

ТЕРАПИЯТА С МЕЗЕНХИМНИ СТВОЛОВИ КЛЕТКИ – НОВИ ВЪЗМОЖНОСТИ И ПРЕДИЗВИКАТЕЛСТВА. ПРОСЛЕДЯВАНЕ НА КЛИНИЧЕН СЛУЧАЙ

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THE MESENCHYMAL STEM CELLS THERAPY – NEW CHALLENGES AND OPPORTUNITIES. CLINICAL CASE REPORT

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ABSTRACT

The treatment with mesenchymal stem cells has proved to be effective in the field of regenerative medicine, especially regarding osteodegenerative diseases. Clinical efficiency of that treatment takes place due to the ability of adipose tissue derived mesenchymal stem cells (MSC) to differentiate into chondroblasts and to modulate immune system via interactions with certain cell populations. The emerging new data give rise to other possible implementation of MSC, such as treatment of spinal chord injuries and chronic renal failure. In this article we try to summarize the recent data in the fast developing field of MSC treatment.

We also present a clinical case report of autologous mesenchymal stem cells transplantation in a 13 years old dog with osteoarthritis.

Key words: mesenchymal stem cells, CRF, osteoarthritis

I. Morphology and characterization of Mesenchymal Stem Cells

Mesenchymal stem cells (MSC) are multipotent stromal cells that can differentiate into a variety of cell types, [6] including: osteoblasts, [3] chondrocytes, [4] and adipocytes. There are evidences that MSC can be differentiated into oligodendrocyte [9] and dopaminergic neurons [18], with further clinical applications.

Because the cells, called MSCs by many labs today, can encompass multipotent cells derived from other non-marrow tissues, such as umbilical cord blood, adipose tissue, adult muscle, corneal stroma [2] or the dental pulp of deciduous baby teeth, yet do not have the capacity to reconstitute an entire organ, the term Multipotent Stromal Cell has been proposed as a better replacement.

The International Society for Cellular Therapy has provided the following minimum criteria for defining multipotent human mesenchymal stromal cells [8]:

- 1) plastic-adherent under standard culture conditions;
- 2) positive for expression of CD105, CD73, and CD90, and absent for expression of hematopoietic cell surface markers CD34, CD45, CD11a, CD19, and HLA-DR;
- 3) under specific stimulus, cells should differentiate into osteocytes, adipocytes, and chondrocytes in vitro.

II. Possible sources, isolation and differentiation capacity of Mesenchymal Stem Cells

Although MSCs are adult stem cells that were initially isolated from bone marrow, subsequent research has shown that other adult tissues also contain MSCs. Most prospective sources of MSC are listed below:

1. **Adipose-Derived Stem Cells (AD-SCs)** ASCs were first isolated by Zuk et al. [22]. ASCs can differentiate into ectodermal and endodermal lineages, as well as the mesodermal

lineage [23]. ASCs can be obtained from either liposuction aspirates or excised fat. Small amounts of adipose tissue can be obtained under local anesthesia. One gram of adipose tissue yields approximately 5,000 stem cells, whereas the yield from BM-derived MSCs is 100 to 1,000 cells/mL of marrow [17].

2. **Periodontal Ligament-Derived Stem Cells (PDL-SCs)** The periodontal ligament, which connects the alveolar bone to the root cementum and suspends the tooth in its alveolus, contains stem cells with the potential to form periodontal structures such as cementum and ligament [39]. Similar to other MSCs, PDL-SCs show osteogenic, adipogenic, and chondrogenic characteristics under defined culture conditions in vitro [7,10]
3. **Muscle-Derived Stem Cells and Satellite Cells (M-MSCs)** - Postnatal skeletal muscle tissue, similar to bone marrow, contains two different types of stem cells, M-MSCs and satellite cells, both of which can function as muscle precursors [16] M-MSCs not only act as muscle precursors but also give rise to a variety of other cell types, including hematopoietic cells [16]. M-MSCs have a high proliferation and self-renewal capacity and are CD34+, Sca1+, CD45-, and c-Kit- [15].
4. **Wharton's Jelly Stem Cells (WJ-MSCs)** - WJ-MSCs are obtained from Wharton's jelly of umbilical cord. Compared to BM-MSCs, WJ-MSCs exhibit a higher expression of undifferentiated human embryonic stem cell (hESC) markers like NANOG, DNMT3B, and GABRB3 [12]; thus, they are more primitive than other types of MSCs and easy to obtain with no ethical considerations. UC-MSCs can be induced into endothelial cells, adipogenic, osteogenic, chondrogenic, neurogenic lineages [5], insulin producing cells [19], and hepatocyte-like cells [21].
5. **Miscellaneous Stem Cells** - In addition to the MSCs discussed in this paper, stem cells have been isolated from liver, perichondrium, periosteum, trabecular bones, pancreas, hair follicles, intestinal epithelium, placenta, and amniotic membranes.

Our attention will remain focused on adipose derived stem cells (ASCs), because of the relatively large amount of stem cells that might be obtained with simple procedure under short-time general or local anesthesia. Isolation protocols for ASCs commonly include homogenization of the sample, enzymatic digestion, collection of adherent cells and subsequent cultivation in DMEM (Dulbecco modified Eagle's medium) supplemented with FBS [11].

