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Original Article

The Effects of Deferoxamine on the Up regulation of Chemokine Receptor 2 in Bone Marrow Stromal Stem Cells

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Abstract

Background: Bone marrow stromal stem cells (BMSCs) are non-hematopoietic, stromal cellsthat can differentiate into mesenchymal and other type of tissues. The BMSCs have properties that make them ideal candidates for tissue engineering. The present study aimed to investigate the effect of deferoxamine (DFO) on homing of bone marrow-derived mesenchymal stem cell, and to examine if DFO can increase migration and subsequent homing of mesenchymal stem cells (MSCs) in vitro.
Methods: BMSCs were isolated from the long bones of NMARI rats through density gradientcentrifugation and adherent cell culture. Next, they were treated using DFO in Dulbecco's modified eagle medium (DMEM) for 24 h. The expression of chemokine receptor 2 (CCR2) were assessed using RT-PCR.
Results: BMSCs expressed CCR2 on a large proportion of cells. In DFO-treated BMSCs,expression of CCR2 ($P<0.005$) significantly increased compared to that in control groups. Elevation and up regulation of CCR2 in DFO-treated MSCs were observed.
Conclusion: Preconditioning of BMSCs using DFO prior to transplantation could increasehoming of BMSCs through affecting some chemokine receptors as well as proteases involved and thus improve the efficacy of cell therapy.

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Introduction

Bone Marrow Stromal Stem Cells (BMSCs)are non-hematopoietic stromal cells capableof differentiating into and contributing to theregeneration of mesenchymal tissues, including bone, cartilage, muscle, ligament, tendon, adipose, and neuron and glial cells [1, 2]. They can also express Chondrogenic phenotypes [3] and are able to differentiate into neural elements in vitro [4].

MSCs are capable of expanding many-fold in culture and still keeping their growth and multilineage potentials. The previous studies have shown immunosuppressive properties for MSCs [5]. They are regarded as non-immunogenic agents; therefore, transplantation of MSCs into an allogeneic host may not require immunosuppression [6]. These properties make MSCs ideal candidates for tissue engineering as well as cellular and gene therapy. More-over, MSCs are shown to be able to migrate into damaged or diseased tissues when transplanted systemically [7, 8], including ischemic brain [9, 10], infarcted myocardium [11], and injured lung [12], where they have proven clinical value. These studies suggest that MSCs possess migratory capacity, yet the mechanisms underlying the migration of these cells have remained unknown. Chemokine receptors and their ligands together with adhesion molecules play an important role in tissue-specific homing of leukocytes [13]; they have also been implicated in trafficking of hematopoieticprecursors into and through tissues [14]. Chemokine presented on endothelial cells trigger integrin activation and arrest of those leukocytes carrying the

corresponding receptors [15]. The extravasation of a leukocyte is tightly controlled by the range of chemokine receptors and adhesion molecules expressed on the leukocyte cell surface, which are known as the cell's address code [16]. The literature review points the functional expression of various chemokine receptors on human MSCs [17-22]. The results of these studies are sometimes inconsistent and the full panel of chemokine receptors is overlooked in many of these studies. Furthermore, various adhesion molecules are known to be expressed on human MSCs; some of these may be functionally important in the adhesion of MSCs to the endothelium [23, 24]. In the present study, we demonstrated the functional presence of chemokine receptors on rat MSCs, and showed that their expression profile exhibited similarities to that of human MSCs.

Patients and Methods

Isolation and expansion of rat BMSCs

Female NMARI rats (Razi Institute, Tehran, Iran), weighing 200-250 g, were housed under standard conditions; the experimental procedures were approved by the Ethics Committee for Laboratory Animals at Shahid Beheshti University of Medical Sciences, Tehran. Bone Marrow was shortly re-moved from the long bones and cells plated out in cell isolation media (DMEM (SIGMA, UK) with 10% FBS) at 37°C, and 5% CO_2. Nonadherent cells were then removed after 24 hours. Next, cells were replated after 4 weeks at 100 cells per cm2 in complete expansion media with 10% FBS to expand BMSCs. Afterwards, cells were passaged and then replated at a density of approximately 2×10^3 cells per cm2 for further expansion.

Flowcytometric analysis

For the purpose of membrane receptor expression, rat BM-SCs were analyzed using a three-step labeling procedure. The cells were incubated at 4°C for 30 minutes with the relevant primary anti-mouse antibodies. After washing, the cells were incubated along with a biotinylated anti-rat Ig, anti-mouse Ig, anti-rabbit Ig or anti-goat Ig antibody, and then with Streptavidin-PE conjugate. Also, as a negative control, the cells were incubated with the same species isotype controls as the primary antibodies. For each analysis, a minimum of 10,000 events were recorded making use of a FAC Scan flow cytometer and then analyzed using Cell Quest software (BD Biosciences, UK). The antibodies used in the present study were as follows: anti-rat CCR2 (1 in 200), and anti-mouse CD105 (all from R & D Systems, UK), CD45 (all from BD Pharmingen, UK), anti-muse CD105 FITC (1 in 50), CD34 (1 in 100), CD45 (1 in 100) and anti-mouse CD34 (1 in 100) (Immunotools, Germany).

