POST-TRANSPLANTATION DISTRIBUTION of CD44+ HUMAN MESENCHYMAL STEM CELLS IN a MOUSE model

Ilea Ioana Cristina[1], Pall Emoke[1], Ciupe Simona[1], Cenariu M[1]., Roxana Roman[2], I.S.Groza[1]

[1]University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Faculty of Veterinary Medicine, Cluj-Napoca, Romania, criss_vet@yahoo.com
[2]University of Medicine and Pharmacy Iuliu Hatieganu, Cluj-Napoca

Abstract

Mesenchymal stem cells (MSCs) are playing an important role in tissue engineering. Because of their properties to differentiate in multiple lineages, these cells became promising materials for the treatment of different types of degenerative disease, including bone disorders. In order to evaluate the distribution of xenogeneic MSCs engraftment, the aim of our study was the screening of human CD44+ MSCs distribution after intraperitoneal transplantation in a mouse model for osteoporosis. Human MSCs were harvested from the palatal subepithelial connective tissue. The cells were grown in DMEM/F12 (Sigma Aldrich) supplemented with 10% fetal calf serum (FCS), 100 U/ml penicillin and 100 mg/ml streptomycin. After i.p. transplantation of 1,1x10^6 CD44+ hMSCs in a mouse model, the screening of donor cells engraftment from blood samples was assessed at 4 and 11 days post-transplantation. The mice were euthanized by cervical dislocation at 14 days, followed by human MSCs engraftment assessment in blood, bone marrow and spleen samples. Results were quantified by immunophenotypic characterization with FACS Canto II flow cytometry system (BD Biosciences, San Jose, CA, USA). Our data confirmed the special homing characteristic of human MSCs in a mouse xenograft model. At 4 days post injection, in blood samples was found a percentage of 0,5% CD44+ cells and at 11 days, a percentage of 0,1% of CD44+ cells. At 14 days, a percentage of 0,1 % CD44+ human MSCs was found in blood as well as in bone marrow, but all spleen samples were negative.

Key words: *human MSCs, mouse model, osteoporosis, engraftment.*

INTRODUCTION

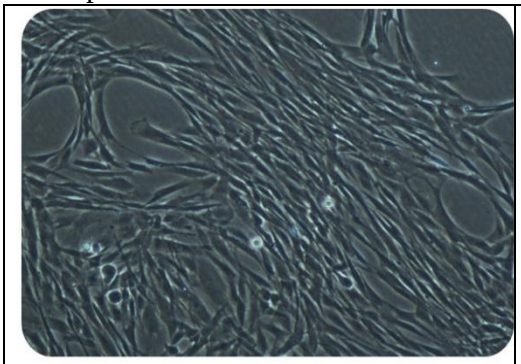
The National Institute of Health resources is defining the stem cells as ‘‘an undifferentiated cell, found among differentiated cells in a tissue or organ that can renew itself and can differentiate to yield some or all of the major specialized cell types of the tissue or organ’’(Dominici et al., 2006, Groza et al., 2009, Diptiman et al., 2010). During the past decades, the multipotent potential property of MSCs was showed in a large number of studies were the culture expended MSCs were differentiated into phenotypic different

lineages, including osteocytes (Bruder et al., 1998), cartilage (Johnstone et al., 1998), adipocytes, muscles (Pittenger et al., 1999) and tendon (Young et al., 1998). Currently, MSCs are most frequently isolated from the bone marrow (Moustapha et al., 2004), but recent studies demonstrated that the mesenchymal stem cells derived from the human palatal subepithelial connective tissue (PDLSCs) and placenta have the same properties as the bone marrow derived MSCs (Pall et al., 2009, Hidefumi et al., 2011).
Because of their unique properties, MSCs are playing an important role in tissue engineering. These cells became promising materials for the treatment of different types of degenerative diseases, including bone disorders due to their capacity to differentiate into osteoblasts (Bruder et al., 1994). It is known that the increased life expectancy leaded to an increased prevalence of post-menopausal osteoporosis and related fractures (Cho et al., 2009). This progressive skeletal disorder, characterized by the reduction of the bone mass as a result of diminished osteogenesis, presents a slow response to therapeutic agents, reason why it is necessary to develop alternative treatments (Kim et al., 2006).
The therapeutic efficacy of transplanted MSCs depends on their mobilization from the place of injection and trafficking through the circulation to the injured tissue. The mechanism of homing after systemic transplantation requires an understanding of the distribution dynamics of injected MSCs (Diptiman et al., 2010).
In order to evaluate the distribution of xenogeneic MSCs engraftment, the aim of our study was the screening of CD44+ human MSCs (hMSCs) distribution after intraperitoneal (i.p.) injection in a mouse model for osteoporosis.

