Review Article

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Tracking mesenchymal stem cells using magnetic resonance imaging

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Abstract:

Recent translational studies in the fields of tissue regeneration and cell therapy have characterized mesenchymal stem cells (MSCs) as a potentially effective and accessible measure for treating ischemic cerebral and neurodegenerative disorders such as stroke, Parkinson's disease, and amyotrophic lateral sclerosis. Developing more efficient cell tracking techniques bear the potential to optimize MSC transplantation therapies by providing a more accurate picture of the fate and area of effect of implanted cells. Currently, determining the location of transplanted MSCs involves a histological approach, but magnetic resonance imaging (MRI) presents a noninvasive paradigm that permits repeat evaluations. To visualize MSCs using MRI, the implanted cells must be treated with an intracellular contrast agent. These are commonly paramagnetic compounds, many of which are based on superparamagnetic iron oxide (SPIO) nanoparticles. Recent research has set out characterize the effects of SPIO-uptake on the cellular activity of *in vitro* human MSCs and the resultant influence that respective SPIO concentration has on MRI sensitivity. As these studies reveal, SPIO-uptake has no effect on the cellular processes of proliferation and differentiation while producing high contrast MRI signals. Moreover, transplantation of SPIO-labeled MSCs in animal models encouragingly showed no loss in MRI contrast, suggesting that SPIO labeling may be an appealing regime for lasting MRI detection. This study is a review article. Referred literature in this study has been listed in the reference part. The datasets supporting the conclusions of this article are available online by searching the PubMed. Some original points in this article come from the laboratory practice in our research centers and the authors' experiences.

Key words:

Cell tracking, human mesenchymal stem cells, hypoxia, ischemia, magnetic resonance imaging, superparamagnetic iron oxide

Introduction

Recent translational studies in the fields of tissue regeneration and cell therapy have characterized mesenchymal stem cells (MSCs) as a potentially effective and accessible measure for treating ischemic cerebral and neurodegenerative disorders such as stroke, Parkinson's disease, and amyotrophic lateral sclerosis.[1-10] Commonly isolated from bone marrow, MSCs are a type of multipotent progenitor cell responsible for the repair and replacement of tissues with mesenchymal origins, such as cartilage, adipose, and bone.[11,12] These cells are readily obtained and exhibit an ease of expansion while also retaining the facility to differentiate into a variety of cellular phenotypes, including chondrocytes, osteoblasts, and neural cells.[13-15] MSCs are appealing not only for their potential to differentiate but also because they are known to produce extracellular stimulatory factors that mollify inflammatory conditions as well as factors that promote neuronal growth when implanted within damaged neural cultures.[16] In fact, the weight of experimental evidence has begun to suggest that the primary role of MSCs may not be to serve as direct replacement cells for injured tissues but rather to generate a conducive microenvironment for tissue regeneration through the secretion of trophic factors.[17] These beneficial properties considered in light of the ability of MSCs to cross the blood–brain barrier afford this cell type immense therapeutic potential.[1,18] Specifically, the neuroprotective, anti-inflammatory, and pro-angiogenic properties of MSCs indicate that their most effective use may be in the repair and regeneration of neural tissues.[2,3,5,6,18-25] Notably, MSC transplantation in ischemic animal models induced by middle cerebral artery occlusions (MCAO) and cardiac arrest resulted in significant therapeutic benefits, including lesion volume reductions and cognitive improvements.[5,19-21,26] However, despite the

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promise of MSC-based therapies, a number of obstacles must still be overcome for them to achieve clinical success, including the improvement of cell survival and delivery.