Differentiation assays

For osteogenic differentiation, rat MSCs were incubated in CEM using ascorbate-2-phosphate (88 ng/ml), dexametha-sone (10−8 M, Sigma-Aldrich, UK), and β-glycerophosphate (10 mM, Sigma-Aldrich) and for adipogenic differentiation, MSCs were incubated in CEM using ITS (Insulin, Transfer-rin, Selenium), Premix (Gibco, UK), dexamethasone (10−6 M), 3-isobutyl-1-methylxanthine (0.5 µM, Sigma-Aldrich), and indomethacin (100 µM, Sigma-Aldrich). After three weeks, cells were fixed and stained using Fast Red TR/naph-thol (Sigma-Aldrich) for alkaline phosphatase activity (osteoblastic differentiation), or using Oil Red O for adipogenic differentiation [25].

RT-PCR

To check the expression of Oct-4, glyceraldehyde 3-phos-phate dehydrogenase (GAPDH), and CCR2 genes, the BMSC at the end of the fourth passage and treated cells were evaluated by DFO. Making use of the RNX plus Kit (Fer-mentas Inc., Maryland, USA), 2 µg of total RNA was treatedfrom each sample with DNase I (Fermentas Inc., Maryland, USA). The extracted RNA was evaluated for the purity and integrity by optical density measurements and electrophore-sis on 1% agarose gel. The First Strand cDNA Synthesis Kit (Ferments Inc. Maryland, USA) was used to convert the ex-tracted RNA (1 µg) to cDNA. A total of 50 ng of cDNA was added to the PCR reaction for 35 cycles with denaturation at 95°C for 45 seconds, annealing at 58°C for 45 seconds, and elongation at 72°C for 30 seconds. After amplification was performed, the products were separated on 2% agarose gel and visualized making use of ethidium bromide under UV light. To ensure reproducibility, each experiment was repeat-ed for a minimum of 3 times [26].

Statistical analysis

Data were analyzed running 1-way analysis of variance (ANOVA), Turkey test, and Student's t test.

Results

After the fourth passage of the isolated BMSC from the rat bone marrow, the viability of the cells was 96.23 ± 1.18% (mean±SEM) (Figure 1). The cellular phenotype was char-acterized by immunocytochemistry for fibronectin, CD90, and CD106. Assays were performed on primary cells from the fourth passages. All BMSC cultures were observed to be CD34−, CD45−, and CD105+ and demonstrated the po-tentials of osteogenic and adipogenic differentiation. The BMSCs were previously shown to differentiate along the osteogenic, adipogenic, and chondrogenic pathways as well as having immunosuppressive properties similar to those of bone marrow-derived primary human BMSCs.