MATERIALS AND METHODS

Human MSCs were harvested from the palatal subepithelial connective tissue with informed consent approved according to the procedures of the institutional review board. The cells were cultured-expanded in DMEM/F12 (Sigma Aldrich) supplemented with 10% fetal calf serum (FCS), 100 U/ml penicillin and 100 mg/ml streptomycin. The multipotency of cultured CD44+ hMSCs was confirmed by immunophenothypic evaluation performed with the BD FACS Canto II flow cytometer.
The enzymatic treatment with 0.25% trypsin-EDTA solution (Sigma-Aldrich) was followed by cell count with a hemocytometer. The hMSCs were centrifuged and suspended at a concentration of $1,1x10^6$ cells/ 0,1ml

PBS. The obtained cells suspension was injected in the intraperioneal cavity (i.p.) in a total volume of 0.1 ml in a mouse model with induced osteoporosis.



A

B

Figure 1. A – Stabile line of hMSCs CD44+
B – Intraperitoneal transplantation of hMSCs

The mechanism of homing after transplantation was evaluated by screening of donor cells engraftment level in blood samples at the 4th and the 11th day post injection. The blood was collected from the tail vein on heparin coated MiniCollect tubes (Greiner). The serum was recovered by centrifugation at 2500 rpm for 10 minutes at room temperature. Samples were stored at -80°C until analysis.

At the 14th day post injection, the mice were euthanized by cervical dislocation and organs, including spleen and long bones, were harvested together with blood samples. The bone marrow was isolated from the femur by removing epiphysis and flushing the shaft with 1 ml PBS after insertion of a 27 gauge needle. The spleen was minced into 2-4 mm pieces using a scalpel blade. An appropriate amount of trypsin EDTA solution was added and the obtained suspension was incubated at 37°C for 30 minutes. The cells were dispersed by gentle pipeting and subsequently were filtered using a cell strainer to eliminate the debris. The cell suspension was centrifuged at 1500 rpm for 4-5 minutes, at 4°C and the supernatant was discarded.

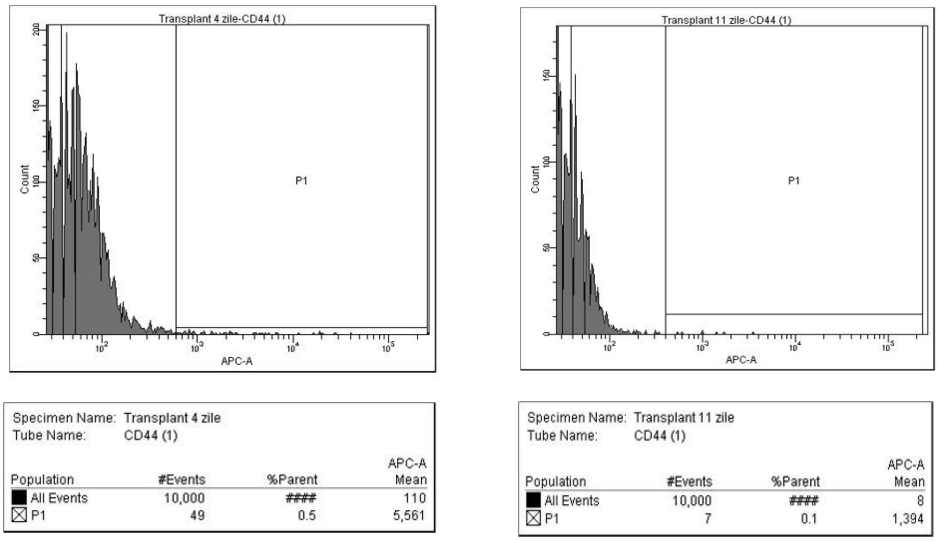
From all cell sources, a final concentration of 2x10^{7} /ml cells was used for immunophenotypic characterization. Anti CD44 antibody (BD Biosciences, San Jose, CA, USA) was used and the quantification was performed with the BD FACS Canto II flow cytometry system.

RESULTS AND DISCUSSIONS

CD44 is a cell surface glycoprotein, being involved in epithelial cell adhesion, reason why is considered to be involved tissue remodeling and stimulation of cell proliferation and migration. Recent studies demonstrated the importance of CD44 expression on MSCs, not only because is a robust marker of pluripotency, but also because plays important functions in cell-matrix interaction, mechanism of homing and apoptosis resistance (Sally et al., 2012; Zoller, 2011).

The percentages of the hMSCs cells in the blood were clinically screened on day 4, 11 and 14 after the i.p. injection, end the engraftment in bone marrow and spleen was evaluated at the 14th day of the study.