Developing more efficient cell tracking techniques bears the potential to optimize MSC transplantation therapies by providing a more accurate picture of the fate of the implanted cells together with potential impact on the lesioned area. Currently, determining the location of transplanted MSCs involves a histological approach, but magnetic resonance imaging (MRI) presents a noninvasive paradigm that permits repeat evaluations.[27-32] To visualize MSCs using MRI, the implanted cells must be treated with an intracellular contrast agent. These are commonly paramagnetic compounds, many of which are based on superparamagnetic iron oxide (SPIO) nanoparticles. SPIOs are nano-sized iron oxides which form single magnetic domains and are fabricated with a surrounding low molecular coating of dextran or carboxydextran. SPIOs induce dephasing of proximal ^{1}H spins when exposed to an external magnetic field due to susceptibility effects. It results in signal loss in and around the location of the particle, increasing the contrast and improving the detectability of cells labeled with these compounds. Accordingly, SPIOs are T_2 or T_2* agents. While previous work has indicated that SPIOs are biologically harmless, the methods used to encourage their uptake and the concentration of the transfected SPIO differ to a significant degree across studies.[33-35] In some cases, this inconsistency has led to conflicting results, such as those produced using SPIO labeling in human MSC (hMSC) osteogenic differentiation.[36-38] For these reasons, the long-term effects of SPIO labeling across various dosages and durations must be examined to determine if SPIOs affect the potential for differentiation and/or survival of hMSCs.

The Concentration of Transfected Superparamagnetic Iron Oxides Affects the Magnetic Resonance Imaging Sensitivity and Potential for Cytotoxic Damage of Labeled Human Mesenchymal Stem Cells

A recent study by Rosenberg *et al*. set out to characterize the effects of SPIO-uptake on the cellular activity of *in vitro* hMSCs and the resultant influence that respective SPIO concentration has on MRI sensitivity.[39] Importantly, cells were transfected via an acute exposure (6-h) to varying concentrations of SPIO without the use of transfection agents or penetrating peptides. Cells were then cultured and allowed to proliferate for up to 14-d, wherein the long-term cell viability, proliferation, and MRI sensitivity of these cultures were investigated. To determine whether SPIOs might encourage further cytotoxicity in already cytotoxic sites of ischemic injury, SPIO loading in hMSCs was examined in low-serum, hypoxic cultures as well. In addition, the researchers employed an animal model, MCAO rats, to evaluate the localization of MSCs using MRI and histological techniques. The results of the study suggest that cellular processes such as proliferation and differentiation were not influenced by any of the SPIO concentrations examined during a cell culturing period of 14 days.[39] In addition, a 6 h incubation time and low SPIO exposure level were sufficient for long-term MRI detectability. Notably, high SPIO exposure opened a cell to higher incidence of calcification and cytotoxicity within *in vitro* ischemic conditions than did low SPIO exposures.[39] Transplantation of SPIO labeled MSCs in animal models encouragingly showed no loss in MRI contrast, suggesting that SPIO labeling may be an appealing regime for lasting MRI detection, with the corollary that high concentrations of SPIO may impact the survival of MSCs in ischemic implantation areas.

Discussion

SPIOs are regularly used as intracellular contrast agents in hMSC labeling and are considered to be appreciably biocompatible, effecting minimal influence on crucial cellular processes such as differentiation and proliferation.[27,30,33,40,41] However, while many studies have examined the success of short-term detection of SPIOs between 0 and 72 h after transfection, few have examined the long-term MRI detectability of SPIO-exposed hMSCs.[27,30,42-44] Importantly, hMSCs may survive more than 7 days after implantation, so long-term studies are important to understanding the full potential of SPIO detection and the efficacy of tracking hMSCs over the course of chronic treatments.[19,45] Moreover, hMSCS are increasingly being used to treat ischemic and cerebral injuries on account of their anti-inflammatory and pro-angiogenic properties.[2,3,5,46,47] Nevertheless, how nutritionally deficient and hypoxic molecular microenvironments at sites of ischemic insult may affect the detectability and survival of SPIO-labeled hMSCs has yet to be resolved. Determining the relative MRI detectability of SPIO-labeled hMSCs after 7 days and their viability within ischemic microenvironments is vital to advancing the current state of hMSC treatments.