MSCs could differentiate into multiple types of tissues including tissues with mesodermal, endodermal and ectodermal origin, their extraction does not raise any ethical issues and they are immunologically compatible in terms of an autologous transplantation.

III. Clinical implementation of Mesenchymal Stem Cells

Multiple clinical implementation of MSC, connected with specific sources and routes of differentiation are thought to be prospective, but we will focus on adipose tissue derived mesenchymal stem cells (AD-MSCs), due to the ease of collection procedure and cultivation protocols. The main possibilities for clinical use of AD-MSCs are listed below:

1. Treatment of osteoarthritis – along with the ability of chondrogenic commitment and differentiation, AD-MSCs can alter inflammation by suppressing T-cell proliferation and secretion of pro-inflammatory cytokine INF γ in a dose-dependent manner. Autologous AD-MSC therapy in veterinary regenerative medicine is commercially available since 2003. Previously reported results from a blinded, controlled trial in dogs with chronic osteoarthritis of the coxofemoral joint demonstrated efficacy of a single intra-articular injection of autologous AD-MSCs [1].
2. Treatment of spinal cord injuries – prospective studies of treatment compression traumas of the spine, using AD-MSCs [13] show, that the introduction of this method in the clinic practice is only a matter of time.

3. Treatment of feline chronic renal disease – although pilot studies are not so promising [14], the ability of AD-MSC to reduce creatinin levels in CKD stage III patients, gives rise to a hope, that this lethal condition will be treated successfully by the means of cellular therapy.

IV. Treatment of severe osteoarthritis in twelve years old German shepherd dog via cellular-substitution therapy with Mesenchymal Stem Cells – case report

Duc, 12 years old German Shepard was presented at the clinic with progressive lameness of the pelvic limbs, which deteriorates in cold days, exercise intolerance and signs of severe pain. He was treated for over a year with NSAID (including selective COX-2 inhibitors), combined with food supplements. The treatment didn't succeed to alleviate symptoms – in fact, when presented at clinic the patient barely could move his legs and have impaired urinary function.

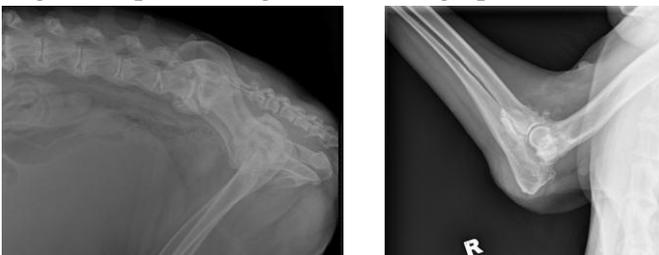
Figure 1. Hip joints radiographic examination



After confirming the diagnosis with a radiographic examination (fig.1), and taking into account the age of the animal, we decided to attempt cellular-based treatment using adipose derived MSCs, rather than total hip replacement.

Biopsy of approximately 20 grams subcutaneous fat was taken and MSCs (CD44+, CD90+, CD105+) were derived, following standard protocol. After two passages they reached target concentration of $3 \cdot 10^6$ cells/ml and were applied intra-articular in both coxofemoral joints. Pain alleviation with sub second improvement of the gait was observed 6 days after treatment, and 4 weeks after application of the MSC the dog tolerates long walks (over 40 minutes) and doesn't show any signs of pain.

Figure 2. Spine and right elbow radiographic examination



Six months after the procedure, Duc visited us again this time with progressive osteoarthritic changes in both elbows and cauda equina syndrome (fig. 2). Another course of cell-based treatment was suggested – via intravenous infusion of stem cells, but the owner preferred topical treatment with hyaluronic acid. It was applied in both elbow

joints, but after mild improvement Duc condition deteriorates (lameness with thoracic limbs, urinary incontinence, severe pain and loss of appetite) and after another 3 months he was euthanized.

It is remarkable that he never experienced pain in hip joints after MSC treatment. That fact and the fast recovery after first intervention were highly appreciated by the owner, but the relatively high cost of the cellular therapy, prevented him for taking the decision for another procedure.

V. Discussion

In the light of aforementioned we have to conclude, that regenerative medicine is one plausible solution in a number of cases, where classic therapy offers only palliative treatment. Treatment of spine injuries, growing self-renewal tissues and in vitro organogenesis are not so far away from broad clinical implementation. With their high proliferation activity, ability to give growth of multiple cell types and accessibility, mesenchymal stem cells are powerful source of growing number of cell-mediated therapies. After successful differentiation to osteoblasts,

chondroblasts and dopaminergic neurons, treatment of insulin deficiency via modified MSCs will be the next big challenge for regenerative medicine [20].

The aim of the regenerative medicine is to provide noninvasive, fast acting treatment with long-term results. This applies especially to the severe hereditary or metabolite conditions, geriatric diseases and severe traumas, where only palliative care is available nowadays.

In the search of new clinic opportunities we have to remember, that only the profound understanding of cellular interactions, will provide us with solid ground for making a next step – in vivo activation, targeting and differentiation of the stem cells, without removing them from the organism.

VI. References

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