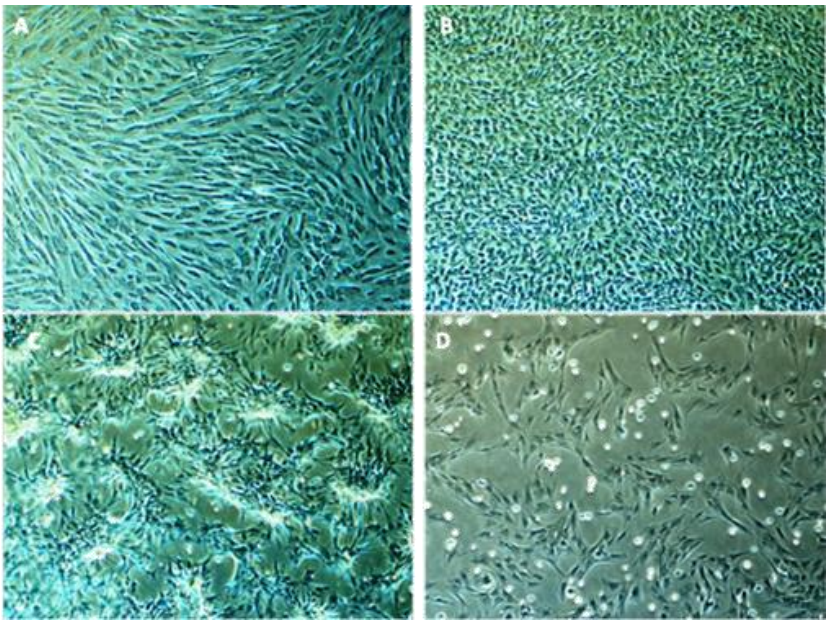

Figure 1. Morphology of BMSC and BMSC after treatment by defroxamine

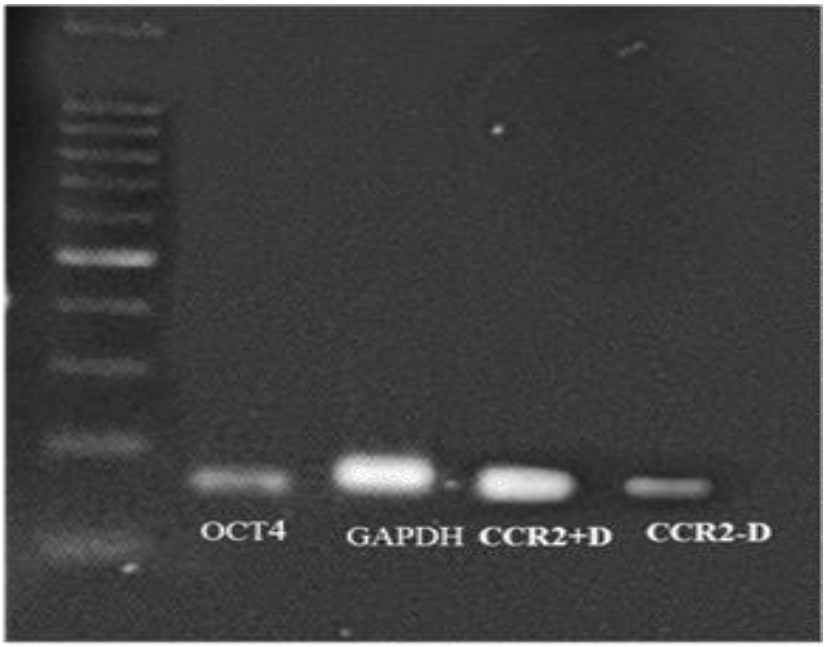

Figure 2. The expression of Oct-4, GAPDH, CCR2+DFO,CCR2-DFO from right to left.

Characterization of BMSCs using flowcytometry

The cell surface expression of chemokine receptors 2 (CCR2) was assessed in BMSC cultures at the fourth pas-sage and DFO treated using flowcytometry as described. The cell surface expression of CCR2 was also assessed in BMSC cultures using flow cytometry. A high percentage of BMSCs treated by DFO (89±14%) were observed to express CCR2 on the cell surface. In addition, a smaller proportion of both BMSC cell types was shown to express CCR2 (28±12% of primary cells).

RT-PCR

The results obtained from RT-PCR of GAPDH, Oct-4, and CCR2 revealed that CCR2 was expressed in induced cells, Oct-4 in BMSCs and BMSC-DFO, and GAPDH in All groups (Figure 2).

Discussion

An increasing number of recent studies have reported the functional expression of different chemokine receptors on human BMSCs [17-22]; nevertheless, the reported results have been contradictory. BMSCs show a huge increase in proportion to the cells expressing chemokine receptors on their surface when removed with EDTA alone rather than trypsin; this fact indicates the sensitivity of some or possibly all chemokine receptors to trypsin digestion.Ample expression of CCR2 on human BMSCs was found in the current investigation, which is in accordance with the findings reported in several other studies [17, 23], where chemotactic responses to CXCL12 were shown, too. However, other studies have reported little or no expression of this receptor on BMSCs [24, 25], which may have been due to different use of trypsin or other experimental conditions. We have also demonstrated functional expression of CCR2 on a large proportion of BMSCs and interestingly, expression of all these four receptors was demonstrated on a proportion of BMSCs. Other groups, too, demonstrated functional expression of one or more of these receptors on human MSCs [26-27].All these four receptors (CCR6, CCR9, CXCR3, and CXCR6) are shown to be involved in recruitment of immune cells to areas of inflammation. CCR6 is involved in mucosal humeral immunity and intestinal T cell homing [28], and it has recently been reported that Th17 cells expressing CCR6.

are preferentially recruited to inflamed joints via its ligand CCL20 in an animal model of rheumatoid arthritis [29]. In other words, both CXCR3 and CXCR6 have also been implicated in the recruitment of T cells to inflamed tissues in autoimmune arthritis [28], as well as other inflammatory conditions. CCR9 is known to be involved in homing of T cells and plasma cells to the intestine, playing a role in inflammatory diseases of the gut, such as Crohn's disease [30].

Considering the known functions of these receptors in relation to recruitment and homing of immune cells to in-flamed tissues, it is reasonable to hypothesize that these re-ceptors may also be involved in the recruitment and homing of rat and human BMSCs to inflamed tissues, either to re-generate tissue or contribute in immunosuppressive activitySome differences were apparent between the spectra of chemokine receptors expressed by human and murineMSCs. CCR3, CCR5, CXCR4, and CXCR5 were present abundantly on human cells while only low levels of these receptors occur on murine cells.

Conclusion:

The present study reported that rat BMSCs demonstrate selective expression of functional chemokine receptors. Thus, these BMSCs would be a useful model to further study the role of particular chemokine receptors in in vivo models of disease and injury, for example in cell therapy.

Conflict of Interest:

The authors declared no Conflict of Interests.

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