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Intraarticular and intravenous administration of ^{99M}Tc-HMPAO-labeled human mesenchymal stem cells (^{99M}*TC-AH-MSC*$_S$): In vivo imaging and biodistribution

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ABSTRACT

Introduction: Therapeutic application of intravenous administered (IV) human bone marrow-derived mesenchymal stem cells (*ah*MSCs) appears to have as main drawback the massive retention of cells in the lung parenchyma, questioning the suitability of this via of administration. Intraarticular administration (IAR) could be considered as an alternative route for therapy in degenerative and traumatic joint lesions. Our work is outlined as a comparative study of biodistribution of ^{99m}Tc-*ah*MSCs after IV and IAR administration, via scintigraphic study in an animal model.
Methods: Isolated primary culture of adult human mesenchymal stem cells was labeled with ^{99m}Tc-HMPAO for scintigraphic study of in vivo distribution after intravenous and intra-articular (knee) administration in rabbits.
Results: IV administration of radiolabeled ahMSCs showed the bulk of radioactivity in the lung parenchyma while IAR images showed activity mainly in the injected cavity and complete absence of uptake in pulmonary bed.
Conclusions: Our study shows that IAR administration overcomes the limitations of IV injection, in particular, those related to cells destruction in the lung parenchyma. After IAR administration, cells remain within the joint cavity, as expected given its size and adhesion properties.
Advances in knowledge: Intra-articular administration of adult human mesenchymal stem cells could be a suitable route for therapeutic effect in joint lesions.
Implications for patient care: Local administration of adult human mesenchymal stem cells could improve their therapeutic effects, minimizing side effects in patients.

1. Introduction

Cell therapy is considered an emerging therapeutic option for various diseases in addition to conventional therapeutic modalities. MSCs have been deeply studied since 70s, when Friednestein et al. [1] first isolated them, and during the last decade, they have attracted much attention because of their capacity for differentiation, migration and proliferation in vivo [2], making them important tools for treatment of immune disorders and tissue repair, with possibilities in both, cell and gene therapy [3–9].

The application of adult human mesenchymal stem cells from bone marrow (*ah*MSCs) is considered a promising treatment for musculoeskeletal injuries, however, their trafficking and homing are still controversial issues with further studies needed [5,10].

As MSCs are a heterogenic population depending on cell source [7], their safety and efficacy must be ensured and studies of dose and biodistribution must be addressed in normal and disease animal models [11]. Furthermore, it is necessary to demonstrate that MSCs do not have unwanted homing that could drive to unsuitable differentiation in some organs or induced cancer development as suggested in some experiments [3]. For this purpose, current noninvasive approaches are utilized for in vivo tracking of transplanted MSCs including direct, nonspecific labeling of cells with magnetic particles, organic fluorescent dyes, encoded fluorescent proteins, quantum dots, optical imaging or magnetic resonance imaging [8,12]. Nuclear medicine imaging is an attractive technique for non-invasive tracking of stem cells in vivo after labeling with radiopharmaceuticals such as ^{99m}Tc- exametazime

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(^{99m}Tc-HMPAO), In-111 oxine/tropolone or F-18-fludeoxyglucose (F-18-FDG) as they offer high sensitivity [8,13,14]. ^{99m}Tc-HMPAO and In-111 oxine/tropolone have been widely used to localize infection in clinical setting through the labeling of leukocytes. ^{99m}Tc-HMPAO is nowadays preferred, despite In-111 having a longer half-life suited for long term tracking, because of other advantages such as better energy for imaging, availability and easiness of handling [4,15,16]. Recent labeling with F-18-FDG has been proposed, even though it has drawbacks as short half-life and radiation burden [16,17].

The therapeutic interest of *ah*MSCs from different sources (synovium or infrapatellar pad) in osteoarticular injuries is based on the hypothesis of their migration potential and capacity to be mobilized to sites of injury [3,5,18]. However, exogenous infused cells are localized differently depending on the model; they are mainly home to the lung in naïve animals whereas they are localized to the injured site in animal models of injuries [5]. So one of the key questions on MSCs biology is whether these cells can migrate toward sites of injury and inflammation. There is some evidence that infused MSCs home to such sites, while other studies indicated that they act from the distance [11,19]. It is not clear how to identify the signals guiding the MSCs to sites of injury, some authors suggest fucosylation of cell surface molecules for homing to the bone marrow [20], other works talk of trophic factors by MSCs [6], and a group of authors link growth factor signals, chemokines and other chemotactic molecules such as SDF-1 with the homing of transplanted bone marrow-derived MSCs [9,21–25].

Other point of controversy is the detection of MSCs in the human vascular system. Some studies try to demonstrate that MSCs can circulate at least under stress conditions, but do not evidence that this occurs under physiological conditions. In bone fracture, MSC may be released into the bloodstream by mechanical disruption of the bone marrow rather than by active migration and recent studies suggest the lymph system as a route for migration [19]. On the contrary, discoveries from Eggenhofer et al. [26] suggest that MSC does not survive long term in the recipient animals and it is likely that previous studies describing MSCs in other tissues, detected MSCs-label (e.g., radioactivity, fluorescence) from MSCs debris or from phagocytosed MSCs rather than living MSCs.

