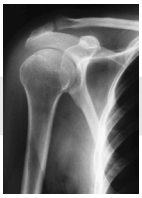

Mesenchymal Stromal Cells: A Novel Treatment Option for Steroid-Induced Avascular Osteonecrosis

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Abstract

Mesenchymal stromal cells are multipotent cells capable of tissue repair and immune modulation. They are primarily found in bone marrow, but are also present in other tissues of mesenchymal origin, such as fatty tissue, muscle, tendons, etc. MSC can easily be obtained by bone marrow aspiration, showing a rapid expansion *in vitro*. New protocols enable cell culture without the use of animal-derived sera and artificial growth factors. Avascular necroses of the bone may have different causes. AVN in autoimmune and hematological diseases show a strong association with corticosteroid treatment, which is often unavoidable in severe cases. Until recently, core decompression of the affected osseous area was the standard approach. Because of their differentiation properties, easy accessibility and proliferative capacity, autologous MSCs could potentially complement AVN treatment by adding fresh "osteogenic cells" to the healing process.

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Mesenchymal stromal cells were long thought to be only the "framework" tissue for hematopoietic stem cells in the bone marrow. However, in 1968 Friedenstein et al. [1] showed that bone marrow cells are also capable of differentiating into bone tissue *in vitro*. The differentiation potential of these cells was further investigated, giving the groundwork for a better characterization of the MSC population [2,3]. Only recently, the minimal criteria for defining MSC as such were elaborated by the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy [4]. MSC application in the treatment of osteogenesis imperfecta [5] and steroid-resistant graft versus host disease [6] may soon gain recognition.

Properties of MSC

The last few years were characterized by a rapid propagation of knowledge in the field of MSC research, including features like immune phenotype, homing, immune modulation, tissue repair, etc. To define MSC as such, certain characteristics have to be present: a) they should be plastic-adherent in standard culture conditions; b) immune phenotype must be expressed, as shown in Table 1; and c) an obvious *in vitro* differentiation potential into osteoblasts, adipocytes and chondroblasts is required [4,7].

On the other hand, MSC seem to be immune privileged as non-immunogenic cells This quality could be of benefit when considering the use of allogeneic MSC for therapeutic purposes [8]. MSC have further immune-modulatory properties and are able to alleviate stimulation and clonal expansion of T cells *in vitro* and *in vivo* by cell contact and secretory molecule mechanisms [9]. Steroid-resistant graft versus host disease is an example of their use in clinical practice [6].

MSC are multipotent and have osteogenic potential

The homing mechanisms of MSC are poorly understood. Based on chemokine/chemokine-receptor interactions and adhesion molecules, MSC are potentially capable of finding the site of injury and, given intravenously, of restoring damaged tissue on site due to their plasticity and/or paracrine properties [10]. However, the efficiency of this process is very variable and depends on

Table 1. Distinct immune phenotype of mesenchymal stromal cells

Cluster of differentiation	73	90	105	14/11b	19	34	45	79α	HLA-DR
Positive	+	+	+						
Negative				–	–	–	–	–	–

MSC = mesenchymal stromal cells
AVN = avascular necrosis

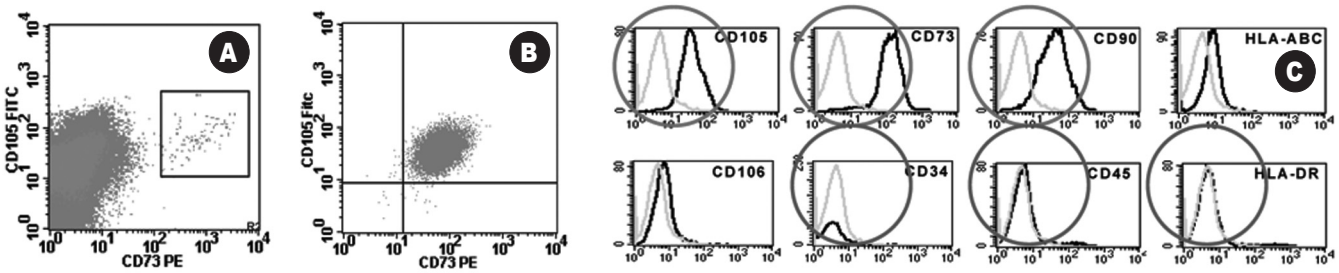

Figure 1. Fluorescence-activated cell sorter analysis of bone marrow. Only a small percentage (typically 0.01–0.1%) conforms phenotypically to MSC. **[A]** Bone marrow cells double-stained for CD73 and CD105. **[B]** MSC after 3 weeks of culture in animal protein-free conditions. **[C]** Immune phenotype of culture-expanded MSC (circles in top panel = positive CD expression; circles in bottom panel = no CD expression).

the diseased tissue. This problem in clinical application can be overcome by local use in certain settings, in particular for unifocal lesions, such as osteonecrosis. MSC are able to form new osseous tissue not only *in vitro* but also *in vivo*, thus giving hope for restoration of damaged bone [1-3,11,12].

Steroid-induced avascular necrosis

Most reports on AVN in the literature refer either to idiopathic conditions or chemotherapy regimens for hematological/oncological diseases. The most commonly affected sites are the femoral head and the knee. In those cases steroids seem to play a major role in disease onset and progression. The particular mechanism of bone necrosis induction is not known. Decrease in local blood flow and probably endothelial cell dysfunction, as well as deteriorated local blood coagulation and thrombus formation could play a role in bone destruction [13,14]. All the latter conditions frequently accompany systemic lupus erythematosus, the most common autoimmune disease associated with AVN when treated with steroids, and could also follow high dose steroid therapeutic regimens for hematological/oncological conditions [15-17].

MSC are easy accessible and animal serum free expandible

MSCs and core decompression to treat AVN

The standard therapy for AVN is the core decompression technique [18]. The procedure shows a satisfactory effect in early-stage AVN. Unfortunately, in many cases the condition is recognized only at a more advanced stage, when little or no effect of core decompression on bone reconstitution can be expected, necessitating surgical intervention with total hip replacement [17].

The osteogenic potential of bone marrow-derived MSC was shown to be sufficient in bone repair in animal models and in humans [11,12,19]. Because of their comparatively small number in bone marrow aspirates [Figure 1], *in vitro* expansion before application during core decompression would have a favourable effect on the final outcome, facilitating reconstitution of bone integrity by providing more "new material" for bone formation. Novel protocols enable the process without usage of animal-derived sera and growth factors, thus making MSC application more secure [20].

Combination therapy with core decompression and MSC may improve AVN outcome

Conclusions

AVN could arise after treatment with steroids in autoimmune and hematological/oncological conditions. Core decompression is a promising procedure in recently acquired AVN, but has little or no effect on progressed stages. New therapeutic strategies are needed to treat more advanced cases.

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