International Journal on Magnetic Particle Imaging
Vol 12, No 1, Suppl 1, Article ID 2603012, 3 Pages

Infinite Science Publishing

Proceedings Article

MPI reveals injection route-dependent biodistribution of human mesenchymal stem cells in EAE mice

Ali Shakeri-Zadeh[a,b] · Kritika Sood[a,b] · Aline Thomas[a,b] · Chengyan Chu[c] · Piotr Walczak[c] · Jeff W.M. Bulte[a,b,*]

[a]The Russell H. Morgan Department of Radiology and Radiological Science, Division of MR Research, The Johns Hopkins University School of Medicine, Baltimore, MD, USA
[b]Cellular Imaging Section and Vascular Biology Program, Institute for Cell Engineering, The Johns Hopkins University School of Medicine, Baltimore, MD, USA
[c]Program on Image-Guided Neurointerventions, Department of Diagnostic Radiology and Nuclear Medicine, University of Maryland, Baltimore, MD, USA
*Corresponding author, email: jwmbulte@mri.jhu.edu

Abstract

Magnetic particle imaging (MPI) enables real-time and quantitative tracking of magnetically labeled therapeutic cells with remarkable sensitivity and no tissue background. Human mesenchymal stem cells (hMSCs) possess potent immunomodulatory and regenerative effects in multiple sclerosis (MS), but their efficacy depends on precise delivery and homing to inflamed neural tissues. Using an experimental autoimmune encephalomyelitis (EAE) mouse model for MS, we dynamically tracked ferucarbotran-labeled hMSCs following four sequential intravenous (IV) or intra-arterial (IA) injections. Minute-timescale MPI acquisitions revealed that IV-injected cells were retained predominantly in the pulmonary space with gradual redistribution to the spleen and liver, consistent with remote immunomodulatory activity via splenic engagement. In contrast, IA delivery achieved immediate cerebral accumulation with strong brain signal and concurrent splenic uptake. These findings demonstrate route-dependent biodistribution patterns and confirm the usefulness of MPI for longitudinal assessment of stem cell delivery.

I. Introduction

MPI offers quantitative tracking of superpara-magnetic iron oxide (SPIO)-labeled cells with high sensitivity, zero tissue background, and unlimited depth penetration, making it a powerful modality for assessing the biodistribution of therapeutic cells [1]. hMSCs have shown promising immunomodulatory and regenerative effects in MS, but their therapeutic efficacy depends critically on successful homing to inflamed neural tissues [2]. IV administration is clinically preferred for its simplicity and systemic delivery, yet it often results in pulmonary trapping and limited brain accumulation. Notably, IV-injected hMSCs can exert remote immunomodulatory effects through splenic accumulation and cytokine modulation [3]. In contrast, IA injection may enhance delivery to the central nervous system but carries a risk of vascular occlusion. We employed MPI for the first time to perform dynamic whole-body tracking with minute-scale temporal resolution of SPIO-labeled hMSCs following four sequential IV or IA injections in EAE mice.

10.18416/ijmpi.2026.2603012

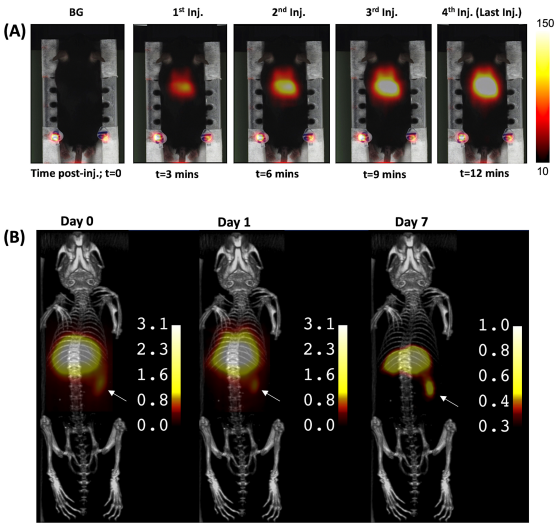

Figure 1: **(A)** Dynamic MPI of SPIO-labeled hMSCs following four sequential IV injections. Time labels indicate minutes elapsed after acquisition of the background (BG) image obtained immediately prior to the first injection. **(B)** Longitudinal 3D MPI/CT overlays demonstrate persistent splenic signal (white arrows) from Day 0 to Day 7. Color bars indicate MPI signal intensity (a.u.).