While the safety of IV infusion has been confirmed in a large number of studies, the entrapment of MSCs in the lungs, explained by their relatively large size and expression of adhesion molecules in the cell membranes [5,15,27,28] appears to be a handicap, related to the small amount, or even the absence, of recirculated infused cells, with consequences in the quality of the tracking imaging study. Besides, the number of infused cells in small animal models is far from therapeutic doses due to the necessity of avoiding lung embolization [3]. Given these drawbacks surrounding the IV administration, for certain clinical applications the intra-articular injection of MSCs may be the key to combine high levels of safety and targeting to specific joints [5,10].

Our work is focused on tracking in vivo biodistribution of *ah*MSCs labeled with ^{99m}Tc-HMPAO after both intra-articular and intravenous administration to check whether the IAR pathway could be more effective for treatment of joint injuries with MSCs.

2. Materials & methods

2.1. Isolation and primary culture of adult human mesenchymal stem cells

Undifferentiated multipotent *ah*MSCs were isolated from the heparinized bone marrow collected from of three healthy male human volunteers (50 ml/patient), undergoing elective surgical procedures, by percutaneous direct aspiration from iliac crest. All the procedures were approved by the Institutional Ethical and Clinical Trials Committee (V. Arrixaca University Hospital of Murcia). Informed consent was obtained from all the volunteers. For the isolation of *ah*MSCs, a mononuclear cell fraction was obtained by ficoll density gradient-based separation and the cell washing closed automated SEPAX™ System (Biosafe, Eysines, Switzerland). Cells number and viability was estimated with the vital dye Trypan blue and the Neubauer chamber.

After seeding the mononuclear cells at a density of 160.000 cells cm^{-2} in basal culture growth medium (GM), they were cultured under standard conditions at 37 °C, 5% CO_2 and 95% of relative humidity atmosphere. At 24 h GM was replaced. The GM consisting of α-Minimum Essential Medium (α-MEM) (Sigma-Aldrich, St Louis, MO, USA) containing 10% fetal bovine serum (FBS) (Sigma-Aldrich) and penicillin G/streptomycin sulfate(100 U ml^{-1} and 100 μg ml^{-1}, respectively; Sigma-Aldrich). During the culture period, the cell morphologies were detected daily using phase-contrast inverted microscopy. The adherent *ah*MSCs were initially cultured up to 80% confluence (approximately, two weeks) in T-75 flask (Becton, Dickinson Co, NJ) replacing the medium every 3 or 4 days.

2.1.1. Subcultures of ahMSCs

After 7–10 days, when the cell culture reached 85–90% confluence in flask, cells were detached using 0.25% *w/v* trypsin–EDTA digestion for 5 min, washed and seeded at 5.0×10^3 cells cm^{-2}. A were expanded into T-175 cm^2 flasks and subcultured twice (P3) until we achieved a sufficient cell number to run our study. Culture medium was changed every 3 days.

2.1.2. ahMSCs characterization prior to implantation

Prior to seeding *ah*MSCs on the discs they were characterized according to minimal defining criteria established by the International Society for Cellular Therapy and implanted/adopted by our center [2]. So, in order to confirm the phenotype, cells were tested for the positive expression of surface markers $CD73^+$, $CD90^+$ and $CD105^+$, and also for the absence of expression of hematopoietic lineage markers, e.g. $CD14^-$, $CD20^-$ $CD34^-$ and $CD45^-$, using specific isotype monoclonal antibodies (all acquired from BD Biosciences. San Diego, CA). Non-specific fluorescence was measured using specific isotype monoclonal antibodies. Samples were acquired in a Beckman Coulter Navios flow cytometer (Fullerton, CA) and analyzed with the FCS Express V3 software. Afterwards, cells were identified as multipotent MSCs (data not shown).

2.2. Radiolabeling adult human mesenchymal stem cells ahMSCs

A cell suspension of *ah*MSCs at a density of $10–40 \times 10^6$ in 1 ml of PBS was incubated with 88–177 MBq of ^{99m}Tc-HMPAO, prepared under manufacturer's instruction (Ceretec, GE Bio-Sciences, Madrid, Spain) [29] in 1 ml of saline, at room temperature under shaking for 15 min, in order to obtain a radiolabelled cell suspension of 4.4–8.8 Bq $cell^{-1}$ (lower than 9.3 Bq $cell^{-1}$) [4,30]. After incubation, suspension was centrifuged at 200 g for 10 min, the supernatant was removed and the pellet suspended in 0.5 ml of saline, ready for administration.

Radiochemical purity (RCP) was assessed by thin layer chromatography as described by manufacturer [29]. Labeling efficiency was calculated upon the formula cell radioactivity in pellet/ (radioactivity in pellet + radioactivity in supernatant)*100. In vitro cell viability was estimated with Trypan blue exclusion test after labeling [31].

Cell efflux of ^{99m}Tc was measured by preparing three aliquots of labeled cells and incubating them at 37 °C during 1 h. Then, aliquots were centrifuged at 250 g for 10 min and the radioactivity in the pellet and supernatant is counted separately. Results are shown as percentage (%).

2.3. Intravenous and intra-articular administration procedures in animals.

Mature New Zealand male rabbits ($n = 6$), weighing between 4.0 ± 0.3 kg were used. A suspension of $17.3 \pm 3.3 \times 10^6$ radiolabeled cells was slowly administered via intravenous to three of them (n = 3). A suspension of $17.6 \pm 3.5 \times 10^6$ of radiolabeled cells in 0.5 ml of PBS was slowly administered in the knee of the three remaining rabbits (n = 3) by intra-articular injection. The joint was approached by the lateral aspect