xenograft) as previously described.[3] Guinea pig (Charles River) infrarenal aortas were decellularized using .1% sodium dodecyl sulfate (Sigma, St-Louis, USA) to obtain intact tubes of aortic ECM which were orthotopically implanted into rats with 10-0 sutures. Fourteen days after xenograft implantation, a chimeric AAA (>50% diameter increase) had developed from the degraded guinea pig ECM infiltrated by cells from rat, with a luminal thrombus,[3] as previously described. AAAs were isolated from blood flow by clamps. The lumen was rinsed with culture medium through a PE10 catheter introduced by an aortotomy performed downstream in the native aorta. Passage 5 to 6 BM-MSCs ($n = 6$ for the time point at one week and $n = 5$ for the time point at one month) or VSMCs ($n = 5$ for a unique time point at one week) suspended in culture medium with 5% FCS were injected into the AAA as previously described.[3] To summarize, animals were perfused 2 min on each side, so perfusion took 8 min. In another set of experiments BM-MSCs were stained before seeding with the fluorescent dye PKH26 (Sigma). These xenograft were harvested at 48 h and one week to get sufficient fluorescent signal. As controls, AAAs were infused with culture medium with 5% FCS with no cell ($n = 5$ at one week and $n = 5$ at one month).

AAA assessment

AAA maximum transverse diameter was measured at endovascular infusion and before euthanasia 1 or 4 weeks later using a graduated scale under dissecting microscopy. Percentage of diameter increase was calculated as follows: (diameter at evaluation time − diameter at seeding) × 100/diameter at seeding. After inclusion into paraffin, 5 µm cross sections were generated from the center of AAAs. Elastin and collagen were stained by orcein and sirius red, respectively. For immunohistochemistry, primary antibodies were mouse anti rat monoclonals: ED1 clone for monocytes/macrophages, RECA for endothelial cells (Serotec, Oxford, England) and smooth muscle alpha-actin (clone 1A4, Sigma). An alkaline phosphatase—antialkaline phosphatase technique was used (Dakopatts). Control sections were generated by omission of the primary antibody and with a nonrelevant primary antibody. Quantitative analyses used Image-Pro Plus Software (Media, Cybernetics, Bethesda, Md). Two blinded observers recorded the percentage of the total area for each section.

mRNA semi-quantification

MMP-9 and TIMP-1 mRNA contents were analyzed using reverse transcription polymerase chain reaction (RT-PCR), comparative to the domestic gene 18S (QuantumRNA™18s Internal Standards kit, Ambion, Montrouge, France) (primers: MMP-9: *forward*: 5′-CTGCGTATTTCCATTCATCTT-3′; *reverse*: 5′-ATGCCTTTTATGTCGTCTTCA-3′; TIMP-1: *forward*: 5′-CCCCAGAAATCAACGAGAGACCA-3′; *reverse*: 5′-ACACCCCA-CAGCCAGCACTAT-3′). Intima, and media plus adventitia were separated by micro dissection and pooled by layers and groups. Total RNA was extracted with TRIzol™(Life Technologies, Paisley, UK) and treated with grade I DNase (Roche Molecular Biomedicals, Rosny, France). Reverse transcription was done with random primers, and Reverse transcriptase M-MLV, dNTP, dithiothreitol, and ribonuclease inhibitor (Eurobio, Les Ulis, France). PCR was performed in a PCR Express thermo cycler (Hybaid, UK) with DNA Taq polymerase (Eurobio, Les Ulis, France). Bands of amplified sequences corresponding to the gene of interest and to 18s were quantified with Gel Analysis (Iconix, Marnes La Coquette, France). Results were expressed as ratios between signals corresponding to the gene of interest and 18s.

Statistical analysis

Results were expressed as mean ± SD. For independent qualitative parameters a Chi-square test was used (biostat TGV). The nonparametric Mann—Whitney *U* and Kruskal Wallis tests (Statview, version 4.5) were used for statistical