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In vivo MPI tracking of ICV-injected mesenchymal stem cells in an EAE mouse model of multiple sclerosis

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Abstract

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system (CNS). Human mesenchymal stem cells (hMSCs) provide therapeutic benefits through their immunomodulatory and neuroprotective effects. This study investigated the biodistribution of magnetically labeled hMSCs following intracerebroventricular (ICV) injection in the chronic experimental autoimmune encephalomyelitis (EAE) mouse model of MS in vivo using magnetic particle imaging (MPI) in combination with magnetic resonance imaging (MRI) and computed tomography (CT). Our imaging analyses revealed increased MPI signal in both the cerebellum and spinal cord by day 7 post-injection, suggesting distribution of hMSCs via cerebrospinal fluid (CSF) flow. These findings highlight the value of MPI as a quantitative and specific non-invasive tool for longitudinal cell tracking in vivo and provide important insights into the optimization of stem cell–based therapies for MS.

I. Introduction

MS is a chronic inflammatory disease of the CNS characterized by demyelination, axonal loss, and gliosis. hMSCs have emerged as a promising cell-based therapy for MS due to their potent immunomodulatory and neuroprotective properties [1]. Clinical trials have demonstrated that MSC therapy is safe, well-tolerated, and can decrease relapse rates and disability progression in MS patients [2]. Despite encouraging outcomes, several challenges remain in developing MSCs into a widespread therapy, particularly with regards to the delivery route. While intravenous (IV) delivery is clinically convenient, it often results in off-target accumulation in organs such as the lungs, liver, and spleen. In contrast, ICV administration permits direct deposition into the CSF, facilitating widespread hMSC dispersal throughout the brain and spinal cord [3,4]. To understand how the delivery route influences the biodistribution, homing, and therapeutic efficacy of cells, it is essential to employ non-invasive, quantitative imaging methods that can monitor cell biodistribution and migration over time [5]. In this study, we investigated the biodistribution of magnetically labeled hMSCs following ICV injection *in vivo* in an EAE mouse model of MS. By combining MPI, MRI and CT, this study aimed to elucidate the homing patterns and migration dynamics of hMSCs to advance the development of optimized cell-based therapies for MS.

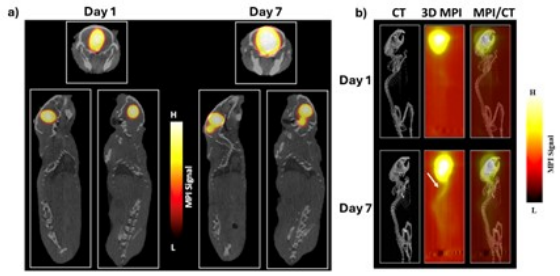

Figure 1: In vivo 3D MPI/CT tracking reveals enhancement in the **(a)** cerebellum and **(b)** spinal cord region at day 7 post ICV injection in EAE mice. Images are of the same mouse in different orientations to highlight signal localization at the cerebellum and spinal cord.

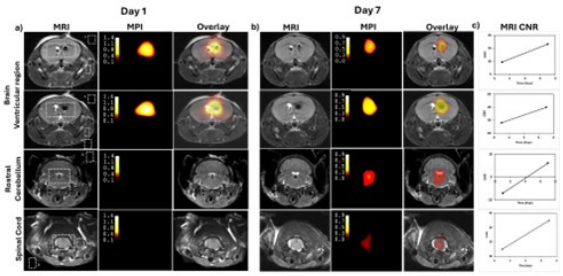

Figure 2: (a-b) *In vivo* 3D MPI/MRI tracking reveals enhancement in the rostral cerebellum and spinal cord region at day 7 post ICV injection in EAE mice. (c) CNR analysis of the MRI data was performed for the corresponding regions over time using the formula CNR = $\frac{R1-R2}{\sigma(B)}$, where R_1= signal intensity in the region of interest, R_2= signal from background brain tissue, σ(B)= standard deviation of signal in the noise region.

II. Methods and materials

EAE was induced in 4 to 6-week-old female C57BL/6 mice using a MOG_{35-55}/CFA Emulsion kit (EK-0111, Hooke Laboratories). Fourteen days post-EAE induction, mice (n=8) received ICV injections of 3.0×10^5 hMSCs labeled with ferucarbotran (25 μg Fe/mL) complexed with poly-L-lysine (390 ng/mL) dispersed in sterile PBS (5 μL) and stereotaxically delivered into the right ventricle (coordinates relative to bregma: AP 0 mm, ML 0.8 mm, DV 2.5 mm) via a 31G Hamilton needle. To prevent immunorejection, mice received daily i.p. injections of 10 mg/kg cyclosporine A, starting one day prior to transplantation and continuing for one-week post-injection. The biodistribution of injected cells was monitored at day 1 and 7 post-injection using a Momentum MPI scanner (Magnetic Insight Inc., USA). Both 2D and 3D MPI data were acquired. The calibration curve for *in vivo* signal quantification was generated using fiducials containing 0, 30,000 and 15,000 labeled hMSCs (y=0.0006x+1.1673, R^2= 0.947). T2-weighted (T2w) MRI and CT imaging were performed on a 11.7T Bruker scanner and IVIS Spectrum CT system, respectively, to provide an anatomical reference. The MPI/CT and MPI/MRI data were co-registered with 3D Slicer software.

III. Results and discussion

MPI/CT (Figure 1) revealed an enhancement of MPI signal intensity in the cerebellum and spinal cord on day 7 compared to day 1 post-ICV injection. This observation suggests the migration of magnetically labeled hMSCs along the neuroaxis during this time-frame.

Our MPI/CT findings were further supported by MPI/MRI co-registered images (Figure 2), which revealed distinct signal localization in the rostral cerebellum and cervical spinal cord region seven days post-injection. The hypointense regions in T2w MRI corresponded to the MPI signal, indicating the presence of labeled hMSCs. Quantitative analysis of the contrast-to-noise ratio (CNR) (Figure 2c) also demonstrated enhanced contrast over time, indicating the migration of labeled cells over time. Cell numbers as measured by 2D MPI decreased from 2.2×10^5 to 1.5×10^5 in the brain and increased from 3.6×10^3 to 9.3×10^3 in the cerebellum/spinal cord region from day 1 to day 7.

IV. Conclusion

This study demonstrates the feasibility of using bimodal imaging (MPI/MRI and MPI/CT) to non-invasively track the biodistribution and migration of magnetically labeled hMSCs following ICV injection *in vivo* in an EAE mouse model of MS. The observed accumulation of hMSCs in the cerebellum and spinal cord over time highlights the potential of the ICV route to facilitate targeted cell delivery along the entire neuroaxis. These findings highlight the value of MPI as a quantitative and sensitive tool for longitudinal *in vivo* cell tracking to optimize stem cell–based therapies.

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Author's statement

Conflict of interest: J.W.M.B is a shareholder of SuperBranche. This arrangement has been approved by Johns Hopkins University. Other authors state no conflict of interest.

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