The Relationship between Superparamagnetic Iron Oxide Uptake and *In vitro* Magnetic Resonance Imaging Detectability

The results of previous studies and recent work by Rosenberg *et al*[39]. suggest that SPIO incorporation in hMCS is exposure-dependent.[25,35,48,49] That is to say, hMSCs incubated in SPIO-infused media exhibited a near linear increase in the amount of SPIO they incorporated as anticipated by the relative concentrations of SPIO in their culture media. Importantly, this result indicates that SPIO-induction in hMSCS is possible without chemical modification of SPIOs or hMSCs. In previous studies, to improve the success of their incorporation in hMSCS, SPIOS have been modified with cell-specific receptors, poly-L-lysine (PLL), dextran, liposomes, lectin, chitosan, starch, and polystyrene.[33,50-58] The studies by Rosenberg *et al*[39]. and other recent investigations reveal that modification of SPIOs with transfection factors or CPP is inessential. Moreover, media-based induction may work to preserve hMSCS functionality, as different CPPs such as PLL are noted as having the potential to alter or interfere with natural cell function by coating cell surfaces.

Defining the specific concentration of SPIO that both allows for long-term detectability while conserving normal cell behavior will help determine the optimal method for media-based labeling of hMSCs. As evidenced by the MRI results in the investigation by Rosenberg *et al*[39], relatively low initial internalized iron concentrations enable hMSC detection over

a complete 14-day detection period. Moreover, the highest SPIO concentration, while producing the greatest initial contrast in cell imaging, appeared to stimulate an increase in cell proliferation. Previous studies have demonstrated that SPIOs may affect the cell cycle of hMSCs leading to observed cases of elevated growth rates in labeled cells.[59,60] Therefore, while a high initial concentration of internalized iron may maintain the long-term detectability of SPIO-labeled hMSCs, this condition may influence cell proliferation in ways that could impact methods of cytotherapeutic monitoring, such as contrast-based *in vivo* cell counts.

Human Mesenchymal Stem Cell Proliferation, Differentiation, and Survival is Minimally Effected by Low Concentrations of Superparamagnetic Iron Oxide

The influence of SPIO labeling on hMSC proliferation and multipotential is vital for selecting the dosage and scheduling of iron labeling for hMSC phenotypes and their functional characteristics. In the study by Rosenberg *et al*[39]., the hMSC proliferation displayed similar growth patterns as nonlabeled control cells, with no statistical significance found between the various dosages of iron for each time point, signifying insignificant long-term effects of SPIO labeling on hMSC proliferation for lengthy culture duration. Kim *et al*. and Arbab *et al*. have reported negligible effects of SPIO labeling in the presence and absence of CPP on hMSC proliferation with an SPIO concentration approximately 12.5-50μg Fe/mL.[35,61] When a colony-forming unit fibroblast (CFU-F) assay was also conducted following the 14 times point, there was also no statistical significance in CFU-F values, implying a marginal effect of SPIO labeling for hMSC progenicity. These results were additionally verified by the real-time polymerase chain reaction results that exhibited similar expression of Oct-4 and Rex-1. Balakumaran *et al*. also showed minimal effects of SPIO labeling on hMSC stemness using *in vitro* and *in vivo* analyses.[62] There are many past studies that have evaluated these surface markers via flow cytometry for internalized SPIO levels at higher levels than introduced by Rosenberg *et al*., generally through the administration of nonspecific and specific CPP.[35,39,63,64] Comprehensively, these investigations have found no variations in positive surface markers related to MSCs and only minute alterations of negative markers (e.g., CD45), which can be affected by extended culture periods independently.[35]

Rosenberg *et al*. showed that labeled hMSCs demonstrate distinctive ALP expression, with a peak at 14 days followed by a decrease from 14 to 21 days.[39] Chen *et al*. recorded decreased ALP expression by hMSCs labeled with SPIO in a dose-dependent manner while Lee *et al*. demonstrated analogous in ALP expression following 7 days in osteogenic induction media.[38,44] ALP expression readies hMSCs for osteogenic differentiation; however, its sole expression is not adequate to conclude the degree of osteogenic differentiation.[65] It is of note that calcification of the SPIO-labeled hMSCs is effected by SPIO labeling at 21 days though statistical insignificance was found between the groups at 14 days. Previous investigations have demonstrated minimal influence of SPIO labeling on calcification, but the induction for these studies was often <2 weeks.[38,44] The outcome of the current study implies that SPIO labeling has minimal effects on hMSC osteogenic commitment; it does, however, advance calcification following long-term exposure to osteoinductive cues.[39] Further studies to explore the exact mechanism are needed because calcification is a primary concern in stem cell therapy for ischemic cardiovascular and cerebral diseases.[66]