II. Methods and materials

EAE was induced in 4 to 6-week-old female C57BL/6 mice using an $MOG_{35–55}$/CFA Emulsion kit (EK-0111, Hooke Laboratories). Fourteen days after induction, mice were catheterized via the tail vein or internal carotid artery ICA) for IV and IA injections, respectively (n=3 per cohort). IA injections were performed using a previously established ICA catheterization protocol [4]. hMSCs were labeled with ferucarbotran (25 µg Fe/mL) complexed with poly-L-lysine (3 µg/mL). Mice received IV injections totaling 5.0×10^5 hMSCs or IA injections totaling 1.2×10^5 hMSCs, each delivered as four equal sequential doses to enable dynamic MPI tracking. Cell numbers for both fiducial calibration and *in vivo* injections were determined using an AO/PI viability assay with automated cell counting (LUNA cell counter). The lower IA dose was selected based on pilot studies establishing a tolerated cell number for carotid artery delivery, whereas higher cell numbers could be safely administered IV. The biodistribution of injected cells was monitored using a Momentum MPI scanner in standard imaging mode. For 2D whole-body mouse imaging, a field of view of 12 × 6 cm was used, with single signal averaging per frame. Three-dimensional MPI was acquired using 21 projection angles, reconstructed over the same field of view as the 2D scans. A calibration curve for signal quantification was generated using two fiducials containing 10,000 and 20,000 labeled hMSCs. CT imaging was performed on an IVIS Spectrum/CT system to provide anatomical reference. MPI/CT data were co-registered with 3D Slicer software.

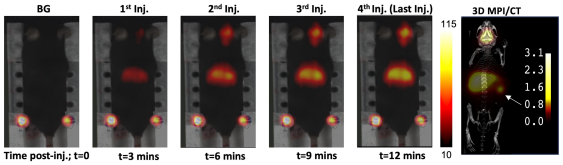

Figure 2: Dynamic MPI of labeled hMSCs following four sequential IA injections. Serial 2D MPI images show progressive signal accumulation in major organs. Time labels indicate minutes elapsed after acquisition of the BG image obtained immediately prior to the first injection. The rightmost MPI/CT image was acquired 30 minutes after completion of the fourth IA injection, confirming splenic localization (white arrow. Color bars indicate MPI signal intensity (a.u.).

III. Results and discussion

Sequential imaging after each of the four incremental IV injections showed progressive signal intensification within the upper-thoracic region (Figure 1A), confirming stepwise cell delivery and minimal systemic redistribution during the injection period. Longitudinal *in vivo* MPI/CT co-registration (Figure 1B) demonstrated gradual clearance of pulmonary, hepatic, and splenic signals over seven days. These findings, also verified by *ex vivo* MPI, suggest that IV-injected hMSCs predominantly localize in the lungs initially, with delayed secondary accumulation in reticuloendothelial system (RES), aligning with their known systemic immunomodulatory activity through splenic engagement [3].

Sequential imaging after each of the four incremental IA injections demonstrated progressive enhancement of signal intensity along the brain and possibly carotid regions (Figure 2), indicating effective arterial delivery and immediate access to the cerebral region. The cerebral signal observed after IA injection in EAE mice likely reflects a combination of transient intravascular sequestration and early tissue-associated localization, with ongoing histological analyses aimed at further resolving these mechanisms. 2D dynamic MPI also captured rapid lower-thoracic distribution within minutes. 3D MPI/CT revealed strong localization of MPI signal in the brain with secondary accumulation in the liver and spleen (Figure 2). These results highlight that IA injection achieves substantially greater early brain delivery of hMSCs compared to IV administration. Both IV and IA delivery led to cell accumulation in the spleen. This was visualized as persistent focal MPI signal in the splenic region, indicating active homing or sequestration of labeled hMSCs within the RES.

10.18416/ijmpi.2026.2603012

IV. Conclusion

MPI enabled real-time visualization of hMSC biodistribution following IV and IA delivery. These results highlight the unique ability of MPI to resolve early biodistribution differences driven by delivery route, which are difficult to capture using conventional imaging modalities that lack the combination of near real-time imaging, whole-body coverage, and linear quantification. Both routes led to splenic accumulation, while IA injection enhanced brain-targeted delivery. Future work will focus on quantitative comparison of IV and IA delivery, including relative signal differences, fold-changes in organ-specific accumulation, and kinetic metrics such as signal decay or apparent residence half-life derived from longitudinal MPI measurements.

Acknowledgments

We thank Dr. Y. Li for experimental assistance. This study was funded by MSCRF (2023-MSCRFL-6270) and NIH (2P41 EB024495, S10 OD026740).

Author's statement

Conflict of interest: J.W.M.B is a shareholder of SuperBranche. This has been approved by JHU.

References

[1] Shakeri-Zadeh A, Kuddannaya S, Chu C, Sood K, Itoo A, Zivko C, et al. Fast dynamic whole-body in vivo cytometry using magnetic particle imaging. bioRxiv 2025:DOI: 10.1101/2025.09.11.675624.

[2] Gavasso S, Kråkenes T, Olsen H, Evjenth EC, Ytterdal M, Haugsøen JB, et al. The therapeutic mechanisms of mesenchymal stem cells in MS—a review focusing on neuroprotective properties. International journal of molecular sciences. 2024;25(3):1365.

[3] Levy O, Kuai R, Siren EM, Bhere D, Milton Y, Nissar N, et al. Shattering barriers toward clinically meaningful MSC therapies. Science advances. 2020;6(30):eaba6884.

[4] Chu C, Jablonska A, Gao Y, Lan X, Lesniak WG, Liang Y, et al. Hyperosmolar blood–brain barrier opening using intra-arterial injection of hyperosmotic mannitol in mice under real-time MRI guidance. Nature protocols. 2022;17(1):76-94.