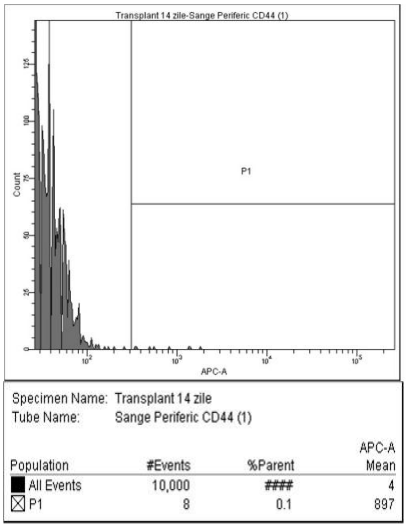
As it is showed in figure 2.A, the circulating number of CD44+ hMSCs peaked at the 4th day post-injection, in the blood samples being found a percentage of 0.5%. The percentage of donor-derived cells had decreased at the 11th day after the treatment, a level of 0.1% CD44+ hMSCs being detected (figure 2.B).



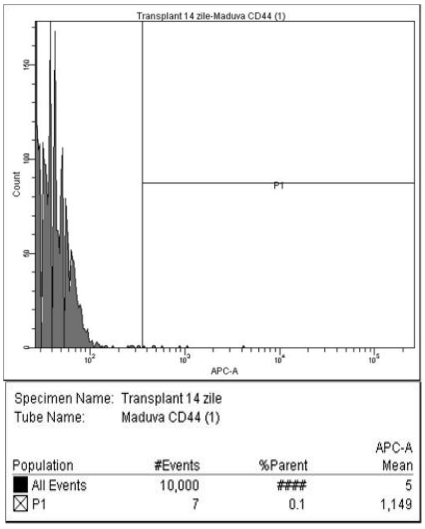
A B

Figure 2. A – CD44+ hMSCs kinetics at 4th day post injection
B - CD44+ hMSCs kinetics at 11th day post injection

When analysing the blood and organ samples on the 14th day, 0.1 % CD44+ hMSCs were found in blood (Figure 3.A), indicating that the maintained level is similar to day 11. The most important finding was the presence of 0.1% hMSCs in bone marrow. (Figure 3.B). All spleen samples were negative (Figure 3.C).



A



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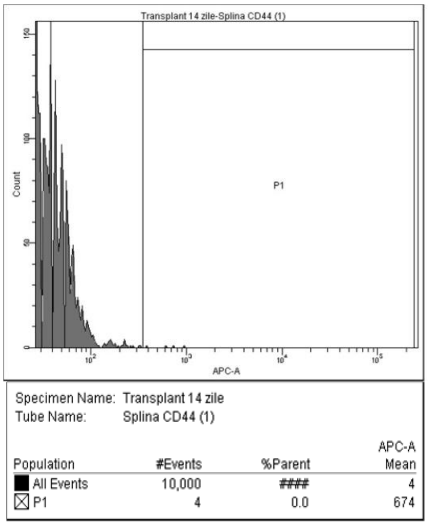

C

Figure 3. A – CD44+ hMSCs kinetics at 14th day in blood
B - CD44+ hMSCs engraftment at 14th day in bone marrow
C - CD44+ hMSCs engraftment at 14th day in spleen

Our data confirmed the special homing characteristic of human MSCs in a mouse xenograft model. The representative results of the FACS profiles which confirm the kinetic data are present in table 1.

Table 1. FACS profile of hMSCs

Screening day	Percentage of hMSCs (%)		
	Blood	Spleen	Bone Marrow
Day 4	0,5%	-	-
Day 11	0,1%	-	-
Day 14	0,1%	0%	0,1%

This data indicate that intraperitoneal injection is an optimal method to realize the bone marrow engraftment of donor derived MSCs after i.p. injection. Compared with other studies, in which the peak of circulating retroviral transducted MSCs was found at 7 days post-injection (Kim et al., 2006), we had the highest percentage at 4 days, which indicate the early engraftment of hMSCs cells after *in vivo* transplantation in an osteoporotic mouse model. There are many reports which suggest that injected MSCs have the potential to differentiate into multiple tissues after transplantation in different animal models (Prockop, 1997; Pereira et al., 1998; Azizi et al., 1998), also being reported that MSCs do not arrive in bone marrow after systemic transplantation (Kim et al., 2006; Murry et al., 2004, Balsam et al., 2004,). Contrary to this findings, in our experiment a percentage of 0.1% donor-derived MSCs were found, which suggest hMSC engraftment in the bone marrow after systemic transplantation via intraperitoneal cavity. The most important aspect of this finding consist in bone marrow engraftment of transplanted MSCs, that have the property to initiate the proliferation of donor hematopoietic stem cells along side with donor derived stromal cells (Kushida et al., 2001).

CONCLUSIONS

Our data confirmed the special homing characteristic of human MSCs in a mouse model with induced osteoporosis, the injection via intraperitoneal cavity being a facile and non-invasive method for engraftment of donor derived cells.

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