In Rosenberg *et al*.'s study, lactate dehydrogenase (LDH) was introduced *in vitro* via SPIO-labeled hMSCs under serum and oxygen depletion that specifies a quantitative measurement for hMSC survival during a simulated *in vivo* setting.[39] Serum removal had negligible effects on hMSC survival, measured by LDH discharge during the first 24 h, followed by a surge at 36 h. Of note, the combination of low-serum and low-oxygen conditions results in a considerable uptick in LDH release and higher SPIO concentrations representing the highest LDH levels at the 24 h period. The definite mechanism of SPIO dosage-dependent for *in vitro* ischemic circumstances has yet to be defined for hMSCs. Yet, Soenen *et al*. demonstrated that stem-like neuroprogenitor cells and escalated SPIO concentrations transfected with external agents, also that internalized dextran-coated SPIOs exhibited an instant and beneficial effect on ROS levels while exhibiting elevated transferrin receptor-1 expression.[59] An ischemic-hypoxic condition would be likely to disturb natural ROS levels, and with the possible effects of internalized SPIOs, labeled hMSCs could be jeopardized when transplanted into the environment. With translation to the clinic in mind for ischemic therapies, further investigations are warranted to assess any possible internal contrasting agent under ischemic-hypoxic influences.

Magnetic Resonance Imaging Detection of Transplanted Superparamagnetic Iron Oxide-labeled Human Mesenchymal Stem Cells Appears Highly Effective

Using rhodamine-conjugated SPIOs for labeling of hMSCs exhibits that particles are found in the perinuclear region, most likely internalized within endosomes or lysosomes, as previously shown.[35] In addition, the covalent bonding, of carboxyflourescein succinimidyl ester (CFSE) treated hMSCs, within the cytoplasm allows for it to remain within the cells for long periods of time. Co-localization of the CFSE and rhodamine signals was evident within the stroke-induced hemisphere and was 2.7 more prevalent in the affected hemisphere.[39] However, nuclei not linked to CFSE will also display rhodamine coloration, signaling the release of SPIOs, which is likely a consequence of hMSC death resulting in endogenous microglia/macrophage uptake of SPIOs *in vivo*.[29] hMSCs were also shown to dispel intracellular iron within 7 days of transplantation, likely a result of asymmetric cell division while the transplanted cells replicated while migrating. As previously stated, hMSCs do not proliferate in the brain for 5–10 days *in vivo*. Therefore, the fading MRI contrast and dispelling of intracellular iron are possibly connected to cell death rather than through proliferation of hMSCs. IA injection provides a more unabated pathway to the ischemic lesion in the brain in comparison to intravenous injection, in which cells can be taken by other systemic organs.[67,68] Walczak *et al*. observed extreme inconsistency in cell transplantation intended for the brain using a similar protocol with SPIO-labeled hMSCs.[69] This indicated

that the current transplantation method has only moderate to low efficiency. Yet, it should be noted that Walczak *et al*. utilized PLL to encourage SPIO uptake which should augment signal voids visible on the MRI.

Conclusion

The abbreviated incubation period and minimal SPIO exposure dose utilized in Rosenberg *et al*.'s investigation was significant for recognition in agarose tissue imitating phantoms for a 2-week period with minimal effects on differentiation and proliferation, excluding the osteogenic cues. However, once the SPIO-labeled hMSCs were introduced to a hypoxic and ischemic environment, there was a significant reduction in viability in comparison to the unlabeled hMSCs. These discoveries need to be considered when using hMSCs in ischemic animal models. Further research must be directed at developing methods to precondition hMSCs to assess the mechanism of cellular function and improve the viability of cell transplantations into ischemic regions.

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Conflicts of interest
There are no conflicts of interest.

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